IntechOpen

Biodegradation and Bioremediation of Polluted Systems

New Advances and Technologies

Edited by Rolando Chamy, Francisca Rosenkranz and Lorena Soler

BIODEGRADATION AND BIOREMEDIATION OF POLLUTED SYSTEMS - NEW ADVANCES AND TECHNOLOGIES

Edited by **Rolando Chamy, Francisca Rosenkranz** and **Lorena Soler**

Biodegradation and Bioremediation of Polluted Systems - New Advances and Technologies
http://dx.doi.org/10.5772/59459
Edited by Rolando Chamy, Francisca Rosenkranz and Lorena Soler

Contributors

Qiuyan Yuan, Mehdi Hassanshahian, Jhosané Pagés-Díaz, Ileana Pereda-Reyes, Ilona Sárvári Horváth, Fan Zhang, Ester Gouveia, Fernanda Vaz, Ednaldo Amaro Santos-Filho, Thatiana Stamford-Arnaud, Newton Stamford, Maria Aparecida Mouco, Suzyane Silva, Andrea Bandeira, Ana Christina Brasileiro-Vidal, Silvany Araújo, Mohamed Hafidi, Loubna El Fels, Mohamed Zamama, Inaya Lima, Simone Pennafirme, Mirian Crapez, Ricardo Lopes, José Augusto Pires Bitencourt, Iheoma Mary Adekunle

Notice

First published in Croatia, 2015 by INTECH d.o.o.
eBook (PDF) Published by IN TECH d.o.o.
Place and year of publication of eBook (PDF): Rijeka, 2019.
IntechOpen is the global imprint of IN TECH d.o.o.
Printed in Croatia

Legal deposit, Croatia: National and University Library in Zagreb

Additional hard and PDF copies can be obtained from orders@intechopen.com

Biodegradation and Bioremediation of Polluted Systems - New Advances and Technologies
Edited by Rolando Chamy, Francisca Rosenkranz and Lorena Soler

p. cm.

ISBN 978-953-51-2238-8

eBook (PDF) ISBN 978-953-51-5418-1

Meet the editors

Rolando Chamy obtained his professional degree in Biochemical Engineering from Pontificia Universidad Catolica de Valparaíso (PUCV), Chile, in 1982. He obtained his PhD in Chemical Engineering from the University of Santiago de Compostela, Spain, in 1991. The same year, he became full-time Professor in the School of Biochemical Engineering at PUCV. He also participated in the creation of the Biotechnology Center (NBC) of PUCV. Currently, Dr. Chamy serves as the Director of NBC and is the main researcher of the bioenergy unit of Fraunhofer Chile Research. His research interests are in the fields of bioenergy, biofuels, environmental engineering, and climate change. Dr. Chamy has authored and co-authored more than 200 scientific publications.

Francisca Rosenkranz obtained his professional degree in Biochemical Engineering from Pontificia Universidad Católica de Valparaíso (PUCV), Chile, in 2009. She obtained her PhD in Biotechnology from Technical University Federico Santa María and Pontifica Universidad Católica de Valparaíso in collaboration with the University of Santiago de Compostela, Spain, in 2013. Currently, Dr. Rosenkranz serves as the Technical Manager of NBC and as the Technical Coordinator of the bioenergy unit of Fraunhofer Chile Research.

Lorena Soler Hyde obtained his professional degree in Biochemical Engineering from Pontificia Universidad Catolica de Valparaíso (PUCV), Chile, in 2006 and her PhD in Biochemical Engineering from the same university in 2013. Dr. Soler worked as Project Engineer for the Interdisciplinary Center for Energy of PUCV in projects related to bioenergy and biofuels. Her PhD thesis was related to the enzymatic hydrolysis of lignocellulose for the production of fuel ethanol and the recycling of enzymes. In 2014, she joined the group of Dr. Chamy at NBC as Academic Assistant. Her research interests are in the fields of bioenergy, biofuels, environmental engineering, and biocatalysis.

Contents

Preface

Pollution of groundwater and soils due to human activities has been an increasingly major concern. Many compounds produced by industrial or agricultural activities, such as pesticides, might accumulate and become serious hazards to human and animal health. For that reason, different research groups worldwide have been studying new technologies to improve the degradation of those hazardous components through the use of the natural abilities of certain microorganisms to degrade or transform those polluting substances.

This book presents a collection of recent research works aimed at the utilization of different microorganisms to degrade organic and inorganic pollutants from water and/or soil, under different conditions, and addressing some of the major problems in biodegradation, such as the toxicity of pollutants to microorganisms and the impact of the presence of different types of pollutants in the degradation performance. Also, some novel technologies in the field are presented.

Rolando Chamy
Biochemical Engineering School,
Pontifical Catholic University of Valparaíso and Núcleo Biotecnología Curauma Center
of the Pontifical Catholic University of Valparaíso,
Chile

Francisca Rosenkranz
Núcleo Biotecnología Curauma Center of the Pontifical Catholic University of Valparaíso,
Chile

Lorena Soler
Núcleo Biotecnología Curauma Center of the Pontifical Catholic University of Valparaíso,
Chile

Section 1

Biodegradation of Organic Pollutants in Solids and Wastewater

Chapter 1

Fungi in Landfill Leachate Treatment Process

Yanan Ren and Qiuyan Yuan

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60863

Abstract

The landfill leachate has high concentration of COD, ammonia and other recalcitrant composition compounds. The amount of eachwhich is mainly largely dependent on the age of the landfill. The conventional leachate treatments can be classified as chemical-physical treatments and biological treatments. Using fungi to treat leachate is an emerging research topic. Fungi, with their excellent recalcitrant compound degradability, have been used to treat industrial wastewater that contains toxic or recalcitrant compound. Due to the complex composition and toxicity of landfill leachate, fungi have showed shown better removal efficiency in terms of COD, toxicity and color removal than the conventional leachate treatment. White rot fungi species and yeast are so far the two species that have been studied in treating landfill leachate. Future research should be extended to the other fungi species as well asand also on the impact of ammonia in landfill leachate on the fungi treatment process.

Keywords: Fungi, landfill leachate, recalcitrant compound, COD removal

1. Introduction

Landfill leachate is produced by the seeping of liquids through landfilled waste. Rain water or melted snow percolating into the waste, as well as the original water content or humidity of the waste itself and the degradation and compaction of the organic fraction, all contribute to the generation of leachate[1,2]. Landfill leachate contains dissolved organic matter, inorganic macro components, heavy metals, and xenobiotic organic compounds such as halogenated organics. These contaminants play an important role in groundwater and soil pollution. Due to the complexity of the pollutants in the leachate, the treatment of landfill leachate is

complicated, usually requiring various processes to reduce COD, nitrogen, and phosphorus all of which make the treatment of landfill leachate expensive.

The conventional landfill leachate treatment includes physico-chemical treatments, and biological treatments. Physico-chemical treatments are usually used to reduce suspended solids, colloidal particles, color, and certain toxic compounds. However the cost associated with this type of treatment usually is high. On the other hand, biological treatment has been shown to be very effective in removing organic and nitrogenous matter from the leachate, especially when the BOD/COD ratio is high (>0.5)[3]. Biological treatments is gaining more popularity due to its relatively low cost and high sustainability. Among the various biological treatment, bacteria are the most common microorganisms that are used. Recently, fungi, with their high tolerance and resistance to toxicity, have been recognized as an excellent candidate for treating leachate.

Fungi were first studied to treatas the treatment for industrial wastewater to remove recalcitrant compounds. Fungi showed excellent degradability of recalcitrant compounds. In addition, good removal of COD and color was achieved. Lately fungi, especially white-rot fungi, have been applied in leachate treatment. Research has shown that white-rot fungi have developed nonspecific mechanisms to degrade an extremely diverse range of very persistent or toxic environmental pollutants [4]. The biodegradation capacity of organic pollutants by white-rot fungi is correlated with their ability to secrete extracellular enzymes such as lignin peroxidases (LiP), manganese peroxidases (MnP), and laccases [5]. Besides white-rot fungi, yeast is the other fungi specie that has been studied. Yeast has a high capacity of to breaking and assimilating assimilate difficult degradation pollutants in leachate. Several genera of yeast have been documented as been able to degrade complex organic compounds [6].

This literature review aimed [1] to understand the unique characteristics of leachate and the current treatment methods, [2] to review the fungi treatment process in wastewater and leachate, and [3] to examine the operating constraints and the important affecting factors of the fungal process. In addition, the future of fungal treatments on leachate will also be discussed.

2. Landfill leachate characteristics

Landfill leachate are any liquid that passes through wastes and different artificial layers that is collected in the bottom of landfill. The leachate flow rate is influenced by precipitation, surface run-off, and infiltration or intrusion of groundwater percolating through the landfill. Leachate production depends on the water content and the degree of compaction of the waste. The production of leachate is generally greater whenever the waste is less compacted, since compaction reduces the filtration rate [7].

Landfill leachate may be characterized as a water-based solution consisting of four groups of contaminants: (1) dissolved organic matter, such as alcohols, acids, aldehydes, and short chain sugars; (2) inorganic macro components, which include common cations and anions (e.g.,

sulfate, chloride, iron, aluminum, zinc, and ammonia); (3) heavy metals (i.e., Pb, Ni, Cu, Hg, etc.); and (4) xenobiotic organic compounds such as halogenated organics (e.g., PCBs, dioxins, etc.)[1].

The composition of landfill leachate mainly depends on the age of the landfill. When water percolates through the waste, it promotes and assists the process of decomposition by microorganisms. During the decomposition process, the by-products are released either in the leachate or as the gas. The decomposition process also rapidly uses up available oxygen creating an anoxic environment followed by anaerobic environment. Young landfill, usually it contains large amounts of biodegradable organic matter, which leads to a rapid anaerobic fermentation. This results in the production of volatile fatty acids (VFA) as the main fermentation products [8]. The early phase of a landfill's lifetime is called the acidogenic phase and leads to the release of large quantities of free VFA, which can be as much as 95% of the organic content [9]. In the mature landfill, the methanogenic phase occurs when the methanogenic microorganisms develop in the waste. In this phase, methanogenic microorganisms convert VFA to biogas (CH_4, CO_2). The organic fraction of leachate decreases as the landfill age increases. Eventually, the main compounds in a matured landfill leachate are nonbiodegradable. Table 1 illustrates characteristics in different landfill age phases [3,10].

	Young	**Intermediate**	**Old**
Age	< 5	5-10	>10
pH	6.5	6.5-7.5	>7.5
COD	>10,000	4,000-10,000	<4,000
BOD/COD	>0.3	0.1-0.3	<0.1
Organic compounds	80% VFA	5-30% VFA + Humic and fulvic acids	Humic and fulvic acids
Heavy metals	Low-medium	Low	Low
Biodegradability	High	Medium	Low

Table 1. Landfill leachate characteristics [1,2]

Leachate, when it emerges from a typical landfill site, is black, yellow or orange in color, and can be slightly cloudy. The smell of leachate is acidic, offensive and pervasive due to the presents of hydrogen, nitrogen and sulfur rich organic species such as mercaptans [11]

3. Conventional leachate treatment

Conventional leachate treatments can be classified as chemical, physical, and biological treatments. However, in order to meet stringent quality standards for direct discharge of leachate into the surface water, an integrated method of treatment is commonly used [12].

3.1. Physical and chemical treatment

Physicochemical treatments are usually used for preliminary leachate treatment and final polishing treatment, including reduction of suspended solids, colloidal particles, color, and toxic compounds.

3.1.1. Coagulation and flocculation

Coagulation and flocculation are widely used as a pretreatment, prior to biological or reverse osmosis step, or as a final polishing treatment step in order to remove nonbiodegradable organic matter. Aluminum sulfate, ferrous sulfate, ferric chloride, and ferric chlorosulfate are commonly used as coagulants [10].

Several studies have been conducted on the examination of coagulation and flocculation for the treatment of landfill leachates. Those studies aimed at performance optimization, i.e., selection of the coagulant, determination of operational conditions, evaluation of the effect of pH, and investigation of the addition of flocculants [13]. Depending on the landfill age and type of coagulant, the COD removal rate is in the range of 20% to 90%.

3.1.2. Chemical oxidation

Chemical oxidation is used to treat leachate that contains soluble organic substance (which cannot be removed by physical separation), nonbiodegradable, and/or toxic substance not suitable for biological oxidation [14].

Recently, there has been growing interest in advanced oxidation processes (AOP). Most of them, except simple ozonation (O_3), use a combination of strong oxidants, e.g., O_3 and H_2O_2, irradiation, e.g., ultraviolet (UV), ultrasound (US), or electron beam (EB), and catalysts, e.g., transition metal ions or photocatalyst [3]. For instance, the efficiency of COD removal by using a Fenton reagent varied from 60% to 75% for mature and biologically pretreated leachate, respectively [15].

3.1.3. Air stripping

Air striping is the most commonly used method for eliminating a high concentration of NH4+-N in the wastewater. High levels of ammonium nitrogen are usually found in landfill leachate. In many applications, air stripping was used successfully in the removal of ammonium nitrogen present in the leachate [16].

However, there are a few drawbacks to this technology. One drawback is the exhausted air which is mixed with NH3 needs to be treated with either H_2SO_4 or HCl before it is released into the atmosphere. Other drawbacks are the calcium carbonate scaling of the stripping tower when lime is used for pH adjustment, and foaming when a large stripping tower is used [17].

3.1.4. Membrane filtration

Membrane filtration is the process that separates solid immiscible particles from the liquid stream. It is based primarily on size difference. It includes microfiltration, ultrafiltration,

nanofiltration, and reverse osmosis (RO). Membrane filtration cannot be used alone in leachate treatment, and usually is used as pretreatment for other membrane processes. Membrane filtration can achieve an over 90% COD removal rate (Table 2) [18–20]; however, cost is a concern. Membrane filtration requires high energy input. In addition, residue needs to be further treated and properly disposed which increases the cost of the treatment.

Process	COD Removal Rate	Reference
Microfiltration	25-35%	[3]
Ultrafiltration	50%	[4]
RO	>90%	[4]
Nanofiltration	60-80%	[5]

Table 2. The performance of different membrane process on leachate treatment

3.2. Biological treatment

Biological processes are very effective in removing organic matters and nitrogen, especially from young landfill leachate when the BOD/COD ratio has a high value (>0.5). When landfill operation time is longer than 10 years, the major presence of refractory compounds (mainly humic and fulvic acids) in leachate tends to limit effectiveness of biological treatment.

3.2.1. Aerobic treatment

Aerobic treatment of leachate can be performed in suspended growth microorganisms in activated sludge as well as attached growth microorganisms. Both systems, which are commonly applied to municipal wastewaters treatment, can be adapted to treat leachate. Aerobic treatment can achieve a partial decrease in biodegradable organic compounds and can result nitrification to transfer ammonia to nitrite and nitrate.

Aerobic biological processes have been widely studied and adopted. There are two types. One is based on suspended-growth biomass, such as aerated lagoons, conventional activated sludge processes and sequencing batch reactors (SBR). The other is based on attached- growth biomass, such as membrane bioreactor and different biofilters. Table 3 summarizes the typical performances of the most commonly used aerobic treatment processes in leachate [21–24].

Process	COD Removal Rate	NH_4^+-N Removal Rate	Reference
Activated sludge	46%-97%	87.5%	[6,7]
SBR	48-91%	>99%	[8]
Trickling Filter	87% BOD	90%	[9]

Table 3. The performance of different aerobic process on leachate treatment

3.2.2. Anaerobic treatment

The anaerobic digestion process involves biological decomposition of organic and inorganic matter in the absence of molecular oxygen. As a result of conversion, a variety of end products, including methanol (CH_4) and carbon dioxide (CO_2), is produced. It is particularly suitable for treating leachate with high strength organic content, such as leachate streams from young landfill [25]. Anaerobic treatment includes suspended-growth biomass processes such as anaerobic digester, up-flow anaerobic sludge blanket reactor (UASBR), and attached-growth biomass processes (anaerobic filter). Table 4 summarizes the typical performances of common anaerobic treatment processes in leachate [8,21,26]

Process	COD Removal Rate	NH_4^+-N Removal Rate	Reference
Digester	20%-96%	---	[10]
UASBR	45-91%	---	[11]
Anaerobic filter	60-95%	87%	[6]

Table 4. The performance of different anaerobic process on leachate treatment

4. Microbiology of fungi

4.1. Characteristic of fungi

A fungus is any member of a large group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. Like other eukaryotes, fungal cells contain membrane-bound nuclei with chromosomes that contain DNA with noncoding regions called introns and coding regions called exons. They are comprised of soluble carbohydrates and storage compounds, including sugar alcohols, disaccharides and polysaccharides [27].

Fungi are heterotrophic organisms and require organic compounds as energy sources. They reproduce by both sexual and asexual means and spores. Some species grow as unicellular yeasts that reproduce by budding or binary fission. The cells of most fungi grow as tubular, elongated, and thread-like (filamentous) structure called hyphae, which may contain multiple nuclei and extend at their tips [28]. In common with some plant and animal species, more than 60 fungal species display the phenomenon of bioluminescence [29]. Dimorphic fungi can switch between a yeast phase and a hyphal phase in response to environmental conditions. Fungi are the only organisms that combine both glucans and chitin structural molecules in their cell wall [28].

4.2. Growth requirements of fungi

4.2.1. Temperature

Most fungi are mesophiles and relatively few can grow at or above 37°C or even above 30°C, whereas many bacteria can grow at this temperature. The upper limit for growth of any fungus

(or any eukaryote) is about 62°C [27]. Temperature affects their growth rate, metabolism, nutritional requirements, regulation mechanisms of enzymatic reactions, and cell permeability. The structure and composition of cytoplasmic membranes in cells are also altered by temperatures that determine the substrate utilization rate of fungi [30]. In addition, temperature also plays a major role in determining fungal spore survival [31].

Most fungi have a maximum growth at a temperature of 25°C with reduced growth at temperatures below 20°C and above 35°C [32]. Thermophilc fungi dominate at a high temperature environment (above 35°C). They are no more efficient in substrate utilization than the mesophiles [27].

4.2.2. pH

Many fungi will grow over the pH range 4.0–8.5, or sometimes 3.0–9.0, and they show relatively broad pH optima of about 5.0–7.0 [27]. Acidophilic fungi, able to grow down to pH 1 or 2, are found in a few environments such as coal refuse tips and acidic mine wastes; many of these species are yeasts. Alkalophilic fungi are able to colonize alkaline environments with pH of 10, and they include specialized species of filamentous fungi. The morphology of fungi is also affected by the pH. Typically, the morphological change attributed to pH variation is in the shape of the fungal pellet. This varies from fluffy to clumpy and compact depend on pH [33]. Fungi can rapidly change the pH of the culture by selective uptake or exchange of ions; therefore, the responses of fungi to pH of the culture need to be assessed in strongly buffered media [27].

4.2.3. Oxygen

Most fungi are strict aerobes; they require oxygen at some, if not all the stages of their life cycle. Therefore, fungi are usually found growing on or near the surface of the substrate that open to the air. Some fungi are facultative aerobes. They can survive in oxygen-limited environments, including sewage sludge and polluted waters. Insufficient oxygen supply increases the nutritional demand and thereby decreases fungal growth [34].

4.2.4. Nutrients

Fungi have quite simple nutritional requirements. They need a source of organic nutrients to supply their energy and to supply carbon skeletons for cellular synthesis [27]. Fungi absorb simple, soluble nutrients through the wall and plasma membrane. In many cases, this is achieved by releasing enzymes to degrade complex polymers to simple nutrients and then absorbing them.

a. Carbon source

Fungi differ widely in their abilities of using different carbon sources. The utilization efficiency of a defined carbon source by fungi may be influenced by the medium composition and the culture conditions. Usually, the carbon sources can be cellulosic, CH_4, monosaccharide, disaccharides, and different types of wastes. The substrates that have different carbon

compositions can be used for growing different type of fungi. For example, although both white- and brown-rot fungi are known for their ability to degrade lignin and cellulose, white-rot fungi perform better in the degradation of both simultaneous and selective lignin, while the brown-rot fungi degrade the cellulose and hemicellulose [35].

b. Nitrogen

Fungi do not fix atmospheric nitrogen, but they can use many combined forms of nitrogen such as nitrates, nitrites, ammonium, or organic nitrogen sources. All fungi can use amino acids as a nitrogen source. Often they need to be supplied with only one type of amino acid such as glutamic acid or glutamine. Then they can produce all the other essential amino acids by transamination reactions [27].

Most fungi can use ammonia or ammonium as a nitrogen source. After uptake, ammonia/ammonium is combined with organic acids, usually to produce either glutamic acid or aspartic acid. Many fungi can also use nitrate as their sole nitrogen source. They produce nitrate reductase and nitrite reductase to convert nitrate to ammonium [27].

c. Phosphorus

Fungi are highly adept at obtaining phosphorus, and they achieve this in several ways: (1) they respond to critically low levels of available phosphorus by increasing the activity of their phosphorus-uptake systems; (2) they release phosphatase enzymes that can cleave phosphate from organic sources; (3) they solubilize inorganic phosphates by releasing organic acids to lower the external pH; and (4) their hyphae, with a high surface area/volume ratio, extend continuously into fresh zones of soil to obtain phosphorus [36].

d. Other nutrients

Essential micronutrients for fungal growth are iron, zinc, copper, manganese, molybdenum, and either calcium or strontium [35]. Different fungal species can have their own specific nutrient needs. Certain fungi require vitamins in trace quantities, whereas others synthesize their own vitamins.

5. Fungi process in wastewater

During the late 1950s to early mid 1960s researchers started to recognize the potential of fungi in wastewater treatment process. Cooke, in the 1976, advocated the use of fungi in wastewater treatment because fungi appeared to show higher rates of degradation and showed a much greater ability to degrade cellulose, hemicellulose, and lignin materials than other microorganisms [37].

In view of the excellent recalcitrant compound degradability of certain groups of fungi, researchers have been focusing on exploring fungal degradation of toxic industrial wastewater. These research have included wastewaters from textile, olive milling, and the food-processing industries, etc. Several studies have been conducted on the ability of fungi to

decolorize specific dyes [38]. Research have shown that the degradation of dye is possibly due to the fact that fungi produce the lignin-modifying enzymes laccase, manganese peroxidase, and lignin peroxidase that mineralize lignin or dyes [39]. Fungi are also very effective in degrading complex aromatic organic compounds present in wastewater. For instance, phenolic compounds present in olive mill wastewater are similar to those derived from lignin degradation [40]; therefore, fungi are an excellent candidate for treating olive milling wastewater. Fungi also have been used to treat wastewater with high COD from the food processing industry [41,42]. Table 5 summarizes the application and performance of fungi in wastewater treatment.

