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Advances in Biofuels and Bioenergy

Edited by Madhugiri Nageswara-Rao and Jaya R. Soneji

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Meet the editors

Madhugiri Nageswara-Rao, PhD, works in the areas of plant breeding, genomics, bioenergy, genetic engineering, disease diagnostics, population, and eco-evolutionary genetics. He is the author of peer-reviewed research articles, book chapters, popular articles and has guest-edited special issues for journals, edited books and newsletters. He was an Adjunct Faculty at the Polk State College, USA. His work has been broadcasted on Fox News, USA. He was invited by CBC Radio, Canada, to speak on air. He has served in the "Executive Committee" of GII. He was recognized as "Young Scientists" by Bioclues, in "Member in Spotlight" of GII and featured in ASPB News. The University of Florida's International Programs appraised his contribution in "International Focus." He has peer-reviewed manuscripts for prominent international journals and grant proposals for international institutions. He obtained his BSc and MSc degrees from the Bangalore University and his PhD degree from FRI, India. He was featured as "Tomorrow's Principal Investigators: Rising Young Investigators" by Genome Technology, USA. He secured "Silver Award" as a team member from the American Museum of Natural History, USA. He was also selected for AAAS/Science Program for Excellence in Science. He has served on the editorial boards of six journals.

Jaya R. Soneji, PhD, works in the areas of plant breeding, genomics, bioenergy, genetic engineering, population, and eco-evolutionary genetics. She is the author of peer-reviewed research articles, book chapters, popular articles and has guest-edited special issues for journals, edited books and newsletters. She was an Adjunct Faculty at the Polk State College, USA. Her work has been broadcasted on Fox News, USA. She was invited by CBC Radio, Canada, to speak on air. She has served in the "Executive Committee" of GII. She was recognized as "Young Scientists" by Bioclues, in "Member in Spotlight" of GII and featured in ASPB News. The University of Florida's International Programs appraised her contribution in "International Focus." She has peer-reviewed manuscripts for prominent international journals and grant proposals for international institutions. Dr. Soneji obtained her BSc (second rank) and MSc (second rank) degrees from the University of Mumbai and her PhD degree from BARC, India. She received many scholarships and awards for her academic achievements. She taught plant genetics, physiology, tissue culture and genetic engineering at the Ruia College, India.

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Preface

The worldwide consumption of fossil fuel continues to increase at unsustainable levels, which will lead to progressive scarcity, if immediate and innovative measures are not taken for its sustainable use. This scarcity necessitates the development of renewable and sustainable alternatives for fossil fuels. A possible solution to today's energy challenges can be provided by biofuels. This book intends to provide the reader with a comprehensive overview of the current status and the future implications of biofuels.

Over the years, it has been observed that grain-based ethanol production is not environmentally sustainable. This led to the exploitation of lignocellulosic biomass from perennial grasses as well as microalgae for biofuel production. Perennial grasses can be grown on marginal lands, while microalgae have no requirement for land and can be easily cultivated in wastewaters. The need for better biomass source and recycling of waste has also led to the utilization of non-edible oils from conversion to biofuel. Initiation of waste-to-energy programs, which utilize anaerobic digestion to convert industrial and residential waste into biofuel, is another example of recycling to create energy and will also be helpful in creating the job opportunities, elevating the environmental merits, and preventing the monoculture of fuel resources. Recently, biohydrogen and biohythane seem to be promising future energy carriers due to their potentially higher conversion efficiency and low-pollutant generation.

However, in order to make biofuels a feasible alternative to satisfy market demand, strategic improvements in the areas of supply chain management need to be made. Handling and feeding of materials represent a substantial challenge in biomass feedstock supply systems and have been a primary factor causing pioneer industrial biorefineries to struggle to achieve their production targets. There is also a need to develop effective, responsive and responsible safety standard and to assess the risks such as biohazard, fires and potentially explosive atmospheres for biorefineries. This will improve public trust in the new biofuel generation plants. There is also a need to emphasize on the importance of developing models that are crucial to the design and performance of combustion engines and cover multicomponent fuel atomization, heating and evaporation modeling, which will improve engine sustainability and reduce emissions.

Such aptly and comprehensive information covered in this book will directly enhance both basic and applied research in biofuels and will particularly be useful for students, scientists, breeders, growers, ecologists, industrialists and policy makers. It will be a valuable reference point to improve biofuels in the areas of ecologically and economically sustainable bioenergy research.

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Chapter 1

Bioenergy from Perennial Grasses

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Additional information is available at the end of the chapter

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Abstract

In recent years, the establishment of perennial grasses as energy crops has emerged as a very viable option mainly due to their comparative ecological advantages over annual energy crops. Nonwoody biomass fuels have a great potential to replace fossil fuels and reduce greenhouse gas emissions. At the same time, their application in small-scale combustion appliances for heat production is often associated with increased operational problems such as slagging in the bottom ash or deposit formation, as well as elevated gaseous and particulate matter emission levels. To mitigate these problems, scope and limitation of blending raw materials owing to critical fuel composition with less problematic biomasses have been systematically studied during combustion experiments in a commercially available small-scale combustion appliance. Apart from traditional use, perennial rhizomatous grasses display several positive attributes as energy crops because of their high productivity and low demand for nutrient inputs, consequent to the recycling of nutrients by their rhizomes and resistance to biotic as well as abiotic stresses. Therefore, they are used to generate heat and electricity. In addition, grasses appear to be an economically and environmentally appropriate fuel for generating some local energy in rural areas. This chapter gives an overview on species characteristics, their soil-climate requirements, cultivation technology, yielding, and energy characteristics of lignocellulosic biomass of giant miscanthus (*Miscanthus* × *giganteus*), reed canary grass (*Phalaris arundinacea* L.), switchgrass (*Panicum virgatum* L.), and giant reed (*Arundo donax* L.).

Keywords: bioenergy, biomass, grasses, giant miscanthus, reed canary grass, switchgrass, giant reed, biomass yield

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1. Introduction

The search for an alternative fuel due to environmental concerns and depletion of fossil fuels has raised interest in sustainable energy systems. The utilization of biomass as renewable energy source is becoming increasingly important in the light of its potential for lowering global warming effects and sustainably securing fuel supply [1]. The main challenge in utilization of biomass as fuels would be a stable supply of raw materials [2]. In Europe, wood fuels (e.g., log wood, wood chips, and wood pellets) are the predominant biomass fuels for small-scale heating. However, in several regions, the rapid increase in wood pellet production resulted in shortage of raw materials [3, 4]. Wood assortments are also considered as promising raw materials for the growing biorefinery sector; therefore, this competition is expected to significantly increase in the future, resulting in an increase in raw material costs [5]. Thus, to fulfill the anticipated growth of biomass utilization, expected worldwide, a wider assortment of raw materials will be required including low-quality wood fuels (e.g., logging residues, short rotation coppice) and nonwoody biomasses [6]. Within the available biomass sources, there has been an increasing interest in the use of perennial grasses as energy crops. In order to achieve a positive energy balance, the condition for a plant species to be a potential energy crop is that its bioenergy yield must be produced with a low level of inputs that require minimal energy for their own production and utilization [7]. In this context, perennial rhizomatous grasses display several positive attributes as suitable energy crops. The characteristics which make perennial grasses attractive for biomass production are their high-yield potential and the high contents of lignin and cellulose of their biomass. The biomass of perennial grasses has higher lignin and cellulose contents than the biomass of annual crops [8]. These characteristics are desirable when used as solid biofuels, mainly because they have a high heating value associated with the high carbon content in lignin and, also, strongly lignified crops have the advantage of remaining stand upright with low water content. Therefore, its biomass has lower water content and a late harvest is possible to improve the quality of the biomass. From the point of view of crop management, high yields of biomass from perennial grasses are possible, but the quality of combustion is lower than that of wood products. Compared to stem wood, all these materials are usually characterized by higher ash content and a large variation in the composition of ash-forming elements. Therefore, the use of perennial grasses as fuel usually requires a greater maintenance of the boiler due to the particular characteristics of this type of biomass [9]. The chemical composition of the biomass is highly influenced by the date of harvest as well as by the procedure to make the bales, the condition of the soil, and the population of the plant. High ash content in the raw material will increase slagging tendency during combustion and also will cause high abrasions during the processes of grinding and densification. High contents of N, Cl, and S are mainly related to technical problems during the combustion process and to the increase of polluting emissions [9].

2. Characteristics and management of perennial grasses for energy production

Compared to other biomass sources, like woody crops and other C3 crops, C4 grasses may be able to provide more than twice the annual biomass yield in warm and temperate regions

because of their more efficient photosynthetic pathway [10]. Furthermore, the need for soil tillage in perennial grasses is limited to the year in which the crops are established, which is an advantage over annual crops. The advantages of the long periods without tilling are reduced risk of soil erosion and a likely increase in soil organic matter content. In addition, due to nutrient recycling by their rhizome systems, perennial grasses have a low nutrient demand [11]. Since they are affected by few natural pests, they may also be produced with little or no pesticide use. Furthermore, there are many environmental benefits expected from the production and use of perennial grasses. The substitution of fossil fuels by biomass is an important contribution to reduce CO_2 emissions.

Perennial grasses have many benefits as an energy crop. They are easy to grow, harvest, and process. Grasses is a "traditional agricultural crop" that does not need any special equipment, and the same could be used as for hay production. Perennial grasses are long-lived and thus do not need to be planted each year. In addition, it is not necessary to plow the soil every year, which leads to less soil disturbance. Grasses have several advantages as raw materials for fuels, since they conveniently occur throughout the world in a wide range of climates, geographies, and types of soils, and additionally, they sequester and store large amounts of carbon in the root systems and in the soil. Grasses can be grown on marginal lands unsuitable for continuous crop production or on open rural lands that currently are abandoned or underutilized. They yield more biomass per hectare and require much fewer inputs compared to annual crops that require more fertilizers, pesticides, and fuels. Perennial grasses are being used as a solid fuel in co-fired coal power plants and are also selected as the raw material for advanced biofuels such as cellulosic ethanol. The dry biomass of perennial grasses can also be densified and transformed into pellets and briquettes, which have uses as heating fuel to replace or supplement fuels made of wood fibers. The inclusion of a thermal component in the use of solid biomass for energy increases the efficiency of the combustion system more than three times [12].

In general, grasses grown as energy crop are managed for biomass yield rather than forage or nutritive quality. Grass biofuel requires minimum management expertise. It is as well suited to small farms as it is to large farming operations, and also works for all levels of management intensity. In fact, lower levels of nutrients such as N, S, K, and Cl may improve fuel quality and reduce emissions. The growth and yield of the grass crop depends significantly on several factors such as soil conditions, fertility, moisture, weed as well as pest control, and the timing of harvest. During the growing season of the grasses, the moderate use of fertilizers may be necessary to maintain soil fertility and to improve crop biomass production [13].

Good weed control in the first year of an establishment is critical to achieve a successful establishment. For example, switchgrass (*Panicum virgatum* L.) seedlings are slow to establish and are susceptible to competition from weeds. Emergence can take several weeks, depending on soil temperatures and moisture. It is critical that perennial weeds are eliminated from the fields prior to planting. To prevent competition from these species, it is important that cultural or chemical weed control is performed to ensure that the field is free of weeds. Nitrogen fertilizer is not recommended in the first year to reduce grass weed competition. Manure nutrients can be applied in the spring or anytime following grass harvest, as long as the grass is still actively growing.

The grass biomass should be harvested once per year, for which standard hay production equipment can be used. Grasses cut in the fall and left to overwinter produce less biomass, but have the advantage of leaching potassium and chlorine, two minerals that may create issues during combustion [13].

3. Grass biomass combustion

The combustion of grasses normally produces more ashes than the combustion of wood. The range in total ash content of grasses can be very wide, from 2% to greater than 20% [14]. Ash values higher than 10% in mature grasses are generally the result of excessive surface soil contamination. The issue of primary concern when burning grass is mineral composition that determines the melting point of ash and the potential for corrosion [15] and also elevated gaseous and particulate emission levels contributing to deposit formation or high-temperature corrosion as well as operational problems resulting from low ash-melting temperatures. High ash content or low ash-melting temperature poses technical issues through deposition, sintering, fouling, slagging, and corrosion. The latter can damage boilers and increase maintenance costs and can cause severe operation problems usually above 850–1000°C [14, 16]. Several indicators affect the ash-melting temperature such as nitrogen fertilizer used on the crop, meteorological conditions, and chemical composition [17]. The ash-forming elements potassium (K), phosphorus (P), chloride (Cl), silicon (Si), calcium (Ca), and sulfur (S) contribute to the abovementioned ash-related mechanical problems [18–20]. Silica is the major component of ash and is found in much higher concentrations in the leaf and inflorescence, compared to the grass stem [21], and the silicon content of the biomass ash may sum up to more than 90 wt% [18, 22, 23]. Silica can combine with alkali metals to form silicates that melt at lower temperatures [16]. K and Cl are the most problematic minerals, and both are consumed in high concentrations by the grasses. K is the most abundant alkali metal in grass biomass [24, 25]. This mineral reduces the melting temperature of the fuel and also contributes significantly to corrosion potential. Chlorine is a particularly undesirable component of grass biomass, as it acts as a catalyst for corrosion reactions and also increases the potential of chlorinated hydrocarbon emissions [26]. Sulfur reacts with alkali metals and forms deposits on heat transfer surfaces, and nitrogen content directly increases NOx emissions. Therefore, reduced concentration of all the abovementioned minerals in grass biomass is highly convenient. To enable and facilitate the utilization of a wide range of grasses in combustion systems, several strategies to mitigate the ash- and emission-related problems have been employed [25]. Appropriate harvesting time and fertilization application can all contribute significantly toward improvement of ash-melting behavior [27]. Potassium and chlorine can be reduced by controlling fertilization of these elements or by leaching them out of grass biomass [28, 29]. The content of some critical elements in fresh grass can be substantially reduced by mechanic dewatering [30]. Nitrogen concentration can be reduced by harvesting mature or overwintered forage. On the other hand, silica can be minimized by using warm-season grasses or by growing grass biomass on a sandy soil. Reduction of ash content and relative amount of critical elements can also be achieved by blending with less problematic biomass fuels such as wood, miscanthus, or peat [31].

Usually, additives are used in addressing the low ash-melting temperatures and the release of critical elements in the flue gas [32]. Using this strategy, slagging is reduced by the introduction of compounds that capture problematic ash components forming higher melting compounds or by diluting the ash with inert, high melting materials [33]. Zeng et al. [34] stated that significant reduction of the slagging risk during combustion of herbaceous fuels can only be achieved for high blending ratios with more than 70 wt% wood.

4. Densification of grasses

Grasses have low energy density (MJ m^{-3}) and low yield per unit area (dry tons ha^{-1}). Volumetric energy content of grasses used for biofuels is considerably lower than traditional fossil fuel sources, and this low energy density is due to low bulk densities of biomass materials [8]. Often, long distances have to be bridged between the biomass place of origin and the place of its utilization, resulting in expensive handling and transportation. Transportation costs of low-density grasses which increase the total cost of biomass processing are an important limitation to their use as an energy source [35]. To increase the bulk density of grasses, they can be densified into pellets using a mechanical process [35, 36]. Therefore, the densification of grasses is an important issue to improve the transport, storage, and handling capabilities of this lignocellulosic material. Densified biomass, especially pellets, has drawn attention due to its superiority over raw biomass in terms of its physical and combustion characteristics. With the international quality standard [37] for nonwoody biomass pellets, the foundation for an increasing commercial utilization of a wide range of biomass such as grasses was laid in 2014. Pellets have multiple end-use applications which range from smaller scale combustion for residential heating to an industrial scale where grass pellets could be co-fired with coal at power plants [38]. The increased demand of pelleted fuel sources in Europe and North America could allow for more nonwoody biomass resources such as perennial grasses to be used for pelletization. One of the most important variables in pellet production is moisture content, since this property will finally determine the durability and density of pellets [36, 39]. A less-expensive method of densification method (higher yield per hour) is by forming the grass into larger briquettes, also called tablets or cubes, which allows to manipulate and store the material easily, and they can also be transported economically and burned efficiently.

5. Description of the main perennial grasses

5.1. Miscanthus

5.1.1. Origin and distribution

It has been largely reported that miscanthus originated in East Asia, where it is found throughout a wide climatic range from tropical, subtropical, and warm temperate areas of Southeast Asia to the Pacific Islands as well as at both high and low altitudes [40]. The genotype widely used in Europe for biomass production is *Miscanthus* × *giganteus*, a natural hybrid

of *Miscanthus sinensis* and *M. sacchariflorus*. This natural hybrid is a giant, perennial warm-season grass native to Asia that is generating much enthusiasm for extremely high yields and very high cold tolerance.

5.1.2. General species description

Miscanthus × *giganteus* is a sterile hybrid that does not produce viable seed and therefore propagates vegetatively underground through its rhizomes (by planting underground stems). The rhizomatous C4 grass has been considered as a strong candidate as an energy crop due to its potential to deliver high biomass yields (up to 30 ton ha^{-1}) under low input conditions, and its economic as well as environmental benefits [41–44].

5.1.3. Ecological demands

Because of its C4 photosynthetic pathway and perennial rhizome, *M. giganteus* exhibits a very good combination of radiation, water, and N-use efficiencies for biomass production [44]. Boehmel et al. [45] compared the N-use efficiency of different annual and perennial energy crops and concluded that *M. giganteus* showed a higher N-use efficiency value of 526 kg DM kg^{-1} when compared to the N-use efficiency of maize (65 kg DM kg^{-1}). *M. giganteus* can be grown on a wide range of soils. The most important soil characteristic is the water holding capacity; therefore, sites with stagnant water are unsuitable. The highest yields have been reported in soils with a good water holding capacity. *M. giganteus* begins growth from the dormant winter rhizome when soil reaches temperatures of 10–12°C [46].

5.1.4. Biomass yields and characteristics

The production of aerial biomass depends on the duration of the growth period. After the first year, the start of the growing season depends on the last frost of spring. On the other hand, the end of the growing season depends on the flowering or the first autumn or winter, according to the date of harvest or location [47].

The lifetime of the crop lasts approximately 20 and 25 years [11], during which biomass is produced during two phases: a yield-building phase, which lasts for 2–5 years, depending on climate and plant densities, and a plateau phase where the yield is maintained [48]. When crop water supplies are not limiting, maximum crop yields are reached more rapidly in warmer climates than in cooler climates [47].

Miscanthus stands need between 3 and 5 years to become fully established and reach the maximum yield level [11]. Biomass yields above 30 t DM ha^{-1} have been reported in southern European locations with a high incidence of annual global radiation and high average temperatures, but only under irrigation conditions. Maximum yields of up to 49 t DM ha^{-1} have been observed in Europe during an autumn harvest of mature crops with irrigation. Harvestable yields in the spring are 27–50% lower than those in the autumn [49].

The main characteristics of miscanthus biomass as a fuel are listed in **Table 1**. The main problem of miscanthus biomass as fuel is its relatively low ash-melting point (1020°C). Biomass

Common name	Giant *Miscanthus*	Switchgrass	Reed canarygrass	Giant reed
Scientific name	*Miscanthus x giganteus*	*Panicum virgatum* L.	*Phalaris arundinacea* L.	*Arundo donax* L.
Photosynthetic pathway	C4	C4	C3	C3
Soils	Wide range. Not tolerant to flooding. No soil compactation	Wide range. Drought tolerant. Does not grow well in wet areas	Wide range. Drought tolerant, tolerant to wet areas	Wide range. Prefers well-drained soils with good water supply; also on saline soils
Day length	Long-day plant	Short-day plant	Long-day plant	Long-day plant
Biomass yields (t ha $^{-1}$)*	5–40	5–34	7–14	3–37
Moisture content at harvest (%)	15–60	15–20	10–23	
High heating value (MJ Kg $^{-1}$)*	17–20	17	17–19	15–19
Ash fusion temperature (°C)	1020	1016	1100–1650	1100
Ash (%)*	1.6–4.0	4.5–10.5	1.9–11.5	4.8–7.8

*Dry matter

Table 1. Perennial grasses species with potential as energy crop.

characteristics and quality of miscanthus are mainly a function of location and genotypes. For example, Lewandowski et al. [11] found that the ash contents of the biomass are correlated with high silt and clay content of the soil. In central Europe, miscanthus is harvested at the beginning of spring because the stems are dried during the winter and part of the ash, Cl, and K are leached by precipitation, which substantially improves the quality of the combustion. The most important management tool to improve biomass quality in miscanthus as a fuel is a delayed harvest.

5.1.5. Miscanthus as a bioenergy crop

The main advantages of *M. giganteus* as an energy crop are exceptional adaptability to different edaphoclimatic conditions; feasibility for growing on poor quality soils; high dry matter yields per unit surface; outstanding disease and pest resistance (application of pesticides is not necessary); very low fertilization requirements; herbicides are applied only during the first 2 years of establishment of the crop; and can be grown without any pest or weed control management once the crop is established [50, 51]. The main constrains of *M. giganteus* are its high establishment costs, its poor overwintering at some sites, and the insufficient supplies of water available in southern regions of Europe. It has been found that *M. giganteus* shows very little genetic diversity due to its sterility and vegetative mode of propagation. Most of the clones found in this species were obtained directly from the "Aksel Olsen" clone, as shown by isozyme and DNA studies [52, 53]. The small genetic base of *M. giganteus* is responsible

for the fact that the same clone has almost always been used in most studies or for cultivation. The sterility of *M. giganteus* is particularly interesting because it prevents the risk of invasion of the species; but on the other hand, it is a limitation to improve biomass production and to adapt it to a wide range of climatic conditions [47]. The sterile hybrid *M. giganteus* has to be propagated asexually using plantlets produced in tissue culture (micropropagation) or by rhizome divisions (macropropagation). The optimal planting density is one to two plants per square meter [11]. It has been reported that irrigation during the first growing season significantly improves the establishment rates.

Miscanthus does not respond to N fertilization at several sites in Europe; therefore, N fertilization is necessary only on soils with low N contents. Weed control in miscanthus in the year of planting is crucial for establishing a successful and healthy stand. The first 2 years are most critical, with little weed management thereafter. There are very few labeled herbicides for use on miscanthus crop, but various herbicides suitable for use in maize or other cereals can be used. It can be harvested only once a year, and the harvest window depends on the local conditions. The later the harvest can be made, the better the quality of the combustion, since it will decrease the moisture content and the mineral content of the biomass.

However, there is a trade-off between improving the quality and yield, since yield losses of up to 35% can occur between maximum yield and late harvest in early spring [54]. From an economic point of view, a late harvest with biomass water content lower than 30% is recommended in order to reduce the costs for harvesting and drying of the biomass [55]. Bilandzija et al. [1] state that harvest delays, from autumn to spring, had statistically significant influence on moisture, C, H, O, N, and S contents. They found that delayed harvest enhanced the quality of biomass in terms of combustion process, primarily through lowering moisture content, which is particularly important if biomass producers do not have drying systems.

Given its potential to be exploited for energy purpose, *Miscanthus* × *giganteus* is presently used mostly for electricity or heat generation in direct combustion [56], mostly in the form of wood chips, pellets/briquettes, and bales [57]. It is estimated that replacing fossil fuels with biomass from *Miscanthus* × *giganteus* can enable reducing the CO_2 emission by 75–93% [48]. However, because there is presently only one commercially available clone, *Miscanthus* × *giganteus*, it has some limitations such as a lack of winter hardiness during the establishment period [7] and it needs to be propagated vegetatively resulting in high field plantation costs.

5.2. Switchgrass

5.2.1. Origin and distribution

Switchgrass (*Panicum virgatum* L.) belongs to the Gramineae family. It is native to the North American tall grass prairies. Although generally associated with the natural vegetation of Great Plains and the western Corn Belt, it occurs widely in grasslands and nonforested areas throughout North America east of the Rocky Mountains and from southern Canada down to Mexico and Central America [58].

5.2.2. General species description

Switchgrass is one of the best herbaceous energy crops due to its habit of perennial growth, high yield potential on a wide variety of soil conditions, and compatibility with conventional agricultural practices [59]. Switchgrass has a deep rooting system that contributes to the accumulation of organic matter in the soil and, therefore, carbon sequestration [60]. In full development of the plant, the underground biomass is similar or even greater than the aerial biomass.

Switchgrass can be established through seeds; therefore, it has lower production costs that make it a practical option among the energy crops. However, the switchgrass biomass yield is considered to be lower than that of miscanthus [11].

Switchgrass can grow to more than 3 m height and develop roots to a depth of more than 3.5 m. The inflorescence is a typical open and diffuse panicle of 15–55 cm long. Each panicle consists of many to hundreds of spikelets at the end of long branches, with two dissimilar florets in each spikelet [61]. The expected life of a pasture would be 10 years or more if properly managed. Switchgrass is a cross-pollinated plant that is largely self-incompatible, and most cultivars are tetraploid or hexaploid [62].

5.2.3. Ecological demands

Switchgrass will grow best on well-drained good quality soils but will also sustain lower quality soils and shallow rocky soils. It can grow on sand to clay loam soils and tolerates soils with pH values ranging from 4.9 to 7.6 [63]. It is drought tolerant, but the grass does not grow in locations where precipitation is below 300 mm per year. Switchgrass can tolerate short-term waterlogging.

Switchgrass can be categorized into two groups or ecotypes classified by their habitat preference: the upland ecotype and the lowland ecotype. Upland ecotypes occur in upland areas that are not subject to flooding, while lowland ecotypes are found on floodplains and other areas that receive run-on water.

The upland ecotype is generally thinner stemmed and shorter than lowland ecotypes, is adapted to drier and wetter environments, and is generally derived from accessions collected in the northern regions of North America. Lowland plants have a later heading date and are taller with larger and thicker stems. Lowland ecotypes are tetraploids, while upland ecotypes are either octoploids or tetraploids. There are ecotypical differences among switchgrass ecotypes for important compositional features, such as fiber, nitrogen, and ash, among others. Dry matter produced by lowland ecotypes has higher cellulose and hemicellulose contents and lower N and ash contents than upland ecotypes, and dry matter produced by upland ecotypes contains higher lignin contents [64]. Upland and lowland tetraploids have been crossed to produce F1 hybrids that have an increase in yield of 30–50% over the parental lines. These hybrids are promising sources of high yield biomass cultivars [64]. Most seedlings of switchgrass will germinate after 3 days at 29.5°C. However, they germinate very slowly when the soil temperature is below 15.5°C [63].

5.2.4. Biomass yields and characteristics

The highest biomass yields per hectare can be obtained when switchgrass is harvested once or twice per year. In fact, one- or two-cut systems often provide similar average yields [65]. Wullschleger et al. [66] compiled 1190 biomass yield observations for both lowland and upland types of switchgrass grown on 39 sites across the USA, from field trials in 17 states, from Texas to North Dakota to Pennsylvania. In this study, it was found that much of the differences in biomass yields could be explained by the variation in the growing season, precipitation, annual temperature, nitrogen fertilization, and the type of switchgrass grown in a specific region. Annual yields averaged 12.9 t DM ha^{-1} for lowland and 8.7 t DM ha^{-1} for upland ecotypes. Some field sites in Texas, Oklahoma, and Alabama reported biomass yields greater than 28 t DM ha^{-1} using the lowland cultivars "Kanlow" and "Alamo."

The main characteristics of switchgrass biomass are listed in **Table 1**. Sladden et al. [67] compared eight switchgrass genotypes that were cut at the same maturity and found the six upland types did not vary much in their biomass composition. However, "Alamo" and "Kanlow" showed significantly lower N contents and higher fiber contents in their biomass which is explained by the later harvest date at maturity instead of differences in nutrient partitioning.

5.2.5. Switchgrass as a bioenergy crop

Switchgrass is established mainly by seeding. Successful stand establishment during the seeding year is essential for economically viable switchgrass as a bioenergy crop [68]. Stand failure as a result of poor seed quality or seedling physiology will have important implications on the cost of switchgrass biomass. However, weed competition is the major reason for switchgrass stand failure. Acceptable switchgrass production can be delayed by one or more years due to poor weed management and deficient stand establishment [69]. Switchgrass is readily established when high-quality seed of an adapted cultivar is used with the appropriate planting date, seeding rate, seeding method, and proper weed control. Switchgrass can be drilled in a conventional seedbed or by direct seeding methods. According to Sladden et al. [67], a row spacing of 80 cm is recommended because this led to higher yields in the second and third years than row spacing of 20 cm. Before planting, soil tests are recommended. N fertilizer is not recommended during the planting year since it will promote weed growth, increase competition for establishing seedlings, and increase economic risk and cost associated with establishment if stands should fail [70]. Economically viable yields will require N fertilization rates between 50 and 100 kg ha^{-1} $yr.^{-1}$ [71]. N fertilizer should be given in late spring. P and K can be applied before seeding to promote root growth and encourage rapid establishment. Switchgrass can tolerate moderately acid soils, but optimum germination of the seed occurs when the soil pH is between 6 and 8 [72].

Weeds can be an important obstacle for switchgrass establishment, especially summer annuals. Spraying herbicides to control broadleaf weeds is usually needed only once or twice every 10 years in established and well-managed switchgrass stands. One year before planting, the field must be plowed or chiseled [63]. A reduction of weed competition can also be achieved by cutting infrequently at 10 cm. In order to control grasshoppers, crickets, and other insects which may affect the new seedlings of switchgrass an insecticide may be needed [63].

Generally, a single harvest during the growing season maximizes biomass recovery, but harvest after a killing frost will ensure stand productivity and persistence, particularly when drought conditions occur, and reduce requirements of nitrogen fertilizers. Delaying the harvest until spring will reduce moisture and ash contents of the biomass; however, the yield loss can be as high as 40% compared to an autumn harvest [73]. With proper management, productive stands can be maintained for more than 10 years. It is not recommended to harvest switchgrass in summer or after flowering when there are drought conditions.

5.3. Reed canarygrass

5.3.1. Origin and distribution

Reed canarygrass (*Phalaris arundinacea* L.) is a member of the Poaceae family. It is a cool-season grass that is less productive than warm-season grasses. It is a sod-forming, perennial wetland grass, native to the temperate regions of Europe, Asia, and North America. It is usually found in wet areas such as lake shores and along the rivers.

5.3.2. General species description

Reed canarygrass is a tall, coarse, and erect grass with a C3 photosynthetic pathway, which reaches a canopy height of up to 300 cm. This grass has vigorous rhizomes that form 1 cm thick and short branches and a root system that reaches to more than 3 m [74].

Its inflorescence is a narrow and compressed panicle. The leaves are wide and flat with prominent nodes. The stems are robust, smooth, and occasionally branching at the nodes. Its ligules are membrane-shaped and obtuse and have a pointed-folded tip. Seeds are shiny brown. The seed production of the species is unreliable due to the seed shattering and occasionally the production of deficient panicles [11]. The presence of several types and concentrations of poisonous alkaloids has restricted the use of reed canarygrass as a forage crop [75]. The estimated life time of a reed canarygrass plantation is approximately 10 years [76].

5.3.3. Ecological demands

Reed canarygrass is a persistent species, which grows well on most types of soils, except droughty sands. It is one of the best grass species for poorly drained soils and tolerates floods better than other cold-season grasses. However, the highest yield can be obtained on organic soils. Reed canarygrass is adapted to and grows very well in a cool temperate climate and has also good winter hardiness. In order to induce flowering, this grass requires exposure to short days (primary induction) followed by long days for initiation of floral primordial and inflorescence development (secondary induction) [77].

5.3.4. Biomass yields and characteristics

There are considerable differences in yield between different soils. Kukk et al. [77] reported that soils with low N content produce yields of almost 1 t DM ha^{-1} in years with unsuitable weather conditions for plant growth. On the other hand, it is possible to achieve an average

dry matter production of up to 6–7 t DM ha^{-1} within limited years on soils with N contents of more than 0.6%. They found that fertilization increases the yield as well as decreases yield variability in soils with low organic matter content, but soils with high N content show an increase in production risks when fertilizer applications increase. Pociene et al. [78] have reported that under favorable climatic conditions reed canarygrass yields are 7–11 t DM ha^{-1}. Moreover, reed canary grass can produce over 15 t DM ha^{-1} in Canada [79], from 6 to 11 t DM ha^{-1} in Sweden [80].

The main biomass characteristics of reed canarygrass are listed in **Table 1**. During the combustion of the reed canarygrass biomass, problems of ash fusion or corrosion have been detected. However, in the delayed harvest system, these problems are almost eradicated. During the winter, there is a decrease in the content of elements such as K, Ca, Mg, P, and Cl. This change in chemical composition is mainly caused by leaching and loss of leaves during the winter, which significantly modifies the chemical and physical characteristics of the ash. It has been reported that the ash content and ash composition show considerable differences between different locations. The type of soil has a great influence on the quality of the biomass. For example, high ash contents have been found in reed canarygrass biomass grown on heavy clay soils and low contents of ash in biomass grown on humus-rich and organic soils [74].

5.3.5. Reed canarygrass as a bioenergy crop

Reed canarygrass is established mainly by seeding. The recommended seeding rate is 15–20 kg ha^{-1}. Seeds of reed canarygrass generally have a slow germination and show varying degrees of dormancy. Therefore, weed competition can reduce crop yields during the first year. Broadleaf weeds can be controlled with common herbicides. From the second year on, an established reed canarygrass stand becomes quite competitive, and as a result, weeds are no longer a problem. The number and timing of harvests during a growing season directly affect biomass yield of reed canarygrass and biofuel quality. Several studies have shown that reed canarygrass has higher than acceptable levels of silica [81], chlorine, and nitrogen [74]. However, delaying harvest of biomass from autumn to late winter or early spring, before regrowth begins can reduce the levels of undesirable components [76].

5.4. Giant reed

5.4.1. Origin and distribution

Giant reed (*Arundo donax* L.), also called giant cane, is a tall perennial grass of the family Poaceae. The area of origin of giant reed has been a subject of debate because the biogeographic and evolutionary origin of this species has been obscured through ancient and widespread cultivation [82]. As a result, there is no agreement on the location of the area where it originated. Botanical and historical evidence supports the hypothesis that the origin started from a pool of wild plants native to the Mediterranean region [83]. On the other hand, some authors suggest that Arundo genus originated in East Asia [84]. However, giant reed has been cultivated in Asia, Southern Europe, Southern Africa, Australia, and the Middle East for thousands of years [85]. The rapid spread of this species is probably attributed to its high productivity and multiple uses.

5.4.2. General species description

Giant reed is a tall, perennial C3 grass, and it is one of the largest of the herbaceous grasses that is widespread in the riparian areas of the Mediterranean and found over a wide range of subtropical and warm-temperate areas of the world [11]. The root system consists of tough, fibrous, lateral rhizomes and deep roots. The rhizomes form compact masses from which arise tough fibrous roots that penetrate deeply into the soil. The rhizomes usually lie close to the soil surface, while the roots are more than 100 cm long [86]. The stems arise during the whole period of growth from the large knotty rhizomes. It is reported that primary reproduction is asexual (sprouts from disturbed stems or rhizomes), due to seed sterility, caused by the failure of the megaspore mother cell to divide [87]. Due to the vegetative reproduction of giant reed, its genetic variability and the chances for finding new genotypes or varieties are low. However, according to the results from electrophoresis tests on some giant reed populations, there was a clustering of the selected populations in relation to their geographical origin, reflecting restricted migration of germplasm [11].

5.4.3. Ecological demands

Giant reed forms dense, monocultural stands and often crowds out native vegetation for soil moisture, nutrients, and space. It tolerates a wide variety of ecological conditions and, however, prefers well-drained soils with abundant soil moisture. It tolerates a pH in the range of 5.5–8.3 and soils of low quality such as saline ones. It can grow in all types of soils from heavy clays to loose sands and gravelly soils, but prefer wet drained soils [88]. Giant reed is a warm-temperate or subtropical species; however, it has little tolerance to survive frost, but when frosts occur after the initiation of spring growth, it is subject to serious damage [89].

Giant reed is commonly known as a drought-resistant species due to its ability to tolerate long periods of severe drought accompanied by low atmospheric humidity. This ability is attributed to the development of thick drought-resistant rhizomes and deeply penetrating roots that reach deep water sources [11].

5.4.4. Biomass yields and characteristics

Biomass yields in a study conducted in Spain showed 45.9 t DM ha^{-1} on average, ranging from 29.6 to 63.1 t DM ha^{-1} [90]. Angelini et al. [91] reported an average biomass yield of 37.7 t DM ha^{-1} in a study conducted in coastal Tuscany (Central Italy), and Di Candilo et al. [92] reported an average biomass of 39.6 t DM ha^{-1} in a study carried out in the Low Po Valley (Northern Italy). In Greece, the recorded average dry matter yields on irrigated plots for the first, second, third, and fourth growing periods were 15, 20, 30, and 39 t ha^{-1}, respectively. The high heating value of different aerial parts of a number of giant reed populations grown in Greece ranged from 14.8 to 18.8 MJ kg^{-1}. Depending upon the population and the growing period, the contents of ash ranged from 4.8 to 7.8%.

5.4.5. Giant reed as a bioenergy crop

Due to seed sterility, giant reed has to be vegetatively propagated from fragments of stems and rhizomes. This may limit large-scale cultivation, since it involves considerable cost and effort

and is time-consuming. Tissue culture is an alternative to conventional methods of vegetative propagation and may represent a useful tool for large-scale propagation in a bioenergy crop [93].

Giant reed has been reported to grow without irrigation under semiarid Southern European conditions [94]. However, it has been reported that irrigation had considerable effects on growth and biomass production since the plant used effectively any possible amount of water [95].

If the nutrient status of the soil is poor, a sufficient amount of K and P should be applied before establishing the giant reed plantation. Otherwise, moderate N fertilization of giant reed is favorable for both economic and environmental reasons. Due to its high growth rates, giant reed does not face significant weed competition from the second year onwards. However, herbicide application is recommended during the first year. Biomass can be harvested each year or every second year, depending on its use [86].

6. Conclusions

Perennial rhizomatous grasses can contribute significantly to the sustainable biomass production due to their high yield potential, low input demands, and multiple ecological benefits. Yields of more than 30 t DM ha^{-1} have been obtained from rhizomatous grasses. However, biomass yields strongly depend on local soil and climatic conditions.

The issue of primary concern when burning grasses is mineral composition that determines the melting point of ash and the potential for corrosion. Ash content needs to be minimized to avoid fouling problems. Appropriate harvesting time and fertilization application can contribute significantly toward improvement of ash content and ash-melting behavior. There is the possibility of using grasses biomass by blending it with other biomasses with low ash, K, and Cl contents. Further research is required to find the optimal blend of biomass.

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Chapter 2

Genetic Improvement of Sorghum for Biomass Traits Using Genomics Approaches

Bushra Sadia, Faisal Saeed Awan, Fozia Saleem, Hafeez Ahmad Sadaqat, Sarmad Frogh Arshad and Haseeb Shaukat

Additional information is available at the end of the chapter

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Abstract

Nonrenewable energy resources deplete with the passage of time due to rapid increase in industrialization and population. Hence, countries worldwide are investing dearly in substitute energy resources like biofuel from miscellaneous set of feedstocks. Among the energy crops, sorghum serves as a model crop due to its drought tolerance, small genome size (730 Mb), high biomass, dry matter contents, quick growth, wide adaptability to diverse climatic and soil conditions and C4 photosynthesis. Sweet sorghum with high sugar content in stalk is an efficient feedstock for advanced biofuels and other bio-based products from sugars. However, high biomass sorghum has the utility as a feedstock for cellulosic biofuels. The enhanced yield of monomeric carbohydrates is a key to cheap and efficient biofuel production. The efficiency of lignocellulosic biofuels is compromised by recalcitrance to cell wall digestion, a trait that cannot be efficiently improved by traditional breeding. Therefore, scientists are looking for solutions to such problems in biomass crop genomes. Sorghum genome has been completely sequenced and hence this crop qualifies for functional genomics analysis by fast forward genetic approaches. This chapter documents the latest efforts on advancement of sorghum for biomass potential at morphological and molecular level by exploiting genomics approaches.

Keywords: biofuel, sorghum, association mapping, lignocellulosic feedstock, genomics, microRNAs, marker-assisted selection

1. Introduction

Though biofuels coevolved with cars, they received less importance in the past, due to abundant availability of fossil fuels at an economical price. Today, the world is experiencing

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higher risks to energy security as well as its efficient utilization. These include disruptions to the supply of imported fossil fuels, limited availability of fossil fuels, and energy price spikes. Carbon dioxide emissions leading to global warming have further worsened the situation. In this context, biofuels are regaining interest, being able to present an attractive alternative to petroleum products since these are known to be biodegradable as well as renewable resources.

While primary biofuels have utility in electricity generation, secondary biofuels generated by biomass processing are used in motorization and several industries. There are first-, second-, and third-generation categories of secondary biofuels depending on the type of raw material used and the processing technology applied. Feedstocks of starch and sugar are being utilized in the production of ethanol. Corn, wheat, and milo are starch-based feedstocks, whereas sugarcane and sugar beet are sugar-based feedstocks. Sugar-based feedstocks contain simple sugars, which can be readily extracted and fermented. Sorghum (Poaceae) serves as both sugar- and biomass-based feedstocks. The crop grows well with minimal input requirements on marginal areas. Sorghum varieties possess diverse phenotypic traits to suit their usage as food, feed, energy, and sugar production. Energy sorghum has high lignocellulosic biomass that can be converted into biofuels. An economic comparison shows that cost per ton of sorghum biomass is lesser than other potential biomass crops including switchgrass [1].

High yield and stress tolerance are two main characteristics attracting the scientists and researchers to sorghum as a promising source of biomass. Efforts for genetic improvement of biomass sorghum directly reduce the overall cost of biomass-to-ethanol conversion, mainly affected by lignin content and its composition. Plant cell walls constituting most of the biomass are mainly composed of cellulose, hemicelluloses, and lignin. Lignocellulosic biofuels are produced via three processes: pretreatment, hydrolysis, and fermentation. The enhanced yield of monomeric carbohydrates leads to cheap and efficient biofuel production. Progress in sorghum feedstock genomics research is a key to enhanced bioenergy production. It relies on the integrated use of breeding and biotechnology. The tremendous source of genetic variability in sorghum world collections has made a significant contribution to sorghum improvement in many countries. Sorghum has a diverse germplasm and a relatively small diploid genome of 760–810 Mbp [2] making it well suited for genomics approaches.

2. Genetics of sorghum biomass traits

The processes like crop phenology, vegetative and productive growth exert a strong influence on biomass yield of energy sorghum. These processes are portrayed by the morphological and physiological characteristics of sorghum [3]. Improvement in sorghum yield depends on the nature and extent of genetic variability, heritability, and genetic advance in the base population. Energy sorghum has characteristic features that are associated with high biomass. These include plant height, flowering time, number of leaves and flag leaves per plant,

leaf length-width area, flag leaf area index, fresh and dry biomass index, brix value, and days to maturity [4].

Significant variability for genotype, general combining ability (GCA), and specific combining ability (SCA) for different components has been observed in sorghum [5, 6]. Genetic variability for biomass-related traits in sorghum has been reported by many scientists [7–9]. Hawkins [10] proposed an ideotype of high yielding and high biomass sorghum being tall, lodging resistant and moderately photoperiod sensitive for maximum vegetation.

Plant height in sorghum is controlled by four independently inherited *Dw* (Dwarf) genes, viz., *Dw1*, *Dw2*, *Dw3*, and *Dw4* [11]. It is determined by the interplay of the internode length and the number of nodes it produces before flowering. The *Dw* genes have partial dominance for tallness, and their effects are additive in nature. Dwarfing genes have been isolated, and dwarf forage hybrids have been developed by incorporating *Dw2* gene into forage seed and pollen parents, leading to 11% increase in leafiness but 30% decrease in forage yield [12]. High heritability has also been reported for plant height [13], number of leaves/plant [14], brix value [15], and leaf area [16] in sorghum. All these traits are under genetic control and improved in early generations.

Lignin is present within plant secondary cell wall. It not only gives rigidity and support to plant cell wall but also enhances water conductance and acts as a protective barrier against microbes [17]. Since lignin lowers the yield of fermentable sugars from cell walls, its higher concentration will negatively affect the morphogenic and industrial potential of lignocellulosic biomass [18]. Higher lignin is also associated with poor forage quality of sorghum owing to reduced access to proteins and other nutrients in the cell wall matrix. Hence, pretreatment of lignocellulosic biomass is essential to degrade lignin material in the cell wall. Researchers are trying to modify biomass composition of sorghum by targeting the genes that encode enzymes of the monolignol biosynthetic pathway [19].

Brown midrib (*bmr*) mutants arose from novel mutations in phenylpropanoid pathway leading to low lignin concentrations. Sorghum *bmr* mutants developed by chemical mutagenesis were characterized by low lignin [20]. Several allelic *bmr* genes, namely, *bmr 12*, *18*, *26*, and *6*, have been introgressed and characterized in sorghum. Most of the sorghum *bmr* mutants exhibited higher yield of fermentable sugars. A decreased caffeic acid O-methyltransferase activity was reported during evaluation of allelic genes *bmr12* and *bmr18*. Similarly, a low cinnamyl alcohol dehydrogenase activity was linked to *bmr6* [21].

In sorghum, the stay-green is a recessive trait causing retention of green leaf area at maturity (GLAM). This character may be functional or cosmetic, indicating continued leaf photosynthesis capacity during grain filling or discontinued photosynthesis from leaf greenness, respectively. High yield potential of sorghum in water scarce environment is governed by the functional stay-green. The pleotropic stay-green leads to arrest protein decline in aging leaves [22]. Sorghum stay-green types have been developed worldwide by conventional breeding. Exploiting stay-green trait in breeding programs may result in genetic enhancement of sorghum yield, industrial value, and biotic and abiotic resistance. Stay-green alleles in sorghum individually enhance grain yield under limited water availability via modification in plant architecture and water

uptake patterns. This is a quantitative trait governed by nuclear genes [23]. Various sorghums have been reported exhibiting different types of stay-green phenotypes [24].

Sorghum is a short-day plant belonging to semiarid region. The photoperiod sensitivity of sorghum is a recessive trait that causes longer vegetative period, which supports plant growth and in turn the green mass production. Sorghum maturity trait is controlled by six genes: *Ma1*, *Ma2*, *Ma3*, *Ma4*, *Ma5*, and *Ma6*. *Ma1* gene is known to be regulated by photoperiod in order to effect height and flowering time. *Ma1* has the largest impact on flowering date of all the maturity genes [25]. The *Ma5* gene, when present in the dominant form together with *Ma6*, very strongly inhibits floral initiation regardless of day length [26, 27].

Sorghum is referred as perennial grass due to its tillering capacity. The number and types of branching in sorghum are genetically controlled. Secondary and tertiary branches termed as vegetative branching are thought to be controlled by the same genetic factors [28], while distinct genetic elements might control tillering or mature branching in sorghum. Tillering enhances accumulation of sugars in sorghum stem for biofuel production [29, 30]. It imparts greater leaf area leading to higher intercepted radiations and thereby also affects biomass accumulation in sorghum. High-tillering sorghum thrives best in appropriate growth conditions where all the resources are best utilized [31]. Sorghum plants with excessive tillers and limited water availability exhibit low biomass and grain yield potential [32].

Sorghum is a C4 plant with characteristic drought tolerance owing to its efficient root system. It is a single-stemmed grass anchored by spreading and fibrous root system having primary, secondary, and supporting roots. The roots may extend from 1.5 to 2.5 m up to 1 m below the soil in all directions. Higher biomass yield is associated with the cloning of genes linked with root loci and improvement in root structure genetic design.

Significant variability in leaf size has been noticed in sorghum. This affects energy-capturing potential, conversion into biomass, and physiological activity. Smaller leaves are adapted to dry and hot regions, whereas in cooler and humid climates, plants with larger leaves are found having insufficient energy conversion capacity. Several other traits are also desirable for energy sorghum, like low grain yield, resistance to lodging, low water content, and biomass quality [32].

3. Genetic diversity evaluation using quantitative biomass traits of sorghum

Sorghum genetic resource characterization is based on morphological, biochemical, and molecular marker approaches. The most economical approach is morphological characterization for diversity evaluation and identifying promising genotypes. The agro-morphological characterization of sorghum is well reported [33]. The genetic diversity of sorghum germplasm comprising of different sorghum types was evaluated [34] by exploiting the quantitative characters. Nine of 14 quantitative traits were selected on the basis of their diverse nature. Panicle width, stem girth, and leaf breadth proved more diverse traits as indicated by principal component analysis. The hierarchical cluster analysis grouped sorghum germplasm into six classes. The clusters 1 and VI contained the maximum and minimum numbers of accessions, respectively,

while the clusters VI and IV were the most distantly related among all the clusters. The accessions grouped in cluster III had the highest average yield and hundred seed weight. The present study indicated that high yielding and diverse accessions can serve as better parents for sorghum variety development.

Disasa et al. [35] conducted a study to characterize the brix degree, grain yield, and some morphological traits of 180 sorghum accessions from Ethiopia and analyzed them under different environments. Greater variability was observed among genotypes collected from different areas. Genotypes collected from northern areas of the country showed high brix value, while the rest of the collection contained high biomass for sugar stalk yield. Cultivars with high-biomass traits and brix value were recommended for utilization in breeding programs to develop sugar-rich sorghum genotypes.

In a recent study, the genetic divergence among 208 Pakistani sorghum genotypes was estimated [36] by evaluating the 14 different quantitative traits for two consecutive years. Multivariate tools like principal component analysis (PCA) and unweighted pair-group method with arithmetic mean (UPGMA) analysis were employed. Study revealed broader variability in fresh biomass, dry biomass, flag leaf area index, leaf area index, and plant height. Broad-sense heritability was reported to be >80% for all traits in both years. The PCA showed that all the biomass-related traits with eigenvalue >1 were contributing significantly in the first three PC axes (75.39 and 71.21% for both years). This indicated the presence of maximum variability among these genotypes. Such a diverse germplasm might be a good candidate for varietal development. Pearson correlation analysis indicated that fresh and dry biomass had a significant positive correlation with leaf area index, number of leaves per plant, flag leaf area index, days to maturity, and 50% days to flowering for 2 years. UPGMA analysis classified the germplasms into 141 morphotypes and 7 classes in the first year and 136 morphotypes and 5 classes in the second year. The genotype P-13-2013 was found to be the best performer with relevance to traits such as the number of leaves per plant, stem thickness, leaf length, fresh biomass, dry biomass, and flag leaf area index. The genotypes Indian-6, BM-726, P-10-2013, and Johar-2013 showed good performance in terms of fresh biomass and days to 50% flowering.

Further, Shokat [37] performed assessment of 1300 diverse USDA sorghum collections on the basis of morphological traits for two consecutive years (2015–2017). The 24 high-biomass sorghum lines selected from the first year trials were further investigated for biomass-related traits in the second year. Out of nine traits evaluated, viz., germination percentage, number of leaves per plant, number of nodes per plant, days to 50% flowering, stem girth, fresh biomass, dry biomass, days to maturity, and plant height, three proved statistically significant, including number of leaves, number of nodes, and stem girth. Sorghum accession number NSL-54978 gave highly significant value for the number of leaves and number of nodes, while sorghum accession number PI-525981-01-SD exhibited significantly higher value for stem thickness. Correlation analysis indicated a significant relationship among stem girth and fresh biomass, days to maturity, and fresh biomass. PCA exhibited 48.9% expression for the number of leaves and number of nodes, while 46.9% expression was recorded for fresh biomass. The biplot analysis showed maximum diversity in fresh biomass, stem girth, days to maturity, and plant height characters (**Figures 1** and **2**).

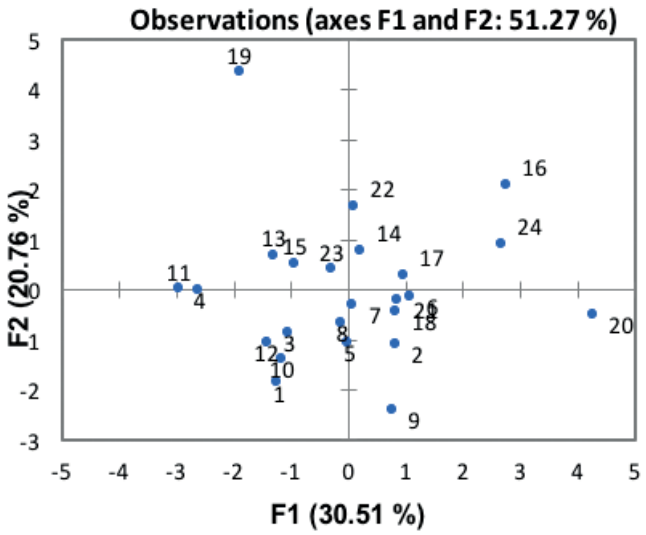

Figure 1. Biplot with sorghum genotypes.

UPGMA analysis generated 9 morphotypes of 24 sorghum genotypes (**Figure 3**). Total of 24 genotypes were divided into five different classes. Cluster analysis revealed that the main cluster was divided into two major clusters. The first subcluster comprised four genotypes (6, 24, 16, and 20), while the second subcluster was further subdivided into four different small clusters. Two genotypes were present in the cluster with blue-colored cluster, and 11 genotypes were placed in light blue-colored cluster. The class represented by red color consists of two genotypes, whereas the class represented by green color includes three genotypes.

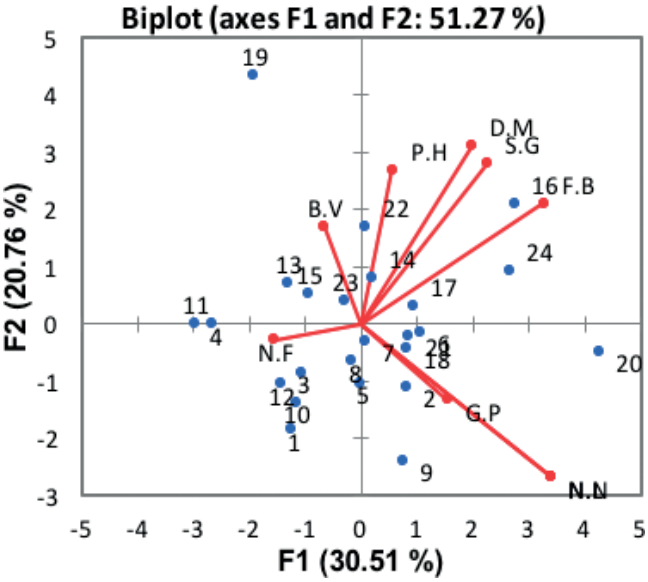

Figure 2. Biplot with cumulative sorghum variables and genotypes.

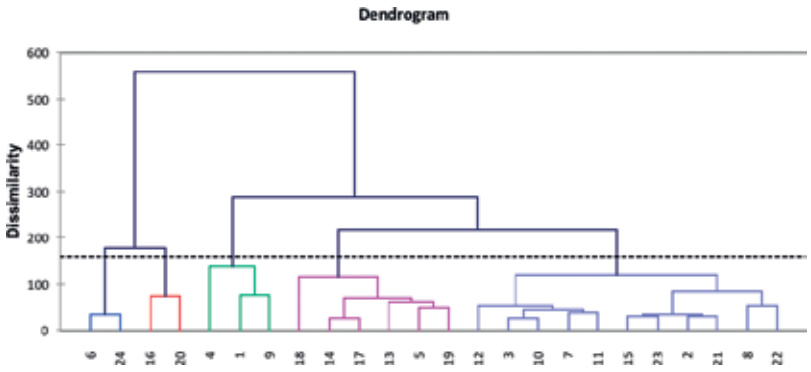

Figure 3. Cluster analysis of 24 sorghum genotypes on the basis of morphological characters.

4. QTL mapping of quantitative traits in sorghum

Germplasm characterization using morphological traits has some limitations. The expression of a phenotype is mostly influenced by the environment and depends upon plant organ as well as plant developmental stages. Owing to these shortcomings, it is the least preferred means of characterizing crop germplasms. Hence, investigating DNA polymorphism is a reliable means of genetic diversity assessment. Molecular markers are extensively used in molecular breeding being reliable, abundant, phenotypically unbiased, and time and stage independent. These markers are helpful in improving breeding programs through different ways. The marker-assisted selection (MAS) technology makes use of an association between the expression of desired characters and markers present in the DNA. Quantitative trait loci (QTL) for many traits can be evaluated by using molecular markers [38]. For a given trait in a particular population, increasing marker density can increase the resolution of the genetic map, thus enhancing the precision of QTL mapping. Genetic mapping studies are based mainly on BTx623 and other grain sorghum types. Widely used polymerase chain reaction (PCR)-based markers are RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), SSRs (simple sequence repeats), STS (sequence-tagged sites), and DArTs (diversity arrays technology) [39–41].

Recently, there has been a growing interest in exploiting QTL mapping for different traits. About >700 QTLs have been identified for several traits in sorghum (http://www.gramene.org). However, fewer studies have been carried out to find out the molecular basis of these traits.

The biomass trait of sorghum depends on stem height and thickness, which are vital for bioethanol production. Taller varieties produce higher biomass with thicker stem and higher sugar contents. Height is positively correlated with biomass production and independent of stem structural composition like cellulose, hemicellulose, and lignin content. The QTL for total dry biomass has been found to localize with height QTLs [42, 43]. In sorghum, height is controlled by few QTLs. Genetic study has identified four loci controlling stem height: *Dw1*, *Dw2*, *Dw3*, and *Dw4* [44]. *Dw3*, which encodes a P-glycoprotein that controls polar auxin transport, has been cloned [45]. This gene is also co-localized with a height QTL on chromosome 7 [42]

and *Dw2* with QTL on chromosome 6 [46]. Another QTL on chromosome 9 was also found for height [42]. Using 377 sorghum accessions and 49 SSR markers, a height QTL (Sb-HT9.1) was mapped. Likewise, Murray et al. [43] used 47 SSR and 322 SNP markers on 125 genotypes of sorghum and identified two associations for height on chromosomes 6 and 9.

Maturity (days to 50% flowering) is also positively correlated with the biomass production [47]. The photoperiod sensitivity in sorghum was initially reported to be controlled by single maturity locus Ma1 [48]. Any genotype with a dominant Ma1 allele will show a photoperiod response, while the homozygous recessive (Ma1) will flower early. Ma1 was cloned and reported as pseudo-response regulator protein 37 [49]. The first maturity cloned locus in sorghum was Ma3 that encoded a phytochrome B [50]. Genotypes with total loss of functional ma3R allele of Ma3 are insensitive to photoperiod and flower early regardless of Ma1 allele and day length. There is an epistatic interaction between Ma1 and Ma3. Few more maturity loci have also been reported in sorghum, e.g., Ma2, Ma4, Ma5, and Ma6, with very little information about their functions. Ma2 is unmapped and shows interaction with Ma1 [51], while Ma4 is thought to be on chromosome 10 [26]. For the production of photosensitive hybrids from two plants, the Ma5-Ma6 interaction has been extensively used by the biomass sorghum seed industry.

Murray et al. [52] identified one QTL for brix (located on chromosome 1) by using 47 SSRs and 322 SNPs for a diverse panel of 125 sweet sorghums. Six marker loci related to plant height and 10 loci to plant maturity were identified [53] by using 14,730 SNPs for sorghum mini core collection. Once identified, QTLs need validation/confirmation in varying experimental conditions prior to exploitation for MAS. Wang et al. [54] used 181 recombinant inbred lines (Shihong137, a dwarf grain sorghum, x L-Tian, a tall sweet sorghum) to validate QTLs controlling plant height, biomass, juice weight, and brix value.

The study identified seven QTLs for biomass-related traits including plant height, juice, and stem fresh weight under four different environmental conditions, while three of these seven QTLs were under strong epistasis. Co-localization of many biomass-related QTLs with previously reported height QTLs confirmed that plant height regulates biomass in sorghum. On the other hand, few QTLs, namely, qSFW1–qSFW2, qSLFW6–qSLFW1, and qSLFW6–qSLFW2, were mapped to chromosomal positions where no height QTLs were located.

5. Association mapping for biomass traits in sorghum

Linkage mapping and association mapping (AM) can both be used to identify QTLs by genotyping and phenotyping the segregating populations. For association mapping (AM), the population screened on the basis of phenotypic performance is subjected to molecular marker analysis, followed by the assessment of population structure and linkage disequilibrium (LD) (**Figure 4**). Linkage mapping requires few markers, due to high linkage disequilibrium (LD), but has low resolution, while association mapping needs a large number of markers to conduct a genome-wide scan of a large number of diverse lines with low levels of LD. Association mapping has

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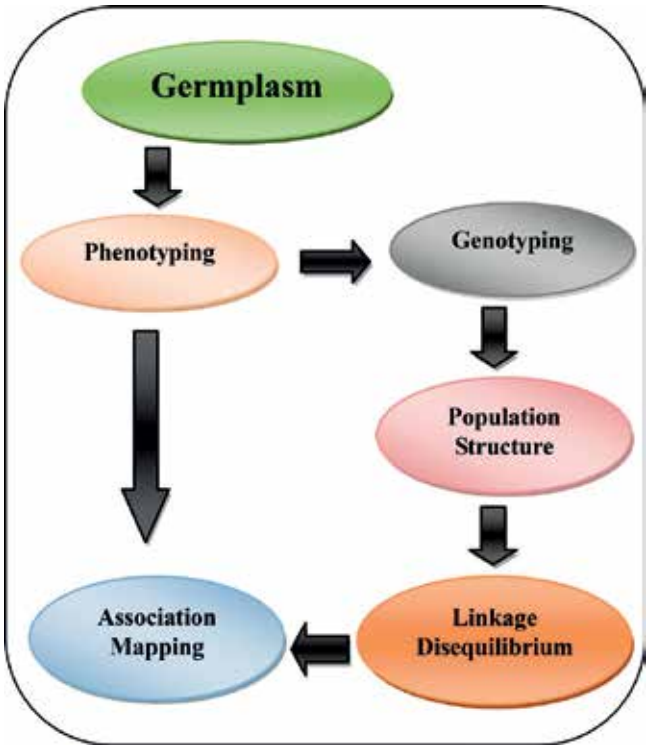

Figure 4. A simplified flow chart showing different stages of association mapping for tagging a gene of interest using germplasm accessions [36].

the ability to evaluate multiple haplotypes. Moreover, association analysis is an efficient strategy to genetically dissect the complex traits that deviate from classical Mendelian pattern of segregation.

Though originally designed for human genetics, exploitation of association mapping is picking momentum in plant improvement. In sorghum, association mapping is being applied for its genetic enhancement by phenotypic evaluation of sorghum germplasm, identifying and mapping QTLs associated with desired traits and selecting the genotypes (parents) that carry favorable alleles for gene introgression through MAS. Using 107 representative sorghum accessions and 98 SSR markers, Shehzad et al. [55] reported the association of 14 SSR loci with four traits including days to heading, days to flowering, number of panicles, and panicle length in sorghum. Another report identified two SSR markers consistently associated with plant height under two different environments [56]. Plant height and maturity date were also reported to be associated with 5 out of 39 SSR markers on chromosomes 6, 9, and 10 in 242 sorghum accessions [57].

About 300 diverse accessions of sorghum were evaluated [58] to conduct association analysis of seedling phenotypic variation during cold and heat stress treatments. They identified and validated 30 and 12 SNPs associated with cold and thermal tolerance, respectively, to determine the haplotypes in sorghum.

Recently, association mapping is performed [36] for biomass-related traits in 208 sorghum accessions of Pakistan. Diversity and structure analysis as well as association mapping analysis were performed on 94 diverse accessions, which were selected through PCA of 208 sorghum accessions. About 215 alleles were detected with an average of 3.47 alleles per locus. The range of alleles varied from 2 to 5. The polymorphic SSR markers were used to identify molecular diversity, population structure, linkage disequilibrium, and marker trait associations (MTAs). Major allele frequency was ranged from 0.13 to 0.74. The average PIC value of primers was 0.51 that ranged from 0.25 to 0.62. The admixture model-based structure analysis revealed four admixture subpopulations, which indicated that all domesticated cultivars had common ancestor with continuous gene flow. The haplotype LD block analysis showed strong linkage between xtxp219 (located at 66.13 Mb) and Xcup37 (located at 61.90 Mb) with the R2 value of 1.00, which depicted that no recombinational event occurred between these two loci on chromosome 6. The markers, Xcup12 (54.22 Mb) and sb4–sb72 (41.44 Mb) were strongly linked with R2 value of 0.90. The markers within the range of R2 value 0.60–0.70 were Xcup36 (47.11 Mb), xtxp127 (44.97 Mb), xtxp045 (49.28 Mb), sb4–sb72 (41.44 Mb), SB3630 (52.81 Mb), and xtxp127 (44.97) on chromosome 6. The LD decay was estimated to be up to 10 Mb in case of chromosome 6 with R2 value of 0.440 by using 23 polymorphic SSRs. The haplotype LD block analysis of chromosome 9 showed strong linkage between SB5111 and Xcup18 with R2 value of 1.00. The pair-wise LD decay analysis revealed LD decay at 50 kb at R2 value of 0.023.

Seven marker trait associations (MTAs) were detected by mixed linear model (MLM) approach with phenotypic variability ranging from 9.13 to 13.9% for the first year and from 6.25 to 23.05% for the second year. Four MTAs were associated with plant height, days to 50% flowering, and leaf length on chromosome 6 and three on chromosome 9 with the same traits. A total of five SSR markers expressed significant MTAs; three of these (Xgap072, Xtxp265, and SB3789) were associated with plant height, days to 50% flowering, and leaf length traits on chromosome 6. Two markers Xtxp283 and SB5040 were associated with plant height, leaf length, and days to 50% flowering on chromosome 9. Hence, chromosomes 6 and 9 appeared to carry important QTLs for biomass-related traits in sorghum.

6. Conclusion

Considering the global drive to explore alternate sources of renewable energy, biomass sorghum stands out for meeting economic demands like greater variability, short development cycle, and high calorific value in boilers. Moreover, sorghum is a good genetic model having integrated genetics-genomics-breeding platform, wide adaptability across varying environments, and diverse germplasm across the globe exploited for food, feed, fiber, and biofuel. Our chapter describes the efforts being carried out to identify and improve biomass in sorghum genotypes with great agronomic and energetic potential, dissecting sorghum genome by using omics approaches to explain the genetics of these traits and documenting the association of these traits with the genetic fingerprints of sorghum crop. The scientific record suggests that there is abundant genetic variation within existing sorghum germplasm to play around for developing high-biomass sorghum.

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Chapter 3

Modeling and Optimization of Quality Variability for Decision Support Systems in Biofuel Production

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Additional information is available at the end of the chapter

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Abstract

Biofuels are a promising alternative to fossil fuel depletion, due to their sustainable production from living or recently living organic matter (i.e., biomass). Biofuel production offers benefits that are not present in non-sustainable resources, like the reduction of air pollution. According to government agencies, biofuel production is expected to increase in the U.S. within the next few years because of government initiatives. In order to become a feasible alternative to satisfy market demand, biofuels require strategic improvements in areas such as supply chain management to deal with the variability within the biomass. Advanced analysis tools might be utilized to integrate biomass physical and chemical properties into the decision processes. This chapter introduces a principal component analysis (PCA) to determine significant factors that affect the operations within the supply chain and, later on, incorporates those factors in an optimization model for the decision analysis. The results show that incorporating quality-related properties has a significant impact in the solution of the optimization program.

Keywords: biofuels, biomass, optimization, principal component analysis, stochastic programming, two-stage problems

1. Introduction

Global population is still increasing, and therefore, more resources are required to fulfill people needs. The demand of energy is growing due to the population increase but also because the presence of new activities such as social networking. Nowadays, most of the vehicles utilize fossil fuels. The fossil fuels are non-renewable resources and they contribute significantly to the global pollution.

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Biofuels are a propitious alternative to the fossil fuels reliance, due to their sustainable production based on using biomass as a raw material. Biomass is an organic matter derived from living or recently living beings. The production of biofuels also has some advantages from those produced from non-sustainable resources, for instance, the reduction of greenhouse emissions (GHG) such as the CO_2. Another benefit of using biofuels is the development of an agricultural industry and the creation of rural jobs to produce and deliver biomass to biorefineries. Bioethanol is one type of biofuel that can be produced from organic resources such as corn stover, miscanthus, and switchgrass, among others. Bioethanol has several applications in a wide variety of industries. According to government dependencies such as the Energy Information Administration (EIA), an increment of biofuels is expected in the U.S. within the coming years as product of the Renewable Fuel Standard [1] that requires more production of fuel utilizing renewable resources (e.g., 16 billion gallons for 2022).

Biofuels are classified according to the raw material utilized to produce them: (1) the first-generation is related to biofuels produced from edible biomass that can be generally used for human consumption (e.g., corn, sugarcane, sugar beet, among others), (2) the second-generation are biofuels generated from a wide range of feedstock, including lignocellulosic biomass (LCB), such as perennial grasses, soft and hard wood, up to municipal solid waste, and (3) the third-generation commonly refers to biofuels produced from algal biomass [2]. Biofuels produced from LCB are a feasible option in the U.S. for the coming years as they utilize non-food feedstock and can be grown in marginal lands or are byproducts produced from the wood industry.

Biofuel production requires improvements in strategic areas such as conversion technologies, genetic manipulation of feedstock breeds, and supply chain management of biomass from harvesting areas to conversion facilities, among others in order to become a plausible alternative to fossil fuel production. Supply chain (SC) improvement represents an important area of opportunity in biofuel production due to the fact that environmental, geographical and economic factors are related to operations like harvesting, handling, storing as well as transportation, which have shown significant impact in biofuel yields/cost. Studies have demonstrated that some factors such as storage time affects the physical and chemical properties of the biomass [3], therefore, biomass properties have an important role in the design of the operations required for the production and distribution of biofuel [4].