Wastewater	Treatment process	Fungi species	Results
Textile wastewater	SBR	*Trametes versicolor*	Color removal : 91-95% [12]
Olive mill wastewater	Airlift reactor	*Pycnoporus coccineus*	COD removal: 20-50% Toxicity removal: 70% [13]
Potato-chip industry wastewater	Batch studies	*Aspergillus niger*	COD removal: 90%[14]
Starch processing wastewater	Airlift reactor	*Aspergillus oryzae*	COD removal: 47-96% [15]

Table 5. The performance of fungi treatment in different industrial wastewater

6. Fungi in leachate treatment process

Although using fungi to treat industrial or municipal wastewater has been studied for decades, it is relatively new to use fungi to treat the landfill leachate. Very few studies have been done in this area. These studies have demonstrated that fungi can effectively decrease high COD, toxicity, and the dark color of leachate.

6.1. Fungi species used in leachate treatment

White-rot fungi are the most commonly used species in landfill leachate treatment. Compared to the other species, white-rot fungi have the ability to degrade an extremely diverse range of very persistent or toxic environmental pollutants. The white-rot fungi have developed very nonspecific mechanisms for degradation [4].

In response to low levels of key sources of carbon, nitrogen, or sulfur nutrients [5], white-rot fungi produce enzymes. These are known as lignin peroxidation and manganese-dependent peroxidases, which can degrade very insoluble chemicals such as lignin or many of the hazardous pollutants. White-rot fungi have an extracellular system that enables them to

tolerate considerably higher concentration of a toxic pollutant such as cyanide. In addition, white-rot fungi possess a very nonspecific nature of mechanisms to degrade very complex mixtures of pollutants. Usually, white-rot fungi do not require preconditioning to a particular pollutant. White-rot fungi can be cultivated from soil using very inexpensive growth substrate such as corn cobs and wood dust. They can also grow in the liquid culture.

Besides the white-rot fungi, yeast is the only other fungi species that is used in landfill leachate treatment. Yeast has a high capacity of breaking and assimilating difficult degradation pollutants in leachate (such as humic substances). Several genera of yeast (e.g., *Candida*, *Rhodotorula*, *Yarrowia*, *Hansenula*, *Saccharomyces cerevisiae*) have been reported to be able to degrade complex organic compounds [6].

6.2. Fungi preparation

Obtaining the desired amount of fungi to treat landfill leachate can be achieved in three steps. First, selected fungi species need to be cultivated. This should be followed by enrichment, which is achieved by mycelial suspension. The fungi will then be ready to be added to the treatment process. The following section describes the detailed technique of cultivation.

6.2.1. Subculture of fungi

The ingredients of culture medium can be different depending on the species and purpose of the experiments. For example, *Trametes trogii* have the ability to produce high activates of laccase, which can remove copper in the leachate. A basal medium can be used to optimize the production of laccase. This medium contains (per liter): glucose, 10 g; soya peptone, 9 g; diammonium tartrate, 2 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; and trace elements solution, 1 ml. The medium is supplemented with $CuSO_4$ (0.3 mM) and ethanol (3% V/V) an inducer of laccases. The cultural medium needs to be buffered to pH 5.5 [43]. Usually, fungi can be cultivated in culture tubes or dishes within the temperature range of 25℃ and 33℃ for several days (7 days)[44].

6.2.2. Mycelial suspension

Fungi should then be further enriched by growing it in mycelial suspension. The common procedure is as follows: (1) 100 ml of sterile potato dextrose broth (PDB) is prepared in a 250-ml Erlenmeyer flask. (2) Four pieces of fungi from the culture tube are inoculated into the PDB medium by using the sterile loop. The flask is plugged with cotton and is agitated for 24 h in a rotary shaker at 150 rpm [44]. The flask is then incubated at 28 °C in an incubator. Usually, after 6–7 days, a dense mycelial mass is formed and the mycelial suspension is ready for further use [45]. Mycelia suspension can be used directly in the leachate treatment process or can be added to the fungi immobilization media.

6.2.3. Immobilization of fungi

Support materials (media) usually play an important role of fungi production and stability in the leachate. Federica Spina et al. [46] studied four inert supports (Figure 1): (A) circle industrial

support; (B) net industrial support; (C) polyurethane foam PUF; and (D) stainless steel scourers. Although Supports A and B are very efficient in bacterial biofilm formation, researchers have found that they were not suitable for hyphal colonization. They also observed that fungi colonized D in a heterogeneous way with great differences in the method of colonization among supports, under an agitated condition homogeneous and persistent biomass colonization was observed on Support C. They therefore concluded that PUF is the most suitable support media in the immobilization of fungi among all the four popular media on the market.

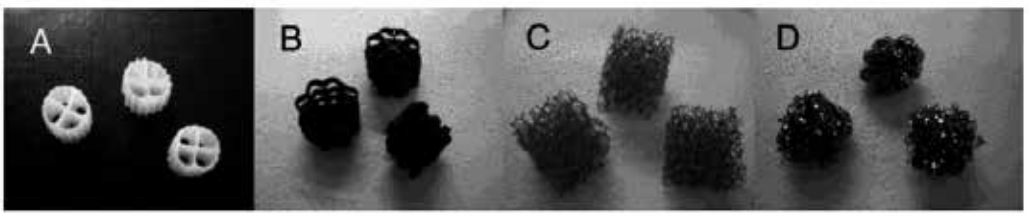


Figure 1. Different inert support materials used in the study conducted by Federoca Spina et al. [46].

The immobilization of fungi can be carried out by adding the media into a liquid fungi growth medium in a flask, which is seeded with a certain amount of mycelial suspension. The flask is then kept at a temperature of 30℃. Usually, it takes 4-7 days for fungi to colonize the media. After this immobilization process, fungi are ready to be used for the leachate treatment.

6.3. Leachate treatment process

6.3.1. Treatment conditions and toxicity test

To promote the growth of fungi, the treatment conditions such as temperature, pH, mixing, etc., are very important. A few studies have been conducted to understand the optimal treatment conditions. Studies have shown that keeping the leachate temperature between 25-33°C, pH of 4-5, is necessary to achieve a desirable treatment outcome. Table 6 illustrates the effect of pH on fungal biomass concentration as well as the COD removal in the wastewater treatment process [47]. Cosubstrates such as glucose willalso result in better treatment performance. Proper mixing and aeration are the other factors that need to be considered.

pH	Fungal biomass (mg VSS/L)	COD removal (%)
3.5	80	34
4.0	625	80
4.5	370	68

Table 6. Effect of pH on fungal biomass concentration in wastewater treatment

It is also necessary to test leachate toxicity, as the raw leachate may contain some toxic compounds that are harmful to the growth of fungi. Common leachate toxicity tests are microtoxicity and phytotoxity test. The microtoxicity test is carried out by measuring the light emission of *Vibrio fischeri* and *Aliivibrio fischeri* [48]. Phytotoxicity is estimated by the determination of the germination index of *Lepidium sativum* and *Sinapis alba* seeds [49].

6.3.2. Leachate treatment methods and results

In the batch study conducted by Kalčíková G et al. [45], they used *Dichomitus squalens* mycelial suspension and beech wood sawdust as cosubstrate. It was found that *D. squalens* was able to grow in the mature leachate from the closed landfill. This resulted in 60% of both DOC and COD removal and decreased toxicity. They also introduced a crude enzyme containing extracellular ligninolytic enzymes filtrate to treat leachate from the active landfill, which contained inhibitory compounds. The removal levels of COD and DOC reached 61% and 44%, respectively [45].

Saetang and Bable [44] studied using immobilized *Tinea versicolor* in a continuous flow system to treat leachate for both color removal and COD removal. In this study, they used glucose as cosubstrate. With 4 days of the initial immobilization of the fungi on polyurethane foam, approximately 78% color removal and 52% of COD removal were observed.

Membrane bioreactor (MBR) was used by Brito et al. [6] to treat the leachate with yeast. In this study, a submerged microfiltration module with hollow fiber membranes of poly was used. Through the study, they gradually increased concentration of leachate while decreasing concentration of broth in the feed. Finally, with 100% raw leachate in the feed, the yeast achieved 70% COD, 82% color, and 67.7% humic substance removal rate.

As of these date, there are very few studies on using fungi to treat leachate. Based on the available literature, the performance of fungi on leachate treatment is summarized in Table 7.

Parameters	Removal Rate Range	Reference
COD	42-79%	[16–19]
BOD	25-52%	[19]
Color	40-82%	[16,19]
Toxicity	40-50%	[16–19]
DOC	40-60%	[18]

Table 7. Performance of leachate treatment process with fungi

7. Conclusion and future challenge

Fungi can be used to treat a variety of wastewaters, ranging from municipal wastewater, industrial wastewater and landfill leachate. In terms of landfill leachate treatment, fungi

showed a better removal efficiency of recalcitrant compounds than the conventional leachate treatment process. This was evident especial in the removal efficiency of recalcitrant compounds which contribute to 1) COD, 2) toxicity and 3) color of leachate. Both white-rot fungi and yeast are capable of producing special extracellular enzymes, and they are the only two species that have been studied so far. There is a need to extend the current research onto other fungi species. This requires a better understand of the characteristics of fungi. The collaboration between the microbiologist and the wastewater engineers is therefore essential.

Besides recalcitrant compounds, high ammonia concentration in the leachate is another concern. However, throughout the literature review, no information was available on the impact of ammonia on the growth of fungi as well as their ammonia removal ability. In addition, how to remove nitrogen along with other pollutants needs to be addressed in the fungi leachate treatment process. Better research and understanding of the role of fungi would help further improve the leachate treatment process. Future research should be done these challenges to develop a leachate treatment technology that is economical and easy to implement.

Author details

Yanan Ren and Qiuyan Yuan*

*Address all correspondence to: qiuyan.yuan@umanitoba.ca

Department of Civil Engineering, University of Manitoba, Winnipeg, Manitoba, Canada

References

[1] Kjeldsen P, Barlaz MA, Rooker AP, Baun A, Ledin A, Christensen TH. Present and long-term composition of MSW landfill leachate: a review. Critical Reviews in Environmental Science and Technology. 2002. p. 297–336.

[2] Kurniawan TA, Lo WH, Chan GYS. Physico-chemical treatments for removal of recalcitrant contaminants from landfill leachate. Journal of Hazardous Materials. 2006. p. 80–100.

[3] Renou S, Givaudan JG, Poulain S, Dirassouyan F, Moulin P. Landfill leachate treatment: review and opportunity. Journal of Hazardous Materials. 2008. p. 468–93.

[4] Barr DP, Aust SD. Mechanisms white rot fungi use to degrade pollutants. Environmental Science and Technology [Internet]. 1994;28(2):78A–87A. Available from: http://pubs.acs.org/doi/abs/10.1021/es00051a002

[5] Gold MH, Kuwahara M, Chiu AA, Glenn JK. Purification and characterization of an extracellular H_2O_2-requiring diarylpropane oxygenase from the white rot basidiomy-

cete, *Phanerochaete chrysosporium*. Archives of Biochemistry and Biophysics. 1984;234:353–62.

[6] Brito GCB, Amaral MCS, Lange LC, Pereira RCA, Santos VL, Machado M. Treatment of landfill leachate in membranes bioreactor with yeast (*Saccharomyces cerevisiae*). Procedia Engineering [Internet]. 2012 [cited 2015 Feb 7];44:934–8. Available from: http://www.sciencedirect.com/science/article/pii/S1877705812036399

[7] Lema J, Mendez R, Blazquez R. Characteristics of landfill leachates and alternatives for their treatment: a review. Water, Air, and Soil Pollution. 1988;40:223–50.

[8] Welander U, Henrysson T, Welander T. Nitrification of landfill leachate using suspended-carrier biofilm technology. Water Research. 1997;31:2351–5.

[9] Harmsen J. Identification of organic compounds in leachate from a waste tip. Water Research. 1983. p. 699–705.

[10] Amokrane A, Comel C, Veron J. Landfill leachates pretreatment by coagulation-flocculation. Water Research. 1997;31:2775–82.

[11] Siddiquea R, Kaur G, Rajor A. Waste foundry sand and its leachate characteristics. Resources, Conservation and Recycling. 2010. p. 1027–36.

[12] Wiszniowski J, Robert D, Surmacz-Gorska J, Miksch K, Weber J V. Landfill leachate treatment methods: a review. Environmental Chemistry Letters. 2006. p. 51–61.

[13] Tatsi AA, Zouboulis AI, Matis KA, Samaras P. Coagulation-flocculation pretreatment of sanitary landfill leachates. Chemosphere. 2003;53:737–44.

[14] Marco A, Esplugas S, Saum G. How and why combine chemical and biological processes for wastewater treatment. Water Science and Technology. 1997. p. 321–7.

[15] Lopez A, Pagano M, Volpe A, Di Pinto AC. Fenton's pre-treatment of mature landfill leachate. Chemosphere. 2004;54:1005–10.

[16] Marttinen SK, Kettunen RH, Sormunen KM, Soimasuo RM, Rintala JA. Screening of physical-chemical methods for removal of organic material, nitrogen and toxicity from low strength landfill leachates. Chemosphere. 2002;46:851–8.

[17] Li XZ, Zhao QL, Hao XD. Ammonium removal from landfill leachate by chemical precipitation. Waste Management. 1999. p. 409–15.

[18] Ozturk I, Altinbas M, Koyuncu I, Arikan O, Gomec-Yangin C. Advanced physicochemical treatment experiences on young municipal landfill leachates. Waste Management. 2003. p. 441–6.

[19] Piatkiewicz W, Biemacka E, Suchecka T. A polish study: treating landfill leachate with membranes. Filtration and Separation. 2001;38:22–6.

[20] Bohdziewicz J, Bodzek M, Górska J. Application of pressure-driven membrane techniques to biological treatment of landfill leachate. Process Biochemistry. 2001;36:641–6.

[21] Bae JH, Kim SK, Chang HS. Treatment of landfill leachates: ammonia removal via nitrification and denitrification and further COD reduction via Fenton's treatment followed by activated sludge. Water Science and Technology. 1997. p. 341–8.

[22] Kettunen RH, Rintala JA. Sequential anaerobic-aerobic treatment of sulphur rich phenolic leachates. Journal of Chemical Technology and Biotechnology [Internet]. John Wiley & Sons Ltd; 1995 Feb [cited 2015 Feb 7];62(2):177–84.

[23] Zaloum R, Abbott M. Anaerobic pretreatment improves single sequencing batch reactor treatment of landfill leachates. Water Science and Technology. 1997. p. 207–14.

[24] Jokela JPY, Kettunen RH, Sormunen KM, Rintala JA. Biological nitrogen removal from municipal landfill leachate: low-cost nitrification in biofilters and laboratory scale in-situ denitrification. Water Research. 2002;36:4079–87.

[25] Pokhrel D, Viraraghavan T. Leachate generation and treatment—a review. Fresenius Environmental Bulletin. 2004. p. 223–32.

[26] Kennedy K. Treatment of landfill leachate using sequencing batch and continuous flow upflow anaerobic sludge blanket (UASB) reactors. Water Research. 2000. p. 3640–56.

[27] Deacon JW. Fungal Biology [Internet]. Wiley; 2005.

[28] Alexopoulos CJ, Mims CW, Blackwell M. Introductory mycology [Internet]. Wiley; 1996.

[29] Desjardin DE, Oliveira AG, Stevani C V. Fungi bioluminescence revisited. Photochemical and Photobiological Sciences. 2008;7:170–82.

[30] Madigan MT, Martinko JM, Brock TD, Parker J. Brock Biology of Microorganisms [Internet]. Prentice Hall International; 1997.

[31] HONG TD, ELLIS RH, MOORE D. Development of a model to predict the effect of temperature and moisture on fungal spore longevity. Annals of Botany. 1997 Feb 1;79(2):121–8.

[32] Hussain A, Iqubal M, Ayub N, Haqqanii AM. Physiological study of *Sclerotium rolfsii* Sacc. Pakistan Journal of Plant Pathology. 2003;2(2):102–6.

[33] Jin B, Van Leeuwen J, Yu Q, Patel B. Screening and selection of microfungi for microbial biomass protein production and water reclamation from starch processing wastewater. Journal of Chemical Technology and Biotechnology. 1999;74:106–10.

[34] Moore-Landecker E. Fundamentals of the Fungi. Prentice Hall; 1996.

[35] Sankaran S, Khanal SK, Jasti N, Jin B, Pometto AL, Van Leeuwen JH. Use of filamentous fungi for wastewater treatment and production of high value fungal byproducts: a review. Critical Reviews in Environmental Science and Technology. 2010. p. 400–49.

[36] Symposium BMS, Boddy L, Marchant R, Read DJ. Nitrogen, Phosphorus, and Sulphur Utilization by Fungi: Symposium of the British Mycological Society Held at the University of Birmingham, April 1988. Cambridge University Press; 1989.

[37] Cooke WB. Fungi in sewage. In: Jones EBG, editor. Recent Advances in Aquatic Mycology. Elek; 1976.

[38] Cing S, Yesilada O. Astrazon red dye decolorization by growing cells and pellets of *Funalia trogii*. Journal of Basic Microbiology. 2004;44:263–9.

[39] Chander M, Arora DS. Evaluation of some white-rot fungi for their potential to decolourise industrial dyes. Dyes and Pigments. 2007;72:192–8.

[40] Jaouani A, Guillén F, Penninckx MJ, Martínez AT, Martínez MJ. Role of *Pycnoporus coccineus* laccase in the degradation of aromatic compounds in olive oil mill wastewater. Enzyme and Microbial Technology. 2005;36:478–86.

[41] Mishra BK, Arora A, Lata. Optimization of a biological process for treating potato chips industry wastewater using a mixed culture of *Aspergillus foetidus* and *Aspergillus niger*. Bioresource Technology. 2004 Aug;94(1):9–12.

[42] Truong QT, Miyata N, Iwahori K. Growth of *Aspergillus oryzae* during treatment of cassava starch processing wastewater with high content of suspended solids. Journal of Bioscience and Bioengineering. 2004;97(5):329–35.

[43] Ellouze M, Aloui F, Sayadi S. Effect of high ammonia concentrations on fungal treatment of Tunisian landfill leachates. Desalination. 2009;246:468–77.

[44] Saetang J, Babel S. Effect of leachate loading rate and incubation period on the treatment efficiency by *T. versicolor* immobilized on foam cubes. International Journal of Environmental Science and Technology. 2009;6(3):457–66.

[45] Kalčíková G, Babič J, Pavko A, Gotvajn AŽ. Fungal and enzymatic treatment of mature municipal landfill leachate. Waste Management. 2014;34:798–803.

[46] Spina F, Anastasi A, Prigione V. Biological treatment of industrial wastewaters: a fungal approach. Chemical Engineering Transactions. 2012;27:175–80.

[47] Jasti N, Khanal S, Pometto A, Leeuwen H. Attached growth fungal system for food processing wastewater treatment and high value protein recovery. Proceedings of the Water Environment Federation. 2002. p. 4049–62.

[48] International Organization for Standardization. Water Quality—Determination of the Inhibitory Effect of Water Samples on the Light Emission of Vibrio Fischeri (Luminescent Bacteria Test) Part 2. ISO; 1998.

[49] Zucconi F, Forte M, Monaco A, de Bertoldi M. Biological evaluation of compost maturity. Biocycle. 1981;22(4):27–9.

Chapter 2

Culture Condition Effect on Bioflocculant Production and Actual Wastewater Treatment Application by Different Types of Bioflocculants

Fan Zhang, Wenju Jiang, Xiangdong Wang, Xiujuan Ji, Yina Wang, Wang Zhang and Jiao Chen

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62114

Abstract

The effect of culture condition on different types of bioflocculant production and its application on actual wastewater treatment were studied in this chapter. The advantages of mixed strain HXJ-1 were as follows: directly using acidic wine wastewater, adapting to wastewater at high concentrations and the presence of less nitrogen. HXJ-1 achieved good flocculating rate when the chemical oxygen demand (COD) was 12,000 mg/L, C/N 20:1. Three kinds of bioflocculants had some good treatment results on starch wastewater, printing and dyeing wastewater and landfill leachate. The treatment effect of XJBF-1 (produced by mixed strain HXJ-1) on the starch wastewater was better than that of traditional polyacrylamide and other bioflocculants produced by a single bacterial (X15BF-1) and yeast strain (J1BF-1). XJBF-1 had better treatment results on three types of wastewater. It also had good removal rate of chromaticity, especially on the starch wastewater , the printing and dyeing wastewater; the removal rate was up to 88%, and the starch wastewater COD removal rate was up to 86%.

Keywords: Alcoholic wastewater, bioflocculant, mixed strains, culture condition, actual wastewater treatment

1. Introduction

Microbial flocculent, the secondary metabolites with flocculating activity and produced by microorganisms [1], is a new water treatment agent with efficient, safe, natural degradation characteristic to flocculate and sediment the solid suspended particles and colloidal particles which are not easily degradable in water [2, 3]. Most bioflocculants are still in the develop-

mental stage and under research because of the high cost of the culture medium and the lack of high production of flocculating engineering fungus. Therefore, the synthesis of microbial flocculants from inexpensive carbon source showed its potential benefit since it could address the critical problems of substrate costs [4]. The use of industrial wastewater, such as soy sauce wastewater, and biological hydrogen production waste as an alternative medium to reduce the cost of production of microbial flocculants has been reported in recent years [5, 6]. China is one of the largest alcoholic beverage-producing and -consuming countries; nutrients in the alcoholic wastewater might be used as free resources for microorganism growth and synthesis of bioflocculants to reduce the cost of culture medium, and the pollutant in the wastewater might also be reduced after it is utilized by microorganisms. Therefore, the idea 'use waste to treat waste' may realize material recycling and will benefit the environment and society. In addition, *Bacillus cereu*s, as feed additives, is widely used in feed production, and a symbiosis relationship was reported between *Bacillus cereu*s and yeast [7]. *Bacillus cereu*s has a strong resistance to adverse environments and is fast growing, and yeasts are widely used in food and wine industry; so it should have good adaptability to wine, but its growth rate is usually slower compared with bacteria. The synergy between different microorganisms has the potential benefit to adapt to a wider range and promote the production of flocculants.