There are many properties that affect the conversion efficiency depending on the type of conversion technology that is being utilized for the production of biofuels. Moisture is a property of biomass that affects both the thermochemical and biochemical conversion technologies and a drying process that diminishes the moisture content in the biomass up to the required level of humidity is needed. The drying process to meet the specification for the selected conversion technology incurs in a cost that could be reduced/controlled with the implementation of logistics processes and infrastructure design that consider the level of humidity in the feedstock. The utilization of the biomass without meeting the expected specifications could lead the production of biofuels to an inefficient conversion process. Another example of the importance of the biomass properties are the carbohydrates. If the level of carbohydrates does not meet the specification, then, the amount of biofuel derived from that

specific batch of biomass will be less than expected. With a shortage of biofuel, acquiring the slack from a third-party supplier to cover the demand could lead the producer to an increment in the overall cost.

Biomass has many physical and chemical properties that need to be considered in order to optimize an objective such as minimization of the total cost or maximization of the profit. This chapter introduces a principal component analysis (PCA) to identify significant factors that affect the design and implementation of logistic processes and infrastructure due to the physical and chemical properties of the biomass. Moreover, the chapter presents a two-stage optimization model that take into consideration quality-related costs in order to set up the biorefinery locations and the flows of biomass from the supplier to the producer. The optimization model can be incorporated into a decision supported system (DSS) to solve several instances of interest and aid the decision-making process.

2. A PCA in a switchgrass composition

This analysis focuses on LCB [specifically, on switchgrass (*Panicum virgatum*)], which is a feedstock derived from organic matter that is mainly composed of cellulose and hemicellulose. Examples of LCB are corn stover, wheat straw and switchgrass and there are many activities involved in the production and distribution of this kind of biomass such as harvesting, extracting, packaging, transporting, handling, among others. There are factors in the aforementioned activities that affect the physical and chemical properties of the biomass, and thus, their quality-related costs. The consideration of those factors has an effect in the design and implementation of the supply chain (SC).

An example of the relationship between the biomass properties and the SC design is the cellulose and hemicellulose (carbohydrates) contents in the LCB. The cellulose and hemicellulose content in the LCB is directly related to the biofuel produced in the conversion process since the carbohydrates are the main component to produce the energy. The more carbohydrates contained in the biomass, the more liters of biofuel obtained from that particular batch of feedstock. Hence, activities that affect the carbohydrate contents need to be improved to minimize the impact on the conversion process.

Densification is one of the processes in the SC of biofuel production that affects some of the properties within the biomass. The densification of biomass consists in conglomerating the organic matter in the form of compact structures such as briquettes and pellets, to improve their handling, storage and transportation but also to reduce the level of dry matter loss (DML) in the feedstock. The DML is directly related with the loss of carbohydrates in the biomass. The less organic matter in the batch, the less amount of cellulose and hemicellulose to produce biofuels. Densification also affects other properties in the biomass such as the moisture content, unit density, durability index, as well as other properties specified for the conversion process according to the implemented technology. Controlling the physical and chemical properties under the specification requirements is vital for an efficient conversion. Delivering

biomass that does not meet the specifications could lead to extra costs as a consequence of re-processing the biomass up to meeting the specifications.

The reduction of the variance within the physical and chemical properties of the biomass helps to avoid extra operational cost due to re-processing of feedstock that is out of the specifications. Identifying the factors, which are involved in production and distribution activities that affect the physical/chemical properties, is necessary to design an efficient SC. Researchers in the field have studied baling effects in feedstock properties [5–7]. In previous works, Aboytes-Ojeda et al. [3] proposes a PCA to detect those factors that have a significant impact on properties of interest and that should be approached by implementing novel operations and strategies in order to fulfill the conversion specifications.

The multivariate methodology proposed by Aboytes-Ojeda et al. [3] intends to: (1) introduce the covariance information analysis to draw systematic insights about the factors under study, and (2) present a novel methodology to identify the contribution of every factor in the analysis with respect to the total system variability. The variables introduced in the analysis were cellulose, hemicellulose, lignin, ash and extractives content; whereas the factors were the particle size in the bale, the wrap material, the days in storage and the weight of the bale.

2.1. Experimental methodology

In the year 2012, an experiment to find insights related to the physical and chemical properties of switchgrass was designed and implemented at the Biomass Innovation Park in Vonore, Tennessee. The type of biomass was from Alamo switchgrass and the samples were harvested and then baled in squared shape (1.2 m $\times$ 0.9 m $\times$ 2.4 m) with a baler machine New Holland BB9080 (New Holland Agriculture, New Holland, PA, USA). New Holland BB9080 is a square baler without a cutter that was used to process the batch of lignocellulosic biomass in the second week of January of that year. After processing the biomass to form square bales, the biomass was transported to other covered location before the beginning of the pre-processing.

A Vermeer TG5000 tub grinder (Vermeer Corporation, Pella, IA, USA) was utilized to unpack and grind the switchgrass in January 2012. Once the biomass was ground, the next step was to sample the moisture content and the chemical composition; the measures for the chemical composition were obtained with a near-infrared (NIR) technology. A machine BT3 industrial baler (TLA Bale Tech LLC, South Orange, NJ, USA) was employed to bale the switchgrass one more time after measuring the chemical properties. Round bales (1.2 m of diameter $\times$ 1.5 m of width) were made with the BT3 and then they were moved to storage before the next phase of the experiment.

Four controllable factors were introduced in the analysis that utilizes a split-split plot design. The factors in the analysis were: (1) the number of days in storage, (2) the particle size of the feedstock, (3) the wrap type of the bale, and (4) the weight of the bale. The number of days has three groups or levels, same as particle size. The wrap type and the weight of the bale have two groups. The database utilized for this study presented the necessary conditions of normality, homogeneity and heteroscedasticity as discussed in Kline et al. [8].

Factors	No. groups/levels	Variables
Particle size	3	Cellulose, hemicellulose, lignin, ash and extractives
Wrap type	2	Cellulose, hemicellulose, lignin, ash and extractives
Storage days	3	Cellulose, hemicellulose, lignin, ash and extractives
Bale weight	2	Cellulose, hemicellulose, lignin, ash and extractives

Table 1. Factors and variables for PCA.

Table 1 identifies the factors and variables included in the analysis. The storage days were classified in three groups or levels: 75, 150 and 225 days of storage. The particle size was defined in three groups or levels: PS1 (243.84 cm), PS2 (7.62 cm) and PS3 (1.27–1.91 cm). The wrap type was categorized in two levels: (i) net mesh and net (excluding the two ending parts of the bale), and (ii) the high tensile strength film wrapping for the complete bale (net and film). The bale weight has two levels for this study; the lower level was for bale with a weight between 957.65 and 1715.20 lb., whereas the high level was for bale with a weight between 1715.21 and 2455.10 lb. The weight in the bale has repercussions in logistic operations such as handling, storage, and transportation.

Five variables were included in the analysis. The cellulose is a glucose polymer linked by glycosidic bonds and the hemicellulose is a branched polymer of carbon sugars. The lignin refers to a structural component of plants, consisting of an aromatic system made of phenyl proposal units. The ash is considered as the inorganic leftover after the combustion process at 550–600°C. The extractives are non-structural components that can include free sugars, proteins, chlorophyll, and waxes. The PCA methodology proposed uses the variability within the variables (i.e., variance and covariance) to create artificial variables and then it groups the data according to their corresponding factor group/level. Finally, a statistical comparison of means is utilized to conclude if there is a significant difference between the means of every factor group/level. **Figure 1** shows the methodology to perform the PCA which consists of five basic steps.

2.2. Principal component analysis (PCA)

In order to implement the PCA [9, 10], it is necessary to test the required data conditions to perform the analysis, then, the covariance/correlation matrix needs to be calculated. A Bartlett's test of sphericity is utilized next to determine if the correlation information for the analysis is significant. With the covariance/correlation matrix, eigenvalues and eigenvectors are computed. The eigenvalue is utilized to determine the portion of variance attributed to the corresponding eigenvector. The components of the eigenvectors known as loadings are used to transform the original data into the components scores. The variance matrix is shown in **Table 2**; there is no need to compute the correlation matrix since all the measures for every variable are in the same scale.

The portion of variance in PCA is calculated according to the eigenvalues obtained in the analysis. The idea behind the PCA is to detect those components with the higher eigenvalues;

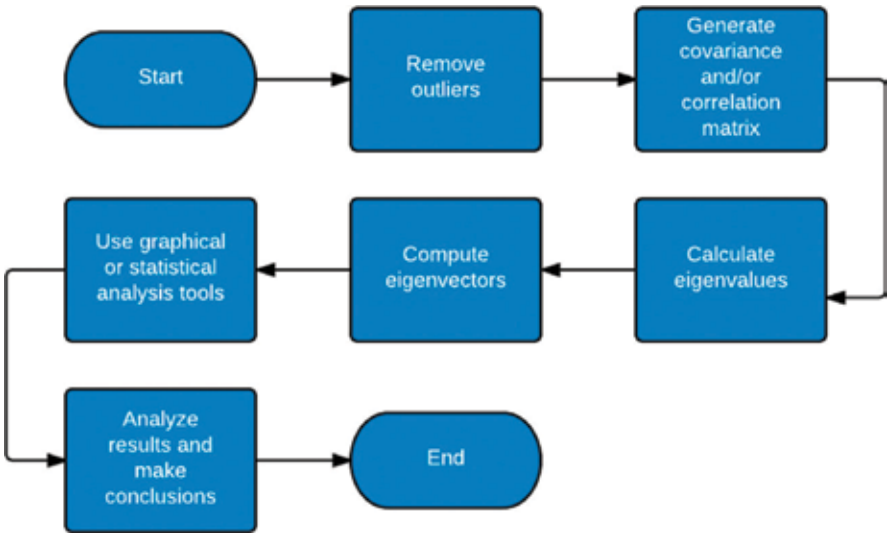

Figure 1. Flow chart for PCA.

Component	Cellulose	Hemicellulose	Lignin	Ash	Extractives
Cellulose	1.344	−0.149	−0.130	−0.325	−0.686
Hemicellulose	−0.149	1.366	−0.467	−0.017	−0.377
Lignin	−0.130	−0.467	0.541	0.198	0.064
Ash	−0.325	−0.017	0.198	0.334	0.226
Extractives	−0.686	−0.377	0.065	0.226	1.100

Table 2. Chemical components covariance.

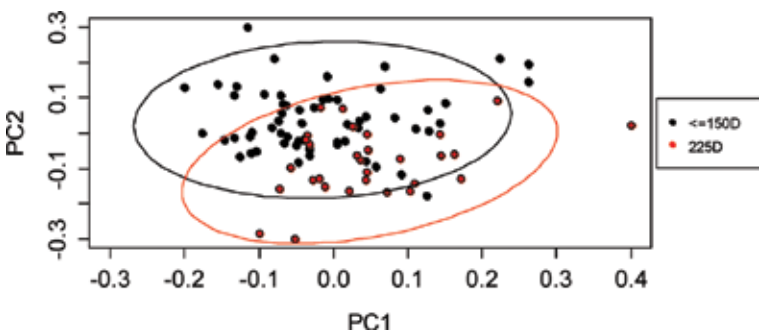

Figure 2. Scree plot for PCA.

therefore, it is possible to identify the principal components due to their share in the total variance. **Figure 2** shows that the first two components contain almost 80% of the total variance; **Table 3** presents the eigenvalues for all the components as well as their share in the total variance. As a rule of thumb, those components above the value of one in **Figure 2** must be considered as the principal components of the analysis.

	Principal component				
	1	**2**	**3**	**4**	**5**
Eigenvalue	1.448	1.266	0.777	0.527	0.318
Variance (%)	45	34	13	6	2
Cumulative (%)	45	79	92	98	100

Table 3. Variance analysis in PCs.

Figure 2 and **Table 3**, PC1 and PC2 are the main components since their eigenvalues are above one and the amount of variance represents up to approximately 80% of the total variance. The eigenvalues are also attached to the eigenvectors which are the directions where the largest variance is presented. The loadings are the values that indicate the correlation between the original value and the score value. A high value means that the original variable and the component are close. **Table 4** represents the loadings values.

With the loadings values, it is possible to transform the original data into scores. Scores are the representation of the original data points under the principal components basis. These scores can be plotted in a graph called bi-plot and then explored (exploratory data analysis) in order to find some insights related to the segregation within the groups of data. In the results section, several bi-plots are presented to show some of the insights found in the analysis.

Sometimes it is not possible to detect any pattern in the exploratory analysis (i.e., segregation in the data cannot be visually identified). For those occasions, a statistical analysis is needed to determine if there is a significant effect in the principal components due to the factors that were previously introduced. The statistical analysis in this work was performed with a t-test; the means for every group/level within a factor are compared to see if there is any significant difference between them, if so, it can be concluded that there is some evidence to claim that there is a significant effect in the data due to the factors. The statistical test has the following assumptions: unknown but equal population variances, known sample means and not equal sample variances. The following equations are defined for the t-distribution and the corresponding estimator is introduced with the expressions:

$$t = \frac{\overline{x}_1 - \overline{x}_2 - \left(\mu_1 - \mu_2\right)}{s_p \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \tag{1}$$

$$s_p = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}} \tag{2}$$

2.3. Exploratory data analysis and statistical test results

The following set of bi-plot graphs are introduced to show the relevant information about the groups/levels within each one of the factors under study. **Figure 3** presents the data classification according to the wrap type that was utilized to wrap the switchgrass. As it can be

	Principal component variables				
	1	**2**	**3**	**4**	**5**
Cellulose	−0.64	0.48	0.30	−0.52	−0.09
Hemicellulose	−0.33	−0.83	−0.05	−0.33	−0.32
Lignin	0.21	0.29	−0.66	−0.26	−0.62
Ash	0.22	−0.07	−0.32	−0.64	0.66
Extractives	0.63	−0.02	0.61	−0.39	−0.29

Table 4. Loadings in PCA.

observed, there is no distinguishable segregation within the groups to claim any possible effect due to this factor.

Like the wrap type, data information was also classified according to its particle size and was shown in a bi-plot graph presented in **Figure 4**. The visual representation of the data does not exhibit any clustering in the plotting area, and therefore, no significant findings can be concluded from the bi-plot. Same analysis occurs with the factor corresponding to the classification according to the weight of the bale that can be observed in **Figure 5**, no segregation is noticeable.

In the bi-plot that corresponds to the classification of the date with respect to the days of storage it is possible to identify a segregation in the data. **Figure 6** exhibits this difference between the bales with more than 150 days of storage and those with less number of days. **Figure 7** has another perspective to visually identify this division.

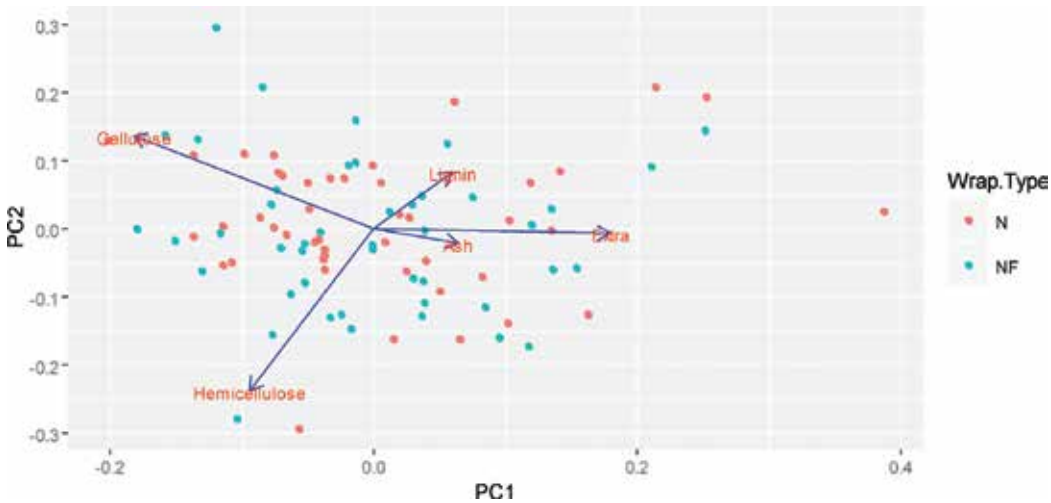

Figure 3. Bi-plot chart for analysis of wrap type.

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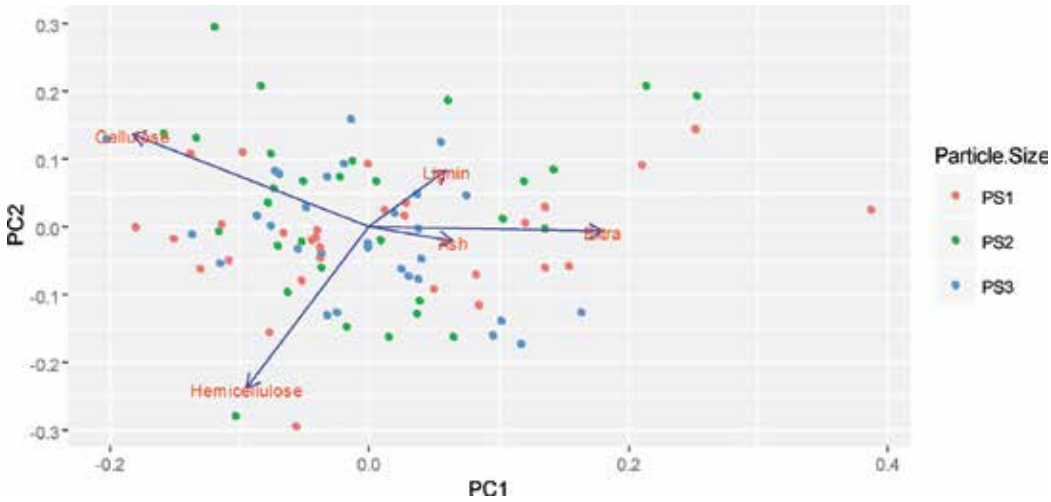

Figure 4. Bi-plot chart for analysis of particle size.

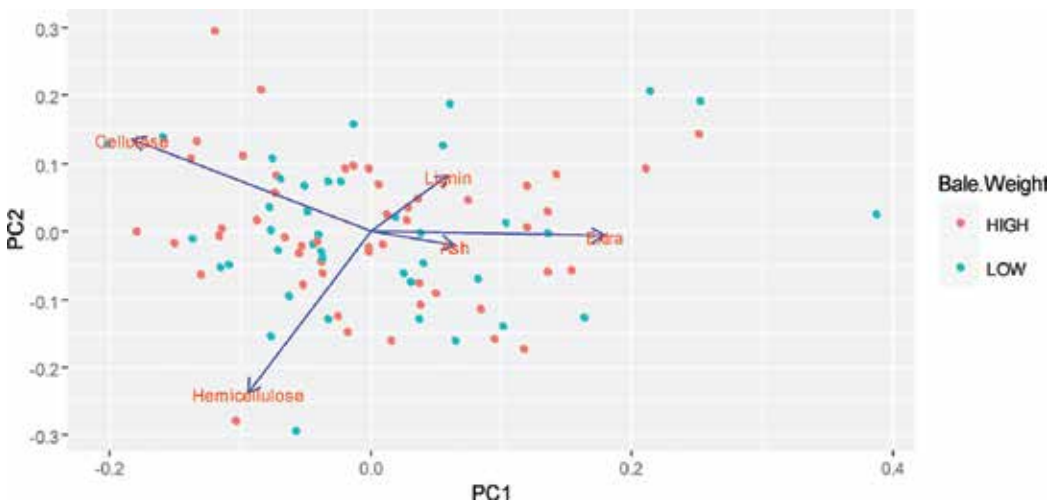

Figure 5. Bi-plot chart for analysis of bale weight.

A t-test was applied for every group/level within every factor presented. The results are shown in **Figure 8**. The storage days is the most important factor since it is the only factor that shows a significant effect due to the statistical difference between the means in the groups. Based on the results, the storage days have repercussion in almost 80% of the variation within the data. Hence, it is relevant in the design of operations that are time dependent.

PCA is a statistical tool that allows the analyst to introduce variance and covariance in the study. Adding the covariance or correlation between the variables could lead the analysis to find some insights that would not be visible with a univariate data analysis tool. Also, PCA allows to sort and classify in a more natural way the significance of every factor analyzed in

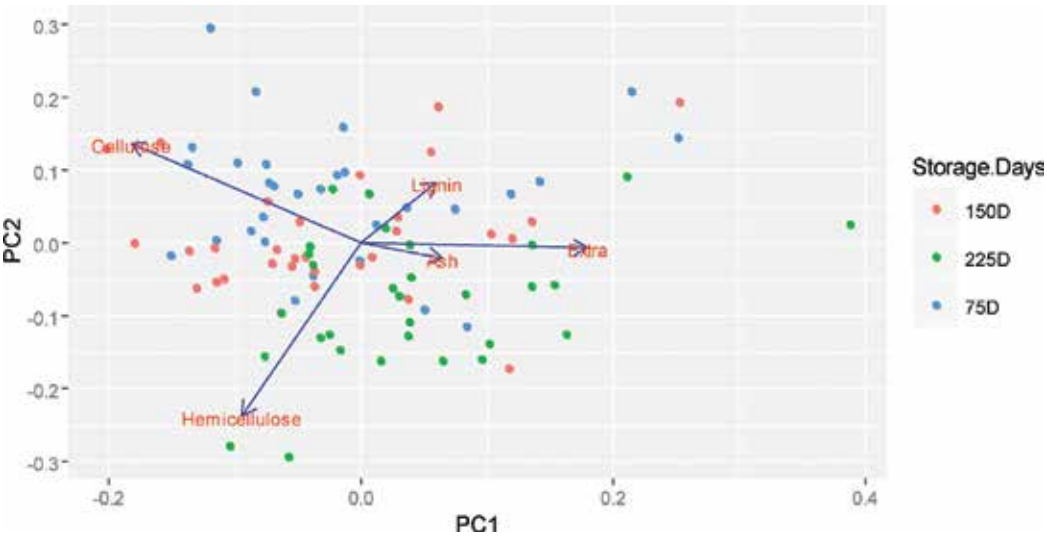

Figure 6. Bi-plot chart for analysis of storage days.

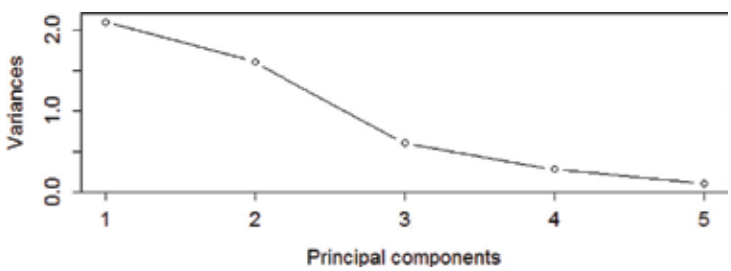

Figure 7. Score plot analysis for PCA.

the database by identifying those factors that have an impact on the main principal components. PCA identifies those factors with a significant effect over the principal components. This information can be utilized to incorporate the relevant factors in stochastic programming models that use the insights found by the multivariate analysis in the optimization problem, and later on, in the decision process. A further discussion on this matter follows.

3. Variable quality-related cost in SC design

The biofuel is an alternative energy resource for the fossil fuels. It is expected that biofuel production increases in the upcoming years due to the increase in the demand. There are some alternatives to approach a mature production of biofuel to support the demand satisfaction. One approach consists in developing new technologies with better conversion processes in order to get an efficient exploitation from the pre-processed biomass for a certain good or

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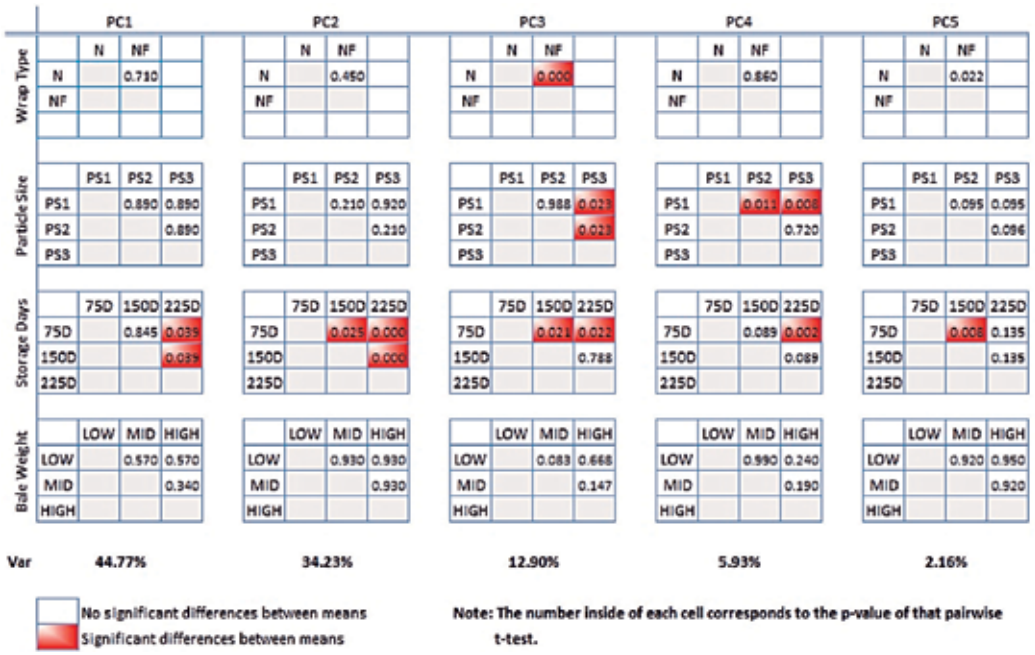

Figure 8. T-test results for all the factors.

product. Another approach considers genetic manipulation with different types of biomass to create hybrids with specific characteristics that suit better for a particular final product. Lastly, another alternative is the improvement of the logistics operations related to the production and distribution of the biofuel.

SCs are being used widely in industry as a system of production and distribution due to the need of integrating processes from suppliers until reaching the end users of any good or service. SCs are presented in diverse scales, from local scale, regional scale up to global scale. Nowadays, large-scale are vastly utilized since they reduce operational costs due to the economies of scale. Large-scale SCs are more challenging since they are more difficult to analyze and solve, so they require the development of new algorithms in order to deal with the complexity of the problem. The introduction of models and algorithms to solve large-scale problems is fundamental in industry since real applications require big data, as well as a large set of constraints related to the problem. Bioenergy industry is a field where large-scale SCs are being implemented.

The production and distribution of biofuels are a big challenge since their commercialization requires a competitive price in the market, and therefore, an efficient SC must be implemented to minimize the operational costs. The production of biofuels deals with the inherent variability in the physical and chemical properties within the biomass because of their impact in some key processes such as the conversion process. The second generation of biofuels (e.g., corn stover, miscanthus, and switchgrass) presents, in general, more variation in properties such as

moisture and ash content than the first generation, and then, quality-related costs must be contemplated in the design and implementation of the SC. However, one of the advantages of using second-generation biomass for the production of biofuels is that local farmers are familiar with the techniques to cultivate and harvest the biomass. For example, harvesting techniques for forage, utilized to feed the livestock, and for the power grass have similar characteristics.

One common objective of the optimization in SC of biofuels is the minimization of costs under the assumption that all types of biomass have similar properties. This assumption can derive in considering only purchasing, logistics and processing costs which is not adequate since every type of biomass possesses different physical and chemical properties that affect the way the SC works. Not including the aforementioned properties could lead companies to have a negative impact on their expected profit since these properties usually experience high levels of variability. Pilot scale biorefineries have experienced a significant difference between the expected and the actual input of biomass [11]. The randomness in the biomass is one of the challenges that biofuel producers must tackle in order to reach profitability and sustainability.

Biomass conversion technologies have their origins in laboratories where specifications of biomass are controlled. The technologies are designed to work under some specifications within the biomass such as the moisture, ash, and carbohydrates contents. When technologies are implemented in large- scale scenarios, it is very likely to receive biomass with specifications that do not meet the requirements, as a result, additional re-work must be done to utilize it. The quality of biofuel is associated with reaching the target levels of physical and chemical properties, for some specific technology, with a low variability.

A poor quality of the biomass results in higher total costs for companies. A quality-related cost can be defined as any cost derived from not meeting the required specifications for a specific conversion technology. The impact of these quality related costs is usually found after the biorefineries have begun operations. The optimization of biofuels [12, 13] considering randomness in the properties of biomass can be approached with stochastic programming [14, 15]. In literature, most of the supply chain models designed for biofuel production are deterministic; then, the stochastic programming is a novel approach to solve instances with variability in the feedstock.

Novel optimization models take into account inherent properties of biomass to lead better decisions that minimize the total cost. It is important to identify the factors that have an influence over the biomass and include those considerations in the optimization model. Castillo-Villar et al. [16] have proposed a stochastic programming model that includes the quality variability in order to make decisions about important aspects of the SC. The work of Castillo-Villar et al. [16] is revisited in this chapter since the authors present a seminal model that integrated biomass variability in the modeling of biofuel supply chains.

3.1. Quality integration in decision models

Moisture and ash content are important properties of the biomass. Castillo-Villar et al. [16] define a random variable, $\varepsilon(t)$ to represent the moisture content corresponding to the mean

value t. A triangular distribution $f_{\varepsilon(t)}$ has been defined for t in the range $[at, bt]$ with a probability density according to the following criteria:

if $at \leq e \leq t$ *then*

$$f_{\in(t)}(e) = \frac{2(e - at)}{(bt - at)(t - at)} \tag{3}$$

if $t < e \leq bt$ *then*

$$f_{\in(t)}(e) = \frac{2(bt - e)}{(bt - at)(bt - t)} \tag{4}$$

Otherwise, 0.

The triangular distribution was proposed due to experimental results in the work of Boyer et al. [17]; in the experiment, a breed of Alamo switchgrass was utilized to test the effect of factors such as storage days, particle size, wrap type and weight of the bale.

Ash content was also represented with a random variable $\vartheta(\delta)$ and the corresponding function is a triangular distribution of the mean value δ. The triangular distribution is defined for the range $[c\delta, d\delta]$. Depending on the technology utilized for the conversion of biomass into biofuel, different requirements are necessary to accomplish the conversion. For example, the conversion of biomass using a thermochemical technology demands at most 10% of moisture content for an efficient process. The moisture target for technology k is defined as t_k. Violating the target for the selected technology will lead to other necessary costs ($\$q$) to compensate not meeting the specifications. The cost of mechanically drying the biomass will be applied to the final good since the content of moisture needs to be reduced up to the target level for conversion purposes. The thermochemical technology also requires at most 10% of ash content for an efficient conversion. The target is defined as δ_k. Again, not meeting the target specification will lead to reprocessing of the biomass with an additional cost.

3.2. Two-stage stochastic model

Stochastic programming introduces randomness into the models where the stochastic variables play a fundamental role in the decision processes. There are several types of stochastic models but probably the most utilized are the two-stage models. In the two-stage models, two types of variables arise, the here-and-now variables and the wait-and-see variables. The here-and-now variables are those that need to be solved in the first-stage because they represent the beginning of the decision and the rest of the decision variables will rely on this step. The wait-and-see variables are presented in the second-stage of the process and depend on the realization of each presented scenario as well as the first-decision stage. The randomness is presented by defining random parameters that will converge to a certain value depending on the scenario, an expected value function for these scenarios in the second-stage plus the value function of the first-stage form the objective function of the stochastic program. A stochastic programming assumes known

distributions in order to set the values for the stochastic parameters in every scenario so the program can be maximized or minimized depending on the objective function.

The two-stage stochastic models for location and transportation define the locations of facilities in the first-stage and the transportation of goods in the second-stage. Castillo-Villar et al. [16] defines a stochastic location-transportation model that introduces the location of the biorefinery as the first-stage variables, and then, the flow of biomass as a second-stage variable. The randomness in the second-stage is included with the inclusion of the stochastic parameters: (1) cost of moisture content, (2) cost of ash content and (3) supply capacity. The aforementioned parameters vary according to the scenario, for example, the level of moisture will be different between a scenario with wet conditions and a scenario with dry conditions. **Table 5** presents the network definitions, **Table 6** shows the parameters definitions and **Table 7** introduces the variables of the model.

$$\text{Min} \sum_{j\in J}\sum_{k\in K} l_{jk}Z_{jk} + \sum_{i\in I}\sum_{j\in J}\sum_{k\in K}\sum_{o\in\Omega} p(o)\left[c_{ij} + c_i'(t_k,o) + c_i(\delta_k,o)\right]X_{ijk}(o) \tag{5}$$

Subject to:

$$\sum_{j\in J}\sum_{k\in K} X_{ijk}(o) \le s_i(o) \quad \forall i\in I, o\in\Omega \tag{6}$$

$$\sum_{i\in I} g_{jk}X_{ijk}(o) \le v_{jk}Z_{jk} \quad \forall j\in J, k\in K, o\in\Omega \tag{7}$$

$$\sum_{i\in I}\sum_{j\in J}\sum_{k\in K} g_{jk}X_{ijk}(o) \ge d \quad \forall o\in\Omega \tag{8}$$

$$\sum_{k\in K} Z_{jk} \le 1 \quad \forall j\in J \tag{9}$$

$$X_{ijk}(o) \in R^+ \quad \forall i\in I, j\in J, k\in K, o\in\Omega \tag{10}$$

$$Z_{jk} \in \{0,1\} \quad \forall j\in J, k\in K \tag{11}$$

Eq. (5) refers to the objective function of the stochastic model which is the minimization of the total cost (investment costs, transportation costs and quality-related costs). Eq. (6) is a constraint

Graph element	Description
N	Set of nodes in supply chain network G(N,A)
A	Set of arcs in G(N,A)
I	Set of suppliers
J	Set of potential locations for biorefineries
T	Set of arcs from I to J

Table 5. Definitions of nodes and arcs in the network graph.

Parameter	Description
l_{jk}	Equivalent annualized investment cost for opening a biorefinery in location $j \in J$ using technology $k \in K$
$p(o)$	Probability of scenario $o \in \Omega$
c_{ij}	Unit cost charged per metric ton shipped along $(i,j) \in T$
$c'_i(t_k, o)$	Quality loss due to moisture content under scenario $o \in \Omega$ for a given t_k
$c_i(\delta_k, o)$	Quality loss due to ash content under scenario $o \in \Omega$ for a given δ_k
$s_i(o)$	Supply capacity for supplier $i \in I$ for scenario $o \in \Omega$
g_{jk}	Conversion factor for biomass supplied to biorefinery $j \in J$ applying technology $k \in K$
d	Total demand of biofuel in the network N
v_{jk}	Production capacity of biorefinery $j \in J$ including technology $k \in K$

Table 6. Definitions of parameters.

Variable	Description
$X_{ijk}(o)$	Flow along arc $(i,j) \in T$ from a supplier location to a potential location for a biorefinery under scenario $o \in \Omega$
Z_{jk}	Binary variable which takes the value 1 if $j \in J$ is used as a biorefinery utilizing technology $k \in K$, and 0 otherwise

Table 7. Definitions of variables.

for the supply capacity, Eq. (7) constraints the biorefinery production capacity and Eq. (8) assures the demand satisfaction of the local market. Eq. (9) selects one technology for every open biorefinery, Eq. (10) is non-negative constraints and Eq. (11) is binary constraints.

3.3. Case study and results

Castillo-Villar et al. [16] solved a case study in the state of Tennessee to test the proposed model. The state of Tennessee has 94 counties that were considered as suppliers in the model, 31 counties were considered as potential locations for biorefineries. The biomass utilized in the model was switch-grass, all the quality information introduced in the model was derived from the experiment of Boyer et al. [17]. Three different triangular distributions (one for moisture and one for ash) were created according to number of groups included in the particle size. **Tables 8** and **9** display the parameters for those distributions.

The available biomass (19,482,102.51 dry tons) for biofuel production was obtained from the U.S. Billion-ton database, and Eq. (12) is utilized to calculate the weight of the biomass in its natural state (i.e., before drying the feedstock).

$$s_i = s_{idry}/(1 - e_i) \tag{12}$$

The scenarios were created from historical data, 11 scenarios were generated utilizing the years 2004–2014. Every region in the state of Tennessee was linked to the closest climate station to

Moisture content (%)	at	t	bt
Distribution 1	26	27	29
Distribution 2	17	19	20
Distribution 3	16	18	23

Table 8. The parameters of triangular distribution for the moisture content.

Ash content (%)	$c\delta$	δ	$d\delta$
Distribution 1	1.33	2.89	4.53
Distribution 2	0.71	2.44	3.79
Distribution 3	0.82	2.18	3.49

Table 9. The parameters of triangular distribution for ash content.

Problem	Moisture	Ash
1	Moisture Level T1	Ash Level T1-Low
2	Moisture Level T1	Ash Level T1-High
3	Moisture Level T2	Ash Level T2-Low
4	Moisture Level T2	Ash Level T2-High
5	Moisture Level T3	Ash Level T3-Low
6	Moisture Level T3	Ash Level T3-High

Table 10. Problem definitions.

Problem	Cost (in $millions)					
	Fixed	Transport	Moisture	Ash	Variable	Total
1	1202	41	28	27	96	1298
2	1202	41	28	37	106	1307
3	1202	46	22	24	92	1294
4	1202	46	22	31	99	1300
5	1202	45	22	23	91	1293
6	1202	45	22	29	96	1298

Table 11. Summary of experimental results.

gather information about the precipitation levels in the region. If the precipitation level of certain region is above the precipitation mean of the state, then, the region is classified as wet; otherwise it is classified as dry. A random number e_i was generated according to the classification of every region in order to calculate the moisture content. The ash content is not

Figure 9. Solution to problem 2, including quality-related costs.

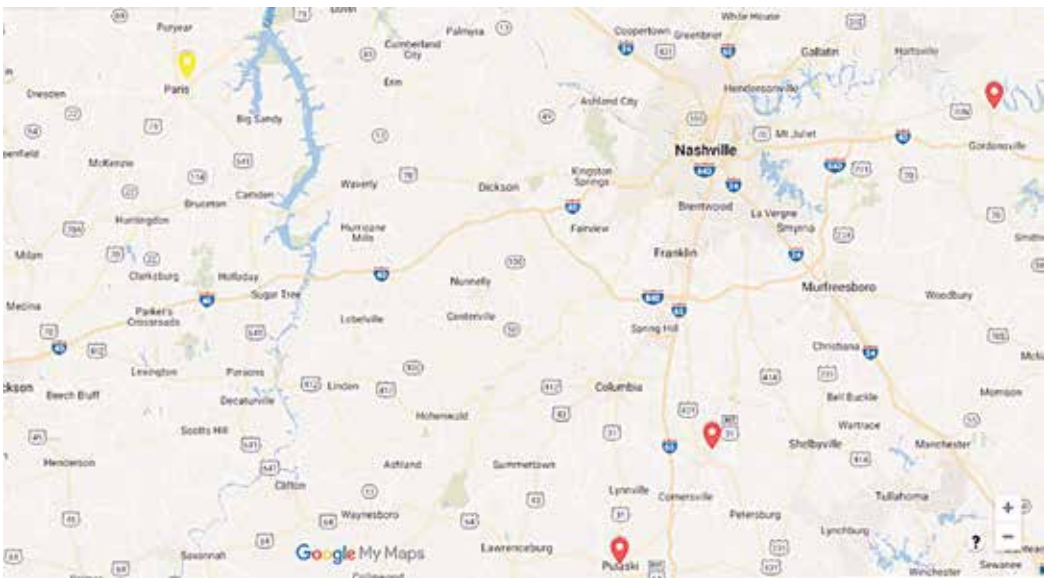

Figure 10. Solution to problem 2, without quality-related costs.

associated with the precipitation level; however, it was also contemplated in the cost calculation for the set of problems shown in **Table 10**.

The study was solved with GUROBI 6.0.0. The experiments were completed in a computer with Intel (R) Core(TM) i7-2600 U CPU @ 3.40 GHz; and 16.00 GB of RAM. The results for every problem described in **Table 10** are shown in **Table 11**. On an average, the quality related

costs are about $52.5 million annually which represents a significant amount of money for an investment. **Figures 9** and **10** shows a different solution for problem 2.

Figure 9 shows the biorefineries locations for the problem 2 (high moisture, high ash) with the inclusion of the quality related costs. **Figure 10** presents the solution for problem 2 without the inclusion of the quality-related costs. The red pin indicates the location of a large capacity plant and the yellow pin shows the location of a smaller capacity biorefinery. It can be noticed that both solutions differ in the position of the pins.

4. Conclusions

This chapter presents a novel approach to incorporate physical and chemical properties of the biomass, which affect the design and implementation of the supply chain for production and distribution of biofuels. The PCA is a tool that allows the decision maker to use the variance plus additional information on how the variables interact with each other (covariance) to infer the effect of the factors included with the analysis. Moreover, PCA has the capability to detect the magnitude of the factor's effect over the variables under analysis. The factors with significant impact on the process can be incorporated into the mathematical models. The stochastic model presented in this chapter shows the impact of moisture and ash content in the production and distribution of biofuel. The results show a difference in the overall cost function**,** but also a different optimal design of the supply chain. Hence, ignoring the quality-related properties might lead to the cost underestimations.

Biomass has other features that impact the logistic system. Investigating new factors such as dry matter loss and integrating these factors into the modeling and algorithmic development is a fruitful future research line.

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Chapter 4

Atomization of Bio-Fossil Fuel Blends

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Additional information is available at the end of the chapter

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Abstract

The importance of modeling multi-component fuel atomization, heating and evaporation has been recognized in many studies. The predictions of these models are crucial to the design and performance of combustion engines. Accurate modeling is essential to the understanding of these processes and ultimately to improving engine sustainability and reducing emission. The interest in bio-fossil fuel blends has been mainly stimulated by depletion of fossil fuels and the need to reduce carbon dioxide emissions that contribute towards climate change. This work presents a review of recent investigations into the heating and evaporation of multi-component blended fuel droplets in real internal combustion engine (ICE) conditions. The models consider the contribution of all groups of hydrocarbons in fossil (gasoline, diesel) fuels, methyl esters in 22 biodiesel fuels, and ethanol fuel. Diffusion of these fuel species, temperature gradient, and recirculation within droplets are accounted for. One important finding is that some fuel blends, for example B5 (5% biodiesel fuel and 95% diesel fuel) and E5 (5% ethanol fuel and 95% gasoline fuel), can give almost identical droplet lifetimes to the ones predicted for pure diesel and gasoline fuels; i.e. such mixtures can be directly used in conventional engines without modification.

Keywords: atomization, biodiesel, diesel, ethanol, fuel blends, gasoline, multi-component

1. Introduction

Biodiesel and ethanol fuels have been of great interest to scientists and public as biofuel resources of energy due to depletion of fossil fuels and impact on global warming [1, 2]. Also, compared to fossil fuel, biodiesel fuel has several advantages: it has less carbon dioxide emissions, higher flash point, higher lubricity and it is cost effective. In addition, diesel fuel can be blended with up to 20% of many biodiesel fuel types and directly injected in

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standard diesel engines with minor tuning or no modification requirement in the ICE processes [3, 4]. According to Tier I and Tier II standards of the U.S.A. Environmental Protection Agency (EPA) (see [5] for details), biodiesel fuels produced within the last decade meet the minimum requirements of health risk [6]. Similarly, ethanol (or bio-ethanol) is commonly used as an alternative source of energy in the form of pure, or gasoline-blended, fuel in sparkling ignition (Otto cycle) engines [7]. Bioethanol is a very promising reactant for petrol engines and for biodiesel production industry, compared to methanol and fossil fuels. Lipids, such as fats or oils, react with ethanol to produce biodiesel. Also, it is renewable, green, and not toxic [8].

The delay in processes preceding the onset of combustion (mainly the spray formulation, and heating and evaporation of fuel droplets) in the internal combustion engines is crucial to the design and performance of these engines [9, 10]. The complexity of modeling evaporation processes should be taken into account as it involves detailed physics of heat transfer, mass transfer and fluid dynamics associated processes. Most of the studies on fuel droplet heating and evaporation analyses have been either based on considering individual components, described as 'discrete component' (DC) approach [11, 12], or on a probabilistic analysis of large numbers of hydrocarbons, described as 'continuous thermodynamics' [13–16] and 'distillation curve' [16–18] approaches. The DC approach is highly accurate and computationally efficient in the cases when a small number of hydrocarbons need to be taken into account. In the second approach, several simplifying assumptions are made; such as the assumption that hydrocarbon species inside droplets are mixed infinitely quickly, described as 'infinite diffusivity' approach, or they are not mixed at all, described as 'single component' approach.

The DC model, based on the analytical solutions to the equations of heat and mass transfer and species diffusion [19], has been verified against numerical simulations and validated against experimental data in [20] (see [9, 21] for more details). Following [22–25], the droplets heating and evaporation processes are analyzed by application of several blends of diesel-biodiesel fuels and ethanol-gasoline fuels.

The DC model is used for this analysis and applied to a broad range of diesel-biodiesel fuel fractions and ethanol-gasoline fractions. The mixture of EU diesel fuel with 22 types of widely used fatty acid methyl ester (FAME) biodiesel fuels have been investigated. These are: tallow (TME), lard (LME), butter (BME), coconut (CME), palm kernel (PMK), palm (PME), safflower (SFE), peanut (PTE), cottonseed (CSE), corn (CNE), sunflower (SNE), soybean (SME), rapeseed (RME), linseed (LNE), tung (TGE), hemp-oil, produced from hemp seed oil in the Ukraine (HME1), hemp-oil, produced in European Union (HME2), canola (CAN), waste cooking-oil (WCO), yellow grease oil (YME), camelina (CML), and jatropha (JME). Droplets with four fractions of diesel-biodiesel blends have been investigated in the DC model. These are 5% biodiesel with 95% diesel fuels (B5), 20% biodiesel with 80% diesel fuels (B20), 50% biodiesel with 50% diesel fuels (B50), pure biodiesel (B100) and pure diesel fuels (PD). For the ethanol-gasoline blends, droplets with five fractions have been investigated in the DC model. These are 5% ethanol with 95% gasoline fuels (E5), 20% ethanol with 80% gasoline fuels (E20),

50% ethanol with 50% gasoline fuels (E50), 85% ethanol with 15% gasoline fuels (E85), pure ethanol (E100) and pure gasoline fuels (E0).

In this work, the discrete component model is utilized to analyze the droplets heating and evaporation of diesel-biodiesel and ethanol-gasoline fuel blends.

2. Model

The diesel-biodiesel blends are represented by a mixture of 22 types of biodiesel fuels with up to 22 species of methyl ester and diesel fuel, formed of 98 hydrocarbons represented by 9 groups (see [26] for more details). The ethanol-gasoline blends are represented by a mixture of ethanol and fuel used in advanced combustion engines, type C (FACE C) gasoline fuel, formed of 20 hydrocarbons, in 6 groups categorized according to their chemical structures and thermodynamic and transport properties (see [27] for more details). The thermodynamic and transport properties of diesel are inferred from [28], and those of biodiesel fuel are inferred from [26, 29] respectively; while properties of ethanol and gasoline fuel are taken from [27, 30] respectively. The contribution of species and average droplet temperatures are taken into account in the calculation of all fuel properties. All units used in our analyses are SI unless indicated otherwise.

2.1. Spray model

Two parameters for the modeling of droplet breakup based on liquid properties have been introduced by Eggers [31]; these are time and length parameters. For the calculation of length parameter (LP), we take into account viscosity, density and surface tension of liquid, as:

$$\mathrm{LP} = \frac{v_f^2 \rho_f}{\sigma} \tag{1}$$

We have proposed to use LP for spray parameters calculation including Sauter mean diameter (SMD) and spray penetration [32, 33]. Our analysis shows that the spray penetration of biodiesels will be proportional to $\mathrm{LP}^{0.1}$:

$$S_{tip} = A_{LP}\left(d_0^{0.5} p_{inj}^{0.36} \rho_g^{-0.29}\right) \mathrm{LP}^{0.1} t_{inj}^{0.5} \tag{2}$$

where d_0 is nozzle diameter, ρ_g is gas density, and p_{inj} is injection pressure.

It has been suggested in [34] that the better prediction of SMD for diesel and biodiesel fuels can be predicted as:

$$\mathrm{SMD} = 3.08\, v_f^{0.385} (\sigma \rho_f)^{0.737} \rho_g^{0.06} \Delta p^{-0.54} \tag{3}$$

We have used the following expression to calculate the middle droplet diameter of biodiesel fuels:

$$\mathrm{SMD} = 23\, d_0^{0.35}\, \mathrm{LP}^{0.1}\, \Delta p^{-0.54}\, \rho_g^{0.06} \tag{4}$$

where Δp is the pressure difference.

For ethanol-kerosene blends for air-blast atomizer, it was found that the SMD can be calculated as [33]:

$$\mathrm{SMD} = 2253\, \mu_l^{0.633}\, p_l^{-0.507}\, p_a^{-4.565\times10^{-3}} \tag{5}$$

2.2. Evaporation model

The DC model is based on the analytical solutions to the heat transfer and species diffusion equations via the effective thermal conductivity (ETC) model and effective diffusivity (ED) model. The importance of these models can be attributed to the fact that they take into account the recirculation, temperature gradients and species diffusion inside droplets. The heat conduction equation for the temperature $T = T(t, R)$ in the liquid phase in a spherical droplet can be presented as:

$$\frac{\partial T}{\partial t} = \kappa_{\mathrm{eff}}\left(\frac{\partial^2 T}{\partial R^2} + \frac{2}{R}\frac{\partial T}{\partial R}\right) \tag{6}$$

where, t is the time, R is the distance from the center of the droplet, T is the temperature and κ_{eff} is the effective thermal diffusivity.

The time evolution of species mass fractions at any R is described by the following Eq. [19, 35]:

$$\frac{\partial Y_{li}}{\partial t} = D_{\mathrm{eff}}\left(\frac{\partial^2 Y_{li}}{\partial R^2} + \frac{2}{R}\frac{\partial Y_{li}}{\partial R}\right) \tag{7}$$

where, $i > 1$, D_{eff} is the effective liquid species diffusivity, $D_{\mathrm{eff}} = \chi_Y D_l$, D_l is the liquid diffusivity and χ_Y is a coefficient that varies between 1 and 2.72 [19, 20]. χ_Y takes into account the recirculation inside droplets.

3. Fuel compositions

The commercial diesel fuel selected in the present work conforms to standard European Union fuel (EN590), formed of 98 hydrocarbons represented by 9 groups, categorized according to their chemical formulae. Molar fractions of various components in this fuel are presented in **Table 1**, inferred from [28].

For gasoline fuel, the number of the components with identical chemical formulae and close thermodynamic and transport properties are replaced with characteristic components leading to reducing the original composition of gasoline fuel (83 components) to 20 components only, represented by 6 hydrocarbon groups as presented in **Table 2** (see [27] for more details). The biodiesel fuels are formed of up to 22 species of fatty acid methyl ester (FAME). These are inferred from [24, 36] and presented in **Table 3**.

Carbon no	N-Alkane	Iso-alkane	Cycloalkane	Bi-cycloalkane	Tri-cycloalkane	Alkylbenzene	Indane & tetraline	Naphthalene	Diaromatic	Phenanthrene
C8	0.308	—	—	—	—	0.497	—	—	—	—
C9	1.0513	1.9807	—	—	—	3.2357	—	—	—	—
C10	1.2635	3.7906	0.6408	0.6926	—	5.3584	1.3157	1.9366	—	—
C11	1.1002	2.0628	1.8745	1.0524	—	0.9492	1.3632	2.5290	—	—
C12	0.9866	1.6290	1.6951	0.9753	—	1.9149	1.1951	1.4012	—	—
C13	0.9646	1.5793	1.2646	0.6611	—	0.6873	1.0652	0.7692	0.3834	—
C14	1.0146	1.6351	1.3633	0.5631	0.0914	0.6469	0.8406	0.4879	0.3217	0.0768
C15	1.2051	1.9595	1.2353	0.4314	0.1799	0.4782	0.7051	0.3843	0.2589	0.2033
C16	1.0442	1.6137	1.0449	0.4921	0.1773	0.4564	0.6684	0.2854	0.2602	0.1705
C17	1.0564	1.8041	1.0162	0.6529	0.4001	0.4204	0.5598	0.2072	—	0.1154
C18	1.0596	2.1807	1.2848	0.6554	0.3304	0.5234	0.5357	0.2358	—	0.0917
C19	1.0916	2.438	1.3566	0.9901	0.2159	0.3226	0.3403	0.2151	—	—
C20	0.7054	1.5284	0.9961	0.1965	0.1696	0.2848	0.3227	0.2256	—	—
C21	0.3756	1.0674	0.5374	0.0935	—	0.2032	0.1638	—	—	—
C22	0.2328	0.5662	0.304	0.0701	—	0.0969	0.0781	—	—	—
C23	0.1083	0.2889	0.109	0.0488	—	0.0494	—	—	—	—
C24	0.0461	0.1442	0.0755	0.0234	—	0.0473	—	—	—	—
C25	0.0221	0.0776	0.0445	0.0169	—	—	—	—	—	—
C26	0.0106	0.0319	0.0214	—	—	—	—	—	—	—
C27	0.0052	0.0257	0.0155	—	—	—	—	—	—	—
Total%	13.65	26.40	14.88	7.62	1.56	16.17	9.15	8.68	1.22	0.66

Table 1. The diesel fuel composition (molar fractions) used in our analyses [28].

http://dx.doi.org/10.5772/intechopen.73180

m	Group	Molar fractions (%)	Number of components
1	N-Alkanes	28.50	5
2	Iso-alkanes	65.18	8
3	Aromatics	4.40	4
4	Indanes/naphthalenes	0.10	1
5	Cycloalkanes	0.33	1
6	Olefins	1.49	1

Table 2. The groups of gasoline fuel molecules, their molar fractions, and the numbers of components within each group, as used in our models [27].

FAME	Biodiesel fuels										
	TME	LME	BME	CME	PMK	PME	SFE	PTE	CSE	CNE	SNE
C8:0	—	—	5.2	6.0	2.6	—	—	—	—	—	—
C10:0	—	—	2.8	8.0	4.0	—	—	—	—	—	—
C12:0	0.2	—	3.4	50.0	50.0	0.3	—	—	—	—	—
C14:0	2.5	1.0	11.0	15.0	17.0	1.3	—	0.5	2.0	1.0	—
C15:0	—	—	—	—	—	—	—	—	—	—	—
C16:0	27.9	26.0	31.7	9.0	8.0	45.1	5.2	8.0	19.0	9.0	5.9
C17:0	—	—	—	—	—	—	—	—	—	—	—
C18:0	23.0	14.0	10.8	3.0	1.7	4.5	2.2	4.0	2.0	2.5	4.2
C20:0	0.4	—	0.4	—	1.5	0.4	—	7.0	—	—	1.4
C22:0	0.4	—	0.4	—	1.5	0.2	—	7.0	—	—	1.4
C24:0	—	—	—	—	—	—	—	—	—	—	—
C16:1	2.5	2.8	2.4	—	0.4	0.2	—	1.5	—	1.5	—
C17:1	—	—	—	—	—	—	—	—	—	—	—
C18:1	40.0	44.0	26.3	7.0	12.0	38.4	76.4	49.0	31.0	40.0	18.5
C20:1	0.3	2.0	1.0	—	—	—	—	—	2.5	1.0	—
C22:1	0.3	2.0	1.0	—	—	—	—	—	2.5	1.0	—
C24:1	—	—	—	—	—	—	—	—	—	—	—
C18:2	2.0	8.0	3.0	2.0	1.3	9.2	16.2	23.0	41.0	44.0	68.3
C20:2	—	—	—	—	—	—	—	—	—	—	—
C18:3	—	—	0.6	—	—	0.2	—	—	—	—	0.3
C20:3	—	—	—	—	—	—	—	—	—	—	—
C18:4	—	—	—	—	—	—	—	—	—	—	—
Others	0.5	0.2	—	—	—	0.2	—	—	—	—	—

FAME	Biodiesel fuels										
	TGE	**HM1**	**SME**	**LNE**	**HM2**	**CAN**	**WCO**	**RME**	**CML**	**JTR**	**YGR**
C8:0	—	—	—	—	—	—	—	—	—	—	—
C10:0	—	—	—	—	—	—	—	—	—	—	—
C12:0	—	—	—	—	—	—	0.2	—	0.4	0.1	0.2
C14:0	—	—	0.3	0.2	—	—	0.7	—	2.6	0.3	0.8
C15:0	—	—	—	—	—	—	—	—	—	—	0.1
C16:0	3.6	6.6	10.9	6.2	6.5	4.5	15.7	4.9	5.8	14.3	16.0
C17:0	—	0.2	—	—	—	0.1	0.2	—	—	0.1	0.1
C18:0	2.6	2.1	4.4	0.6	2.5	2.0	6.1	1.7	2.7	5.9	6.9
C20:0	—	0.5	0.4	—	0.9	0.6	0.4	0.6	1.3	0.2	0.3
C22:0	13.1	0.3	—	—	—	0.4	0.4	—	0.9	0.2	0.4
C24:0	—	0.2	—	—	—	0.2	0.3	—	0.7	2.5	0.2
C16:1	—	0.3	—	—	—	0.4	0.7	—	—	1.0	0.9
C17:1	—	—	—	—	—	—	—	—	—	—	0.1
C18:1	10.1	11.9	24.0	18.0	11.9	59.7	42.8	26.6	15.9	38.9	43.2
C20:1	0.8	0.3	—	—	0.9	1.5	0.6	—	13.7	0.1	0.5
C22:1	—	0.2	—	—	—	0.4	0.2	22.3	2.9	0.1	0.1
C24:1	—	0.2	—	—	—	—	—	0.8	0.2	0.1	4.3
C18:2	13.8	56.6	52.8	16.0	54.7	20.8	29.4	24.8	16.0	34.8	24.3
C20:2	—	—	—	—	—	—	—	—	1.4	—	—
C18:3	51.6	20.6	7.2	59.0	20.1	9.4	2.0	9.7	33.8	0.3	1.1
C20:3	—	—	—	—	—	—	—	—	0.8	—	—
C18:4	—	—	—	—	—	—	—	—	—	—	0.5
Others	4.4	—	—	—	2.5	—	0.3	8.6	0.9	1.1	—

Table 3. Biodiesel fuel compositions [24, 36].

4. Results

4.1. Atomization

The importance of spray breakup associated phenomena for various applications is well recognized and has been extensively investigated experimentally and numerically by engineers, environmentalists, automotive industrialists, pharmaceutics, and agriculturists [21, 37–41]. A rigorous representation of spray breakup is very complicated procedure as it would involve accurate estimation of nozzle flow, initial formation of ligaments, instabilities, cavitation, and droplets associated physics and their subsequent breakup, heating, evaporation, the entrainment of air and the effects of turbulence [21, 40]. The efficiency of the combustion process and emission reduction in internal combustion engines depends on the atomization characteristics;

the most important characteristics of which are droplet Sauter mean diameter (SMD), cone angle, droplet size distributions. The SMD of biodiesel and diesel fuel droplets at temperature 80°C, as reported in [34], are shown in **Table 4**.

Reference	PME	HME1	HME2	RME	SME	Diesel
Eq. (3)	25.1	—	—	28.8	25.7	17.7
Eq. (4)	—	23.55	23.55	26.69	23.87	18.3

Table 4. The SMDs (in μm) of typical biodiesel and diesel fuel droplets at 80°C.

The average value of biodiesel fuel droplet SMDs (25.32 μm) is larger than those of diesel fuel droplets, which can be attributed to the higher viscosity of biodiesel fuels [34].

4.2. Probability density function for biodiesel spray

It is very important to know how biofuel droplets distribute/spread by size after the atomization. **Figure 1** shows the drop-size probability density for diesel and biodiesel fuels when experimental data [42] are fitted by maximum entropy method [43].

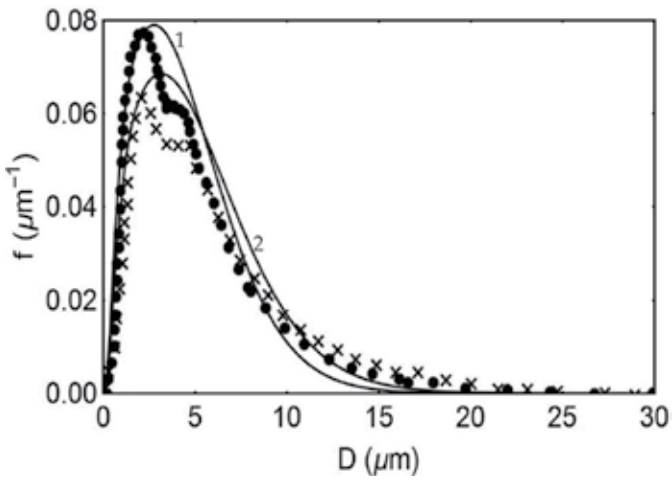

Figure 1. Probability density functions of the droplet diameters at distance of 15 mm from nozzle exit [43]; 1- for diesel fuel, 2 - for biodiesel fuel. Experimental data for diesel (•) and biodiesel (×) fuels are inferred from [43, 44].

The case shown in **Figure 1** is close to realistic diesel engine conditions with an injection pressure of 100 MPa. In this case, diesel fuel has emerged from the nozzle orifice with a velocity of about 100 m/s as ultra-high-speed videos shown in [40]. We assumed that biodiesel has a lower mean injection velocity than diesel, but this difference is compensated by the higher value of middle droplet diameters for biodiesel.

4.3. Blended diesel-biodiesel fuel droplets

The DC model is facilitated for the analysis of heating and evaporation of diesel-biodiesel fuel droplets of initial radius R_{d0} = 12.66 μm and temperature T_0 = 360 K. The droplets are moving at U_d = 10 ms^{-1} in still air of pressure and temperature equal to p_g = 30 bar and T_g = 800 K, respectively. The evolutions of droplet surface temperatures (T_s) and radii (R_d) for three mixtures of diesel-biodiesel fuels (B5, B20, B50) and pure biodiesel fuel (B100) of 22 types of biodiesel fuels are analyzed. Typical examples of these results are presented in **Figures 2–7**.

In **Figures 2–7** (some examples of the analyzed blends of diesel-biodiesel fuels), one can see that increasing the concentration of biodiesel from B5 to B100 has a noticeable effect on the evolution of R_d and T_s. In addition, the predicted surface temperature of the droplet for B100 is higher than that of B5 during the initial heating period. According to [22], the droplet break-up process can be enhanced as a result of the increase in droplet surface temperature. This can be attributed to the decrease in droplet surface tension. A full illustration of the results provided in **Figures 2–7** are shown in **Table 5**. The droplet lifetimes of 22 types of biodiesel fuel mixtures with PD fuel and their differences from the one predicted for PD fuel (2.25 ms) are presented in this table.

As can be seen from **Table 5**, the droplet lifetime for B100 of RME fuel is 6% less than that of PD. This reduction does not exceed 0.4% for the B5 fuel blend for the same fuel. Also, droplet lifetime of TGE biodiesel fuel droplet is noticeably close to that of PD droplet; it is less than 8 and 0.5% for B100 and B5 mixtures, respectively. The maximum difference in droplet lifetimes for these fuels is up to 21.6% (B100 CME), which cannot be sacrificed in any engineering application, and it is always higher than 5.29% (RME) compared to PD, which may be tolerated in some limited engineering applications.

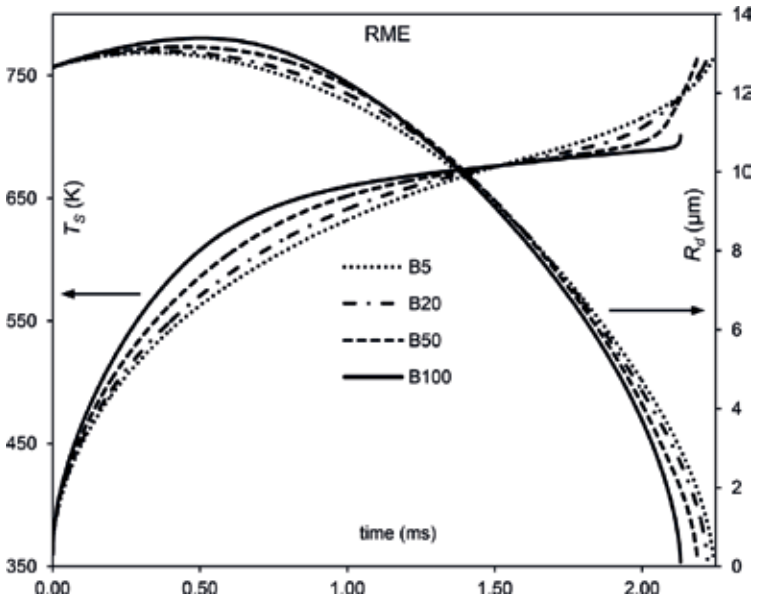

Figure 2. Droplet surface temperatures T_s and radii R_d versus time for four fractions of diesel-RME biodiesel fuels: B5, B20, B50 and B100.

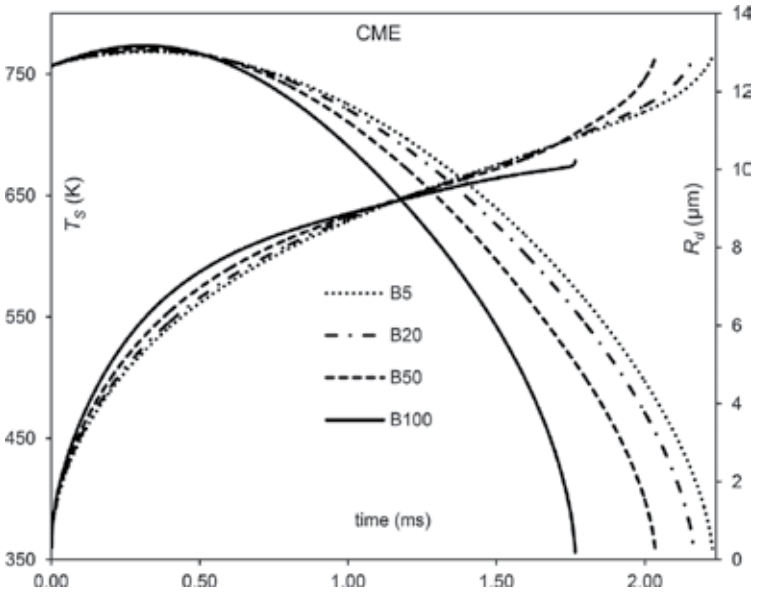

Figure 3. Droplet surface temperatures T_s and radii R_d versus time for four fractions of diesel-CME biodiesel fuels: B5, B20, B50 and B100.

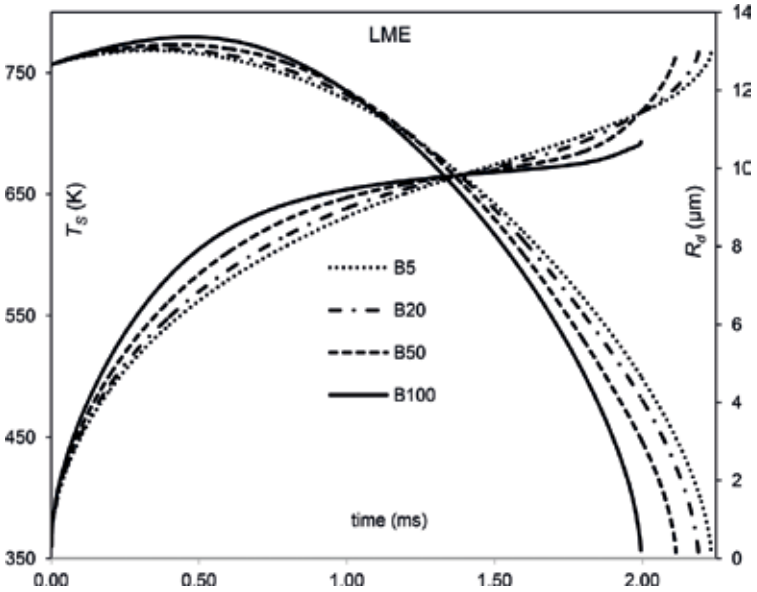

Figure 4. Droplet surface temperatures T_s and radii R_d versus time for four fractions of diesel-LME biodiesel fuels: B5, B20, B50 and B100.

In some previous studies (for example, see [22, 23]) the heating and evaporation of PD fuel droplets and their comparison to the results of diesel-biodiesel blends were analyzed. For instance, in [23] the droplet lifetime for B100 of WCO was shown to be 11% less than that of PD. While in [22], the droplet lifetime for B100 of SME fuel was shown to be 6% less than that

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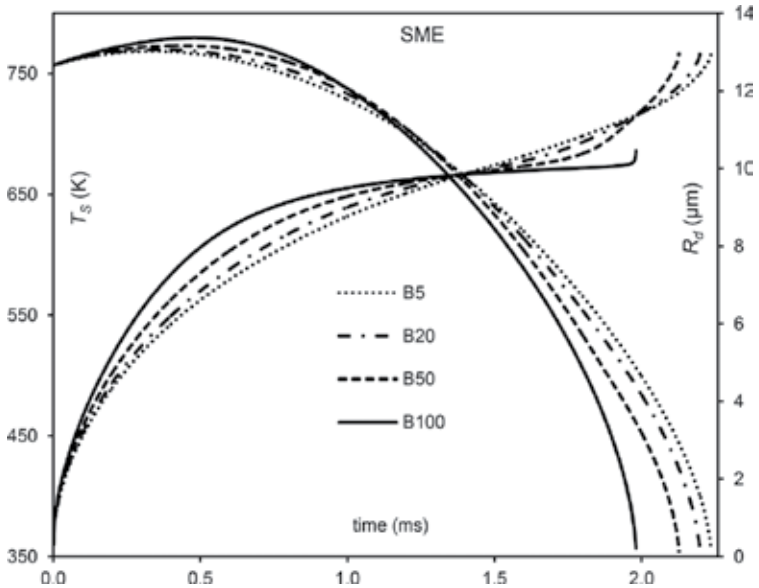

Figure 5. Droplet surface temperatures T_s and radii R_d versus time for four fractions of diesel-SME biodiesel fuels: B5, B20, B50 and B100.

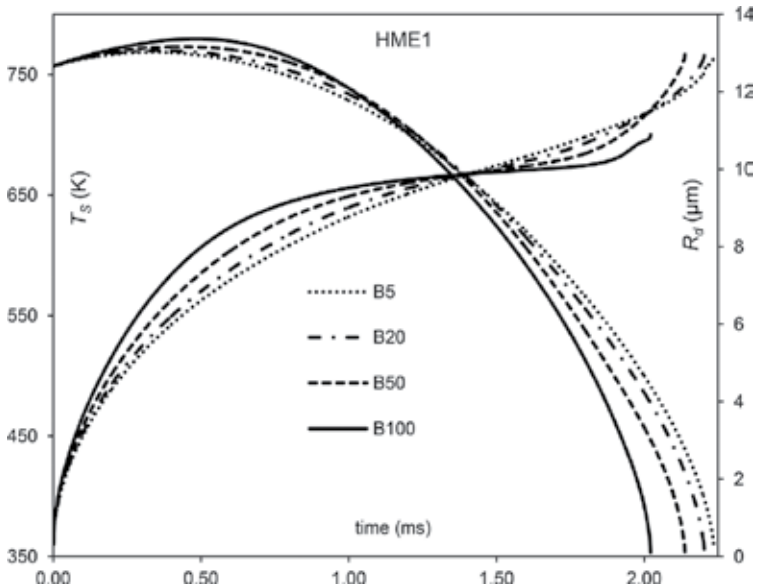

Figure 6. Droplet surface temperatures T_s and radii R_d versus time for four fractions of diesel-HME1 biodiesel fuels: B5, B20, B50 and B100.

for PD. In this study, similar trends were predicted for the same fuels. This prediction, however, was different for the other types of biodiesel fuel presented in this work. For example, the B100 droplet lifetimes for CME and PMK biodiesel fuels showed deviations of 21.6 and 18%, respectively, from that of PD fuel.

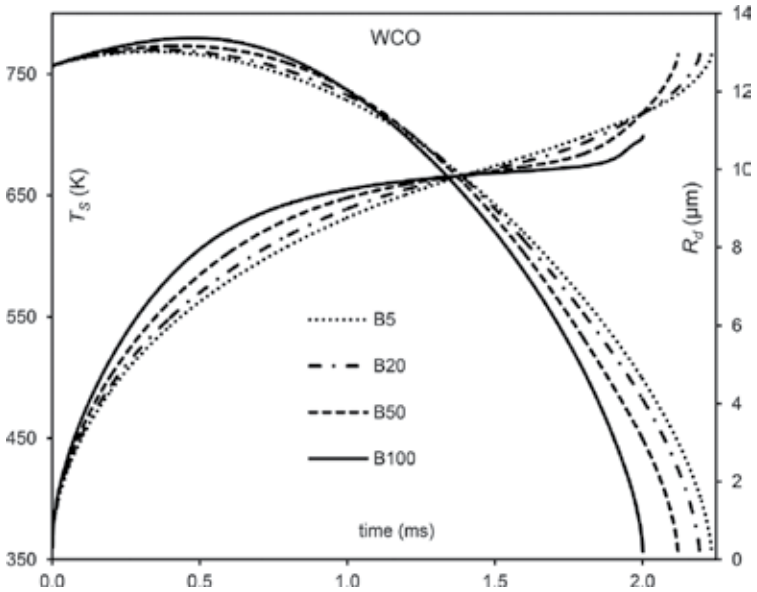

Figure 7. Droplet surface temperatures T_s and radii R_d versus time for four fractions of diesel-WCO biodiesel fuels: B5, B20, B50 and B100.

A general trend shows that droplets' lifetimes of all 22 types of B5 diesel-biodiesel blends that are used in this study deviate with less than 1% from the one predicated for PD droplets. This concludes the possibility of labeling diesel-biodiesel blends, with up to about 5% biodiesel concentration, without modifying the automotive system is achievable. For some fuel blends (for example B20 RME, TGE, LNE, and HME1), this deviation (up to 2%) is still relatively negligible to mix higher biodiesel concentrations (for example, 20% biodiesel and 80% diesel fuels) without losing the main feature of these processes (i.e. droplet lifetime).

The difference in thermodynamic and transport properties between hydrocarbons and methyl esters is the main reason for the influence of biodiesel fuel fractions on the heating and evaporation of diesel fuel droplets. For instance, when increasing the biodiesel fractions, the droplet surface temperature tends to reach a plateau during the evaporation process, which is similar to the case of single component model (see [20, 28]). Also, the significance of such behavior can change depending on the input parameters and ambient conditions.

A typical example of time evolutions of mass fractions at the surface of droplets (Y_{lis}) of selected nine species of B50 fuel mixture of diesel with RME is shown in **Figure 8**; in which, the curves 1, 2 and 3, refer to alkane hydrocarbons of $C_{27}H_{56}$, $C_{25}H_{52}$ and $C_{23}H_{48}$, respectively; and the curves 4, 5 and 6, refer to cycloalkane hydrocarbons of $C_{27}H_{54}$, $C_{25}H_{50}$ and $C_{23}H_{46}$, respectively; and the curves, 7 and 8, refer to rapeseed methyl esters of $C_{19}H_{36}O_2$ and $C_{19}H_{34}O_2$, respectively, under the same conditions used in **Figures 2–7**.

As can be seen from **Figure 8**, the diffusion of mass fractions of components at the surface of droplets is typical and similar to those presented in previous studies. The mass fractions of the heavy components, for example $C_{27}H_{56}$ (1) and $C_{27}H_{54}$ (4), increase with time at the

Biodiesel fuels	B100		B50		B20		B5	
	Lifetime (ms)	Diff. (%)	Lifetime (ms)	Diff. (%)	Lifetime (ms)	Diff. (%)	Lifetime (ms)	Diff. (%)
TME	1.967	12.6	2.102	6.6	2.184	2.9	2.232	0.80
LME	1.995	11.3	2.114	6.0	2.190	2.7	2.234	0.71
BME	1.943	13.6	2.089	7.2	2.180	3.1	2.232	0.80
CME	1.765	21.6	2.036	9.5	2.166	3.7	2.229	0.93
PMK	1.846	18.0	2.050	8.9	2.169	3.6	2.230	0.89
PME	1.944	13.6	2.097	6.8	2.183	3.0	2.232	0.80
SFE	1.980	12.0	2.122	5.7	2.195	2.4	2.235	0.67
PTE	2.052	8.8	2.138	5.0	2.199	2.3	2.236	0.62
CSE	2.014	10.5	2.128	5.4	2.197	2.4	2.236	0.62
CNE	2.002	11.0	2.128	5.4	2.197	2.4	2.236	0.62
SNE	2.011	10.6	2.132	5.2	2.200	2.2	2.237	0.58
SME	1.981	12.0	2.127	5.5	2.198	2.3	2.236	0.62
RME	2.131	5.3	2.188	2.8	2.222	1.2	2.242	0.36
LNE	1.991	11.5	2.141	4.8	2.206	2.0	2.239	0.49
TGE	2.085	7.3	2.160	4.0	2.211	1.7	2.240	0.44
HME1	2.022	10.1	2.138	5.0	2.203	2.1	2.237	0.58
HME2	1.994	11.4	2.135	5.1	2.202	2.1	2.238	0.53
CAN	2.014	10.5	2.130	5.3	2.199	2.3	2.236	0.62
WCO	2.002	11.0	2.121	5.7	2.194	2.5	2.235	0.67
CML	2.064	8.3	2.153	4.3	2.209	1.8	2.239	0.49
JTR	2.047	9.0	2.133	5.2	2.198	2.3	2.236	0.62
YGR	2.077	7.7	2.149	4.5	2.203	2.1	2.237	0.58

Table 5. Estimation of biodiesel fuel droplets lifetimes and their differences compared with those of PD fuel (2.25 ms), under the same conditions shown in **Figures 2–7**.

expense of the lighter ones leading to different properties of duel near the evaporation time. The impacts of ambient pressure on the estimated droplet lifetimes of various LME biodiesel-diesel mixtures are shown in **Figure 9**.

It can be seen from **Figure 9** that the impacts of increasing ambient pressure (20–60 bar) at a relatively high ambient temperature (800 K) on reducing the estimated droplet lifetimes are proportional with almost the same effect for all mixtures (B5 – B100), but with lower droplet lifetimes for B100 and higher ones for B5. One can see that the difference in droplet lifetimes for different blends increase with increasing ambient pressure. Typical ambient pressure at diesel injection time is about 32 bar, however, it can be concluded that minimizing the pressure to 32 bar is better for high blend ratios, as the less the pressure the less the expected deviation in droplet lifetimes.

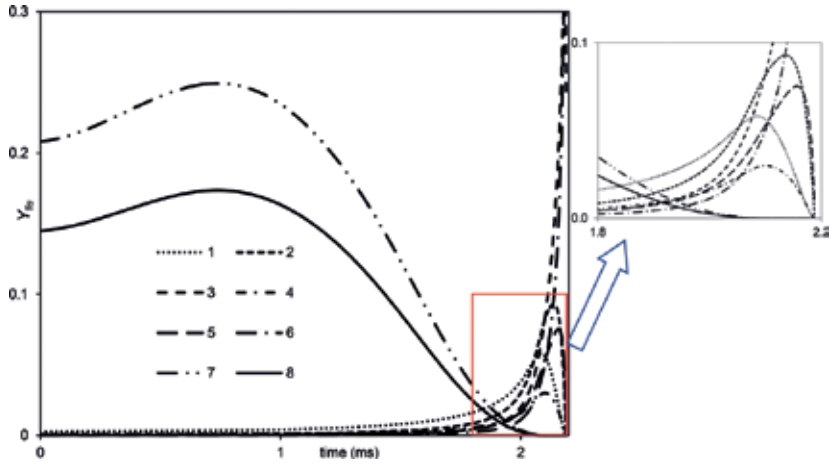

Figure 8. The liquid mass fractions at the surface of droplet (Y_{lis}) versus time for selected 8 components of 106 components of B50 (50% diesel hydrocarbons and 50% rapeseed methyl ester (RME)) fuel mixture.

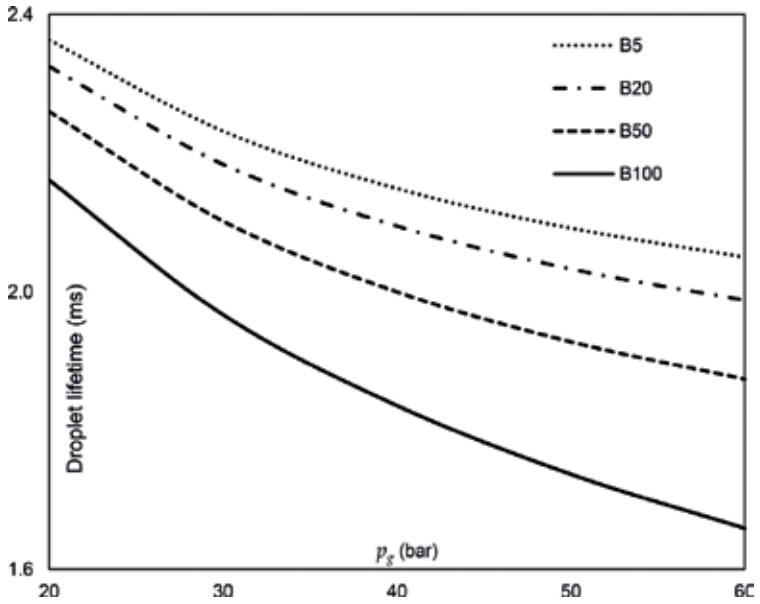

Figure 9. The effect of ambient pressure on diesel-biodiesel (LME) droplet lifetimes.

4.4. Blended ethanol-gasoline fuel droplets

The DC model is facilitated for the analysis of heating and evaporation of ethanol-gasoline fuel droplets of initial radius R_{d0} = 12μm and temperature T_0 = 296 K. The droplets are assumed to be moving at U_d = 24 ms^{-1} in still air of pressure and temperature equal to p_g = 9 bar and T_g = 545 K, respectively. The evolutions of droplet surface temperatures (T_s) and radii (R_d) for the ethanol-gasoline fuel mixtures are analyzed. The mixtures are: E0 (pure gasoline), E5 (5% ethanol, 95% gasoline), E20 (20% ethanol, 80% gasoline), E50 (50% ethanol, 50% gasoline), E85 (85% ethanol, 15% gasoline) and E100 (pure ethanol).

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In **Figures 10–11**, the plots for droplet radii and transient surface temperatures are shown, respectively, for six mixing ratios of ethanol-gasoline fuel blends (E0 – E100).

In **Figure 10**, the droplet lifetime for pure gasoline fuel (E0) is the smallest. This increases with the increase of ethanol fraction from E0 to E100. The error in predicted droplet lifetime of E100 is

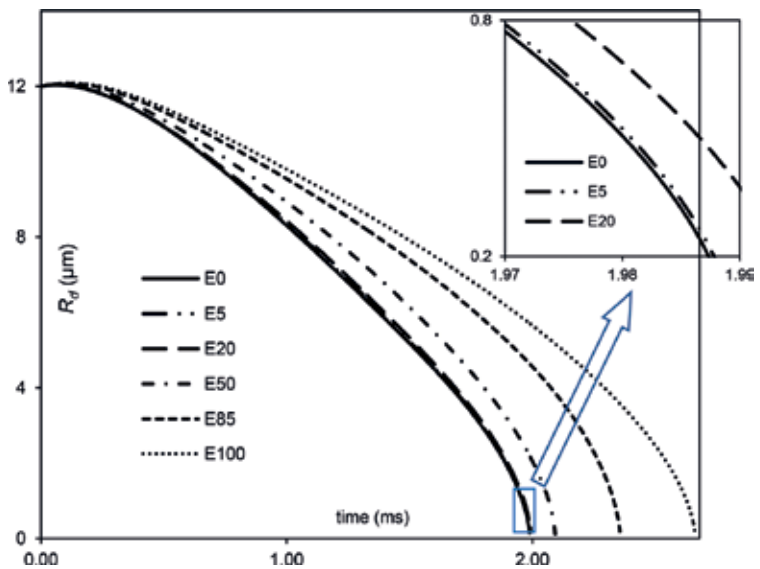

Figure 10. Droplet radii R_d versus time for six fractions of ethanol-gasoline fuels: E0, E5, E20, E50, E85 and E100.

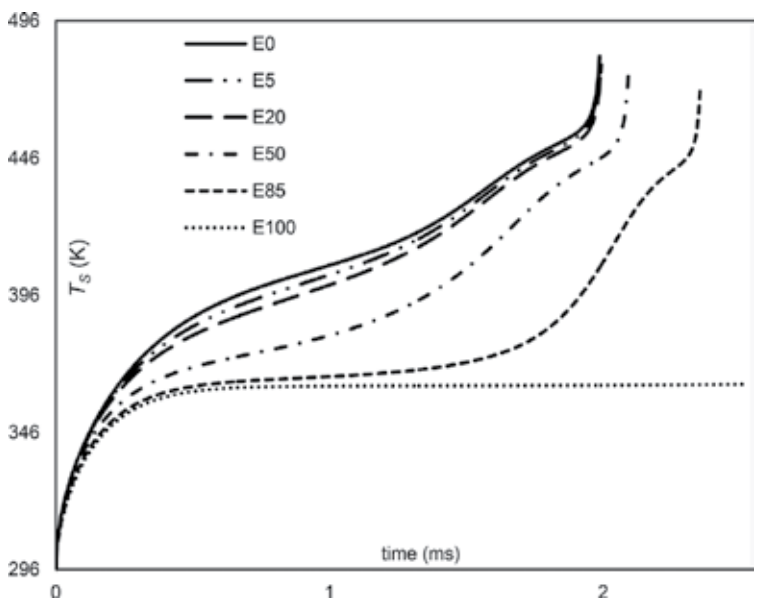

Figure 11. Droplet surface temperatures T_s versus time for six fractions of ethanol-gasoline fuels: E0, E5, E20, E50, E85 and E100.

Blends	Time (ms)	Diff%	
E0	1.988	—	
E5	1.989	0.050	*Note:*
E20	1.994	0.302	$\text{Diff}\,\% = \frac{\left\|(tim\,e_{EN} - tim\,e_{E0})\right\|}{tim\,e_{E0}} \times 100\%$
E50	2.093	5.282	
E85	2.356	18.511	
E100	2.662	33.903	

Table 6. The impact of ethanol/gasoline fuel blends on the estimated droplet lifetimes.

33.9% compared to the one predicted for E0. In **Figure 11**, the impact of increasing the ethanol/gasoline fraction from E0 to E100 is seen to be significant. The deviation in the predicted droplet surface temperature for E100 is 24.3% compared to the one predicted for E0. The impacts of different ethanol/gasoline fuel mixtures on droplet lifetimes are presented in **Table 6**.

The droplet lifetimes of ethanol-gasoline fuel mixtures (**Figures 10–11**) have been estimated under standard engine conditions. The impact of different ambient conditions on these predictions is presented in **Figures 12** and **13**.

As can be seen from **Figures 12** and **13**, increasing the ambient temperature (500 to 650 K), or pressure (3 to 20 bar), leads to a proportional reduction in estimated droplet lifetimes with almost the same effect for all ethanol-gasoline blends.

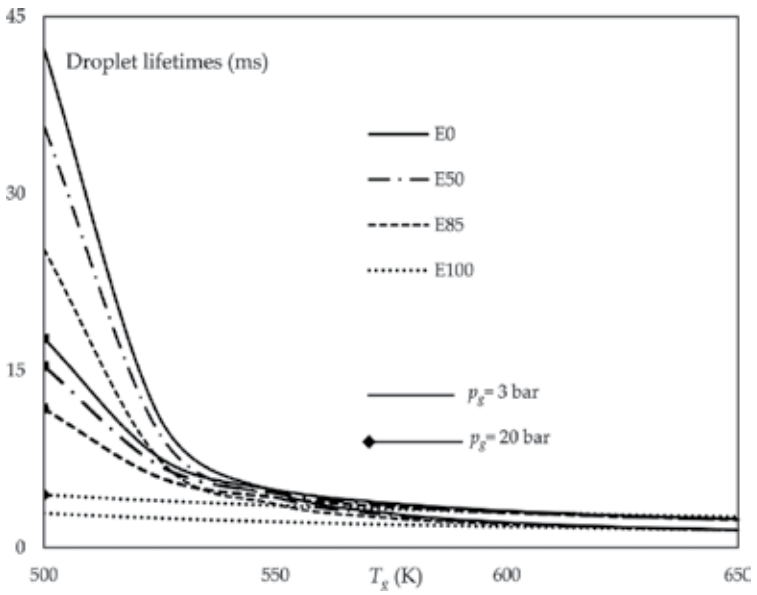

Figure 12. The impact of ambient temperatures on droplet lifetimes for E0, E50, E85 and E100 fuel blends, estimated at ambient pressures 3 and 20 bar.

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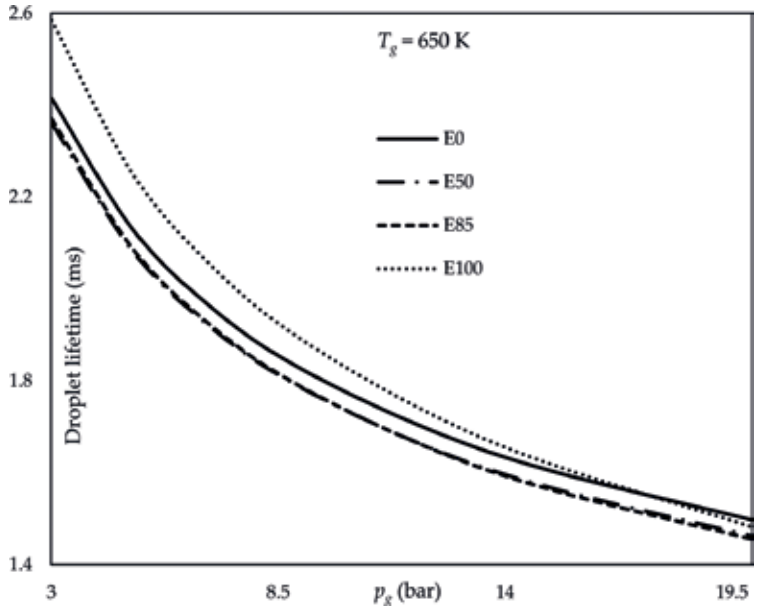

Figure 13. The impact of ambient pressures on droplet lifetimes for E0, E50, E85 and E100 fuel blends, estimated at ambient temperature 650 K.

5. Conclusion

In this chapter, the maximum entropy method was applied for the droplet distribution of diesel and biodiesel fuel sprays in conditions relevant to diesel internal combustion engines. The droplet distribution for biodiesel was more skewed to the right compared to the predicted diesel spray. The theoretical distribution indicated that biodiesel fuel droplets are larger than those of diesel fuel. The model was validated against available experimental data to show a reasonable agreement between both results.

The discrete component model was used to analyze the heating and evaporation of blended diesel-biodiesel fuel sprays and droplets using 22 types of biodiesel, European standard diesel, gasoline FACE C, and ethanol-gasoline fuels. The full compositions of diesel, biodiesel and gasoline fuels were considered. The diesel and gasoline fuels consisted of 98 and 20 hydrocarbons respectively, while the 22 biodiesel fuels consisted of 4 to 18 components of methyl esters.

The effect of increasing biodiesel fuel concentration on the evolutions of droplet surface temperatures and evaporation times was clearly illustrated. The predicted B5 fuel droplet lifetimes for the 22 types of biodiesel fuel were only 1% less than that of pure diesel (PD) fuel. The RME biodiesel fuel droplets were observed to have lifetimes close to that of PD fuel, where their predicted lifetimes for B5 and B100 droplets were up to 0.4 and 0.6%, respectively, less than the one estimated for PD fuel droplet. However, for ethanol, the predicted E5 fuel droplet lifetimes were only 0.05% greater than that of pure gasoline (E0) and only 0.3% greater for E20.

To conclude, the B5 fuel droplet lifetimes for all 22 types of biodiesel fuels used in this study are almost identical to the one predicted for PD fuel; i.e. diesel fuel can be possibly blended with up to 5% biodiesel fuel without any noticeable effect on the evolutions of their droplet radii or surface temperatures. Similarly, the E5 and E20 fuel droplet lifetimes are almost identical to the one predicted for E0 fuel; i.e. gasoline fuel can be possibly blended with up to 20% ethanol fuel without/minimal modifications to the gasoline engine. Also, increasing the ambient pressure, or temperature, will lead to a faster evaporation of E0-E100 droplets regardless of their blending ratios.

Acknowledgements

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Nomenclature

B#	#% biodiesel/diesel fraction
BME	butter methyl ester
CAN	canola methyl ester
CME	coconut methyl ester
CML	camelina methyl ester
CNE	corn methyl ester
CSE	cottonseed methyl ester
DC	discrete component
E#	#% ethanol/gasoline fraction
FAME	fatty acid methyl ester
HME1	hempseed methyl ester (Ukrainian oil production)
HME2	hempseed methyl ester (EU standard)
JTR	jatropha methyl ester
LME	lard methyl ester
LNE	linseed methyl ester

LP	length parameter
PD	pure diesel fuel
E0	pure gasoline fuel
PME	palm methyl ester
PMK	palm kernel methyl ester
PTE	peanut methyl ester
RME	rapeseed methyl ester
SFE	safflower methyl ester
SMD	Sauter mean diameter
SME	soybean methyl ester
SNE	sunflower methyl ester
TGE	tung methyl ester
TME	tallow methyl ester
WCO	waste cooking oil
YGR	yellow grease methyl ester

Symbols

A	spray penetration coefficient
d	nozzle diameter [m]
D	diffusion coefficient [$m^2\ s^{-1}$]
k	thermal conductivity [$W\ m^{-1}\ K^{-1}$]
p	pressure [Pa]
T	temperature [K]
R	radius [μm]
t	time [ms]
U	velocity [ms^{-1}]
Y	mass fraction
κ	thermal diffusivity
μ	dynamic viscosity [Pa s]
ρ	density [$kg\ m^{-3}$]

σ	surface tension [N m^{-1}]
ν	kinematic viscosity [m^2 s^{-1}]
χ	recirculation coefficient

Subscripts

d	droplet
eff	effective properties
f	fuel
g	gas
i	liquid species
inj	injection
l	liquid
0	initial condition
s	droplet surface

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Chapter 5

Biohythane Production from Organic Wastes by Two-Stage Anaerobic Fermentation Technology

Sompong O-Thong, Chonticha Mamimin and Poonsuk Prasertsan

Additional information is available at the end of the chapter

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Abstract

The combination of biohydrogen and biomethane production from organic wastes via two-stage anaerobic fermentation could yield a biohythane gas with a composition of 10-15% H_2, 50-55% CH_4 and 30-40% CO_2. Biohythane could be upgraded to biobased hythane by removing of CO_2. The two-stage anaerobic fermentation process is based on the different function between acidogens and methanogens in physiology, nutrition needs, growth kinetics, and sensitivity to environmental conditions. In the first stage, the substrate is fermented to H_2, CO_2, volatile fatty acids (VFA), lactic acid and alcohols by acidogens with optimal pH of 5–6 and hydraulic retention time (HRT) of 1–3 days. In the second stage, the remaining VFA, lactic acid, and alcohols in the H_2 effluent are converted to CH_4 and CO_2 by methanogens under optimal pH range of 7–8 and HRT of 10–15 days. The advantage of biohythane over traditional biogas are more environmentally, flexible of H_2/CH_4 ratio, higher energy recovery, higher degradation efficiency, shorter fermentation time, and high potential to use as vehicle fuel. This chapter outlines the general approach of biohythane production via two-stage anaerobic fermentation, principles, microorganisms, reactor configuration, process parameters, methods for improving productivity as well as technical challenges toward the scale-up process of biohythane process.

Keywords: biohythane, microbiology and biochemistry, physicochemical parameters, reactor configuration, improvement methods, two-stage anaerobic fermentation, organic wastes

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1. Introduction

Currently, development of biofuels to replace fossil fuels by the biological process has been attracting attention as an environmentally friendly process. Among the various processes, biohydrogen and biohythane are the promising future energy carriers due to their potentially higher conversion efficiency and low pollutants generation [1]. Dark fermentation shows high H_2 production rate under realistic conditions, which is approaching practical levels [2]. In addition, the major advantages are rapid bacterial growth rates, relatively high H_2 production capacities, operation without light sources, no oxygen limitation problems, and low capital cost of at least at small-scale production facilities [3, 4]. The dark fermentation process can utilize organic materials for H_2 gas production, such as cellulose and starch-containing agricultural and food industry wastes, and some food industry wastewaters, such as cheese whey, olive mill, palm oil mill, and baker's yeast industry wastewaters [5]. H_2 yields from dark fermentation of organic wastes such as food waste, apple processing wastewater, starch wastewater, palm oil mill effluent, and potato processing wastewater were 57, 92, 92, 115, and 128 mL H_2/gCOD, respectively [6–9]. However, dark fermentation has low substrate conversion efficiency as only 7.5–15% of the energy contained in organic wastes are converted to H_2 and the rest of the energy still remains in the liquid (H_2 effluent) as VFA (mainly butyric acid and acetic acid), lactic acid, and alcohols [1]. The disadvantage of dark fermentation must be overcome before biohydrogen can become economically feasible. The conversion of VFA, lactic acid, and alcohols to CH_4 through anaerobic digestion (AD) [10] is faster and simpler than the conversion of these components to H_2 by photo-fermentation and microbial-electrolysis process [1]. In addition, it has been shown to be an energy efficiency strategy for the production of a mixture of H_2 and CH_4, known as biohythane, via two-stage anaerobic fermentation [11, 12].

Biohythane has attracted growing attention worldwide due to its potential use as vehicle fuel, high potential to produce from conversion of organic wastes and probably an alternative to the fossil-based hythane [10]. Normally, hythane gas was produced from a thermo-chemical process using natural gas as a starting material. This process is a high-energy consumption and still depends on fossil fuel. Biohydrogen and biomethane production from organic wastes by fermentation process and anaerobic digestion process, respectively, are already established. The combination of these two processes via two-stage anaerobic fermentation processes could yield a H_2 and CH_4 gas with a composition like hythane (10–15% H_2, 50–55% CH_4, and 30–40% CO_2) called biohythane [13], which could be upgraded to biobased hythane by removing of CO_2. The two-stage anaerobic fermentation for biohythane production is involved with the fermentation of organic wastes to H_2, CO_2, VFA, lactic acid, and alcohols in the first stage and conversion of these substances in H_2 effluent to CH_4 and CO_2 via anaerobic digestion process in the second stage (**Table 1**). The optimum condition for the first stage is a pH range between 5 and 6 and a hydraulic retention time (HRT) range of 1–3 days that are suitable for acidogens for the conversion of organic wastes to H_2 via the acetate and butyrate pathways. In the second stage, the acetic acid in the H_2 effluent is converted to CH_4 and CO_2 by acetoclastic methanogens under an anaerobic condition with optimal pH range of 7–8 and optimal HRT of 10–15 days [11]. Others VFA, lactic acid, and alcohols in the H_2 effluent are anaerobically converted by acetogens to H_2 and CO_2, which are consequently converted to CH_4 by hydrogenotrophic methanogens [14].

Technology	Processes	Substrates	Products
Hythane	Thermo-chemical	Natural gas	5–7% H_2, 90% CH_4 and 5% CO_2
Biomethane	Anaerobic digestion (AD)	Organic wastes	50–60% CH_4 and 40–50% CO_2
Biohydrogen	Fermentation	Organic wastes	40–60% H_2 and 40–60% CO_2
Biohythane	Two-stage fermentation/AD	Organic wastes	5–10% H_2, 60% CH_4 and 30% CO_2

Table 1. Biohythane technology development from two-stage anaerobic fermentation technology.

The two-stage anaerobic fermentation process could increase energy recovery, degradation efficiency, reactor stability, CH_4 production rates, and purity of gas products when compared to one-stage H_2 or CH_4 fermentation [15]. In addition, the two-stage process has advantages of improving negative impacts of inhibitive compounds in feedstocks (such as wheat hydrolysate, molasses, and skim latex serum), operated at high organic loading rates and reduced fermentation time with total HRT of 10–18 days for overall processes. Advantages of biohythane over traditional biogas are improved energy recovery, shortened fermentation time, flexible H_2/CH_4 ratio, and more environmentally benign and process robustness for handling the organic wastes [10, 16]. Integrated biohydrogen with biomethane process worth for commercialization could get the biogas in the form of biohythane. Typically, the suggested H_2 content in biohythane is 10–15% by volume. Biohythane is considered to be a clean fuel for vehicles compared to gasoline or diesel due to low greenhouse gas emission from the combustion process [17].

Biohythane via two-stage anaerobic fermentation using organic wastes could be a promising technology for higher energy recovery and cleaner transport biofuel than biogas. Various types of organic wastes can be used as substrate for biohythane production such as starch wastewater, wheat straw hydrolysate, palm oil mill effluent, food waste, and organic solid waste [13, 18–20]. Wheat straw hydrolysate was used for biohythane production by *Caldicellulosiruptor saccharolyticus* with maximum H_2 production rate of 5.2 L H_2/L·d and maximum CH_4 production rate of 2.6 L CH_4/L·d. The maximum energy output of the process was 10.9 kJ/g of straw with energy recovery of 57% of energy contained in the wheat straw [20]. Biohythane production of starch wastewater achieved H_2 and CH_4 yields of 130 mL H_2/gCOD and 230 mL CH_4/gCOD, respectively [18]. Biohythane production of food waste achieved H_2 and CH_4 yields of 205 mL H_2/gVS and 464 mL CH_4/gVS, respectively [21]. Biohythane production of palm oil mill effluent (POME) was achieved with H_2 and CH_4 yields of 201 mL H_2/gCOD and 315 mL CH_4/gCOD, respectively [13]. Nathao et al. [22] obtained two-stage process for biohythane production from food waste with H_2 and CH_4 yields of 55 and 94 mL/gVS at F/M of 7.5. Kongjan et al. [11] used UASB reactors for extreme thermophilic H_2 and thermophilic CH_4 production from wheat straw hydrolysate via a two-stage anaerobic fermentation process. Specific H_2 and CH_4 yields of 89 mL H_2/gVS and 307 mL CH_4/gVS, respectively, were achieved. Successful continuous biohythane production from POME by two-stage thermophilic fermentation and mesophilic anaerobic digestion was reported by Mamimin et al. [13]. The continuous biohythane production rate of 4.4 L/L·d was achieved with biogas containing 51% CH_4, 14% H_2, and 35% CO_2. Energy analysis suggested that the two-stage fermentation process for biohythane production had greater net energy recovery than the single H_2 fermentation

and CH_4 fermentation process. This chapter provides the information on general approach of biohythane via two-stage anaerobic fermentation, principles of biohythane process, microorganisms involved in H_2 and CH_4 production, reactor configuration for biohythane production, methods for improve biohythane production, process parameters affecting biohythane production and technical challenges toward the scale-up process.

2. Principles of biohythane process

Most of wastewater and organic wastes were usually treated in an anaerobic process for CH_4 recovery as energy. Regarding clean energy of H_2, anaerobic process was modified for H_2 production by suppression of methanogenic activity. To harvest H_2 from the first stage, the H_2-consuming pathway has to be inhibited [23]. Most H_2-producing bacteria can form endospores in stress environment. Various selection methods can be used to enrich H_2-producing bacteria [24]. The most common selection methods are heat treatment and pH control. However, some researchers reported the invalidity of such selection methods [25], because not all H_2-producing bacteria are associated with the ability to form endospores. In addition, there are many H_2-consuming bacteria that can form endospores, such as acetogens and sulfate-reducing bacteria [26]. The pH control is an important method for maintaining H_2-producing bacteria in continuous systems of first stage. The pH varies depending on the microbial species, microbial activities, reactor configuration, feedstock characteristics, organic loading rate, buffer capacity, and temperature. The change of pH is due to acetic acid and butyric acid production accompanies with H_2 production, whereas the low pH influences on the shift of metabolic products from acidogenesis to solventogenesis [27]. Low pH is also critical strategies to inhibit the activity of methanogenesis. The suggestion for optimal pH of H_2 production could range from 5.0 to 6.5. From the perspective of thermodynamics, changes of Gibbs free energy during H_2 production were much larger than those of methanogenesis. This means faster rates for microbial growth in biohydrogen fermentation. On the basis of this characteristic, the manipulation of hydraulic retention time (HRT), temperature, and oxidation-reduction potential (ORP) can achieve microbial H_2 process feasible in continuous operation.

Continuous biohythane production by integrating biohydrogen with biomethane process worth for commercialization could get the biogas that has composition like hythane gas. In the first stage, substrate is fermented to H_2, CO_2, VFA, lactic acid, and alcohols whereby the non-gas metabolites are converted to CH_4 and CO_2 in the second stage [10]. The fermentation products from H_2 production process are very important for the whole biohythane system performance because they can affect the loading, degradation efficiency, and operating stability of the methanogenesis stage [28]. The conversion rate from VFA to acetic acid will affect the methanogenic archaea quantity, and subsequently affect the degradation rate of acetic acid and CH_4 yield. The basic principle of a two-stage process is shown in **Figure 1**. The first stage includes hydrolysis and acidogenesis where hydrolytic and fermentative bacteria excrete enzymes to break down complex organic compounds of carbohydrate, protein, and lipid into single molecules of mono sugar, amino acid, and long chain fatty acids and/or glycerol respectively. The acidogenesis, fermentative, and acidogenic bacteria convert the hydrolysis products into CO_2,

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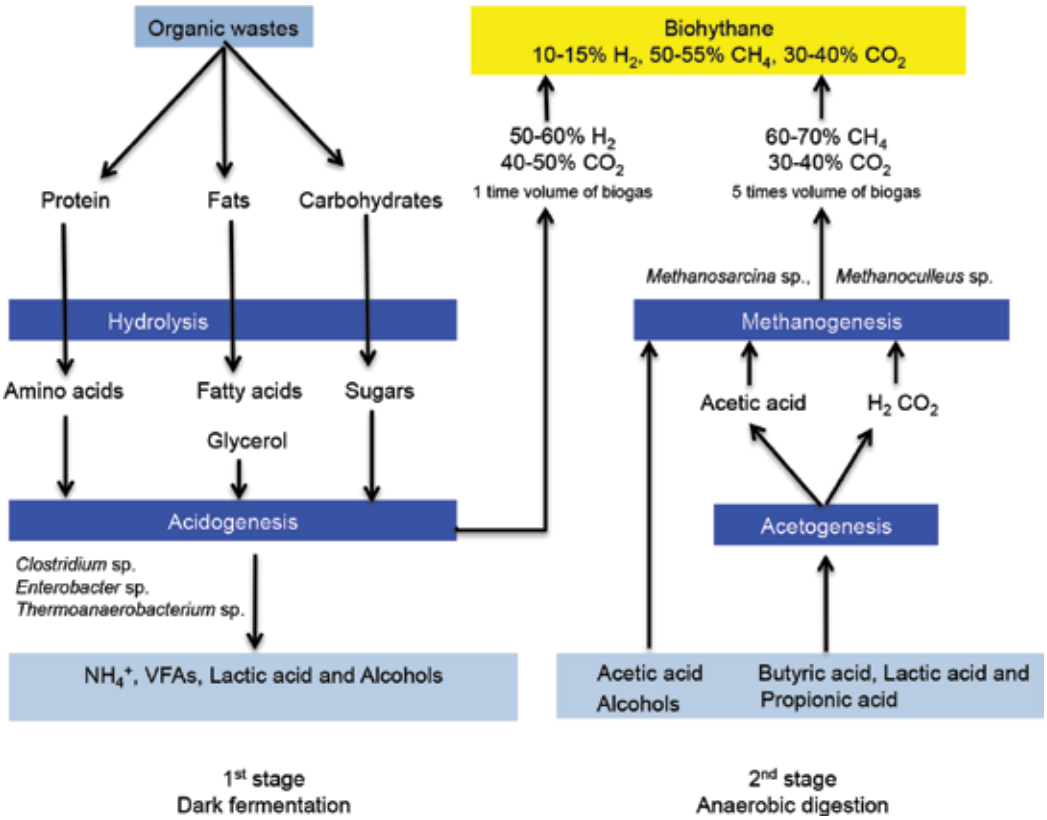

Figure 1. Modification of anaerobic digestion for biohythane production from organic wastes via two-stage anaerobic fermentation process.

H_2, VFA, lactic acid, and alcohols. High H_2 production was achieved by fermentative bacteria via acidogenesis process under pH range of 5-6 and operating at short HRT of 1-3 days. Under the optimum condition, acidogenic bacteria could convert carbohydrate to H_2 and CO_2 via the acetate and butyrate pathways and competition to other microorganisms. In the second stage, the acetic acid in the H_2 effluent is anaerobically converted to CH_4 and CO_2 by acetoclastic methanogens. The acetogenic bacteria could produce acetic acid along with additional H_2 and CO_2 from butyric acid, propionic acid, and lactic acid. H_2 and CO_2 are consequently converted to CH_4 by hydrogenotrophic methanogens [29]. These reactions occur under an optimal pH range of 7–8 and HRT of 10–15 days [30]. The two-stage anaerobic fermentation process is also characterized by a significantly reduced fermentation time with overall fermentation time of 13–18 days [10].

The two-stage anaerobic fermentation process is based on two physiologically different groups of microorganisms. One group of acidogenic bacteria that converts organic matter into H_2, CO_2, soluble VFA, lactic acid, and alcohols, is fast growing, prefers a slightly acidic environment of pH 5–6, and is less sensitive to environmental changes. A large number of microbial species, including strict and facultative anaerobic bacteria such as *Clostridium* sp.,

Enterobacter sp., *Caldicellulosiruptor* sp., *Thermotoga* sp., and *Thermoanaerobacterium* sp., are efficient H_2 producers, while degrading various types of carbohydrates [31]. The other group in second stage is methanogenic archaea, which converts VFA, lactic acid, and alcohols into CH_4 and CO_2, is slow growing, prefers neutral to slightly alkaline environments, and is very sensitive to environmental changes. *Methanosarcina* sp. and *Methanoculleus* sp. were dominant and played an important role in second stage [14, 15]. *Methanosarcina* species were reported to be dominant at high acetate concentration (>1.2 mM), and the results were consistent with the high acetate concentrations in H_2 effluent that feed to CH_4 reactors. *Methanoculleus* species were responsible for hydrogenotrophic methanogenesis that convert H_2 and CO_2 to CH_4 [11]. Obtaining the optimum environmental conditions for each group of organisms by the two-stage anaerobic fermentation process provides several advantages over the conventional single stage [32–34], e.g., high net energy efficiencies, more stable operation, allowing higher organic loading rate operation, smaller-size reactor (40–60% smaller), thus better economics for construction cost and higher CH_4 content in the biogas (65–75%) [15, 35]. High CH_4 content and production was found in the second stage due to CO_2 in the second stage is mainly generated by aceticlastic methanogen and then consumed partly by hydrogenotrophic methanogen also existed in the second stage. The higher CH_4 content is definitely a better fuel value for on-site use and higher digestion efficiency, thus more CH_4 is recovered [36].

3. Microorganisms in biohythane process

The two-stage anaerobic fermentation process is based on the differences between acidogens and methanogens in physiology, nutrition needs, growth kinetics, and sensitivity to environmental conditions. The acidogens and methanogens are enriched separately in two tanks enabling optimized growth by maintaining proper environmental conditions in each reactor [37]. Microorganisms involved in the first stage H_2 production and in the second stage CH_4 production via two-stage anaerobic fermentation process are shown in **Table 2**. First stage (H_2 reactor) involved with the several bacterial strains is capable to produce H_2 through dark fermentation of various carbohydrates. Obligate anaerobic *Clostridia* are potential H_2 producers and are well known for high H_2 yield [38]. *C. butyricum*, *C. welchii*, *C. pasteurianum*, and *C. beijerinckii* were used for H_2 production [39]. *Clostridium* sp. is capable of utilizing a wide range of carbohydrates such as xylose, arabinose, galactose, glucose, cellobiose, sucrose and fructose with a H_2 yield of 2.1–2.2 mol H_2/mol sugars [40]. Facultative anaerobes *Enterobacteriaceae* are H_2 producers that are resistant to trace amount of dissolved oxygen. *Enterobacter* sp. has lower yield (1.0 mol H_2/mol sugars) when compared to *Clostridium* sp. [41]. *Citrobacter* sp. also belongs to family *Enterobacteriaceae* known to produce H_2 from CO and H_2O by water-gas shift reaction under anaerobic condition [42]. *Escherichia coli* is capable of producing H_2 and CO_2 from formate in the absence of oxygen. The H_2 yields of *E. coli* were 0.6–1.3 mol H_2/mol glucose [43]. *Bacillus* sp. also has been identified as H_2 producers such as *B. licheniformis* [44] and *B. coagulans* [45]. Its H_2 yield was 0.5 mol H_2/mol glucose with lactic acid as main soluble metabolites. Dark fermentation at thermophilic temperatures (55–60°C) showed favorable kinetics and stoichiometry of H_2 production compared to the mesophilic systems. Metabolism at higher temperatures becomes thermodynamically more favorable

Stages	Mesophilic condition (30–35°C)	Thermophilic condition (55–60°C)	Extreme thermophilic condition (70–90°C)
1st hydrogen production (Bacteria)	*Clostridium* sp.	*Thermoanaerobacterium* sp.	*Caldanaerobacter* sp.
	Enterobacter sp.	*Clostridium* sp.	*Caloramator* sp.
	Citrobacter sp.	*Thermoanaerobacter* sp.	*Thermotoga* sp.
	Bacillus sp.		
2nd methane production (Bacteria)	*Clostridium* sp.	*Clostridium* sp.	*Caloramator* sp.
	Bacillus sp.	*Thermoanaerobacterium* sp.	
	Desulfobacterium sp.	*Desulfomicrobium* sp.	
2nd methane production (Archaea)	*Methanobacterium* sp.	*Methanothermobacter* sp.	*Methanothermus* sp.
	Methanoculleus sp.	*Methanosarcina* sp.	*Methanothermococcus* sp.
	Methanospirillum sp.		
	Methanococcus sp.		
	Methanobacter sp.		

Table 2. Microorganisms involved in the first stage H_2 production, and the second stage CH_4 production via two-stage anaerobic fermentation process.

and less affected by the partial pressure of H_2 in the liquid phase. Dark fermentation under thermophilic condition was involved with *Thermoanaerobacterium* sp., *Thermoanaerobacter* sp., and *Clostridium* sp. [15]. *Thermoanaerobacterium thermosaccharolyticum* has an optimal growth at moderate thermophilic temperature (60°C) and can convert carbohydrate to H_2 via butyrate- and acetate-type fermentation [46]. *Thermoanaerobacterium* species are well known as good H_2-producing bacteria [8, 47]. *Thermoanaerobacterium* sp. represents anaerobic spore forming thermophilic microorganisms previously found in thermophilic H_2-producing reactors [8, 9]. Genus *Thermoanaerobacterium*, especially *Tbm. thermosaccharolyticum*, is capable of H_2 production from various types of substrate under the thermophilic conditions. Various *Tbm. thermosaccharolyticum* strains have been isolated such as strain PSU2 [46], strain GD17 [48], strain W16 [49], strain KKU19 [50], and strain IIT BT-ST1 [51]. In addition, *Tbm. thermosaccharolyticum* can grow on various organic wastes including hemicellulosic waste and lignocellulosic waste [48, 52]. *Thermoanaerobacter* sp. has optimal growth at moderate thermophilic temperature (60°C) and can convert carbohydrate to H_2 via ethanol- and acetate-type fermentation, but cannot degrade cellulose. These species produce H_2, ethanol, lactate, acetate, and CO_2 as the major products, but no butyrate production. Thermophilic *Clostridium* sp. was found to degrade cellulose using cellulase enzymes and can ferment the lignocellulosic biomass to H_2 with the yield of 1.6 mol H_2/mol hexose [53]. Dark fermentation at extreme thermophilic temperatures (70–90°C) showed more favorable kinetics and stoichiometry of H_2 production compared to the thermophilic and mesophilic systems. Dark fermentation under extreme thermophilic condition was involved with *Thermotoga* sp. and *Caldicellulosiruptor* sp. [54]. The H_2 production ability of *Caldicellulosiruptor* sp. was explored at extreme temperatures. These microbes are known to have various kinds of hydrolytic enzymes that can utilize a wide range of substrate such as cellulose, cellubiose, and xylan. *Caldicellulosiruptor* sp. has high poten-

tial to use lignocellulosic waste for H_2 production with the yield of 3.3 mol H_2/mol hexose. The predominant metabolites formed by these organisms are acetic acid and lactic acid [55]. *Thermotoga* sp. was isolated from geothermal spring and capable to grow and produce H_2 at temperatures of 90°C. *Thermotoga* sp. can use elemental sulfur as electron source with H_2 yield of 3.5 mol H_2/mol hexose [56]. The soluble metabolites of these strains are mostly acetic acid, H_2, CO_2, and trace amount of ethanol [57].

Microbial consortium or mixed cultures are providing more enzymes for the utilization of complex substrate than pure cultures. Mixed microbial consortium can be developed from various sources such as anaerobic digested sludge, soil samples, and wastewater by heat treatment and load-shock treatment [58]. These two treatments could eliminate unwanted microorganisms such as methanogens and H_2-consuming bacteria while enriching an H_2-producing bacterium. Heat treatment inhibits the activity of the methanogens and H_2 consumers, while the spore forming H_2-producing bacteria was survived. Additionally, continuous operation at a low hydraulic retention time (1–2 days) helps in washing out slow-growing methanogens from H_2 reactor. Industrially, the use of mixed cultures for H_2 production from organic wastes in the first stage could be more advantage than pure cultures. Enriched H_2-producing bacteria from anaerobic sludge could utilize cellulose as a substrate for H_2 production with the yield of 2.4 mol H_2/mol hexose [59]. The fermentation of various organic wastes by mixed cultures gave the H_2 yields in the range of 57–128 mL H_2/gCOD, depending on type of waste [6–9]. This indicates the practical potential to commercialize H_2 production from organic wastes by mixed microbial consortium.

The second stage CH_4 reactor involved with several archaea strains is capable to produce CH_4 through anaerobic fermentation of VFA, lactic acid, and alcohols. The order *Methanobacteriales* comprises of two families (*Methanobacteriaceae* and *Methanothermaceae*) is CO_2, H_2, and methanol consuming methanogens. The family *Methanobacteriaceae* including *Methanobacterium* sp., *Methanothermobacter* sp., *Methanobrevibacter* sp., *Methanothermus* sp., and *Methanospaera* sp. are commonly found in CH_4-producing reactor. *Methanothermobacter* sp. is a thermophilic *Methanobacteriaceae* that is commonly found in thermophilic CH_4-producing reactor. *Methanothermus* sp. is an extreme thermophilic *Methanobacteriaceae* that is commonly found in extreme thermophilic CH_4-producing reactor. *Methanothermus* sp. grows at a temperature of 83–85°C and assimilates CO_2 and H_2 [60]. The order *Methanococcales* consists of *Methanocaldococcus* sp., *Methanothermococcus* sp., and *Methanococcus* sp. These archaea produces CH_4 from CO_2 and H_2 or formate as the energy source. [61]. The order *Methanomicrobiales* consists of *Methanomicrobium* sp., *Methanocorpusculum* sp., *Methnanoplanus* sp., *Methanospirillum* sp., and *Methanoculleus* sp. These archaea produce CH_4 from acetic acid and exception of *Methanocorpusculum* sp. and *Methanoculleus* sp. using CO_2 and H_2 for CH_4 production [62]. The order *Methanosarcinales* consists of *Methanosarcina* sp., *Methanohalobium* sp., *Methanohalophilus* sp., *Methanolobus* sp., and *Methanosaeta* sp. *Methanosarcina* sp. are hydrogenotrophic or acetoclastic and thus can reduce CO_2 to CH_4 or can utilize acetic acid to CH_4 and CO_2. *Methanosarcina* sp. also can convert methyl-group-containing compounds such as methanol, methylamines, and methyl sulfides to CH_4 and CO_2. *Methanosaeta* sp. utilizes acetic acid as the energy source through acetoclastic reaction.

Acidogenic H_2 producers grow faster than methanogens and eventually produce VFA in effluent. Major genuses related to acidogenic H_2 production are *Enterobacter* sp., *Clostridium*

sp., *Citrobacter* sp., *Thermoanaerobacterium* sp., and *Caldicellulosiruptor* sp. After H_2 production, effluents rich in VFA such as acetic acid, butyric acid, lactic acid, and ethanol would be consumed by methanogenic archaea at neutral pH. High acetic acid concentration promotes the growth of *Methanosarcina* sp. On the contrary, lower acetic acid concentration is preferred by *Methanosaeta* sp. For acetoclastic methanogens such as *Methanosarcina* sp., the minimum thresholds for acetate utilization are typically in the range of 0.5 mM and higher. The minimum thresholds for acetic acid utilization of *Methanoseata* sp. are in the micromole range. The presence of *Clostridium*, *Bacillus,* and *Desulfobacterium* in CH_4 production stage is in accordance with the significant removal of lactic acid in the H_2 effluent since *Clostridium* and *Desulfobacterium* spp. are able to degrade lactic acid to acetate and/or H_2 [63]. Meanwhile, some acidogenic bacteria, *Thermoanaerobacterium* sp., *Clostridium roseum*, and *Clostridium isatidis,* which are H_2 producers [64–66] were also detected in CH_4 stage, confirming that some H_2 and CO_2 were also produced. However, the presence of the hydrogenotrophic methanogens of *Methanothermobacter defluvii* and *Methanothermobacter thermautotrophicus* could possibly consume H_2; thus, no H_2 could be detected when the methanogenic stage reached stable conditions [67].

4. Process parameters affecting biohythane production

Biohythane production processes are greatly influenced by complex biochemical and physical parameters. The process parameters such as inoculum properties, complexity of substrate, nutrient, alkalinity, H_2 concentration, hydraulic retention time (HRT), and toxic compounds have influence on biohythane process (**Table 3**). Inoculums and feedstocks compositions greatly affect first stage H_2 fermentation when using mixed cultures and non-sterile feedstocks [1, 70, 74]. Environmental and physical factors greatly affect the second stage CH_4 production [75, 76]. To stabilize and maximize H_2 production, it is necessary to direct the metabolic pathway toward acetic acid and/or butyric acid and also to maintain the right H_2-producing bacteria during first stage operation. The performance of microorganisms in the conversion of substrate to H_2 is also dependent on the efficiency of its enzymatic machinery. The main factors affecting two-stage anaerobic fermentation are described as follows.

4.1. Feedstocks

Biohythane can be produced from various substrates mainly carbohydrate. In terms of H_2 rate and yields, carbohydrates are the most suitable feedstock followed by protein and peptides, while fat is considered very limited [77]. Most of dark fermentation for H_2 production has been conducted with glucose or sucrose. Glucose is the monomeric unit of cellulose and starch which is a major component in organic wastes [78]. Carbohydrate-rich organic waste is a favorable substrate for H_2 fermentation [79, 80]. The H_2 yield from bean curd manufacturing waste was significantly low compared to carbohydrate-rich substrates [80]. For stable H_2 fermentation, a carbon/nitrogen (C/N) ratio of feedstock greater than 20 is recommended [81]. The H_2 fermentative microorganisms showed improvement in H_2 production when they were grown in a fermentation media having a C/N ratio greater than 20. The C/N ratio of 20–30 also has positive effect on CH_4 production stage. Phosphate concentration in feedstock is also

Factors	Effects on biohythane process	References
Feedstocks	• Fermentation metabolism, microbial activity, and microbial community	[68]
Inoculum	• Fermentation metabolism and microbial community	[69]
pH and Alkalinity	• Fermentation metabolism, microbial activity, and microbial community • Cell membrane charge • Metabolic shift to solvent production	[70]
Temperature	• Fermentation metabolism, microbial activity, and microbial community	[71]
HRT	• Fermentation metabolism, microbial activity, and microbial community • Microbial growth rate	[72]
H_2 Partial Pressure	• Fermentation metabolism and activity • Activity of acetogens and methanogens	[70]
Trace element	• Essential for cell growth, • Enzyme activity	[73]

Table 3. Main factors affecting the two-stage anaerobic fermentation for biohythane production from organic wastes.

important in dark fermentation. Phosphate helps in maintaining buffered condition during fermentation and provides the building blocks of nucleic acid and ATPs. In dark fermentation, an increase in phosphate concentration leads to enhancement of the H_2 production [47].

4.2. Inoculums

Developing an enriched inoculum is very important for obtaining H_2 in first stage fermentation. In the enrichment process, selection procedure was applied to selectively promote H_2-producing bacteria and eliminate H_2 consumers. Different selective procedures such as heat, acid, ultrasonic, ultraviolet, organic and alkali treatment were commonly used [58]. Most of H_2-producing bacteria are spore forming, while H_2-consuming bacteria and methanogens are non-spore forming, which get eliminated with selection methods. The selection methods are promoting endospores formation in a certain group of bacteria that also include H_2-producing bacteria. Thus, under favorable conditions, the endospores germinate and the H_2-producing bacteria dominate in the system. The H_2-producing inoculum might consist of sporulating bacteria like *Bacillus* sp. and *Clostridium* sp. Furthermore, the bacteria capable of producing H_2 widely exist in natural environment in the form of mixed cultures such as anaerobic sludge, municipal sewage sludge, hot spring sediment, compost and soil have been widely used as inoculum for fermentative H_2 production [82–84]. Using mixed cultures is more practical than using pure cultures due to the easy operating and control under the non-sterile condition. Mixed cultures also have a broader source of feedstock [85]. The selection of H_2-producing bacteria suitable for introduction into H_2 reactor may be regarded as inoculum preparation. It should consider the revival of bacteria from the stock, successive of subculturing to active bacteria, short lag phase and high active

cells [86]. Inoculum size for dark H_2 fermentation was varied in the range of 10–20% (v/v). This depends on the characteristics of the species and medium used. Obligate anaerobes produce very less amount of biomass; thus, larger inoculum volume and concentration are required. The inoculum age also matters during the fermentation. Cells growing at the exponential phase have the entire enzymatic machinery active which is required for H_2 and CH_4 production.

4.3. Hydrogen partial pressure

The H_2 partial pressure in the liquid phase is the major factor affecting H_2 production, as high H_2 partial pressure causes deactivation of hydrogenase enzyme. Decreasing H_2 partial pressure by intermittent nitrogen sparging of batch reactor headspace could enhance H_2 production during thermophilic fermentation [87]. In addition to a high H_2 partial pressure, the NADH, which is an electron carrier in the cell, will be oxidized mainly to lactate during extreme thermophilic fermentation with *Caldicellulosiruptor saccharolyticus* [88]. The formation of lactate during the overloading or unstable conditions might be caused by a high H_2 partial pressure.

4.4. Hydraulic retention time (HRT)

The total time that cells and soluble nutrients reside in the reactor is called the HRT. H_2 production occurring at low HRT is dependent on the volume of the reactor and the flow rate of feed. It is generally well known that the H_2-producing bacteria are fast growing [70]. By applying this principle, Liu et al. [48] produced H_2 free of CH_4 in continuously CSTR feeding with household solid waste at acidic pH range of 5.0–5.5 and a short HRT of 3 days without any pretreatment to inhibit methanogens contained in the initial digested manure. HRT is the main optimization parameters of continuous H_2 dark fermentation bioprocesses. In the CSTRs, short HRTs or high dilution (D) rates can be used to eliminate methanogens, which have significant low growth rate [70, 89]. However, HRT is needed to be maintained in a proper level that still gives a D value less than specific growth rate of H_2-producing bacteria. Generally, short HRT is considered to favor the H_2 fermentation metabolism [3]. On the other hand, too high loading rates may result in substrate inhibition effects, improper food to microorganism (F/M) ratios of H_2 producers or washout of microorganisms [90]. These shock loads could reduce the H_2 production metabolism through decreasing of pH and metabolite inhibition (accumulation of intermediates). The HRT could also help in the enrichment of microbial consortium, since it directly affects the specific growth rate of bacteria. By manipulating the HRT, slow-growing microbes like methanogens and H_2-consuming microbes can be expelled out of the reactor, thus leading to selective enrichment of H_2-producing bacteria [91]. This approach of using short HRT for suppressing methanogens led to improvement in H_2 production [92]. In second stage, the HRT is a measure to describe the average time that a certain substrate resides in a digester. If the HRT is shorter, the system will fail due to washout of microorganisms. HRT for anaerobic digestion process are typically in the range of 15–30 days at mesophilic conditions and 10–20 days at thermophilic conditions [13]. Long retention times also benefit hydrolysis of the particulate matter of complex structure such as lignocellulose biomass [93]. On the other hand, organic loading rate (OLR) or amount of organic matter in the system is relative with HRT. The shorter HRT will achieve high OLR that leads to the accumulation of VFA which consequently leads to a pH drop and inhibition of methanogenic

activity. This causes a system failure. During methanogenesis, the HRT should be kept two-fold greater than the generation time of the slow-growing microbes [94]. The HRT should be held for a suitable duration so that the dead zones get eliminated, and it would also help in promoting an efficient syntrophy among the microorganisms present in the mixed culture.

4.5. pH and alkalinity

Among all the chemical factors influencing dark fermentation, pH is considered the most influential. It influences the stability of the acid-producing fermentative bacteria and acetoclastic CH_4-producing archaea. It plays a major role in the oxidation-reduction potential of the anaerobic process. Thus, it directly impacts the metabolic pathway. In most of literature reports, a pH of 5.5 has been considered to be the optimum pH for H_2 production [3, 47, 70, 95]. The optimal initial pH range for the maximum H_2 yield or specific H_2 production rate is between pH 5.5 and 6.5 [95]. The optimal pH is highly dependent on the microorganism. The control of pH and alkalinity of a substrate is essential for first stage dark fermentation since organic acids produced tend to decrease the pH. The pH lower than 4.5 trends to inhibit the activity of hydrogenases. Low pH also causes in shift of metabolic pathways of dark fermentation microorganisms away from H_2 production. H_2-producing bacteria like *Clostridium acetobutylicum* can change metabolism from H_2 (acetate and butyrate pathway) to the production of solvents (acetone and butanol pathway) when the pH is decreased to less than 5.0. Alternatively, depending on the organism, low pH can shift the metabolism toward ethanol production [72]. Carbohydrate-based substrates provide good carbon and energy sources for H_2-producing bacteria. The fermentation process needs buffering of the growth medium, and to be supplemented with nutrients to enhance the growth of microorganisms and resist the pH change caused by organic acids produced [9, 55, 96]. CH_4 production is favored at alkaline pH exhibiting maximum activity at pH of 7.8–8.2 [97]. The rate of CH_4 production may decrease if the pH is lower than this optimal range. The pH is also an important factor for the stability of CH_4 production. The H_2 effluent which is rich in VFA, may cause a drop in pH if fed with high OLR. The pH adjustment can be achieved by an addition of alkali chemical, typically calcium carbonate or sodium hydroxide. A cheap material like ash was used to adjust the pH in an anaerobic reactor [98]. A stable CH_4 production process is characterized by the bicarbonate alkalinity in the range of 1000–5000 mg/L as $CaCO_3$. The ratio between VFA and alkalinity should be in the range of 0.1–0.25.

4.6. Temperature

Temperature is one of the most important factors affecting the growth of microorganisms. The operating temperature influences the growth rate of bacteria by influencing the biochemical reactions responsible for the maintenance of homeostasis and their metabolism. H_2-producing dark fermentation reactors can be operated in various temperature ranges from mesophilic (35–45°C), thermophilic (55–60°C) to extreme thermophilic (70–80°) conditions. Most of the H_2 dark fermentation studies have been conducted at temperature range of 35–45°C. Many mesophilic bacteria such as *Clostridium* sp. and *Enterobacter* sp. showed optimal H_2 production in the temperature range of 35–45°C [99]. A thermophilic H_2-producing bacterium gave higher H_2 yield compared to mesophilic bacteria [100]. When temperature rises, microbial growth rates increase due to the increase in the rates of chemical and enzymatic reactions in

their cells. Thermophilic temperature makes the H_2 production process thermodynamically favorable with the H_2 yield of ~2.1 mol H_2/mol glucose, while mesophilic H_2 production gave the yield of ~1.7 mol H_2/mol glucose [101]. Although the H_2 yield from thermophilic temperature was slightly higher than that for mesophilic temperatures, the specific H_2 production rate (mmol H_2/h·gVSS) for thermophilic temperatures was 5–10 times higher than that from the mesophilic temperatures. Thermophilic H_2-producing bacteria has certain operation advantages such as low solubility of H_2 and CO_2, less influenced by the H_2 partial pressure, better solubility of the substrate, improved hydrolysis reaction as well as thermodynamic efficiency. Temperature is also a very important operation factor in the second stage for anaerobic digestion process. It determines the rate of anaerobic digestion process, particularly the rate of hydrolysis and methanogenesis. The thermophilic process could accelerate the biochemical reactions and give higher degradation efficiency as well as higher CH_4 production rates compared to mesophilic condition [102]. As temperature increases, the rate of retention time process is much faster and this results in more efficient operation and lowers the retention time requirement [97]. Thermophilic condition also increases in thermodynamic favorability of CH_4-producing reactions, decreases solubility of CH_4 and CO_2, and destruction of pathogens in the reactor effluent. Methanogens are extremely subtle to change in temperature and even a small temperature variation (2–3°C) can lead to VFA accumulation [103]. This decreases the CH_4 production rate for methanogens, especially at the thermophilic conditions. Maintaining the stable temperature is important for biohythane production.

4.7. Trace elements

Biohydrogen and biomethane production required various types of metal ions as micronutrients. These metal ions play a critical role in the metabolism of microorganisms. Metal ions such as Fe^{2+}, Zn^{2+}, Ni^{2+}, Na^{+}, Mg^{2+}, and Co^{2+} play a pivotal role in both biohydrogen and biomethane process. Metals are essential to supplement in media for dark fermentation. These micronutrients might be required in trace amounts but they have an influential role as cofactors, transport processes facilitators, and structural skeletons of many enzymes (Fe-Fe hydrogenase and Ni-Fe hydrogenase) involved in the biochemistry of H_2 formation [104]. Therefore, several researchers have studied the effect of supplementation of Fe ion on biohydrogen production. For example, Lee et al. [105] studied the effect of Fe ion concentration (0–4000 mg/L) on H_2 fermentation and found that the H_2 production increased with iron concentration of 200 mg/L. The addition of Fe ion 200 mg/L influences the system positively with increasing H_2 production from 131 to 196 mL H_2/g sucrose. Ferchichi et al. [106] suggested that the supplementation with Fe^{2+} ions (12 mg/l) led to a shift in their metabolic profile, for example, supplementation with Fe^{2+} ion concentration of 12 mg/l caused a metabolic shift from lactic acid fermentation to butyric acid fermentation. Magnesium ions function as a cofactor of many enzymes such as kinases and synthetases. In glycolysis, many enzymes require magnesium ions as a cofactor. The activation of hexokinase, phosphofructokinases, glutaraldehyde-3-phosphate dehydrogenases, and enolases helps bacteria to metabolize substrate and produce energy component ATP [107]. Fe ion also plays a critical role in biomethane stage. The Fe ion is required by methanogenic archaea like *Methanosarcina barkeri* to synthesize protocheme via precorrin-2, which is formed from uroporphyrinogen III in two consecutive methylation reaction utilizing S-adenosyl-L-methionine [108]. Nickel is also an

essential metal which plays a critical role in functioning of many enzymes that are responsible for CH_4 production such as monoxide dehydrogenase, hydrogenase, and methyl coenzyme M reductases.