Only a few references on actual wastewater treatment by bioflocculant could be found at present [8]. In order to put bioflocculant into use on actual wastewater treatment, based on previous study [9, 10], different culture conditions on single strains and mixed strains were studied in this chapter. Three kinds of bioflocculants produced by different strains were used in actual wastewater treatment. These results will provide reference for complex bioflocculant research and future applications.

2. Materials and Methods

2.1. Materials

2.1.1. Strains

The flocculant-producing strains used in the experiments were isolated and selected from activated sludge from Chengdu wastewater treatment plant. They were primarily identified to be *Bacillus cereus* and *Pichia membranifaciens* according to morphological and genetic sequence identification, and then kept in the Lab.

2.1.2. Wastewater quality parameters

Wastewater came from Chengdu alcoholic production plant. The water quality parameters are reducing sugar 20.80 g/L, chemical oxygen demand (COD) concentration of 90,000 mg/L and PH3.6.

http://dx.doi.org/10.5772/62114

2.1.3. Wastewater fermentation medium

Appropriate dilution of alcoholic wastewater and pH adjustment according to the testing requirements, adding nitrogen (urea as the nitrogen source) according to a certain C/N ratio, and sterilization at 121℃ after 30 min were performed.

2.1.4. Actual wastewater for treatment

The landfill leachate: from Chengdu landfill plant. The water quality parameters: COD concentration: 1944 mg/L, turbidity: 1440 degrees, chromaticity: 512 times, SS: 11.04 g/L and pH: 6.5.

The starch wastewater: from Chengdu medicine production plant. The water quality parameters: COD concentration: 9660 mg/L, turbidity: 2098 degrees, chromaticity: 320 times, SS: 1.094g/L and pH: 2.3.

The printing and dyeing wastewater: from Chengdu textile plant. The water quality parameters: COD concentration: 760 mg/L, turbidity: 165 degrees, chromaticity: 1200 times, SS: 0.348g/L and pH: 8.7.

2.2. Methods

2.2.1. Measurement of flocculating activity

The flocculating activity was measured using the previous method [11] with a slight modification [12]. Ninety-three mL Kaolin suspension (1g/L), 5 mL of 1% $CaCl_2$ solution and 2 mL of bioflocculant were taken in a 200 mL beaker, and the pH was adjusted to 7.0. Then the beaker was placed in an electric mixer for 250 r/min of fast stirring for 1 min, 60 r/min of slow stirring for 2 min and then kept unstirred for 15 min at room temperature. The supernatant was carefully transferred to a utensil, and the optical density of the supernatant (OD_{550}) was measured by spectrophotometer, keeping an equal volume of distilled water as a control. The flocculating rate (FR) indicates the flocculating activity, which is calculated as follows:

$$FR = \frac{A-B}{A} \times 100\%$$

where A is the absorbance at 550 nm of control and B is the absorbance at 550 nm of treatment.

2.2.2. The effects of culture conditions on microbial flocculant production and flocculating activity

Ten percent relative inoculum size (V/V cell concentration: 10^8/L) *Bacillus cereus, Pichia membranifaciens* and mixed strains of these 1:1 (V/V) (referred to as HXJ-1) were seeded in different wastewater concentrations (COD concentration), different C/N ratios, initial pH of wastewater fermentation medium, shaking speed of 120 r/min and at a temperature of 30℃. After fermentation for 24 h, 10 mL of fermentation broth by centrifugation was taken, and the flocculating rate to kaolin suspension was measured from the supernatant. The flocculating

rate was used to investigate the effect of wastewater concentration, C/N ratio and initial pH value of *Bacillus cereus, Pichia membranifaciens* and HXJ-1-producing flocculants.

2.2.3. Different bioflocculants applications on actual wastewater treatment

After 93 mL starch wastewater, printing and dyeing wastewater and landfill leachate were poured into three 200 mL beakers respectively, 5 mL of 1% $CaCl_2$ solution and 2 mL of four different types of flocculants were added in each of the 200 mL beaker, and the pH was adjusted to 7.0. Then the beaker was placed in an electric mixer for 250 r/min of fast stirring for 1 min and 60 r/min of slow stirring for 2 min and then kept unstirred for 15 min at room temperature. Variations in wastewater COD, turbidity, chromaticity and SS were measured before and after the treatment. The COD speed-measuring device was used to measure COD [13], the spectrophotometric method was used to measure turbidity [14], the dilution factor method was used to measure chromaticity [15] and the constant weight method was used to measure SS [14].

3. Results and discussion

3.1. The effect of wastewater concentration on bioflocculant production

The wastewater concentration of the fermentation liquid with the effect of different microbial flocculant production is shown in Figure 1. *Bacillus cereus* could grow and produce bioflocculants in the wastewater at a low COD concentration, which was corresponding to the characteristics of low nutritional requirements of *Bacillus cereus* [7]. Higher COD concentrations were suitable for the growth of *Pichia membranifaciens* and flocculant production; the growth and bioflocculant production of HXJ-1 needed more carbon source; therefore, HXJ-1 reached a maximum flocculating rate of 90.0% at a higher COD concentration (COD concentration of 12,000 mg/L). However, if COD concentrations are higher than 12,000 mg/L, the lack of oxygen may cause an incomplete substrate oxidation and a large amount of acidic substance accumulation, thereby affecting the physiological activity of microorganisms, as well as reducing the capacity of producing flocculants. Other results also could be seen from Figure 1, which showed that HXJ-1 had the adaptability to higher wastewater concentration and better flocculating activity than a single strain. This result suggested that during flocculant production, yeast fermentation could quickly reduce the COD concentration in the fermentation broth, so that the role of bacteria is enhanced. *Bacillus cereus* and *Pichia membranifaciens* adjusted to niche separation to avoid disorderly competition; hence, it is possible not only to fully use the organic materials in wastewater and improve the synthetic efficiency of the flocculants but also to improve the utilization efficiency of the wastewater to achieve the purpose 'use waste to treat waste' by the utilization of alcoholic wastewater.

3.2. The effect of C/N on bioflocculant production

The effect of C/N in the fermentation broth on bioflocculant production by different strains is shown in Figure 2. It could also be seen that the flocculating rate of HXJ-1 increased rapidly

http://dx.doi.org/10.5772/62114

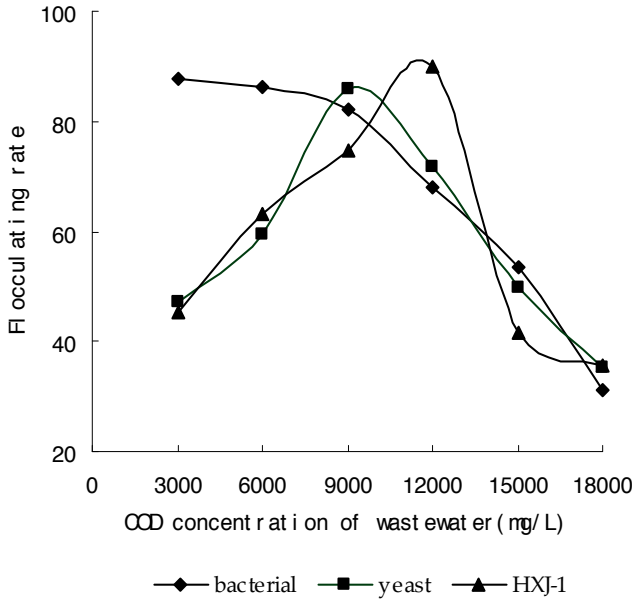


Figure 1. The effect of COD on the flocculating rate of different strains.

with the increasing C/N ratio and achieved the maximum rate 89.3% at C/N 20:1 and then declined slightly. *Pichia membranifaciens* achieved the maximum flocculating rate at C/N 20:1 and then its rapid decline started. C/N had a less effect on the bioflocculant production of *Bacillus cereus*, which achieved the maximum flocculating rate at C/N 15:1 and C/N 10:1–30:1 and could maintain a good rate of flocculation; the results were in line with the strong resistance characteristics of *Bacillus cereus* to adverse environmental conditions [7]. The flocculating rate of *Pichia membranifaciens* decreased rapidly when C/N > 20:1 and indicated that the N source demand of *Pichia membranifaciens* was greater than that of *Bacillus cereus* and HXJ-1. The following results could be observed: HXJ-1 had the same lower demand for N source characteristics as that of *Bacillus cereus*; good flocculating effect was observed when C/N > 20:1; hence, a favourable benefit condition had been set up to reduce the cost of bioflocculant production by mixed strains.

3.3. The effect of initial medium pH on bioflocculant production

The effect of initial pH of alcoholic wastewater fermentation medium on bioflocculant production by different strains is shown in Figure 3. The flocculating rate of *Bacillus cereus* increased with the increase in initial pH and achieved the maximum at pH 7.6; the flocculating rate of *Pichia membranifaciens* was the highest at pH 3.6, and it showed a downward trend with an increase in pH. The flocculating rate of HXJ-1 with the trend of change in pH was more complex than that of a single strain. A good flocculating effect appeared either in acidic or

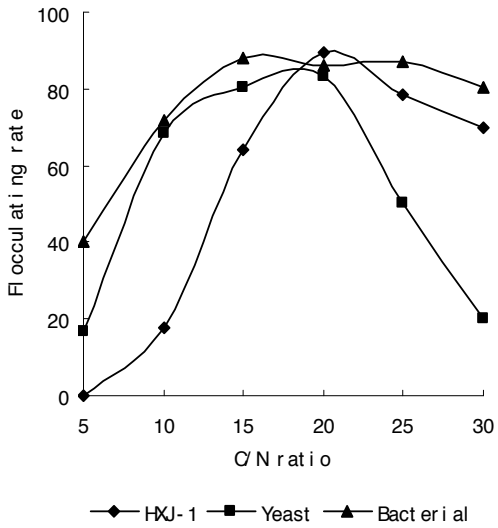


Figure 2. The effect of C/N on the flocculating rate of different strains.

alkaline conditions, but the flocculating rate was low at pH 4.0–6.0. This may be due to conducive acidic conditions for the growth and reproduction of *Pichia membranifaciens* in HXJ-1, in which *Pichia membranifaciens* played a major role, while in the alkaline conditions, *Bacillus cereus* played a major role. However, the flocculant synthesis enzyme of *Bacillus cereus and Pichia membranifaciens* had different activities in the optimum pH range, pH 4.0–6.0, which were not conducive to flocculant production, leading to a reduction in the flocculating rate. The observation of the strain number in the HXJ-1 fermentation broth showed that 82.8% strains were of *Pichia membranifaciens* with an initial pH of 3.6, but 71.6% of the strains were *Bacillus cereus* when the initial pH was 7.6 in the fermentation medium. The observation results further confirmed the correctness of the above inference.

3.4. Treatment effect of different flocculants on the starch wastewater

The starch wastewater normally has a high COD concentration and turbidity. Its main component of starch wastewater is starch, protein and carbohydrate. The results of starch wastewater treatment of four different bioflocculants are shown in Table 1. The COD removal rate was very high, reaching 81% and 86% by X15BF (produced by bacterial strains) and XJBF-1 (produced by mixed strains of bacteria and yeast) respectively, much higher than that of polyacrylamide (PAM) removal rate of COD. At the same time, the removal rate of SS in the starch wastewater and three kinds of microbial flocculants was equal to PAM but was quite superior to turbidity and chromaticity removal in the starch wastewater compared with PAM. The results also showed that the removal rates of all indicators in the starch wastewater by XJBF-1 were the highest, and excellent wastewater treatment results were achieved compared

http://dx.doi.org/10.5772/62114

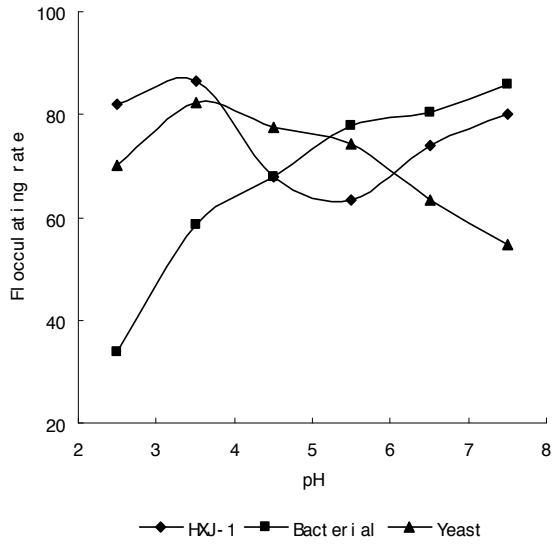


Figure 3. The effect of initial pH on the flocculating rate of different strains.

with the traditional flocculant and other two kinds of bioflocculants in starch wastewater treatment.

Type of flocculants	Water quality before treatment				Water quality after treatment				Removal rate (%)			
	COD mg/L	T NTU	C degree	SS g/L	COD mg/L	T NTU	C degree	SS g/L	COD mg/L	T NTU	C degree	SS g/L
J1BF	9660	2098	320	0.194	4021	966	136	0.128	58	54	57	34
X15BF	9660	2098	320	0.194	1780	856	98	0.124	81	59	69	36
XJBF-1	9660	2098	320	0.194	1350	713	36	0.114	86	66	88	41
PAM	9660	2098	320	0.194	4108	1011	120	0.125	59	55	62	36

J1BF: bioflocculant produced by yeast

X15BF: bioflocculant produced by bacteria

XJBF-1: bioflocculant produced by mixed strains (bacteria and yeast V/V 1:1)

PAM: polyacrylamide

T: turbidity

Table 1. Treatment results of starch wastewater by different kinds of flocculants

3.5. Treatment effect of different flocculants on landfill leachate

The composition of landfill leachate is very complex, with variations in water quality and quantity and a high concentration of organic matter. The landfill leachate treatment results of

four kinds of flocculants are shown in Table 2, which indicated that the removal rates of turbidly, chromaticity and SS by three bioflocculants were not as high as PAM, especially the X15BF and J1BF produced by a single bacterial and yeast strain. However, the COD removal rate reached 73% by XJBF-1 (produced by mixed strains), while the PAM COD removal rate was only 58%. Although the removal rates of turbidity, chromaticity and SS of landfill leachate by XJBF-1 were not as good as PAM, the treatment effect had significantly improved compared with the bioflocculants produced by a single strain. The removal rates of chromaticity and SS have been more than 70%, except for the turbidity removal rate which is only 50%. It is difficult to treat landfill leachate because of its complex composition. Therefore, relatively speaking, these types of bioflocculants played a certain role in landfill leachate treatment, especially XJBF-1 produced by mixed bacteria and yeast showed good results on landfill leachate treatment.

Type of flocculants	Water quality before treatment				Water quality after treatment				Removal rate (%)			
	COD mg/L	T NTU	C degree	SS g/L	COD mg/L	T NTU	C degree	SS g/L	COD mg/L	T NTU	C degree	SS g/L
J1BF	1944	1440	512	11.04	1125	793	302	6.15	42	44	41	51
X15BF	1944	1440	512	11.04	1070	747	216	4.48	45	48	58	59
XJBF-1	1944	1440	512	11.04	512	716	154	2.99	73	50	70	74
PAM	1994	1440	512	11.04	439	331	77	1.44	58	55	85	77

J1BF: bioflocculant produced by yeast

X15BF: bioflocculant produced by bacteria

XJBF-1: bioflocculant produced by mixed strains (bacteria and yeast V/V 1:1)

PAM: polyacrylamide

T: turbidity

C: chromaticity

Table 2. Treatment results of landfill leachate by different kinds of flocculants

3.6. Treatment effect of different flocculants on the printing and dyeing wastewater

The printing and dyeing wastewater has a high chromaticity degree and complex composition; the wastewater has greater biological toxicity, which contains dyes, sizing additives, oils, acids, alkalis, fibre impurities and inorganic salts, the structure of dyes, nitro and amino compounds, copper, chromium, zinc, arsenic and other heavy metals. The treatment effects by four kinds of bioflocculants on the printing and dyeing wastewater are shown in Table 3. From the experimental results, COD removal rates on the printing and dyeing wastewater by four flocculants were low, only 45–57%, but there was a high removal rate of suspended particles. The turbidity and chromaticity removal rates by X15BF (produced by a single bacterial strain) and XJBF-1 (produced by mixed bacterial and yeast strains) achieved 70–80%. The results were

http://dx.doi.org/10.5772/62114

far better than those of J1BF produced by yeast and traditional PAM. Especially in XJBF-1, the chromaticity removal rate was high as 88%, much higher than those of PAM and the other two microbial flocculants produced by bacteria and yeasts respectively. The SS removal efficiency by PAM was slightly better than that of the three types of microbial flocculants.

Type of flocculants	Water quality before treatment				Water quality after treatment				Removal rate (%)			
	COD mg/L	T NTU	C degree	SS g/L	COD mg/L	T NTU	C degree	SS g/L	COD mg/L	T NTU	C degree	SS g/L
J1BF	760	165	1200	0.348	415	83	576	0.146	45	49	46	58
X15BF	760	165	1200	0.348	382	44	352	0.146	49	73	70	58
XJBF-1	760	165	1200	0.348	328	36	124	0.128	57	78	88	63
PAM	760	165	1200	0.348	350	40	108	0.108	54	56	69	69

J1BF: bioflocculant produced by yeast

X15BF: bioflocculant produced by bacteria

XJBF-1: bioflocculant produced by mixed strains (bacteria and yeast V/V 1:1)

PAM: polyacrylamide

T: turbidity

C: chromaticity

Table 3. Treatment results on the printing and dyeing wastewater by different kinds of flocculants

4. Conclusion

The advantages of mixed strain HXJ-1 were as follows: directly using acidic wine wastewater, adapting to wastewater at high concentrations and the presence of less nitrogen. Three kinds of bioflocculants had some good treatment results on the starch wastewater, printing and dyeing wastewater and landfill leachate, respectively. The treatment effect of XJBF-1 (produced by mixed strains) on the starch wastewater was better than that of traditional PAM, and XJBF-1 had better treatment results on the three types of wastewater than those of X15BF-1 and J1BF-1 produced by single bacterial and yeast strain respectively. XJBF-1 had good removal rates for three kinds of wastewater chromaticity, especially for the starch wastewater and dyeing wastewater.

Acknowledgements

This study was supported by the Sichuan basic application project (Fund No. 05JY029-026-2). The authors are grateful to Dr. Chi Chang (Agriculture and Agri-Food, Canada), Prof. Yang

Jinxia (Sichuan University, China), Mr. Zhang jing (General Manager of Chengdu Hen Shi English Translation Company) and Ms Song Jinling for discussion and English editing.

Author details

Fan Zhang[1,2*], Wenju Jiang[3], Xiangdong Wang[3], Xiujuan Ji[3], Yina Wang[3], Wang Zhang[3] and Jiao Chen[3]

*Address all correspondence to: jhj94@hotmail.com

1 Institute of Plant Protection, Sichuan Academy of Agricultural Science, Chengdu, Sichuan, P.R. China

2 Key Laboratory of Integrated Pest Management on Crops in Southwest, Ministry of Agriculture, Chengdu, Sichuan, P.R. China

3 College of Architecture and Environment, Sichuan University, Chengdu, Sichuan, P.R. China

References

[1] Zhang T., Lin Zh. Microbial flocculant and its application in environmental production. Journal of Environmental Sciences, 2003, 1(1): 1–1

[2] Huang X.W., Cheng W., Hu Y.Y. Screening of flocculant producing strains by NTG mutagenesis. Journal of Environmental Sciences, 2005, 17(3): 494–498.

[3] Shih I.L., Van Y.T., Yeh L.C., Lin H.G., Chang Y.N. Production of a biopolymer flocculant from *Bacillus licheniformis* and its flocculation properties. Journal of Bioresource Technology, 2001, 78: 267–272.

[4] Rhee Y.H., Jiang J.H., Rogers P.L. Production of copolymer consisting of 3-hydroxybutyrate and 3-hydroxyvalerate by fed-batch culture of Alcaligenes sp. SH-69. Biotechnology Letters, 1993, 15: 377–382.

[5] You Y., Ma F., Ren N.Q., Wang A.J., Dong S.S., Peng Y.Z. Screening of flocculant-producing bacteria by the effluent of bio-hydrogen production reactors and its flocculant-producing conditions. China Water & Wastewater, 2006, 22(17): 29–33.

[6] Wang S.G., Liu X.W., Gao B.Y., Li J., Yue Q.Y., Feng G.Y. Production and properties of bioflocculant by Agrobacterium sp. LG5-1 using soy sauce brewing wastewater. Chinese Journal of Applied and Environmental Biology, 2006, 12(4), 74–576 (in Chinese).

http://dx.doi.org/10.5772/62114

[7] He Y.Y., Zeng D.N., Hu S.F., Ning L.Z., Tian K.X., Yan M.Q. Isolation and identification of *Bacillus cereus*. Journal of Hunan Agriculture University (Nature Science), 2005, 31(2): 203–205.

[8] Deng S.B., Bai R.B., Hu X.M., et al. Characteristics of a bioflocculant produced by *Bacillus mucilaginosus* and its use in starch wastewater treatment. Journal of Applied Microbiology and Biotechnology, 2003, 60(5): 588–593.

[9] Zhang F., Jiang W.J., Wang X.D., et al. Study on microbial flocculant produced by mixed strains via distillery wastewater. China Water & Wastewater, 2008, 24(11): 93–96.

[10] Zhang F., Jiang W.J., Wang X.D., et al. A new complex bioflocculant produced by mix-strains in alcohol wastewater and the characteristics of the bioflocculant, Fresenius Environmental Bulletin, 2011, 20(9): 2238–2245.

[11] Kurane R., Hatamochi K., Kakuno T., Kiyohara M., Hirano M. Production of a bioflocculant by *Rhodococcus erythropolis* S-1 grown on alcohols. Bioscience, Biotechnology, and Biochemistry, 1994, 58: 428–429.

[12] Ma F., Zhang J.F., Yuan L.J., Wang W., Wang Q., Wang A.J. Flocculating mechanism and ingredient analysis of compound bioflocculant. Acta Scientiae Circumstantiae, 2005, 25 (11): 1491–1496.

[13] Xi D.L., Sun Y.S, Liu X.Y. Environmental monitoring [M]. Beijing: High Education Press, 1996.

[14] Xie X.Z., Zhang Y.L., Yu R.S., Hu T.T. Water analytical chemistry [M]. Beijing: China Electric Power Press, 2007.