5. Reactors configuration for biohythane production

The bioreactors in which the microorganisms are grown also play a crucial role. The design and the configuration of the fermenter help in the improvement of mixing characteristics and manipulation of overhead gas partial pressure. Parameters such as HRT and recycle ratio are influenced by the bioreactors configuration. The progress on two-stage system was presented based on the type of feeding substrates, classified as sugar-rich biomass, food/municipal waste, cellulose-based biomass, and palm oil mill effluent (POME). Over 20% of the publications reported so far focused on a system using sugar-rich synthetic wastewater. The most commonly used sugars were glucose and sucrose [10]. The maximum biohythane production was 3.21 mol H_2/mol hexose and 3.63 mol CH_4/mol hexose from glucose and acetic acid (synthetic wastewater) in CSTR reactor [109]. The summarized H_2 and CH_4 yield from various two-stage reactors configuration used for biohythane production is shown in **Table 4**. The schematic flow diagrams of each two-stage anaerobic fermentation systems for biohythane production are shown in **Figure 2**. The two-stage anaerobic fermentation is suitable for individual optimization of the H_2 and CH_4 production processes. For example, temperature-dependent process will be favored by the two-stage process, where high yield of H_2 could be achieved under thermophilic conditions, and stable maintaining of CH_4 production might be achieved under mesophilic conditions [13, 15, 21, 110]. Solubilization and saccharification of organic wastes with high solid content can be realized simultaneously during the first stage H_2 production [17, 74]. The two-stage anaerobic fermentation systems by integrated continuous stirred-tank reactor (CSTR) with anaerobic baffled reactor (ABR), CSTR with UASB, CSTR with CSTR, UASB with UASB, ASBR with UASB and stepped anaerobic baffled (SAB) were used for biohythane production (**Figure 2**.). The system with a CSTR and an upflow biofilter reactor for H_2 and CH_4 production from sucrose was established [89]. This system inoculated with heat-treated sludge as inoculum achieved a maximum H_2 yield of 1.62 mol H_2/mol hexose. The second stage reactor inoculated with raw anaerobic sludge achieved a maximum CH_4 yield of 323 L CH_4/kg COD. The analysis of COD balance showed that 13.5% of the influent COD was transformed to H_2 and 70% of the influent COD was transformed to CH_4. A CSTR H_2 and CSTR CH_4 system fed with synthetic glucose medium using the same anaerobic sludge as inoculums was reported [18]. By optimizing the inoculums-to-substrate ratio (2:1) in this CSTR-CSTR system, the H_2 yield and the methane yield increased to 2.75 and 2.13 mol/mol hexose, respectively, with 10 g/L glucose as a substrate, which corresponded to a total energy recovery of 82%. A similar reactor configuration was also used by Lee et al. [25] and Hafez et al. [109]. A synthesis wastewater containing glucose and acetic acid produced 2.6 mol H_2/mol hexose and 426 mL CH_4/kg COD via continuous fermentation in CSTR [109]. The stable H_2 production in the CSTR was possibly due to the introduction of a gravity settler after the H_2 CSTR for H_2-producer retention. A complete CSTR system for H_2 and CH_4 production from cassava stillage was developed [12]. The gas yields under thermophilic conditions with high

Reactors (H_2 and CH_4)	Feedstock and conditions	H_2 production yield (L-H_2/kg VS)	CH_4 production yield (L-CH_4/kg VS)	Biogas composition	References
CSTR and CSTR	Olive pulp, temperature of 35 and 35°C, pH of 5 and 7	190	160	1.6% H_2 38.3% CO_2 60% CH_4	[110]
UASB and UASB	Desugared molasses, temperature of 70 and 55°C, pH of 5 and 7	89	307	16.5% H_2 38.7% CO_2 44.8% CH_4	[11]
CSTR and UASB	Sugarcane syrup, temperature of 37 and 30 °C, pH of 5.5 and 7.5	88	271	19.6% H_2 62.6% CO_2 10.9% CH_4	[111]
ASBR and UASB	POME, temperature of 55 and 35°C, pH of 5.5 and 7.5	210	315	14% H_2 32% CO_2 51% CH_4	[13]
CSTR and UASB	POME, temperature of 55 and 35 °C, pH of 5.5 and 7.5	135	414	13.3% H_2 32.2% CO_2 54.4% CH_4	[15]
CSTR and CSTR	Biowaste, temperature of 55 and 35 °C, pH of 5.5 and 8	41	102	6.7% H_2 40.1% CO_2 52.3% CH_4	[112]
CSTR and UASB with gas upgrade systems	Wheat straw, temperature of 70 and 37°C, pH of 6.9 and 7.5	270	179	46–57% H_2 0.4% CO_2 43–54% CH_4	[113]
CSTR and ABR	Food waste, temperature of 55 and 35°C, pH of 5.5 and 7.5	205	464	15% H_2 54.5% CO_2 30.5% CH_4	[21]
SAB	Petrochemical wastewater, temperature of 21 and 21°C, pH of 5.5 and 7.5	88	318	16% H_2 27% CO_2 52% CH_4	[114]

Table 4. Hydrogen and methane yield from various reactor configurations used for two-stage biohythane production.

organic loading (13 g COD/L·d) were 56.6 L H_2/kg TS, and 249 L CH_4/kg volatile solid (VS), respectively. Chu et al. [21] developed a two-stage thermophilic CSTR reactor and a mesophilic ABR reactor with the heat-treated digested sludge to recirculation to first reactor for H_2 and CH_4 production from organic fraction of municipal solid wastes (OFMSW). The separation of H_2 and CH_4 production was successful by operating the H_2 reactor at a controlled HRT of 1.3 days, and pH of 5.5. Kongjan et al. [11] established a biohythane process from wheat straw hydrolysate by two-stage extreme thermophilic UASB and thermophilic UASB. Specific

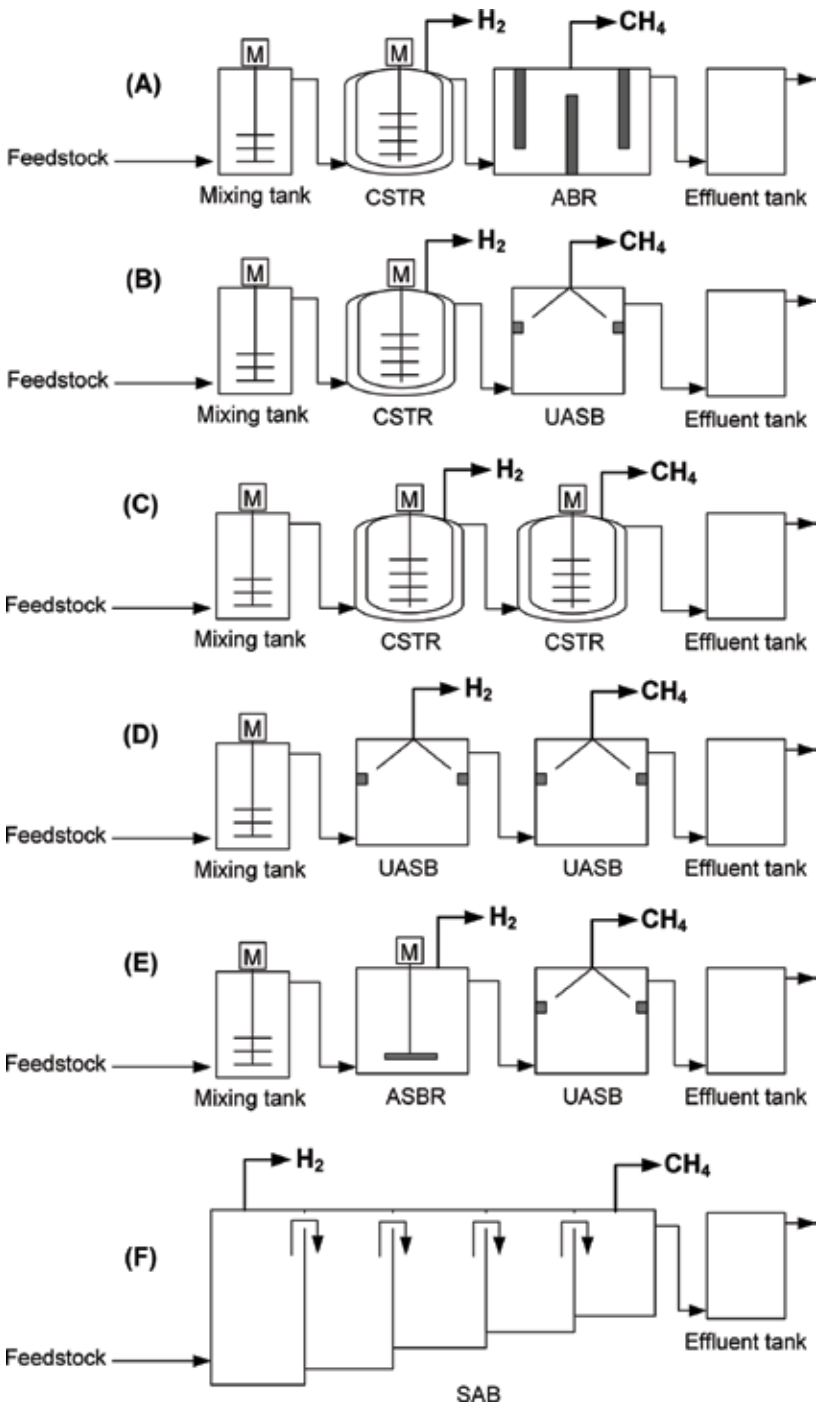

Figure 2. Schematic flow diagrams of two-stage anaerobic fermentation systems for biohythane production by integrated CSTR with ABR (A), CSTR with UASB (B), CSTR with CSTR (C), UASB with UASB (D), ASBR with UASB (E) and SAB (F).

H_2 and CH_4 yields of 89 mL-H_2/g-VS (190 mL H_2/g sugars) and 307 mL CH_4/gVS, respectively were achieved simultaneously with the overall VS removal efficiency of 81% by operating with total HRT of 4 days. A biohythane gas with the composition of 16.5% H_2, 44.8% CH_4, and 38.7% CO_2 could be produced at high production rates (3.5 L/L·d). *Thermoanaerobacter wiegelii*, *Caldanaerobacter subteraneus*, and *Caloramator fervidus* were responsible for H_2 production in the H_2-UASB reactor. Meanwhile, the CH_4-UASB reactor was dominated with methanogens of *Methanosarcina mazei* and *Methanothermobacter defluvii*. Successful biohythane production from palm oil mill effluent (POME) by two-stage thermophilic ASBR followed by mesophilic UASB was achieved by Mamimin et al. [13]. The continuous biohythane production rate of 4.4 L/L·d with biogas composition of 14% H_2, 51% CH_4 and 35% CO_2 was achieved. O-Thong et al. [15] established two-stage thermophilic CSTR and mesophilic UASB with methanogenic effluent recirculation to H_2 reactor for biohythane production from POME. The 30% recirculation rate of methanogenic effluent could keep pH at optimal pH with two times increase in H_2 production when compared with non-recirculation systems. The H_2 and CH_4 yields were 135mL H_2/gVS and 414 mL CH_4/gVS, respectively. Biohythane gas composition was composed with 13.3% H_2, 54.4% CH_4, and 32.2% CO_2. *Thermoanaerobacterium* sp. was dominated during H_2 production from POME, whereas archaea belonging to *Methanosarcina* sp. and *Methanoculleus* sp. were dominated in the CH_4 reactor. A two-stage process with methanogenic effluent recirculation flavored *Thermoanaerobacterium* sp. in the H_2 reactor and efficiently for energy recovery from POME. Elreedy et al. [114] established biohythane production from petrochemical wastewater containing mono-ethylene glycol by a novel stepped anaerobic baffled (SAB) reactor. The reactor was continuously operated for 5 months at constant hydraulic retention time (HRT) of 72 h with hydrogen and methane yield of 88 mL H_2/gVS and 318 mL CH_4/gVS, respectively.

Reactors are considered to be practical and economical for industrial H_2 production, particularly via mixed culture fermentation [70, 100]. The two main bioreactor configurations: suspended and attached, or immobilized, growth types have been applied to optimize fermentation process for H_2 production through advancements in active biomass concentration and substrate conversion efficiency [101, 115]. Most studies on H_2 production from carbohydrate rich substrates have been conducted in suspended CSTRs, which are simple to construct, easy to regulate both acidity and temperature, and give complete homogeneous mixing for direct contact between the substrate and active biomass [1, 70, 72]. Furthermore, the CSTR is very suitable for substrates with a high-suspended solid (SS) content, typically with a volatile solid (VS) content of 2–12% [48]. However, in CSTR reactor, HRTs must be greater than the specific growth rate of the microorganisms in order to control the proper concentration of microbial biomass, but faster dilution rates risk active biomass washout [1, 67] leading to process failure. In addition, cell density retained in CSTR is limited, since the active biomass has the same retention time as HRT, resulting in process instability caused by the fluctuation of environmental parameters, including acidity and then having the consequence of limiting substrate degradation and H_2 production. To overcome the above mention problem, a new configuration of a continuous flow reactor is required to decouple the cell mass retention from HRT and subsequently retain higher cell densities in the reactor, such as UASB and ASBR, which can be achieved through granules and biofilm [47, 91, 115, 116]. Cells immobilization can be

employed successfully by using a diluted waste stream with relatively small reactor volumes in ASBR, SAB, and UASB reactors. However, such a reactor configuration has a poor mass transfer system, which is mainly caused by a lack of mixing; this can lead to gases accumulating in the biofilm or granular sludge that risk losing H_2 by H_2-consuming bacteria [92, 101]. Mass transfer can be improved by mechanical stirring or liquid recirculation, depending on the reactor type and configuration. Also, applying proper bioreactor shapes and optimizing reactor dimensions such as the height to diameter ratio can help to improve mass transfer efficiency [91, 98, 117–119].

The anaerobic conversion of VFA to CH_4 is mainly associated with sequential stages of acetogenesis and methanogesis. When optimizing a methanogenic process using VFA rich, soluble organic matters, the goal is to maximize both CH_4 production and VFA degradation, while keeping the reactor stable [37]. The acetogenesis is limited mainly by VFA degradation, especially propionate that is the rate-limiting factor in the second stage anaerobic process. The investigation into optimizing the methanogenic reactor is mostly carried out by varying OLRs via increasing the substrate concentration or decreasing the HRTs to obtain satisfactory performance [25, 120]. The main signs of methanogenic reactor instability or overloading are decrease in pH [121]. As a drop of pH actually corresponds to VFA accumulation, pH below 6.3 has an impact on enzyme activity in the microorganisms involved in the second stage anaerobic digestion. Methanogenic archaea can function properly in a pH range between 6.5 and 7.8 [122]. Thus, a buffering solution is needed in order to resist a pH drop from VFA accumulation in the methanogenic process and maintain stability. The main buffer in the anaerobic digester is bicarbonate (HCO_3), which is usually added to carbohydrate rich substrates before feeding them to the first stage of H_2 fermentation because the first stage needs to be controlled with pH within the favorable range of 5–6 for H_2-producing bacteria [123, 124]. Lee et al. [25] found that the pH drop below 6.4 caused by the accumulation of 122 mM VFA in the attached growth reactor operated at 55°C and fed with 11.0 gVS/L·d (5.13 d HRT) of the food waste fermentation. The pH could inhibit the bioactivity of methanogenesis. Meanwhile, the maximum CH_4 production rate of 2100 mL CH_4/L·d with a CH_4 content of 65% was obtained at pH around 7.5, where the reactor was operated at a 7.7 day HRT (7.9 gVS/L·d OLR) and almost VFA degradation was achieved. For the high rate anaerobic reactor, UASB reactor was operated at double OLR comparing to CSTR at thermophilic temperature (55°C) which providing better VFAs degradation than mesophilic temperature (35°C) [125]. This is mainly attributed to the increase of chemical and biological reaction rates for operating temperature of thermophilic condition and the organic acid oxidation reactions become more energetic at higher temperature [126, 127]. Because the H_2 reactor effluents are in soluble form of organic matters as the consequence of hydrolysis and acidogenesis in the first stage, the reactor type used to convert these soluble organic matters to CH_4 in the second stage are based on high rate biofilm systems as reviewed by Demirel et al. [27]. Cell mass is retained well in the biofilm/granular aggregates in biofilm systems, leading to have much higher sludge retention time (SRT) compared to HRT, which provides the advantage that the reactor can run at a higher flow rate and can tolerate higher toxic concentrations [128]. Various types of high rate biofilm systems such as UASB, ABR, and SAB can be operated by continuous feeding with the H_2 reactor effluent, with HRTs of less than 5 days [114, 125, 129, 130]. Among the high rate reactor types, the UASB is the most popular for anaerobic treatment of soluble organic matters

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due to the large surface area of granular sludge, which provides fast biofilm development and improves methanogenesis. Also clogging and channeling occur less in the UASB reactor than other biofilm systems [121].

6. Application of biohythane process

Methane is being commonly used, not only in the chemical industry but also in transport as compressed natural gas (CNG), which has been regarded as the clean energy carrier in comparison to gasoline or diesel. By combining the advantages of H_2 and CH_4, biohythane is considered one of the important fuels involved in achieving the transition of technical models from a fossil fuel-based society to renewable-based society. CH_4 used as a fuel for vehicle has weak points on its narrow range of flammability, slow burning speed, poor combustion efficiency as well as requirement for high ignition temperature of CNG-powered vehicles. Interestingly, H_2 perfectly complements the weak points of CH_4 such as the hydrogen/carbon ratio which is increased by adding H_2, which reduces greenhouse gas emissions. Adding H_2, thus, improves the fuel efficiency and can extend the narrow range of flammability of CH_4. The flame speed of CH_4 can be greatly increased by adding H_2, eventually reducing combustion duration and improving heat efficiency. The quenching distance of CH_4 can be reduced by the addition of H_2, making the engine easy to ignite with less input energy. A two-stage process technique, combining acidogenesis and methanogesis appears to give more efficient waste treatment and energy recovery than a single methanogenic process [13]. As the results reported by Kongjan and Angelidaki [129], mixed gas of CH_4, CO_2, and H_2 with the volumetric content of 44.8, 38.7, and 16.5%, respectively, containing approx. 10% H_2 on energy basis could be achieved. This specification was found to be most suitable for burning directly in the internal combustion engines [131] and could be biohythane. In addition to economical concern, the two-stage thermophilic anaerobic process has been previously evaluated that the payback time is around 2–6 years, depending on the disposal costs of organic wastes/residues [28].

Various types of organic wastes can be used as substrate for biohythane production such as starch wastewater, palm oil mill effluent (POME), biowaste, sugarcane syrup, olive pulp, desugared molasses, food waste, and organic solid waste [13, 18, 19]. H_2 and CH_4 yield from two-stage biohythane production of palm oil mill effluent (POME) was 201 mL H_2/gCOD and 315 mL CH_4/gCOD, respectively [13], which were higher than those of starch wastewater (130 mL H_2/gCOD and 230 mL CH_4/gCOD, respectively) [18], sugarcane syrup (88 mL H_2/gCOD and 271 mL CH_4/gCOD, respectively) [111], and biowaste (21 mL H_2/gCOD and 55 mL CH_4/gCOD, respectively) [112]. H_2 and CH_4 yield from two-stage biohythane production of olive pulp (190 mL H_2/gVS and 160 mL CH_4/gVS, respectively) [110] was lower than that of food waste (205 mL H_2/gVS and 464 mL CH_4/gVS, respectively) [21]. Successful biohythane production from POME by two-stage thermophilic H_2 reactor and mesophilic CH_4 reactor was achieved with biohythane production rate of 4.4 L/L·d with biogas composition of 51% CH_4, 14% H_2, and 35% CO_2 [13]. POME is a suitable substrate for H_2 production in terms of high biogas production volume. Energy analysis of two-stage anaerobic fermentation

process has greater net energy recovery than the single stage H_2 production and single stage CH_4 production process. O-Thong et al. [15] applied two-stage thermophilic fermentation and mesophilic methanogenic process with methanogenic effluent recirculation to H_2 reactor for biohythane production from POME. The pH two-stage reactor was control by recirculation of methanogenic effluent with H_2 and CH_4 yield of 135 mL H_2/gVS and 414 mL CH_4/gVS, respectively. Flow diagram of successful thermophilic two-stage anaerobic fermentation for biohythane from POME at lab scale 5 L CSTR and 25 L UASB, semi-pilot scale 50 L CSTR and 250 L UASB and industrial scale 5 m^3 CSTR and 25 m^3 UASB are shown in **Figure 3**.

Improvement methods such as effluent recirculation to mix with feedstock in H_2 reactor, biomethane gas recirculation to H_2 reactor, and the combined effluent recirculation to H_2 reactor with biomethane gas sparging to CH_4 reactor were reported to enhance biohythane production (**Figure 4**). The two-stage anaerobic fermentation process with methanogenic sludge recirculation (two-stage recirculation process) could be successfully operated and maintained at pH around 5.5 in H_2 reactor without any alkaline addition [21]. The recirculation of part of the methanogenic sludge to a H2 reactor was provided as the buffer for the first stage. Kim et al. [132] also reported the recycling of a methanogenic effluent to a H_2 reactor with H_2 production increased from 1.19 to 1.76 m^3 H_2/m^3·d, and decreased the requirement for alkali addition. H_2 yield from the two-stage anaerobic fermentation with the recirculation process was 2.5–2.8 mol/mol hexose [25], which was relatively high comparing to 4 mol/mol hexose from the maximum theoretical H_2 yield. The recirculation of the CH_4 effluent to hydrogen reactor could protect the H_2 fermentation process from a sharp drop in pH or organic overloading. Operations with the circulation of heat-treated sludge performed considerably better than those with the recirculation of raw sludge with respect to both the H_2 production rate and yield [19]. Lee et al. [25] improved two-stage anaerobic fermentation for biohythane production by biomethane gas sparging to second stage and recirculation biomethane effluent for pH adjustment in H_2 reactor. The gas yields were 2.3 mol H_2/mol hexose and 287 L CH_4/kg COD, respectively, while TS of food waste was kept at 10%. The recirculation of methanogenesis effluent provides ammonia-rich buffer, which flavors H_2-producing bacteria eventually and improves the performance of the H_2 reactor. Liu et al. [34] were the first group to develop a two-stage CSTR-CSTR system for mesophilic H_2 and CH_4 production using household solid waste as both inoculum and substrate. The yields of H_2 and CH_4 were 43 and 500 L/kg VS, respectively, while the TS of the H_2 CSTR was maintained at 10%. CH_4 production was over 20% higher than that in single-stage CH_4 fermentation. Cavinato et al. [120] established a two-stage CSTR-CSTR reactor under thermophilic condition for biohythane production from municipal solid waste. The H_2 and CH_4 gas yields were 52 L H_2/kg VS and 410 L CH_4/kg VS, respectively. Willquist et al. [113] proposed a biohythane process from wheat straw including pretreatment, H_2 production using *Caldicellulosiruptor saccharolyticus*, CH_4 production using a methanogenic consortium, and gas upgrading using an amine solution. The first reactor was extreme thermophilic CSTR and the second reactor was mesophilic UASB applying for biohythane production. A biohythane gas with the composition of 46–57% H_2, 43–54% CH_4, and 0.4% CO_2 could be produced at high production rates (2.8–6.1 L/L·d), with 93% chemical oxygen demand (COD) reduction, and a net energy yield of 7.4–7.7 kJ/g dry straw. The CO_2

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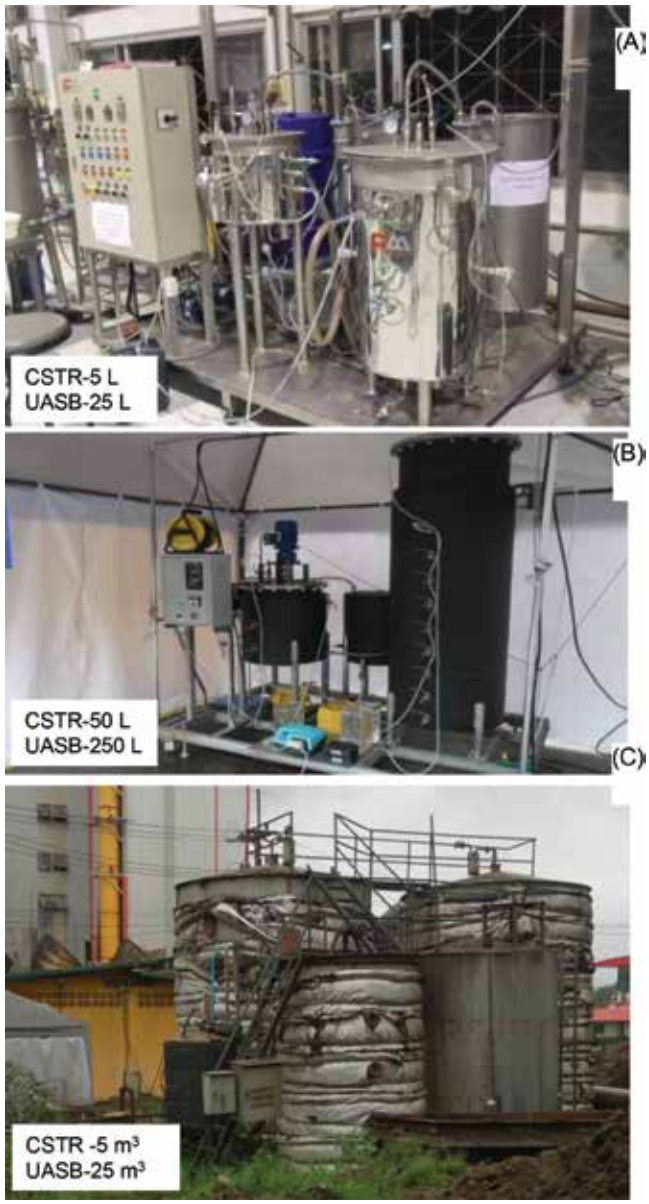

Figure 3. Flow diagram of scaling-up of the two-stage anaerobic fermentation for biohythane production from POME; a lab scale 5 L CSTR and 25 L UASB (A), semi-pilot scale 50 L CSTR and 250 L UASB (B), industrial scale 5 m^3 CSTR and 25 m^3 UASB (C).

has to be removed before the biogas can be used as hythane by an amine solution, consisting of a mixture of 40% N-methyldiethanolamine (MDEA), 10% piperazine (PZ) and 50% water, by weight. This is a solvent commonly used in industry for the removal of CO_2 in various mixtures of gases, including biogas.

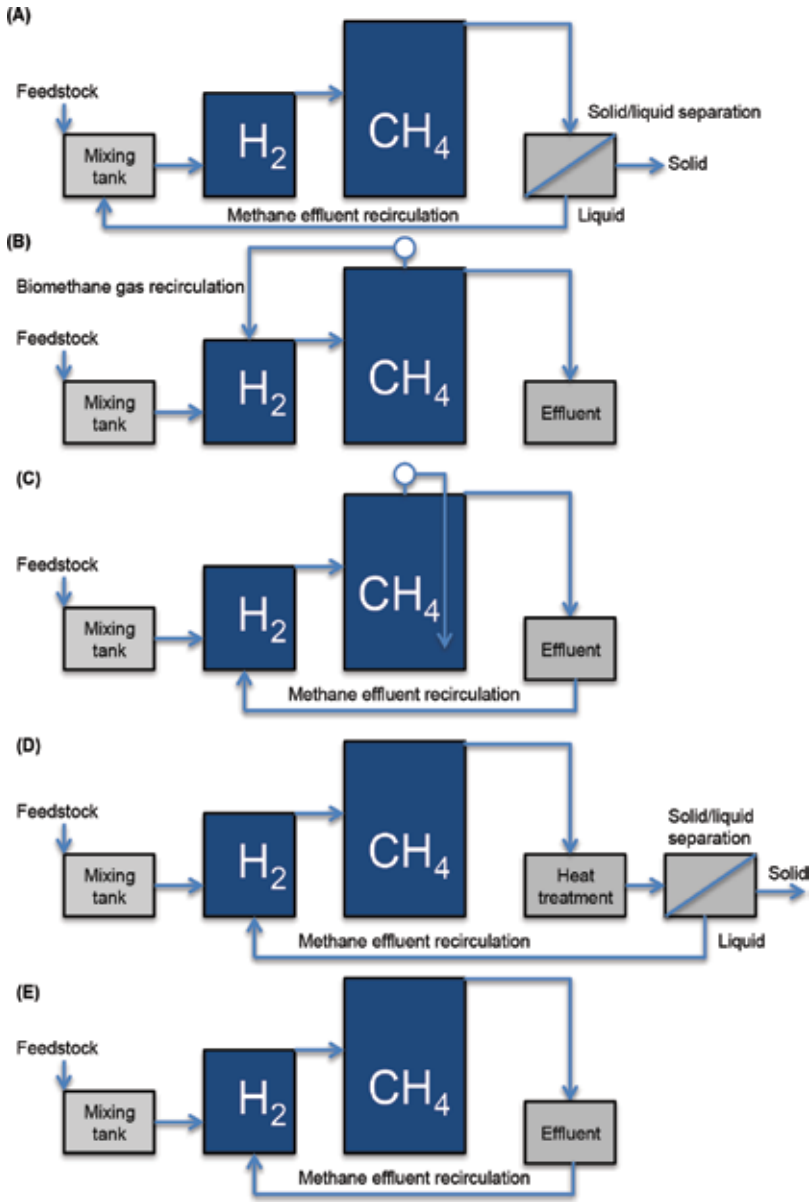

Figure 4. Schematic flow diagrams of gas yield improving for two-stage anaerobic fermentation for biohythane production by liquid methane effluent recirculation method (A), biomethane gas recirculation method (B), the combine liquid methane effluent recirculation and biomethane mixing method (C), liquid methane effluent heated recirculation method (D), and mixed solid and liquid methane effluent recirculation (E).

7. Conclusions

Biohythane via two-stage anaerobic fermentation using organic waste could be a promising technology for higher energy recovery and a cleaner transport biofuel than the biogas.

The H_2/CH_4 ratio of range 0.1–0.25 is suggested for biohythane. A flexible and controllable H_2/CH_4 ratio afforded by two-stage fermentation is of great importance in making biohythane. Biohythane can be achieved by two-stage anaerobic fermentation; in the first stage, organic wastes is fermented to H_2, CO_2, VFA, lactic acid and alcohols. Effluents from first stage containing VFA, lactic acid, and alcohols are converted to CH_4 in the second stage by methanogens under a neutral pH range of 7–8 and HRT of 10–15 days. The pH of 5–6 and an HRT of 2–3 days are optimized for first stage that flavor acidogenic bacteria to convert organic wastes to H_2. *Clostridium* sp., *Enterobacter* sp., *Caldicellulosiruptor* sp., *Thermotoga* sp., and *Thermoanaerobacterium* sp., are efficient H_2 producers in the first stage. *Methanosarcina* sp. and *Methanoculleus* sp. played an important role in the second stage CH_4 production. The combination of biohydrogen and biomethane production from organic wastes via two-stage anaerobic fermentation could yield a gas with a composition like hythane (10–15% of H_2, 50–55% of CH_4, and 30–40% of CO_2) called biohythane. Biohythane could be upgraded to bio-based hythane by removing CO_2. The two-stage anaerobic fermentation could increase COD degradation efficiency, increase net energy balance, increase CH_4 production rates as well as high yield and purity of the products. In addition, the two-stage process has advantages of improving negative impacts of inhibitive compounds in feedstock, increased reactor stability with better control of the acid production, higher organic loading rates operation, and significantly reducing the fermentation time.

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Chapter 6

Biomass Handling and Feeding

Tyler L. Westover and Damon S. Hartley

Additional information is available at the end of the chapter

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Abstract

Handling and feeding of materials represent a substantial challenge in biomass feedstock supply systems and have been primary factors causing pioneer industrial biorefineries to struggle to achieve their production targets. The focus of this chapter is handling and feeding within the plant prior to conversion. The dominant material properties that impact biorefinery operations are presented, and biomass flow patterns and behavior in silos, bins and hoppers are briefly explained. Methods to measure key properties are reviewed, including the Jenike method as well as the efficacy of newer ring shear methods. Finally, areas are identified in which future effort should focus to have the greatest impact to alleviate the challenges that currently plague the emerging biomass industry.

Keywords: biomass, feedstock, handling, feeding, flowability

1. Introduction

Feeding and handling of materials represent a substantial challenge in biomass feedstock supply systems. Conventional feeding, conveying, and storage systems for dry bulk solids are generally not suitable for lignocellulosic feedstocks because of their low densities and elastic nature. Reports indicate that industrial plants that handle bulk solids operate on an average at 77% of their design capacity, which is considerably lower than that of plants that handle liquids or gases [1]. Importantly, however, many of the surveyed plants handled simple powders for which there are decades of handling experience in multiple industries, including food, pharmaceutical, powder metallurgy, ceramics and plastics. Such powders often have favorable properties, such as low cohesion, small particle sizes and distributions, high densities and low compressibilities that facilitate feeding and handling. In contrast, feedstocks for lignocellulosic biofuels production tend to be cohesive, have large particle size variations, low

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densities and are highly elastic, leading to greater challenges. It has been estimated that in 2016, the biofuels production achieved only 7% of the total active 58 million gallons per year of nameplate capacity [2]. Delayed startup times and operation below the designed capacity can have serious consequences in terms of the cost of the final product and missed business opportunities. As indicated in **Figure 1**, an increase in plant down time by 10% (decrease in the minimum on-stream factor) can increase the minimum fuel selling price by nearly a USD per gallon. Achieving 80% time on-stream compared to design capacity, even for a short time period of 2 weeks, is still considered a cellulosic biofuel breakthrough as evidenced by a recent press release by POET-DSM [3].

Several factors underlie the difficulty of feeding and handling biomass. Particulate solid materials belong to the family of yield stress materials that have flow behavior that is intermediate between those of solids and liquids [4]. These materials can support imposed stresses without significant deformation until the stress attains a threshold value. At that threshold value, permanent deformation occurs that can include complex localized elastic and plastic behavior due to discrete particle effects that are not present in liquids, creams, or gels. The threshold stress value is closely related to inter-particle friction, which depends strongly upon stress and deformation histories. The complexities associated with the flow of bulk solids make them much more challenging to handle than traditional solids, liquids, or gases. Common problems include uncontrollable flow that takes the form of plugging, obstructed or limited discharge, and erratic flow, as well as particle segregation and loss of live storage due to material adhering to container walls. Under extreme cases, flow problems can even cause high transient stresses that result in structural failure.

In addition to numerous research articles, several books and book chapters have been written on the topic of biomass handling and feeding [5, 6]. As explained by Bell [7], there have been significant, though relatively few, contributions from various researchers in powder mechanics, solids flow, and related topics over the last 100 years. This chapter is divided into three sections. The first section briefly summarizes the primary topics of biomass handling

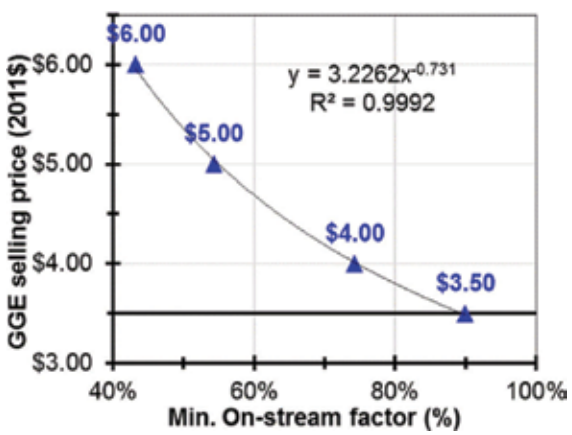

Figure 1. Gallon of gasoline equivalent (GGE) selling price as a function of minimum on-stream factor. Adapted from [2].

and primary impacts of dominant material properties. The second section presents a brief explanation of biomass flow in silos, bins and hoppers and includes key analyses needed to understand biomass flowability in the context of shear deformation, which is how bulk solids flow. The third section focuses on recent advances that have been made in biomass handling and briefly points to areas in which future effort should focus to have the greatest impact to alleviate the challenges that currently plague the emerging biofuels industry.

Biomass energy systems are typically considered to consist of several processes, including resource production, collection, transportation, storage, feeding, conversion and transmission of biofuel or energy to end users. Production and collection include farming and forestry activities, as well as collection of waste materials that are suitable for bioenergy production. Transportation of biomass to conversion plants is usually performed by truck, barge or rail car. Short-term and long-term storage at the plant is necessary to ensure that sufficient material is on-hand to keep the plant operating through small disturbances in supply or to mitigate exceedance, in which spikes in biomass supply exceeds conversion capacity, such as could occur in the fall for agricultural residues. The topics associated with research production through storage are wide-ranging and too voluminous to be considered in this chapter. Basu [5] has provided a brief summary of those activities and their implications. The primary concern of this chapter is biomass handling at the plant prior to conversion.

The ability of a real feedstock material to flow through a particular assembly system is a function of the design of the structure and the rheological properties of the biomass material. These properties include bulk density, moisture content, compressibility, elasticity or spring back, particle size and shape distributions, cohesive strength, unconfined yield strength, adhesive strength (shear stress required to initiate motion on a surface), angle of internal friction (theoretical angle describing stress at failure), wall friction angle (shallowest angle at which a material slides on a surface), and permeability (ability of a material to allow gas or liquid to pass through it). These physical properties are important for both biochemical and thermochemical conversion processes [8, 9]. Thermochemical reactions are known to be sensitive to particle geometry, especially for fast reaction rates and short particle residence times. Biochemical conversion processes are generally more tolerant of variations in particle sizes and shapes, such that constraints on physical properties are primarily imposed by requirements of the feeding and handling systems [10, 11].

A wide range of feedstock particle-size requirements have been reported for supplying feedstocks for biofuels conversion applications [12]. Feedstocks for fast pyrolysis are approximately 2 mm in size [13, 14], while those for biochemical conversion processes are much larger, varying from 6 to 75 mm. Excessive quantities of fine particles contribute to nuisance dust, clogged filters, and reduced permeability of the bulk solid to gases and liquids. Oversized particles create a different set of problems, such as incomplete conversion as well as plugging of air locks and pneumatic transfer lines. Particle grinding and other preprocessing steps have a strong impact on feeding behavior. For example, Idaho National Laboratory reported that replacing a hammer mill with a knife mill with the same nominal screen size solved a blowout problem with a pressure seal [12]. The level of fines can also increase as particles pass through consecutive unit operations and can cause negative impacts on the

Performance aspect	Governing parameters/mechanisms	Impacts
"Bulk flowability"	Particle-particle interactions, bulk density, chemical composition, moisture, temperature, and trapped gases	Easily flowing materials • facilitate emptying and cleaning equipment to prevent spoilage • readily fill containers to minimize storage and transportation volumes • feed uniformly for processes that requires consistent flow • tend to be easier to mix and blend • if overly aerated, may flow too freely and flood equipment
Time consolidation or caking (increase in strength after prolonged storage times)	Can be due to many different effects, such as crystallization, material creep, capillary condensation, and fungal growth	• Loss of live storage space because material adheres to storage container walls • Risk of loss of perishable material • Erratic flow with large dynamic forces on containing structures • Material bridges over outlet preventing flow
Handling properties in a slurry for enzymatic conversion	Particle-particle and particle-slurry interactions through particle shape, size, and ploy-dispersity	• Lower volatility resulting in increased conversion efficiency • Acidity of product bio-oil may be reduced
Reactivity for thermochemical and biochemical conversion processes	Particle sizes and shapes affect surface area to volume ratios	• Small particles have much faster thermochemical reaction kinetics as compared to large particles • More reactive particles can be substantially larger than less reactive particles. For example, biomass particles can be larger than coal particles in co-fired gasifiers
Permeability of bulk solid to flow of gases or liquids	Pore spaces between particles that allow gases and liquids to flow through bulk solid	• Low permeability restricts chemical access to material's interior, slowing reactions • Low permeability can limit discharge rates from outlets

Table 1. Noninclusive summary of feedstock performance related to particle physical and mechanical properties (adapted from [15]).

feeding and conversion performance [7]. Thus, even if ideal specifications are achieved, care must be taken so that the subsequent unit operations do not unintentionally modify material properties.

The behavior of biomass feedstocks in handling and feeding equipment is affected by many factors beyond traditional rheological properties. These factors include chemical composition of particles, temperature, presence of trapped gases and the unique stress and deformation histories of the bulk solid. The impacts of specific parameters are summarized in **Table 1**. Particle size and moisture content often receive the most attention, and it is important to recognize that in some cases the particle size "specification" is based on the screen size of a laboratory mill, rather than a thorough classification of particle-size distribution. Such a screen size specification is often misleading because in most cases the mean product particle size is

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significantly smaller than the screen size. Many parameters actually affect the particle size distribution and its mean. For example, the mill type strongly affects particle size and shape. Typically, hammer mills produce more fine particle sizes than knife mills using the same screen size and also result in wider particle size distributions. This is particularly important because knife mills are typically used to prepare samples for laboratory tests, while hammer mills are often used in high-throughput industrial-scale applications. The impacts of moisture content, incoming particle size, and tool speed also vary for different mill types [12].

2. Silos, bins and hoppers for storing and discharging

2.1. Flow obstructions and patterns

Proper storage and retrieval of biomass is critical to maintain quality in terms of both chemical properties for conversion and physical properties for feeding and handling. Retrieval or reclamation of biomass from storage is one of the most trouble-prone processes of biomass plant operation [5]. Silos are common in the agricultural and grain industries to store large quantities of material in a protective environment and can be large in diameter (4–15 m) and quite tall. Material is usually augured into the top of the silo and removed at the bottom. Very cohesive materials require specialized and expensive sweeping reclaimers to extract material from the entire bottom cross-section of the silo, where compressive pressures and material strengths can be very high. These systems require extensive engineering and are not discussed further here; however, additional information can be found at http://www.laidig.com/reclaimers.

Less cohesive materials and shorter storage systems in which compressive forces are lower often use less expensive hoppers or chutes to funnel biomass to a small feed discharge mechanism. In the 1960s, Andrew Jenike developed the first complete methodology for the flow of bulk solids within the framework of hoppers, bins, and feeders. His work included test equipment and procedures for measuring the necessary material properties, a theory of bulk solids flow within hoppers and bins, and a procedure to determine the hopper slope and outlet dimensions required for unobstructed gravity flow [6, 16]. The development presented here closely follows the formalism that Jenike advanced.

As described by Jenike [17], the primary issues in the design of hoppers and chutes are: (1) solid flow pattern, (2) slope angle of discharge, and (3) size of the discharge opening. Although there are a number of flow obstructions that may develop in a bin, two primary types are analyzed here: arching or doming as illustrated in **Figure 2(a)** and ratholing or piping as illustrated in **Figure 2(b)**. Most particulate solids are easily flowable when they are well-aerated but become cohesive and strong when compacted. For example, fluidized bulk solids have very low shear strengths and typically flow with carrier gases; however, the same bulk solids can be made into rigid briquettes or pellets by subjecting them to high compressive stresses, especially in the presence of moisture or binders. The increasing strength of bulk solids with increasing compressive stress allows them to form arches and bridge over openings. In the case of large bins and hoppers, the weight of material in upper layers compresses the lower

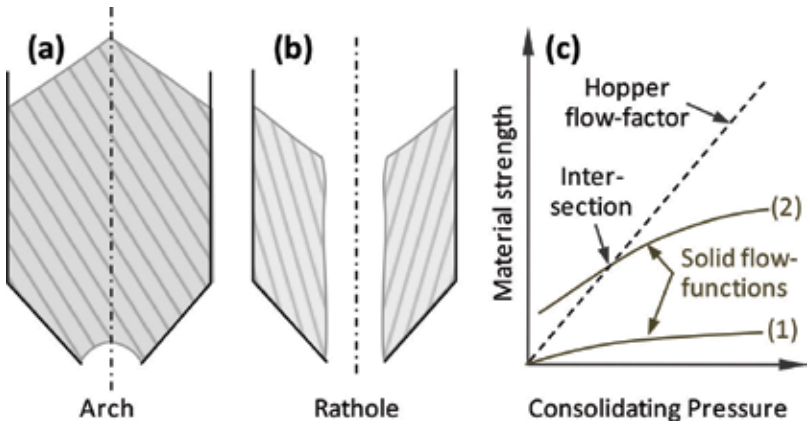

Figure 2. Schematics showing (a) arching, (b) ratholing, and (c) flow functions FF for two materials and a representative hopper flow factor.

material, causing it to gain strength and become cohesive. The cohesive strength, typically referred to as unconfined yield strength f_c, of a solid resulting from its stress history is the cause of the arch shown in **Figure 2(a)**. The strength-pressure curve of a solid is known as its flow-function FF and typically increases rapidly with increased pressure in the low pressure range and then increases more slowly at higher pressures [18]. The strength developed by different bulk solids as a function of consolidation pressure varies from solid to solid as exemplified by the flow-functions of materials (1) and (2) in **Figure 2(c)**. It is evident that material (2) is much stronger and less free flowing than material (1).

Approximating the downward pressure across an arch to be nearly uniform, the total force acting to break the arch scales with the area ($\pi d^2/4$ for a circular outlet, where d = diameter), while the material's ability to maintain the arch only scales with the perimeter (πd for a circular outlet) of the hopper outlet. Thus, if the size of the hopper outlet is steadily increased, eventually the strength of the material becomes insufficient to support the arch and the bulk solid flows. Flow of a bulk solid material can be assured for a hopper with specified geometry and wall material, as long as the strength of the material is maintained below a certain value, giving rise to the concept of a hopper flow-factor, which is also shown in **Figure 2(c)**. The intersection of the material's flow-function with the hopper's specific flow-factor usually determines the minimum outlet size needed to assure consistent gravity flow. Similar reasoning also applies to the formation of ratholes or pipes.

The flow pattern in a hopper affects all aspects of its performance, including not just the outlet size required to assure reliable discharge, but also the order in which the contents are discharged [19] and the loads acting on the structure [20, 21]. Two recent standards identify some flow patterns that have been identified [ISO 11697 (1992) and Eurocode 1 part 4 prEN 1991–4 (2002)]. For most purposes, flow patterns can be classified into two categories, mass flow and funnel or plug flow, as illustrated in **Figure 3(a)** and **(b)**. Perfect mass flow requires that all the material moves downward when material is removed from the outlet. Typically, smooth and steep walls are required to achieve mass flow. In contrast, funnel flow hoppers

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allow some material to remain at rest while only a portion of the material moves through the hopper. In funnel flow, a moving channel of material is formed within the central region of the hopper, while material outside the channel is at rest. A distinguishing feature of funnel flow is that the material flows primarily on itself, such that the walls of the container do not influence the shape of the channel or the velocities of moving particles.

Mass flow hoppers have many advantages over funnel flow hoppers. Mass flow hoppers preserve the first-in-first-out flow sequence, allow powders to deaerate, minimize segregation, and supply uniformly densified material to the feeder (see **Figure 3(a)** and [22]). Funnel flow hoppers have the opposite characteristics: the flow sequence is first-in-last-out, ratholes may form, powders have a strong tendency to flood, segregation problems are exacerbated, and the compaction of material fed to the hopper is nonuniform (see **Figure 3(b)**). Materials that are suitable for mass flow hoppers and not funnel flow hoppers include cohesive solids, fine powders, degradable materials, and solids which segregate [22]. The primary advantage of funnel flow hoppers is that they can have shallow hopper angles and, consequently, require much less headroom. A third common flow pattern, denoted expanded flow, is illustrated in **Figure 3(c)**. In this flow pattern, the lower portion of the hopper is designed to ensure mass flow and prevent arching while the central and top portions are designed solely to prevent ratholing (funnel-type flow is allowed in the central portion of the hopper). Expanded flow designs are practical for hoppers with large diameters filled with solids that exhibit strong tendencies to rathole in funnel flow bins, but flow well in mass flow bins.

2.2. Yield locus and effective yield locus

As a particle moves downward through a bin and hopper, the pressure field around the particle first increases as the height of material above it increases and then in the hopper section, the pressure decreases as the cross-section size decreases toward the outlet. At an open outlet,

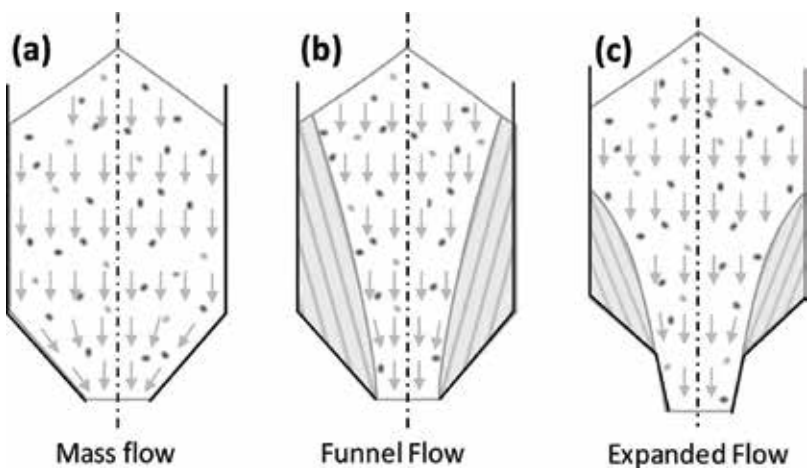

Figure 3. Schematics showing (a) mass flow, (b) funnel flow, and (c) expanded flow. Note that for funnel flow, the shape of the flow channel is independent of the shape of the hopper.

the stresses perpendicular to the material surface are nearly zero (i.e., the material is unconfined). At some point in this process, the major and minor principal stresses (pressures), σ_{major} and σ_{minor}, respectively, experienced by neighboring particles pass through maximum values, labeled as σ_1 and σ_2, respectively. For materials with low spring back or elasticity, the final bulk density ρ_b of the material depends only on σ_1 and σ_2, which are the dominant consolidation pressures.

Importantly, the shear strength of a static mass of material depends not only on the instantaneous principal stresses, σ_{major} and σ_{minor}, but also on their maximum values, σ_1 and σ_2, that are attained within the "memory" of the material (i.e., the shear strength is a function of σ_{major}, σ_{minor}, and ρ_b). This relationship is depicted in **Figure 4** as a yield locus curve, which represents the collection of points in consolidation-shear stress space that results in failure of the material at a specified bulk density $\rho_b(\sigma_1, \sigma_2)$. This follows the well-known flow-no flow criteria, which is that flow in a bulk solid occurs if the applied stress at a location exceeds the material's yield strength. If the stress in the material is below the yield locus (i.e., the stress is less than material shear strength), then flow does not occur. The point marked "0" denotes the end of the yield locus. If a neighborhood of particles is subjected a pressure greater than that at point "0," then the consolidation pressures, σ_1 and σ_2, necessarily increase, which also increases the bulk density, and a new yield locus is formed that is typically higher on the τ axis. Another important point to note in **Figure 4** is that a Mohr stress semi-circle through the point "0" and tangent to the yield locus determines the maximum major and minor consolidation pressures, σ_1 and σ_2, (as described in nearly any strength of materials text book). The unconfined yield stress $f_c(\sigma_1, \sigma_2)$ can also be determined using the yield locus because it is the major consolidation pressure that corresponds to zero minor consolidation pressure (corresponding to an unconfined surface). Thus, the Mohr-stress semi-circle that defines f_c is also tangent to the yield locus but is subject to the additional constraint that it pass through the origin (i.e., the minor stress is zero), as depicted in **Figure 4**.

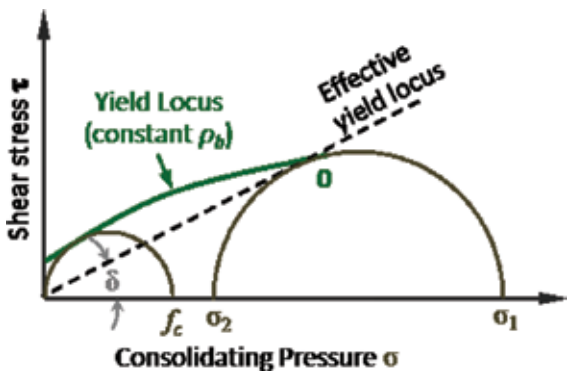

Figure 4. Schematic showing typical yield locus and effective yield locus for a material that has been subjected to maximum consolidation pressures σ_1 and σ_2 (resulting in a steady bulk density ρ_b, assuming that spring back is negligible).

The local cohesion of the material, which is a measure of the inter-particle binding strength in the absence of applied pressure (i.e., the shear strength with zero consolidation pressure) is the intercept of the yield locus with the shear stress axis. A fourth parameter that can be found from the yield locus is the effective angle of internal friction δ, which is the angle between the σ axis and the tangent to the Mohr's circle passing through point "0." δ defines the straight line termed the "effective yield locus" and is a measure of the internal friction at steady flow.

2.3. Jenike shear tester and test method

To measure the yield locus curves of finely divided materials (i.e., powders) at specified values of bulk density ρ_b, Jenike developed a special shear cell test apparatus, shown schematically in **Figure 5**. The shear cell is closely modeled after simple direct shear cells used to measure the shear strength of soils (A direct shear tester is one in which the design of the tester controls the location of the shear zone. In an indirect shear tester, the shear zone is allowed to develop according to the applied state of stress). The primary difference between the Jenike shear cell and simple shear cells used in soil analysis is that Jenike's cell is designed to be much more sensitive to small normal loads N and provision is made to ensure that the sample experiences similar maximum consolidation pressures, σ_1 and σ_2, before different points on the yield locus are measured.

The process to measure a point on a yield locus actually consists of two steps, referred to as (1) "preconsolidation" or "preshear" and (2) "shear." The objective of the first step is to preconsolidate the sample to the point "0" in **Figure 4**. The exact procedure to fill the ring and preconsolidate the sample is described in an ASTM and other standards [ASTM D-6128-06; Institution of Chemical Engineering, UK, 1989]. After uniformly filling the cell with material, a vertical force N_0 is applied to preconsolidate the sample. A horizontal shear force S is then applied to the bracket to move the lid and ring at a slow constant velocity relative to the base. The sample is slowly sheared in this manner until a steady state flow with constant force S is observed, indicating that the sample is preconsolidated to point "0" in **Figure 4**. This short preshearing step helps establish a uniform stress state throughout the sample. The force S is then removed, and the normal load N_0 is replaced with a smaller load N_1. The second step of

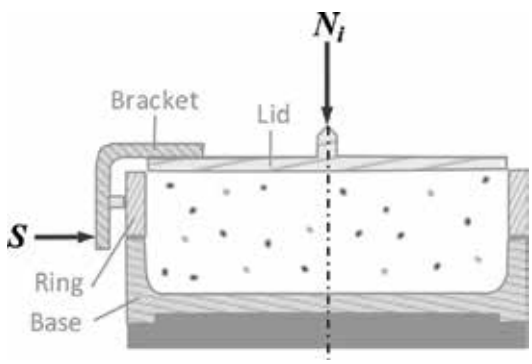

Figure 5. Schematic of Jenike's shear cell showing base, ring, lid and bracket.

the shear process, referred to as "shear," is accomplished by again applying a force S on the bracket and recording the maximum force required to shear the sample. The normal load N_1 and the maximum recorded shear force S are then converted to a consolidating pressure and yield shear stress, respectively by dividing each value by the horizontal area of the shear cell. The two values obtained in this manner define a single point on the desired yield locus. To obtain additional points on the yield locus, the two steps "preshear" and "shear" are repeated with different normal loads N_2, N_3, etc. The entire process is shown schematically in **Figure 6**.

It is critical that the first step ("preshear") be performed in as nearly as identical a manner as possible before each point on the yield locus is measured to ensure that the preconsolidation stresses are the same for each measurement (i.e., each measurement shares the same maximum principal stresses σ_1 and σ_2). After a sufficient number of points are obtained to define a yield locus, the unconfined yield stress f_c for the specific maximum principal stress σ_1 is found as described above. A plot of several values of f_c versus corresponding values of σ_1 yields the material flow function featured in **Figure 2(c)**. The measured flow-function FF is used with design charts developed by Jenike to quantitatively design systems to handle flowing bulk solids, such as determining the minimum outlet of a hopper that is required to ensure that an arch or rathole cannot form.

The flow-function is also used to classify the flowability of bulk solids. Jenike warns that several numbers and curves are required to precisely define the flowability of a bulk solid [20]; yet, for the sake of convenience, Jenike offered a simple flowability scale based on the flow function. The classification is accomplished by picking a point on the flow-function and determining the ratio of the major principal stress σ_1 to the unconfined yield strength f_c, denoted as $ff_c = \sigma_1/f_c$. The flowability of the material is then defined by the following scale:

$0 < ff_c < 2$—Very cohesive and non-flowing

$2 < ff_c < 4$—Cohesive

$4 < \text{f}\, ff_c < 10$—Easy-flowing

$10 < ff_c$—Free-flowing

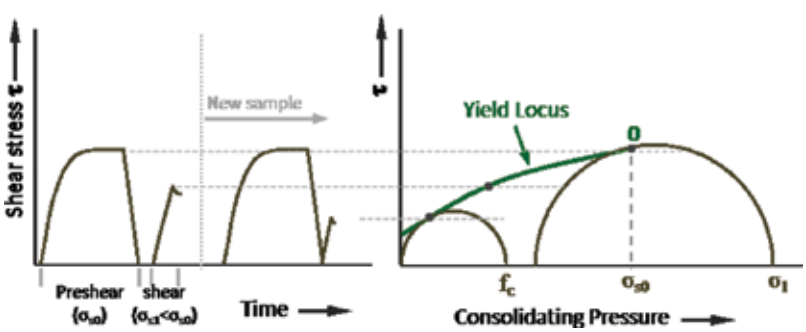

Figure 6. Procedure to measure three points on a yield locus using the Jenike shear tester.

This classification scheme is most useful for materials for which the flow-function is approximately a straight line. If the slope of the flow function of a material is not approximately constant, then the ratio σ_1/f_c is not constant and the material can exhibit behavior ranging from very cohesive to free-flowing depending on the consolidation pressure it is exposed to. Classification of such a material requires choosing a point on the flow function that is representative of conditions that exist when the material is required to flow.

Although Jenike's approach offers proven principles for designing systems to handle bulk solids, it also has drawbacks, which have greatly hindered its widespread adoption by industry [20, 23]. First, ensuring that the preconsolidation stresses at the point "0" in **Figure 4** are consistently and properly attained before measuring each point on the yield locus is not trivial and requires a high level of skill and training. Second, the tests are very time-consuming and expensive. It has been estimated that obtaining a flow-function curve for a material requires approximately 15 h for a skilled technician. The time cost is further exacerbated if multiple flow functions are required to understand a material's flow behavior at different moisture contents, temperatures, or after prolonged periods of consolidation (the shear strength of many materials increases with temperature, moisture, and after prolonged consolidation times). A third drawback is that measurements are not possible at small normal stresses, so that it is necessary to extrapolate the yield locus to find its intersection with the τ axis (cohesion). A fourth drawback is that the Jenike tester has very limited travel (approximately 7 mm) to minimize the reduction of the shear cross-sectional area during the test. The small amount of travel is sometimes insufficient to ensure that a consistent stress state is attained during the preshear step. Materials that are particularly problematic are those with large particles, high moisture content, and/or a high elastic limit (large spring back). The final drawback to the Jenike tester is that substantial variability often exists in the measured values, increasing the error in the extrapolation of the yield locus to find its intersection with the τ axis (cohesion) and making it necessary to employ conservative designs for hoppers to promote flow.

2.4. Other shear testers capable of determining the flow function

Despite the drawbacks of the Jenike shear tester, it remains one of the very few testers that is capable of measuring the unconfined yield stress f_c (and hence the flow function) of a material without additional uncertain assumptions. Other instruments that can also measure f_c include biaxial shear testers, uniaxial shear testers, and ring shear testers. Biaxial shear testers are uncommon due to their complexity, and are not practical for the measurement of flow properties for routine design of bulk solids handling systems. So-called "uniaxial" testers are very simple, and underestimate f_c because the consolidation pressure is applied in the same direction as the shear stress [23]. A further disadvantage of uniaxial testers is that they can only be used to test bulk solids that are sufficiently cohesive that they retain their consolidated shape when lateral support is removed. A result of the last observation is that uniaxial testers cannot perform tests at low consolidation pressures. The primary advantage of uniaxial testers is their simplicity-tests can be performed quickly. It is worth noting that a simpler and quicker procedure can be followed with the Jenike shear tester to obtain approximate results

for quality control or product development. This method employs only a single test (preshear and shear) and a repetition test to determine an estimate for the yield locus and a single point on the flow-function [20].

The last type of instrument capable of measuring unconfined yield stress f_c is a rotational ring shear tester. Early ring tester models were only partly successful in accurately measuring the unconfined yield stress f_c of materials, and it was not until an improved unit was developed by Schulze in 1994 that the superiority of ring testers over the Jenike shear tester became apparent [23]. The test procedure with a ring shear tester is equivalent to that described above—the sample is still sheared in two steps including "preshear" and "shear." The primary advantage of ring shear testers is the unlimited rotary travel that they offer, making it possible to measure a complete yield locus without changing the sample or refilling the shear cell. Unfortunately, however, ring shear testers do not overcome all of the limitations of linear shear testers. In particular, ring shear testers have difficulty evaluating the flow performance of compressible materials because the stress fields of those materials are highly non-uniform during the test [24, 25]. An automated commercial version of the Schulze ring shear tester is now available that increases the speed of the test process and reduces the dependence of measured flowability properties on the skill level of the operator [6]. Of course, these improvements come at a substantial cost: the base price of a commercial Schulze ring tester is greater than $70,000 USD. However, even with these features, ring shear tests are not always reliable for biomass as shown in **Figure 7**, which compares predicted minimum hopper opening sizes to ensure consistent flow based on shear test results of various ground pine materials to experimentally measured values [26].

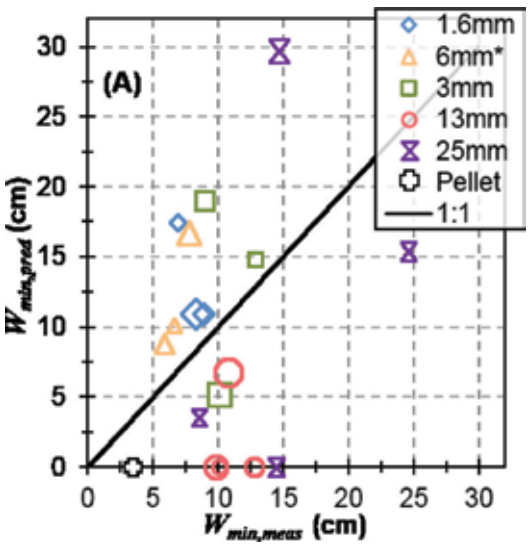

Figure 7. Predicted minimum hopper outlet widths versus the values measured using the hopper tests. Symbol size indicates moisture content with larger symbols representing higher moisture content (10–40% wet basis).

2.5. Conveying and feeding

Silos and bins serve to store material, which is then discharged through reclaimers or hoppers as explained above. After material is reclaimed from storage, it is subjected to final evaluation for suitability, including excessive moisture or unacceptable sizes of particles. Foreign materials, such as rocks and metals are also removed. The material is then conveyed using belt, chain, or pneumatic conveyors to the conversion reactor and is fed into the reactor. There are six primary types of biomass feeders: (1) gravity chute, (2) screw conveyor, (3) pneumatic injection, (4) rotary spreader, (5) moving-hole feeder, and (6) belt feeder. Proper design of the reclaiming, conveying, and feeding equipment is essential to ensure uninterrupted flow from storage to feeder. The design principles are based upon the material properties discussed above and, overall, share similar considerations with the design of silos, bins and hoppers summarized above. For detailed analyses of the various options, the reader is referred to specialized texts, such as those by [5–7, 23]. One topic that is of note here is the cost of biomass handling systems. Material handling represents a significant portion of the capital and operating costs of a biomass conversion facility even if all of the components operate exactly as intended. **Table 2** shows an example of relative costs of handling equipment for two biomass pelleting facilities, one for herbaceous feedstocks and one for woody feedstocks. The herbaceous facility is designed to handle baled material while the woody facility is designed to handle wood chips. For both feedstocks, the drying operation is the single largest cost with grinding and densification being the next most expensive. Overall, handling and processing bales incurs approximately $1.2 million more in total direct costs than handling and processing wood chips.

System type	Herb.	Woody
Material receiving	5%	4%
Separator/screener	2%	1%
Primary grinder	16%	—
Dryer	31%	41%
Secondary grinder	10%	13%
Densification	13%	16%
Dust collection	6%	7%
Buffer storage	2%	2%
Controls	2%	2%
Equipment installation and electrical	4%	4%
Civil/structural work	9%	9%
Total direct cost	$5.4 M	$4.2 M

Table 2. Relative estimated costs in 2011 USD ($) of herbaceous and woody biomass handling and preprocessing systems, each operating at 9 tons/h (adapted from [27]).

3. Solutions to biomass handling challenges

3.1. Co-optimization of feeding equipment and material properties

Failure to recognize the extent of material variability during equipment and process design is a common cause of feeding and handling problems. Systems can be designed to accommodate the full range of material variability; however, costs often increase as systems are made more robust. In the end, the selected design becomes a trade-off between increased capital costs for more robust systems (which is a near term, well-defined expense) and increased operating expenses due to additional down time if less expensive equipment fails. Importantly, the impact of increased operating costs is farther in the future and is rarely well defined. Relying on uniform bulk densities for gravity feed, low moisture and consistent particle-size distributions allows equipment designs to be simple and low cost. As long as the material meets the desired specifications, no problems are anticipated, but when material properties deviate outside narrow design specifications, equipment efficiency and reliability suffer, often dramatically.

There are two primary approaches to addressing material handling problems. First, the equipment systems may be engineered to anticipated material properties, or second, the feed material may be engineered to perform properly in the equipment systems. The first approach follows traditional engineering design concepts and tends to gain the most attention. In truth, a balanced approach that carefully considers both methods is usually best, especially for processes that are intended to handle different feedstock materials or materials that do not have well-defined and controlled properties.

Figure 8 depicts how this dual approach of more robust equipment design and better control of feedstock material properties can improve the reliability of a hypothetical operation,

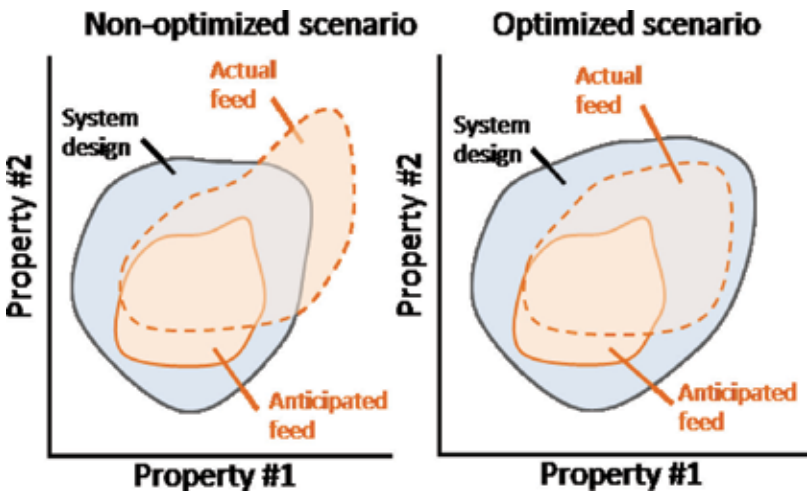

Figure 8. The combined approach for solving biomass handling and feeding problems through improved system design and improved preprocessing operations that control feedstock properties to meet to specifications. The scenario on the right in which the equipment systems and feedstocks have been optimized will likely exhibit superior and more reliable performance.

such as a bin/auger feeder. The range of anticipated material properties and the corresponding design specifications of the hypothetical equipment are illustrated in the regions labeled "anticipated feed" and "system design," respectively. Variation of material properties, due to unavoidable diversity of sources and supply conditions, including seasonal and weather effects, over the course of operation, often breaches equipment design specifications as depicted by the region labeled "actual feed." Ensuring that the reliable operational envelope of the process completely encompasses the actual operating conditions requires consideration and control of both the equipment design and material properties, such as bulk density and moisture content, as well as particle size/shape distributions and roughness. The combination of improved equipment design and better control of material properties is illustrated at the right side of **Figure 8** by the expanded system design envelope and the reduced envelope of actual feed properties that is achieved by actively managing the variation of raw material properties. The objective of this holistic approach is the simultaneous optimization of both cost and performance.

Achieving an optimal balance between minimizing the cost and complexity of equipment and managing the variation of feedstock properties requires a comprehensive understanding of the material properties and the factors that impact those properties. A common mistake identified by Bell [7] is believing that feeding and handling problems can be readily solved during start up. In truth, retrofitting equipment and processes can be very expensive and drawn-out because problems are often discovered one at a time as successive pieces of equipment come online. Actions that are taken to solve one problem may have unintended consequences that ripple through downstream operations and can add to the confusion between causes and effects. Fully characterizing all potential feedstocks and carefully managing material properties to match handling and conversion equipment is crucial to minimizing the probability of unexpected operating inefficiencies and failures.

3.2. Recommended future research directions

Solving biomass feeding and handling challenges will require a combination of techniques and capabilities, including numerical simulation, comprehensive material characterization, and mechanical tests. Numerical simulations to date have not had great impact in evaluating the flowability of biomass in handling equipment because of the extreme complexity of the flow problem. It is recognized that Cauchy equations of force and momentum conservation are insufficient to simulate solids flow because of the interactions of the various forces, including wet and dry friction, capillary, gravity, Coulomb, and elastic windup [28]. However, attempting to empirically solve solids flow problems through a series of tests to classify or rank biomass materials in all possible flow situations is not practical. Tests would have to be conducted for each equipment geometry at all scales using all types of biomass materials and all types of biomass preprocessing options that impact the dominant flow properties of bulk density, particle size and shape distributions, particle surface friction, particle rigidity, and moisture content. The number of tests that would be required is prohibitive, and correctly interpreting the large database of properties and test results would be daunting if not infeasible.

In contrast, a close coupling between instrumented lab and pilot scale tests and multiscale modeling may be able to elucidate the appropriate constitutive relations that are needed to augment the Cauchy equations of force and momentum conservation for successful continuum modeling. The powerful outcome of empirically-based numerical simulations is that the results would be scalable within any reasonable equipment size and the impact of specific material properties, such as those described above, could be determined to understand the operational envelope of specific processes. The multiscale models would operate as a direct transfer function to translate microscopic and macroscopic material properties that can be measured in the laboratory to material flow performance in biomass feeding and handling systems. The flow simulations could be used to identify cost effective approaches to modify the biomass materials and/or the transportation and handling equipment to reduce supply chain costs and also to minimize the equipment down-time due to material feeding problems. Continuum models may also be augmented by discrete element method (DEM) modeling that can simulate the motion and even the deformation of each particle in a flow field. **Figure 9** show an example of DEM model of a material that consists of particles with different shapes flowing in a wedge-shaped hopper. Simulating each individual particle in the flow offers the possibility of realistically capturing particle size and shape effects that cannot be directly incorporated into continuum models; however, such models have very high computational costs, so they are typically limited to simulations that involve not more than a few million particles with relatively simple shapes.

A final need that should be addressed is real-time, inline feeding and handling quality assurance (QA) and quality control (QC). Even with near perfect understanding of how material

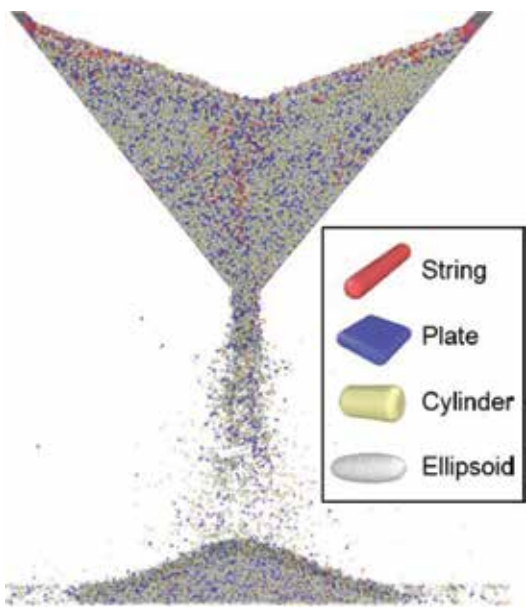

Figure 9. DEM model of flow in a wedge-shaped hopper. The material consists of particles with different shapes as indicated by particle color. Image courtesy of Hai Huang and Yidong Xia at Idaho National Laboratory.