[15] Jing C.H, L.M., Zhu D. X., Li K.X. Environmental monitoring [M]. Tiangjing: Tiangjing University Press, 2007

Chapter 3

Anaerobic Biodegradation of Solid Substrates from Agroindustrial Activities — Slaughterhouse Wastes and Agrowastes

Ileana Pereda Reyes, Jhosané Pagés Díaz and
Ilona Sárvári Horváth

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60907

Abstract

Solid wastes from the meat industry are produced in large amounts resulting in a negative impact on the environment if not properly treated. Due to their high content of proteins and fats, these residues are excellent substrates for anaerobic digestion which holds high potential for methane yield. However, possible toxic compounds may be formed during its biodegradation with a consequent failure of the process under long-term operation. The anaerobic co-digestion of such residues with other co-substrates as those generated in agricultural activities has been proposed as a good alternative to overcome these problems. Nevertheless, today there is very little knowledge to assess on mixture interactions connected to wastes composition, biodegradability, and the kinetics of the anaerobic process when complex materials are utilized in ternary and quaternary mixture, specifically when co-digesting solid cattle slaughterhouse waste with agrowaste. It is therefore important to select the right combination of substrates and ratios to obtain synergy instead of antagonism in those mixtures. This chapter aims to provide an overview of the anaerobic digestion of solid slaughterhouse waste and agrowaste, as well as the influence of mixture interactions on its biodegradation.

Keywords: Agrowaste, anaerobic digestion, co-digestion, synergy, slaughterhouse waste

1. Introduction

The agriculture sector belongs to one of the most important human activities, but at the same time, it is considered as one of the most residue-producing sector in the world. Farmer activities have a huge impact on the environment, and moreover, industries related to agriculture, such as the meat processing industry, generate a large amount of high-strength residues. Due to the growing demand of meat in the world, the amount of organic solid wastes from meat producing industries is increasing every day. There are several attempts to improve the biodegradation of such residues, such as the anaerobic process, the preferred technology to diminish the organic load with an adequate efficiency [1-6]. It is well known that anaerobic digestion (AD) provides both environmental solutions and renewable energy production in rural areas, in most cases, with the corresponding autonomy.

Because of the high content of proteins and fats, slaughterhouse residues are holding high biogas potential and hence are interesting for the anaerobic digestion process. However, potential inhibitory compounds can be formed during the degradation of proteins and lipids, which make this process sensitive and prone to fail [7-9]. A possible way to overcome these problems is the co-digestion with carbon-rich co-substrates, i.e., a mixture of agrowastes with low protein/lipid content. This will lead to a better nutritional balance together with an improvement in the methane yield due to positive mixture interactions. Today, there is very little knowledge to assess mixture interactions connected to wastes' composition, biodegradability, and the kinetics of the anaerobic process when complex materials are utilized. The aim of this chapter is to describe the behavior of the anaerobic process when slaughterhouse residues are interacting with agro wastes, to provide data on its optimal mixture ratios, methane yield improvement, and the kinetics of the biodegradation process.

2. Characteristics of slaughterhouse wastes and agrowastes

Organic wastes are produced as an integral part of human life. Many anthropologic activities are responsible for the generation of organic wastes, such as the agriculture, the food processing, and the drinks manufacturing industry as well as domestic waste [10]. Agricultural wastes is a wide definition for residues resulting from numerous agricultural activities, such as the production of animals for slaughter (slaughterhouse residues), dairy products, the operation of feedlots, and planting and harvesting of crops [11]. This chapter will focus on both slaughterhouse residues and agrowaste residues.

Specifically, slaughterhouse residues are the result of abattoir operation in which solid and liquid wastes as well as wastewater are generated in larger amounts. In such activities, both the liquid and solid fractions are lumped together [12]. Depending of the slaughterhouse operation, there is a wide range of sources of residues that exist during meat processing. They are determined by the degree of further processing of the slaughtered animals, particularly by the degree of processing of the rumen, stomachs, and intestines in the tripery. Besides, the composition of these fractions also depends on the quality of actions to retain the solid and

http://dx.doi.org/10.5772/60907

liquid slaughter residues. The organic matter contained in abattoir effluents is the result of water-cleaning operation from all areas (the slaughtering wastewater, the tripery wastewater, and the washing-down and cleaning water) of the plant, where water comes in contact with manure, carcasses, offal, blood, and waste meat. The principal components of the organic matter presented in abattoir effluents are feces, gut contents, fat, and blood. Other components as coarse separable materials as well as suspended, colloidal, and dissolved organic materials are also presented, including the degradation products of fat and proteins, such as volatile organic acids, amines, and other organic nitrogen compounds. Carbohydrates occur in the wastewater in dissolved or colloidal forms.

Agrowastes are derived from biomass, which is usually comprised of lignocellulosic materials, and they have therefore high contents of cellulose, hemicellulose, and lignin. Table 1 shows a summary on the characterization of diverse animal wastes and agrowaste residues. Agrowastes are considered as the main renewable natural resources utilized widely in the world. The general composition of agrowastes is wood residues (leftover from forestry operations), municipal solid wastes (MSWs), and agricultural and food wastes. Today, 64% of the biomass energy is produced from wood and wood wastes, followed by 24% from MSW, 5% from agricultural waste, and additional 5% from landfill gases [13].

In the last 20 years, the energy crops and their subproducts, mainly in Europe, became and still are a very common feedstock for biofuel production. Governmental regulations, specifically in Germany, provided a scenario, which is quite attractive for energy crops exploitation [14, 15]. Nevertheless, plant wastes and manures have also a high potential to produce biogas cost-effectively [16] without compromising soil utilization for food production.

Substrates	**pH**	**TS (%)***	**VS (%)**	**Total nitrogen (%)***	**Lipids (%)***	**Proteins** (%)*	**Carbohydrates (%)***	**C/N**	**References**
Animal waste									
Cow rumen	6.1	14.9	89.4	0.3	n.a	n.a	n.a	n.a	[1]
Swine punch waste	5.9	31.7	82.7	0.3	n.a	n.a	n.a	n.a	[1]
Cow blood	7.4	19.8	75.0	2.9	n.a	n.a	n.a	n.a	[1]
Poultry offal, feed, and head	n.a	22.4	68.6	n.d	54	32	n.d	n.d	[17]
Iberia pig slaughterhouse waste	6.24	n.a	n.a	n.a	n.a	n.a	n.a	4.7	[18]
Solid cattle slaughterhouse waste	5.8–6.8	13–26	92–95	2.1–4	17.5–43	13–24	0.1	14.4	[19, 20]

Substrates	pH	TS (%)*	VS (%)	Total nitrogen (%)*	Lipids (%)*	Proteins (%)*	Carbo-hydrates (%)*	C/N	References
Poultry trimmings and bones	n.a	22.4	68	15.4	4.9	11.42	n.a	n.a	[17]
Solid cattle meat and fat	n.a	88.5	96.5	0.3	76.2	1.9	n.a	n.a	[21]
Solid pig meat and fat	n.a	56.4	98.7	1.4	46.7	8.3	n.a	n.a	[21]
Pig stomach	n.a	18.2	98.3	1.2	8.7	6.7	n.a	n.a	[21]
Rumen content	n.a	11.6	93.1	0.1	1.8	0.8	n.a	n.a	[21]
Bovine slaughterhouse waste	n.a	53.2	98.8	0.6	46.1	3.5	n.a	n.a	[2]
Agrowaste									
Horse manure	n.a	81.5	75.8	1.7	1.6	11.0	49.2	n.a	[22]
Cattle manure	n.a	23	78.6	0.8	0.3	4.8	13.0	n.a	[22]
Swine manure	n.a	55	63.6	1.8	n.a	n.a	n.a	10.2	[23]
Mixture of animal manure	8.4	35	40	0.4	0.4	2.6	18	n.a	[20]
Sugar cane press mud	6.3	9.1	80.84	n.a	n.a	n.a	n.a	26.4	[24]
Rice husk	6.6	89.2	77.8	n.a	n.a	n.a	n.a	99	[25]
Rice straw	6.5	87.8	79.6	n.a	n.a	n.a	n.a	43	[25]
Maize crops	n.a	67.2	95.8	0.6	n.a	n.a	n.a	64.7	[23]
Various crops	4.2	24	90	0.3	0.2	2.1	28.7	n.a	[20]
Tomato processing waste	4.4	n.a	n.a	n.a	n.a	n.a	na	16.8	[18]
Potato pulp	3.7	13.5–17.8	96–97	n.a	n.a	n.a	n.a	42–60	[26]
Fruit and vegetable wastes	4.2	8.3	93	0.2	n.a	n.a	n.a	34.2	[27]

n.a., not available; * based on fresh matter; TS, total solid; VS, volatile solid (based on dry matter); C/N: carbon/nitrogen ratio.

Table 1. Characterization data on diverse animal waste and agrowaste fractions

http://dx.doi.org/10.5772/60907

2.1. The impact of final disposal of slaughterhouse residues and agrowastes

Taking into account that the food and agroindustries usually produce large amounts of wastes, in those places where suitable treatment systems are unavailable, the environmental problemsassociated to such waste streams became an emergency issue to solve. The slaughtering process in the meat industry is the major contributor to liquid waste [28]. Furthermore, large amounts of water is used in dairy plants and slaughterhouses counting up to approximately 40×10^6 m^3 $year^{-1}$, which is an equivalent of the demand of water required for 500,000 people. In general, the wastewater from the meat industry is very difficult to decontaminate due to its high content of organic, mineral, and biogenic matter and the irregular discharge [5]. In order to reduce adverse ecological effects, the direct disposal of both liquid and solid abattoir wastes is not permissible, and a waste treatment prior to landfill is essential.

Slaughterhouse wastewater is a concern from the epidemiological point of view since it can also contain disease-causing agents [29]. Together with the blood, the rumen, and the stomach contents, these are at the focus of the disposal problems. Even after the slaughter of healthy cattle, the rumens have been found to contain somewhat rare *Salmonella* types, as well as other bacteria, viruses, and parasites (e.g., worms) in concentrations that are alarming from epidemiological point of view [30, 31]. In order to diminish such negative environmental impacts, several technologies have been introduced around the world. Composting and bioremediation are alternatives to the disposal of untreated residues, taking into account that the materials are biodegradable and can provide nutrients to soil, if land application is considered [32].

In addition, agrowastes are one of the major contributors of greenhouse gas emissions. The necessity to reduce this adverse effect and to develop a reliable alternative to the fossil fuel-dependent economy has raised the interest in agrowastes as a renewable energy sources. When applying this concept, a double effect can be achieved: the reduction of fossil fuels' consumption together with solving the above-mentioned environmental problems [33, 34]. Therefore, anaerobic digestion of agricultural wastes should be considered as one of the main alternative for treating these types of waste streams in an environmentally friendly scheme. It is well known that AD technology is one of the most useful decentralized sources of energy supply, especially when considering that all substrates utilized are easily available in many farms. Moreover, the capacity of AD process to reduce the organic content of biowastes provides a low-CO_2 emission, taking into account the overall waste-to-energy transformation.

Accordingly, the AD process stands for a promising solution to the problem from both energy conservation and pollution control points of views [5]. Besides energy production, the AD process generates a pathogen-free effluent and produces a stabilized material to be utilized as fertilizer in land applications [35].

3. Anaerobic digestion

Biological transformations can generally be classified as either aerobic or anaerobic processes. Each organic waste has a constant ultimate biodegradable fraction, and the final outcome of

its biodegradation is severely affected by different factors such as temperature, pH, alkalinity, nutrient requirements and bioavailability, digestion time (under anaerobic conditions), and particle size. Therefore, all aspects related to biodegradability should be taken into account to finally describe the degradation of different substrates and the performance of biological transformation processes [25, 36].

AD is a process by which the complex organic matter (proteins, lipids, and carbohydrates) are broken down by the action of different groups of microorganisms, i.e., *Bacterias* and *Archaeas* in the absence of oxygen, and a mixture of gases (mainly CH_4 and CO_2), called biogas, is produced. The final effluent with lower organic content can be utilized as a high-quality biofertilizer. The biodegradation process involves several serial and serial-parallel reactions in which each group of microorganisms is linked to another group and working together. The main steps of degradation are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In the hydrolysis phase, the complex particulate materials are disintegrated by the action of several extracellular enzymes into amino acids, long chain fatty acids (LCFAs), and sugars. The activity of the main extracellular enzymes (i.e., proteinases, lipases, and cellulases) involved in this phase is dependent on the characteristic of the substrates to be degraded [14]. Further on, the soluble compounds, produced during the hydrolysis step, are converted to volatile fatty acids (VFAs) and alcohols with carbon chain units less than five by the action of facultative bacteria in the acidogenesis step. However, carbon dioxide, hydrogen, and ammonia are also produced [37]. During this step, the accumulation of some intermediate compounds, such as acetate, propionate, butyrate, or ethanol, may occur in the system depending on the hydrogen production [38]. Then, in the acetogenesis step, the previous intermediates are converted into acetic acid, hydrogen, and carbon dioxide. The last step of the process is called methanogenesis, and it is driven by methanogens. Additionally, in the presence of sulfate, it is possible to obtain H_2S, ranging from 1% to 2% v/v in the biogas, which is produced by the action of sulfate-reducing bacteria [39].

The end products of the previous phases are converted into CH_4 and CO_2 via the acetotrophic or hydrogenotrophic pathways. The acetotrophic pathway is well known to be responsible for about 70% of the methane produced [40]. The other 30% is produced by the hydrogenotrophic pathway, in which H_2 and CO_2 are converted to CH_4 by *Methanobacteriales* and *Methanomicrobiales* (order level). In this step, the hydrogen-consuming microorganisms play an important function in order to keep low hydrogen partial pressure in the system.

Many factors affect the AD process, and temperature is one of the most important physical parameters since it directly affects the kinetics of the degradation and the growth of the microorganisms. However, AD can be carried out in a wide range of temperatures (i.e., between 10°C and 65°C); for industrial applications, mesophilic (35°C-37°C) and thermophilic (50°C-55°C) temperatures are the most applied ones. Several biogas plants operate today under mesophilic conditions due to higher process stability and lower energy requirements [41]; however, when it comes to increase the reaction rates and to achieve higher reduction of pathogens, thermophilic conditions have got an increasing attention [42]. Nevertheless, the operation at thermophilic temperatures might result in a less stable process due to accumulation of inhibitory compounds [43].

http://dx.doi.org/10.5772/60907

Alkalinity and pH are also important factors to take into account since each group of microorganism has a different optimum pH range. In AD, acid-producing microorganisms live at pH<5.0, while most methane producers require neutral pH. Neutral and stable pH values in the reactor require high alkalinity values, which primarily depend on the presence of bicarbonate ions in equilibrium with carbon dioxide. Nevertheless, ammonia released from protein degradation can also provide alkalinity to the system as it often reacts with carbon dioxide and water to form ammonium carbonate. Usually, the anaerobic digestion of slaughterhouse waste results in higher alkalinity values in comparison with processes treating sewage sludge [44]. However, pH is not a good indicator to control the process. Relatively slight fluctuations in the pH values might lead to process imbalance and instability. In that sense, rather the use of VFA/alkalinity ratio to monitor the system will give a fast and good indicator to detect stress conditions in the process [45].

As a biological treatment, the biodegradability of the substrates and the efficiency of AD are strongly affected by several other environmental and operation conditions, which will not be fully discussed in this chapter. Nevertheless, prior information about how some of these parameters may affect the AD process can be obtained by the use of a biodegradability test. One of the most relevant and useful tools for assessing the biodegradability of wastes is the investigation of a parameter known as biochemical methane potential (BMP), also called "biomethanation" or "biomethane potential". It can be determined by an experimental assay called BMP test. Performing this assay will not only lead to the determination of the BMP value, which is the ultimate amount of methane produced under anaerobic conditions from a certain substrate, but will also give information about the kinetics of the degradation process. Both the yield of methane and the degradation rate are very important factors when designing and operating full-scale anaerobic digesters and give the basics for defining operation parameters, like organic loading rate (OLR) or hydraulic retention time (HRT). Other important results, which can be obtained during the BMP test, are the identification of microbial inhibition, overloading, and possible adaptation of the microbial community to certain conditions [46, 47].

3.1. Biodegradability of slaughterhouse and agrowaste

The proper development of the anaerobic digestion is highly dependent on the type and composition of the material to be digested [48]. The breakthroughs, when it comes to deal with reactor design and operating conditions, succeeded in overcoming the initial limits of AD implementation. Today, AD can be operated with shock loads and can deal with different feed compositions, sensitivity to possible toxicants, instability, and different temperature requirements [49]. Although biological treatments remove organic compounds and pathogens from the effluents using microorganisms, in the case of slaughterhouse residues, the AD treatment is often complicated due to the presence of particulates and fats [50]. Indeed, AD is still a promising alternative for the treatment of these materials, since, just because of their high protein and fat content, these types of residues have a high potential to produce biogas.

Slaughterhouse waste can be considered as a protein-rich waste. During the anaerobic digestion of such wastes, the concentration of ammonia nitrogen can considerably increase due to the degradation of proteins. Accordingly, ammonia toxicity represents a major problem

during the anaerobic treatment of such wastes [7, 51]. With excess of ammonia concentrations (i.e., above 4 gN L^{-1}), the methanogenesis can be inhibited [52]. Furthermore, the digestion process becomes unstable, and the biogas production will drop as nitrogen concentration increases [10].

Due to the presence of higher amounts of lipids floating scum and the accumulation of long chain fatty acids (LCFAs), other problems during the AD of slaughterhouse wastes are presented [17, 53, 54]. Consequently, the methanogenesis will be inhibited, and the increased hydrogen levels will affect the propionate- and butyrate-degrading acetogens [55]. In general, the mechanisms responsible for the LFCAs accumulation are adsorption, precipitation with divalent ions, and entrapment in the flocculent structure of the sludge [5].

In the case of agrowastes, both cellulose and hemicellulose are the principal biodegradable components, which are linked with lignin in rigid lignocellulose complexes. On the one hand, due to the sheltering effect of lignin and the low biodegradability of lignin under anaerobic conditions, the degradation of these organic complexes is limited to yield of at most 50% of methane (<200 mL of CH_4 dry g^{-1}) compared to that produced from pure carbohydrates. On the other hand, several agrowastes can be degraded up to 80% of their fiber content, making them feasible for the AD treatment, e.g., paper [56] and rice residues from drying processes [25]. Nevertheless, at large-scale commercial farms, there is a lack of knowledge on biodegradability of agrowastes, and they are therefore not aware to utilize fruit and vegetable wastes for biogas production. However, a cost-effective operation, for example, using the codigestion of these residues, would drive the practical conditions to promote biogas technology.

3.2. Monodigestion of slaughterhouse wastes and agrowastes

In general, all sorts of biomass containing carbohydrates, proteins, lipids, cellulose, and hemicelluloses, as main components, are suitable to be used as substrates for biogas production. Among these residues, slaughterhouse wastes and agrowastes are of major importance due to both the amounts in which they are generated and the high organic content, as discussed before. The theoretical gas yield varies with the content of carbohydrates, proteins, and lipids, declared as the main volatile components that can be degraded under anaerobic conditions. The presence of carbohydrates and proteins provide faster conversion rates but lower gas yields, whereas the lipid content provides the highest biogas yield, however, with unfavorable kinetics of the overall process due to the requirement of a long retention time as a consequence of a slow biodegradability of lipids [56].

In the case of slaughterhouse wastes and agrowastes, diverse results are obtained whendigesting each of them alone as a sole substrate. Table 2 summarizes the results from severalresearch studies conducted either with slaughterhouse residues or agrowastes in batch orsemicontinuous operations.In most of the cases, the efficiency of VS reduction is low and is attributed to LCFA, protein, or lignocellulosic material (LCM) content of the different residues. In the next sections, the effect of LCFA, protein, and LCM content on the biodegradability of slaughterhouse wastes and agrowastes will be briefly discussed.

http://dx.doi.org/10.5772/60907

Substrates	T (C°)	Operation modeand conditions	Methane yield (Y_{CH4})	k_0 (d^{-1})	Degradation efficiency (%)	References
Solid cattle slaughterhouse waste	55	Batch (2L)	582 (mL gVS^{-1})	0.09	n.a	[19]
Solid cattle and swine slaughterhouse waste	35	CSTR (2L), HRT= 30 days	60 (mL gVS^{-1})	n.a	34.5 (VS red)	[1]
Iberian pig slaughterhouse waste	38	CSRT (2L), HRT=23.5 days	17.84 (m^3 m^{-3})	n.a	78.59 (COD red)	[18]
Fruit and vegetable wastes	35	CSTR (2L), HRT = 30 days	0.2 (mL gVS^{-1})	n.a	19.2	[1]
	55	ASBR (2L)	278 (mL gVS^{-1})	n.a	79 (VS red)	[27]
Horse manure	55	Batch (118 mL)	279 (mL gVS^{-1})	0.071	n.a	[22]
Cattle manure	55	Batch (118 mL)	250 (mL gVS^{-1})	0.041	n.a	[22]
Swine manure	35	Batch (250 mL)	357 (mL gVS^{-1})	n.a	n.a	[23]
	35	CSTR, HRT = 30 days, OLR = 1.2 kgVS m^{-3} d^{-1}	330 (mL gVS^{-1})	n.a	36.6 (VS red)	[23]
Organic fraction of municipal solid waste	55	Batch (2L)	537 (mL gVS^{-1})	0.33	n.a	[19]
Various crops	55	Batch (2L)	504 (mL gVS^{-1})	0.29	n.a	[19]
Sugar cane press mud	37	Batch (500 mL)	160 (mL gVS^{-1})	0.138	39 ($Y_{CH4}/Y_{CH4Theoric}$)	[24]
Rice straw	37	Batch (2 L)	226 (mL gVS^{-1})	0.078	62 ($Y_{CH4}/Y_{CH4Theoric}$)	[25]
	55	Batch (2 L)	281 (mL gVS^{-1})	0.168	74 ($Y_{CH4}/Y_{CH4Theoric}$)	[25]
	55	Batch (118 mL)	45 (mL g^{-1})	n.a	n.a	[25]
Rice husk	37	Batch (2L)	19 (mL gVS^{-1})	0.101	6 ($Y_{CH4}/Y_{CH4Theoric}$)	[25]
	55	Batch (2L)	44 (mL gVS^{-1})	0.111	11 ($Y_{CH4}/Y_{CH4Theoric}$)	[25]
Tomato processing waste	38	CSRT (2L), HRT = 8days	5.76 (m^3 m^{-3})	n.a	60.76 (COD red)	[18]
Potato pulp	35	Batch (5L)	n.a	0.073	80 (VS red)	[26]
Maize crops	35	Batch (250 mL)	350 (mL gVS^{-1})	n.a	n.a	[23]

n.a., not available; CSTR, continuous stirring tank reactor; ASBR, anaerobic sequence batch reactor; HRT, hydraulicretention time; COD, chemical oxygen demand; OLR:, organic loading rate; k0, the observed first-order kinetic constantof the overall process.