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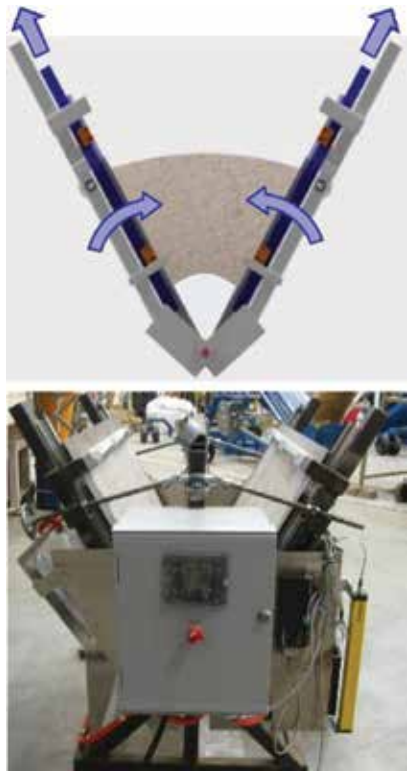

Figure 10. Wedge-shaped flow hopper with rotating and sliding walls for monitoring flow properties in real-time.

attributes impact flowability performance, feeding and handling problems can still arise if variation in harvest, storage, or preprocessing results in localized material that does not meet the specifications. Data recently obtained at Idaho National Laboratory, Idaho in which the author participated indicates a manner in which an in-line test can be rapidly performed [29] using a custom V-shaped hopper with sliding walls as shown in **Figure 10**. The proposed apparatus offers real-time, inline measurement of material flow performance. Installing this or similar QA/QC equipment in biomass feeding and handling systems can prevent out-of-spec material from causing expensive down-time and potential damage to processing equipment.

4. Conclusions

Feeding and handling of biomass has been a primary factor causing pioneer industrial bio-refineries to struggle to achieve production targets. The primary biomass properties that impact feeding behavior include bulk density, moisture content, compressibility, elasticity or spring back, particle size and shape distributions, cohesive strength, unconfined yield strength, internal friction angle, and wall friction angle (a property shared with the container surface). The primary issues in the design of hoppers and chutes are: (1) solid flow pattern, (2)

slope angle of discharge, and (3) size of the discharge opening. Comprehensive methodologies have been developed to test material properties and design equipment systems for well-behaved particulate materials, such as the Jenike method and tester. However, these methods are not always reliable for compressible, elastic, and anisotropic materials, such as biomass. Solving biomass feeding and handling challenges will require a combination of techniques and capabilities including numerical simulation, comprehensive material characterization, and mechanical tests. Numerical simulations to date have not had great impact in evaluating the flowability of biomass in handling equipment because of the extreme complexity of the flow problem. However, a close coupling between instrumented lab tests and multiscale modeling may be able to elucidate the appropriate constitutive relations that are needed for successful continuum modeling. These models could operate as a transfer function to translate microscopic and macroscopic material properties that can be measured in the laboratory to material flow performance in biomass feeding and handling systems at lab, pilot, and industry scale to understand the impact of variation in key flow properties on process reliability. This combination of experiments and flow simulations could be used to identify cost effective approaches to modify the biomass materials and/or the transportation and handling equipment to reduce supply chain costs and also to minimize equipment down-time due to material feeding problems.

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Chapter 7

Production of Microbial Lipids from Lignocellulosic Biomass

Mirela Ivancic Santek, Suncica Beluhan and Bozidar Santek

Additional information is available at the end of the chapter

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Abstract

The current industrial production of the biodiesel relies mainly on vegetable oils that could result in the shortage of edible oils in food markets and increase in their prices. Microbial lipids produced by oleaginous microorganism have attracted a lot of attention in the recent years as a source of high-value polyunsaturated acids as well as alternative feedstock for the production of biodiesel. However, the production of microbial oils faces a number of problems concerning the costs of lipid extraction, carbon source and operational cost for microbial cultivation in conventional stirred tank bioreactor which makes production economically unfeasible. Non-food feedstocks, lignocellulose biomass and different waste streams containing lignocellulose, are low-cost sources of renewable carbon that could significantly reduce the production cost of microbial lipids. This review analyses the current production of microbial lipids from lignocellulose feedstocks and gives an overview of the main stages in the process of lipid production, pretreatment and hydrolysis of the feedstock and microbial cultivation. Cultivation of oleaginous microorganisms has been conducted by submerged cultivation and solid state fermentation. Three process configurations have been used in the lipid production including, separate hydrolysis and lipid production (SHLP), simultaneous saccharification and lipid production (SSLP) and consolidate bioprocessing (CBP). Implementing the biorefinery concept that includes co-production of different value-added products (polyunsaturated fatty acids, amino acids, lignin and pigments) could improve the feasibility of lipid production bioprocess.

Keywords: biodiesel, cellulase, lignocellulose, microbial lipids, value-added products

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1. Introduction

Biodiesel is renewable, biodegradable and non-toxic transport fuel composed of fatty acid methyl esters. It is produced by transesterification of triacylglycerols with alcohol (mostly methanol and ethanol) in the presence of alkaline catalyst (e.g. sodium hydroxide). Common feedstocks for the production of biodiesel are different vegetable oils including rapeseed oil, palm oil, cooking oil, soybean oil and sunflower oil [1]. Production of biodiesel increased steadily in the last few years. In year 2016, the United States and Brazil were the world's biggest biodiesel producers with a production volume of around 5.5 and 3 billion liters, respectively [2]. All existing diesel engines, vehicles and infrastructure can run on pure biodiesel (B100) or blends with petroleum diesel fuel without any change [1]. Use of biodiesel has positive environmental impact, improves energy supply security, stimulates economic development and generates employment especially in the rural areas [3]. It reduces harmful emission characteristic for diesel exhaust such as particulate matter, carbon monoxide and total unburned hydrocarbons. Additionally, emission of toxic compounds including vapor-phase hydrocarbons from C1 to C12, aldehydes and ketones up to C8 and polyaromatic hydrocarbons and nitrated polyaromatic hydrocarbons are also decreased [4].

Microbial lipids are viewed as an alternative feedstock for the biodiesel production because fatty acid compositions of accumulated lipids are similar to vegetable oils currently used as feedstock for the production of first generation biodiesel. Microbial lipids are also known as single cell oils (SCO), and are produced by heterogeneous group of oleaginous microorganisms that include less than hundred species of different microbial species including yeasts, fungi, bacteria and algae [3, 4]. Oleaginous microorganisms have the ability to accumulate significant amounts of intracellular lipids (more than 20% of their dry weight), mostly triacylglycerols, under certain cultivation conditions. Yeast strains such as *Lipomyces starkeyi, Rhodosporidium toruloides, Rhodotorula graminis, Rhodotorula glacialis* and *Trichosporon oleaginosus* can accumulate intracellular lipids from 50 to 80% (w/w) under certain cultivated conditions [5–8]. The fatty acid composition of lipids depends on the microbial strain and the cultivation conditions used. The most common fatty acids are palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids [9]. Microbial lipids of some oleaginous microorganisms are valuable source of polyunsaturated fatty acids that are used as additive for different food products and in nutriceuticals. Following omega-3 and omega-6 fatty acids are commercially produced using different wild-type and genetically modified oleaginous microorganisms such as γ-linolenic acid (GLA, C18:3, n^{-6}) by *Mucor circinelloides*; dihomo-gamma-linoleic (DGLA) (20:3, n−6) by *Mortierella alpina* 1S-4; eicosapentaenoic acid (EPA) (20:5, n−3) by *Mortierella alpina* ST1358 and *Yarrowia lipolytica*; docosahexaenoic acid (DHA, 22:6, n−3) by *Crypthecodinium cohnii*, *Schizochytrium* and *Ulkenia* sp. and arachidonic acid (ARA, 20:4, n−6) by *Mortierella alpina* [10–17]. Microbial lipids from oleaginous yeast strains can be used as substitute for cocoa-butter and shea butter [18]. In comparison to vegetable oils, biodiesel production from microbial lipids have a number of advantages such as heterotrophic oleaginous microorganism grow much faster than the terrestrial crops; no need for arable land for cultivation; growth as well as cultivation does not depend on whether conditions and elimination of conflict between food and food supply chain [19]. Yeasts and fungi are favored oleaginous

microorganism since they grow much faster than the microalgae. Unlike microalgae, they can use more diverse sugars and other carbon sources for their growth and lipid synthesis monosaccharides (glucose and xylose), amino sugars (N-acetylglucosamine), disaccharides (lactose, galactose, mannose, cellobiose and sucrose), alcohols (glycerol, ethanol and methanol), polysaccharides (starch and pectin) and organic acids (volatile fatty acids and acetic acid) [5, 8, 20–24].

In order to increase economic feasibility of the process production, different low-cost substrates have been used for the production such as crude glycerol, lignocellulose biomass (hydrolysate sweet sorghum bagasse, rice straw hydrolysate and corn stover hydrolysate), molasses waste, waste streams from food industry (whey permeate, olive pomace oil and olive oil mill wastewaters) and waste spent yeast from the brewing industry [5, 25–31].

2. Biochemistry of lipid accumulation

The fatty acid biosynthetic pathway in most of oleaginous microorganisms is similar to non-oleaginous microorganisms. Two features of oleaginous microorganisms make them an efficient producer of lipid such as ability to efficiently produce precursor acetyl-CoA and cofactor NADPH needed for fatty acid synthesis. Process of lipogenesis could be divided in two steps, synthesis of precursor acetyl-CoA followed by biosynthesis of triacylglycerols (**Figure 1**). Nitrogen starvation in the presence of excess of carbon sources triggers *de nov*o synthesis of lipids in oleaginous microorganisms. Depletion of other media component like phosphorus or sulfur can efficiently induce lipogenesis [16]. Exhaustion of the nitrogen source induces a series of the consecutive biochemical reaction in the cell. The activity of AMP deaminase (AMPD) is upregulated. It cleaves the AMP to inosine monophosphate (IMP) and ammonia ions that cell can use as a nitrogen source. Consequently, concentration of AMP is reduced and the activity of NAD^+ ($NADP^+$)-depended isocitrate dehydrogenase (ICDH) is downregulated. This enzyme in oleaginous microorganisms is allosterically regulated by its activator AMP. Isocitrate accumulates in mitochondria and isomerized to citrate by aconitase (A). Accumulated citrate is transported into the cytoplasm in exchange for malate (citrate/malate translocase, CMT). In the cytoplasm, ATP citrate lyase (found only in oleaginous microorganisms) converts citrate to acetyl-CoA and oxaloacetate [32]. The acetyl-CoA is used for fatty acid synthesis while oxaloacetate is converted to malate by malate dehydrogenase (MDH) and exported to mitochondria *via* CMT [16, 32].

The synthesis of lipids depends on efficient supply of NADPH, which is used for acetyl group reduction in the growing acyl chain. For the synthesis of 1 mol of a C18 fatty acid, 16 mol of NADPH is required. There is no unique metabolic route for generating NADPH in the oleaginous microorganism. Ratledge [33] described several routes for the synthesis of NADPH in the cytosol during lipogenesis. Transhydrogenase cycle which includes three enzymes pyruvate carboxylase (PC), MDH and malic enzyme (ME) has been proposed as a major route for the NADH production in the oleaginous microorganism. However, presence of ME in cytosol was not confirmed in some yeast species [16, 33]. In yeast *Y. lipolityca,* ME is located in the mitochondria and therefore cannot participate in the lipid synthesis [34].

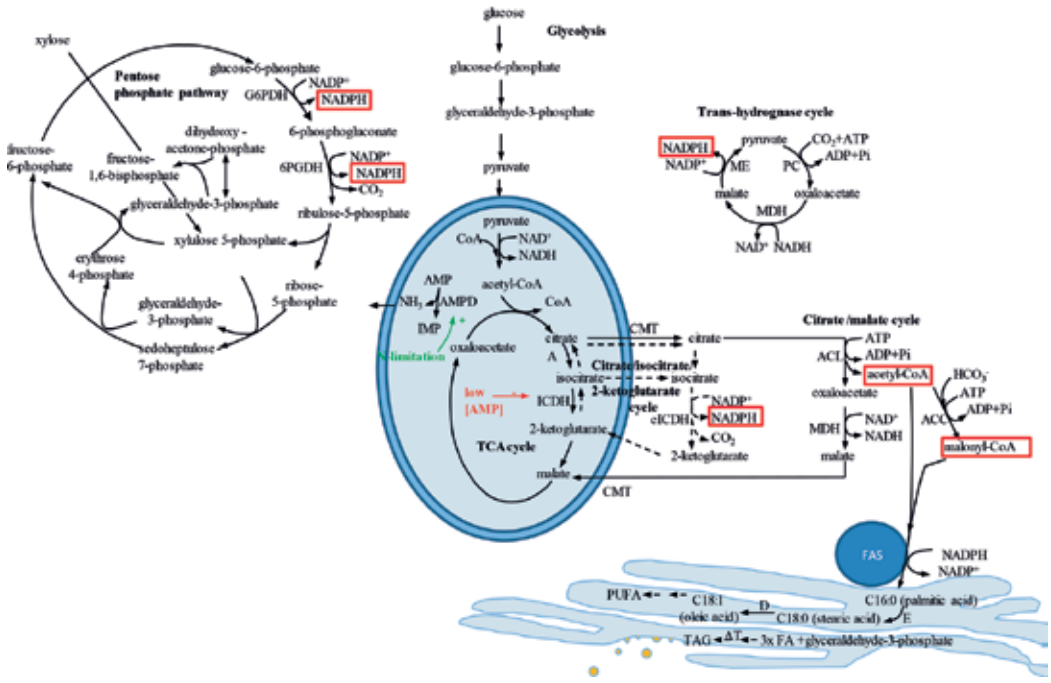

Figure 1. Overview of major metabolic pathways involved in lipid synthesis. The precursors for fatty acid synthesis, acetyl-CoA, malonyl-CoA and NADPH, are highlighted (red rectangles). Green and red arrows indicate upregulation and downregulation of the key enzyme for lipid accumulation. Abbreviations used for enzymes and metabolic intermediates: 6PGDH: 6-phosphogluconate dehydrogenase; AT: acyltransferase, cytosolic; ACC: acetyl-CoA carboxylase; ACL: ATP:citrate lyase; AMPD: AMP deaminase; cICDH: $NADP^+$-dependent isocitrate dehydrogenase; CMT: citrate-malate translocase; D: desaturase; E: elongase; FAS: fatty acid synthetase; G6PDH: glucose-6-phosphate dehydrogenase; ICDH: isocitrate dehydrogenase; MDH: malate dehydrogenase; ME: malic enzyme; PC: pyruvate carboxylase; FA: fatty acid; IMP: inosine monophosphate; PUFA: polyunsaturated fatty acid; TAG: triacylglycerol. Adapted from [16, 33].

Furthermore, expression of this enzyme is not changed upon limitation of the cell growth by nitrogen source [35]. Recent studies confirmed that primary source of NADPH for lipid synthesis in *Y. lipolityca* is the Pentose phosphate pathway [34, 36]. The NADPH is generated by enzymes glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH). Additional NADPH could be also provided by the cytosolic $NADP^+$-dependent isocitrate dehydrogenase (cICDH) present in some eukaryotic organisms (citrate/isocitrate/2-ketoglutarate cycle) [16, 32, 33].

The *de novo* fatty acid biosynthesis takes place in cytosol on a multifunctional enzyme complex called fatty acid synthetase (FAS). FAS is fed by three precursors needed for the fatty acid synthesis such as acetyl-CoA, malonyl-CoA and NADPH. Malonyl-CoA is synthetized by carboxylation of acetyl-CoA with the enzyme acetyl-CoA carboxylase (ACC). The end products are saturated fatty acids C16 (palmitic cid) or C18 (stearic acid) depending on the microorganism. Fatty acid are further elongated and desaturated by specific elongases (E) and desaturases (D) in the endoplasmic reticulum leading to fatty acid of different chain length and degree of unsaturation. The final step is triacylglycerol formation from glycerol-3-phosphate and fatty acids catalyzed by specific acyltransferases (AT). Neutral lipids including

triacylglycerols form lipid droplets on the lumenal and/or cytoplasmic side of the endoplasmic reticulum membrane [16].

3. Lignocellulose biomass as carbon source for microorganism growth

Lignocellulose is complex biopolymer composed of the polysaccharides (cellulose and hemicellulose), amorphous polymer lignin and a remaining smaller part including pectin, protein, extractives and ash. The structural carbohydrates, which accounts for approximately two thirds of the total dry weight of the lignocellulosic biomass, can be used as carbon source for microbial production of biofuels after hydrolysis to fermentable sugars. Composition of lignocellulosic biomass varies depending on the plant source. For example, the agriculture residues like rice, rye and wheat straw contains less cellulose (approximately 30%) than hardwood including poplar, pinewood and spruce (>40%) [37–42]. However, digestibility of carbohydrates in the native lignocellulosic biomass by cellulases is low due to its structural features. Structural features of lignocellulosic biomass are determined by its chemical composition (content of lignin, hemicellulose and acetyl groups bound to hemicellulose) and physical characteristics (accessible surface area, i.e., porosity, crystallinity and degree of cellulose polymerization, the physical distribution of lignin in the biomass matrix, pore volume and biomass particle size) [43]. Lignocellulosic biomass is subjected to pretreatment process which breaks down the native structure and exposes cellulose fibers to hydrolytic enzymes improving the yield of fermentable sugars. For the past three decades, various methods for the pretreatment of the lignocellulosic biomass have been developed. The pretreatment process is considered as one of the most expensive steps in the production of lignocellulosic biofuels. The estimated cost of pretreatment process in bioethanol production is approximately 30 US cent per gallon of ethanol [44]. The pretreatment processes are classified in the following groups: physical (milling, grinding, pyrolysis, extrusion and gamma-ray irradiation), chemical (alkali hydrolysis, dilute acid hydrolysis, organosolv process and oxidative delignification), physicochemical (steam explosion/autohydrolysis, ammonia fiber explosion, CO_2 explosion and SO_2 explosion), biological (biochemical degradation using white-, brow- and soft-rot fungi and lignin-degrading enzymes) and combination of these methods. During pretreatment process, a number of degradation products are formed: furan aldehydes (furfural and 5-hydroxymethyl furfural), aromatic compounds (vanillin, syringaldehyde and 4-hydroxybenzoic acid), aliphatic acids (acetic, formic and levulinic) and inorganic compounds [44–46]. During the pretreatment process at high temperature and pressure, hemicellulose is hydrolysed mainly to xylose and lesser extent to glucose. Furan and 5-hydroxymethyl furfural (HMF) are formed by dehydration of released xylose and glucose, respectively [45, 47, 48]. Acetic acid is formed by hydrolysis of acetyl groups in hemicellulose. Formic and levulinic acid are derived from furan aldehydes during prolonged exposure to high temperature in an acidic environment. Formic acid is formed by furfural and HMF degradation, while levulinic acid is generated from HMF. Concentration of HMF in lignocellulosic hydrolysate is much lower than the furfural due to limited hydrolysis of hexose from lignocellulosic biomass. The third group of degradation product includes diverse phenolic compounds which are derived from lignin

and extractive compounds present in the lignocellulosic biomass [45, 49–51]. The most common aromatic compounds in the lignocellulose acid hydrolysate are vanillin, syringaldehyde, 4-hydroxybenzoic acid, ferulic acid, etc. [45, 51]. Formation of degradation by-product strongly depends on the plant source and pretreatment process (temperature, pressure, reaction time and presence of catalyst) [46–48, 51].

4. Production of microbial lipids from lignocellulose biomass

The bioconversion of lignocellulose to the microbial lipids includes following steps: pretreatment of lignocellulose biomass, hydrolysis of structural carbohydrates to fermentable sugars, microbial production of lipids and isolation and purification of the product. Since most of the oleaginous microorganisms lack cellulase and hemicellulase activity, structural polysaccharides in lignocellulosic biomass has to be hydrolysed to fermentable sugars (mainly xylose and glucose) which microorganism can use as a carbon source. The structural polysaccharides are hydrolysed using cellulolytic enzymes or thermochemical process conduct at elevated temperature in the presence of concentrate acid catalyst. Enzymatic hydrolysis is preferred over thermochemical route since the reaction is carried out under mild conditions (pH and temperature) in non-corrosive environment. Furthermore, inhibitors that could potentially inhibit the microorganism are not formed [65–67]. The major drawbacks of enzymatic hydrolysis are longer hydrolysis time, higher price of enzyme and inhibition by end products [67–70]. Production of the oleaginous lipids from lignocellulosic biomass is carried out using three process configurations such as separate hydrolysis and lipid production (SHLP), simultaneous saccharification and lipid production (SSLP) and consolidate bioprocessing (CBP, **Figure 2**). The production of the lipids by SHLP involves two separate steps, enzymatic hydrolysis of lignocellulose followed by lipid production, while in SSLP these steps are integrated and carried out simultaneously in one vessel. In SHLP both steps are run under optimal conditions for microorganism (pH = 4.8–6.0, T = 25–30°C) and cellulases (pH = 4.5–6,0; T = 50–60°C) [25, 71, 72]. However, inhibition of cellulase by accumulated glucose and cellobiose decreases the yield of fermentable sugars. In SSLP, sugars released by hydrolysis are simultaneously assimilated by microorganism minimizing the inhibition effect by the end-product. Elimination of enzyme inhibition enhances the rate of carbohydrate hydrolysis and shortens the process time. Since the enzyme hydrolysis and microorganism growth are carried out in one vessel, the number of vessels needed for the process is reduced, decreasing the capital costs. The main disadvantage of SSLP in comparison to SHLP is the necessity of running the process at temperature favorable for the microbial growth (T = 30–32°C) which is usually suboptimal for the cellulase hydrolysis [67]. To compensate lower activity at the process temperature, enzyme loading is increased. Alternatively, lipids could be produced in a process known as 'Consolidate bioprocessing', which gain much attention in the production of lignocellulosic bioethanol [73]. CBP integrates cellulase production, carbohydrate hydrolysis and lipid production in one step. Besides high lipid productivity and titer, the industrially viable CBP-strain has to efficiently secrete cellulases for hydrolysis of carbohydrates. Suitable microorganism for the CBP could be isolated from nature or alternatively designed by genetic engineering using two strategies already used in development of CBP yeast strain for the lignocellulosic bioethanol production [74]. The first strategy includes a heterologous expression of the cellulose degrading genes in the oleaginous

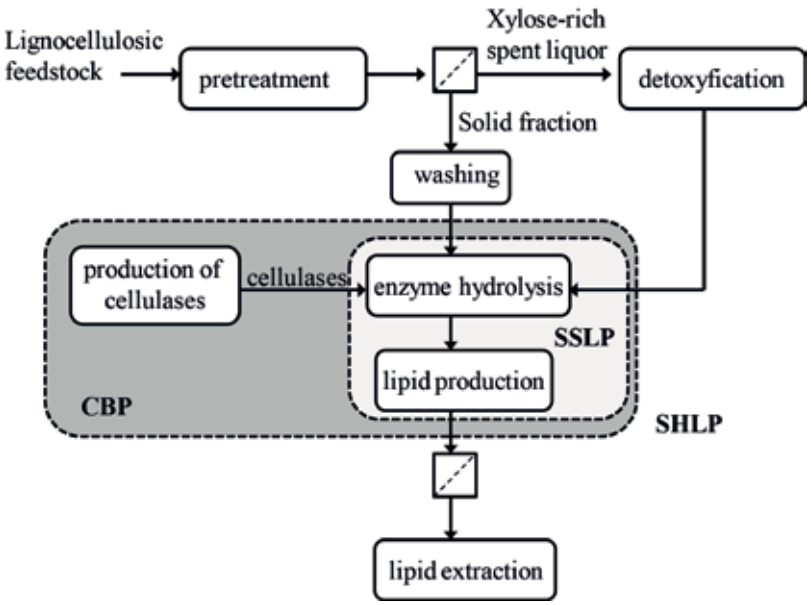

Figure 2. Production of microbial lipids from lignocellulosic biomass by separate hydrolysis and lipid production (SHLP), simultaneous saccharification and lipid production (SSLP) and consolidate bioprocessing (CBP).

microorganism and the second strategy includes a metabolic engineering of cellulolytic microorganism for improved lipid accumulation.

Microbial production of oleaginous lipids from lignocellulosic biomass is carried out either by submerged or by solid state cultivation.

4.1. Submerged production of lipids

Tables 1 and **2** summarize processes of lipid production by SHLP and SSLP in submerged culture. Most of the researches have been done in shake-flask cultures at 30°C, pH between 5 and 6, using 10% (v/v) of inoculum and buffer to maintain constant pH [57, 75–77]. The most favorable feedstocks for lipid production are agriculture waste, corn stover (stalks, leaves and cob) and corn cobs. Other lignocellulosic feedstocks used for lipid production include energy crops (*Panicum virgatum* and *Jerusalem artichoke*), forest residue (*Douglas fir*) and agriculture waste (sweet sorghum bagasse). The performance of the process depends on the cultivation mode (batch and fed-batch), the pretreatment method, method for carbohydrate hydrolysis, the substrate loading and type of microorganism. Acid and alkali pretreatments are the most often used methods for improving the digestibly of lignocellulose by cellulase [57, 75, 78–84]. Hydrolysis of structural polysaccharides is commonly carried out using enzymatic hydrolysis [27, 57, 75–77, 84–86]. The efficiency of cellulase hydrolysis mostly depends on pretreatment method, but also on used commercial cellulase. For efficient hydrolysis, at least 10 different enzymes are needed including enzymes from the glycoside hydrolase families 7 (CBHI, EGI), 6 (CBHII), 5 (EGII), 10 and 11 (xylanases) and 3 (ß-glucosidases) as well as the acetyl xylan esterases. Commercial cellulase preparations are constantly improved and their prices are being reduced. Thus, Cellic CTech2 and Cellic CTech3 from Novozymes (www.novozymes.com)

Pretreatment process	Effect on lignocellulosic biomass	Disadvantage
Dilute acid hydrolysis	Hydrolysis of hemicellulose and amorphous cellulose, increase of crystallinity, increase of porosity of biomass [52–54]	Toxic and corrosive process, formation of inhibitors [40, 52, 55, 56]
Mild alkaline hydrolysis	Delignification, partial hydrolysis of hemicellulose, increase the surface area, reduction of degree of polymerization and crystallinity of cellulose [52, 53, 57–59]	Less corrosive and expressive process than dilute acid hydrolysis, formation of inhibitors, less efficient for feedstock with high lignin content [44, 52, 53, 58]
Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL)	Complete hemicellulose and minimal lignin removal, cellulose depolymerization [60]	
Ammonia fiber expansion (AFEX)	Depolymerization and deacetylation of hemicellulose, depolymerization and cleavage of lignin-carbohydrate bonds [63, 64]	Is not effective for biomass with higher lignin content, formation of some inhibitors [46, 61, 62]
Hydrothermal process	Partial hydrolysis of hemicellulose, redistribution of lignin in biomass, deacetylation of hemicellulose [56]	Does not use chemical catalyst, less corrosive, minimal formation of inhibitor [56]

Table 1. Overview of various pretreatment methods.

have improved cellobiohydrolases, endoglucanases, ß-glucosidases and additional oxidative activity (auxiliary activity family 9, formerly known as GH61) for enhanced sugar yield especially at the high substrate loading [87, 88]. Spent liquors from acid pretreatment of lignocellulosic biomass are also used as a carbon source [57, 75, 78–84]. Unlike the enzymatic hydrolysate, spent liquor obtained by acid pretreatment of a lignocellulosic biomass contains lignocellulose-derived products that can inhibit microorganism growth and synthesis of product as well as the enzyme activity [47, 48].

Economically feasible process for industrial cellulosic lipid production requires high final lipid titer (**Table 2**). Most of the research has been done in batch SHLP using different oleaginous strains of yeasts. Concentration of lipid and productivity of batch SHLP process depends on lignocellulose feedstock, microorganism, pretreatment method, detoxification method and type of carbohydrate hydrolysis. As shown in **Table 2**, in most of the batch SHLP under optimized culture conditions, lipid concentration and lipid productivity was below 20 g/L and 0.15 g/L h, respectively.

Harde et al. [86] cultivated *Mortierella isabellina* on pretreated biomass and detoxified spent liquor obtained by SPORL pretreatment of *Douglas fir*. For lipid production, three strategies were investigated. First two strategies included separate processing of pretreated biomass and spent liquor. Lignocellulosic biomass was subjected to separate hydrolysis and lipid production and simultaneous saccharification and lipid production with prehydrolysis step. Third strategy included hydrolysis of whole lignocellulosic slurry, detoxification and lipid production (**Tables 2** and **3**). Lipid yield produced from whole lignocellulosic slurry was lower than those from other two strategies, where pretreated biomass and spent liquor were

Feedstock	Microbial strain	Pretreatment	Cultivation media	Fermentation mode	X[a] (g/L)	L[b] (g/L)	w_L[c] (%)	$Y_{L/S}$[d] (g/g)	Pr[e] (g/L/h)	Reference
Corn stover	*Trichosporon cutaneum* AS 2.571	H_2SO_4 (0.1–1%, 140–180°C, 5–10 min)	Spent liquor from acid pretreatment	Batch	—	7.6	39	0.15	0.078	[78]
Sweet sorghum bagasse	*Cryptococcus curvatus* ATCC 20509	Microwave radiation (100°C, 4 min h)	Enzymatic hydrolysate of pretreated sweet sorghum bagasse (endoglucanase 778–1022 CMC U/g DM, ß-glucosidase126–186 pNG/g DM, xylanase 625–950 ABXU/g DM)	Batch	15.5	—	64	0.11 [d*]		[26]
Corn stover	*Mortierella isabellina* ATCC 42613	NaOH (1%, 121°C, 2 h)	Enzymatic hydrolysate of pretreated corn stover (26 FPU/g DM, substrate loading 5%)	Batch	10.9	2.48	29	—	0.027	[57]
		H_2SO_4 (1%, 121°C, 2 h)	As above		14.1	4.78	34	—	0.05	[57]
Corn stover	*Trichosporon cutaneum*	Pre-soaking with H_2SO_4 (190°C, 3 min)	Enzymatic hydrolysate of pretreated corn cobs (cellulase 7 FPU/g DM, substrate loading 10%)	Batch	—	0.97	—	—	0.014	[75]
Corn stover	*Cryptococcus curvatus*	Ionic liquid (1-ethyl-3-methylimidazolium acetate, 140°C, 1 h)	Enzymatic hydrolysate of pretreated corn stover (cellulase10 FPU/g DM, cellobiase 20 CBU/g DM, xylanase 10 mg/g DM, substrate loading 5%)	Batch	16.5	7.2	43	0.138[d*]	—	[79]
Jerusalem artichoke	*Cryptococcus* sp.	HNO_3 (0.57%, 117°C, 49 min)	Spent liquor from acid pretreatment	Batch	6.1	—	28	—	0.072	[80]
Corn cobs residue	*Trichosporon cutaneum* ACCC20271	-Unknown pretreatment conditions	Enzymatic hydrolysate of pretreated corn cob residue (cellulase 15 FPU/g DM) substrate loading 15%)	Batch	38.4	12.3	32	0.131	0.047[f]	[76]
Corn stover	*Cryptococcus curvatus* ATCC 20509	NaOH (0.5 M, 80°C, 75 min)	Enzymatic hydrolysate of pretreated corn stover (cellulase: 20 FPU/g DM, ß-glucosidase: 40 CBU/g DM, xylanase 140 U/g DM, 10% substrate loading)	Batch	27.7	—	44	0.155	0.156	[81]

Feedstock	Microbial strain	Pretreatment	Cultivation media	Fermentation mode	X[a] (g/L)	L[b] (g/L)	w_L[c] (%)	$Y_{L/S}$[d] (g/g)	Pr[e] (g/L/h)	Reference
Sweet sorghum stalks	*Lipomyces starkeyi* CBS 1807	No pretreatment	Enzymatic hydrolysate of sweet sorghum stalks cellulase (8 FPU/g, Celluclst 1.5 L: Novozyme 188 (ß-glucosidase) at a1:5 (vol/vol)	Batch	6.4	—	29	0.077	0.033	[77]
Corn cobs	*Rhodotorula glutinis* CGMCC 2.703	Mixed acids (0.5% H_2SO_4 + 1.5% H_3PO_4, 123°C)	Undetoxified spent liquor form acid pretreatment	Batch/65 h/ bioreactor cultivation	15.1	5.5	36	0.129	0.09	[82]
	As above	As above	As above	Fed-batch with constant C and N feed	75.4	30.6	39	0.146	0.15	[82]
	As above	As above	As above	Fed-batch with two stage feeding strategy (1st C + N-source, 2nd C-source)	70.8	33.5	47	0.159	0.17	[82]
Switchgrass	*Lipomyces tetrasporus* Y-11562	H_2SO_4 (0.936%, 160°C, 15 min, 20% solids)	Undetoxified spent liquor form acid pretreatment	Batch	53.4	29.0	53	0.156	0.215	[83]
	Lipomyces kononenkoae Y-7042	As above	As above	As above	47.7	28.1	59	0.161	0.179	[83]
	Rhodosporidium toruloides Y-1091	As above	As above	As above	42.6	26.2	61	0.159	0.128	[83]
Corn stover	*Mortierella isabellina*	steam explosion (200°C, 7 min)	Enzymatic hydrolysate of pretreated corn stover (cellulase 30 FPU/g, substrate loading 30%)	Batch	36.1	18.7	52	0.05[f]	0.039	[85]
Douglas fir forest residue	*Mortierella isabellina* NRRL 1757	SO_2 (11 g/L, 140°C, 60 min)	Enzymatic hydrolysate of pretreated lignocellulose biomass (cellulase 14.6 FPU/g glucan, substrate loading 10%)	Batch	25.5	14.4	—	0.18	0.120[g]	[86]
	As above	SO_2 (11 g/L, 140°C, 120 min)	As above	Batch	25.7	11.9	—	0.18	0.120[g]	[86]

Feedstock	Microbial strain	Pretreatment	Cultivation media	Fermentation mode	X[a] (g/L)	L[b] (g/L)	w_L[c] (%)	$Y_{L/S}$[d] (g/g)	Pr[e] (g/L/h)	Reference
	As above	SO_2 (11 g/L, 140°C, 60 min)	Detoxified spent liquor	Batch	16.05	8.4	—	0.18	0.050[h]	[86]
	As above	SO_2 (11 g/L, 140°C, 120 min)	As above	Batch	11.6	7.7	—	0.16	0.046[h]	[86]
	As above	SO_2 (11 g/L, 140°C, 60 min)	Detoxified enzymatic hydrolysate of whole pretreated slurry (cellulase 14.6 FPU/g glucan, substrate loading 10%)	Batch	35.4	18.55	—	0.17	0.086[i]	[86]
	As above	SO_2 (11 g/L, 140°C, 120 min)	As above	Batch	38.2	17.6	—	0.18	0.081[i]	[86]
Corn stover	*Rhodosporidium toruloides*	NaOH (0.4%, 80°C, 2 h) and H_2SO_4 (0.8%, 160°C, 10 min)	Enzymatic hydrolysate of pretreated corn stover (cellulase 40 mg protein/g cellulose- substrate loading 20%)	Batch	36.2	21.4	59	0.19	0.28	[84]
	As above	As above	As above	DO-stat fed-batch	42	25.2	60	0.23	0.33	[84]
	As above	As above	As above	Pulse fed-batch	43	26.7	62	0.24	0.35	[84]
	As above	As above	As above	Online sugar monitoring fed-batch	54	32	59	0.29	0.4	[84]

[a]X: Biomass concentration, g cell/L.

[b]L: Lipid concentration, g lipids/L.

[c]w_L: Lipid content, g lipid produced/g dry cell weight.

[d]$Y_{L/S}$: Lipid yield, g lipid/g of consumed carbon source; [d*]$Y_{L/S}$: Lipid yield, g lipid/g pretreated lignocellulosic biomass.

[e]Pr: Lipid productivity, g lipid produced/h L

[f]Lipid productivity was calculated based on time for prehydrolysis (3 days) and fermentation (8 days): lipid concentration/time.

[g]Lipid productivity, lipid concentration (L)/time of cultivation (216 h).

[h]Lipid productivity, lipid concentration (L)/time of cultivation (120 h).

[i]Lipid productivity, lipid concentration (L)/time of cultivation (168 h).

Table 2. Production of lipids by separate hydrolysis and lipid production (SHLP) from lignocellulosic hydrolysate.

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processed separately. Despite the lower process efficiency, this approach is attractive from the economic point of view since it reduces the operational and capital costs for lipid production. Development of the strain with high tolerance toward inhibitors in spent liquor could reduce the number of steps in production and production cost. Harde et al. [86] developed sulfite tolerant strain of *M. isabellina* by gradual adaptation of the strain to inhibitors from spent liquor. The sulfite-adapted strain was able to grow in the presence of 2.0 g/L of sulfite in synthetic media and spent liquor [86].

Slininger et al. [83] designed two step screening assay for the detection of the highly productive yeast strains with high tolerance to lignocellulose-derived inhibitors. Growth media contained undetoxified enzyme hydrolysate of corn stover pretreated by ammonia fiber expansion (AFEX) and acid pretreated switchgrass. Three yeast strains, *Lipomyces tetrasporus*, *Lipomyces kononenkoae* and *Rhodosporidium toruloides* were identified. Yeast strains were able to grow on the undetoxified switchgrass hydrolysate and accumulate 25–30 g/L lipids at the rate of 0.128–0.215 g/L h with lipid yield of 0.156–0.161 g/g of consumed substrate [83]. Those values are the highest values reported in literature for batch cultivation of oleaginous microorganisms using lignocellulosic hydrolysate. Contrary to expectation, performance of the isolated oleaginous yeasts was significantly better than other used yeasts in SHLP with detoxified spent liquor (**Table 2**). Some oleaginous microorganisms show high tolerance to most of lignocellulose-derived inhibitors. Indeed, yeast strain *R. toruloides* tolerates acetate, 5-hydroxymethylfurfural and syringaldehyde at concentrations below 70, 14.7 and 12 mM, respectively. Negligible effect on growth and lipid production showed the presence vanillin and *p*-hydroxybenzoate at concentrations below 10 mM. The strongest inhibitory effect on growth and lipid accumulation had furfural. At concentration of 1 mM, biomass and lipid concentrations dropped by 45.5 and 26.5% [89].

Fed-batch mode of cultivations in production of microbial lipids has already been proved to be superior to batch cultivation. High cell and lipid concentration of 106.5 and 71.9 g/L (67.5%), respectively, were obtained in pilot scale fed-batch in a 15 L stirred tank bioreactor cultivation by yeast *R. toruloides* using glucose as a carbon source with the productivity of 0.54 g/L h [90]. Fei et al. [84] applied fed-batch cultivation mode to improve the efficiency of lipid production by *R. toruloides* using lignocellulosic hydrolysate. Different feeding strategies of the culture were investigated including dissolved oxygen-stat (DO-stat) feeding mode, pulse feeding mode and online sugar control mode. All three fed-batch strategies improved processes performance in comparison to the batch cultivation in terms of cell concentration, lipid yield and process productivity. The highest lipid yield of 0.29 g/g and lipid productivity 0.4 g/(L h) was obtained using the online sugar control feeding mode. Those values are the highest reported in the literature obtained by using concentrated enzymatic hydrolysate of lignocellulose biomass. This study represents major breakthrough in the research of lipid production from lignocellulosic biomass that could improve feasibility of the bioprocess. However, production of concentrated lignocellulosic hydrolysate (~ 550 g/L) used in research relies on the cost-intensive evaporation [84].

Therefore, developing new methods for preparation of concentrated lignocellulosic hydrolysate could improve the process economics. Fed-batch cultivation was applied in process of the

lipid production by yeast *R. glutinis* using the undetoxified spent liquor from acid pretreatment of corn cobs as a carbon source [83]. In this study, the lipid productivity was remarkably improved using two feeding strategies regarding the dynamics of nitrogen supplementation. Since yeast *R. glutinis* showed high tolerance toward inhibitors, a corn cob acid hydrolysate was used without detoxification. First strategy included feeding with concentrated undetoxified spent liquor (790.2 g/L xylose and 40.5 g/L glucose) supplemented with the nitrogen source. The second strategy included feeding of the culture for the first 80 h of cultivation with concentrated undetoxified spent liquor supplemented with nitrogen source and afterwards only with the carbon source. The highest biomass concentration of 75.4 g/L was obtained using first feeding strategy, while second feeding strategy resulted with the highest lipid concentration of 33.5 g/L, which is the highest value of lipid concentration reported in literature for culture grown on lignocellulose hydrolysate [82].

Still most of the studies on lipid production have been done by SHLP using hydrolysate of lignocellulosic biomass as a carbon source. Research by Gong et al. [79, 81] showed that the efficiency can be improved by integrating the enzyme hydrolysis and microbial process applying SSLP (**Table 3**). Two SSLP processes were conducted in cultivation media with and without the addition of nitrogen source. In cultivation media-containing alkaline pretreated corn stover without nitrogen, cells did not grow due to the lack of nitrogen and carbon sources was used for lipid production. To obtain high lipid productivity, the culture media was inoculated at high inoculums size (7.2 g/L), while control culture supplemented with nitrogen was inoculated at average inoculums size (10% v/v). The highest lipid productivity of 0.195 g/L h was obtained in SSLP without nitrogen source and this is the highest value reported for the SSLP using the lignocellulose as a carbon source. In comparison to the SHLP using the same pretreated lignocellulosic biomass (**Table 2**), the productivity of SSLP was improved and loading of cellulase and xylanase was reduced for 50%, while ß-glucosidase was not used. The major disadvantage of this strategy is increased cost for cultivation of larger quantities of inoculum using enriched growth media [81]. Gong et al. [79] applied similar strategy using the corn stover pretreated with ionic liquids as a carbon source. However, lower lipid yields of 0.125 and 0.135 g/ g DM were obtained for SSLP and SHLP, respectively [79]. SSLP was also conducted with fungus *M. isabellina* using pretreated *Douglas fir* forest residue but without success. After prehydrolysis step, fungus was able to grow in semi-solid media obtaining 17.0 g/L of lipids. However, the productivity of lipid synthesis with fungus was half of those obtained in SHLP with detoxified enzymatic hydrolysate of whole pretreated Douglas fir [86].

All cultivations were carried out at substrate loading of 10% (w/w) or lower suggesting possible problems with the enzyme hydrolysis and microorganism growth at higher substrate loadings. The product titer could be improved by increase of the substrate loading conducting so-called high-gravity fermentations which have been successfully applied in the bioethanol production from starch and lignocellulosic feedstock by simultaneous hydrolysis and fermentation. Significant savings in energy input, decrease of waste discharge, distillation costs and capital costs increased the competiveness of the process [91]. However, running the SSLP under high-gravity conditions imposes a number challenges with respect to the lignocellulose-derived inhibitors and mixing and mass transfer in cultivation broth. Due to high substrate loading, the concentration of inhibitory by-products are increased and consequently lead to the

Substrate	Microbial strain	Pretreatment	Enzyme hydrolysis	Fermentation mode/time/ note	L[a] (g/L)	$Y_{L/S}$[b] (g/g)	Pr[c] (g/L/h)	Reference
Corn stover	*Trichosporon cutaneum*	Pre-soaking with H_2SO_4 (190°C, 3 min)	Prehydrolysis for 6 h, cellulase 7 FPU/g DM, substrate loading 10%	Batch/80 h/bioreactor	3.03	—	0.042	[75]
Corn stover	*Cryptococcus curvatus*	Ionic liquid (1-ethyl-3-methylimidazolium acetate, 140°C, 1 h)	Cellulase4 FPU/g DM, cellobiase 8 CBU/g DM, xylanase 5 mg /g DM, substrate loading 5%	Batch/2 days/ no nitrogen source	6.0	0.112	0.125[d]	[79]
Corn stover	*Cryptococcus curvatus* ATCC 20509	NaOH (0.5 M, 80°C, 75 min)	Cellulase 10 FPU/g DM, xylanase 80 U/g DM, substrate loading 10%	Batch/3 days/	11.9	0.129	0.168	[81]
	Cryptococcus curvatus ATCC 20509	As above	As above	Batch/3 days/ high inoculums concentration of 7.2 g/L, media without nitrogen source	15.9	0.159	0.195	[81]
Douglas fir forest residue	*Mortierella isabellina* NRRL 1757	SO_2 (11 g/L, 140°C, 60 min)	Prehydrolysis for 24 h, cellulase 14.6 FPU/g glucan, substrate loading 10%	Batch/168 h	17.0	0.21[b*]	0.101[e]	[86]
	Mortierella isabellina NRRL 1757	SO_2 (11 g/L, 140°C, 120 min)	As above	Batch/ 168 h	11.7	0.18 [b*]	0.070[e]	[86]

[a]L: Lipid concentration, g lipids/L.

[b]$Y_{L/S}$: Lipid yield, g lipid/g pretreated lignocellulosic biomass; [b*] $Y_{L/S}$: Lipid yield, g lipid/g theoretical sugar yield from pretreated biomass.

[c]Pr: Lipid productivity, g lipid produced/h L.

[d]Lipid productivity, lipid concentration (L)/time of cultivation (48 h).

[e]Lipid productivity, lipid concentration (L)/time of cultivation (168 h).

Table 3. Production of lipids by simultaneous saccharification and lipid production (SSLP) from lignocellulosic hydrolysate.

decrease or complete inhibition of growth and product accumulation along with their enzyme activity. Furthermore, increased viscosity of the lignocellulose slurry prohibits the efficient mixing, decreasing the heat and mass transfer (substrate, enzyme and oxygen) in bioreactor. Increasing the stirring rate in a conventional stirred tank bioreactor provides even mixing directly around the impeller, while the solid substrate settles down to the bottom and toward to the bioreactor's wall. To avoid the above motioned problems, cultivation should start with lower substrate loadings. The substrate should be gradually fed keeping the viscosity of culture broth sufficiently low (fed-batch cultivation). Kinetics of substrate additions depends on the activity of cellulolytic enzymes and substrate consumption by working microorganism and it should be experimentally optimized. Gradual addition of substrate should enable the working microorganism to adapt to increasing inhibitors concentrations and convert some them to less toxic compounds (furfural and HMF into less toxic compounds such as furfuryl alcohol and 2,5-bis-hydroxymethylfuran, respectively) [92]. Using this strategy Elliston et al. [93] produced 11.6% (vol/vol) ethanol from waste paper in a bioreactor with high shear mixing. Gradual addition of substrate resulted in cumulative substrate loading of 65% [93].

4.2. Production of lipids by solid state fermentation

Solid state fermentation offers a number of advantages over submerged cultivation in the production of microbial biomass and specific products of microbial metabolism. This technique of cultivation has been successfully used for the production of food (fermented sausages and sea food), products of microbial metabolism including antibiotics, giberellinic acid, aflatoxines, pigments, alkaloids, organic acids and plant growth factors, enzymes, biopesticides, including mycopesticides and bioherbicides, biosurfactants, biofuel, aroma compounds, etc. [94–96]. The major benefit of the solid state cultivation is higher bioprocess productivity, lack of catabolic repression, higher product concentration and low water and energy demands. In comparison to submerged culture, the risk of contamination is decreased due to lower water content in growth media. Furthermore, in comparison to the submerged culture, the product isolation is simpler and also less cost effective. The major drawback of solid state fermentation includes engineering problems with control of process parameters (temperature, water content, pH, substrate and oxygen concentration, etc.) and the scale-up of process to industrial size [96].

Several researches on lipid production by solid state fermentation using lignocellulose biomass have been described in literature (**Table 4**). Production of lipids by this type of cultivation depends on oleaginous microorganism's ability to hydrolyse the carbohydrates from lignocellulosic biomass to fermentable sugars. This bioprocess of lipid production is also called consolidate process (CBP). Desirable characteristics of CBP-strain are efficient lipid accumulation, high lipid productivity, high cellulase and hemicellulase activity and the ability to grow on insoluble substrate in the absence of free water. The oleaginous microorganisms used in the submerged production of lipid are not able to grow on the solid substrate or secrete cellulase and hemicellulase. Several fungi strains were isolated with 20–35% (w/w) of accumulated lipids in cell dry weight. Low lipid yield in solid state fermentation is a consequence of insufficient cellulolytic activity of isolated CBP-strains and low efficiency of lipid accumulation [97]. The cellulolytic activity in submerged cultivation was between 10 and 20 FPU/g and 4–15 FPU/g of dry matter of lignocellulosic biomass in SHLP and SSLP, respectively (**Tables 2** and **3**). The

unrestricted carbon source supply is required for the efficient growth and lipid accumulation. Therefore, enhancement of the cellulase activity in cultivation media was recognized as crucial for the improvement of bioprocess performance. Enhancement of cellulase activity was obtained by the optimization of moisture content of solid substrate, cultivation temperature, addition of complex substrates (e.g. wheat bran) and addition exogenous cellulase [97, 98]. The most promising CBP-strain for solid state cultivation is fungus *A. tubingensis* TSIP9 with high cellulase activity and moderate lipid content of 20.5% [99, 100]. Different modes of the solid state fermentation were applied to improve the lipid yield including batch, fed-batch and batch with repeated substrate replacement. Simple strategy of substrate addition in fed-batch cultivation (0.0719 g/g DM) did not improve the lipid yield in comparison to the batch cultivation (0.0799 g/g of substrate dry matter). The batch cultivation with repeated substrate replacement was the most efficient strategy for the production of lipids on the solid substrate. Repeated cycles of the batch cultivations with replacement of 90% fermented substrate with fresh one shortened the process time in comparison to the batch cultivation. Furthermore, cleaning and sterilization of the bioreactor between the batches and inoculum preparation was avoided that additionally saved the time, energy as well as labor [99]. Regardless the fermentation mode, the bioprocess efficiency of solid state fermentations was lower than in the submerged culture (**Tables 2** and **3**). Lipid yields in solid state fermentations were at least two times lower than the submerged cultures. In addition to strain characteristic, significant impact on process efficiency have concentration gradients of hydrogen ions, oxygen, fermentable sugars, products of metabolism formed in the layer of solid substrate during cultivation that inhibited growth of microorganism and cellulase activity.

5. Future perspective of lipid production from lignocellulose biomass

Microbial lipids are promising feedstock for biodiesel production, but the development of lipid production is still far from ready to be commercialized. The process of microbial lipid production is still uncompetitive with agricultural production of vegetable oils which market price is significantly lower. Techno-economic study of biodiesel production with *R. toruloides* (Pr = 0.54 g/L h and $Y_{L/S}$ = 0.23 g/g) using glucose as a carbon source pointed out the main obstacles in commercialization of this process. Estimated costs for biodiesel production and microbial lipids using glucose as a carbon source are US$5.9/kg biodiesel and US$5.5 / kg lipids, respectively. The glucose cost accounts for 80% of the raw material used for production of biodiesel and for approximately 35% of the overall cost of biodiesel produced. Furthermore, the main generators of capital and energy costs are connected to production of microbial lipids using stirred tank bioreactors [103]. The production cost could be reduced by using low-cost substrates such as lignocellulose instead of glucose. The replacement of glucose with the lignocellulose feedstock as a carbon source reduces the cost for the raw material but also brings number issues including technical problems connected to complexity of process production and high capital costs. Due to the lack of investor interests and government assistance, the progress in development of this technological process is still very slow. The production of microbial lipid could become more economically feasible, if the biorefinery concept of co-production of different value-added products is applied. To obtain additional

Substrate	Microbial strain	Pretreatment	Fermentation mode/ time/note	Enzyme activity	$Y_{L/S}$ (g/g)[a]	Reference
Wheat straw and wheat bran mixture	*Microsphaeropsis* sp.	Steam exploded (121°C, 1 h)	Batch (75% moisture, 10 days, 27°C ratio of wheat straw to wheat bran 4:1 g/g)	Cellulase 0.31–0.54 FPU/g DM[b]	0.024–0.042[e]	[97]
	Sclerocystis sp.	As above	As above	Cellulase 0.34–0.52 FPU/g DM[b]	0.019–0.028[b]	[97]
	Phomopsis sp.	As above	As above	Cellulase 0.32–0.56 FPU/g DM[b]	0.021–0.027[b]	[97]
	Cephalosporium sp.	As above	As above	Cellulase 0.39–0.58 FPU/g DM[b]	0.026–0.034[b]	[97]
	Nigrospora sp.	As above	As above	Cellulase 0.069 FPU/g DM[b]	0.023[e]	[97]
Wheat straw and wheat bran mixture	*Microsphaeropsis* sp.	Steam exploded (15% water, 1.5 MPa, 10 min)	Batch (75% moisture, 30°C, 10 days, 27°C, ratio of wheat straw to wheat bran 4:1 g/g)	Cellulase: 0.32 FPU/g DM[c]	0.042[c]	[98]
	Microsphaeropsis sp.	As above	As above	Addition of exogenous cellulase 10 FPU/g DM[c]	0.074 [c]	[98]
	Microsphaeropsis sp.	As above	Batch (75% moisture, 30°C, 10 days, 27°C, ratio of wheat straw to wheat bran 9:1 g/g)	Addition of exogenous cellulase 10 FPU/g DM[c]	0.08 [c]	[98]
Rice straw and wheat bran	*Colletotrichum* sp.	—	Batch	Cellulase 1.84 FPU/g DM	0.0682	[101]
	Colletotrichum sp.	—	Batch	+ Exogenous cellulase 10 FPU/g DM	0.0843	[101]
	Alternaria sp.	—	Batch	Cellulase 1.21 FPU/g DM	0.0603	[101]
	Alternaria sp.	—	Batch	+ exogenous cellulase 10 FPU/g DM	0.0817	[101]
Wheat straw and wheat bran mixture	*Aspergillus oryzae* A-4	Acid (0.7% H_2SO_4, 121°C, 1 h)	Batch (50–80% moisture, 6 days, 30°C, weight ration of wheat straw to wheat bran 2:8 g/g)	Cellulase: 1.69 FPU/g DM[d]	0.06287	[102]
Palm pressed fiber and palm empty fruit bunches	*Aspergillus tubingensis* TSIP9	Acid (0.5% H_2SO_4, 121°C, 1 h)	Batch, (65% moisture, 28°C 5 days)	Cellulase: 26.1 U/g DM[b] xylanase 59.3	0.0885	[100]

Substrate	Microbial strain	Pretreatment	Fermentation mode/ time/note	Enzyme activity	$Y_{L/S}$ (g/g)[a]	Reference
Palm empty fruit bunch and palm kern cake	*Aspergillus tubingensis* TSIP9	Alkali (10% NaOH, 100°C, 15 min)	Batch (28°C,5 days)	Cellulase: 11.1 U/g DM	0.0799	[99]
	Aspergillus tubingensis TSIP9	As above	Fed-batch(28°C,12 days)	Cellulase: 19.0 U/g DM xylanase: 65.6 U/g DM	0.0719	[99]
	Aspergillus tubingensis TSIP9	As above	Repeated-batch (28°C, 12 days/ substrate was added every 3 days)	Cellulase: 18.4 U/g DM xylanase: 119 U/g DM)[c]	0.0919	[99]

[a]$Y_{L/S}$: Lipid yield, g produced lipid/g of dry matter of pretreated lignocellulosic biomass.
[b]Cellulase activity was determined on 6th and lipid content on 10th day of cultivation.
[c]Cellulase activity on 10th and lipid content on 9th day of cultivation.

Table 4. Production of lignocellulosic lipids by solid state fermentation.

income, microbial fatty acids should be fractionated depending on their price; low value fatty acid should be used for biodiesel production, while high value (GLA, DHA and ARA) should be used as food supplement and in production of nutriceuticals [11–18]. Therefore, oleaginous microorganism with high content of unsaturated fatty acids such as fugus *Mortierrella* sp. would be favorable for process of microbial lipids production. Other value-added products such as pigments or sophorolipids could also give additional revenue. Oleaginous yeast such as *R. glutinis*, *R. rubra* and *S. ruberrimus* accumulate valuable pigments, ß-carotene, torulene and astaxanthin [13, 104–107]. Sophorolipids can be used as biosurfactants instead of classical chemical-derived surfactants in cosmetics, food, cleaning and petroleum industry. Unlike chemical surfactants, sophorolipids are biodegradable and also have interesting biological activities including anti-microbial, anti-cancer, anti-HIV, anti-inflammatory and anti-viral activities [108–110]. Lignin is by-products generated during pretreatment that should be separated and sold. In biorefinery, lignin can be converted to heat and power for the processing steps. Building blocks derived from lignin can be used for production of vanillin, carbon fiber, bio-oil, resin, adhesives, polymer fillers, coating agents, bioplastics, paints, soil amendment, slow nitrogen release fertilizers, rubbers, elastomers and microbial agents. Proteins from lignocellulosic biomass and microbial biomass after lipid isolation could also be used as animal feed or after acid hydrolysis to amino acids could be used as building blocks for the synthesis of different chemicals [111–114]. Significant influence on production cost of biodiesel has process of lipid recovery from cell biomass. Lipid isolation on laboratory scale is based on laborious and expensive isolation protocols that include cell harvesting by centrifugation, energy-intensive step of biomass drying and lipid extraction using an organic solvent.

Therefore, a new cost-effective method for isolation of lipids from wet cell biomass is needed to improve the competiveness of the process [115].

6. Conclusions

The current production of microbial lipids from lignocellulose biomass faces a number obstacles associated with low lipid yield of producing strains, low tolerance of microbial strains to lignocellulose-derived inhibitors, insufficiently high substrate concentration in lignocellulose hydrolysate and high costs of product isolation. In order to reduce production cost and improve feasibility of the bioprocess, research efforts must be focused on: (1) optimization of oleaginous microorganism applying genetic engineering methods and adaptive evolution to obtain higher lipid concentrations and tolerance to inhibitors from pretreatment process, (2) new effective method of pretreatment and hydrolysis of lignocellulosic biomass that provide high concentration of fermentable sugars in growth media, (3) novel innovative designs of bioreactor should improve the productivity of the process and reduce the production cost, (4) optimization of lipid isolation from wet cell biomass and (5) generation of valued-added products that could provide additional income and improve economic feasibility of the bioprocess (**Figure 3**) .

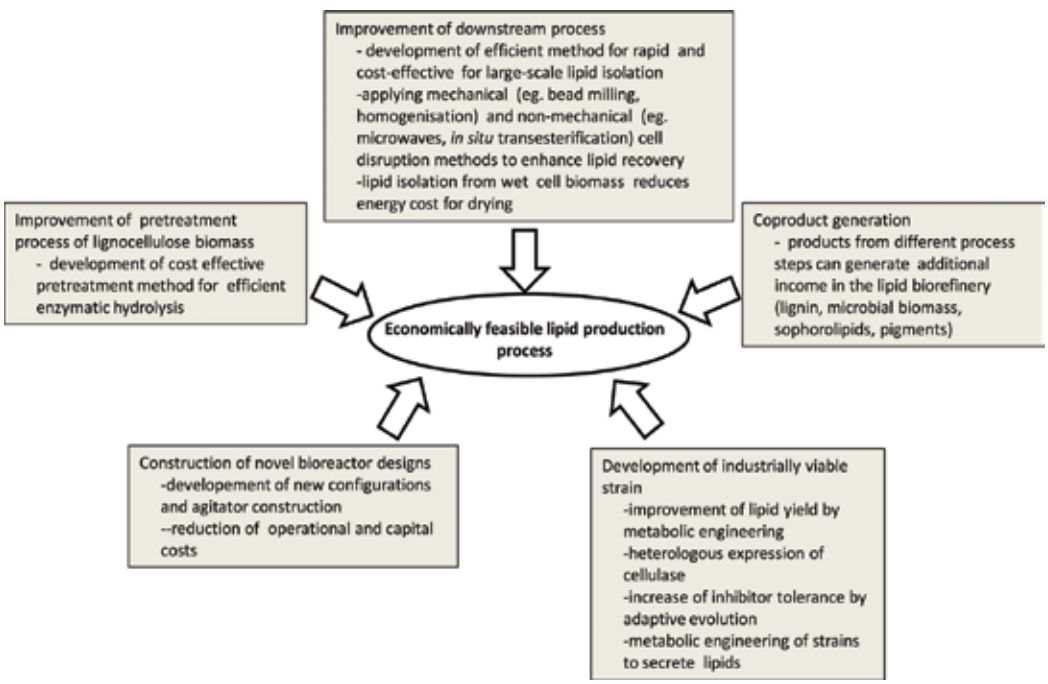

Figure 3. Strategies for improvement of lipid production process.

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Chapter 8

Glycoside Hydrolases in Plant Cell Wall Proteomes: Predicting Functions That Could Be Relevant for Improving Biomass Transformation Processes

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Abstract

Glycoside hydrolases (GHs) are enzymes that are able to rearrange the plant cell wall polysaccharides, being developmental- and stress-regulated. Such proteins are used in enzymatic cocktails for biomass hydrolysis in the second-generation ethanol (E2G) production. In this chapter, we investigate GHs identified in plant cell wall proteomes by predicting their functions through alignment with homologous plant and microorganism sequences and identification of functional domains. Up to now, 49 cell wall GHs were identified in sugarcane and 114 in *Brachypodium distachyon*. We could point at candidate proteins that could be targeted to lower biomass recalcitrance. We more specifically addressed several GHs with predicted cellulase, hemicellulase, and pectinase activities, such as β-xylosidase, α and β-galactosidase, α-N-arabinofuranosidases, and glucan β-glucosidases. These enzymes are among the most used in enzymatic cocktails to deconstruct plant cell walls. As an example, the fungi arabinofuranosidases belonging to the GH51 family, which were also identified in sugarcane and *B. distachyon*, have already been associated to the degradation of hemicellulosic and pectic polysaccharides, through a peculiar mechanism, probably more efficient than other GH families. Future research will benefit from the information available here to design plant varieties with self-disassembly capacity, making the E2G more cost-effective through the use of more efficient enzymes.

Keywords: *Brachypodium distachyon*, cell wall proteomes, glycoside hydrolase (GH), second-generation ethanol, sugarcane

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1. Introduction

The production of second-generation ethanol (E2G) provides an additional source of energy in the sugar and ethanol sector by increasing the biofuel yield without expanding the crop area, thus leading to a sustainable production system. However, for the process to become financially competitive in comparison with first-generation ethanol (E1G), it is necessary to reduce the costs related to the lignocellulosic biomass processing required to recover and break the sugars present in plant cell walls [1]. The principal barrier to the conversion of lignocellulosic biomass into bioethanol or chemicals is the insoluble lignin network that surrounds and shields the cellulose microfibrils from degrading enzymes. The high energy and environmental costs of the treatments necessary to overcome these drawbacks constitute major hurdles to commercial E2G production [2]. To overcome these limitations, many studies have focused on the identification of enzymes related to biomass pretreatment and hydrolysis processes. The majority of the enzymes that compose enzymatic cocktails are proteins prospected from fungi that belong to different glycoside hydrolase (GH) families [3].

The raw material for E2G production is plant fiber, which is mainly composed of cell walls. Nevertheless, less work has been devoted to study plant cell wall proteins (CWPs), and thus, to understand how the plant mechanisms themselves function to loosen and tighten up back the cell wall in order to promote cell growth and adapt to their changing environment. Accordingly, a common opinion today is that it is important to understand how cell walls are built up to improve the biomass deconstruction processes [4, 5].

Plant cells are surrounded by a wall characterized by its specific structure and composition [6]. Cell walls are mainly composed of polysaccharides, lignin, suberin, waxes, proteins, calcium, boron, and water, and have the ability to self-assemble [7]. Plants have two different kinds of cell wall deposition. Primary cell walls are synthesized in still-growing cells, whose form is not definitive, and thus, they can undergo growth and expansion. Secondary cell walls are synthesized in already fully expanded cells which are differentiating to perform specific functions, like xylem and fibers cells. In lignified secondary walls, the proportion of cellulose is higher than in primary cell walls with a higher degree of polymerization and crystallinity specificities [8]. Cellulose, hemicellulose, and pectins are the cell wall polysaccharides, and the biogenesis of cell walls involves their synthesis in intracellular compartments or at the plasma membrane, secretion, assembly, and rearrangement *in muro*. The primary cell walls of grasses have specific characteristics, and they are called type II cell walls [9]. They have a low content in pectins and xyloglucans, but a high content in mixed linked β-D-glucan [also named (1 → 3, 1 → 4)-β-D-glucans] during growth and in glucuronoarabinoxylans (GAXs). They also present ferulate and p-coumarate esters formed by attachment to the arabinosyl units of GAXs that are absent in gymnosperms, dicots, and other monocots. It has been assumed that plants devote more than 10% of their genome to the biogenesis of cell walls [10]. The cell wall is a dynamic structure involved in several physiological processes such as: cell growth [11], defense against pathogens [12], or signaling [13]. In sugarcane, the cell wall also plays a key role in the distribution of sucrose [14].

Displaying roles in cell growth, enzymes are part of the cell wall proteome. Glycosidases and glycanases have exo- and endo-GH activities, respectively, while trans-glycosidases and trans-glycanases perform exo- and endo-transglycosylation, respectively. Pectin methylesterases

and pectin acetylesterases control the degree of homogalacturonan methylesterification and acetylation, respectively [15]. Class III peroxidases (Prxs) can either form covalent bonds by oxidizing aromatic compounds such as monolignols or aromatic amino acids or produce reactive oxygen species that participate in non-enzymatic breakage of covalent bonds of polysaccharides [16]. All these proteins belong to multigene families and their genes are differentially regulated during plant development and in response to environmental changes.

GHs are of special interest, since they can hydrolyze the glycosidic bonds from two carbohydrates or from a carbohydrate and a non-carbohydrate moiety, thus actively contributing to cell wall polymer rearrangements. In *Arabidopsis thaliana*, about 379 GHs have been identified, belonging to 29 different families, among which approximately 52% were predicted to be cell wall GHs [17]. A great number of plant cell wall GH families have been identified so far in cell wall proteomes (see [18, 19]). Associations between structure and function can be predicted to point to candidate genes prone to manipulation. Among others, GHs comprise β-glucosidases, β-galactosidases, β-mannosidases, β-glucuronidases, β-xylosidases, β-D-fucosidases, exo-β-1,4-glucanases, lactases, β-glycosidases, α-L-arabinofuranosidases, glucan 1,3- and 1,4-β-glucosidases, β-amylases (for a complete repertoire, see [20]).

In this chapter, we will describe cell wall GHs identified in the cell wall proteomes of sugarcane and *Brachypodium distachyon*, two monocots from the grass family. Sugarcane is already largely used for E1G production and could be used for E2G production [21], whereas *B. distachyon* is a model plant amenable for genetic transformation [22]. Up to now, there were 49 and 114 GHs identified in sugarcane and *B. distachyon* cell wall proteomes, respectively (see [18]). Based on their amino acid sequences, we have made bioinformatic predictions of functional domains and phylogenic analyses. GH families possibly relevant for improving biomass transformation processes to E2G are highlighted.

2. Plant GH families

In plants, several strategies have been used in order to extract and identify CWPs with a high number of GHs, such as vacuum-infiltration protocol with saline solution and identification of proteins predicted by bioinformatics to be targeted to the secretory pathway [23–25]. In *A. thaliana*, around 200 GHs, belonging to 13 families, are assumed to be involved in polysaccharide modification and cell wall reorganization [8].

Conversely, in the monocot rice, GH17 is the one that presents the highest number of members, followed by GH28 [26]. The cell wall particularities of dicots (e.g., *A. thaliana*) vs. monocots, among which grasses, are also reflected in the distribution of GH families. For example, the lower proportion of pectins in monocot cell walls relates to a lower number of polygalacturonases (GH28) [17]. Thus, the range of GH families depends on plant species, and each of them has to be studied separately.

By bioinformatic analyses of amino acid sequences, it is possible to classify newly identified GHs into families. We have done it for the GHs identified in the sugarcane and the *B. distachyon* cell wall proteomes. However, assigning a GH to its family as defined in the CAZy database [20] does not necessarily provide a clear picture of its function, since proteins from a given GH

family can display different roles. For example, GH1 family members can be involved in cell wall metabolism, lignification, signaling, or defense [17]. Phylogenic analyses can help getting more information regarding the functions of specific plant GHs. Since monocots could be a major source of raw material for E2G production, it is interesting to study their cell wall metabolism and outline possible strategies to increase biomass production or lower cell wall recalcitrance to deconstruction [27]. In addition, it might be possible to find interesting analogies by comparing the plant cell wall assembly/disassembly mechanisms to those of microorganisms.

3. GHs identified in sugarcane and *B. distachyon* cell wall proteomes

In sugarcane, 49 GHs have been identified in cell wall proteomes [28, 29]. They are distributed in 16 GH families. The GH3 family is the best represented (~20%), followed by GH17 (~16%), GH18 (~12%), and GH1 (~8%) (**Figure 1**). This distribution varies according to organ and developmental stage. In cell suspension cultures, only 4 GH families were identified among which GH3 was the most populated [28]. In 2-month-old stems, 7 GH families were found, GH3 also being the most represented [29]. Leaves recovered few GHs, from families 19, 27 (young leaves only), 28, and 31 (young leaves only). Apical internodes mainly contained GH3 members, whereas mostly GH17 members were found in basal ones [30]. Noteworthy, it should be mentioned that the absence of some GH families in a given cell wall proteomes could be due to technical limitations or differential accessibility as a consequence of differences in cell wall structure.