Table 2. Biodegradability of several substrates from agriculture/agroindustrial activities in anaerobic monodigestion

3.2.1. Effect of LCFA on the biodegradability of slaughterhouse wastes and agrowastes

Lipids are characterized as fats, liquid (oils), and solid (greases), commonly presented in slaughterhouse wastes and food wastes [57]. Lipids are quite attractive for biogas production since they have high theoretical methane potential due to the high number of C and H atoms present in their molecules. Nevertheless, the AD of lipids leads to several problems, such as their adsorption onto the cell wall of microorganisms and the inhibition of methanogenic consortium provoking sludge flotation and washout [58]. Usually, such inhibition problems occur when semicontinuous operation is applied.

Under anaerobic conditions, lipids are hydrolyzed into long chain fatty acids and glycerol by extracellular enzymes, i.e., lipases. Glycerol is easily degraded into biogas, while the degradation of LCFAs is more complicated. LCFAs are organic acids that contain long carbon chains of 8 to 18 units.

They are compounds, like lauric, myristic, palmitic, stearic, oleic, linoleic, caprylic, and capric acids, and usually occur in saturated or unsaturated fats (Table 3). Saturated fats are more difficult to degrade because of their high melting point compared with unsaturated fats. Their degradation is usually the rate-limiting step in the AD of solid slaughterhouse waste due to the slow growth of bacteria which consume LCFAs (i.e., with growth rate below 0.5 d^{-1}) in thermophilic reactors [59].

Fatty acids	Carbon units	Number of double bonds	Saturated/unsaturated
Caprylic	8	0	Saturated
Capric	10	0	Saturated
Lauric	12	0	Saturated
Myristic	14	0	Saturated
Palmitic	16	0	Saturated
Palmitoleic	16	1	Unsaturated
Stearic	18	0	Saturated
Oleic	18	1	Unsaturated
Linoleic	18	2	Unsaturated

Table 3. Common long chain fatty acids present in anaerobic digesters (adapted from [60])

The pathway for catabolism of LCFAs is referred as ß-oxidation (Figure 1) because the oxidation occurs at the ß-carbon (C-3) once LCFAs go into the cell [61]. In general, fatty acids can be found with an odd number of carbons or even number of carbons. The odd numbers of carbon are quite abundant in lipids of plants and some marine animals, while the even numbers of carbon are found in the rumen of cows. The oxidation of even or odd chains takes place in similar manner, but the final products are different. When even chains of carbon are oxidized, two carbon units of acetyl-CoA are released in each cycle; meanwhile, propionyl-

http://dx.doi.org/10.5772/60907

CoA is the final product when odd chains of carbon units are oxidized (Figure 1). In that sense, acetate and hydrogen or acetate, hydrogen, and propionate are produced [61], which are further converted to methane and carbon dioxide in the AD process. The degradation of the propionic acid released is thermodynamically dependent on low H_2 partial pressure in the reactor and hence on the activity of H_2 consuming methanogens. This means that if these compounds are not efficiently degraded, their accumulation can cause several disturbances and will lead to an instable process. Acetogenic microorganisms degrading LCFAs are closely related to *Syntrophomonadaceae* and *Clostridiaceae* families living together with H_2 consuming methanogens [62, 63], although few species are described to use LCFAs with more than 12 carbon units [64].

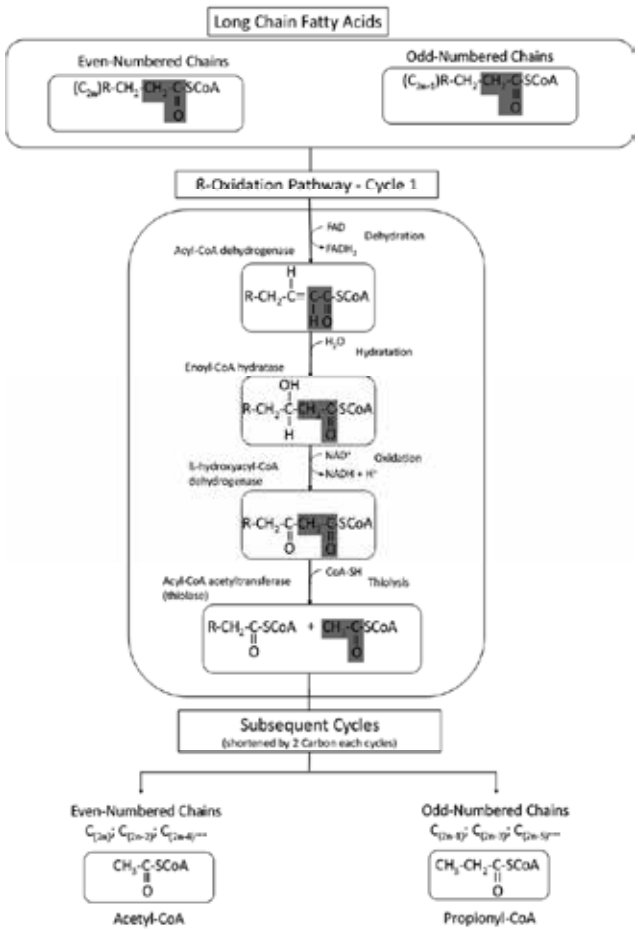


Figure 1. The degradation pathway for long Chain fatty acids (adapted from [61]).

The accumulation of LCFAs is well known to inhibit the methanogenic activity of both acetotrophic and hydrogenotrophic methanogens causing several operational and microbio-

logical problems in biogas plants treating slaughterhouse waste [65-67]. The mechanism of inhibition has been attributed to the adsorption onto the cell wall creating a physical barrier and affecting the transport of nutrients, substrates, and products [68]. This is strongly dependent on the type of microorganisms present in the system, the specific surface area of the sludge, the length and complexity of the carbon chain (i.e., the number of double carbon bonds), and the adaptation of the biomass [69]. Anaerobic sludge with higher specific surface area, i.e., suspended and flocculent, was observed to be more susceptible to inhibition than granular sludge [69]. In addition, process failure has been observed in up-flow anaerobic sludge blanket reactors fed with a mixture of LCFAs at concentrations below of the toxicity level [70] because of the washout of the anaerobic biomass [64]. Sludge flotation is caused by the adsorption of LCFAs on the cell surface and mainly depends rather on the loading rates of LCFAs than on their concentration [70]. Concentrations over 50-75 mg L^{-1} for oleic acid have been reported inhibitory [65, 69], while inhibitory levels of 1 100 mg L^{-1} and 1 500 mg L^{-1} where reported for palmitic [68] and stearic [71] acids, respectively. However, other studies have proved that the inhibition is a reversible process and the system may recover after a lag phase and adaptation period [72].

Another problematic associated with lipids' degradation is their high tendency to form floating aggregates and foam in biogas reactors leading to severe operational problems, such as obstruction of the piping gas collection and pump failure. Recent studies have linked the foam formation with substrate composition, especially in cases of fatty and protein-rich materials [73-75]. Furthermore, specific microorganisms, such as *Dialister*, *Pseudonocardia*, *Thermoactinomyces*, *Pseudomonas*, and *Thermotoga*, were found to increase their abundance in foaming biogas reactors overloaded with lipid-rich substrates [75]. During the metabolic activity of these microorganisms, natural biosurfactant products are released to the medium decreasing the surface tension and contributing to foaming. Intermittent feeding operations have been proposed as an effective strategy to allow biological degradation of LCFAs coupled with inhibition phenomenon [76].

3.2.2. Effect of proteins on the biodegradability of slaughterhouse wastes and agrowastes

Slaughterhouse wastes as well as other agrowaste residues, such as swine and poultry manure, are protein-rich materials. Proteins, like fats, are also energy-rich materials that provide high gas production. Proteins are composed of long chains of amino acids joined together by peptide bonds each containing an amino group (-NH_2) and a carboxyl group (-COOH) [60]. In the course of decomposition, proteins are first hydrolyzed by extracellular enzymes, i.e., proteases, into amino acids. One particular element to consider, when analyzing protein-rich wastes, is the low C/N ratio in contrast to the high organic content and the high biological oxygen demand (BOD) [77, 78].

During degradation of proteins, the nitrogen is released in the form of ammonia (NH_3) or ammonium (NH_4^+). Acetate and butyrate are also produced. NH_3 and NH_4^+ are present in equilibrium with each other, and the predominance of which will depend on prevailing conditions inside the digester, such as pH and temperature. The described inhibitory specie is NH_3 rather than NH_4^+. So far, the most accepted mechanism of inhibition has been

http://dx.doi.org/10.5772/60907

attributed to the fact that uncharged NH_3 may easily diffuse into the cell, where it is further converted to NH_4^+ consuming hydrogen ions and causing proton, sodium and potassium imbalance (Figure 2) [79]. As a consequence, the cell must then use energy to recover the transmembrane ion gradient, which is used for various energetic purposes and to drive several biochemical reactions [80-82]. Some studies have shown that acetoclastic methanogens are more affected by ammonia than hydrogenotrophic methanogens [52, 53]; however, the reported inhibitory concentrations vary in a wide range depending on the source of inoculum and the adaptation of the microorganisms, as well as on substrates' characteristics, pH, and temperature [79]. It was reported previously that free ammonia inhibits methanogenesis in nonadapted sludge at concentrations ranging from 0.1 to 2.0 gN L^{-1} [52, 53, 83-85]. However, in the presence of adapted biomass, higher inhibitory concentrations, i.e., up to 4 gN L^{-1}, have been observed [52]. The adaptation of methanogens to arising ammonia concentrations has been reported [86, 87] and explained by the growth of new biomass rather than the metabolic changes in the methanogens [52]. However, differing from LCFAs' inhibition, ammonia inhibition does not lead to failure and instability of the biogas process. The interaction that takes place between volatile fatty acids, pH, and ammonia will lead to a so-called "inhibited steady state," in which the process is running under apparent stable condition but with lower methane production [88].

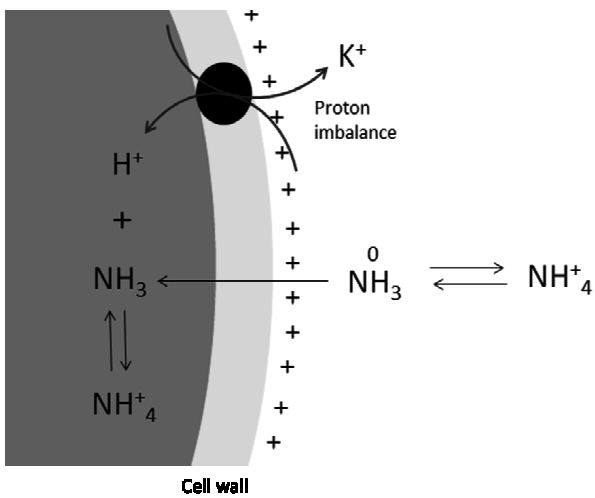


Figure 2. The mechanism of ammonia inhibition in nonionized form NH_3 can easily enter the cell causing proton imbalance (adapted from [79]).

3.2.3. Effect of Lignocellulosic Materials (LCM) on the biodegradability of slaughterhouse wastes and agrowastes

Carbohydrates are the main components of organic wastes from anthropogenic activities including the organic fraction of municipal solid waste (MSW) from households and markets. In addition, agrowaste with a high content of lignin, cellulose, and hemicellulose are produced

in several activities from the agriculture sector. Typical substrates are green residues of fruits and vegetables remaining after the harvest, leaves of sugar beets, energy crops, straw from animal feeding, and animal manure consisting a large fraction of straw, among others. As it was point out earlier, the composition of feedstock used for biogas production is highly important, as the biogas yield will be strongly linked with its biological degradability. Obtaining high biodegradability will also lead to a better final disposal of such residues, as well as higher energy production per unit of mass of substrates [25].

LCM is a carbohydrates-rich substrate mainly composed of cellulose (40%-50%), hemicellulose (25%-35%), and lignin (15%-20%) [89]. Lignin protects the lignocellulosic structure and is linked with cellulose and hemicellulose by different chemical bonds, making this structure extremely resistant to enzymatic digestion. The rate and the biodegradability (extension) of lignocellulosic substrate are one of the most important factors to be considered, when applying them for anaerobic digestion. Depending on the ratio between the rate of acidification and the rate of methanogenesis, the anaerobic degradation can be considered to be successful or not. VFAs tend to be accumulated as a result of a faster acidification step than methanogenesis step, provoking a drop in pH, which leads to inhibition of the methanogenic activity [90].

Since carbohydrates vary in their nature, they are anaerobically converted at different rates. A fast biodegradation gives more methane per unit of feed biomass per time and also reduces the reactor's size, making the process economically more attractive. However, the easily biodegradable fraction present in fruit and vegetable wastes with high moisture content may be converted too fast, increasing the volatile fatty acid content in the digester. This is a major limitation of treating these kinds of wastes, as they are very prone to acidify the system, decreasing the pH and making the process unstable [91, 92]. On the other hand, complex agrowaste fractions, such as straw, contain a large amount of recalcitrant structures that hamper the degradation [22, 25]. Straw is a cellulose-rich substrate. Cellulose consists of linear polymer chains of glucose molecules linked by β-glucosidic bonds, which makes this structure hard to digest. Hence, hydrolysis has proved to be the rate-limiting step during anaerobic degradation because of this complex structure of cellulosic materials [92, 93]. Enzymes involved in the hydrolysis of cellulose-rich materials have difficulty to access the structure, especially in the case of lignocelluloses, making the process slow. In order to improve the accessibility providing higher methane yield and increasing biodegradation rate, some pretreatments (chemical, physical, and enzymatic) have been used to open up the structure and to disrupt cellulose crystallinity [24, 94-99]. For example, methane yields reaching up to 88% of the theoretical values have been achieved after a chemical pretreatment using *N*-methylmorpholine-*N*-oxide pretreatment on barley straw [100].

3.3. Anaerobic codigestion (AcoD) of slaughterhouse waste and agrowaste

Codigestion is not a new concept in anaerobic processes. It has been applied within research and practice for more than 20 years, especially in Europe [101]. Codigestion appears to be "the solution" to obtain an increase in methane production, to avoid inhibition, and to operate profitable biogas plants. In order to achieve these objectives, an appropriate mixture of substrates, containing proper percentages of different kinds of organic matter to be degraded,

http://dx.doi.org/10.5772/60907

must be determined. A lot of studies have been investigating such factors [102-108]. This chapter will give a brief summary of these results.

The beneficial effect of the codigestion has been widely studied from different points of views, as the nutrient balance, mainly when mixing nitrogen-rich wastes with carbon-rich ones, as well as pH, the presence of inhibitors/toxic compounds, biodegradable organic matter, and dry matter [109]. Hence, at early stage of different studies and applications related to codigestion the principal variable was the C/N ratio.

Codigestion can be defined as the treatment of a mixture of at least two different waste streams under anaerobic condition with the intention of improving the efficiency of the anaerobic process. Additional benefits of AcoD are the dilution of toxic compounds if present or developed during the process, the supply of required buffer capacity, as well as the adjustment of the moisture content and the augmentation of bacterial strains taking part in the process [108, 110].

Thus, why is the codigestion of slaughterhouse waste and agrowaste preferable to apply? As we described it above, several technological and economic advantages are established when codigestion is performed. Due to these benefits, an increasing number of full-scale codigestion plants treating manure and industrial organic wastes are in operation, mainly in Denmark and in Germany [111, 112]. Higher biogas output and therefore better anaerobic process performance and profitability can be attained by codigesting of, for example, animal manure or sewage sludge with 10% to 20% of solid waste fractions from agroindustry and food industry, i.e., slaughterhouse, pharmaceutical, kitchen, fermentation, or municipal wastes [49]. In this way, an increase of 50% to 200% in the methane (CH_4] production of manure digesters can be achieved [10, 113, 114]. Manure is widely accepted as the basic substrate in codigestion. This substrate is easily available in many farms all over the world; however, the low biogas yield of manure usually does not justify the investment costs for farm-scale plants. Nevertheless, by introducing energy-rich cosubstrates, this aspect can overcome.

The effect of temperature on codigestion has also been widely studied due to the problems that are faced in case of temperature fluctuations, which provoke instability and disturbances in all the other main parameters of the process [115, 116] with corresponding decreasing levels of yields. As a result of several experiences, the thermophilic operation is preferred over the mesophilic one, mainly due to its kinetics improvements and sanitization capability [117].

Still, the optimal operational conditions in terms of mixture composition must be investigated for each specific case [78, 118-120]. In addition, the amounts of wastes available in site should also be evaluated. The amounts of residues with high organic content that are generated at a particular location usually are not enough for a cost-effective anaerobic process. If codigestion concept is applied, designing a proper mixture with other locally generated residues might improve the economy of the overall process.

3.3.1. Effect of the C/N ratio on the biodegradability of slaughterhouse wastes and agrowastes

As explained before, during the codigestion process, protein-rich wastes can provide the buffering capacity and a wide range of nutrients, while carbon-rich wastes provide a high

carbon content. This will balance the C/N ratio for all substrates decreasing the risk for inhibition [84]. The C/N ratio is one of the most controlled parameter for an efficient biodegradation of residues. Higher or lower values than the optimal ones of this parameter diminish the reaction rate of microorganisms involved in each step of degradation, and in some cases, the anaerobic process can be inhibited. As carbon and nitrogen are the main macronutrients for microorganisms, established levels of those provide certain insurance for the nutrient supply during the process. Nevertheless, a wide range of values is reported in the literature, showing the proposed optimal range, and in some cases with inconsistency in the boundaries of these values. The bioavailability of the carbon seems to play an important role in this apparent contradiction. Numerous investigations found that the C/N ratio values ranging from 20 to 35 are optimal for the anaerobic process [79, 99, 121-123]; meanwhile, optimal intervals as between 12 and 16 [123] and between 20 and 70 [124] have also been suggested. Moreover, lower values of C/N ratios (i.e., 6-9) have been also reported as suitable values for the anaerobic digestion of nitrogen-rich wastes [123, 125]. Some authors revealed the relevance of temperature not only for the nitrogen balance [88] but also for the carbon bioavailability [25]. The biodegradation capability of certain substrates will also influence the C/N ratio due to the release of NH_4^+ [107]. As demonstrated, there is no a unique criteria for C/N boundaries to define an optimal ratio for the anaerobic process. Hence, substrate characteristics as well as operational parameters should also be considered in order to ensure a proper development of the anaerobic process.

Generally, we can conclude that the codigestion concept is widely applied, and in most cases, it is related to positive effects and rarely with negative interactions for the process itself. Modeling of anaerobic codigestion has been therefore a focus of many researchers in order to predict the expected methane yield [126, 127]. However, the mathematical tools are still unable to properly predict synergy and antagonisms effects [128]. The necessity to clarify adverse effects when mixing substrates and cosubstrates has not gained enough attention yet, as it can be seen from the topics in recent literature [129].

3.4. Recent developments in AcoD of slaughterhouse waste and agrowaste mixtures

As it was pointed out in the previous chapter, using a codigestion concept will lead to several advantages. However, it is important to access the mixture interactions affected by the waste composition in order to determine the responses and optimize the process. The composition of substrate will influence the activity of the microbiological population [21, 130], which in turn will largely affect long-term process stability, the degradation rate of the solids, and consequently the biogas yield. When larger yields than the predicted ones can be detected, it is usually because of synergy occurring in the mixture, while lower yields are caused by antagonism. The recent developments in AcoD of slaughterhouse waste and agrowaste mixtures were therefore focused on the evaluation of these synergistic or antagonistic interactions [20]. To be able to investigate the mixture interactions between several fractions of substrates, it is preferable to use a statistically designed experimental setup. Meanwhile synergy and antagonism can be detected using this methodology; the actual explanation of why those effects are presented cannot be explained by a simple statistical evaluation. In this

http://dx.doi.org/10.5772/60907

section, the biological influence of different substrate compositions on AcoD of solid cattle slaughterhouse wastes mixed with different residues from agricultural activities will be discussed.

The use of slaughterhouse waste for biogas production gained an increasing attention in Europe during the past years; however, it is still not very common in Latin America and in developing countries. As it was earlier mentioned, this waste fraction can increase the economic feasibility of biogas production in codigestion plants due to its high methane potential. Nevertheless, slaughterhouse waste is a complex material, which cannot be easily treated because of its high potential to inhibit the process owing to ammonia and long chain fatty acids' accumulation [9, 21, 121]. Therefore, the use of this waste fraction in codigestion processes was investigated in different studies, and several surveys have proved that this concept will improve the biological degradation [1, 19, 20]. However, it is still a little knowledge available when it comes to its biodegradation and mixture interactions using several cosubstrates from agricultural activities. The decision of which cosubstrates should be used for the codigestion had been simplified so far, based on determining an optimal C/N ratio together with a balanced lipids/proteins/carbohydrates composition; however, the bioavailability of these materials must also be considered.

A brief summary on the investigations and the obtained results considering the use of different slaughterhouse waste fractions in codigestion processes are shown in Table 4. When investigating codigestion processes, most of the studies are focused so far on manure and sewage sludge as the main raw materials used in agricultural and agroindustrial sector [129], and two components have been typically used in the codigestion processes. Considering slaughterhouse waste as the main substrate used in the mixtures, poultry and swine animal wastes are the most reported waste fractions found in the literature [2, 18, 131].