In *B. distachyon*, 114 CW GHs were identified in cell wall proteomes [31–34]. They are distributed into 21 families. The most populated one was GH17 (~17%), followed by GH28 (~13%), GH1 (~9%), GH3 (~8%), and GH35 (~8%) (**Figure 1**). GH28, followed by GH1, GH3, and GH16, had the highest number of members in young leaves. In mature leaves, GH17, GH18, and GH28 were the most represented. In internodes, they were GH28 and GH17. In seedlings and seeds, GH17 was the most populated family.

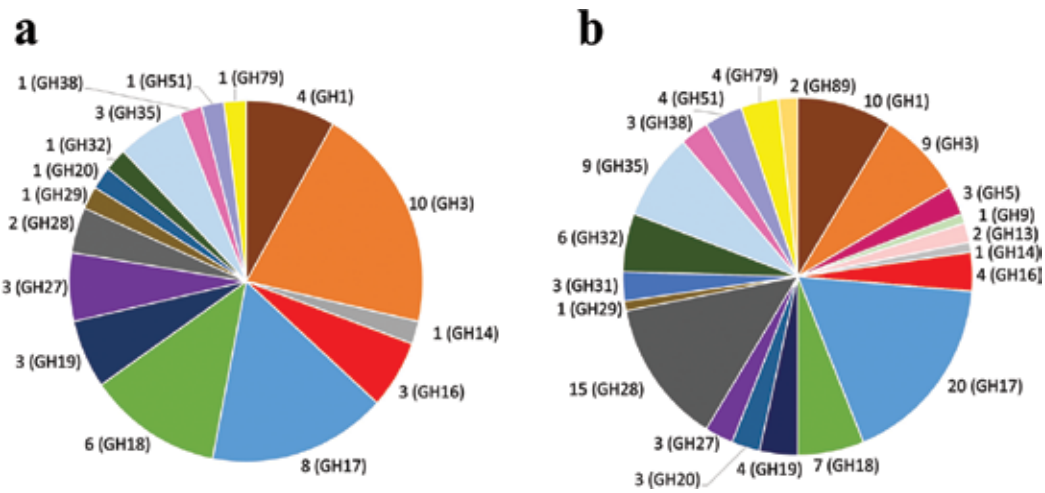

Figure 1. GH family distribution of presently known cell wall proteomes in sugarcane (a) and *B. distachyon* (b). The number of family members identified in each of them is indicated. Same colors indicate same GH families in both species.

The large size of the GH1, GH17, and GH28 families is probably linked to their roles in the assembly and in the rearrangement of cell wall polysaccharides [17]. Usually the GH1, GH16, GH17, and GH35 families are less represented in dicots than in monocots [31]. GH17 display glucan-1,3-β-glucosidase activity and possible substrates could be mixed (1,3)(1,4)-β-D-glucans [8]. This is consistent with the fact that only type II grass cell walls present this kind of hemicellulose.

After a survey of the cell wall proteomes described so far and collecting information regarding microorganism enzymes used for biomass deconstruction, we decided to focus this review on the GH1, GH3, GH17, GH27, GH35, and GH51 families. We have predicted functional and structural domains in newly identified CWPs using the PredictProtein bioinformatic software and grouping them in families [35]. Since plant cells perform cell expansion themselves by involving cell wall polysaccharide rearrangements, the plant mechanisms could be mimicked by the enzymes used in cocktails. The comparison of plant and microorganisms enzymes presently used for biomass hydrolysis could contribute to determining their common characteristics and which specificities of plant enzymes could be copied in order to improve industrial cell wall deconstruction processes. Conversely, this comparative study could help in identifying which of the characteristics of microorganism enzymes could be engineered in plant species in order to obtain biomass with less recalcitrant cell walls.

4. GH1, GH3, and GH51

The GH1 family mainly comprises β-glucosidases, which are found in several organisms performing different functions. In plants, they are involved in cell wall catabolism, signaling, lignification, defense, symbiosis, and secondary metabolism. Putative β-glucosidase genes have been shown to be induced by biotic and abiotic stresses and they were considered critical for the success of plant development in stressful environments [36–40]. Accordingly, plants are the organisms that have the highest number of GH1s, e.g., 48 in *A. thaliana* [41] and 40 in *Oryza sativa* [42]. Over the years, various β-glucosidases hydrolyzing cell wall oligosaccharides have been characterized mainly in monocots, such as in germinating seedlings of barley where they show preference for manno-oligosaccharides in endosperm cell walls [43], and in rice seedlings, where they hydrolyze different oligosaccharides [42]. Extracellular β-glucosidases can also contribute to the production of toxic compounds, such as hydroxamic acids and cyanide [44–46]. For this process to occur correctly, the defense molecules are stored in non-active glucosylated forms in the vacuole, while the β-glucosidases are stored in the apoplast or in protein bodies in dicots or in plastids in monocots. The enzyme and its substrate get into contact when the cell is damaged during plant-microorganism interaction.

The plant cell wall is a large polysaccharide repository that contains a large amount of glucosyl residues. β-glucosidases play important roles in cell wall formation and plant development, because they participate in cell wall polysaccharide turnover [47]. In sugarcane [30] and *B. distachyon* [31], more GH1 were found in cell wall proteomes of growing organs, such as young leaves and apical internodes, than in mature organs. In addition, as suggested by bioinformatic predictions, several of the identified GH1 (e.g., SCCCCL3001B10.b, SCJFLR1017E03, SCEQLB1066E08, Bradi1g10940, Bradi1g10930, Bradi1g10940, and Bradi2g59650) have a β-glucosidase activity (GO:0008422).

Ten GH3s have been identified in sugarcane [28–30] and nine GH3s have been identified in *B. distachyon* [31, 32, 34]. Half of the sugarcane GH3 are predicted to have a β-glucosidase activity (GO:0008422) (e.g., SCEZLB1007A09, SCEQLR1093F09, and SCQSLR1089A04). However, some GH3 are predicted to have xylosidase (e.g., Bradi5g23470) or α-L-arabinofuranosidase (AFase) activity (e.g., SCCCCL4009F05, SCCCSB1003H06, and Bradi3g59020).

The fungi β-glucosidases can degrade cellulose together with other kinds of enzymes, like endoglucanases and cellobiohydrolases. They separate the molecules of glucose from cellobiose, thus being used in enzymatic cocktails to produce cellulosic bioethanol [48]. In barley, the structure of the GH3 β-D-glucan exohydrolase ExoI was determined through X-ray crystallography, showing a two-domain globular structure being different from that of GH1 [49]. Besides the catalytic site, this enzyme has another binding site for (1 → 3, 1 → 4)-β-D-glucans only identified in monocots. Xylan 1,4-β-D-xylosidases hydrolyze xylose from xylo-oligosaccharides. These enzymes have several uses, such as in the industrial processes related to bread dough, animal feed digestibility, and deinking of recycled papers. In enzymatic cocktails, they are considered the most efficient enzymes to break glycosidic bonds of hemicelluloses [50]. The few GH51 members identified in sugarcane and *B. distachyon* were also assumed to be AFases (EC 3.2.1.55, GO:0046556). AFases catalyze the hydrolysis of α-L-arabinofuranoside in α-L-arabinosides. They act together with hemicellulases and pectinolytic enzymes to achieve hemicellulose and pectin hydrolysis. Several AFases used commercially belong to the GH51 family, generally originating from fungi. Such enzymes are of special interest for monocots

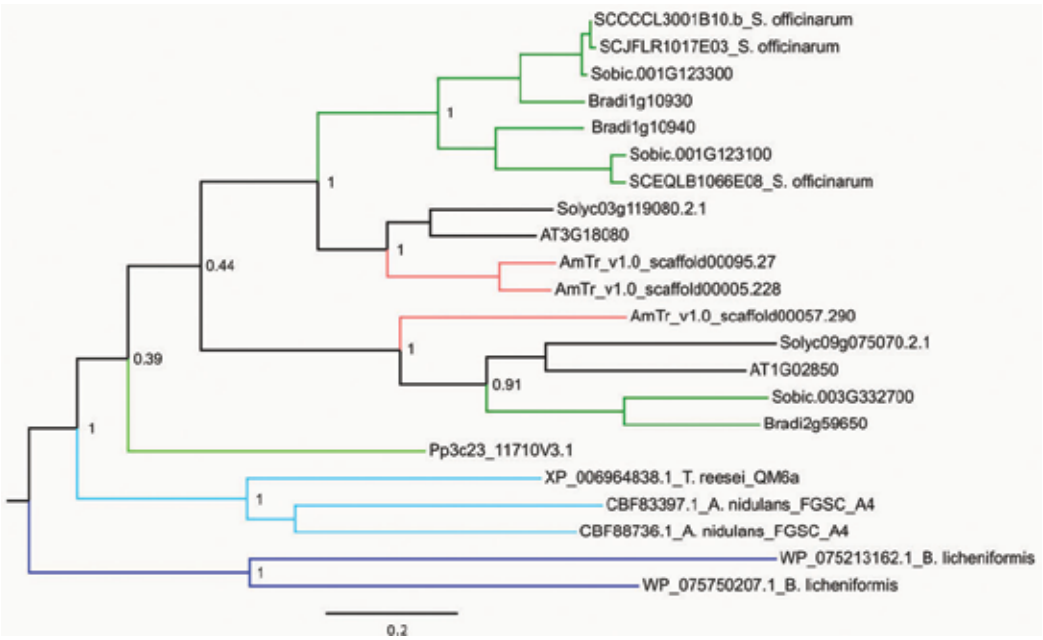

Figure 2. Phylogenic tree of the GH1 subfamily comprising the GH1 identified in sugarcane and *B. distachyon* cell wall proteomes. All the plant sequences have been retrieved from Phytozome v12.1 (phytozome.jgi.doe.gov), whereas the microorganism sequences originate from the NCBI website (www.ncbi.nlm.nih.gov/protein/). The MEGA6 software (www.megasoftware.net) was used to generate the tree with the following options: MUSCLE for amino acid sequence alignments and maximum likelihood tree with 500 bootstraps. Visualization was done with FigTree (tree.bio.ed.ac.uk/software/figtree/).

biomass hydrolysis, since this material is particularly rich in arabinoxylans, which need to be degraded with AFases in addition to endoglucanases [51].

To compare plant and microorganism GH1 and GH3, we have performed phylogenic analyses. Some GH1 and GH3 identified in cell wall proteomes of sugarcane and *B. distachyon* have been selected. For plants, we have chosen species at critical evolutionary nodes since terrestrialization like a moss (*Physcomitrella patens*), the common ancestor to higher plants (*Amborella trichopoda*), *Sorghum bicolor* as the closest plant to sugarcane having a sequenced genome, and two dicots (*Lycopersicon esculentum* and *A. thaliana*). The *B. distachyon* and sugarcane sequences of several GHs identified in cell wall proteomes have been included in this analysis. Regarding microorganisms, we have retained GHs usually used in enzymatic cocktails for cell wall deconstruction. They belong to bacteria like *Bacillus licheniformis*, or to fungi like *Aspergillus nidulans* or *Trichoderma reesei* [52, 53]. As expected, the selected GH1 and GH3 share common ancestors. The two *B. licheniformis* GH1 root the GH1 tree (**Figure 2**). *A. nidulans* and *T. reesei* GH1 come next, prior to the *P. patens* GH1.

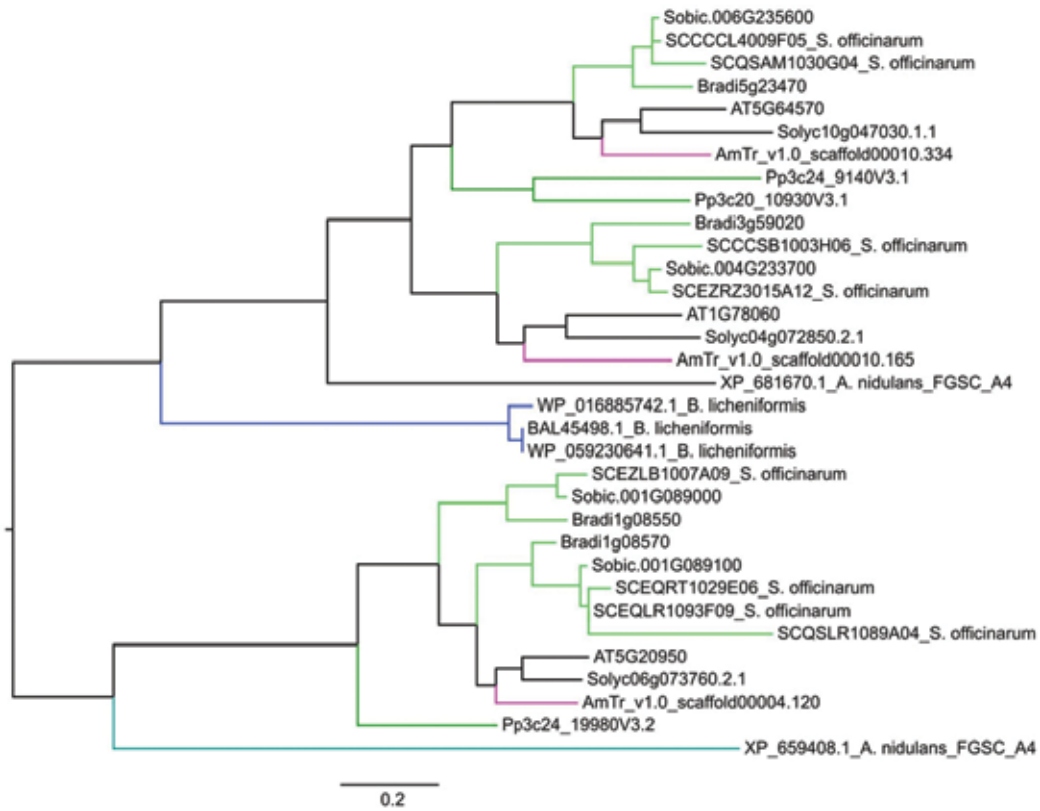

Figure 3. Phylogenic tree of the GH3 subfamily comprising the GH3 identified in sugarcane and *B. distachyon* cell wall proteomes. All the plant sequences have been retrieved from Phytozome v12.1 (phytozome.jgi.doe.gov), whereas the microorganism sequences originate from the NCBI website (www.ncbi.nlm.nih.gov/protein/). The MEGA6 software (www.megasoftware.net) was used to generate the tree with the following options: MUSCLE for amino acid sequence alignments and maximum likelihood tree with 500 bootstraps. Visualization was done with FigTree (tree.bio.ed.ac.uk/software/figtree/).

Finally, the tree is split into two distinct clades, each containing either one or two closely related *A. trichopoda* GH1. Monocot and dicot GH1 are finally separated in sub-clades. Regarding the GH3, the situation is more complex (**Figure 3**). Two clades are separated at the basis of the tree: clade A is rooted by three *B. licheniformis* GH3, followed by an *A. nidulans* GH3, whereas clade B is only rooted by an *A. nidulans* GH3. As for the GH1 tree, each sub-clade comprises an *A. trichopoda* GH3, whereas monocot and dicot GH3 form distinct groups. Similar results could be obtained for the other GH families. This phylogenic analysis shows the close relationships between microorganism and plant GH1 or GH3. Additional work is required to define precisely their specificities with the aim of generating new tools for industrial processes of biomass deconstruction.

5. GH 17

GH17 are encoded by large gene families in plants. In *O. sativa*, GH17 is the largest GH family [17]. In *A. thaliana* and *Populus trichocarpa*, it comprises 50 and 100 members, respectively [54]. The GH3 family includes β-1,3-glucanase (glucan endo-1,3-β-D-glucosidase, E.C 3.2.1.39), glucan 1,3-β-glucosidase (E.C 3.2.1.58), licheninase (EC 3.2.1.73), glucan 1,4-β-glucosidase (EC 3.2.1.74) activities (enzyme.expasy.org/). All the GH17 found in sugarcane and *B. distachyon* cell wall proteomes are predicted to have a glucan endo-1,3-β-D-glucosidase activity (GO:0042973).

β-1,3-glucanases have been shown to be important proteins involved in plant defense reactions against pathogens and are considered as pathogenesis-related proteins of the PR-2 family [55]. Their role is hydrolysis of the β-1,3-glucan bonds, an important structural component of fungal cell walls, resulting in their destabilization and in the release of elicitors that further stimulate defense responses [55]. This antifungal activity was shown both *in vitro* [56] and *in vivo* [57]. In sugarcane, GH17 SCQSRT2031D12 identified in basal internodes was considered similar to the *A. thaliana* At4g16260 β-1,3-endoglucanase that has been associated with increased resistance to pathogen attack [58]. Noteworthy, β-1,3-glucanases can accumulate in vacuoles of root cells or mature leaf cells in response to pathogen infection, whereas others are secreted to the extracellular space, but they can also be secreted in the absence of pathogen infection [55]. They are, thus, also important during plant development, being involved in cell division, pollen development, seed germination, and maturation as well as in signaling [55, 59, 60].

According to phylogenic analyses, the GH17 family is divided into three distinct clades (denoted α, β, and γ) [61, 62], with 10% of its members having cell wall-related functions [61]. GH17 of the α clade are more related to stem elongation, but also responsive to gibberellin, those of the β and γ clades are more related to stress response and defense against pathogens [62–65]. In addition to the GH17 domain *per se*, proteins of the GH17 family comprise other domains as shown by [61] studying the β-1,3-glucanase sequences of *A. thaliana.* They noted that all the sequences had a predicted N-terminal signal peptide linking them to the secretory pathway. Half of them had a C-terminal extension, being first classified as an X8 domain [66]. Previously, the X8 domain was identified as the cellulose binding module 43 (CBM43) responsible for the interaction with β-1,3-glucans [67]. The other GH17 had either a C-terminal glycosylphosphatidylinositol (GPI)-anchor [66, 68] or a vacuolar targeting peptide [55]. The absence or gain of these domains could be related to ancestral traits. All the γ clade members and more than half of the α clade members retained the CBM43 domain, whereas all the members of the

β clade lost it through evolution. It is thought that the loss of this domain facilitates the extracellular secretion induced by biotic stresses, thus improving the response to pathogens [61, 62].

Other studies also revealed the antifungal effects of plant extracellular chitinases (GH18 and GH19) in combination with those of GH17 [69]. Indeed, fungi cell walls are composed of chitin and of branched β-(1,3):β-(1,6) glucans [57, 70–73]. Thereby, transgenic plants overexpressing a chitinase and/or a ß-l,3 glucanase became less susceptible to fungal attack [74, 75].

6. GH27 and GH35

The GH27 identified in cell wall proteomes of both sugarcane and *B. distachyon* was predicted to have α-galactosidase activity (EC 3.2.1.22, GO:0004557). α-galactosidases break galactosidic linkages in galactose-containing oligosaccharides, galactolipids, and galactomannans [76]. Since galactomannans are hemicelluloses, α-galactosidases could be used in enzymatic cocktails to enhance the cell wall hydrolysis process by acting as a hemicellulase.

GH35 are mainly β-galactosidases (EC 3.2.1.23), but exo-1,4-β-D-glucosaminidase (E.C 3.2.1.165) and exo-β-1,4-galactanases (EC 3.2.1.-) also belong to this family. β-galactosidases are found in microorganisms such as bacteria, fungi, and yeast, as well as in animals and plants [77]. They catalyze the hydrolysis of terminal non-reducing β-D-galactose residues in different molecules, like glycoproteins, oligosaccharides, glycolipids and lactose (www.cazy.org). β -galactosidases are classified in two families: GH2 are predominantly found in microorganisms (around 70%), and GH35 are found in plants [78, 79].

GH35 can be distributed into two main groups according to their preferred substrates: hydrolysis of pectic β-1,4-galactans, cleavage of β-1,3- and β-1,6-galactosyl linkages of *O*-glycans of arabinogalactan proteins [80]. In plants, they are associated with secondary metabolism or polysaccharide degradation, performing important roles in physiological events, including cell wall degradation and expansion during plant development, and turnover of signaling molecules [79–83]. They were also shown to be involved in ripening and abscission of mango, papaya, and orange fruits [84–86]. The GH35 found in the cell wall proteomes of sugarcane [30] and *B. distachyon* [31] is predicted to have a β-galactosidase activity (GO:0004565). In *B. distachyon*, they were only identified in leaves and in seedlings, whereas they were mostly found in sugarcane internodes. GH14 are very close to GH35 due to sequence similarity, perhaps playing similar roles, and they have only been identified in sugarcane internodes. Interestingly, the SCUTAM2089E05 GH14 was found to be differentially expressed in ancestral genotypes of sugarcane showing differential carbon allocation to lignin or sucrose [87].

7. Concluding remarks

Microorganisms use an arsenal of GHs to degrade plant cell walls, in order to establish themselves in their host. Similar mechanisms are thought to be used in their own plant cell wall modification, since plant cell walls embrace several types of carbohydrates with a variety of structures and biological functions. For sugarcane biomass deconstruction, the first step

proposed is the use of pectinases to release pectins, such as endopolygalacturonases, AFases, and β-galactosidases, along with pectin methylesterases. Lichenases are used to hydrolyze mixed linked β-D-glucan. The remaining polymers, cellulose, and hemicelluloses, would have to be treated with a mixture of enzymes like endo-β-xylanases, α-arabinofuranosidases, xyloglucanases, α-xylosidases, and β-galactosidases. Finally, cellulose could be the substrate of endo-β-glucanases, cellobiohydrolases, and β-glucosidases [88].

Besides many studies focusing on microorganism enzymes to optimize E2G production, this work has evaluated the plant enzymes that are assumed to display similar activities. Since plant GHs perform cell wall breakage and expansion, a deeper investigation of their structure could be performed in order to produce more efficient chimeric enzymes to be used in enzymatic cocktails. It is difficult to establish GH functions from their amino acid sequences because proteins from the same GH family may have diverse substrates and roles [8]. However, we were able to predict functions for the GHs identified in the cell wall proteomes of sugarcane and *B. distachyon*. Thus, the mentioned GH1, some GH3 and GH17 were predicted to have a β-glucosidase activity. Other GH3 had possible β-xylosidase and AFase activities, the latter also predicted for GH51. The GH27 and GH35 families were predicted to have α- and β-galactosidase activity, respectively. Nevertheless, it is crucial to mention that in order to precisely characterize the function of a given protein, one should perform biochemical analyses, involving purification, characterization of substrates, as well as genetic studies on mutants in well-characterized model plants.

Therefore, this work has contributed to provide target proteins that could possibly be used in future research to facilitate cheaper E2G production, besides allowing a more detailed analysis of the cell wall proteomes of the grasses, sugarcane and *B. distachyon*.

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Chapter 9

Biogas Production Plants: A Methodological Approach for Occupational Health and Safety Improvement

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Additional information is available at the end of the chapter

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Abstract

Existing lessons on public safety, referred to as new biotech plants, suggest that the development of effective, responsive and responsible safety standard can improve the trust of the public in the new generation plants such as biorefineries. This implies the need for specific risk assessment aimed at defining the mitigation measures, which can minimize the impact of hazards on workers' health. The main hazards, referred to biogas production process, are biohazard, fires and potentially explosive atmospheres. In particular, the last two hazards strictly depend on the presence of methane in the biofuel. This chapter presents the results of a work aimed at providing the biogas industry with a practical tool, which can be used to carry out the analysis of hazards of biogas plants. The adopted method for developing the tool is based on the well-known checklist approach. The checklist is a valuable support for the plant operator to evaluate periodically the actual effectiveness of the overall safety measures and ensure a safer management of the biogas plant. The checklist can meet these requirements. This chapter reports the main preventive, protective and managerial measures, which can be adopted to decrease the hazardous outcomes on workers' health and safety.

Keywords: biogas, biohazard, potentially explosive atmospheres, fire, checklist

1. Introduction

The renewable energy sources, that have been well developed in Italy, include biogas, which is mainly obtained from the anaerobic digestion (AD) of agricultural and livestock biomasses. Italy is the second biogas producer in Europe, after Germany, and by the end of 2015, about 1555 biogas plants were operating, of which 77% were powered by agricultural matrices [1]. Biomethane production from biowaste is also an important contributor to reach the objectives established by the European Directive 2009/28/EC on renewable energies [2]. The Italian

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decree on incentives for producing biomethane opens new development perspectives for renewable energies from biowaste, as this biofuel could be used in vehicles as a substitute for fossil fuel and replace the natural gas dependence in domestic, commercial as well as industrial consumptions [3]. Most of the biogas production plants are of small or medium size, therefore, falling below the thresholds for the application of legislation aimed at the control of major accidents, as the Seveso Directive [4]. In Italy, in 2010, there were approximately 100,000 workers in green industries, and it is expected that the number will reach 250,000 units in 2020 and most of them will be involved in bioenergy industry. Green jobs are activities characterized by previously evaluated risks but with a different scope and exposition in connection with newly applied technology [5]. However, it is important to complete an evaluation process with respect to new or re-emergent risks in the biorefineries [6]. The transformation of such plants, from only agricultural and livestock to energy production, introduced a different risk profile for the operators. This aspect is generally underestimated for occupational health and safety (OHS) management, and the large number of plants maintain the same individual risk evaluation that they followed for the operators before transforming the plant. A European database of accidents (mainly explosions), related to biogas production, has been recently created and data on about 170 accidents have been collected from different literature sources [7]. It is necessary to integrate the OHS issues at an early stage of development of the industrial process [8]. The principal reason behind any OHS risk assessment activity is to undertake a proactive and systematic analysis of health hazards in the workplace in order to appropriate the control measures. The management of OHS must be in accordance with the general principles, which should be applied to control workplace hazards in order to:

(a) eliminate the risks;

(b) assess the risks, which cannot be avoided;

(c) reduce the risk at source;

(d) give priority to collective protective measures over individual protective measures;

(e) adapt the work to the individual, especially with regard to the design of workplaces and the choice of work equipment and production methods;

(f) adapt working methods to technical progress;

(g) develop a coherent overall prevention policy, which covers technology and work organization and

(h) give appropriate instructions to employees.

Even though biogas plants are considered quite simple in installation, they feature a variety of items. As there are many feedstock types, which are suitable to anaerobic digestion (AD) in the biogas plants (biomass from dedicated crops, vegetable waste, sludge, residues of livestock farming such as manure or slurry, organic fraction of municipal solid waste, etc.), there are various techniques for treating these feedstock types and different digester constructions and systems of operation. This implies the need to carry out a specific risk assessment in order to define risk prevention and mitigation measures aimed at minimizing the impact of biohazard on workers' health. In the biogas production supply chain, various work-linked

risks can be identified such as explosion, fire and biological risks. In connection with used materials, including vegetables, food production residuals and animal biomasses, as well as with the properties of fermentation, biological risk deserves particular attention. Fermentation biomass is rich in microorganisms, including pathogens and opportunistic pathogens, and anaerobic processes could lead to the selection of microbial flora, which can promote the presence of anaerobic microorganisms, for example, *Clostridia*, that initially are less represented [9]. Epidemiological data in the field of workers' exposure to the organic dust showed specific occupational diseases, such as respiratory tract disorders (airways inflammation, bronchitis, asthma); gastrointestinal problems (from nausea to diarrhoea) and skin, eyes, nose and airways allergic reactions. In the 1990s, for example, it was found that gastrointestinal diseases were more common among workers of refuse-derived fuel plants [10]. This chapter presents the results of a work aimed at providing the biogas industry with a practical tool, which is able to protect its workers. Biological contamination, fire and potentially explosive atmospheres are the main hazards referred to the biogas production. On this subject, it is essential to take into account that the typical culture of farming is far enough from industrial approach and therefore it requires clear and useful tools, which are able to address both elements—maintenance and operation. The work has allowed to define technical and organizational measures aimed at preventing and mitigating the hazards. From this analysis, a structured safety checklist has been derived. This checklist is a valuable support for the plant operator to evaluate periodically the actual effectiveness of the overall safety measures and to ensure a safer management of the biogas plant.

2. Material and methods

The adopted method for developing the required tool is based on the well-known checklist approach. At the beginning, the initial events (biological contamination, fire and explosion), which could cause an adverse effect on workers (injuries or diseases), were identified. The next step was focused on identification of measures aimed at preventing the workers from getting affected by a potential 'initial event'. In succession, protection measures were subsequently identified to reduce the 'dose', which is received by the worker exposed to the initial event. The event mitigation was aimed at:

- minimizing the amount of hazardous agent;
- protecting workers from hazardous phenomenon and
- minimizing the duration of exposure.

The 'safety checklist' has been derived from organizational and procedural measures and technical systems. The discrimination of protective and preventive measures are highly valuable to define the safety devices' importance; assess and monitor safety levels and take adequate decisions about training, maintenance schedule and safety investments. The checklist has been divided into three sections referred to as biohazard, fire and explosion risk. Each section reports preventive, protective and managerial measures. The checklist has to be considered as a very important tool aimed at evaluating the actual efficiency of safety measures.

3. Biohazard

Biohazard in the production of biogas may be related to feedstock and digestate. Wastes of animal and human origin contain various pathogenic bacteria (e.g. *Salmonella*, *Enterobacter*, *Clostridia*, *Listeria*), parasites (e.g. *Ascaris*, *Trichostrongylidae*, *Coccidae*), fungi, viruses [11, 12] and could represent an occupational biohazard. In the biogas production from co-digestion of animal manure and biogenic wastes, the microbiological quality of raw materials of animal origin is guaranteed only through the application of specific veterinary and sanitary measures (e.g. control of livestock health, hygiene control of raw materials entering the digester). High-risk biomasses such as those from sick animals must be excluded from use; for biomass categories such as slaughterhouse residues, pre-sanitation measures are required through pasteurization or sterilization as stipulated by European Regulation EC 1069/2009 [13]. In case of feedstock categories, which do not require separate pre-sanitation, the combination of AD process temperature and a minimum guaranteed retention time provides an effective pathogen reduction/inactivation in the digestate [14]. In Italy, the digestate quality standard is monitored by several checkpoints [15]. In a biogas plant, exposure levels to biological agents are highly dependent on site activities and tasks undertaken by workers. It is the site operator's responsibility to identify potential hazards, carry out suitable risk assessments and provide adequate protection to their workforce to control such risks. During AD, the microbial reactions take place inside the digester under containment conditions and, therefore, there is no workers' exposure. However, activities such as inoculation, sampling and harvesting the microbial flora during the monitoring of the fermentation process, could involve worker exposure and, therefore, the workers' activities should be checked to define the exposure characteristics. According to European classification, the microorganisms with infection potential, which take part in the anaerobic fermentation process, are mainly assigned to the risk group 1 and to a small extent to the risk group 2 [16]. Some of these microorganisms should be considered opportunistic agents, which do not cause any infections in healthy employees, but they can lead to diseases when body defences are defective. In general, good work practices and simple but effective personal hygiene measures are sufficient to prevent workers from infection risk, including provision of adequate hand-washing facilities. Biological risk assessment should take into account that specific activities, such as biomass reception, temporary storage, biomass handling, digestion drainage and maintenance work, may pose exposure risks to organic dust, bioaerosol and biological components conveyed such as particulates (i.e. bacterial endotoxins, fungal spores). Evidence from epidemiological data shows that these biological agents can cause allergic reactions such as hypersensitivity pneumonitis, allergic rhinitis, some types of asthma and organic dust toxic syndrome (ODTS) [17]. In Italy, the biogas industry expansion is quite recent, and there are not many data available on biological risk in these plants. Recent findings on airborne workers' exposure in two full-scale plants of anaerobic digestion in North Italy showed different biological contamination levels in relation to the involved biomasses (silage, vegetable waste, animal slurry and biomass from dedicated crops) and to the technological and building characteristics [18]. This evidence suggests that every biogas plant requires a specific approach. Contamination and occupational risk must be evaluated individually for each plant, because numerous variables influence risk magnitude, with particular regard to digested sludge treatments, such as input biomass nature, storage,

movement conditions, building configuration and technological processes [18]. The results of the air microbiological monitoring, performed during the biomass movement in some biogas plants investigated in Italy, showed that organic dust (PM_{10}) and its endotoxin content are limited [18] and widely below the occupational safe guidelines [19, 20]. The particulate is not a relevant risk for workers in the plants monitored, because it reached rural environmental levels recorded in North Italy [18].

3.1. Biological risk assessment

The assessment of biological risks is seriously hampered, since neither universally approved criteria for assessing exposure to biological agents nor agreed dose-response estimates and occupational exposure limits (OELs) are yet available. Lack of a standardized sampling methodology has made it difficult to compare data derived from different studies and relate exposure levels to effects on health. Potential seasonal variation of microbial exposures also adds difficulties in comparing data. Establishing the prevalence and incidence rates of diseases related to exposure to biological agents is not easy: data on occupational diseases from biological agents are difficult to collect, because the infections could often be in subclinical form, with atypical incubation periods and/or transmission routes [21]. Moreover, the exact role which, is played by biological agents in the development or aggravation of symptoms and diseases, is only poorly understood. Human response to exposure to biological agents depends on the organic material involved and individual's susceptibility to infections and allergies. In addition, microorganisms constantly interact with the environment and are able to modify their pattern of gene expression rapidly in response to the environmental signals [21]. A variable human response has also been described, following the exposure to organic dust in different workplace settings, and it was shown that the composition of the dust may play an important role in determining the potency [22]. The assessment of biological risk in the biogas sector is a complex task, even considering that the biogas industry is still in its infancy in some countries such as Italy. Limited public domain information is also available from ongoing health and injury surveillance of biogas workers, particularly for health outcomes of highest concern (e.g. respiratory, irritation, sensitization). There is a need for improving the collection of work-related diseases in the biogas sector, and an ad-hoc accident reporting system should be created.

The proposed approach for biological risk assessment is that certain areas or activities, resulting from the biogas industry, could be categorized using fairly simple descriptive expressions of risk and a corresponding set of control measures, which depend on the perceived risk associated with the area or the activity. The qualitative checklist approach can represent a reasonable tool in order to overcome the current knowledge gaps in establishing agreed monitoring protocols and developing reliable dose-response data. In absence of such information, the potential risk should be managed in a precautionary manner. Exposure levels to biological agents are highly dependent on site activities and tasks undertaken by workers, and an adequate workers' protection requires a detailed site and task risk assessment. Potential exposure can be controlled by changing the work process to minimize the generation of bioaerosols or dustiness. In order to achieve compliance, employers should demonstrate that adequate control measures have been developed in accordance with the hierarchy of controls, detailed

in the Directive 2000/54/EC [16]. Examples of control measures are exhaust ventilation to prevent exposure, adequate filters on the air intakes of vehicles (such as tractors used to move biomass) and personal protective equipment, such as suitably fitted respiratory devices, when working in areas close to where bioaerosols are generated.

3.2. Prevention and protection measures for occupational biohazard

Design of workplaces and work processes, the choice of adequate equipment and working methods allow the control of occupational biohazard in the biogas plants. Any activities involving the movement of biomass and/or waste should be controlled, and site design and activities should be managed to avoid organic dust and/or bioaerosol release in the workplace. In particular, the biomass, such as silage, should be stored in closed silos or in platforms provided with containment walls and covered by a plastic material wrap. Livestock slurry storage tank should be equipped with immersion agitators to avoid air contamination, and moreover, the automatic transfer of slurry into the digester should be guaranteed by a pumping system. Working areas, where biomass is moved, should be considered as potential high exposure zones. An efficient system of forced ventilation is required if high-exposure activities are conducted within a confined space and, where practicable, employees should only work in these areas within a suitably controlled environment, such as a vehicle cab, or wear appropriate respiratory protective equipment (RPE). It is recommended that for exposure to bioaerosols, RPE is provided with the highest efficiency filters (P3). The replacement of the filters in the vehicle cabs' air handling system, cleaning of vehicle cabs and the instructions given to operators not to open cab doors and windows and remain in the vehicle have a significant effect on workers' exposure levels. These rules should be applied within a radius of 50 m from the operational areas, considering that bioaerosol levels typically return to background concentrations within this distance [23]. Such requirements clearly have an impact on site design and layout. In order to achieve these targets, the employers should amend working practices and operations and relocate office accommodation and welfare facilities to an area outside the potentially high-exposure zones. Dust control from the movement of vehicles is also recommended, and roadways should be properly constructed so that they can be cleaned and a vehicle wheels washing system should be planned. The workplace should be provided with adequate hand-washing and shower facilities and 'clean areas' in order to ensure that no contamination can affect external places. Employers should undertake an appropriate health surveillance of their workforce to ensure that early signs and symptoms of diseases, related to exposure to biological agents, are managed and reported. This may involve simple health screening or more detailed assessments, involving health questionnaires, lung function and blood serum test. All employees, who have undertaken health surveillance, should have a personal health record and the information must be kept for a period of 40 years and the findings of any health surveillance should be communicated to employees and any adverse findings should be deeply investigated and appropriate controls should be adopted. The training of site managers and personnel is a fundamental topic in order to verify the design and implementation of these prevention measures. It must be stressed that appropriate instructions, information and training, referred to the potential risks to their health and how they should be controlled, must be given to employees. Employers should also develop procedures for people who do not comply with the procedures and site rules.

4. Explosion risk: formation of potentially explosive atmospheres

Because of the presence of methane in its composition, biogas in combination with air can form potentially explosive atmospheres (**Table 1**). In Europe, safety measures against explosion risk are stipulated in Atex Directives 99/92/EC [24] and 2014/34/EU, which have inspired the preparation of checklist section, referred to the explosion risk. A crucial topic, reported in safety checklist, is the classification of plant areas [25], where explosive mixtures could be generated by biogas releases. This classification has to be carried out in terms of zones (Zone 0, Zone 1 and Zone 2), geometrical characterization (extent and volume) of hazardous areas [26] and persistence time:

1) **Zone 0**: an area in which an explosive gas atmosphere is present for long periods;
2) **Zone 1**: an area in which an explosive gas atmosphere can periodically occur during the normal operation and
3) **Zone 2**: an area in which an explosive gas atmosphere is not expected during the normal operation, but if it should occur, it would exist for a short period.

Directive 99/92/EC states that, places where potentially explosive atmospheres can occur are marked with specific signs (**Figure 1**), which are characterized by the following distinctive features:

- triangular shape and
- black letters on a yellow background with black edging

In **Figures 2** and **3**, the classification procedure of hazardous areas (outdoor and indoor place) is shown. It may be used as a basis to support the proper selection and installation of work equipments in hazardous zones. Classification of indoor places is particularly important because ventilation system design plays a fundamental role in order to dilute the potentially explosive atmosphere in the shortest times.

The first step of classification procedure consists of locating the potential sources of biogas release. On this subject, it has to be remembered that catastrophic elements failures are not considered as potential sources because they are beyond the concept of abnormality [27], reported in Technical Standards.

A plant component, such as valves, flanges, pumps, compressors, and so on, is considered as a potential source when its failures are expected during the operation. Zone classification depends on source release grade, ventilation degree and availability. Release grade

	Unit	Biogas (60% CH_4, 40% CO_2)	Methane	Natural gas
Heat value	kWh/m^3	6	10	10
Ignition temperature	°C	700	600	650
Explosion range	Vol (%)	7.3–28.3	4.4–16.5	4.4–15

Table 1. Properties of gases.

Figure 1. Sign (zone where potentially explosive atmospheres can occur).

(continuous, primary, secondary) is determined by the analysis of element operating conditions [28]. On the contrary, ventilation degree depends on the volume of explosive atmosphere, which is strongly influenced by biogas mass flow. This last parameter depends on gas outflow typology (sonic or subsonic), which is determined by the comparison between critical pressure (p_{cr}) and atmospheric pressure (p_a):

- $p_{cr} > p_a \rightarrow$ (sonic outflow)
- $p_{cr} < p_a \rightarrow$ (subsonic outflow)

Ventilation degree can be high or medium for outdoor places, whereas it can be high or medium or low for indoor places. Three levels of ventilation availability are reported in Technical Standard (EN 60079-10-1):

Good: ventilation is continuously present;

Fair: ventilation is expected to be present during normal operation and its discontinuities are permitted, but they have to occur infrequently or for short periods and

Poor: ventilation, which does not meet the standard of fair or good.

Ventilation availability can be good or fair for outdoor zones, whereas it can be good or fair or poor for indoor areas. For outdoor places, this parameter depends on local minimum wind speed. If wind speed is bigger than 0.5 m/s, ventilation availability can be considered as good. For indoor areas, in order to assess ventilation availability, reliability of artificial ventilation system and presence of standby fans or an emergency ventilation plant has to be ensured. In case of fan failure, good availability usually requires automatic start-up of standby fan(s). Indoor areas are the most

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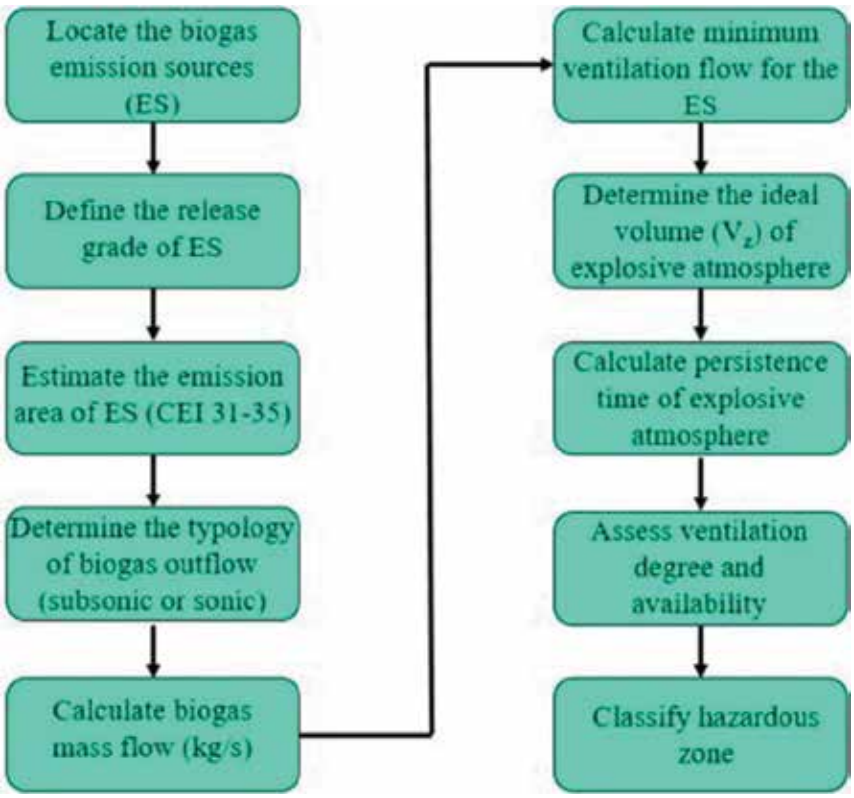

Figure 2. Classification of an outdoor place.

hazardous places with regard to formation of explosive mixtures. In a biogas production plant, a potentially dangerous zone is the container (indoor place), which includes combined heat and power (CHP) unit (**Figure 4**). In indoor places, in order to assess the ventilation degree, sources emission contemporaneity must be considered. This is a necessary condition aimed at calculating average biogas concentration (X_m%) in indoor areas. X_m% depends on source release grade and can be calculated according to IEC 31-35 (Technical Standard). In case of continuous grade emissions (temporary period can be negligible), X_m% is calculated by the following equation:

$$X_m\% = \frac{M_{gas}}{Q_a \cdot \rho_{gas}} \cdot 100 \quad (1)$$

where:

- M_{gas} (kg/s) is biogas mass flow;
- Q_a (m^3/s) indicates ventilation air flow; and
- ρ_{gas} (kg/m^3) is the biogas density.

In case of primary and secondary grade releases (temporary period is considered), X_m% is calculated by the following expression:

$$X_m\,\% = \frac{M_{gas}}{Q_a \cdot \rho_{gas}} \cdot (1 - e^{-C \cdot t_E}) \cdot 100 \quad (2)$$

where:

- C (s^{-1}) represents the number of fresh air changes per time; and
- t_E (s) is the release duration.

Table 2, which is reported [26] in EN 60079-10-1, is used to classify the hazardous zones.

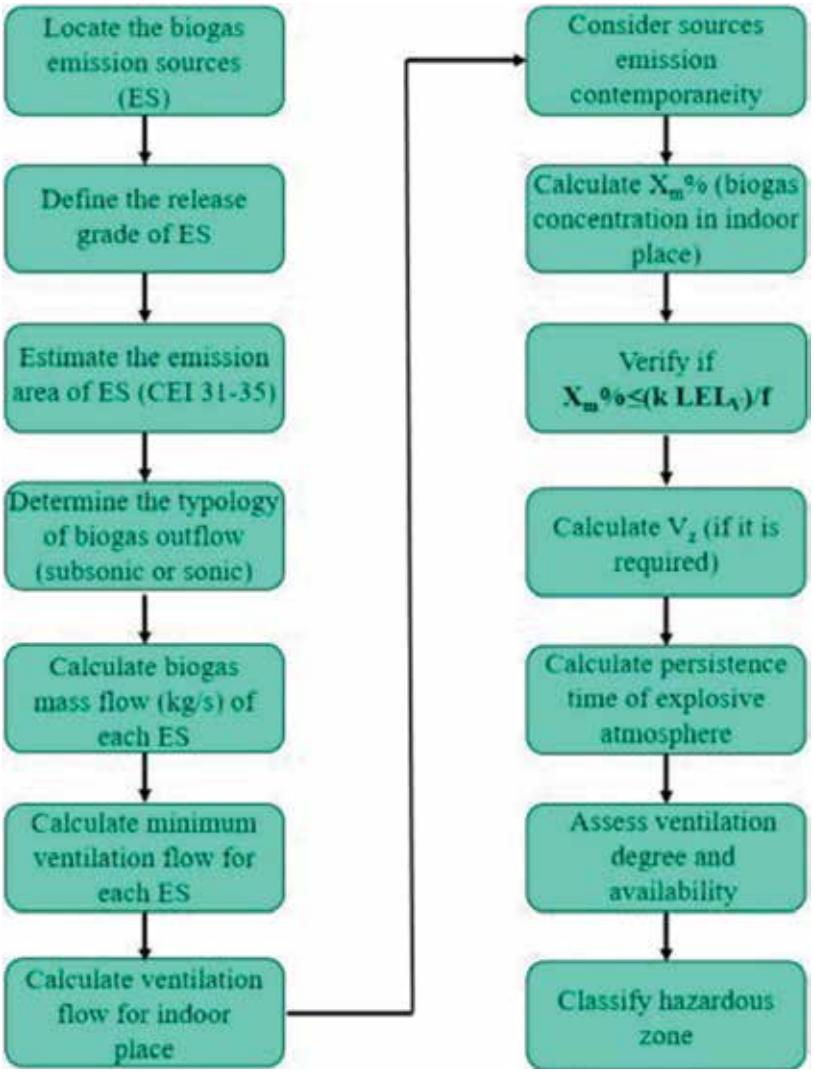

Figure 3. Classification of an indoor place.

Figure 4. Combined heat and power unit (indoor place)—Source: Maccarese S.p.a.

	Ventilation						
Release grade	**Degree**						
	High			**Medium**		**Low**	
	Availability						
	Good	**Fair**	**Poor**	**Good**	**Fair**	**Poor**	**Good, fair or poor**
Continuous	(Zone 0 NE)	(Zone 0 NE)	(Zone 0 NE)	Zone 0	Zone 0	Zone 0	Zone 0
	Non-hazardous zone[a]	Zone 2[a]	Zone 1[a]		+	+	
					Zone 2	Zone 1	
Primary	(Zone 1 NE)	(Zone 1 NE)	(Zone 1 NE)	Zone 1	Zone 1	Zone 1	Zone 1 or Zone 0[b]
	Non-hazardous zone[a]	Zone 2[a]	Zone 2[a]		+	+	
					Zone 2	Zone 2	
Secondary	(Zone 2 NE)	(Zone 2 NE)	Zone 2	Zone 2	Zone 2	Zone 2	Zone 1 or Zone 0[b]
	Non-hazardous zone[a]	Non-hazardous zone[a]					

Glossary: '+' means 'surrounded by'.

[a]Zone 0 NE, 1 NE or 2 NE indicate areas, which have negligible extents.

[b]Zone 0 can be generated in poor states of ventilation.

Table 2. Classification of hazardous zones.

5. Fire risk

In order to reduce the fire risk, the safety checklist suggests several recommendations and actions, which consist of prevention, protection and managerial measures. By reason of space, the checklist only shows the most important points related to fire risk. In order to minimize the fire effects, biogas production plant has to be divided into fire protection sectors [29], for example, the anaerobic digester, the biogas holder and CHP unit. Certain distances must be maintained among these sectors. In particular, during the biogas holder construction, specific safety distances must be ensured (internal and external safety distance and protection distance).

6. Results and discussion

By reason of space, the safety checklist only reports the most important bullet points referred to as the three examined hazards (**Tables 3–5**).

The safety checklist for the biogas industry can support the hazards identification and the definition of the prevention and protection measures and, if used in the right way, forms a basic part of risk assessment. It is essential that the checklist is used as a means of development

Biohazard assessment
Are there operations/tasks, which may result in bioaerosol, organic dust or particulate dispersion (biomass reception, its storage, grinding, shredding or other pre-processes of the biomass, digester loading operations, digestion drainage, sampling activity or maintenance work)?
Are high-exposure activities performed within indoor places?
Are the work processes designed to reduce the releases of organic dust and bioaerosol in the workplace?
Is the biomass stored in closed containers/tanks?
Are there leakages of solids or leachate during the handling phases of the materials entering and leaving the system?
Do workers have direct contact with manure, slurry or other organic waste?
Are there risks of splashes and spills contaminated with biological agents?
Are workers particularly subject to the risk of infective or immunological diseases (workers with particular allergies or asthma, low immune system, pregnant women)?
Prevention and protection measures
In indoor places, are collective protection measures applied to the source of the biohazard, such as ventilation systems and appropriate work organization procedures?
Is the workplace regularly cleaned? Are operating procedures defined?
Is the **workplace** provided with hand-washing and shower facilities and 'clean areas'?
Is eating and drinking forbidden in the workplace?
Are warning and safety signs used at the workplace? Do workers have difficulty of national language understanding?
Do workers receive information on biohazards and protective measures before assuming their tasks?
Are vehicles, circulating in the biogas plant, subjected to regular washing?

Is the vehicle cab equipped with dedicated ventilation systems? Is the monitoring of the door seals and the filter maintenance provided?
Are workers provided with respiratory protective equipment (RPE) during high-exposure activities?
Do workers carry out trainings focused on the right use of individual protection devices?
Is it verified that the defined procedures are actually observed by the workers?
Are workers under health surveillance?
Are workers informed of the significance of health assessments and their outcomes?
Is there a system which reports the accidents and records the episodes of contamination with biological agents (even mild)?
Are workers aware of the importance of recording any contamination episodes?

Table 3. 'Safety checklist' extract: biohazard.

Explosion risk
Prevention measures
Are places with explosion risk classified into zones (0, 1 and 2) according to the probability of occurrence of potentially explosive atmosphere?
Are hazardous zones (0, 1 and 2) characterized in terms of volume and extent?
Is persistence time of explosive atmosphere calculated?
Are suitable ventilation rates ensured in indoor places in order to dilute biogas concentration below lower explosive limit?
Are standby fans or an emergency ventilation system installed in indoor workplaces (container of CHP unit)?
Are there adequate openings aimed at ensuring a good natural ventilation in indoor workplaces?
Are wind action and stack effect taken into account for dimensioning the openings of indoor workplaces (natural ventilation)?
Are work equipments and protective devices selected on the basis of categories set out in Directive 2014/34/EU?
Is all process control equipment classified according to European Standards?
Is the air flow, injected for biological desulphurization, matched with the current rate of biogas production (max. 6% volume)?
Protection measures
Can a biogas release be diverted **or** removed to a safe place or, if that is not practicable, safely contained by other suitable methods?
Are flame arresters installed in biogas pipes?
Are **biogas holders** equipped with positive (hydraulic seal) and negative pressure protection devices?
Is the water filling of pressure safety devices daily controlled and is the correct water level maintained?
Are all closed tanks, in which fermentation can occur, provided with pressure safety devices?
Managerial measures
Are workers equipped with working clothes which do not generate electrostatic charges?

Explosion risk
Are hazardous areas indicated by specific signals?
Is there an obligatory journal **for the documentation** of all daily measurements, controls and maintenance works as well as failures?
Is there a plan indicating the explosion protection zones?
Is it certain that an operational manual is available before any work is done?
Is it established in the manual that safety devices have to be checked at least once a week and after any failure?
Is the engine (CHP unit) maintained according to the timetable given by the manufacturer?
Is the CHP unit maintained or checked by specialized companies?
Are all parts of the biogas plant, containing a gas flow, regularly checked and submitted to a pressure test at least every year?
Are operating instructions readily available, easy to see and read by the operators during their work?
Is artificial ventilation system of container, which includes CHP unit, maintained and checked according to the timetable given by the manufacturer and if necessary is it maintained or checked by specialized companies?

Table 4. 'Safety checklist' extract: explosion risk.

Fire risk
Prevention measures
Are biogas holder membranes made of fire-resistant materials?
Do biogas holder membranes avoid the formation of electrostatic charges?
Are electrical equipments designed in accordance with Regulations and Technical Standards?
Are electrical equipments provided with protective grounding?
Are biogas holders protected from lightning?
Is the storage of flammable materials, flammable liquids and gases limited to small amounts?
Protection measures
Are biogas pipes insulated to give protection against fire and provided with fire protection flaps?
Is the protection distance respected during the biogas holder placing?
Is the internal safety distance respected during the biogas holder placing?
Is the external safety distance respected during the biogas holder placing?
Are there enough fire extinguishers on plant site?
Are there gas/fire detectors, which sound an alarm in case of fire?
Are the hydrants chosen in accordance with Technical Standards in terms of flow and pressure?
Is an additional generator, aimed at ensuring electric delivery in case of failures, installed?
Managerial measures
Is a responsible person designated for all fire protection measures?
Are fire protection exercises regularly carried out?

Fire risk
Are smoking, naked flames and storage of flammable materials forbidden in the plant area?
Is firefighting system maintenance regularly carried out in accordance with the reported directions?
Are maintenance operations reported in a specific register?
Is electrical equipment maintenance regularly carried out?
Are there adequate and well-marked routes for fire brigade vehicles?
Are fire protection posts set up and suitable fire extinguishers made available when works (welding, abrasive cutting, etc.), which involve a fire risk, are carried out?
Are firefighting systems periodically checked?
Are gas sensors/fire detectors periodically checked?
Is it certain that the operation and maintenance of biogas plant is done by reliable and qualified persons?

Table 5. 'Safety checklist' extract: fire risk.

support and not simply as a 'tick off-the-box' exercise. A specific sector guidance, referred to the potential risks to biogas plants workers health and their assessment and management, is required, and it is likely that site operators will be in need of specialist advice to carry out an effective risk assessment and develop risk control procedures. In this context, the reported checklist can improve the safety culture in the biogas field. In conclusion, it has to be stressed that the safety checklist has been tested in some biogas production plants, where inspections and audit activities were simulated in order to verify its real feasibility.

7. Conclusions

Countries of the European Union (EU) have agreed on a new 2030 Framework for climate and energy, which includes targets and policy objectives for the period between 2020 and 2030. These targets are aimed at achieving a more competitive, secure and sustainable energy system. A specific target has established that at least a 27% share of renewable energy consumption must be achieved. In this context, biogas/biomethane production plants can be strategic, and therefore particular attention has to be paid for their safe operation. In fact, biogas industry is experiencing fast growth worldwide. However, the number of accidents in biogas production is growing even faster. The estimated risk profile of biogas production confirmed that its production process presents a non-negligible risk. Accident analysis can improve the safety of such plants. In particular, creation of an accidents report can be strategic in order to individualize the more hazardous operations and elements which require a specific maintenance schedule. Indeed the decrease of number of accidents, which occurred in the biogas production plants, could be easily achieved by adapting the process safety experience acquired in other industrial sectors. With regard to this topic, it is important to remember that the typical culture of the farming is far enough from industrial approach and therefore it requires clear and useful tools, which are able to address both elements—maintenance and operation. The safety checklist can meet these requirements, because it is a practical tool, which can be used

to carry out the analysis of hazards of biogas plants. Starting from a scientific analysis of preventive and protective measures, the checklist has been designed to assess the actual safety levels of the biogas plant and to support the operators in order to improve the safe process management. Furthermore, the development and application of specific safety standards to the biogas sector would be beneficial to avoid design and operational errors.

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Chapter 10

Non-Edible Vegetable Oils as Renewable Resources for Biodiesel Production: South-East Asia Perspective

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Additional information is available at the end of the chapter

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Abstract

Biodiesel derived from plant species has been a major renewable source of energy and has received global interest mainly due to climate change issue. It has increasingly received worldwide attention as a promising alternative fuel. Growing interest in biodiesel production from edible oil brings scarcity in food supply. To overcome this problem, utilization of non-edible oils could be explored. Non-edible oil as biodiesel feedstock impressed in many factors such as energy sustainability and independence in certain areas, especially in rural community, creating job opportunities, elevating environmental merits, and avoiding monoculture of fuel resources. The present chapter reviews several such potentials, including fatty acid methyl ester (FAME) or biodiesel production process of non-edible oil resources as biodiesel feedstock in South-East Asian geographical region. The South-East Asian countries fall in the tropical region of the world and have many species as non-edible oil, viz., jatropha, karanja, polanga, neem, rubber, and mahua. The oils derived from these species have shown considerable potential as biodiesel feedstock.

Keywords: biodiesel, oil properties, renewable energy, potential crop, FAME

1. Introduction

Biodiesel has energy sustainability benefit for most of the countries in Asia, aiming to reduce nations' dependence on fossil fuels, who still import the oil and other petroleum product [1, 2]. It is also considered as the final strategy for clean development mechanism (CDM). Utilization of edible oil as feedstock for biodiesel production has increased its price and created demand in the world market [3]. More than 60% of the world's population resides in Asia leading to a

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higher demand for food and energy. Utilizing non-edible oils as a biodiesel feedstock assists the sustainability of biodiesel production and minimizes the impact directly on food supply [1].

2. Non-edible oil merits as biodiesel feedstock

Deforestation and destruction of ecosystem due to urbanization and plantation expansion is a big issue in utilizing edible oil as biodiesel feedstock. Furthermore, the boundary line between food and fuel is blurred since both the fields are competing for the same resources. Debating over food versus fuel is still a dilemma. There are large surplus of food crops in developed countries. However, millions of people in developing countries still face the scarcity of food. Conversion of food crops such as palm oil, coconut oil, corn, soybean, and sugarcane to biodiesel could lead to serious food shortage. Countries in South-East Asia fall in the tropical belt and have many species of crops both edible as well as non-edible ones. Developing biodiesel production based on non-edible oil is one of the scenarios for fuel security without interfering with the food supply. This is especially true for palm oil which makes up about one-third of vegetable oil as biodiesel feedstock and has become the hottest environmental topic in South-East Asia.

High price of high quality refined edible oil makes them not feasible as tent to uneconomic for developing countries like India due to high production cost of methyl or ethyl ester from the edible oil. The cost is about four times higher compared to the cost of diesel [4]. Valuable nutrient elements in edible oil such as essential amino acids, β-carotene, α-carotene, vitamin-E, lycopene, tocotrienols, and carotenoids will be neglected, if this oil is converted to fuel.

Non-edible crops can grow in waste and unproductive land which may be helpful for reclaiming the land [1, 5, 6]. Furthermore, non-edible oils may contain toxic substances such as triterpenoids and strong odor in neem oil, and furanoflavones, furanoflavonols, chromeno-flavones, flavones, and furanodiketones in karanja oil [7]. Rubber seed oil contains cyanogenic glucoside that yields poisonous prussic acid (HCN) due to enzymatic reaction [8]. Jatropha oil contains toxic phorbol esters (0.03–3.4%) [9] or curcain [10], depending on the variety. Hence, it is better to exploit these non-edible oils as feedstocks for biodiesel production.

2.1. Non-edible oil crops in South-East Asia

Jatropha (*Jatropha curcas*) was not the only prominent non-edible oil crop. Other crops such as karanja (*Pongamia pinnata*), polanga (*Calophyllum inophyllum*), and neem (*Azadirachta indica*) were also found to be promising as alternative biodiesel feedstocks [11]. Rubber (*Hevea brasiliensis*) and mahua (*Madhuca indica*) have also shown potential and needs to be explored as biodiesel feedstock (**Figure 1**). The oil from the seed of these plants (**Figure 2**) has been considered as a waste material as it is non-edible; hence, they have the significant potential for biodiesel production.

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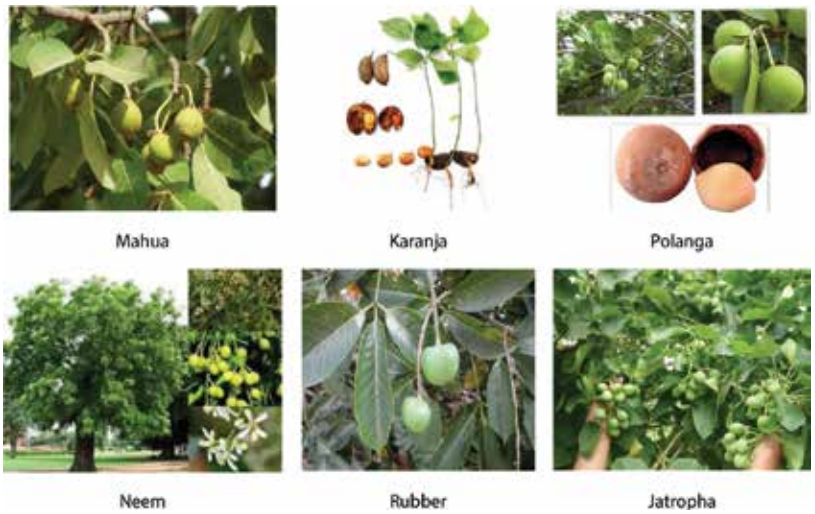

Figure 1. Six different species of non-edible vegetable oils as renewable sources for biodiesel.

Figure 2. Seeds of six different species of non-edible vegetable oils as renewable sources for biodiesel.

*2.1.1. Jatropha (*Jatropha curcas *L.)*

Jatropha curcas L., a member of the family Euphorbiaceae, is a large drought-resistant multipurpose shrub with several attributes and considerable potential, and has evoked interest all over the tropics as a potential biofuel crop [12]. The lifespan of this perennial bush is more than 50 years, and it can grow on marginal soils with low nutrient content [12, 13]. Seed

production is from 0.2 kg to more than 2 kg per plant [5]. The average weight per 1000 seeds is about 500–800 g, which is equivalent to 1333 seeds per kg. The seed coats constitute about 35–40% of the total seeds. The oil content in seeds ranges from 35 to 40% and the kernels 55–60% (www.jartropha.org) [14]. Many investigations have been done on the composition and content of the jatropha seeds [15]. Optimal seed yield in good condition area is around 5 t dry seed $ha^{-1}y^{-1}$ [16]. Seed oil contain 22.50% saturated fatty acid (16.00% palmitic acid, 6.50% stearic acid) and 78.70% unsaturated fatty acid (43.50% oleic acid, 34.40% linoleic acid, 0.80% linolenic acid) [6].

*2.1.2. Karanja (*Pongamia pinnata *(L.))*

Pongamia pinnata (L.) Pierre (Family Fabaceae – Papilionoideae) is native to India and commonly known as karanja [17]. It has been introduced to humid tropical lowlands in the Philippines, Malaysia, Australia, the Seychelles, the United States of America, and Indonesia [18]. In fertile soil, *P. pinnata* can produce 10–50 kg of seeds/tree and reach yearly production of around 200 t (metric ton) [19]. Seeds of *P. pinnata* are heavy, contain greater food reserves and around 800–1200 seeds are found to weigh 1 kg. As a legume, it is also able to fix its own nitrogen from the soil thus minimizing the need for adding fertilizer. Further, it has positive bio-ameliorative effect on the nitrogen, phosphorous, potassium, and organic carbon content of soil. The oil seed consists 19.15% saturated fatty acid (11.65% palmitic acid, 7.50% stearic acid) and 70.70% unsaturated fatty acid (51.59% oleic acid, 16.46% linoleic acid, 2.65% linolenic acid) [20].

*2.1.3. Polanga (*Calophyllum inophyllum *L.)*

Calophyllum inophyllum L., a member of Clusiaceae family, is native to Australia and has many attributes to be used as a biodiesel feedstock [21]. It fruits profusely (3000–10,000 seeds $tree^{-1}$ $season^{-1}$) and requires little maintenance [22]. Productivity of polanga is 3744 kg/ha (dry weight) within density 400 trees/ha [11]. Seed yields 65% oil that contains 24.96% saturated fatty acid (12.01% palmitic acid, 12.95% stearic acid) and 72.65% unsaturated fatty acid (34.09% oleic acid, 38.26% linoleic acids, 0.30% linolenic acid) [6].

*2.1.4. Neem (*Azadirachta indica*)*

Azadirachta indica is a member of the family Meliaceae and is native to Indian subcontinent. It can be grown by seeds in rainy season and reach the maximum productivity after 15 years. The adult plants reach at the height of 25–30 m and bears fruit at the age of 3–5 years. Neem tree produce around 40–50 kg fruits per plant per year equivalent to 25–30 kg seeds in its full growth [23]. Average seed yield of neem is 2.67 ton/ha at density 400 plants/ha [11]. The neem oil yield that can be obtained from the seed kernels varies from 25 to 45% [11].

*2.1.5. Rubber (*Hevea brasiliensis*)*

The rubber tree is a perennial plantation crop, indigenous to South America and cultivated as an industrial crop since its introduction to South-East Asia around 1876. Rubber tree can grow in hot and moist regions. Its productivity starts from eighth year onwards. The yield of the

seeds is about 300–500 kg/ha/year [24]. Rubber seed kernels (50–60% of seed) contain 40–50% of pale yellow oil [25–27]. Rubber seed oil does not contain any unusual fatty acids, but is rich in polyunsaturated fatty acids C18:2 and C18:3 that make up 52% of its total fatty acid composition [28]. Rubber seed oil contains 18.90% saturated fatty acid (10.20% palmitic acid, 8.70% stearic acid) and 80.50% unsaturated fatty acid (24.60% oleic acid, 39.605 linoleic acid, 16.30% linolenic acid) [29].

*2.1.6. Mahua (*Madhuca indica*)*

Madhuca indica is a member of the family Sapotaceae and is commonly known as Butter Tree. Mahua oil is obtained from the seed kernel. The tree is medium to large and found in Asia. Average productivity of mahua seed is about 1.6 kg/tree [30]. The seeds contain 30–40% fatty oil, which is non-edible and used in the manufacture of various products such as soap and glycerin. It contains 31.80% saturated fatty acid (17.80% palmitic acid, 14.00% stearic acid) and 64.20% unsaturated fatty acid (46.30% oleic acid, 17.90% linoleic acid) [11].

3. Characteristic of non-edible oils

Characteristics of vegetable fats and oils depend on the length and degree of un-saturation of the fatty alkyl chains. Thus, the fatty acid plays an important role in determining biodiesel characteristics. Amount of each fatty acid, chain length, and number of double bond present in the hydrocarbon chain influences the biodiesel properties [31]. The stability of biodiesel also depends on the feedstock properties used for biodiesel production. The most abundant fatty acids in the oil samples were oleic, linoleic, linolenic, palmitic, and stearic fatty acid. Oleic acid comprises of a major portion of the total fatty acid irrespective of non-edible oil summarized in **Table 1**. All oils have high unsaturated fatty acids (up to 80%) which mean they have good low

Properties	Jatropha	Karanja	Polanga	Rubber	Mahua	Neem	Diesel
Saturated fatty acid							
Palmitic ($C_{16:0}$)	16.0	11.7	12.0	10.2	17.8	14.9	—
Stearic ($C_{18:0}$)	6.5	7.5	12.9	8.7	14.0	14.4	—
Total	22.5	19.2	24.9	18.9	31.8	29.3	—
Unsaturated fatty acid							
Oleic ($C_{18:1}$)	43.5	51.6	34.1	24.6	46.3	61.9	—
Linoleic ($C_{18:2}$)	34.4	16.5	38.3	39.6	17.9	7.5	—
Linolenic ($C_{18:3}$)	0.80	2.7	0.30	16.3	—	—	—
Total	78.7	70.8	72.4	80.5	64.2	69.4	—
Cetane number	52.3	55.8	57.3	—	40	57.8	46
Oilseed content, %w	55	33	65	40–50	50	44.5	—
FFA %w	14	2.5	22	17	20	—	—
Specific gravity	0.92	0.91	0.90	0.91	—	—	0.84

Properties	Jatropha	Karanja	Polanga	Rubber	Mahua	Neem	Diesel
Viscosity 40°C (mm^2/s)	18.2	27.8	72.0	76.4	24.6	—	7.50
Flash point	174	205	221	198	232	—	50
Calorific value (Mj/kg)	38.2	34.0	39.3	37.5	36	—	42.25
Iodine value	93.0	80.9	93.8	135.3	74.2	69.3	38.3

Sources: Azam et al. [11], Ghadge and Raheman [35], Karmee and Chadha [42], Puhan et al. [30], Ramadhas et al. [5, 29], Tiwari et al. [16], Sahoo and Das [20], Islam et al. [53].

Table 1. Characteristic and composition of several non-edible oils compared to diesel.

temperature properties and are suitable as biodiesel feedstocks (**Figure 3**). Higher concentrations of saturated fatty acids can increase cloud point (CP) and cold filter plugging points (CFPP), which makes them undesirable as liquid fuel [31, 32]. On the other hand, unsaturated fatty acids helps to maintain oil in liquid form, but if the concentration of polyunsaturated fatty acids exceeds certain limit they can form polymers under heat which can block the fuel system of a vehicle [11]. The oils with larger proportion of saturated fatty acids will be more stable than those having larger portion of unsaturated fatty acids. But again, higher proportion of saturated fatty acids lowers the temperature for becoming solid even in the room temperature.

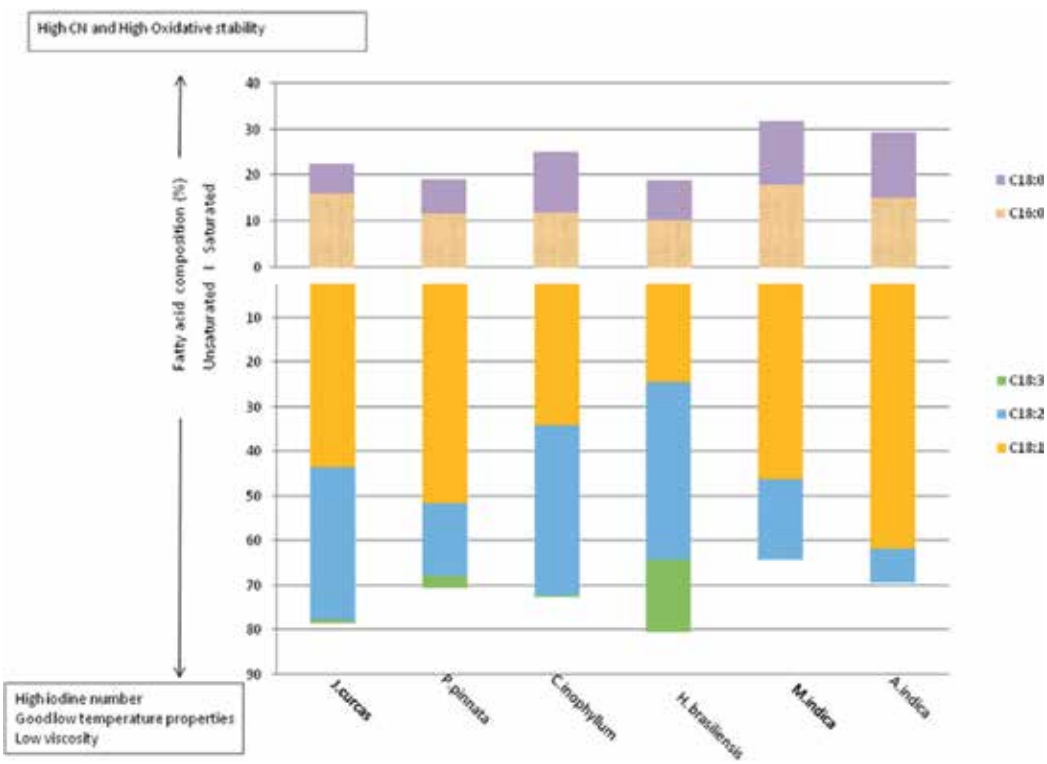

Figure 3. Distribution of fatty acid and its influence on the characteristics of biodiesel in different non-edible oils.

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The presence of double bond in the fatty acid brings diesel on poor stability. This decomposition occurs very fast at an exponential rate.

The amount and type of free fatty acid (FFA) in the biodiesel determines the viscosity, one of the most important characteristics of biodiesel. Due to the presence of higher amount of long chain FFA, polanga (72.0 mm^2/s) and rubber (76.4 mm^2/s) seed oils may have a slightly higher viscosity compared to others. Karanja and mahua oil has similar viscosity, due to the presence of same FFA. Iodine value represents the degree of unsaturation and relatively high iodine value is reported in range 69.3 in neem to 135 in rubber seed oil (**Table 1** and **Figure 1**). These properties are relatively applicable in cold climates. From calorific value, non-edible oils have potential to be biodiesel feedstocks.

Cetane number (CN) is widely used as diesel fuel quality parameter related to the ignition delay time and combustion quality [14, 32]. Higher cetane numbers in all vegetable oils listed in **Table 1**, will give better ignition properties. Cetane number increases with the increase of saturated fatty acid, and increases linearly with the chain length, decrease with number of double bonds and carbonyl groups move toward the center of the chain. High level of saturated fatty acid (C14:0, C16:0, C18:0) raise cloud point, cetane number, NOx, and improve stability, while more polyunsaturated (C18:2, C18:3) reduce cloud point, cetane number, stability, and raise NOx.

4. Barrier of transesterification process for non-edible oils

Generally, non-edible oil has high free fatty acid content of 2.53–22% in weight basis. Alkaline transesterification is not feasible for oil containing high free fatty acid for producing biodiesel [33, 34]. It generates soap, consumes more catalyst and reduces the effectiveness of catalyst. Subsequently, soap causes the solution to be more viscous, and leads to the formation of gel and foam that inhibits purification of biodiesel from glycerol [35]. To overcome this dilemma, biodiesel production from non-edible oils that has high free fatty acid was conducted by several methods; two/three stages reaction, acid-catalyzed esterification and alkaline-catalyzed transesterification; enzymatic process; and supercritical methanol [36, 37]. In enzymatic process, water content in the raw material does not interfere to the reaction conducted in low temperature. Lipase reaction occurs at the interface between the aqueous and oil phase [38] which generates alkyl ester with high purity and easy separation [39]. Due to deactivation of catalyst and time cut in a few minutes, supercritical transesterification in high temperature and pressure can tolerate presence of high percentage of water in the feedstock [40–42].

5. Biodiesel production from non-edible oil

Several studies have shown that there exists an immense potential for the production of plant-based oil to produce biodiesel. Azam et al. [11] studied the prospects of fatty acid methyl esters (FAME) of some 26 non-traditional plant seed oils as potential biodiesel feedstocks. Among

them, *J. curcas*, *A. indica*, *C. inophyllum*, and *P. pinnata* were found to be the most suitable for use as biodiesel and they met the major specification of biodiesel for use in diesel engine.

5.1. Jatropha

Most of the researchers [20, 36, 43, 44] used two stage (acid catalyzed and alkaline catalyzed) esterification for biodiesel production from *J. curcas* oil due to its high free fatty acid content. Ortho-phosphoric acid is used as catalyst and degumming agent in acid esterification stage [20]. Pre-esterification is the first stage that uses sulfuric acid prepared by calcination of metatitanic acid as a catalyst. The conversion of FFAs was higher than 97% under the reaction conditions of 90°C, 2 h, 4% solid acid, and molar ratio of 20:1 of methanol to FFA. Then alkaline catalysis was carried out for 20 min, 64°C using 1.3% KOH as catalyst and molar ratio of 6:1 of methanol to oil. Berchmans and Hirata [43] achieved 90% yield in 2 h alkaline transesterification and Sahoo and Das [20] achieved 93% yield. Lu et al. [44] produced biodiesel and achieved yield up to 98% from jatropha oil with FFA over 20%. Complete conversion and the highest yield was conducted in supercritical methanol [36] within 4 min with temperature at 320°C, pressure 8.4 MPa, molar ratio absolute methanol to oil was 43:1 as optimum ratio [45]. Supercritical was success at temperature above 327°C and pressure above 8 MPa since ester yield increase rapidly at that state. From technical and environmental point of view, supercritical is appropriate for biodiesel production due to less glycerol waste but from economic analysis point of view; this method is not appropriate due to its high operating skill and cost. Two stages process can be a good choice, since it can reduce FFA to proper amount below to 1%.

5.2. Karanja

Two-stage process was conducted in producing biodiesel (up to 20% FFA) from *P. pinnata* seed oil [19]. Acid-catalyzed esterification was adopted by using 0.5% (w/w) H_2SO_4, molar ratio of alcohol to oil of 6:1 at 65°C. Next step was alkali-catalyzed transesterification by 1% (w/w) KOH, molar ratio of methanol:oil of 6:1, which was the optimum condition [46]. The yield of biodiesel (96.6–97%) was achieved at 65°C.

5.3. Polanga

Crude polanga oil generally has 22% free fatty acid. Hence, it must be carried out in three-stage process for producing appropriate biodiesel [6, 20, 47]. Three-stage transesterification process was zero catalyzed transesterification, acid catalyzed transesterification, and alkaline catalyzed transesterification. The oil was purified from organic matter and other impurities by mixing 0.5%v toluene and 35%v methanol as reagent. The reaction was carried out at 65°C for 2 h. Acid catalyzed esterification was conducted by 0.65% v H_2SO_4, molar ratio of alcohol to oil of 6:1 for 4 h. This process reduced FFA less than 2%. Sahoo and Das [20] added 0.5%v orthophosphoric acid as a reagent that reduced FFA from 14.5 to 1.62%. Alkaline catalyzed transesterification process was conducted by using 1.25% w KOH, molar ratio of methanol to oil of 8:1, 60°C for 2 h. Biodiesel from polanga still gets unsatisfactory yield below 90%. Further

research needs to be done to get better yield in order for polanga to be more acceptable in the biodiesel production.

5.4. Neem

Neem seed contains 20–30% and kernel contains 30–52% oil [23]. The oil of neem seed has several uses from making soap, pesticides, and pharmaceuticals to biodiesel. The seed oil contains 29.30% saturated fatty acid (14.90% palmitic acid, 14.40% stearic acid) and 69.40% unsaturated fatty acid (61.90% oleic acid, 7.50% linoleic acid) [11]. Biodiesel production from neem seed oil is quite the same with other non-edible oil resources in order to get appropriate product.

5.5. Rubber

Several researchers produced biodiesel from rubber seed oil [25, 29, 48]. Ikwuagwu et al. [48] produced biodiesel from fresh rubber seed oil, where the FFA in crude oil was 2% and in refined oil 0.5%. The reaction carried out under condition of molar ratio of methanol to oil was 6:1 and 1% NaOH as catalyst, ester yield from crude seed oil was just 76.64% compared to refined oil (84.46%). Ramadhas et al. [29] produced biodiesel from rubber seed oil with high free fatty acid by two stages reaction. Acid esterification reduced FFA of oil from 17% to less than 2% when reaction with 0.5% v H_2SO_4, methanol to oil molar ratio of 6:1, temperature at 50°C for 20–30 min. Final stage was alkaline transesterification, where the oil mixture with methanol to oil molar ratio of 9:1 and 0.5%w of NaOH at temperature 40–50°C during 30 min for achieving conversion efficiency almost 100%. Result from Ramadhas et al. [29] showed rubber seed oil is appropriate as biodiesel feedstock. The viscosity of biodiesel obtained is close to diesel, although yield that Ikwuagwu et al. [48] achieved was considered uneconomical. However, further research is needed for fostering the biodiesel quality as well as more acceptability.

5.6. Mahua

Ghadge and Raherman [35, 49] studied the process optimization for biodiesel production from *M. indica* oil using response surface methodology. FFA content can be reduced from 27% to less than 1% by 0.32 v/v methanol using 1.24% w/v H_2SO_4 as catalyst under reaction condition at 60°C for 1.26 h. Next step was conducted by adding 0.7% w/v KOH, 0.25 v/v methanol to oil molar ratio of methanol 6:1. The biodiesel yield was achieved 98%.

6. Characteristic of biodiesel from non-edible oils

Table 2 represents the fuel properties of methyl esters (biodiesel) from various plant-based oils. Specific gravity of biodiesel methyl esters of six non-edible oils meet the standard biodiesel ranging from 0.86 to 0.89.

Properties	JME	KME	PME	MME	NME	RME	Diesel	Biodiesel standard	
								EN 14214	ASTM 6751–09
Specific gravity	0.86–0.88	0.88–0.89	—	—	—	0.87	0.84	0.86–0.90	0.87–0.90
Calorific value (MJ/kg)	42.7	42.1	41.4	37.0	40.1	36.5	42.5	—	—
Viscosity (mm^2/s) at 40°C	4.2	4.4	4.0	4.0	8.8	5.8	3.8	3.5–5.0	1.9–6.0
Flash point (°C)	148	163	140	208	—	130	45	≥120	≥130
Cloud point (°C)	10.2	14.6	13.2	—	—	4.0	−1.0	—	—
Pour point (°C)	4.2	5.1	4.3	6.0	—	−8.0	−16.0	—	—
Cetane number	60.7–63.3	59.7–60.9	52.5	—	—	—	—	—	47 min

Source: Shaoo and Das [20]; Ghadge and Raherman [49]; Ramadhas et al. [29].
Note: JME = Jatropha Methyl Ester, KME = Karanja Methyl Ester, PME = Polanga Methyl Ester, MME = Mahua Methyl Ester, RME = Rubber Methyl Ester, NME = Neem Methyl Ester.

Table 2. Biodiesel properties from various non-edible oil feedstock.

Viscosities of all non-edible oils were ranging from 3.8 to 8.8, which comply with the standard biodiesel of EN 14214 and ASTM 6791–09, except neem methyl ester (**Table 2**). The neem oil was the most viscous one among the six oils. Consequently, the viscosity of neem methyl ester was the highest in their respective series. Biodiesel derived from jatropha, karanja, polanga, rubber, mahua, and neem were found to comply with the industrial standards.

7. Status of biodiesel production in South-East Asia

Recently, biodiesel production from non-edible oil has risen. In South-East Asia, countries such as Indonesia, Malaysia, Philippines, and Thailand have taken initiatives to develop biodiesel from non-edible oil generally using *J. curcas*. Indonesia has taken several steps in biodiesel roadmap including target to use 10% of diesel fuel consumption of 2.41 million kL within 2005–2010, spread out over Indonesia including Sumatera (Riau, Medan, South Sumatera, Jambi, and Dumai), Banten, West Kalimantan, Balikpapan, Papua, and Merauke in 2007–2011. Biodiesel utilization will increase in 15% of diesel fuel consumption of 4.52 million kL by 2011–2015 and finally 20% of diesel fuel consumption of 10.22 million kL within 2025 [50]. Indonesia developed *J. curcas* and *C. inophyllum* as biodiesel feedstock. The country plans to breed 10 million of *C. inophyllum* seeds on 10,000 ha in Madura [51]. The Ministry of Forestry, Republic of Indonesia reported that, the engine runs well without problem on a road test with 370 km mileage by using biodiesel obtained from *C. inophyllum*.

Malaysia started with breeding high quality *J. curcas* seeds, sets-up the country policy, proper process and invests in the land for jatropha cultivation for biofuel production since 2005. Malaysia has also invested in processing plants and *J. curcas* [52, 53] plantations. On the other

hand, Malaysia is also arranging partnership with private sectors in further expansion of jatropha plantations. Philippines has also developed *J. curcas* plantations through Philippinea National Oil Company (PNOC) and expect at least 700,000 ha jatropha plantations in the Mindanao area with yield of 300 gallons of biodiesel per acre [54]. The government arranges the mandate of utilizing biodiesel B2 in 2011. Jatropha is prominent biodiesel feedstock in Thailand [55] as well. Thailand started using biodiesel B2 in Cheiang Mai area and plans to increase the use of B5, B10 in 2011 and 2012, respectively. Thailand also plans to collaborate with Laos, Myanmar, and Cambodia in biofuel development.

8. Conclusion

Production of biodiesel from edible oil such as palm, coconut, soybean, corn, rape seed oils, or other food crops like sugarcane will lead to severe shortage in food and its security. High price of edible oil makes them not feasible for the production biodiesel. In this situation, use of oils from non-edible sources may increase fuel security without interfering with the food security. South-East Asian countries fall in the tropical region and have many species of crops of non-edible oil. The non-edible crops can grow in waste as well as in marginal lands which may be helpful for reclaiming the unproductive areas. It is better to exploit these non-edible oils as feedstocks for biodiesel production. Major non-edible plants in this region are jatropha, karanja, polanga, neem, rubber, and mahua have shown significant potential as biodiesel feedstock. Biodiesel properties and fatty acid composition has in-direct correlation because transesterification cannot change the fatty acid composition. Fatty acid compositions give paradoxal properties among cetane number, low temperature properties, and stability of the products. Optimal characteristics could not be achieved within this current time. At present the end usage production is low and utilization of these oils are limited. Exploitation and utilization of these non-edible oils as biodiesel feedstock can save foreign currency, fossil fuel dependency, and equally improve the rural economy as well as future job opportunities.

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Chapter 11

Sustainable Waste Management and Waste to Energy Recovery in Thailand

Warangkana Jutidamrongphan

Additional information is available at the end of the chapter

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Abstract

In Thailand, the municipal solid waste (MSW) generated is currently about 71,700 tons a day. Moreover, solid waste management (SWM) is an interdisciplinary issue. The concept of WM has been embraced by Thailand through the setting of a national master plan for SWM. Several waste to energy (WTE) projects have been initiated. The anaerobic digestion WTE power plant in Rayong municipality was selected for performance evaluation. It is able to treat 70 tons of organic waste per days but its actual throughput has decreased to 20 tons per day based on limited amount of waste separation effected to isolate organic waste. In addition, a better digester design is required for the actual organic waste generated. Thermal processes such as gasification and incineration in Hatyai have been applied for mixed waste. However, they suffer from the limitation that high moisture content waste can cause fluctuating heating values. Also, the environmental impact on nearby communities is an important concern. Meanwhile, investment in WTE project has been encouraged by the introduction of the feed-in tariff (FiT) rate paid for electricity generated through sustainable processes to promote energy recovery from MSW. The keys to success for WTE technologies are waste separation at source and development of machine innovation.

Keywords: anaerobic digestion (AD), feed-in tariff (FiT), solid waste management (SWM), waste incineration, waste to energy (WTE)

1. Introduction

Thailand is located in the center of South East Asia and has a total area of about 514,000 km^2 (200,000 mile^2). The Tourism Authority of Thailand [1] noted that looking at a map of Thailand (**Figure 1**) shows a country whose borders form the rough shape of an elephant's head, the head and ears forming the mostly landlocked northern and eastern provinces and

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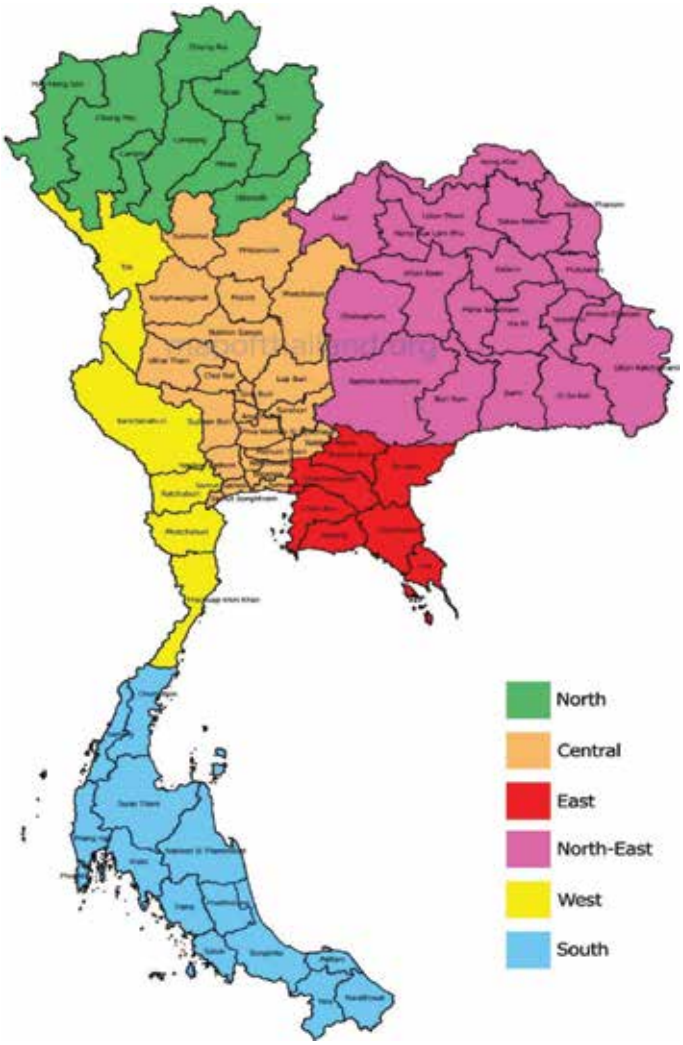

Figure 1. Provincial administration in Thailand [4].

the trunk extending down the Malaysian peninsula between the Andaman Sea and the Gulf of Thailand. Thailand had a population of approximately 66 million in 2017 [2] and the provincial administration of Thailand is divided into 77 provinces. Thailand's climate is influenced by tropical monsoons and the weather in Thailand is generally hot and humid across most of the country throughout the year. The central, northern, and northeastern Thailand, especially the landlocked provinces have three different seasons, summer, the rainy season, and a cool season, whereas the southern and the coastal regions of Thailand have only two seasons which are the hot and rainy seasons. Generally, it is relatively hot most of the year. The cool season and the summer occur from November to February and March to May respectively. Between February and May the weather is mostly hot and dry. The rainy season, lasts from May to November and is dominated by the southwest monsoon, during which time rainfall in most of Thailand is at its heaviest [3].

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2. Waste situation in Thailand

Currently, the increases in the generation of municipal solid waste (hereafter MSW) caused by population growth and increasing economic development in developing countries has become one of the most serious environmental issues. Solid waste management (hereafter SWM) is difficult to implement as part of integrated management strategies and sustainable development in developing countries. A large amount of mixed waste is usually dumped in open landfills or burned outdoors. Many Asian countries have poor practical waste separation [5]. Despite the adoption of waste management strategies such as the so called 3Rs strategies (reduce, reuse, recycle), which have been widely implemented throughout the world during the last decade, a complete solution to the problems of SWM has not been found. Developing countries such as Thailand still produce large amounts of MSW which for some time have been overwhelming the available landfill sites. MSW generation has continuously increased over the years and in 2016, the total amount of MSW in the country, as reported by the Pollution Control Department (hereafter PCD), Ministry of Natural Resources and Environment (hereafter MNRE), Thailand, was 27.06 million tons which represented an increase of 0.2 million tons since 2015. As an illustration, the amount of waste generated annually is equal to a hundred times the volume of the highest building in Thailand, "Baiyoke Tower II" which is 328 metres high. Of this waste only 14.81 million tons was placed in disposal sites, with waste recovery accounting for a further 4.64 million tons with the remaining 6.91 million tons accounted for by other means [6]. The efficiency of waste collection is about 80% countrywide but only 36% of the total generated waste is disposed of using acceptable processes such as incineration, composting or landfilling [7]. However, there are only 2500 waste recovery entrepreneurs nationwide of which only one fifth use proper waste disposal methods, the remaining four fifths use illegal waste disposal methods such as open dumping, and direct burning resulting in environmental contamination through leaching, and leakage of substances. Several landfills have been burned as has been reported in newspapers [8]. Only a small fraction of the MSW collected is managed by proper treatment or disposal and there is a need for a sustainable solution to these problems as well as the implementation of public participation strategies [9]. It inevitably involves municipalities, scavengers, investors, teachers, and people.

2.1. Legislation affecting waste management

Generally, local authorities in Thailand are responsible for SWM on their administrative zones and the collection, treatment, and disposal of municipal, industrial and infectious waste is under the control of those local authorities. The MSW generated by the population of 66 million people is currently about 71,700 tons a day. The government of Gen. Prayut Chan-o-cha, the Prime Minister (hereafter PM) in power in Thailand since 24 September 2014, has recognized that the amount of waste generated and its management constitutes a serious issue and the government has adopted policies relating to SWM including the management of solid and hazardous waste. As part of this policy, the government has directed that each province should identify locations for the construction of waste facilities including proper landfill disposal sites and facilities to convert waste into renewable energy. This national strategy was incorporated into a solid and hazardous waste management roadmap which was drafted by the MNRE and represents a significant step in defining the national agenda relating to the solid waste issue.

Further regulations relating to this national agenda on SWM were announced by the office of the PM in the form of an act of parliament, published in the royal gazette, in September 2014 [10]. Under the decentralization strategy adopted, responsibility for SWM was passed to each province under the authority of the provincial governor in collaboration with the MNRE, who will provide back-up on technical and management issues to support the provincial administrations. At the beginning of the fiscal year 2015, the MNRE assigned national SWM policy to local provincial administrative and local government organizations in order to implement this policy [11]. The main concept of the national roadmap for solid and hazardous waste management is divided into four categories;

2.1.1. Residual waste

1. There will be no more open dumping and residual waste must be properly treated including urgently removing it from landfills

2. Survey and improve all illegal and improper landfills including both sites within local administrative control and private sites

To comply with the national waste management roadmap, the following steps should be applied:

- Survey and assess landfills with a view to closing or rehabilitating them.
- Renovate existing landfills to render them sanitary.
- Dispose of waste in private areas or convert it to refuse derived fuel (RDF) and promote private investment in waste treatment technologies.
- Enforce the law in relation to the operation of private illegal dumping sites.

2.1.2. Emerging waste

1. Reduce and separate waste at household sources

Reducing waste at source is a simplified methodology to save costs and natural resources. It represents a sound business concept as well as encouraging social responsibility. If the entire country adopted source reduction practices, the pressure on natural resources would be significantly decreased. Several organizations and businesses have already recognized that source reduction and waste separation and recycling will result in financial savings and will yield an enhancement of productivity as well as promoting corporate social responsibility.

2. Apply a clustering waste management system using combination technologies with the emphasis on waste to energy (WTE) and maximizing waste recovery

In order to centralize waste management systems, each province should aggregate SWM based on district and sub-district clustering taking into consideration the amount and condition of waste. The waste treatment pattern should be divided into three levels as follows:

- Model S clusters waste disposal sites into those that receive a daily input of waste of up to 50 tons per day. The provinces in which this model was initiated were Nakorn Ratchasima, and Buriram
- Model M clusters waste disposal sites into those that receive a daily input of waste of between 50 and 300 tons per day. Model M was piloted in municipalities in Nan and Rayong provinces.
- Model L clusters waste disposal sites into those that receive a daily input of waste of more than 300 tons per day. The pilot provinces for the L model were Nonthaburi, Phuket, Songkhla (including both Songkhla and Hatyai municipalities), Chiang Rai, and Bangkok [12]

Under the M and L models waste would be processed by the following procedures:

- Promote the separation of waste at source.
- Screen hazardous waste out of MSW and gather it at a transfer station before sending it to private contractors.
- Implement a combination WMS and convert waste to electricity.
- Rehabilitate existing landfills into sanitary landfill sites for continued dumping.

Under model S the same processes would be operated as those under models M and L but a different approach to waste recovery would be adopted with composting being utilized instead of electricity generation at small scale sites and employing private contractors for hazardous waste management instead of landfill rehabilitation.

3. Reinforce the role of the private sector in waste management and increase investment especially in waste incinerators

2.1.3. Waste management measures and policy

1. Henceforth, governors are the provincial regulatory waste management administrators

2. Legislation to introduce and standardize procedures including waste reduction, separation, collection and transportation, and, to standardize waste disposal fees for solid, hazardous and infectious waste

The roadmap focuses on policy and legislation while the practical implementation of measures on waste management have been decentralized to provincial administration organizations in three stages.

In the first stage covering a period of 6 months, governors meet with provincial waste management committees selected from related stakeholders e.g., local administration representatives, academics, NGOs and the private sector, to brainstorm and discuss SWM with a view to drafting a provincial solid and hazardous waste management model. From this an overview of the implementation of the provincial SWM model is prepared incorporating an assessment of local preparedness and how collaboration between all the stakeholders can systematically

and sustainably resolve the waste problem. The provincial waste management committee also plays an important role in the selection of alternative waste technologies at a centralized waste disposal site selected depending on the size of their area and the likely inputs of waste. It is also important to deal with political issues which may arise during the bidding process for concessions and to ensure that this process is transparent and fair.

Additionally, governors have authorization to adopt a flexible approach where necessary, for example, in conducting environmental impact assessments (EIA), allowing joint ventures between private companies and government organizations under the Government Business Act (2013), the City Plan Act (1975), and the Environmental and Safety Assessment Act (ESA, 2009), as well as adopting measures to support private sector investment in solid and hazardous waste management.

In addition, provincial administration organizations have the authority to pass local regulations to set up, operate, control, and monitor waste disposal sites. These regulations may also cover waste separation (general waste, organic waste, recyclable waste, and hazardous waste) and the prohibition of the mixing of hazardous waste with general MSW.

Following this initial period, there follows an intermediate period of a year during which the policy highlights the disposal of products and packaging material which may include permitting the use of waste disposal sites and the disposal of electric appliances and electronic waste under pilot projects involving the extended producer responsibility (EPR) principle. Recycling of packaging material will be enforced in industrial sectors and measures will be taken to prevent illegal dumping of hazardous wastes. Waste treatment plants, disposal sites and recycling plants will be progressively introduced where they do not already exist and their use will be promoted. Local legislation relating to waste separation and the prohibition of the mixing of hazardous waste with MSW will also be enforced as well as other enactments to support the integrated solid and hazardous waste management program.

In the longer term beyond that year, measures will tend to focus on international directives that will make producers responsible for waste electrical and electronic equipment (WEEE) at the end of its useful life [13] based on the EPR concept and the polluter pays principle (PPP). These strategies are designed to promote the integration of environmental costs of goods throughout their life cycle into the market price. Therefore, the duty to eliminate WEEE should be allocated to producers in order to encourage them to adopt sustainable production methods [14].

2.1.4. Encouraging civil discipline, public education, and enactments for sustainability

These strategies relate to improving civic quality by both gentle and strict methods. People illegally dumping solid, hazardous, and infectious waste including industrial and radioactive waste should be traced and strictly punished. Public relations, education, and awareness-raising are necessary to ensure public participation in integrated MSW management from cradle to grave including a reduction in the use of plastics and the promotion of substitute materials. It is important that overall awareness of the issue of waste management should be raised and that the practice of waste separation should be promoted particularly among students and adolescents as this is essential to ensure sustainable MSW [15]. Awareness of

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Figure 2. Handbags from coffee pouches [17].

these issues should be promoted at every school and academic institution [6] and higher educational institutions should be requested to introduce youth awareness programs for the environment and waste management. Several schools have already adopted such waste management approaches. For instance, Benjamarachutit School, a public high school in Nakorn Sri Thammarat province, applied food waste from canteen to produce effective microorganisms used in the organic hydroponic culture of Chinese mustard greens in their agricultural learning area with both economic and environmental benefits [16]. Similarly, Boonpeng [17] noted that the Director of Baan Don Kha School, a small primary school in Si Sa Ket province, initiated a waste separation campaign in which students separated waste into four types; glass bottles, plastic bottles, milk pouches, and paper. These waste products are recycled or sold to provide revenue applied to school learning activities. Similarly, this primary school also taught their students to create value-added products from waste for daily utilization such as making handbags from waste coffee pouches (**Figure 2**).

3. Modern holistic waste reduction and recovery policies

June 5, 2015, was World Environment Day, and the PM chose that day to launch a plastic-bag-free campaign to encourage Thai people to reduce the volume of trash they generate. In particular, the PM stated that the government would seek cooperation from retailers, convenience stores, department stores and shopping centers in avoiding the use of plastic bags on the 15th day of each month and instead to use cloth bags or to reuse plastic bags in order to reduce the volume of plastic waste. Meanwhile, the MNRE initiated the "Green Card" project

to convince producers, suppliers and customers to produce and consume environmentally-friendly products and packaging. Card holders will gain green points whenever they purchase eco-friendly products at participating stores which will be redeemable for special vouchers or other rewards [18]. The PM also promoted strategic planning for alternative materials to substitute for polystyrene packaging with a view to banning this form of packaging which would achieve cost reductions as well as reducing waste and also announced a feasibility study into the use of biodegradable plastic bags [19].

4. Waste to energy (WTE) technologies

The increasing amount of mixed household waste has become a national problem in Thailand and elsewhere, the resolution of which may be to turn a crisis into an opportunity and reap benefits from garbage. The Thai government has instructed provincial authorities to find locations for constructing waste management facilities capable of using garbage to produce renewable energy. It is not only Thailand which lacks proper waste management systems and throughout the world peoples' attitudes to finding suitable sites to dump garbage have led to continuing conflicts and protests based on the "Not in my Backyard" (NIMBY) attitude. Therefore, it would be preferable to solve the problem by employing suitable waste management strategies as alternatives to disposal. The PM has declared that every province should build a WTE facility to convert waste to electricity which will support the country's efforts to reduce its dependence on natural gas and other fossil fuels. However, to-date, Thailand has just 3 WTE incinerators [19–21].

Nowadays, the use of WTE technologies is gaining momentum as a favorable waste management strategy. Unquestionably, WTE seems to be a viable option for diminishing the volume of waste as well as offering the additional benefit of producing alternative energy from waste recovery [21]. Already there has been increased recovery of recyclable materials from MSW rather than continued dependence on sanitary landfilling as the primary conventional method of solid waste disposal [22]. But the benefits of energy recovery from MSW are potentially more valuable, both as an alternative energy source and for the positive environmental implications, mainly relating to the saving of non-renewable energy derived from fossil fuels [23]. WTE or energy from waste refers to any waste treatment that transforms waste resources into electricity, steam, or heat energy. These include, for example, anaerobic digestion (hereafter AD), incineration, pyrolysis, gasification, plasma arc, and RDF. WTE technologies usually reduce the volume of original waste by as much as 90%, depending on the waste composition and the type of energy derived. A waste management hierarchy generally follows the pattern of waste avoidance or reduction, reuse, recycling, recovery treatment, followed by disposal. An integrated approach to WTE that practices waste segregation and pre-treatment of waste does not by-pass the waste hierarchy but precedes or replaces the disposal step which is a more sensible approach to WTE recovery than simply burning or converting raw unsorted waste. Nonetheless, the choice of WTE technology is important and the conversion plant itself may incorporate waste pre-treatment units to facilitate this approach.

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Thailand has a recent history of developing projects in WM in terms of sanitary landfills and managing waste through WTE facilities. Moreover, Thailand has experience of WTE projects which have been developed locally and which date back at least a decade and from these, significant lessons related to both thermal and non-thermal technologies have been learned. Thermal treatments have involved both incineration and gasification processes and under the heading of non-thermal processes, biogas has been produced from, for example, waste fermentation or AD and the following sections will examine and compare case studies relating to both these technologies.

5. Anaerobic digestion (AD) power plant: a case study of Rayong municipality

The advantages of AD systems were set out by Spuhler [24] who noted that biogas and sludge were produced, respectively, for electricity generation and the production of fertilizer. Greenhouse gas emission can be reduced through methane recovery and efficient AD treatment systems reduce excess sludge by separating out heterogeneous organic waste, leachate, and wastewater. However, AD technologies may need to be modified to be appropriate for small and medium-scale facilities in developing countries. The high sensibility of methanogenic microorganisms need to be carefully investigated. Sulphuric compounds generated during methane production can cause erosion of equipment in AD facilities, and in order to protect AD equipment, the biogas produced may need to be significantly purified. The design and construction of AD power plants on a commercial scale needs to be under the supervision of experts, and professional operational and maintenance skills are also required to deal with fluctuations in the AD process.

Bearing in mind that Thailand is located in a tropical area and that the main economic income is derived from agricultural activities, the composition of MSW in Thailand is largely from food waste (40–60%) which has a high moisture content. Therefore, organic waste in Thailand seems to have a high potential as a raw material for producing biogas which can be converted into electricity. The case study which follows is of a biogas power plant located in Rayong municipality and includes some lessons-learned from its installation in 2004 and its operation since then.

Rayong municipality, is located in the coastal industrial zone on the eastern seaboard of Thailand. Rayong province is 179 km from Bangkok, approximately 3552 km^2 in area and is separated into eight districts, 58 sub-districts called Tambons in Thai and 440 villages. The population of Rayong municipality in December 2007 was 56,085: 27,110 males and 28,975 females [25].

Rayong municipality is a largely commercial city and in the late 20th Century generated increasing volumes of MSW due to the growth of the population. Between 1995 and 1997 the volume of waste grew from 57.47 tons to 63 tons per day. In 2000, the MSW contents were noted to be composed mainly of organic waste (67.77%) [26] and the bulk density of the MSW was 220 kg per m^3, as illustrated in **Table 1**.

The WTEF plant constructed in 2004 had the potential to treat 70 tons of organic waste per day. However, during 2004–2005, the organic waste fed to the AD system were separated in

2 main streams of about 12 and 3.3 tons per day of source sorted organic waste (SSOW) and mechanically-sorted organic fraction of MSW (OFMSW), respectively. The solid contents of the organic waste were 18% of total solids (TS) and 36% of volatile solids (VS) [27]. From 2006 to 2008, the organic waste collected and fed into the AD system was between 14.55 and 25.85 tons per day, with an average of 20.5 tons per day. However, this amount of organic waste was far less than the design capacity of the WTEF plant of 70 tons per day and represented only 29.3% of full capacity. A survey of the organic waste resources in Rayong municipality (**Table 2**) showed that marketplaces were the biggest source of organic waste representing 70% of the total. Other sources of organic waste were restaurants, hotels, and department stores. Nowadays, the organic waste treated in the AD plant is less than 20 tons a day.

The overall waste treated consisted of two waste streams (SSOW and OFMSW). The MSW collected is firstly processed in the front-end treatment (hereafter FET) unit then fed into the AD facilities to produce electricity and fertilizer [29]. There was a significant effect from the low amount of organic waste input and a lack of operation management which affected the loading capacity which has a maximum capacity of 70 tons of organic waste per day. The AD substrates were mostly, derived from food waste. The food waste treated at the WTE facility

Parameter	Unit, %
Composition	
Food waste	42.70
Paper	9.24
Rubber and leather	1.06
Clothes	2.25
Green waste (Wood and Leaves)	12.52
Plastic	17.13
Glass	0.74
Metal	4.26
Miscellaneous	10.1
Total	**100**
Chemical characteristic	
Moisture content	46.70
Carbon	18.16
Hydrogen	2.18
Nitrogen	1.20
Ash	20.62
Combustible fraction	32.68
C/N ratio	15.13/1

Table 1. MSW characteristics of Rayong municipality [26].

was separated at its sources: communities, restaurants, hotels, marketplaces, and department stores in the Rayong municipal area. On the environmental aspect, the recovered CH_4 from the AD process that was used in electricity generation resulted in GHG reduction of about 0.34 Gg CH_4 per year, equivalent to 7.15 Gg CO_2 eq of total GHG emission per year [30].

In order to raise people's awareness of and participation in waste management, various facilities were provided and activities conducted, such as recycling banks, recycling markets, and Tung Khaw Moo which is a process in which food waste is separated and gathered before being used as animal feed. The Rayong Municipality Office approached local residents by setting up public relations teams and providing information to the community about collaborating in separating food waste from schools, households, restaurants, hotels, department stores and marketplaces. These activities need to be conducted continuously and required proper monitoring systems to be successful. However, cooperation from government departments, the administrative organization was not forthcoming and local communication was poor.

There are a number of lessons to be learned from the AD project at Rayong municipality. Firstly, the characteristics of the organic waste intended to be used should be comprehensively identified in terms of its availability, and chemical and physical characteristics. Further, the climate and also the culture, and lifestyle of the people in the area should be established and taken into consideration in assessing how much organic waste will be available. Furthermore, the small amount of organic waste produced was also a significant problem in the AD process. This might be solved by finding other sources of additional substrate such as night soil, manure, and shredded pineapple peel which could be put into the AD process to improve the biogas yield. Secondly, the facilities in the AD process should be properly designed, durable and flexible. Thirdly, AD microbial activity should be increased by means of chemicals and

Resource	The percentage of organic wastes	
- Marketplaces		**63.45**
Star market	41.38	
Maedaeng market	6.90	
Saroch's fresh-food market	4.83	
Middle place night market	3.45	
Clock tower night market	3.45	
Tedsabaan 1 fresh-food market	3.45	
- Restaurants		**19.31**
- Hotels		**10.34**
- Department stores		**6.90**
Lotus	3.45	
Big C	3.45	
Total		**100**

Table 2. Survey of organic waste resources in Rayong municipality (modified from [28]).

adjustment or improvement of the anaerobic microbial activity of enzymes. Finally, the feasibility of investing in this kind of project should be carefully considered taking into consideration social awareness and people's willingness to participate since both are essential for the sustainable development of such projects.

6. Waste incinerator: a case study of Hatyai municipality

Hatyai, the largest district of Songkhla province is situated near to the city of Songkhla and is the main gateway to Malaysia and Singapore. Hatyai is an attractive tourism city affording a variety of shopping centers and duty-free stores and has been identified as the major southern business center. Hatyai is located on the eastern side of Southern Thailand close to the Thai Gulf coast and it is strategically located only 60 km from the land entry port at Sadao. Hatyai has undergone significant development in the recent past and has rapidly been transformed into the commercial, transportation, communication, educational, and tourism hub of the southern part of Thailand with consequent economic growth [31]. Hatyai municipality is approximately 21 km^2 in area and is separated into 15 sub-districts, 104 communities and 4 administrative zones (**Figure 3**). Hatyai has a tropical climate, which is hot and humid, like other parts of Thailand but it has only two seasons; wet and dry. The wet season, which is influenced by the monsoon and rainstorms, extends from May to December, while the dry season extends only from January to April. Additionally, there have been occasional floods in Hatyai due to heavy rain; it is not unknown for it to rain for twenty-two days in November with more than 300 mm of precipitation (Wikipedia, 2015). The population of Hatyai municipality in 2016 was a little under 160,000 of whom around 74,000 are male and more than 85,000 are female (December 2016) [32].

In regard to waste management in Hatyai, the total amount of MSW in Hatyai was 164, 182, and 158 tons per day in 2012, 2013, and 2014, respectively. In the fiscal year, 2014, the highest monthly amount of waste generated was in October 2013, during the rainy season (**Table 3**).

The waste collection system has been divided into four zones, as shown in **Figure 3**, the air being to efficiently serve each individual zone. As an illustration, the waste composition in Pom-Hok, was investigated as a pilot community between 2012 and 2014 (**Table 4**) and it was found that food waste and plastic made up the largest portion of mixed waste [33]. Fees were paid to contractors with respect to waste collection amounting to between 500 and 1,000 kg of waste per day.

The waste generated is transported to a 0.22 km^2 sanitary landfill in Kuan Lang community which is separated into two parts, a landfill of about 0.13 km^2 (**Figure 4**), and a WTE plant occupying 0.02 km^2 (**Figure 5**). The MSW input is treated in the WTE facility, which is operated by a private company, by means of ash melting gasification technology. It operates on a guaranteed daily capacity of 250 tons of waste and has a maximum capacity of 400 tons per day with a generating capacity of 6.7 MW of electricity per day which is sold to the Provincial Electricity Authority (PEA) at 6.4 Bht per unit. The Hatyai Municipality Office (HMO) has to pay a waste disposal fee of 290 Bht per ton to a private company for waste treatment [33].

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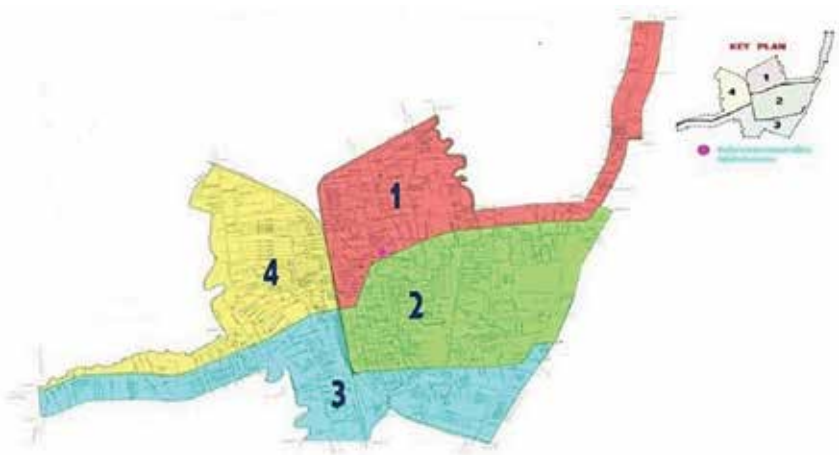

Figure 3. Waste collection zones in Hatyai municipality [32].

Since the provincial administration and regional environmental office 16 set up a collection center for hazardous waste in 2013–2014, hazardous waste in Hatyai municipality has been collected from communities prior to disposal by private companies.

The WTE facility consists of a FET facility, combustion machines, a boiler, a gas cleaning system, a controller system, an electricity generator, and a pollution control system (wastewater treatment, slag and sludge treatment, and an air pollution emission control system). Waste

Month	**Generated waste**
	Amount (tons)
October 2013	5,777.38
November 2013	5,253.81
December 2013	4,988.00
January 2014	4,558.15
February 2014	4,203.72
March 2014	4,610.77
April 2014	4,510.68
May 2014	4,723.88
June 2014	4,715.25
July 2014	4,292.63
August 2014	4,887.37
September 2014	4,769.37
Total	57,291.01

Table 3. Waste generated in fiscal year, 2014, Hatyai municipality [33].

Figure 4. Hatyai municipality landfill [34].

transported from the municipality is stored in an open-area in front of the FET facility. The waste is moved by a waste pusher to a conveyer through a shredder and a dryer in the FET prior to being fed into the combustion zone which is equipped with an air supply system.

Figure 5. Layout of Hatyai municipality WTE power plant [34].

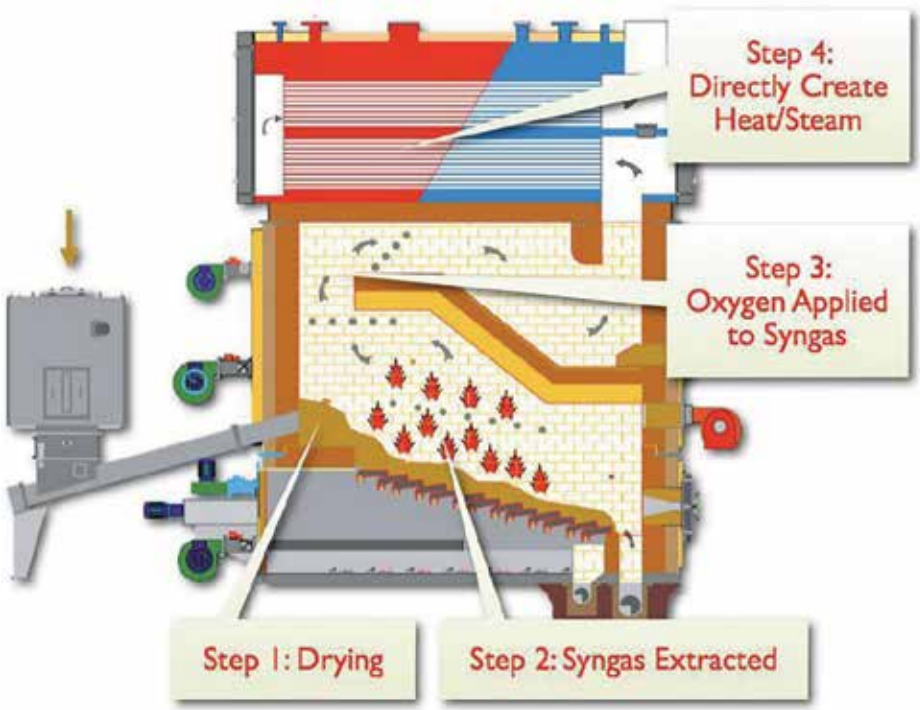

Figure 6. Gasification process [35].

Composition	Unit (%)	
	Year 2012	**Year 2014**
Paper	12.31	4.46
Food waste	39.90	31.28
Clothes	1.89	6.70
Plastic	19.94	32.43
Napkins	0.0	4.47
Leather and rubber	1.73	0.0
Metal	10.45	0.56
Glass	11.56	5.59
Stone and ceramic	0.05	5.59
Shells	0.0	2.23
Hazardous waste	0.08	0.56
Other	2.17	6.14
Total	100.0	100.0

Table 4. Waste composition in Pom-Hok community, Hatyai municipality [33].

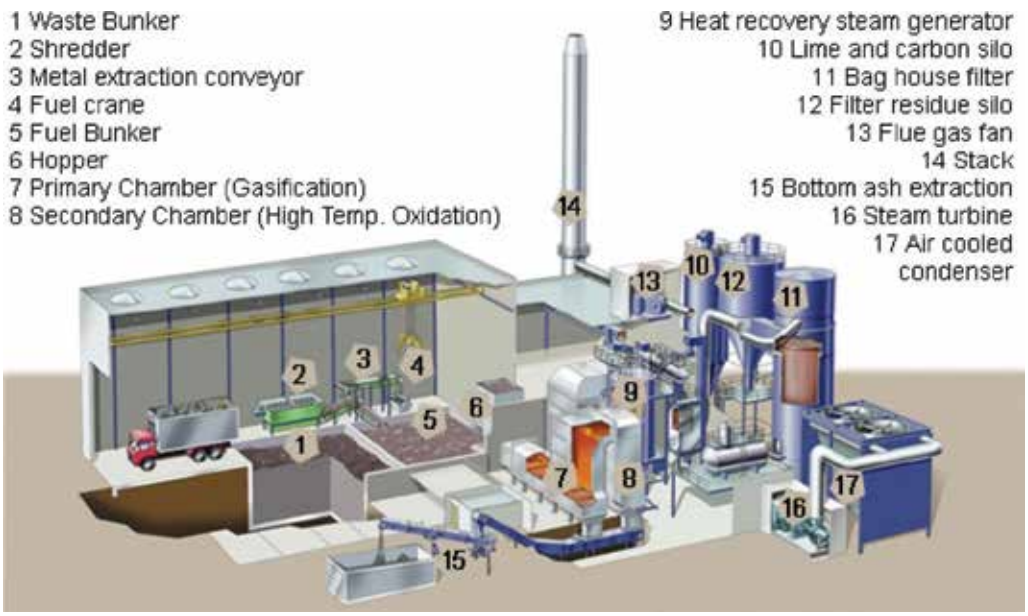

Figure 7. Structure of the WTE gasification power plant [36].

The mixed waste is ignited and burned in a gasification process (**Figure 6**). The schematic layout of the WTE gasification plant is illustrated in **Figure 7**.

The technology employed is focused on solving the problem of the increasing amount of waste generated in Thailand [37]. However, the heterogeneous composition of the waste which has a high moisture content has resulted in varying heating values especially in the

Figure 8. Wood chip mixed with residual waste before gasification.

rainy season and wood chips were mixed with the MSW before combustion to increase the heating value (**Figure 8**). Recently however, the FET has been under renovation with a view to improving its operation.

7. WTE promotion strategies and FiT incentive

The Ministry of Energy has targeted WTE production of 160 MW of electricity and 100 kilotons oil equivalent (ktoe) of thermal energy by 2021 a substantial increase from the amount of 44.324 MW reported by the Department of Alternative Energy Development and Efficiency (DEDE) in 2015. The 10-year (2012–2021) Alternative Energy Development Plan focuses on increasing the ratio of alternative energy use to one-fourth of overall use. Currently, about 22 MW electricity is produced from landfill biogas, 20 MW is generated from waste gasification and incineration, and 2 MW from AD from waste. From the total thermal energy produced of 78.6, 77.3 ktoe was derived from RDF, and the remaining small amount of 1.3 ktoe was from using methane biogas instead of fossil-fuel-based cooking gas. In addition, some cement kilns also utilize waste as a substitute fuel instead of coal [36].

To support WTE productivity, the government has promoted campaigns to encourage public participation in waste separation and WTE conversion, as well as providing information and conducting meetings with local administrative organizations, communities, municipalities, academic institutes, and students to enhance awareness and understanding about municipal waste management for a sustainable environment and energy security. It has also initiated measures to promote WTE production beginning with a 3.50 Bht per kWh subsidy for power generated from waste incineration and gasification, and a 2.50 Bht per kWh subsidy for landfill gas converted to electricity and AD from waste fermentation. The Energy Service Companies (ESCO) revolving fund for the energy support project from Thai government was also established to support energy conservation and investment in renewable energy, with investments in facilities and equipment being eligible for financial support from the Board of Investment (BOI) and the machinery import tariff being waived. Moreover, an exemption from corporation tax for 8 years with a further 5-years at a 50% reduction will apply to alternative energy projects. To motivate energy-from-waste production in 2014, a 4.54 million Bht budget has been allocated to study and enhance the efficiency of potential WTE projects [36]. Latterly, the National Energy Policy Commission (NEPC) has replaced its policy of applying additional rate payment structures with a "feed-in tariff" (FiT) system based on actual cost. For renewable energy from MSW, the FiT rate will be varied based on the annual cost of fuel. In particular, very small power producers (VSPPs, i.e., power producers generating less than 10 MW per year) have now been converted from the additional rate to FiT in power purchase agreements (PPAs) for 20-year project lifetimes. The new VSPP PPAs will apply a competitive bidding model instead of a first-come first-served process. Besides, an FiT premium for all project lifetimes privilege will be provided at a rate of 0.50 Bht per unit extra above the regular FiT in order to provide an incentive in the Southern border provinces to support energy security in those areas. WTE projects located in southern border provinces are eligible for a higher FiT incentive with regards to logistic and location. Details of these schemes are shown in **Table 5** [36].

Capacity (MW)	FiT (Bht/kWhr)			Support duration (Years)	FiT premium (Bht/kWhr)	
	FiT_F	FiT_V, 2017	FiT[(1)]		Bio-fuel projects (First 8 years)	Projects in border area[(2)] (Entire project lifetime)
1. Waste (Integrated waste management)						
Installed capacity >1 MW	3.13	3.21	6.34	20	0.70	0.50
Installed capacity >1–3 MW	2.61	3.21	5.82	20	0.70	0.50
Installed capacity >3 MW	2.39	2.69	5.08	20	0.70	0.50
2. Waste (Landfill)	5.60	—	5.60	10	—	0.50
3. Biomass						
Installed capacity >1 MW	3.13	2.21	5.34	20	0.50	0.50
Installed capacity >1–3 MW	2.61	2.21	4.82	20	0.40	0.50
Installed capacity >3 MW	2.39	1.85	4.24	20	0.30	0.50
4. Biogas (Wastewater/Manure/Solid waste)	3.76	—	3.76	20	0.50	0.50
5. Biogas (Energy crop)	2.79	2.55	5.34	20	0.50	0.50
6. Hydropower						
Installed capacity >200 kW	4.90	—	4.90	20	—	0.50
7. Wind	6.06	—	6.06	20	—	0.50

[(1)]Remark: This FiT rate applies to projects that supply power into the grid system in the year 2017. After 2017, FiT_V will be continually increased depending on the core inflation rate. This rate applies to waste fuel (integrated waste management), biomass, and biogas (energy crop) projects only [36].

[(2)]Projects located in Southern Border Provinces i.e. Yala, Pattani, Narathiwat provinces, and only 4 districts in Songkla province (Chana, Tepha, Sabayoi, and Natawee) [36].

Table 5. Feed-in tariff (FiT) rate of renewable energy for very small power producers (VSPP) [36].

8. Conclusion

The current rapid growth in the generation of MSW due to population growth and increasing economic development in developing countries has become one of the most serious environmental issues. In 2016, the total amount of MSW in Thailand increased to 27.06 million tons. The efficiency of waste collection is about 80% countrywide but only 36% of the total generated waste is disposed of through acceptable processes such as incineration, composting and landfilling. Moreover, SWM is an interdisciplinary issue. It inevitably involves municipalities, scavengers, investors, teachers, and people. The concept of WM has been embraced by Thailand through the setting of a national master plan for SWM, waste separation at source, clustering waste disposal sites, terminating open dumping, rehabilitating landfills, promoting investment in waste businesses, and building up civil discipline to support sustainable SWM. The increasing amounts of household mixed waste has created a national problem, the resolution of which is to turn a crisis into an opportunity and to reap benefits from garbage. The Thai government has directed provincial authorities to discover new locations for constructing waste management

facilities in order to manage the increasing amounts of garbage and to produce renewable energy. WTE is primarily aimed at waste treatment, with the additional benefit of recovering energy and materials from the process. Nowadays, WTE technologies are gaining momentum as a favorable means of managing waste. AD is the most favorable technology for organic waste separated at source. To enhance the AD performance, the retrofitting AD unit to minimize biogas leakage and increasing microbial activities by improving tank mixing is recommended [38]. Thermal processes such as incineration can also be used to treat mixed waste with a low moisture content but this technology has limitations when dealing with high moisture content waste which may cause fluctuated heating values.

However, the environmental impact on nearby communities is an important concern. Nevertheless, motivation for investment in WTE projects has been provided by the FiT rate paid for electricity generated with the aim of promoting energy recovery from MSW. Furthermore, a FiT premium rate for all project lifetimes of 0.50 Bht per unit above the regular FiT is now applicable as an incentive in the southern border provinces. In conclusion however, sustainable SWM can only succeed through the improvement of WTE technology and with public participation.

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Chapter 12

Biofuels from Microalgae

Archana Tiwari and Thomas Kiran

Additional information is available at the end of the chapter

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Abstract

Biofuels are the most awaited products of scientific research. The fossil fuels are being exhausted, and pollution is increasing globally. Algal biofuels are one of the promising options. They are wonderful tiny factories that yield a variety of substances that have the property to act as sources of ecofriendly fuels. More attention has been focused on microalgae-derived biomass for generating diverse renewable energy sources. The distinct features that microalgae possess include high biomass yield, abundant oil content, no requirement for land and easy cultivation in wastewaters coupled with carbon dioxide mitigation. Microalgae are tiny reservoirs of a plethora of biofuels. The diverse algal biofuels range from biodiesel, straight vegetable oil (SVO), lipids, ethanol and hydrogen. Biofuels are the need of today, and researchers around the globe are exploring the options for biological fuel production.

Keywords: microalgae, biofuels, renewable energy, algal biomass, biorefinery

1. Introduction

The choice for the most suitable energy carrier to be produced from algae is a promising option. Algae have been explored for their unique potential to yield a variety of biofuels concomitantly with generation of value-added products and phycoremediation of wastewater (**Figure 1**). Many algal strains like *Chlamydomonas*, *Chlorella*, *Scenedesmus*, *Botryococcus braunii*, and so on have been reported to produce biofuels (**Table 1**). The selection of algal strains is exclusively dependent on various factors like oil content, production yield and downstream processing and also on adaptability of microalgae toward high oxygen concentration, temperature variations and water chemistry [1].

The algal metabolism consisting of photosynthetic potential, which makes it unique in comparison to other microorganisms when it comes to processing sugars from cellulosic sources

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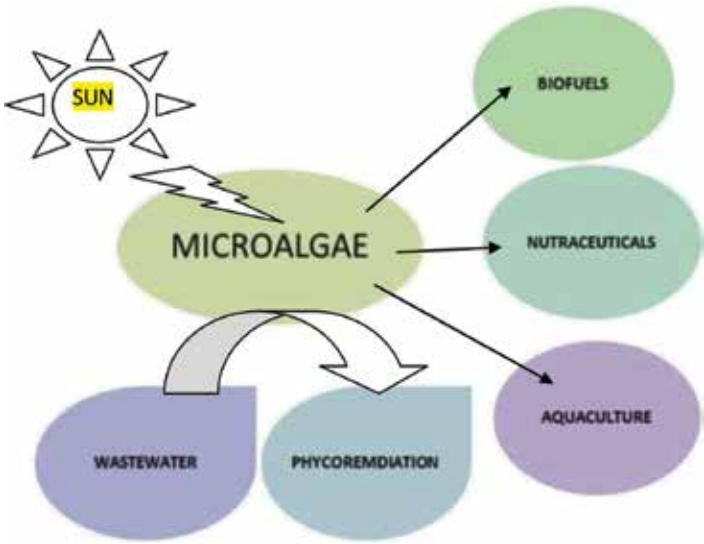

Figure 1. Potentials of microalgae.

such as grass and wood chips. After algal biomass degradation into sugar, there are substances like lignin associated with it, which are toxic to microorganisms. Removal of lignin is, thus, essential to promote further microbial growth leading to processing of sugar. Algae are tolerant to the presence of lignin, which makes the processing convenient coupled with reduction in the economic cost. In addition to this, there are other applications of algae like aquaculture, high-value products and nutraceuticals, which can be extracted from algae [2]. The microalgae require minimal inputs for metabolic processes—namely sunlight, CO_2 and water, with few required mineral nutrients. Sunlight is the most readily available and inexpensive source of energy on earth. The efficiency of microalgae in converting captured solar energy into biomass exceeds the potential of terrestrial plants. Microalgae do not compete with terrestrial plants for land or water supply as they can be grown in wastewater, leading to their remediation coupled with biomass production. The acumen of microalgae to inhabit diverse habitats could be exploited to allow for the production of compounds near the site of use, which could reduce the transportation costs [3].

1.1. Advantages of microalgae as source of biofuels

Microalgae are one of the most promising candidates for plethora of biofuels owing to their easy, inexpensive and simple cultivation system. They grow easily with basic nutritional requirements like air, water and mineral salts with light as the only energy source. They grow on liquid media, so diverse wastewater can also be utilized, which can be efficiently remediated by algae coupled with biofuel production.

The optimal use of light energy through photosynthesis is very efficiently executed by microalgae. They possess higher photosynthetic levels and growth rates and can be used for the production of desired biofuels. They can contain considerable amounts of lipids that are mainly

http://dx.doi.org/10.5772/intechopen.73012

Species	Product	Yield
Chlamydomonas reinhardtii(CC124)	**Biohydrogen**	102 mL/1.2 L
		0.58 mL/hL
		0.30 mol/m^2
		0.6 mL/L h
Chlamydomonas reinhardtii(Dang 137C mt+)		175 mL/L
		4.5 mmol/L
		71 mL/L
Chlorella vulgaris MSU 01		26 ml/0.5L
Scenedesmus obliquus		3.6 ml/μgChl a
Platymonas subcordiformis		11,720 nL/h
		7.20 mL /h
		0.339 mL/hL
Dunaliella tertiolecta	**Bio-oil**	43.8%, 34 MJ/Kg
		42.6%, 37.8 MJ/Kg
		25.8%, 30.74 MJ/Kg
Chlorella protothecoides		52%
		57.9%
Chlorella sp		28.6%
Chlorela vulgaris		35.83%
Nannochloropsis sp.		31.1%
Chlorella vulgaris	**Biogas**	0.63-0.79 LCH_4/gVS
Dunaliella salina		0.68 LCH_4 /gVS
Euglena gracilis		0.53 LCH_4 /gVS
Scenedesmus		140 LCH_4 /KgVS
Scenedesmus(Biogas from lipid-free biomass)		212 LCH_4 /KgVS
Scenedesmus(Biogas from amino acids-free biomass)		272 LCH_4 /KgVS
Scenedesmus obliquus		0.59-0.69 LCH_4/gVS
Botryococcus braunii	**Lipid content for Biodiesel**	25-75%
Chlorella sp.		28-32%
Chlorella vulgaris		56%
Crypthecodinium cohnii		20%
Monallanthussalina		20-70%
Nannochloris sp		20-35%
Nannochloropsis sp		31-68%
Neochlorisoleo abundans		35-54%
Nitzschiasp		45-47%
Scenedesmus dimorphus		6-40%
Scenedesmus obliquus		11-55%
Schizochytrium sp.		77%
Chlorella pyrenoidosa	**Carbohydrates content for Bioethanol**	26%
Chlorella vulgaris		12-17%
Dunaliella salina		32%
Scenedesmus obliquus		10-17
Porphyridium cruentum		40-57%
Euglena gracilis		14-18%

Table 1. Biofuel yields from microalgae [27].

present in the thylakoid membranes. Their biofuels are nontoxic and highly biodegradable. They are essentially free-living chloroplasts and are the pinnacle of minimizing structural component. They have high carbon dioxide sequestering efficacy thereby, reducing GHG emissions.

They reduce nutrient load in wastewater as they can utilize nitrogen and phosphorous present in agricultural, industrial and municipal wastewater owing to their phycoremediation acumen. They can be cultivated in areas like seashore, desert, and so on, which is not suitable for agricultural plants and not competing with cultivable land. Their cultivation is independent of seasons as they can be cultivated round the year and have minimal environmental impact. The cultures can be facilitated to produce high yields through technological interventions of genetic engineering, synthetic biology, metabolic engineering, and so on as algal systems are readily adaptable.

The biofuels from algae are diverse in nature. Carbohydrate component of biomass is used for bioethanol production, while algal oil for biodiesel and the residual biomass can be utilized for methane, fuel gas or fuel oil production. The biomass after biofuel production can further be used as source of many value-added products like eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), nutraceuticals, protein supplements, therapeutics, biocontrol agents, fertilizers, animal feed and aquaculture.

1.2. Biofuels derived from microalgae

A plethora of biofuels are derived from microalgae by virtue of their unique potential (**Figure 2**). The biofuels include alcohols, which are produced through fermentation, processing of algal biomass through dual approach of hydrolysis and fermentation, traditional method of transesterification, gasification of biomass or Fischer-Tropsch synthesis [4].

1.2.1. Biodiesel

Biodiesel has comparable engine performance to petroleum diesel fuel, while reducing sulfur and particulate matter emissions [5, 6]. Biodiesel is a biodegradable alternative fuel derived from renewable sources and is nontoxic in nature [7]. During the manufacturing process, triacylglycerols (TAGs) are transesterified with an acid or alkali catalyst to produce biodiesel and glycerol [8].

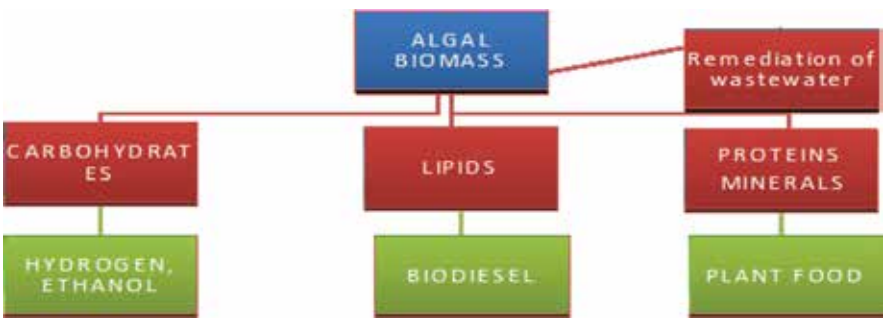

Figure 2. Biofuels derived from microalgae.

The algal biodiesel production processes fatty acid methyl esters (FAME). The chemical composition of biodiesel is generally produced by transesterification of algal oil in the presence of acid or alkali as a catalyst [5]. The biodiesel from algae can be derived directly from transesterification of algal biomass [9]. Alternately, it can also be produced by two-step process wherein the lipids are initially extracted and later on transesterified, though either of the processes involves lipid extraction through solvents and alcohols like methanol, isopropanol and petroleum ether [8, 10]. The process of direct transesterification is fast and cost-effective technology. Biodiesel generated from microalgae can be an excellent alternative to current diesel crisis, but in order to efficiently produce biodiesel from microalgae, strains with a high growth rate and oil content have to be selected [11].

1.2.2. Biogas

The anaerobic digestion of organic matter leads to formation of fuel called biogas or biomethane. Biogas is mainly formed by methane (55–75%) and CO_2 (25–45%). There are four stages of anaerobic digestion [12], which are described as follows:

- Biopolymer hydrolysis to monosaccharaides mediated by hydrolytic bacteria.
- Conversion of monosaccharaides to acids via fermentation.
- Action of acetogenic bacteria leading to the formation of acetate.
- Methane and carbon dioxide formation by methanogenic bacteria.

Microalgae has been reported to produce biogas as source of fuel, although the yield of biogas formation is quite low because of the sensitivity of algal cells to bacterial degradation and low carbon and nitrogen (C:N) ratio, which leads to the formation of inhibitor (ammonia). In *Scenedesmus* spp., residual biomass free from lipids and amino acids was investigated for biogas production, and results exhibited that residual biomass gives better biogas yield compared to raw biomass [13].

1.2.3. Hydrocarbons

The microalga species are capable of producing hydrocarbons, which can further be converted to diesel, kerosene and gasoline. The microalga, *Botryococcus braunii,* has been reported to produce hydrocarbons with excellent oil yield [14]. The habitat of *B. braunii* is freshwater, which can be one of the factors leading to its adaptation to varied salt concentration. In addition to this, the hydrocarbons from *B. braunii* get deposited outside the cell, thus the extraction becomes relatively easier and convenient [15–17].

1.2.4. Hydrogen

Microalgae can directly produce hydrogen from sunlight and water, only in the complete absence of oxygen. Hydrogen is a promising future energy source because it does not emit greenhouse gases and releases water as a by-product [18]. There are limitations existing regarding the large-scale production of hydrogen as fuel. At present, hydrogen is produced by stream reformation, photofermentation [19] and photolysis of water mediated by photosynthetic algae

[20]. Purple non-sulfur bacteria derive hydrogen from diverse substrates, while green sulfur bacteria get hydrogen gas from hydrogen sulfide (H_2S). Other microalgae can make hydrogen directly from sunlight and water, although only in the complete absence of oxygen.

Exploring new organisms for hydrogen production, optimization of growth conditions and use of biotechnological techniques can open new doors in making hydrogen a viable fuel for future [21, 22].