Substrates	**Mixture ratio (%)**	**T (°C)**	**Operation mode and conditions**	**Methane yield (Y_{CH4})**	***k*0 (d^{-1})**	**Degradation efficiency (%)**	**References**
Binary mixture combinations							
Solid cattle/swine slaughterhouse waste + fruit/vegetable waste	50:50 (wet basic)	35	CSTR (2L), HRT = 30 days	40 (mL gVS^{-1})	n.a	53.8 (VS red)	[1]
Solid cattle/swine slaughterhouse waste + solid cattle/swine manure	50:50 (wet basic)	35	CSTR (2L), HRT = 30 days	0.26 (m^3kgVS^{-1})	n.a	51.7 (VS red)	[1]
Solid cattle slaughterhouse waste + animal manure (pig, cow, horse)	64:36 (VS basic)	55	Batch (2L)	613 (mL gVS^{-1})	n.a	n.a	[20]

Substrates	Mixture ratio (%)	T (°C)	Operation mode and conditions	Methane yield (Y_{CH4})	*k*0 (d^{-1})	Degradation efficiency (%)	References
Solid cattle slaughterhouse waste + organic fraction of municipal solid waste	62:38 (VS basic)	55	Batch (2L)	647 (mL gVS^{-1})	n.a	n.a	[20]
Solid cattle slaughterhouse waste + various crops (straw and fruit/vegetable waste)	53:47 (VS basic)	55	Batch (2L)	461 (mL gVS^{-1})	n.a	n.a	[20]
Liquid poultry slaughterhouse waste + organic fraction of municipal solid waste	17:83 (wet basic)	34	CSTR (3L), HRT = 50 days, OLR = 1.85 kgVS m^{-3} d^{-1}	400 (mL gVS^{-1})	n.a	80.6 (VS red)	[134]
Abattoir waste + fruit/ vegetable waste	70:30 (wet basic)	35	ASBR (2L), HRT = 20 days, OLR = 1.2 gVS L^{-1} d^{-1}	191 (mL gVS^{-1})	n.a	84 (VS red)	[27]
Abattoir waste + fruit/ vegetable waste	70:30 (wet basic)	55	ASBR (2L), HRT = 20 days, OLR = 1.28 gVS L^{-1} d^{-1}	453 (mL gVS^{-1})	n.a	86.2 (VS red)	[27]
Slaughterhouse waste (hog and cow stomach content) + sewage sludge	25:75 (wet basic)	37	CSTR ($2m^3$), HRT = 17 days, OLR = 2.9 kgTS m^{-3} d^{-1}	0.23 (m^3 $kgTS^{-1}$)	n.a	n.a	[136]
Cattle/swine slaughterhouse waste + sewage sludge	1:7 (wet basic)	35	CSTR (3L), HRT = 50 days, OLR = 1.85 kgVS m^{-3} d^{-1}	430 (mL gVS^{-1})	n.a	38 (VS red)	[137]
Ternary mixture combinations							
Solid cattle slaughterhouse waste + cattle manure + various crops (straw, fruit/vegetable waste, animal feed)	25:37.5:37.5 (wet basic)	55	Batch (2L)	499 (mL gVS^{-1})	0.32	n.a	[19]
Solid/liquid cattle slaughterhouse waste + cattle manure + various crops	25:37.5:37.5 (wet basic)	37	Batch (500 mL)	208 (mL gVS^{-1})	0.169	n.a	[4]

http://dx.doi.org/10.5772/60907

Substrates	Mixture ratio (%)	T (°C)	Operation mode and conditions	Methane yield (Y_{CH4})	$k0$ (d^{-1})	Degradation efficiency (%)	References
Solid cattle/swine slaughterhouse waste + solid cattle/swine manure + fruit/ vegetable waste	67:17:17 (wet basic)	35	CSTR (2L), HRT = 30 days	270 (mL gVS^{-1})	n.a	67.3 (VS red)	[1]
Solid cattle slaughterhouse waste + various crops + organic fraction of municipal solid waste	40:35:25 (VS basic)	55	Batch (2L)	614 (mL gVS^{-1})	n.a	n.a	[20]
Slaughterhouse waste + pig manure + a mixture of industrial waste	12:71:17 (wet basic)	35	CSTR (3L), HRT = 28 days, OLR = 3.1 kgTS m^{-3} d^{-1}	624 (mL gVS^{-1})	n.a	n.a	[48]
Quaternary mixture combinations							
Solid cattle slaughterhouse waste + cow manure + various crops + organic fraction of municipal solid waste	25:25:25:25 (wet basic)	55	Batch (2L)	664 (mL gVS^{-1})	0.20	n.a	[19]
Solid cattle slaughterhouse waste + cow manure + various crops + organic fraction of municipal solid waste	22:22:45:11 (wet basic)	55	Batch (2L)	491 (mL gVS^{-1})	0.34	n.a	[19]
Slaughterhouse waste + pig manure + vegetable waste + various kinds of industrial waste	12:66:5:17 (wet basic)	35	CSTR (3L), HRT = 36 days, OLR = 2.6 kgTS m^{-3} d^{-1}	682 (mL gVS^{-1})	n.a	n.a	[48]

n.a., not available; CSTR, continuous stirring tank reactor; ASBR, anaerobic sequence batch reactor; HRT, hydraulic retention time; COD, chemical oxygen demand; OLR, organic loading rate; k_0, the observed first-order kinetic constant of the overall process.

Table 4. Biodegradability of slaughterhouse waste in anaerobic codigestion with residues from agricultural/ agroindustrial activities

Taking a look into the literature, there are only few studies investigating the codigestion of solid cattle slaughterhouse waste with residues from agriculture activities in ternary or quaternary mixtures (Table 4). It is proved that a considerable improvement in methane yield can be achieved, when treating several waste fractions together at the same time, due to the positive synergistic interactions that will lead to an increase in the biogas yield [132].

In addition, temperature also plays an important role in increasing the methane yield, probably due to higher bioavailability of carbon and nitrogen sources and a better hydrolysis performance at higher temperatures [19, 20, 27].

Recent investigations in anaerobic codigestion have evaluated the biodegradability in binary, ternary, and quaternary mixture combinations of solid cattle slaughterhouse waste with agrowastes in terms of methane yield and the specific methane production rate [20]. The experiments were performed in batch assays at thermophilic conditions (55°C±1°C) using a four factor mixture design to evaluate the two and three factor mixture interactions (i.e., synergy or antagonism). The biodegradability of every individual fraction was also assessed. As shown in Figure 3, the response variables, i.e., methane yield (Y_{CH4}) and methane production rate (r_{sCH4}), can be predicted as a function of any component in the blend. These results show that high methane yield can be attained with the presence of slaughterhouse and municipal solid wastes in the mixture. On the other hand, the presence of manure and various crops, even though they do not contribute with high values of methane yield (Figure 3A), is needed for a proper balance of macro- and micronutrients. This was proved when analyzing the biodegradability in terms of specific methane production rates (Figure 3B), in which the optimal result was found when the slaughterhouse waste was codigested with various crops, manure, and municipal solid wastes. The mixture that includes all of the four substrates resulted in an increase in methane yield by 31%, compared to the expected yield, which was calculated on the basis of the methane potential of each individual fraction. This clearly demonstrates a synergistic effect. Moreover, when combining cattle slaughterhouse waste in ternary mixtures, an increment of 15% in the methane yield was achieved compared to that in binary mixtures [20]. Mixtures in similar combinations were also investigated in another studies. When slaughterhouse waste was codigested with pig manure, vegetable waste and food industrial waste a biogas yield of 0.9 to 1 m^3 $kgVS^{-1}$ was obtained together with a stable operation [48]. Furthermore, anaerobic digestion of mixtures of rumen, stomach/intestinal content, food waste, and manure showed also stable performance working at OLR exceeding 2.5 kgVS m^{-3} d^{-1} with a hydraulic retention time of less than 40 days under mesophilic conditions (37ºC) [133].

The binary mixture combination of liquid poultry slaughterhouse waste and MSW has shown to give a feasible process after an acclimatization period comparing with the results observed when these fractions were digested individually [134]. HRT could be decreased from 50 to 25 days with the corresponding increase in OLR up to 3.70 kgVS m^{-3} d^{-1}, and an increase of volatile fatty acid reduction efficiency from 80.6% to 82.6% was also found [134]. Moreover, the codigestion of slaughterhouse waste (i.e., either cattle or swine) with animal manure (i.e., either pig or cow) has also proved to be successful [1, 20, 135]. High methane yields and stable process performance have been observed for these mixtures both during thermophilic and mesophilic

http://dx.doi.org/10.5772/60907

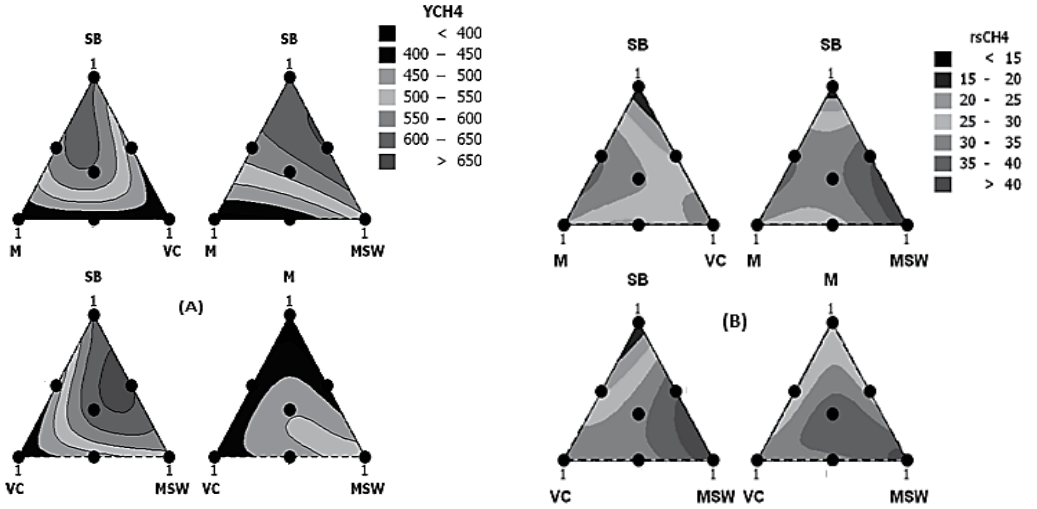


Figure 3. Mixture contour plots for methane yield (A) and for specific methane production rate (B). Letters on the apex of the triangle correspond to the following: SB, solid cattle slaughterhouse waste; M, a mixture of animal manure (pig, horse, and cow); VC, a mixture of fruit/vegetable waste and straw; MSW, the organic fraction of municipal solid waste (adapted from [20]).

semicontinuous operations [1, 135] (Table 4). This behavior has been mainly attributed to the characteristics of the animal manure itself (i.e., high fraction of fibers, nutrients, and good buffer capacity), which led to significant synergetic interactions during the codigestion process with solid cattle slaughterhouse waste [20].

In Figure 4, the performance of the semicontinuous anaerobic codigestion of slaughterhouse waste and manure is compared with the digestion of slaughterhouse waste as a single substrate. In accordance with the results attained previously in batch mode [20], the combination of slaughterhouse waste and a mixture of animal manure showed the best performance even during semicontinuous operation with an OLR of 3 gVS L^{-1} d^{-1} and HRT of 25 days. Meanwhile, the digestion of cattle slaughterhouse waste as sole substrate failed at much lower OLR (i.e., 0.9 gVS L^{-1} d^{-1}), and moreover, strong foam formation was also observed in this reactor (Figure 4A). When these results were compared with those found in the literature, a stable semicontinuous operation was reported for waste combinations of solid cattle/swine slaughterhouse waste and solid cattle/swine manure compared with that when slaughterhouse waste was digested with fruit and vegetable wastes at mesophilic conditions [1].

Similarly, the binary mixture combination of solid cattle slaughterhouse waste and various crops, including fruits/vegetables and straw, showed previously to be a blend with antagonistic interactions during the thermophilic batch assays [20], and this binary mixture combination led to unstable operation performance even during the semicontinuous operation with accumulation of VFAs leading to a drop in pH [1, 135]. The OLR of 2 gVS L^{-1} d^{-1} led to overloading with a consequent gradual decline in the methane production [135], probably due to the high biodegradability of fruit and vegetable wastes, leading to a fast acidification of the

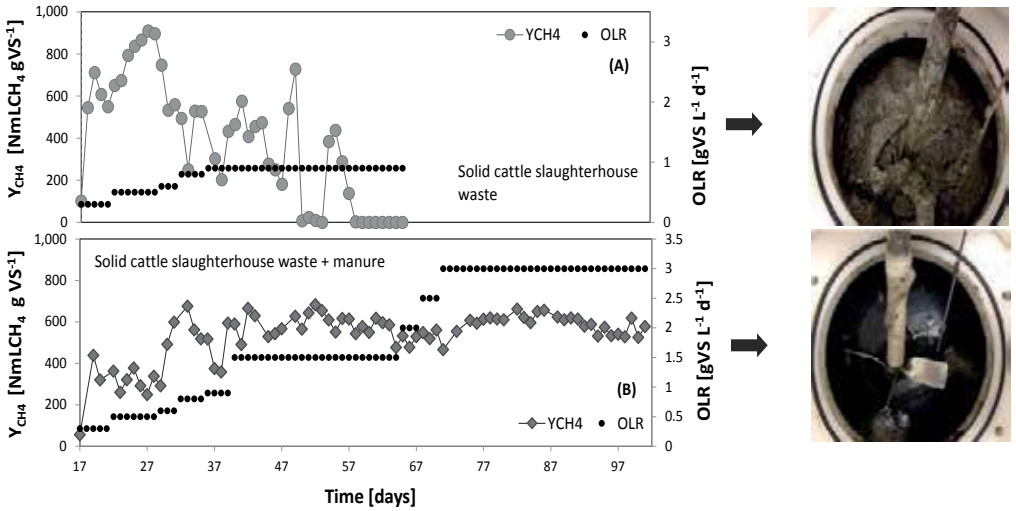


Figure 4. Daily methane yield (Y_{CH4}) and applied organic loading rate (OLR) during semicontinuous digestion of slaughterhouse waste as a single substrate (A) and its codigestion with animal manure (B) (adapted from [135]).

system [107]. Furthermore, on investigating kinetic parameters, this mixture composition showed the lowest degradation rate, resulting in the lowest value of methane production rate when compared with other mixtures of slaughterhouse waste and cosubstrates examined in batch assays [20].

These results clearly show that it is very important to choose the right cosubstrates' combinations and ratios in order to avoid failure at industrial level and get those technological, economical, and biological advantages of the codigestion technology mentioned above. Since the economic feasibility of AD plants is directly linked with the methane potential of the treated waste, it is important to investigate mixture interactions between substrates that may enhance or attenuate the degradation rate and the methane yield.

Hence, it is necessary to recall that the biodegradability of complex substrates, as slaughterhouse residues and agrowastes, is highly dependent on the relative quantities of fats, proteins, and LCM. The actual bioavailability of carbon and nitrogen sources, as well as temperature, organic loading rate, moisture content, pH and alkalinity, and many other parameters are interrelated and provoke either positive or negative effects when mixing the waste fractions in different ratios.

4. Conclusions

Agricultural residues, as slaughterhouse and agrowastes, are produced in large amounts with a high organic content holding an important potential for biogas production. As the organic composition of such residues can lead to the development of inhibitory effects during the

http://dx.doi.org/10.5772/60907

anaerobic process, one possible way to overcome this problem is applying codigestion for an appropriate mixture of substrates. One aspect to be analyzed when designing a codigestion process is the availability of different waste fractions generated by local industries or communities. Nevertheless, the codigestion of slaughterhouse residues with agrowastes does not necessarily result in only positive effects in terms of methane yield and degradation rate. It has been shown that antagonistic effects can also be obtained with certain mixture ratios. It was also shown that applying BMP assays had provided a good way to detect synergy or antagonism in different mixtures, when the experiments were designed and the results were evaluated using statistical methods. However, it is essential to make further efforts to study the long-term effects of these interactions deeply and find possible impacts on the microbial community structure developed during the process. This will give the necessary information for engineers to develop and promote environmental-friendly technologies, such as biogas production, for the management of locally produced residues at or close to abattoir sites.

Author details

Ileana Pereda Reyes[1], Jhosané Pagés Díaz[1,2*] and Ilona Sárvári Horváth[2]

*Address all correspondence to: jhosane@quimica.cujae.edu.cu

1 Study Center of Process Engineering (CIPRO), High Polytechnic Institute "José Antonio Echeverría" (Cujae), 119 #11901 e/ Rotonda and Ciclovía, Marianao, Havana, Cuba

2 Swedish Centre for Resource Recovery, University of Borås, Borås, Sweden

References

[1] Alvarez R, Liden G. Semi-continuous co-digestion of solid slaughterhouse waste, manure, and fruit and vegetable waste. Renewable Energy. 2008;33(4):726-734.

[2] Bayr S, Rantanen M, Kaparaju P, Rintala J. Mesophilic and thermophilic anaerobic co-digestion of rendering plant and slaughterhouse wastes. Bioresource Technology. 2012;104:28-36.

[3] Franke-Whittle IH, Insam H. Treatment alternatives of slaughterhouse wastes, and their effect on the inactivation of different pathogens: a review. Critical Reviews in Microbiology. 2013;39(2):139-151.

[4] Pagés-Díaz J, Sárvári-Horváth I, Pérez-Olmo J, Pereda-Reyes I. Co-digestion of bovine slaughterhouse wastes, cow manure, various crops and municipal solid waste at thermophilic conditions: a comparison with specific case running at mesophilic conditions. Water Science and Technology. 2013;67(5):989-995.

[5] Arvanitoyannis IS, Ladas D. Meat waste treatment methods and potential uses. International Journal of Food Science and Technology. 2008;43(3):543-559.

[6] Hejnfelt A, Angelidaki I. Anaerobic digestion of slaughterhouse by-products. Biomass and Bioenergy. 2009;33(8):1046-1054.

[7] Flotats X, Palatsi J, Ahring BK, Angelidaki I. Identifiability study of the proteins degradation model, based on ADM1, using simultaneous batch experiments. Water Science and Technology. 2006;54(4):31-39.

[8] Vidal G, Carvalho A, Mendez R, Lema JM. Influence of the content in fats and proteins on the anaerobic biodegradability of dairy wastewaters. Bioresource Technology. 2000;74(3):231-239.

[9] Palatsi J, Illa J, Prenafeta-Boldu FX, Laureni M, Fernandez B, Angelidaki I, Flotats X. Long-chain fatty acids inhibition and adaptation process in anaerobic thermophilic digestion: batch tests, microbial community structure and mathematical modelling. Bioresource Technology. 2010;101(7):2243-2251.

[10] Callaghan FJ, Wase DAJ, Thayanithy K, Forster CF. Co-digestion of waste organic solids: batch studies. Bioresource Technology. 1999;67(2):117-122.

[11] Weiland P. Biomass digestion in agriculture: a successful pathway for the energy production and waste treatment in Germany. Engineering in Life Sciences. 2006;6(3): 302-309.

[12] Liu YY, Haynes RJ. Origin, nature, and treatment of effluents from dairy and meat processing factories and the effects of their irrigation on the quality of agricultural soils. Critical Reviews in Environmental Science and Technology. 2011;41(17): 1531-1599.

[13] Demirbas MF, Balat M, Balat H. Biowastes-to-biofuels. Energy Conversion and Management. 2011;52(4):1815-1828.

[14] Weiland P. Biogas production: current state and perspectives. Applied Microbiology and Biotechnology. 2010;85(4):849-860.

[15] IEA. Task 37 Biogas Country Overview (Country Reports). 2014.

[16] Gali A, Benabdallah T, Astals S, Mata-Alvarez J. Modified version of ADM1 model for agro-waste application. Bioresource Technology. 2009;100(11):2783-2790.

[17] Salminen EA, Rintala JA. Semi-continuous anaerobic digestion of solid poultry slaughterhouse waste: effect of hydraulic retention time and loading. Water Research. 2002;36(13):3175-3182.

[18] Gonzalez-Gonzalez A, Cuadros F, Ruiz-Celma A, Lopez-Rodriguez F. Energy-environmental benefits and economic feasibility of anaerobic codigestion of Iberian pig slaughterhouse and tomato industry wastes in Extremadura (Spain). Bioresource Technology. 2013;136:109-116.

[19] Pagés-Díaz J, Pereda Reyes I, Lundin M, Sárvári Horváth I. Co-digestion of different waste mixtures from agro-industrial activities: kinetic evaluation and synergetic effects. Bioresource Technology. 2011;102(23):10834-10840.

[20] Pagés-Díaz J, Pereda-Reyes I, Taherzadeh MJ, Sárvári-Horváth I, Lundin M. Anaerobic co-digestion of solid slaughterhouse wastes with agro-residues: synergistic and antagonistic interactions determined in batch digestion assays. Chemical Engineering Journal. 2014;245(0):89-98.

[21] Palatsi J, Vinas M, Guivernau M, Fernandez B, Flotats X. Anaerobic digestion of slaughterhouse waste: main process limitations and microbial community interactions. Bioresource Technology. 2011;102(3):2219-2227.

[22] Aslanzadeh S, Taherzadeh MJ, Horváth IS. Pretreatment of straw fraction of manure for improved biogas production. Bioresources. 2011;6(4):5193-5205.

[23] Cuetos M, Fernández C, Gómez X, Morán A. Anaerobic co-digestion of swine manure with energy crop residues. Biotechnology and Bioprocess Engineering. 2011;16(5):1044-1052.

[24] González LML, Vervaeren H, Pereda-Reyes I, Dumoulin A, Romero OR, Dewulf J. Thermo-chemical pre-treatment to solubilize and improve anaerobic biodegradability of press mud. Bioresource Technology. 2013;131:250-257.

[25] Contreras LM, Schelle H, Sebrango CR, Pereda-Reyes I. Methane potential and biodegradability of rice straw, rice husk and rice residues from the drying process. Water Sience and Technology. 2012;65(6):1142-1149.

[26] Kang H, Weiland P. Ultimate anaerobic biodegradability of some agroindustrial residues. Bioresource Technology. 1993;43(2):107-111.

[27] Bouallagui H, Rachdi B, Gannoun H, Hamdi M. Mesophilic and thermophilic anaerobic co-digestion of abattoir wastewater and fruit and vegetable waste in anaerobic sequencing batch reactors. Biodegradation. 2009;20(3):401-409.

[28] Benka-Coker M, Ojior OO. Effect of slaughterhouse wastes on the water quality of Ikpoba river, Nigeria. Bioresource Technology. 1995;52:2-12.

[29] Böhm R. Air, exit air and wastewater, requirements and treatment processes from the point of view of hygiene. Paper at the ATV Seminar 3/90 on the Treatment of Wastewater from Slaughterhouses and Meat Processing Plants, 6-7 February (Germany) 1990.

[30] No 1771/2002. European Community Regulatin (EC) no. 1774/2002 of the European Parliament and of the Council of 3 October 2001 laying down health rules concerning animal by-products not intended for human consumption.