1.2.5. Biosyngas

Biosyngas is produced by the biomass gasification in presence of oxygen, water vapor or air, produces carbon monoxide, hydrogen, methane, water, other hydrocarbons and ashes. For gasification, high temperature (800–1200°C) is essential, and the feedstock needs to have not more than 20% water content in the biomass [23]. Electricity can be produced by burning in boilers and turbines and subsequently, kerosene, wax, naphtha and gasoline can be obtained [24].

1.2.6. Ethanol

Ethanol production from or by microalgae has very interesting prospects, but is currently only in the preliminary phase of research. Bioethanol can be used as a biofuel, which can replace part of the fossil-derived petrol. More development is needed to analyze a full-scale production system. Currently, bioethanol is produced by fermenting sugars, which in the case of corn are derived from hydrolyzing starch. Microalgae species with starch content of over 50% have been reported. With new technologies, cellulose and hemicellulose can be hydrolyzed to sugars [25]; thereby, facilitating formation of ethanol from major part of dry algal biomass. Compared to the traditional use of woody biomass, microalgae hold better options some of which are enlisted below [26]:

- Microalgae lack lignin, so the processing becomes easier.
- The microalgal cellular composition is very simple and biomass can be utilized readily.
- Microalgal cells consist of copious amounts of polysaccharides, which can be converted to sugar.
- Microalgae can be genetically engineered to produce ethanol.

Ethanol production from or by microalgae has very interesting prospects, but is currently only in the preliminary phase of research. More development is needed to analyze a full-scale production system. **Table 1** highlights the biofuels produced from different species of microalgae round the globe.

1.3. Cultivation of microalgae for biofuel production

The development of dedicated culture systems for microalgae started in the 1950s when algae were investigated as an alternative protein source for the increasing world population. Subsequently, the diverse products and the bioremediation of wastewater potential of algae

were explored. The initiation of research on algae as a source of renewable energy began by virtue of the energy crisis in the 1970s. The cultivation of algae requires few relatively simple conditions: light, water, carbon source, micro- and macronutrients and optimum temperature. Over the years, different culture systems have been developed keeping in mind the optimum conditions for microalgal growth, although it is a challenging task. The cultivation system for the growth of algae is an important requirement to aid in enhanced production of biofuels which includes open air ponds and closed controlled systems. The development of profitable algae-based fuel generation technology is yet in transition state wherein the final configuration is still to be explored and demonstrated at the industrial scale [29].

The cultivation of microalgae is a significant factor leading to enhanced biofuel production. The choice of cultivation system has to be emphasized because the phycoremediation efficiency and the yield of biofuels and other value-added products would largely depend on it. Broadly, the cultivation systems meant for microalgae are either open systems or closed systems. Hybrid systems, which are a combination of an open system and a closed system, can be used to achieve high biomass productivity with high nutrient removal [28].

1.3.1. Open microalgal systems

The open microalgal pond systems are commonly used for cultivation of microalgae as they have good opportunity to utilize the atmospheric carbon dioxide readily available in the atmosphere. There are several configurations of microalgae cultivation systems for biomass production and enhanced phycoremediation of industrial, domestic and agricultural wastewaters. The most commonly used systems for research and industrial microalgal cultivation are as follows:

- The raceway pond
- The circular pond tank
- The shallow big pond
- The closed pond

For open systems, location is an important criterion keeping in mind, the sufficient sunlight availability and the requirement of the algae to be cultivated. The open ponds can be natural or artificial in nature and usually include natural lagoons, circular ponds, tanks and raceway ponds. Cultivation of *Chlorella* sp. was traditionally done in circular ponds, which are usually made up of concrete. They are also equipped with rotating arm to ensure mixing of the culture and prevention of sedimentation of algal biomass. Generally, the raceway ponds comprise race track or oval channel made up of concrete, and they are meant to circulate nutrients and carbon dioxide regularly to the algal cultures [30].

1.3.2. Closed algal systems

The closed systems (photobioreactors (PBRs)) have well-controlled growth conditions. Generally, these reactors are designed to increase the light accessibility. They also allow

perfect mixing to permit the light to be within an optimum value for cell growth and to improve gas exchange. Since photobioreactors solve many problems of the open cultivation, researchers have focused on designing photobioreactors for large microalgal biomass production [30]. There is a wide variation in the design of the photobioreactor depending upon their geometry and construction. Photobioreactors can be built as bags tanks, and towers. Photobioreactors can be plates or tubular and made up of plastic or glass. Tubular photobioreactors seem to be the most suitable. Bubble columns and airlift photobioreactors can also be considered since they produce a relatively high concentration of microalgal biomass product [31]. An auxiliary tank is used to separate the oxygen produced from the photosynthesis. This is important considering that excessive oxygen can negatively affect the microalgae growth [32]. Despite the advancements in the design of photobioreactors for enhancing biofuel productivity in algae cultivation, bottlenecks are yet to be addressed efficiently considering the cost economics of biofuels and their productivity.

1.3.3. Hybrid algal systems

The hybrid systems are cost-effective and can be used for large algae cultivation [33]. Hybrid systems overcome the limitations of open systems and the high initial and operating cost associated with closed systems. In the hybrid system, the microalgae are initially cultured in closed and controlled photobioreactor system and then shifted to open system in order to enhance the biomass yield [34]. This system offers promising options for algal cultivation toward biofuel production.

2. Conclusion

Alternate energy sources are to be explored because nonrenewable energy sources are getting depleted, and environmental pollution is increasing globally. The economic viability of microalgal biofuel production should be significantly enhanced by a high-value coproduct strategy, which would, conceptually, involve sequentially the cultivation of microalgae in a microalgal farming facility (CO_2 mitigation), extracting bioreactive products from harvested algal biomass, thermal processing and utilizing residual biomass for extraction of high-value products. The synergy approach toward biofuel production can make the technology more viable and economically more feasible [35].

Enhanced production of these biofuels will help conserve our natural resources and save our environment. Algae technology has enormous potential not only for algae-based biofuels but also for food, feed, renewable chemicals and many other products that are critical for a more sustainable society. Major technological challenges like effective design of photobioreactors, innovative upstream processing and downstream processing ought to be addressed before commercialization of microalgae as a source of biofuels for a better future.

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Chapter 13

Biogas Recovery from Anaerobic Digestion of Selected Industrial Wastes

Pengchong Zhang

Additional information is available at the end of the chapter

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Abstract

Treating industrial wastes requires large amount of capital investment and also creates environmental concerns from several aspects. One of the techniques to reduce these concerns is anaerobic digestion. By applying anaerobic digestion technique, the organic waste from various industries could be removed and recovered to renewable energy, mostly in the form of biogas (methane); therefore, waste treatment process shifted from a cash negative process to an economic beneficial process. In this chapter, various kinds of industrial wastes were selected and descripted, followed by a gradually progressive order. The selected waste streams include paper mill wastes, brown grease, and corn ethanol thin stillage. Due to their dissimilar properties, the motivations of treating these wastes are also different. Paper mill effluents and solid wastes contain large portion of refractory or toxic chemicals and fibers; their bio-treatability, organic removal efficiency, and substrate utilization rate have been investigated and the results showed good anaerobic treatability. Brown grease is already well-known as a treatable substrate; therefore, the economic effort by using a high-rate anaerobic digester will be more important. For thin stillage, a systematic design of incorporated anaerobic digestion process was analyzed; the cost analysis was also conducted; and the possibility of using this technique as an add-on system was discussed.

Keywords: anaerobic digestion, biogas, brown grease, corn-to-ethanol, paper mill waste

1. Introduction

Among the increasing energy consumption, natural gas (mainly methane) demand is increasing. Methane (CH_4) is created both in the natural environment and through various human activities. Derived from the decay of organic material, CH_4 is easily produced and abundant. Although in most cases CH_4 created from human activity cannot completely replace significant energy needs, it could lower the costs and decrease a facility's reliance on the electrical grid [1].

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Therefore, biogas as a sort of renewable energy is gaining more attraction throughout several nations of the world [2]. Biogas is the gaseous emission produced by the breakdown of organic matter in the absence of oxygen. It is a mixture of CH_4 and carbon dioxide (CO_2) along with other trace gases (H_2S, H_2, SO_2, etc.). CH_4, the primary component of natural gas (98%), makes up 55–90% by volume of biogas (depending on the source of organic matter and conditions of degradation). CH_4 is the only constituent of biogas with significant energy value. The inert diluents of CO_2 and nitrogen lowers the calorific content of the gas, while the corrosive nature of hydrogen sulfide (H_2S) wears down the anaerobic digester and pipes involved in the gas distribution. Biogas has a very wide industrial application range, includes heat combustion systems, motors, turbines, and fuel cells, and it can also be sold as a by-product separately. Biogas can be generated by anaerobic treatment of organic wastes. In the past decades, researchers have been conducting massive experiments on evaluating the conversion of miscellaneous wastes such as animal manure, municipal solid waste, energy crops, municipal biosolids, and food waste to biogas [3, 4]. In this chapter, three kinds of wastes were selected for investigation: bleaching and pulping effluent from paper mill, brown grease from food waste, and corn stillage from the bio-ethanol plant.

1.1. Introduction of anaerobic digestion (AD) process

Anaerobic digestion (AD) is the consequence of a series of metabolic interactions among various microorganisms. It occurs in three stages: hydrolysis/liquefaction, acidogenesis, and methanogenesis [3, 5]. In these three stages, complex organic materials are converted to CH_4 and CO_2 in the absence of O_2 *via* activity of several groups of anaerobic microorganisms. Firstly, fresh organic matter was hydrolyzed to soluble particles. Afterwards, soluble organic matter was biodegraded to volatile fatty acids (VFAs) and alcohols by a heterogeneous microbial population called acidogens. Finally, a limited number of organic compounds were used as carbon and energy sources and to be transferred to CH_4 by microbes called methanogens. AD process is an effective proven technology for handling and treating municipal or industrial wastes and effluents, for the generation of district heating and electricity supplies, as well as for clean environment.

1.2. Important operating parameters in AD process

During the AD process, many operating parameters must be controlled to optimize the microbial activity and keep the system efficiency stable and superior. Ideally, the performance of an AD process should be evaluated by observing those important parameters.

pH and temperature are important factors for keeping functional AD process. Generally, anaerobic process happens in neutral pH range (pH 6.5–7.6) [6], because anaerobic bacteria, especially the methanogens, are sensitive to the acid concentration within the digester and their growth can be inhibited by acidic conditions. Based on pH level, two temperature ranges, named mesophilic (30–45°C) and thermophilic (45–65°C), were commonly applied in industrial fields [7]. Redox potential (ORP) is a parameter to reflect changes in oxidizing or reducing agents; it represents the oxygen inhibition situation during the AD process. At the same time, the concentration of dissolved oxygen (DO) could also be monitored as an indicator of oxygen inhibition.

The concentration of specific volatile fatty acids (VFAs) and total alkalinity (ALK) can give vital information on the status of AD processes. VFA is an important intermediate product in the AD process, which should be converted to CH_4 finally, and proper amount of ALK is used to offset the excess VFA to keep the pH value at the stable level. System retention time, including hydraulic retention time (HRT) and solid retention time [(SRT), in solid digesters] may influence the system performance. A longer retention time comes with a higher organic mass removal, but it can also lead to possible VFA accumulation and decrease of system treatment efficiency. The required retention time for the completion of AD reactions varies with different reactor types, temperature, and waste composition.

Organic loading rate (OLR) is the measurement of the biological conversion capacity of the AD system. Feeding the system above, its sustainable OLR will result in low biogas yield due to the accumulation of inhibiting substances such as VFA [8]. Generally, OLR was calculated based on the concentration of chemical oxygen demand (COD) of volatile solids (VSs). The composition of OLR may contain biodegradable organic loading and refractory organic loading; this composition will affect the biogas yield and quality and the organic removal efficiency as well.

Biogas production is one of the main purposes of AD process. Tracking the biogas production is a widespread online measurement in AD control systems. A low biogas production may indicate accumulation of some inhibitive intermediate compounds. The measurement of CH_4 is important because it is the major energy output of the AD system. The concentration of tract gases such as H_2S in produced biogas reflects the current presence and degradation of sulfide-containing compounds. H_2S has a certain amount of toxicity; thus, its concentration needs to be cautious to not reach inhibiting levels when treating rich H_2S substrates.

1.3. Current research background

The technology of AD has developed in many aspects [8]. There are a lot of studies that use AD to treat different kinds of municipal, agricultural, and industrial wastes. Gunaseelan [9] has summarized the application of AD to over 100 kinds of wasted biomass to recover CH_4. Appels et al. [10] have applied AD technology in wastewater treatment plant (WWTP) to treat the waste-activated sludge and successfully recovered CH_4 from those discarded organic matters to save over 50% of the WWTP cost. Hansen et al. [11] have used a continuous stirred tank reactor to treat high-ammonia swine manure and obtained a CH_4 yield of 0.022–0.188 m^3-CH_4 kg-VS-1. Bouallagui et al. [12] and Zhang et al. [13] also applied a batch AD reactor to food waste. Angelidaki et al. [14] have defined the measurement protocol for biomethane potential (BMP). Furthermore, in the study of De Baere [15], the anaerobic treatment capacity of solid waste in Europe was over 1 million tons in the year 2000.

To develop and extend the application of AD technique to large-scale fabrication plants and industries, three kinds of industrial wastes were selected to be treated in a pilot-scale AD process. The waste substrates include paper mill effluents (comes from different paper making processes), brown grease (a kind of common food waste), and thin stillage (a kind of intermediate from corn grain-to-ethanol process). All the selected waste substrates come from real industries and practical plants. The reason for choosing these industrial wastes included, firstly, for these selected wastes, the traditional treatment technique seems inefficient. For example, for paper mill effluents, the traditional treatment technique is activated sludge process, which could

remove up to 90% of biochemical oxygen demand (BOD) but the chemical oxygen demand (COD) removal efficiency is just in the range of 20–50% [16–19]. Secondly, the United States Environmental Protection Agency (US EPA) have strict and specific policies about these industrial wastes, such as the US EPA CMOM (capacity, management, operation, and maintenance program, including grease control program), the US EPA final pulp and paper cluster rule and amendments, the US EPA CWA (Clean Water Act), the FOG ordinance/FOG management policy, and so on. That could be considered as the driving force to push industries to treat these wastes before discarding. Finally, these materials from industrial waste contain high organic content, which means they have the potential to be treated anaerobically as the energy feedstock.

2. Substrates

2.1. Paper mill effluents

Pulp and paper industry produces a large quantity of wastewater of high organic strength [20, 21]. Even with the most modern operations, about 60 m^3 of wastewater is generated for every ton of paper produced [22]. In the paper manufacturing processes, pulping and bleaching processes creates most of the wastewater streams [23, 24]. These wastewaters typically have high organic content (COD 800–4400 mg^{-1}) [24–26], high biological content (BOD 300–2800 mg^{-1}) [24–26], and high dye content (1200–6500 color unit) [24–26]. Several steps of treatment process were generally involved, including a primary clarification process to remove the suspended solids, a secondary treatment process to remove most of the air lagoons, and a final biological treatment process (aerobic) to remove the biological content (BOD5) [25, 26]. However, due to the recalcitrant chemical properties, the final effluent always still contains large amount of high molecular weight organic compounds [25].

Anaerobic treatment technique has not been widely used in the pulp and paper industry yet [27, 28]. One major advantage of anaerobic treatment is that the process is capable of treating high-organic strength streams that are not suitable for aerobic processes [30, 31]. Furthermore, it has the added benefit of lower treatment cost because the produced biogas can be diverted to energy generation [32]. Traditional treatment technique of energy-rich wastes should be avoided as far as possible mainly because of their low energy recovery efficiency [33], but the recovered biogas from anaerobic digestion process has a high methane content (60–80%) and can be directly used as fuel [34]. One of the current research issues is most of the evaluations for pulp and paper wastewater are only focused on synthetic waste stream in the lab-scale environment (reactor size 5–50 L) [29, 35–40], which makes the results less representative to large-scale industrial fabrications, a research utilizing pilot-scale system, and practical waste streams directly from the paper mill would be more helpful and relevant.

2.2. Brown grease

Using biogas as an alternative source of energy is gaining more attention globally in recent decades [41, 42]. There have been an increasing number of studies performed to evaluate the

conversion of waste streams such as animal manure, municipal solid wastes, energy crops, municipal biosolids, and food wastes to biogas [43–46]. In Europe, there are over 50 waste treatment plants using these materials to produce biogas [16, 45, 46]. For instance, ~15% of organic wastes are being converted annually in Germany [47]. The practice of converting wastes to energy provides a two-fold benefit of environmental protection and energy recovery.

Brown grease (BG) is a mixture consisting of trapped grease, sewage grease, and black grease collected in grease interceptors (traps) of restaurants and food industries [48]. In the United States, there are 1.84 million tons of BG produced every year [49]. Most collected BG eventually ends up in landfills. The landfill cost for BG is ~5 cents per pound [50]. This results in a very high direct disposal cost. In addition, the moisture content in BG can lead to soil and water pollution, making the soil sterile and unable to support plant life [51]. Because of these drawbacks, the European Union enacted a general ban on landfilling organic waste in 2005 [52]. An earlier study suggested that 14×10^6 m^3 of CH_4 could be produced in the United States annually by converting the generated BG into biogas [53]. This is a substantial amount of renewable bioenergy. Recovering the energy and eliminating the waste input to landfills yields both economic and environmental benefits [54, 55].

AD is a treatment process capable of producing biogas from organic wastes. The benefits of anaerobic digestion include smaller reactor size in terms of organic loading, lower air emissions, and a smaller amount of generated sludge compared to aerobic biological treatment [55]. Greasy wastes such as BG have been added as a lipid-rich cosubstrate in earlier AD studies for sewage sludge [56–58], municipal wastewater [59–61], and the digestible fraction of municipal solid wastes [62]. Typically, it is blended at 2–50% of the primary substrate's organic loading to improve the biogas yield and methane content [56–62]. However, higher lipid loading (>50% of the substrate) can cause long-chain fatty acid (LCFA) inhibitions [55, 61, 62], scum and foam formation, and fat clogging problems [56]. To our knowledge, there are few studies devoted to investigating the degradability and biogas production using BG alone.

2.3. Stillage from corn-to-ethanol process

Based on the increased demand of renewable energy, bio-ethanol as an alternative energy source was considered and has enormous economic and strategic advantages. In the past decade, the national total annual fuel-grade ethanol production has increased from 1.77 billion gallons (in 2001) to 13.95 billion gallons (in 2011). In 2005, 67% of this ethanol was produced from dry mill corn [63], and this percentage has kept on increasing because of the low cost of this technology [64].

In a typical bio-ethanol production process, corn mash has been fermented and distilled to produce high purity ethanol, and the fermentation residue is called whole stillage, which is centrifuged to produce wet cake (precipitate) and thin stillage (supernatant). About 50% of the thin stillage is recycled as backset. The remainder is further concentrated by evaporation to produce syrup and blended with dried wet cake to create a feed product known as distiller's dry grain with soluble (DDGS). The effluent of evaporation process was purified and recycled as water reuse.

Stillage handling is the most energy consuming process in the life cycle of corn to ethanol process. The drying and evaporation of stillage will take more than 35% of the total energy consumption [65], which makes the stillage treatment technique a main limitation of bio-ethanol making process [66]. Except for energy consumption, thin stillage is also a kind of high strength wastewater, which exhibits a considerable pollution potential [67]. Up to 20 l of stillage will be produced for each l of corn ethanol [68, 69], and the pollution potential of generated stillage can reach to a chemical oxygen demand (COD) of over 100 g L^{-1} [69].

The problems described in the previous paragraph have a significant negative impact on the industrial cost of the corn to ethanol process. Thus, a gate-to-gate life cycle assessment for thin stillage treatment was needed to provide a synergistic effect for energy recovery and cost saving. AD technique could be used to remove COD from thin stillage and also to convert the organic fraction of thin stillage into methane, which is a readily in-plant-usable energy source for ethanol industries [69]. Once this AD process was linked as a gate-to-gate life cycle to the ethanol production chain, the efficiency of the complete cradle-to-gate evaluation will be improved and the total cost will be reduced.

3. Biogas production for different substrates

3.1. Anaerobic treatability of paper mill effluents

As mentioned in Section 2.1, most of the current researches related to paper mill effluent treatment are focused on lab-scale experiments; therefore, an upscaling research is necessary to predict more comprehensive and representative results. In this study, a pilot scale sequential reactor system was introduced to evaluate the biotreatability of paper mill waste stream. Various waste streams from different paper making process were used, including liquid waste from bleaching process (DO), liquid discharge from alkaline extraction operating process (EOP), foul condensate from chemical pulping process (FC), and screw press liquor from dewatering operation process (SPL). For pH adjustment purpose, as well as improve the biodegradability, a small volume of wasted sugar water (SW) from a food processing plant was also blended in, as a co-digestion substrate used in this study.

The entire pilot system was established on a property outside of a pulp and paper mill, waste streams were obtained from the paper on a daily basis. The whole system consists of an equalization tank with a volume of 2.1 m^3 to blend all substrates equalized. Before sending to the packed-bed AD column, a 0.95 m^3 continuous stirred tank reactor was used for predigestion. The AD column is a cylindrical column with 1.07 m in diameter and 2.60 m in height, 85% of the AD column was packed with commercial ceramic bio-packing media. The discharge of the AD column will be fed to a 0.95 m^3 aerobic tank for final aeration, and the sample was taken on each tank on a daily basis.

The evaluation lasted for 156 days and was divided into six periods according to different feeds and operating conditions. Initially, the packed-bed column was operated as a downflow digester. From the 80th day, the flow direction was changed, and the AD column is operating

as an upflow flooded-bed reactor, the HRT is about 1.7–2.4 days. The feeding and operational characteristics are summarized in **Table 1**.

The evaluation lasted for 156 days, and was divided into six periods according to different feeds and operating conditions. Initially, the packed-bed column was operated as a down-flow digester, with a recirculation ratio of 5.0. Note for this stage, there is no water retention. Beginning with the 80th day, the AD column was operated upflow direction; the HRT was kept at 1.7–2.4 days. The entire operation is built on neutral pH range (6.92–7.60, see **Table 1**) and slightly mesophilic condition (T = 31.5–34.5°C, measured for effluent, see **Table 1**).

Table 2 listed the initial characteristics of each kind of substrates. The COD concentrations for each type of substrate ranged from 2800 to 4500 mg L^{-1}. In this study, the waste streams from paper mill are mostly in liquid phase and have relatively very low solid content (TS < 1 wt%, see **Table 2**). As mentioned above, a sugar water substrate was used to adjust the pH of the mixed substrate. The sugar water (SW) is a high organic content and slightly acidic substrate (COD = 408,000 mg L^{-1}, pH = 3.99). In this study, the sugar water was blended for about 0.5 wt%.

Figure 1 shows the plots between cumulative CH_4 production and the cumulative COD digested (mass basis) against the time axis. Note the system start-up and recovery during substrate changes were not included in the figure. There are totally six linear stages (Stage I–Stage VI, see **Figure 1**) that the system has a stable and consistent CH_4 production rate; these six periods were considered as steady state periods. The CH_4 yield was calculated as the ratio of the slopes of the two curves in **Figure 1**. The values range from 0.22 to 0.34 m^3-CH_4 kg-COD-1 for the substrates evaluated.

Based on the treatability study listed above, all waste streams are readily treatable. The anaerobic treatment removed 50–65% of substrate COD. Coupled with the aerobic treatment using a CSTR ASP, the overall COD removal efficiency was 55–70%. The application of anaerobic treatment has the potential of significantly improving the energy footprints of the pulp and paper industry.

Operating periods	**1**	**2**	**3**	**4**	**5**	**6**
Duration (days)	1–36	45–81	82–135	136–142	143–148	149–156
Substrate	FC + SW	EOP + SW	EOP + SW	EOP + DO + SW	EOP + DO	EOP + DO + SPL
Flow scheme	Downflow	Downflow	Upflow	Upflow	Upflow	Upflow
OLR (kg-COD/m^3d)	2.96 ± 0.70[1]	3.02 ± 0.38[1]	2.25 ± 0.81[2]	2.75 ± 0.70[2]	1.59 ± 0.48[2]	1.44 ± 0.48[2]
HRT (d)	—	—	2.44 ± 0.83	1.72 ± 0.51	2.12 ± 0.91	1.82 ± 0.55
pH in digester	6.92 ± 0.39	7.23 ± 0.11	7.42 ± 0.10	7.60 ± 0.48	7.25 ± 0.02	7.26 ± 0.09
Temperature of effluent (°C)	32.7 ± 2.6	34.3 ± 1.6	31.5 ± 3.1	34.5 ± 1.6	32.2 ± 1.8	33.3 ± 0.9

[1]Based on the volume of packing media.

[2]Based on total volume of the packed-bed digester.

Note: Day 37–44 was in maintenance and recovery mode.

Table 1. Pilot-scale feeding activities and conditions during six operating periods.

Parameters	Foul condensate (n = 11)*	DO filtrate (n = 8)	EOP filtrate (n = 4)	Screw press liquor (n = 13)
COD (mg L^{-1})	2973 ± 142	2886 ± 381	3901 ± 1940	4498 ± 2020
dCOD (mg L^{-1})#	2740	2445 ± 151	2890	609 ± 189
TS (mg L^{-1})	406 ± 104	4718 ± 522	4744 ± 532	8768 ± 7957
VS (mg L^{-1})	210 ± 14	2497 ± 346	1903 ± 136	3742 ± 1666
VS/TS ratio	0.53 ± 0.1	0.53 ± 0.02	0.4 ± 0.02	0.5 ± 0.1
TSS (mg L^{-1})	357 ± 577	868 ± 365	388 ± 127	4048 ± 1750
VSS (mg L^{-1})	339 ± 461	758 ± 339	296 ± 204	1997 ± 875
TSS/VSS ratio	0.83 ± 0.25	0.86 ± 0.04	0.79 ± 0.23	0.49 ± 0.06
Alkalinity (mg L^{-1} as $CaCO_3$)	205 ± 50	—	915 ± 263	—
pH	9.28 ± 0.18	5.19 ± 1.04	9.29 ± 0.29	8.44 ± 0.83
TN (mg L^{-1})	52.2 ± 4	4 ± 1.3	27 ± 43.3	2.3 ± 0.1
TP (mg L^{-1})	0.24 ± 0.09	6.33 ± 0.18	3.98 ± 5.22	0.41 ± 0.04
Conductivity (ms cm^{-1})	5 ± 5.7	—	4.6 ± 0.4	—
Sulfide (mg L^{-1})	52.2 ± 18.1	<0.5	<0.5	—
Sulfate (mg L^{-1})	<40	—	106 ± 23	—
Chloride (mg L^{-1})	—	—	335 ± 39	—

*n stands for sample size, i.e. testing times for raw industrial waste streams.

#dCOD stands for dissolved COD concentration.

Table 2. Initial characteristics of the evaluated substrates.

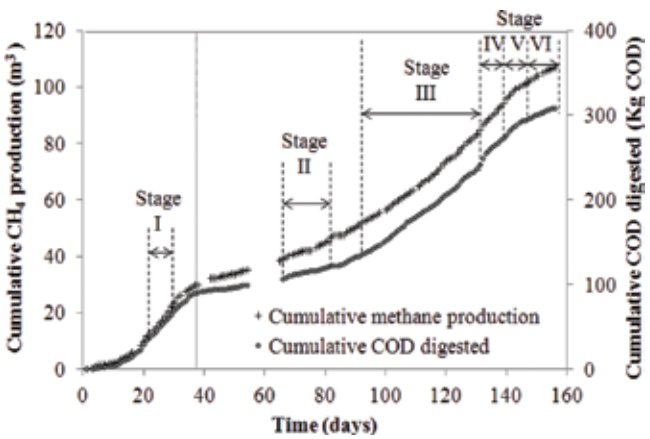

Figure 1. Cumulative CH_4 production at STP and cumulative COD mass digested during the evaluation period. There are six linear stages (I–VI) during which the data were used for calculating the CH_4 yield.

3.2. High-rate anaerobic digester to treat brown grease

The high-rate anaerobic digestion system employed in this work comprises three CSTRs and a clarifier: a balance tank (BAL), a facultative tank (FAC), an anaerobic digester (AD), and a final sedimentation tank (ST). The BAL and FAC are rectangular shaped tanks having an adjustable volume of 0.2–1.0 m^3. AD is a cylindrical tank with a total volume of 7.6 m^3 (1.6 m in diameter and 3.8 m in height) with adjustable reaction volumes of 4.3, 5.8, and 7.6 m^3. It has a Plexiglas window at the top for observing the mixed liquor in the digester. Various liquid waste streams from paper mill wastewater including foul condensate (FC) and screw press liquor (SPL) were blended as an effort to minimize the water use in the feed. The sedimentation tank (1.5 m^3) has a cylindrical shape with a conical bottom at 1:1 slope.

The evaluation period lasted for 343 days. Excluding the system start-up, maintenance, and feeding transition periods, process data were collected for 238 days. The evaluation was divided into five intensive evaluation periods (I–V). During each operating period, a steady stage (S1–S5) defined as a state with relatively consistent biogas production and organic removal) was selected for intensive measurement and data analysis. **Table 3** summarizes the evaluation schedule and the corresponding operating parameters in each stage.

	System start-up	I	II	III	IV	V
Date	4/13/11–7/26/11	7/27/11–8/7/11	8/8/11–10/24/11	10/25/11–12/7/11	12/8/11–2/29/12	3/1/12–3/21/12
Days of operation	/	1–12	13–90	91–135	136–217	218–238
Days of intensive evaluation[a]	/	1–12 (S1)	34–45 (S2)	107–133 (S3)	184–217 (S4)	218–238 (S5)
Sedimentation tank	No	No	Yes	Yes	Yes	Yes
Feeding	BG	BG	BG	BG + FC	BG	BG + SPL
Influent COD (mg L^{-1})	/	34,510 ± 2557	56,570 ± 3894	26,570 ± 6264	33,881 ± 9176	30,200 ± 1503
Influent VS (mg L^{-1})	/	13,965 ± 1262	23,937 ± 1625	10,139 ± 754	13,224 ± 3236	13,225 ± 1891
OLR[b]	/	2.0 ± 0.2	2.7 ± 0.3	0.8 ± 0.2	0.6 ± 0.2	0.9 ± 0.3
HRT[b]	/	7.3 ± 0.6	8.9 ± 0.9	15.2 ± 1.1	15.8 ± 1.9	11.0 ± 0.1
Activity	Seeding and initiating	Establish BG steady state	Add ST	Establish BG + FC steady state	Back to BG steady state	Establish BG + SPL steady state

[a]S1–S5 stands for five selected stages with intensive evaluation and stable data consistency.

[b]OLR and HRT in S1 and S2 were calculated based on AD only, while in S3–S5 were calculated based on AD + ST.

Data were collected in five different periods for analysis.

Table 3. Feeding characteristics and reactor configuration during the evaluation.

The characteristics of BG feedstock, FC, and SPL are shown in **Table 4**. Since the BG has an extremely high organic content (~1 kg-COD kg-BG-1, **Table 4**), the feeding stream was diluted to the range of 25,000–50,000 mg L^{-1} COD. FC and SPL have a relatively low COD concentration and solid content compared with BG (**Table 4**). In addition, their mild alkalinity (**Table 4**) effectively offset the mild acidity in BG. FC is a liquid substrate with relatively low solid content (TS = 400 mg L^{-1}), its major organic content is in the dissolved phase (dCOD is >90% of total COD, **Table 4**). SPL has a TS content less than 1.0 wt%. Its dCOD concentration is <20% of total COD concentration (**Table 4**), which indicated the major organic content is in the solid phase.

The daily biogas production during the evaluation is summarized in **Figure 2**. In S1, the biogas production is 5–6 m^3 d^{-1}. The biogas production (~7 m^3 d^{-1}) was higher in S2 because of the higher organic removal. During S3–S5, the average daily biogas production was lower than S1 and S2 since the system OLR was reduced. In S3, the COD removal efficiency was higher than S4 and S5, leading to higher biogas production (~5.6 m^3 d^{-1}) compared to that in S4 and S5 (~3.5 m^3 d^{-1}) (**Table 5**). The easily digested dCOD in FC may account for this increase. Another reason for the lower biogas production was the lower OLR applied in S4 and the slightly lower organic removal in S5. Generally, the biogas production trend in S3–S5 was

Parameters	Brown grease (BG)[a] ($\mu \pm \sigma$, n = 17)	Foul condensate (FC) ($\mu \pm \sigma$, n = 11)	Screw press liquor (SPL) ($\mu \pm \sigma$, n = 13)
COD (mg L^{-1})	910,634 ± 229,993	2973 ± 142	4498 ± 2020
dCOD (mg L^{-1})	/	2740 ± 125	609 ± 189
TS (mg L^{-1})	437,778 ± 91,348	406 ± 104	8768 ± 7957
VS (mg L^{-1})	372,111 ± 77,646	210 ± 14	3742 ± 1666
VS/TS ratio	0.85 ± 0.06	0.53 ± 0.1	0.5 ± 0.1
TSS (mg L^{-1})	/	357 ± 577	4048 ± 1750
VSS (mg L^{-1})	/	339 ± 461	1997 ± 875
VSS/TSS ratio	/	0.83 ± 0.25	0.49 ± 0.06
Alkalinity (mg L^{-1} as $CaCO_3$)	/	205 ± 50	/
pH[b]	6.51 ± 0.77	9.28 ± 0.18	8.44 ± 0.83
TN (mg L^{-1})	/	52.2 ± 4	2.3 ± 0.1
TP (mg L^{-1})	/	0.24 ± 0.09	0.41 ± 0.04
Sulfide (mg L^{-1})	/	52.2 ± 20.5	/
Sulfate (mg L^{-1})	/	<40	/
Moisture content (wt%)	56 ± 9	/	/

[a]Here BG stands for pretreated brown grease in solid phase, thus the unit of COD, TS, and VS is mg kg^{-1}.

[b]pH of brown grease was measured by suspending 100 g brown grease in 1 L tap water. Tap water has pH of 8.05 and alkalinity of 55 mg L^{-1} as $CaCO_3$.

Brown grease was used as the primary substrate and the other two liquid wastes were used as co-substrates in part of the evaluation.

Table 4. Substrate characteristics.

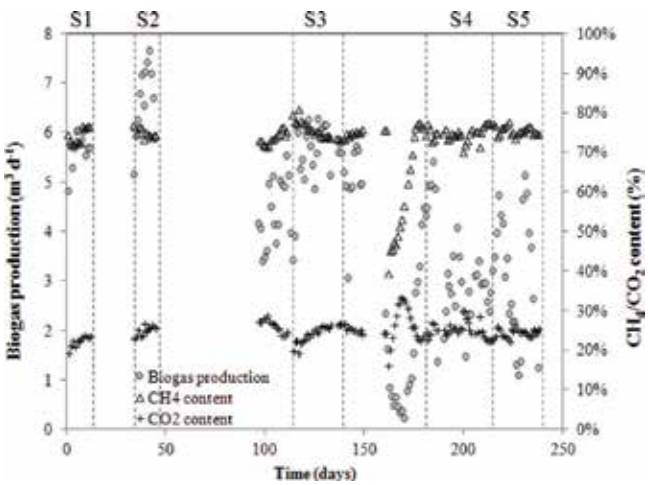

Figure 2. Measured daily biogas production and CH_4/CO_2 content.

consistent with the organic removal (**Table 5**), suggesting that the biogas production was not significantly affected by the addition of co-substrate.

The pilot-scale system produced biogas of excellent quality (75% CH_4 content), with a CH_4 yield in the range of 0.40–0.77 m^3-CH_4 kg-VS^{-1}. The addition of paper mill waste streams (FC and SPL) as co-substrate did not adversely affect the CH_4 yield. BG has the industrial potential to be anaerobically treated as a biofuel feedstock and there has been an ongoing commercial effort to build large-scale digesters using BG as the primary substrate. Using BG for biofuel recovery could serve as a profitable model for converting waste to renewable energy.

3.3. AD addendum unit to improve corn-to-ethanol process

The integrated anaerobic-aerobic system employed in this work contains three CSTRs, two transfer tanks, two clarifiers, and one serious CSTR aeration basin. The receiving tank (REC) is a rectangular CSTR with total volume of 4.5 m^3 (1.2 m width × 2.5 m length ×1.5 m height). Facultative tank (FAC) is a cylindrical CSTR with total volume of 0.35 m^3 (0.6 m diameter and 1.5 m height), the operating level is adjustable from 0.15 to 0.30 m^3. Anaerobic digester (AD) is a cylindrical CSTR whose volume is 10.4 m^3 (2.1 m diameter and 3 m height) and the operating level is 7.2–9 m^3. Two transfer tanks were respectively set between FAC and AD (0.04 m^3) and between AD clarifier and aerobic basin (0.15 m^3). The aerobic basin is rectangular whose volume is 2.5 m^3 (0.7 m width × 3 m length × 1.2 m height), three baffle plates were placed inside to divide the whole basin into four equal-sized serious tanks (0.6 m^3 for each). Two clarifiers were set after AD (anaerobic clarifier) and after aerobic basin (aerobic clarifier), respectively, both of them have a volume of 0.7 m^3.

Stillage feedstock was obtained daily from the ethanol plant. Generally, the raw stillage has a COD concentration of ~100 g L^{-1} [66]. In this study, to make the experimental results more comprehensive, the feeding concentration was adjusted to different ranges, which will be discussed later. Homogenized feeding substrate from REC was pumped to FAC for predigestion.

Stages	1	2	3	4	5	Typical range [a]
pH	7.34 ± 0.05	/	7.12 ± 0.08	7.10 ± 0.07	7.01 ± 0.17	6.5–8.5 [33][b]
T (°C)	36.0 ± 0.7	36.3 ± 0.7	34.3 ± 1.8	34.3 ± 2.1	37.9 ± 1.0	35–40 [33][b]
DO (mg L^{-1})	0.01 ± 0.00	/	0.06 ± 0.04	0.15 ± 0.05	0.10 ± 0.03	/
ORP (mV)	−209 ± 14	−228 ± 24	−243 ± 40	−247 ± 37	−263 ± 23	−400–−150 [31]
TN (mg L^{-1})	591 ± 83	409 ± 37	237 ± 74	314 ± 50	306 ± 46	60–1000 [32]
TP (mg L^{-1})	3.4 ± 2.4	1.5 ± 0.4	0.9 ± 0.4	2.3 ± 1.1	2.2 ± 0.4	6–50 [32]
Alkalinity (mg L^{-1} as $CaCO_3$)	3087 ± 282	/	1455 ± 457	2478 ± 291	2204 ± 222	1500–5000 [33][a]
VFA (mg L^{-1} as HAc)	274 ± 97	/	199 ± 76	394 ± 84	469 ± 378	<1800 [31]
COD removal efficiency (%)	42.1 ± 6.7	50.6 ± 5.8	73.8 ± 11.0	61.7 ± 12.3	53.5 ± 8.7	/
VS removal efficiency (%)	26.8 ± 7.9[b]	37.1 ± 4.3[b]	72.7 ± 7.4	57.9 ± 13.2	56.4 ± 9.9	/
CH_4 content (%)	74.3 ± 2.0	74.6 ± 1.0	75.9 ± 1.9	74.6 ± 1.8	75.4 ± 1.0	/
CO_2 content (%)	22.3 ± 1.3	/	23.9 ± 1.9	25.2 ± 1.8	24.2 ± 1.0	/
H_2S content (ppm)	38.2 ± 4.1	/	147.2 ± 34.8	185.2 ± 28.1	371.7 ± 127.6	/
CH_4 yield (m^3-CH_4 kg-VS^{-1})	0.40–0.49	0.58–0.77	0.49	0.48	0.45	0.11–0.42 [52][a], [72]

[a]Typical value of operating parameters including pH, T, ORP, TN, TP, VFA and alkalinity were based on the description of typical anaerobic digestion systems. Typical values of CH_4 yield were based on earlier literature.

[b]For comparison purpose, VS removal efficiency in S1 and S2 has not been corrected by biomass calculation.

Table 5. Anaerobic digestion operating parameters and system performance in five selected stages.

This predigestion process will initially introduce a series of microbial strains, and to eliminate potential process inhibitors as reported earlier [55–57]. Afterwards, the predigested substrates were pumped continuously to AD for digestion. The effluent of AD was transferred via gravity to clarifier for sedimentation. The upper flow of clarifier was pumped to aerobic basin for aerobic treatment.

The evaluation period lasted for 171 days. Excluding the system set-up period, process data were obtained mainly from day 100 to day 171. Two intensive evaluation periods (day 100–116, period I, and day 161–171, period II) were applied in the study. These two intensive periods were corresponding to two different scenarios of anaerobic treatment for stillage in this study. In period I (day 100–116), the system organic loading rate was 8.54 kg COD m^{-3} day^{-1}, the raw thin stillage (~120,000 mg L^{-1} as COD) was directly fed into the REC without dilution, and no aeration process was added. The purpose of this period was to maximize the methane production by anaerobic treatment. In period II (day 161–171), the system organic loading rate was reduced to 40% of the period I, which is 3.50 kg COD m^{-3} day^{-1}. The thin stillage was diluted to ~50,000 mg L^{-1} as COD before fed to the REC, and the aeration process was operated to further polish the AD effluent and to produce another economic product, single cell protein (SCP). These two scenarios will be discussed later.

The cumulative CH_4 production and digested VS in stage I and II are shown in **Figure 3**. The CH_4 yield was calculated as the ratio of the two slopes. The CH_4 yield was reported based on VS

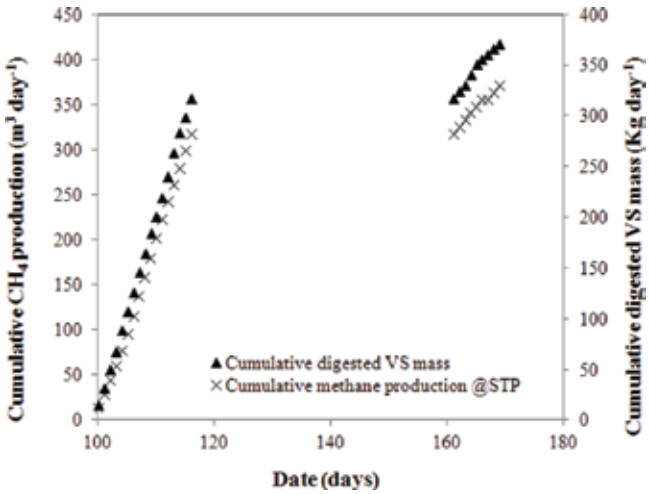

Figure 3. Cumulative CH_4 production at STP and cumulative VS digested during two intensive evaluation periods (I and II). CH_4 production yield was calculated based on the ratio of the two slopes.

removal because the organic loading of the stillage was mainly in the solid phase (VS 67,224 mg L^{-1}, COD 122,743 mg L^{-1}). The calculated CH_4 yield was 0.790 m^3-CH_4 kg-VS digested^{-1} (at standard temperature and pressure, STP). All the gas volumes mentioned hereafter have been normalized to STP) in period I, and 0.824 m^3-CH_4 kg-VS digested^{-1} in period II. Based on the measured VS concentration in thin stillage and the mean VS removal efficiency, the CH_4 yield could be converted to 0.507 m^3-CH_4 kg-VS fed^{-1} in period I and 0.414 m^3-CH_4 kg-VS fed^{-1} in period II.

AD process could be integrated to traditional corn-to-ethanol process as a treatment technique to the thin stillage product. The generated methane will partially replace the nonrenewable fuels and a large amount of energy could be saved from the removed evaporation process. Based on the lower heating value (LHV) of CH_4 (50.00 MJ kg^{-1}) and our CH_4 yield, the total energy output in this anaerobic system is 16.8 MJ kg-VS fed^{-1} in period I and 13.7 MJ kg-VS fed^{-1} in period II.

The energy saving is calculated based on several areas. In traditional process, the stillage treatment process including evaporation and syrup flash drying will take 38 MJ for each gallon of 95% ethanol produced [65], and DDGS treatment process will take 8.4 MJ [70]. In periods I and II, the evaporation process was removed to save 38 MJ, and DDGS productivity was decreased by 45.2 and 39.8% (SCP was considered as the same quality animal feed with DDGS); thus, the saved energy from these two processes was calculated and listed in **Table 6**. Energy recovered from produced methane was calculated based on the CH_4 yield and it is LHV. The consumed energy of applied anaerobic system was mainly focused on three mixing pumps in REC, FAC, and AD, respectively and the aeration activity in the aerobic system during period II. The energy cost of transfer pumps is negligible.

The power of the mixing pump was calculated based on the Camp-Stein equation for mixing with an impeller:

$$P = G^2 \mu V \quad (1)$$

Scenario		I	II	III
Description		Traditional EtOH process	High thin stillage feeding to maximum methane production, all the generated thin stillage will be treated	Low thin stillage feeding to produce methane and single cell protein, 40% of the generated thin stillage will be treated
Raw material		Corn grain 0.357 Bushel (12.6 L)		
Main product		95% ethanol 1 gallon (3.785 L)		
By-products	Biosolids (pound)	DDGS 6.43	DDGS 3.52	DDGS 3.52 and SCP 0.35
	Methane (m^3@STP)	N/A	0.568	0.464
Energy saved (Megajoule)	From stillage treatment process	N/A	38	38
	From operation of applied AD system	N/A	−18.3 from three mixing pumps	−4.5 from three mixing pumps, and −0.02 from air diffuser
	From DDGS treatment process	N/A	3.8	3.4
	From methane recovered	N/A	18.7	15.3
	Total	N/A	42.2	52.2
Industrial cost saving (US cents)	From power saved by stillage treatment process	N/A	68.7	68.7
	From power saved by DDGS treatment process	N/A	6.9	6.1
	From operation of AD system	N/A	−33.1	−8.2
	From methane produced	N/A	9.9	8.2
	From biosolids produced	N/A	−40.2	−35.2
	Total	N/A	12.2	39.6

Three scenarios (traditional, high thin stillage feeding, and low thin stillage feeding) were applied.

Table 6. Summary of energy and industrial cost saving in traditional ethanol making process and integrated processes for producing one gallon of 95% ethanol.

where P is the power requirement (W), G is the average velocity gradient (S^{-1}), μ is the dynamic viscosity (N S m^{-2}), and V is the reactor volume (m^3). In this study, the applied G was a typical value in rapid mixing operations reported by Metcalf and Eddy [72], which is 1000 S^{-1}. μ was water dynamic viscosity at 60°C, 4.66×10^{-4} N S m^{-2}. V was calculated based on the flow rate (1.9×10^5 L h^{-1} thin stillage in period I and 7.6×10^4 L h^{-1} thin stillage in period II) and the applied HRT. HRT in the system is 5 days for REC, 1.5 days for FAC, and 12.6 days for AD in period I. In period II, the HRT for REC and FAC was kept the same, the HRT for aerobic basin is 1 day. To

save the dilution water usage and the operation cost, generally the system will use less flow rate rather than dilution in real industries. Thus, the applied HRT in period II should be the calculated result, which is mentioned in kinetic analysis section (5.34 days). The calculated energy consumption in mixing pumps is listed in **Table 6**. For each pump, a 70% pump efficiency was assumed.

The power of the aerator (mostly an air diffuser) is the main operation cost of the aerobic section. The power requirement was estimated based on the reported typical energy requirement form Metcalf and Eddy [72], which is 30 kW/10^3 m^3, the estimated result is shown in **Table 6**. To sum up, compared with the traditional ethanol making process, the anaerobic integrated process could save 42.2 MJ (period I) or 52.2 MJ (period II) for each gallon of 95% ethanol produced.

The calculation of industrial cost saving was similar to the energy saving. The power cost saved by thin stillage treatment process was calculated based on electricity (price based on US EIA report 2013). The operation cost of anaerobic system was calculated based on the energy consumption. The price of CH_4 comes from US EIA report 2013, and the price of DDGS comes from USDA livestock and grain market report (2013). The price of the SCP was assumed to be the same with DDGS. Since the anaerobic system cost and DDGS productivity reduction is the capital of the integrated system, in **Table 6**, they were shown in cash-negative format. After the calculation, the integrated system saved 12.2¢ (period I) or 39.6¢ (period II) for each gallon of 95% ethanol produced. Period II has a higher cost saving because the system applied in period II has just treated 40% of the generated stillage; thus, the energy consumption was lower. For a typical ethanol plant with 100 million gallon 95% ethanol yr^{-1} productivity, by applying this AD integrated system, the cost saving of the plant could reach $ 12.2 million by completely treating thin stillage with AD, or $ 15.8 million by partially (40%) treated. This amount is higher than the reported amount ($ 7–17 million, most likely 10 million) in the study of Schaefer and Sung [71] because the gate-to-gate life cycle assessment was more comprehensive in this study.

By changing the influent condition, two different scenarios of anaerobic digestion were studied in this research. For each gallon of 95% ethanol produced, when thin stillage was fed directly to the anaerobic digester without dilution, the produced CH_4 will be 0.568 m^3 at STP, system energy saving was 42.2 MJ, and industrial saving will be 12.2 cents compared with traditional dry mill process, which means a typical ethanol plant could save 12.2 million dollars per year. When thin stillage was partially (40%) fed to a smaller sequential anaerobic-aerobic system, the produced CH_4 will be 0.464 m^3 at STP, and system energy saving was 52.2 MJ. The industrial saving will be 39.6 cents, which means a typical ethanol plant could save 15.8 million dollars with this 40% of thin stillage. This study shows that thermophilic AD is a better use of thin stillage and is applicable to practical dry mill ethanol plants.

4. Benefit of anaerobic digestion process in biofuel recovery

In this chapter, three kinds of waste streams from real industries were selected to investigate their anaerobic treatability, economic feasibility, and applicability to the practical plants. Generally, these selected waste streams were applied to a pilot-scale anaerobic-aerobic biological treatment system to convert their organic fraction into renewable energy in the form of CH_4.

For paper mill effluents, the improved COD removal efficiency (55–70%) and the substrate utilization rate (0.28–0.46 d^{-1}) indicated that it is anaerobically treatable. The CH_4 yield (0.22–0.34 m^3-CH_4 kg-COD^{-1}) showed that the application of anaerobic technique has the potential to improve the energy footprints of the pulp and paper industry. For brown grease, the COD removal efficiency had somewhat been sacrificed (58%) to the maximum methane yield (0.40–0.77 m^3-CH_4 kg-VS^{-1}). The obtained high CH_4 yield showed that using brown grease for biogas production could serve as a profitable model for converting waste to renewable energy. For thin stillage, based on the high CH_4 yield (0.464–0.568 m^3 CH_4 @ STP per gallon 95% ethanol produced) and reducing energy consumption, a typical ethanol plant (producing 100 million gallon 95% ethanol per year) could save 12.2–15.8 million dollars per year, which indicated anaerobic digestion is a better use of thin stillage and is applicable to practical dry mill ethanol plants.

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Chapter 14

Jatropha Curcas L. Biomass Waste and Its Utilization

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Additional information is available at the end of the chapter

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Abstract

Jatropha curcas L. is cultivated for its oil utilization as fuel feedstock. This main purpose is achieved with the biomass waste after oil extraction. The biomass wastes are leaf and stem from pruning, fruit hull, seed husk, and oily-cake. This paper discusses the utilization of the waste in order to achieve zero waste of jatropha and develop the jatropha utilizations. Jatropha waste is also utilized as fertilizer, briquettes, adsorbent, resin, and bioactive compost. It can also be utilized as feedstock for production of polymer composite, combustion for gasifier, biogas, liquid oil, and dye. These wide utilizations make jatropha very suitable for biofuel proposes.

Keywords: biomass, Jatropha cake, utilization, environment, waste management

1. Introduction

Jatropha curcas L. is one of growing interest in bioenergy resource. It belongs to the family Euphorbiaceae. It is perennial bush, easy to grow in tropical and subtropical regions, resistance to drought and produce crimson color flower (**Figure 1**). It is native to Mexico and Central America, but can be suitably cultivated in tropical and subtropical areas such as South East Asia. It is a small tree with height of about 6 m. *J. curcas* L. is planted principally as a hedge to prevent crop plant from the cattle, sheep and goats. Currently, jatropha is popularized as bioenergy plant due to high content of oil in the seeds. The oil from this crop is a promising alternative in biodiesel production. Utilization of jatropha oil as biodiesel feedstock is increasing as its oil is non-edible and does not compete with food crops. It helps the food security in biodiesel production. Some advantages using it as biodiesel resource are continuity resources (renewable) and ecofriendly energy.

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Figure 1. *Jatropha curcas* L.

The oil can be converted to biodiesel through transesterification easily [1]. Utilization is associated with the biomass that is left behind after the oil extraction. Large number of waste impacts the environment. In this chapter, we present several ways of processing the waste to make it a valuable product.

2. The biomass waste of *Jatropha curcas* L. oil production

The waste biomass from oil production is shown in **Figure 2** below.

Waste biomass from *J. curcas* L. plantation and its oil production has nutrient and mineral content as presented in **Table 1**.

Although there are small amount of nutrients in leaves but these compounds are good for the soil fertility. When the plant sheds off its leaves, they decompose and their minerals go back

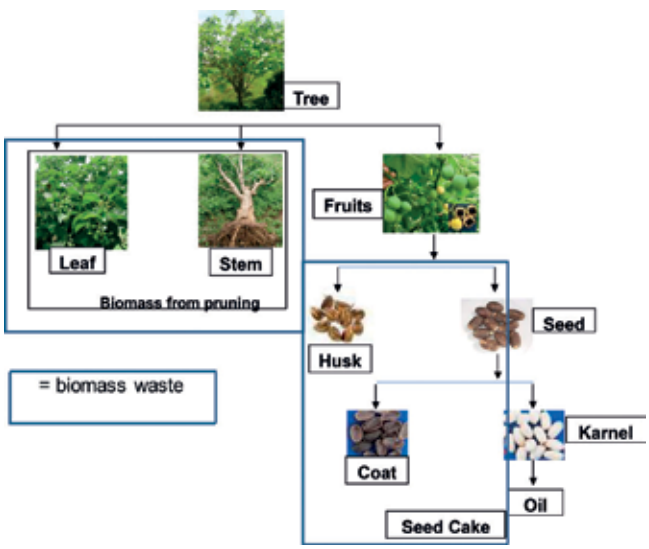

Figure 2. Biomass waste from *Jatropha curcas* L. oil production.

Nutrients	Leaf[a]	Wood[b]	Fruit			Seed cake[b]
			Hull[b]	Seed husk[b]	Kernel[c]	
N (%)	6.40	3.34	2.15	0.19	4.39	4.90
P (%)	0.34	0.09	0.05	0.01	1.10	0.90
K (%)	2.45	2.87	0.73	0.31	0.94	1.75
Ca (%)	1.40	0.30	0.44	0.28	0.34	0.31
Mg (%)	0.53	0.26	0.30	0.06	0.53	0.68
S (%)	0.19	0.12	0.10	0.01	0.21	0.24
Zn (ppm)	28	55	22	1	47	55
Fe (ppm)	168	99	40	8	73	772
Cu (ppm)	6	2	11	3	18	22
Mn (ppm)	117	605	25	13	28	85
B (ppm)	71	10	4	2	5	20
Na (ppm)	808	134	28	20	17	—

Refs: [a]Pacheco et al. [2]; [b]Saturnino et al. [3]; [c]Wan et al. [4].

Table 1. Nutrient composition of *Jatropha curcas* L. plant.

to the soil. The fruit hull constitutes 30% of the fruit. Calorific value of fruit hull is dependent on the humidity. With humidity 15%, the calorific value being considered is 11.1 MJ/kg [5]. Calorific value will increase with increase in its humidity.

Considering nutrients and minerals above (**Table 1**), waste biomass from *J. curcas* L. has its own peculiarity. Jatropha seed cake has proven as fertilizer [6]. Leaf has nutrient and mineral content higher than other parts especially seed cake, while stem also has potential nutrients and minerals too. Seed cake cannot be used as animal feed because it contains toxins such as phorbol ester curcasin and cursin [2].

The leaves, stems, fruit hull and seed oilcake could be used as potential fertilizer. Jatropha hull has very high ash content, around 13% [7] and ash will melt at temperature above 750°C. Gasifier operates around 900–1000°C. Hence, the hull is not suitable for being used as a fuel for gasifier. It creates melted ash that can deposit in the bed fluidization. However, the hull can be converted to biogas using biological conversion process.

Theoretically, the best use of press cake is for energy purposes first, and then as a fertilizer. Even when digested to obtain biogas for energy, the nutritional value remains intact. The presence of milky substance (sap) in the stem makes it hard to burn the stem without drying it first.

The largest biomass from jatropha is from seed cake, which is around 59.24%w of fruit (wet basis), fruit hull 22.05%w of fruit (wet basis), seed husk 27.16%w of fruit (wet basis) [8]. Around 95% oil can be extracted using chemical extraction, while only 85% can be obtained by mechanical extraction [9]. It means the seed cake still contains 5–15% oil. Considering the amount of energy in jatropha seed cake, it should first be utilized for energy purposes such

as biogas production and second purpose may be as a fertilizer. The seed cake of jatropha can be used as organic fertilizer because it still has nutrients and mineral needed by the plant. Jatropha seed cake could be used in combustion process but considering air emission produced by burning, its use as a fertilizer would be a better choice.

2.1. Jatropha as a fertilizer (green manure)

In this section, use of jatropha as a fertilizer or green manure has been presented. Green manure can reduce loss of nutrients from soil and give sustainable nutrient supply for long period as compared to chemical fertilizer. Chemical fertilizers can get solubilize in water and will drift together with water especially during the rainy seasons. Patolia et al. [10] reported after 2 years that N_{45} application could increase the dry matter compared to N_o application. Ghosh et al. [6] also reported that dry matter of sandy loam soil could be increased upto 120% by adding 3 tons of seedcake/ha as manure. *J. curcas* L. seedcake contain 2% N, 1.2% P_2O_5 and 1.4% K_2O.

2.2. Biogas production

Biogas (methane) is produced by anaerobic digestion. Biogas has wide utilities as it can be applied directly for cooking, heating and stationary engine operation in dual fuel mode. The biogas is purified, compressed and stored in cylinder as CNG (Compressed Natural Gas) for automotive transport purposes, power generation as well as in agricultural unit operation.

Jatropha seed cake has good potential as biogas feedstock due to confer 60% higher biogas and also better calorific value than the cattle dung [11]. Chandra et al. [11] reported jatropha seed cakes have biogas generation potential in the range of 220–250 and 240–265 L/kg of cake respectively (under mesophilic temperature range of anaerobic digestion). C, H, and N composition was 48.8; 6.20 and 3.85% with C/N ratio of 12.70. The methane content of biogas derived from non-edible oil seed cakes has been found to range between 65 and 70% against 55% from the cattle dung. The best dilution ratio of cake is at 1:4 (cake:water) for *J. curcas* seed cakes. Production of biogas from *J. curcas* seed cakes is one of easy ways for waste management. It can also be used to fulfill energy need for rural areas. According to Kumar [12], in India ~2550 million cubic meters of biogas has been produced from 10.2 lakh metric tons of *J. curcas* seed cakes.

Visser and Adriaans [13] studied anaerobic digestion of *J. curcas* press cake. The cake was from cold pressing jatropha seed including the husk. Digestion was carried out at temperature 20°C, pressure 1 bar during 60 days. Jatropha cake that pressed with nozzle (aperture size of 7 mm) contain 33% hull produced a cumulative biogas yield of 0.95 m^3/kg dry matter, with 85% carbon conversion. Gunaseelan [8] reported energy biomass from part of fresh *J. curcas*. The feedstocks were dried at 60°C before use and subsequently grained to become 2 mm mesh. Other parts of *J. curcas* plant as a biogas feedstock is presented in **Figure 3**.

Figure 3 suggests not only jatropha cake, but also all parts of the jatropha plant could be utilized for producing biogas (methane). The highest yield was achieved from seed kernel 0.969 L/g Volatile Solid added. If compared to the yield from de-oiled cake, which is just 0.23 L/g VS added, it shows oil has potential with 0.739 L/g Volatile Solid added.

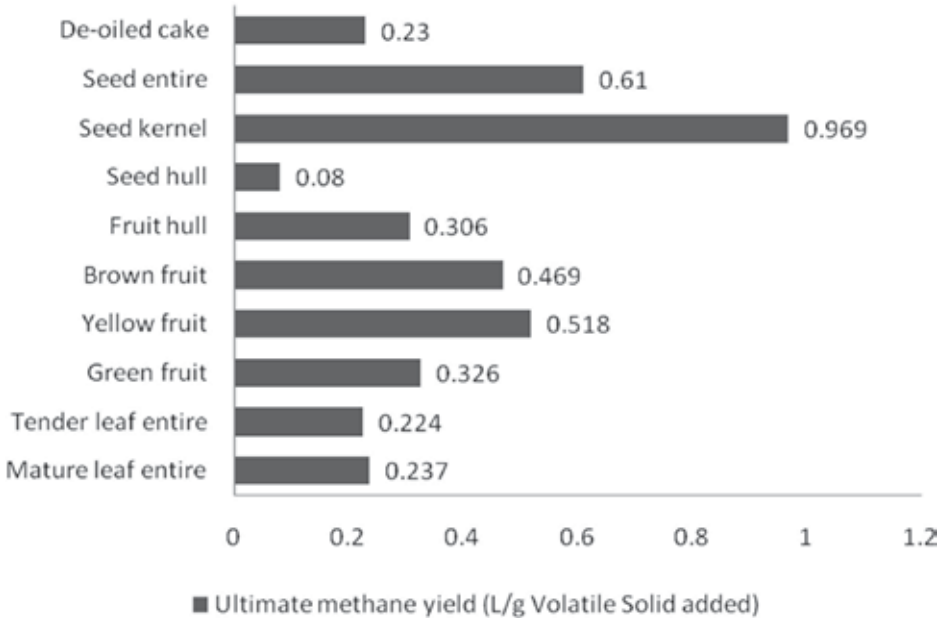

Figure 3. Production of methane from parts of *Jatropha curcas* L. [8].

2.3. Feedstock combustion for gasifier

Vyas and Singh [14] reported that jatropha seed husk could be used successfully as feedstock for open core down draft gasifier, although the gasifier has to be induced by 1 kg charcoal and 3.2 kg wood. Jatropha seed husk analysis contains 3.97% dry basis ash, 71.04% dry basis volatile matter and 24.99% dry basis fixed carbon. Gasifier was able to run on jatropha seed husk for 340 minute at 2 flow rates (4.5 and 5.5 m^3/h) without any problems. Gasification efficiency of 68.31% was achieved at gas flow rate of 5.5 m^3/h and biomass consumption rate of 2.2 kg/h. Gasification efficiency can possibly be increased 2.35% by increasing 1 m^3/h of gas flow rate and by adding 0.4 kg/h biomass consumption rate.

2.4. Bioactive compost production

Bioactive compost can be produced from *J. curcas* hull. Sharma et al. [15] produced bioactive compost from jatropha hull biomass by using lingocellulolytic fungi. Bioactive compost is for increasing the added value compared to ordinary manure. Within 1 month, carbon to nitrogen (C/N) ratio of hull decreases from 66.93 to 12–16. From C/N ratio point of view, composting of jatropha hull in 1 month has indicated a better composition of bioactive compost. However, it takes nearly 4 months for complete compost maturation. After 4 months, phytotoxicity of compost can be reduced, thus compost will be ready to use. Bioactive compost from jatropha hull is alkaline, so it is suitable for acidic soil. It can balance the pH of the acidic soil.

2.5. Liquid oil from pyrolysis of jatropha cake

Pyrolysis is a thermal decomposition without oxygen that converts biomass into solid (charcoal), liquid (tar and other organics, such as acetic acid, acetone and methanol) and gaseous products (H_2, CO_2, CO). *J. curcas* seed cake is lignocellulosic biomass that consists of cellulose, hemicellulose and lignin contents. Lignocelluloses decompose at different temperatures. Hemicelluloses decompose at temperatures of 470–530 K. Meanwhile, cellulose decomposes at the temperature range between 510 and 620 K and lignin being the last component to pyrolyze at temperatures of 550–770 K [16]. Thus, pyrolysis of *J. curcas* seed cake is being carried out at elevated temperature [17]. The liquid oil as pyrolysis product has a reddish-brown color with an irritant odor [18].

J. curcas seed cake has empirical formula of $CH_{1.53}O_{0.4}N_{0.007}S_{0.0008}$ with H/C ratio 1.53. The gross energy value of seed cake was found to be 17.7 MJ/kg. Pyrolysis of seed cake can obtain maximum yield of oil (31.17% by wt) at 500°C [17]. Fast pyrolysis without catalyst (thermal pyrolysis) produced wide range of organic compounds. Purification needs to be addressed such as liquid–liquid extraction into aqueous and organic phases. This oil is considered as another source for biofuel. The sludge obtained after biogas can be used as fertilizer.

2.6. Briquettes

Briquetting can be used as one of the solution to handle the jatropha press cake. The seed cake still has energy content of around 25 MJ/kg [18]. Since the briquette produces a lot of smoke, it is better to use the product outdoors. It can be used indoors with proper ventilation [19]. The carbonized process should be maintained at average time 10 minutes per 5 kg in order to prevent cake from complete combustion. Complete combustion will produce ash instead of charcoal briquette. Cassava and corn starch were used as binders for bonding the carbonized cake. Pandey et al. [20] reported that using 10% binder (cassava and corn starch) for pressed cake produced good jatropha charcoal briquette. The briquette from jatropha seed cake has emission of CO and CO_2 are lower than briquette from wood. However, briquette from jatropha seed cake has much higher emissions of NO and NO_2 than wood pellet. It is because of the presence of residual oil and higher nitrogen content [20].

2.7. Dye productions

Dye can be produced from leaves, stem, bark, wax, and roots of jatropha plant [21–23]. The leaves and stem of jatropha produced brownish dye when boiled in water as an extraction process [20]. It is based on experience of Tharu tribes, Devipatan division. The dye has no irritative and/or toxic effect on skin. Bark contains tannins that produce purple color in dye production [22]. Meanwhile, combining wax and bark produced dark blue dyes. Roots of jatropha could produce yellow dye. The dye is used to dye domestic threads, ropes and clothes during ceremonial occasions [23].

2.8. Polymer composite production

Polymer composite has strong and stiff fibers in a matrix which is weaker and less stiff compared to fiber. The quality of polymer composite is determined by their characteristic properties

such as modulus of rupture (MOR), modulus of elasticity (MOE). The cake of jatropha and the shell were utilized for polymer composite production by Hrabě [24] and Raju [25]. Jatropha cakes were in two forms, *viz*., continuous (as epoxy adhesive) and discontinuous phase (as reinforcing particles). The filler moisture was 4.59 ± 0.22% wet basis. Raju [25] produced polymer composite by using jatropha shell as reinforcement and reported that 20 wt% of shell has maximum MOR of 40.57 MPa and 60 wt% of shell as reinforcement has maximum MOE of 8.204 GPa.

2.9. Adsorbent

Adsorbent (activated carbon) was produced by using jatropha pods (hull). It is used as an adsorbent for the removal of reactive dye, Remazol Brilliant Blue R (RBBR) [26]. It adsorbed 24.5 g dye by using 0.1 g activated carbon from jatropha pods. Further, the adsorbent removed almost 245 g dye per g activated carbon. Another study [26] reported that the hull of jatropha produced active carbon that had the potential to remove heavy-metal ions, such as zinc and cadmium from waste water and dye (malachite green) and has shown a remarkable adsorption capacity. Its adsorption capacity can reach up to 11.89 mg/g for cadmium removal. Biosorption of Zinc (II) from waste water was also supported by Abidin et al. [27] by using aqueous solution of jatropha press cake (kernel part only). Abidin et al. [27] reported that ~40 mg/L Zinc (II) was removed from 1 L waste water by using 0.5 g jatropha press cake in about 100 minutes. It revealed that jatropha has potential for adsorbent production either in the form of activated carbon or deoiled-press cake itself without any prior treatment [27].

2.10. Resin

Resin is one of the essential chemical in polymer industries. One of resins, alkyd, is widely applied in coatings and paint industries. Usually, resin is produced by using palm oil and other edible oil such as rapeseed, coconut and soybean oil. However, utilization of edible oil affects food security. Thus, non-edible oil such as jatropha oil has potential for substituting the edible oil in resin production. The jatropha seed oil-based epoxy acrylate synthesized has a potential to be used in formulations for the pre-polymer resin for UV curable coating applications [28, 29]. Further, phenolic resin derived from jatropha seed-husk lignin is used as phenol substitute.

3. Conclusion

Jatropha curcas L. is a potential biofuel plant especially in tropical and subtropical land. It is resistant to environmental factors such as low nutrient and low moisture soil. All of the plant parts and also its waste have multipurpose uses to generate valuable commercialized product. Moreover, high non-edible oil content in jatropha seed makes this plant popular as a renewable resource for bioenergy and food security. Thus, this plant is considerable rising star for solution of better future in the energy security demand.

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The worldwide consumption of fossil fuel continues to increase at unsustainable levels, which will lead to progressive scarcity, if immediate and innovative measures are not taken for its sustainable use. This scarcity necessitates the development of renewable and sustainable alternatives for fossil fuels. A possible solution to today's energy challenges can be provided by biofuels. This book intends to provide the reader with a comprehensive overview of the current status and the future implications of biofuels. Diverse and aptly covered comprehensive information in this book will directly enhance both basic and applied research in biofuels and will particularly be useful for students, scientists, breeders, growers, ecologists, industrialists and policy makers. It will be a valuable reference point to improve biofuels in the areas of ecologically and economically sustainable bioenergy research.

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