[31] Tritt WP, Schuchardt F. Materials flow and possibilities of treating liquid and solid-wastes from slaughterhouses in Germany—a review. Bioresource Technology. 1992;41(3):235-245.

[32] Mittal GS. Treatment of wastewater from abattoirs before land application—a review. Bioresource Technology. 2006;97(9):1119-1135.

[33] Cecchi F, Battistoni P, Pavan P, Fava G, Mata-Alvarez J. Anaerobic-digestion of OFMSW (organic fraction of municipal solid-waste) and BNR (biological nutrient removal) processes—a possible integration—preliminary-results. Water Science and Technology. 1994;30(8):65-72.

[34] Cecchi F, Traverso PG, Mata-Alvarez J, Clancy J, Zaror C. State of the art of R-and-D in the anaerobic-digestion process of municipal solid-waste in Europe. Biomass. 1988;16(4):257-284.

[35] Salminen E, Rintala J. Anaerobic digestion of organic solid poultry slaughterhouse waste—a review. Bioresource Technology. 2002;83(1):13-26.

[36] Kayhanian M. Biodegradability of the organic fraction of municipal solid-waste in a high-solids anaerobic digester. Waste Management and Research. 1995;13(2):123-136.

[37] Deublein D, Steinhauser A. Biogas from Waste and Renewable Resources. Germany: Wiler-VCH Verlag GmbH & Co. KGaA; 2008. pp. 87-147.

[38] Schink B. Energetics of syntrophic cooperation in methanogenic degradation. Microbiology and Molecular Biology Reviews. 1997;61(2):262-280.

[39] Choi HB, Hwang KY, Shin EB. A mechanical pretreatment of waste-activated sludge for H2S decrease on anaerobic digestion. Biotechnology Letters. 1997;19(2):101-104.

[40] Batstone DJ, Keller J, Angelidaki I, Kalyuzhnyi SV, Pavlostathis SG, Rozzi A, Sanders WTM, Siegrist H, Vavilin VA. The IWA anaerobic digestion model no 1 (ADM1). Water Science and Technology. 2002;45(10):65-73.

[41] Astals S, Venegas C, Peces M, Jofre J, F. L, Mata-Alvarez J. Balancing hygienization and anaerobic digestion of raw sewage sludge. Water Research. 2012;46:6218-6227.

[42] Gavala HN, Yenal U, Skiadas IV, Westermann P, Ahring BK. Mesophilic and thermophilic anaerobic digestion of primary and secondary sludge. Effect of pre-treatment at elevated temperature. Water Research. 2003;37(19):4561-4572.

[43] Palatsi J, Gimenez-Lorang A, Ferrer I, Flotats X. Start-up strategies of thermophilic anaerobic digestion of sewage sludge. Water Science and Technology. 2009;59(9): 1777-1784.

[44] Nordberg A, Jarvis A, Stenberg B, Mathisen B, Svensson BH. Anaerobic digestion of alfalfa silage with recirculation of process liquid. Bioresource Technology. 2007;98(1): 104-111.

[45] Ahring BK, Sandberg M, Angelidaki I. Volatile fatty-acids as indicators of process imbalance is anaerobic digesters. Applied Microbiology and Biotechnology. 1995;43(3):559-565.

[46] Lesteur M, Bellon-Maurel V, Gonzalez C, Latrille E, Roger JM, Junqua G, Steyer JP. Alternative methods for determining anaerobic biodegradability: a review. Process Biochemistry. 2010;45(4):431-440.

[47] VDI-4630. Vergärung organischer Stoffe Substratcharakterisierung, Probenahme, Stoffdatenerhebung, Gärversuche (Fementation of Organic Materials Characterisation of the Substrate, Sampling, Collection of Material Data, Fermentation Tests). Germany: VDI-Handbuch Energietechnik; 2006.

[48] Murto M, Bjornsson L, Mattiasson B. Impact of food industrial waste on anaerobic co-digestion of sewage sludge and pig manure. Journal of Environmental Management. 2004;70(2):101-107.

[49] Verstraete W, Vandevivere P. New and broader applications of anaerobic digestion. Critical Reviews in Environmental Science and Technology. 1999;29(2):151-173.

[50] Batstone D, Keller J, Newell B, Newland M. Model development and full scale validation for anaerobic treatment of protein and fat based wastewater. Water Science and Technology. 1997;36(6-7):423-431.

[51] Angelidaki I, Ellegaard L, Ahring BK. A comprehensive model of anaerobic bioconversion of complex substrates to biogas. Biotechnology and Bioengineering. 1999;63(3):363-372.

[52] Angelidaki I, Ahring BK. Thermophilic anaerobic-digestion of livestock waste—the effect of ammonia. Applied Microbiology and Biotechnology. 1993;38(4):560-564.

[53] Hansen KH, Angelidaki I, Ahring BK. Anaerobic digestion of swine manure: inhibition by ammonia. Water Research. 1998;32(1):5-12.

[54] Angelidaki I, Ahring BK. Effects of free long-chain fatty-acids on thermophilic anaerobic-digestion. Applied Microbiology and Biotechnology. 1992;37(6):808-812.

[55] Angelidaki I, Ellegaard L, Ahring BK. A mathematical-model for dynamic simulation of anaerobic-digestion of complex substrates—focusing on ammonia inhibition. Biotechnology and Bioengineering. 1993;42(2):159-166.

[56] Esposito G, Frunzo L, Panico A, Pirozzi F. Model calibration and validation for OFMSW and sewage sludge co-digestion reactors. Waste Management. 2011;31(12): 2527-2535.

[57] Li C, Champagne P, Anderson BC. Biogas production performance of mesophilic and thermophilic anaerobic co-digestion with fat, oil, and grease in semi-continuous flow digesters: effects of temperature, hydraulic retention time, and organic loading rate. Environmental Technology. 2013;34(13-14):2125-2133.

[58] Neves L, Oliveira R, Alves MM. Fate of LCFA in the co-digestion of cow manure, food waste and discontinuous addition of oil. Water Research. 2009;43(20):5142-5150.

[59] Angelidaki I, Ahring BK. Establishment and characterization of an anaerobic thermophilic (55-degrees-C) enrichment culture degrading long-chain fatty-acids. Applied and Environmental Microbiology. 1995;61(6):2442-2445.

[60] Gerardi HM. The Microbiology of Anaerobic Digesters. New Jersey: Waste Water Microbiology Series. John Wiley and Sons; 2003. pp. 17-30.

[61] Madigan MT, Martinko JM, Stahl D, Clark DP. Brock: Biology of Microorganisms. San Francisco, USA: Benjamin Cummings; 2010.

[62] Sousa DZ, Pereira MA, Smidt H, Stams AJM, Alves MM. Molecular assessment of complex microbial communities degrading long chain fatty acids in methanogenic bioreactors. FEMS Microbiology Ecology. 2007;60(2):252-265.

[63] Wu CG, Liu XL, Dong XZ. *Syntrophomonas erecta* subsp *sporosyntropha* subsp *nov*., a spore-forming bacterium that degrades short chain fatty acids in co-culture with methanogens. Systematic and Applied Microbiology. 2006;29(6):457-462.

[64] Alves MM, Pereira MA, Sousa DZ, Cavaleiro AJ, Picavet M, Smidt H, Stams AJM. Waste lipids to energy: how to optimize methane production from long-chain fatty acids (LCFA). Microbial Biotechnology. 2009;2(5):538-550.

[65] Alves MM, Vieira JAM, Pereira RMA, Pereira MA, Mota M. Effects of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors. Part II: oleic acid toxicity and biodegradability. Water Research. 2001;35(1):264-270.

[66] Hwu CS, Lettinga G. Acute toxicity of oleate to acetate-utilizing methanogens in mesophilic and thermophilic anaerobic sludges. Enzyme and Microbial Technology. 1997;21(4):297-301.

[67] Kim SH, Han SK, Shin HS. Kinetics of LCFA inhibition on acetoclastic methanogenesis, propionate degradation and beta-oxidation. Journal of Environmental Science and Health Part A—Toxic/Hazardous Substances and Environmental Engineering. 2004;39(4):1025-1037.

[68] Pereira MA, Pires OC, Mota M, Alves MM. Anaerobic biodegradation of oleic and palmitic acids: evidence of mass transfer limitations caused by long chain fatty acid accumulation onto the anaerobic sludge. Biotechnology and Bioengineering. 2005;92(1):15-23.

[69] Hwu CS, Donlon B, Lettinga G. Comparative toxicity of long-chain fatty acid to anaerobic sludges from various origins. Water Science and Technology. 1996;34(5-6): 351-358.

[70] Hwu CS, Tseng SK, Yuan CY, Kulik Z, Lettinga G. Biosorption of long-chain fatty acids in UASB treatment process. Water Research. 1998;32(5):1571-1579.

[71] Shin HS, Kim SH, Lee CY, Nam SY. Inhibitory effects of long-chain fatty acids on VFA degradation and beta-oxidation. Water Science and Technology. 2003;47(10): 139-146.

[72] Pereira MA, Sousa DZ, Mota M, Alves MM. Mineralization of LCFA associated with anaerobic sludge: kinetics, enhancement of methanogenic activity, and effect of VFA. Biotechnology and Bioengineering. 2004;88(4):502-511.

[73] Boe K, Kougias PG, Pacheco F, O-Thong S, Angelidaki I. Effect of substrates and intermediate compounds on foaming in manure digestion systems. Water Science and Technology. 2012;66(10):2146-2154.

[74] Kougias PG, Boe K, Angelidaki I. Effect of organic loading rate and feedstock composition on foaming in manure-based biogas reactors. Bioresource Technology. 2013;144:1-7.

[75] Kougias PG, De Francisci D, Treu L, Campanaro S, Angelidaki I. Microbial analysis in biogas reactors suffering by foaming incidents. Bioresource Technology. 2014;167:24-32.

[76] Coelho NM, Rodrigues AA, Arroja LM, Capela IF. Effect of non-feeding period length on the intermittent operation of UASD reactors treating dairy effluents. Biotechnology and Bioengineering. 2007;96(2):244-249.

[77] Cuetos MJ, Gomez X, Otero M, Moran A. Anaerobic digestion and co-digestion of slaughterhouse waste (SHW): influence of heat and pressure pre-treatment in biogas yield. Waste Management. 2010;30(10):1780-1789.

[78] Callaghan FJ, Wase DAJ, Thayanithy K, Forster CF. Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure. Biomass and Bioenergy. 2002;22(1):71-77.

[79] Kayhanian M. Ammonia inhibition in high-solids biogasification: an overview and practical solutions. Environmental Technology. 1999;20(4):355-365.

[80] Deppenmeier U. The membrane-bound electron transport system of *Methanosarcina* species. Journal of Bioenergetics and Biomembranes. 2004;36(1):55-64.

[81] Deppenmeier U, Muller V, Gottschalk G. Pathways of energy conservation in methanogenic archaea. Archives of Microbiology. 1996;165(3):149-163.

[82] Blaut M. Metabolism of Methanogens. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology. 1994;66(1-3):187-208.

[83] Wiegant WM, Zeeman G. The mechanism of ammonia inhibition in the thermophilic digestion of livestock wastes. Agricultural Wastes. 1986;16:243-253.

[84] Hashimoto AG. Ammonia inhibition of methanogenesis from cattle wastes. Agricultural Wastes. 1986;17:241-261.

[85] Koster IW, Lettinga G. The influence of ammonium-nitrogen on the specific activity of pelletized methanogenic sludge. Agricultural Wastes. 1984;9:205-216.

[86] Koster IW. Characteristics of the pH-influenced adaptation of methanogenic sludge to ammonium toxicity. Journal of Chemical Technology and Biotechnology. 1986;36:445-455.

[87] van Velsen AFM. Adaptation of methanogenic sludge to high ammonia-nitrogen concentrations. Water Research. 1979;13:995-999.

[88] Angelidaki I, Ahring BK. Anaerobic thermophilic digestion of manure at different ammonia loads—effect of temperature. Water Research. 1994;28(3):727-731.

[89] Kaparaju P, Serrano M, Thomsen AB, Kongjan P, Angelidaki I. Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept. Bioresource Technology. 2009;100(9):2562-2568.

[90] Siegert I, Banks C. The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. Process Biochemistry. 2005;40(11): 3412-3418.

[91] Bouallagui H, Touhami Y, Cheikh RB, Hamdi M. Bioreactor performance in anaerobic digestion of fruit and vegetable wastes. Process Biochemistry. 2005;40(3-4): 989-995.

[92] Gunaseelan VN. Anaerobic digestion of biomass for methane production: a review. Biomass and Bioenergy. 1997;13(1-2):83-114.

[93] Zhang B, He PJ, Lu F, Shao LM, Wang P. Extracellular enzyme activities during regulated hydrolysis of high-solid organic wastes. Water Research. 2007;41(19):4468-4478.

[94] Kabir MM, Forgács G, Sárvári Horváth I. Enhanced methane production from wool textile residues by thermal and enzymatic pretreatment. Process Biochemistry. 2013;48:575-580.

[95] Forgacs G, Lundin M, Taherzadeh MJ, Sárvári Horváth I. Pretreatment of chicken feather waste for improved biogas production. Applied Biochemistry and Biotechnology. 2013;169(7):2016-2028.

[96] Jeihanipour A, Niklasson C, Taherzadeh MJ. Enhancement of solubilization rate of cellulose in anaerobic digestion and its drawbacks. Process Biochemistry. 2011;46(7): 1509-1514.

[97] Purwandari FA, Sanjaya AP, Millati R, Cahyanto MN, Sárvári Horváth I, Niklasson C, Taherzadeh MJ. Pretreatment of oil palm empty fruit bunch (OPEFB) by *N*-methylmorpholine-*N*-oxide (NMMO) for biogas production: structural changes and digestion improvement. Bioresource Technology. 2013;128:461-466.

[98] Teghammar A, Yngvesson J, Lundin M, Taherzadeh MJ, Horvath IS. Pretreatment of paper tube residuals for improved biogas production. Bioresource Technology. 2010;101(4):1206-1212.

[99] Yadvika, Santosh, Sreekrishnan TR, Kohli S, Rana V. Enhancement of biogas production from solid substrates using different techniques—a review. Bioresource Technology. 2004;95(1):1-10.

[100] Kabir MM, Niklasson C, Taherzadeh MJ, Sárvari Horváth I. Biogas production from lignocelluloses by *N*-methylmorpholine-*N*-oxide (NMMO) pretreatment: effects of recovery and reuse of NMMO. Bioresource Technology. 2014;161:446-450.

[101] Mata-Alvarez J, Mace S, Llabres P. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresource Technology. 2000;74(1):3-16.

[102] Goberna M, Camacho MD, Lopez-Abadia JA, Garcia C. Co-digestion, biostimulation and bioaugmentation to enhance methanation of brewer's spent grain. Waste Management and Research. 2013;31(8):805-810.

[103] El-Mashad HM. Kinetics of methane production from the codigestion of switchgrass and Spirulina platensis algae. Bioresource Technology. 2013;132:305-312.

[104] Kabouris JC, Tezel U, Pavlostathis SG, Engelmann M, Todd AC, Gillette RA. The anaerobic biodegradability of municipal sludge and fat, oil, and grease at mesophilic conditions. Water Environment Research. 2008;80(3):212-221.

[105] Macias-Corral M, Samani Z, Hanson A, Smith G, Funk P, Yu H, Longworth J. Anaerobic digestion of municipal solid waste and agricultural waste and the effect of co-digestion with dairy cow manure. Bioresource Technology. 2008;99(17):8288-8293.

[106] Fongsatitkul P, Elefsiniotis P, Wareham DG. Effect of mixture ratio, solids concentration and hydraulic retention time on the anaerobic digestion of the organic fraction of municipal solid waste. Waste Management and Research. 2010;28(9):811-817.

[107] Lin J, Zuo JE, Gan LL, Li P, Liu FL, Wang KJ, Chen L, Gan HN. Effects of mixture ratio on anaerobic co-digestion with fruit and vegetable waste and food waste of China. Journal of Environmental Sciences (China). 2011;23(8):1403-1408.

[108] Alvarez JA, Otero L, Lema JM. A methodology for optimising feed composition for anaerobic co-digestion of agro-industrial wastes. Bioresource Technology. 2010;101(4):1153-1158.

[109] Hartmann H, Angelidaki I, Arhing BK. Co-digestion of the organic fraction of municipal waste with other waste types. In: Biomethanization of the Organic Fraction of Municipal Solid Wastes. UK: IWA Publishing; 2003.

[110] Mata-Alvarez J, Dosta J, Mace S, Astals S. Codigestion of solid wastes: a review of its uses and perspectives including modeling. Critical Reviews in Biotechnology. 2011;31(2):99-111.

[111] Angelidaki I, Ellegaard L. Codigestion of manure and organic wastes in centralized biogas plants—status and future trends. Applied Biochemistry and Biotechnology. 2003;109(1-3):95-105.

[112] Weiland P. Anaerobic waste digestion in Germany—status and recent developments. Biodegradation. 2000;11(6):415-421.

[113] Soldano M, Fabbri C, Piccinini S. Co-digestion plant in dairy cattle farm in Emilia Romagna region (Italy). In: Proceedings of the International Conference Progress in Biogas Stuttgart, Germany; 2007. pp. 95-99.

[114] Amon T, Amon B, Kryvoruchko V, Bodiroza V, Pötsch E, Zollitsch W. Optimising methane yield from anaerobic digestion of manure: effects of dairy systems and of glycerine supplementation. International Congress Series. 2006;1293:217-220.

[115] Lettinga G. The anaerobic treatment approach towards a more sustainable and robust environmental protection. Water Science and Technology. 2005;52(1-2):1-11.

[116] Masse DI, Masse L, Croteau F. The effect of temperature fluctuations on psychrophilic anaerobic sequencing batch reactors treating swine manure. Bioresource Technology. 2003;89(1):57-62.

[117] Cavinato C, Fatone F, Bolzonella D, Pavan P. Thermophilic anaerobic co-digestion of cattle manure with agro-wastes and energy crops: comparison of pilot and full scale experiences. Bioresource Technology. 2010;101(2):545-550.

[118] Kaparaju P, Luostarinen S, Kalmari E, Kalmari J, Rintala J. Co-digestion of energy crops and industrial confectionery by-products with cow manure: batch-scale and farm-scale evaluation. Water Science and Technology. 2002;45(10):275-280.

[119] Ponsa S, Gea T, Sanchez A. Anaerobic co-digestion of the organic fraction of municipal solid waste with several pure organic co-substrates. Biosystems Engineering. 2011;108(4):352-360.

[120] Lehtomaki A, Huttunen S, Rintala JA. Laboratory investigations on co-digestion of energy crops and crop residues with cow manure for methane production: effect of crop to manure ratio. Resources Conservation and Recycling. 2007;51(3):591-609.

[121] Chen Y, Cheng JJ, Creamer KS. Inhibition of anaerobic digestion process: a review. Bioresource Technology. 2008;99(10):4044-4064.

[122] Habiba L, Hassib B, Moktar H. Improvement of activated sludge stabilisation and filterability during anaerobic digestion by fruit and vegetable waste addition. Bioresource Technology. 2009;100(4):1555-1560.

[123] Mshandete A, Kivaisi A, Rubindamayugi M, Mattiasson B. Anaerobic batch co-digestion of sisal pulp and fish wastes. Bioresource Technology. 2004;95(1):19-24.

[124] Burton C, Turner C. Manure Management. Silsoe Research Institute; 2003. pp. 281-282.

[125] Chamy R, Poirrier P, Schiappacasse MC, Alkalay D, Guerrero L. Effect of ammonia content in the biodegradability of the salmon industry wastes. Bioprocess Engineering. 1998;19(1):1-5.

[126] Astals S, Ariso M, Gali A, Mata-Alvarez J. Co-digestion of pig manure and glycerine: experimental and modelling study. Journal of Environmental Management. 2011;92(4):1091-1096.

[127] Zhou HD, Li H, Wang FF. Anaerobic digestion of different organic wastes for biogas production and its operational control performed by the modified ADM1. Journal of Environmental Science and Health Part A—Toxic/Hazardous Substances and Environmental Engineering. 2012;47(1):84-92.

[128] Batstone DJ, editor Modelling and control in anaerobic digestion: achievements and challenges. 13th World Congress on Anaerobic Digestion, Recovering (bio) Resources for the World; 2013; June 25th-28th, Santiago de Compostela, Spain.

[129] Mata-Alvarez J, Dosta J, Romero-Güiza MS, Fonoll X, Peces M, Astals S. A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renewable and Sustainable Energy Reviews. 2014;36:412-427.

[130] De Vrieze J, Verstraete W, Boon N. Repeated pulse feeding induces functional stability in anaerobic digestion. Microbial Biotechnology. 2013;6(4):414-424.

[131] Cuetos MJ, Gómez X, Martínez EJ, Fierro J, Otero M. Feasibility of anaerobic co-digestion of poultry blood with maize residues. Bioresource Technology. 2013;144:513-520.

[132] Carucci G, Carrasco F, Trifoni K, Majone M, Beccari M. Anaerobic digestion of food industry wastes: effect of codigestion on methane yield. Journal of Environmental Engineering (ASCE). 2005;131(7):1037-1045.

[133] Edström M, Nordrerg A, Thyselius L. Anaerobic treatment of animal byproducts from slaughterhouses at laboratory and pilot scale. Applied Biochemistry and Biotechnology. 2003;109(1-3):127-138.

[134] Cuetos MJ, Gomez X, Otero M, Moran A. Anaerobic digestion of solid slaughterhouse waste (SHW) at laboratory scale: influence of co-digestion with the organic fraction of municipal solid waste (OFMSW). Biochemical Engineering Journal. 2008;40(1):99-106.

[135] Pagés-Díaz J, Westman J, Taherzadeh MJ, Pereda-Reyes I, SárváriHorváth I. Semi-continuous co-digestion of solid cattle slaughterhouse wastes with other waste

streams: interactions within mixtures and methanogenic community structure. Chemical Engineering Journal. 2015;273:28-36.

[136] Rosenwinkel KH, Meyer H. Anaerobic treatment of slaughterhouse residues in municipal digesters. Water Science and Technology. 1999;40(1):101-111.

[137] Luste S, Luostarinen S. Anaerobic co-digestion of meat-processing by-products and sewage sludge—effect of hygienization and organic loading rate. Bioresource Technology. 2010;101(8):2657-2664.

Section 2

Biodegradation of Complex Pollutants

Chapter 4

Organic Matter Biodegradation by Bacterial Consortium under Metal Stress

Simone Pennafirme, Inaya Lima, José Bitencourt, Mirian Crapez and Ricardo Lopes

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60980

Abstract

Organic matter biodegradation proceeds via multiple enzymatic reactions, involving different oxidants as well as a number of intermediate compounds. Microbial reworking of organic matter can result in a substantial microbial contribution to the total organic matter pool. The investigations of the mechanisms, which can alter the microbial metabolism in marine sediments, are essential for understanding diagenetic processes, especially at those environments with toxic metal concentrations. Metals can bind with cells components, affecting their functioning. Consequently, the organic matter oxidation in the cellular metabolism may be affected. By contrast, the carbon sources are discriminated between labile and refractory organic compounds. The labile portion of organic matter mainly consists of biopolymers and includes carbohydrates, lipids, and proteins. The aim of this chapter is to present the main results of 10 years of studies regarding the organic matter oxidation by bacterial consortia under toxic metal levels on a tropical estuarine environment surrounded in part by mangrove areas. As the main find, the chronic dominance of lipids and carbohydrates at estuaries and mangroves systems may change the bacterial trophic state from aerobic to anaerobic metabolism. This alteration may reflect on decreasing both bacterial efficiency of organic matter degradation and bacterial productivity. Further, when these systems show high levels of metals at the sediment, the metabolic efficiency is even lower because, although bacteria consortia is able to produce extracellular polymeric substances (EPS) as defense mechanism, multimetal contam-

ination may hinder bacterial organic matter oxidation through dehydrogenase activity inhibition.

Keywords: Organic matter, biopolymers, bacterial consortium, dehydrogenase, metals

1. Introduction

Bacteria are cosmopolitan unicellular microorganisms that show great metabolic capacity, allowing them to live in a wide range of environments [1,2]. Although bacteria size can range between 0.2 and 2.0 μm [2,3], their tiny body do not limit their main features such as rapid growth, metabolic versatility, genetic plasticity, and ability to rapidly adapt to environmental changes [1,2]. These features allow a wide earth colonization by prokaryotes, estimated of 4–6 × 1030 cells [4], influencing biomass production with an amount of 350–550 Pg carbon (1 Pg = 1015 g). Thus, they correspond to 60%–100% of all carbon produced by plants [4] and comprise 30%–50% of the particulate matter on sea [5]. Furthermore, they are the main producer in some estuarine regions [1] and show capacity to live in places with recalcitrant elements, like metals and industrial effluents [6,7].

Bacteria play an important role in recent diagenesis of organic matter and on nutrient recycling [8–11]. Originally, the organic matter content is naturally occurring as organic compounds such as carbohydrates, amino acids, polypeptides, pigments, phenolic substances, lipids, and other constituents of living organisms [12.13]. During the sedimentation process, only a small portion of the initial organic matter reaches the bottom sediments because pelagic organisms can easily take nutrients and food from organic matter in this process, being left refractory elements in higher proportion [12]. Some microorganism can act transforming highly resistant enzymatic hydrolysis elements (refractory elements) into polypeptides or fatty acids [14] along diagenesis [15–19].

Furthermore, bacteria can enhance organic matter quality, transforming refractory elements (e.g., cellulose) into organic nitrogen compounds (e.g., ammonia, nitrate, and proteins) and vitamins [20]. During diagenesis, bacteria play an important role on biogeochemical process of metals, by transforming inorganic to organic forms or by recycling them due to energy production [15–19]. In this sense, bacteria are important link between biogeochemical cycles and environment, by taking part in recycling nutrients that pass through the ecosystems, especially those rich in organic matter.

1.1. Biochemical pathways of microbial organic matter biodegradation

The benthic degradation of organic matter proceeds via multiple enzymatic reactions involving different organisms and oxidants as well as a number of intermediate compounds. Although microbial biomass is only a small fraction of total organic matter in sediments, the continuous processing of organic matter by benthic microbes, combined with turnover of

http://dx.doi.org/10.5772/60980

microbial biomass, results in a continuous flux of microbial detritus into the sediment organic matter pool. In this way, microbial reworking of organic matter can result in a substantial microbial contribution to the total organic matter pool and may also alter its composition and thereby change its long-term fate [21]. Furthermore, aerobic and anaerobic biogeochemical cycles driven by microbial processes in marine sediments are essential for understanding diagenetic processes [22,23]. These diagenetic mechanisms play a fundamental role on, among others, the recycling of inorganic carbon and nutrients, the flux of organic carbon, and ultimately the burial of organic carbon in the sedimentary record.

The microbial community exerts an important influence on the degradability of organic matter. Microbes can respond to chemical changes across millimeter distances in marine sediments, thus altering the subsurface chemistry of those sediments, and the chemical changes are transmitted through sedimentary layers [24]. In this sense, the degradation of the deposited material is thermodynamically and/or kinetically controlled by the different abilities of the physiological groups that compete for the common substrate. In cases where the molecular weight of the substrate is equal or higher than 600 Da, bacteria may release extracellular enzymes, such as esterases. These enzymes reduce the size of the molecules by hydrolysing ester bond, allowing the entrance of intracellular carbon source [25].

Organic matter oxidation is coupled to the sequential utilization of terminal electron acceptors, typically in the order of O_2, NO_3^-, Mn(VI), Fe(III), andSO_4^{2-} followed by methanogenesis and/or fermentation. Depending on the degradation pathway, organic matter is directly oxidized to CO_2, partly oxidized to intermediate compounds, or reduced to CH_4 [20, 26, 27].

Marine sediments typically become anoxic because the bacteria within them consume oxygen as it diffuses down from the overlying water. This process establishes a series of sedimentary layers, within which a different kind of molecule (or ion) accepts electrons from oxygen and is consumed to form a chemically reduced product. In one of the lower layers, sulfate (SO_4^{2-}) is converted to highly toxic hydrogen sulfide (H_2S), which accumulates and poisons the environment for most oxygen-consuming microorganisms. The hydrogen sulfide can, in turn, be converted into sulfate or other oxidized forms of sulfur by bacteria that use inorganic material as an energy source [24]. The sulfate-reducing bacteria do not require low H_2 concentration for their growth, which gives them a thermodynamic advantage. Besides, in absence of sulfate, they can use fermentative or acetogenic pathways to produce energy. They are the main competitors of methanogenic bacteria for the electron donor, such organic matter and H_2, and they release hydrogen sulfide as an excretion, which is an important methanogenesis inhibitor. The methane formation is performed through chemosynthesis by methanogenic *Archaea* that fix CO_2 and excrete methane. The same microorganisms can reduce nitrate and iron oxides, with a preference of nitrate. The reduction of iron oxides, sulfate, and carbon dioxide is made by different physiological groups of bacteria with specific affinities for electron donors such as acetate and hydrogen. Thus, the reduction of iron oxides occurs preferentially to the reduction of sulfate and carbon dioxide [28].

Nitrogen is the fourth most common element found in cells and includes the microbially catalyzed processes of nitrogen fixation, ammonium oxidation, assimilatory and dissimilatory

nitrate reduction, ammonification, and ammonium assimilation. The biological nitrogen removal is achieved by nitrification followed by a denitrification process, i.e., (i) aerobic nitrification of NH_4^+ by chemolithoautotrophic bacteria to NO_2^- or NO_3^- with O_2 as the electron acceptor, and (ii) anoxic denitrification of NO_2^- or NO_3^- to gaseous N_2 by heterotrophic microorganisms using organic matter as carbon and energy source [29].

Manganese acts as a catalyst that shapes chemical gradients in the oxygen-deficient zones because it exists in multiple oxidation states and is recycled rapidly between these states by bacterial processes. These transformations serve as an electron-transfer system for other chemical cycles. The manganese may play a dominant role as a catalyst in chemical cycles like HS^-/O_2, and the reduction of nitrate by Mn(II), and the oxidation of ammonia by MnO_2. These cycles are thermodynamically favorable in suboxic marine environments [30].

Dissimilatory iron-reducing bacteria are a group of microorganisms that can oxidize organic matter and reduce Fe(III) to Fe(II) under anoxic conditions. They include both facultative and obligate anaerobes and can be classified as fermentative, photosynthetic, organic acid-oxidizing, and hydrogen-oxidizing bacteria [31].

1.2. Bacterial mechanisms of metal resistance and tolerance

Microorganisms can be resistant or tolerant to metal stress. While the resistance is the ability of a bacteria or bacterial consortium to continue growing in the presence of multimetal combination in a toxic level, the tolerance is their ability to survive at these conditions [32].

Although some metals are essential elements for organisms, playing a fundamental role on cellular functioning, they can become toxic depending on their intracellular concentrations. Metals are important switched elements, and the exposition and natural selection over thousands of years have made the association between organism and metal so close that bacteria need these elements in their biological process such as on proteins formation, enzymes function, and energy production [33, 34]. However, on environments with high metal concentrations, it may bind with nucleic acids, proteins, and enzymes, among others cells components, affecting their functioning [35]. Consequently, the organic matter oxidation in the cellular metabolism may be affected. Once the metal ions concentration are elevated intracellularly, they may bind with enzymes such as the dehydrogenases (DHA), which act on the oxidation–reduction reactions in the electron transport system activities (ETSAs) [36].

In this sense, cells may show regulatory mechanisms that control metals intracellular levels, keeping it at the minimal inhibitory concentrations values. One of these mechanisms is the production of a matrix of exopolymeric polymeric substances. The bacteria are embedded in a biofilm or an extracellular organic matrix that consists extracellular polymeric substances (EPS) produced by them. Generally, the EPS are composed of carbohydrates, heteropolysaccharides, proteins, and nucleic acids. Due to their negatively charged carboxyl and phosphoryl functional groups, EPS can bind with extracellular metals [37,38]. Thus, the EPS reduce the bioavailability and activity of the ionizable metal forms, such as Pb, Cd, Co, Ni, Zn, and Cu, being a survival strategic against toxic flux of metals [37–40].

http://dx.doi.org/10.5772/60980

Once within the EPS, bacteria may form a complex consortium, with intra- and interspecific species [1,15,41]. The community helps bacteria to resist against environmental stress due to connection of different physiological features of these members. Consequently, they spend less energy on the competition for space, nutrients, or defense [9,10,29,42]. Due to intercellular communication throughout biofilm, the consortium becomes complex and multicellular, favoring syntrophic life, where two or more individuals combine their metabolic capacity or one species lives off the products of another species [41]. In this context, the consortia formation may help bacteria cells to colonize substrates [15,43], affecting the cycling of both organic matter and metals [15,18,19,43]. The bacterial consortium is more resistant toward multimetals than the pure cultures [44].

2. Aim

This chapter is going to present a temporal series compilation of the main results of 10 years of studies regarding the organic matter oxidation by bacterial consortia under toxic metal levels on a tropical estuarine environment surrounded in part by mangrove areas.

3. Methods

3.1. Study case

The sample sites of the study case presented in this chapter are located at the Guanabara Bay, Rio de Janeiro State, Brazil, between 22° 40′–23° 00′S and 043° 00′–043° 18′W. This bay is a complex tropical estuarine environment and one of the largest bays along the Brazilian coastline with an area of approximately 384 km^2. In the past, each of the 55 rivers might create a single estuary, each one distinct from the other [45]. These estuaries originally made the estuarine system of Guanabara Bay ecologically diverse [46]. However, human and industrial occupation caused the loss of the bay's diversity and its natural characteristics, as suggested by data collected in [45, 47–49]

The drainage basin of Guanabara Bay has an area of 4080 km^2, and it comprises 32 separate subwatersheds [50]. However, although 55 rivers carry 4,000,000 t year−1 of solid material [45,47], only 6 rivers are responsible for near 85% of the 100 $m^3 s^{-1}$ total mean annual freshwater input [47]. Besides, these few rivers carry tons of untreated sewage directly into the bay [46,48].

Not only sewage but also hazard pollutants are carried and deposited in Guanabara Bay. Along its drainage basin and its surroundings, there are more than 12,000 industries and two oil refineries. Also, the bay is the homeport of two naval bases, which release large amounts of organic and inorganic pollutants into the subwatersheds [51]. To better analyse and compare the main features, the Guanabara Bay was divided in this present chapter into four main areas (Figure 1).

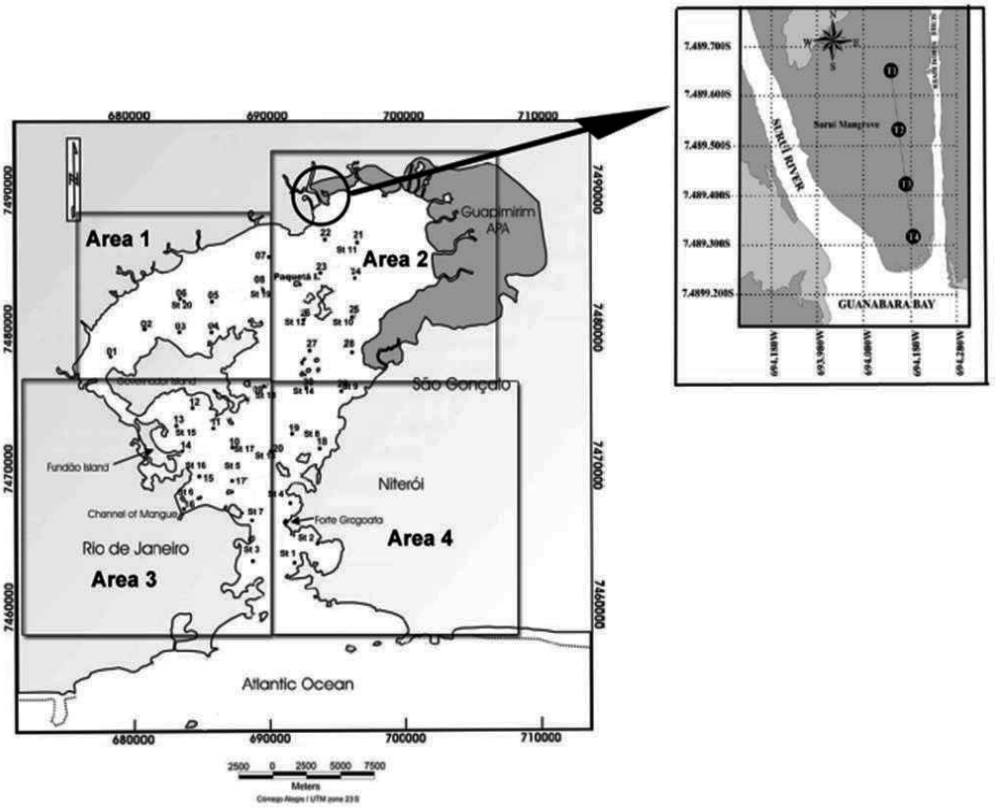


Figure 1. Map of Guanabara Bay divided into four areas. Numbers represent sample points of Sobrinho da Silva et al. [73]; St points represent samples sites of Sabadini-Santos et al. [78]. Suruí mangrove area is detached.

3.2. Sampling

At Guanabara Bay, the sediment samples were obtained using an Eckman sampler for the mud and a VanVeen grab sampler for the sand. Another sampling was performed at Suruí mangrove, located at the Bay background, inside the Guapimirim Environmental Protection Area (EPA). This mangrove area is situated in Magé Municipality and shows an area ranging of 80,000–100,000 m^2 [13]. Four sediment samples were collected in triplicate, using PVC soil cores (4.5 × 30.0 cm), and sliced in sections. The study of Suruí and surrounding site is important to understanding the dynamics of organic matter and metals.

3.3. Analysis

Total organic matter was estimated in triplicates by the calcinations method. Approximately 50 g of wet samples were dried to a constant weight and calcinated. Organic matter was determined as the differences between sediment dry weight (60°C, 24 h) and weight of the residue after combustion (450°C, 4 h) [52–54].

http://dx.doi.org/10.5772/60980

Concentrations of different sources of carbons (carbohydrate, lipids, and proteins) were determined in separate triplicates using 1 g of sediment samples. All determinations were done by spectrophotometric methods. Carbohydrates were quantified using glucose as a standard [55,56]. For lipid analysis, samples were extracted with chloroform and methanol, and tripalmitin was used as the standard [57]. Proteins were determined with standard of bovine albumin, fraction V (Sigma) [58,59]. The sum of protein, carbohydrate, and lipid carbon equivalents was referred to as biopolymeric carbon [60]

The bioavailable organic carbon (%) was determined according to the equation:

$$(\text{total biopolymeric carbon} \times 100) / \text{total biopolymers})$$

The unavailable organic carbon (%) was determined according to the equation:

$$(100 - \text{total biopolymeric carbon})$$

Esterase enzyme activity (EST) was based on fluorogenic compounds, which are enzymatically transformed into fluorescent products that can be quantified by spectrophotometric assay to [61]. These enzymes act on biopolymers and transform them into low-molecular-weight organic carbon. Triplicates of 1 g of each sample were analyzed. The results were expressed in l μg fluorescein h^{-1} g^{-1} of sediment. Electron transport system activity (ETSA) was based on dehydrogenase enzyme activities measurements [62,63]. These enzymes provide equivalents for ATP synthesis in the electron transport systems. Triplicates of 1 g of each sample were used. Results from this assay were expressed in μL O_2 h^{-1} g^{-1} of sediment. Metabolic bacterial activity on sediment such as aerobic, facultative anaerobic, denitrification, and sulfate reduction was measured using triplicates of 1 g of each sample on assay tube and specific selective liquid medium [64].

Bacterial cell was enumerated by epifluorescence microscopy (Axiosp 1, Zeiss, triple filter Texas Red—DAPI—fluorescein isothiocyanate, 1000× magnification) and using fluorochromes such fluorescein diacetate or acredine orange and irradiated with UV wave [65].

For metal analyses, the selective extraction analysis was used with both shaking and heating techniques [66], where 0.500 g of prepared sample was weighed into acid washed polypropylene tubes and blanks were prepared by taking each extractant, without the sample through all the preparation procedures prior to analysis. Water-soluble ions were extracted using a modified version of the [67] technique, where a smaller sample weight (0.5 g) was extracted with a lower volume of deionized water (2.5 ml), diluted to 10 ml and membrane filtered (0.2 μm), prior to analysis. This extraction protocol was modified to include the organic phase to ensure better oxidation of organic matter (Lefort aqua regia). An elemental analysis was carried out using a Perkin Elmer Model 3100 atomic absorption spectrometer.

In order to investigate the metal retention by bacterial biofilm, samples of resistant bacterial consortia biofilm from bioassays were analyzed through X-ray fluorescence microscopy (XRF).

The XRF is a nondestructive semiquantitative method that allows to identify as well as to estimate the concentrations of the elements presents in a multielemental sample at once analysis. It is based on the evaluation of the energies and intensities (number of detectable X-rays per unity time) of the characteristic X-rays (fluorescent photons) emitted by the elements that constitute the sample. Once a sample is irradiated with X-rays, its constitutive elements can emit fluorescent photons afterward. The element identification is possible because each element emits fluorescent photons at a given energy. Further, the energy intensity of these fluorescent photons is related to the element concentration at the sample [68]. The bacteria consortia used on bioassays were isolated from metal contaminated mangrove sediments. The bacterial consortia resistant to zinc was incubated at 37°C for 15 days in a liquid medium (sea water, yeast extract (2 g L^{-1}) and urea (2 g L^{-1}) with 50 ppm of sulfate of zinc). The bacterial consortium resistant to cupper was cultivated at same condition but with 50 ppm of sulfate of cupper. As a control, other two cultures with respective consortia were prepared without metal. After 10 days of incubation, 5 ml of each culture was vacuum filtered through a membrane filter 0.22 μm, fixed on a support and positioned on a XZ table controlled by a microcomputer. These analyses were performed through synchrotron X-ray fluorescence microscopy (microXRF) at the Synchrotron Light National Laboratory (LNLS), São Paulo State, Brazil (HPGe detector, resolution of 150 eV at 5.9 keV, white beam, pixel size of 20 μm diameter, 45°/45° geometry). To confirm the biofilm formation, the membrane filters were analyzed through scanning electron microscopy (SEM; Jeol, JSM6460 model; LV, Japan; EDS: Noran System Six; 20 kV).

4. Results and discussion

Changes in the trophic state of the sediments can be more evident in terms of labile organic matter composition (proteins, carbohydrates, and lipids) than in terms of organic matter/organic carbon concentrations [60]. The labile organic matter is the carbon source for benthic organisms. The oxidation of organic matter, which is an overall estimate of metabolism (aerobic and anaerobic), can be obtained by measuring the activity of the dehydrogenase enzymes. However, generally, at polluted environments, the cell exposure to metal ions results in decrease or inhibition of dehydrogenase activity. Thus, heavy metals distribution within sediments results from diagenetic processes linked to organic decomposition [69].

In this context, in order to investigate the organic matter oxidation by benthic marine bacteria exposed to metal stress, the dehydrogenase activity, biopolymer concentrations, and cell number were tracked from 2003 to 2011 at Guanabara Bay (Tables 1 and 2). As the central result, at first glance, the ETSA was maintained at high levels, despite high metal concentrations, indicating the presence of tolerance or resistance mechanisms by bacterial consortia. Bioassays showed that EPS formation and metal retention by biofilm can be one of these strategies. However, afterward, both cell number and ETSA decreased along the years. Meanwhile, the input of organic matter and metals continues to rise. In addition, the anaerobic metabolism was characterized as the main process of organic matter degradation. Once the anaerobic metabolism is less efficient than aerobic, the observed decreased on ETSA may be a

http://dx.doi.org/10.5772/60980

consequence of the also observed decrease on the metabolic rate, which is at least one order of magnitude on the bacterial cell numbers. The chronic sewage discharge and the metal release in the Bay are contributing to changes on the bacterial community physiology and biomass, bringing serious consequences for energy production.

In 2003, at Boa Viagem Beach, located at the east margin of the entrance of Guanabara Bay (area 4), seasonal variations were observed on ETSA activity. During summer, bacteria reached 10^8 cells, ETSA achieved highest level (7.48 μl O_2 h^{-1} g^{-1}), followed by the same pattern for organic matter (1.764 g g^{-1} of sediment). The mean metal concentrations evaluated are presented in Table 1. At this point, although the metal concentrations were already higher than natural background, these levels were not sufficient to inhibit the dehydrogenase activity, and thus, the bacteria were able to continuing oxidizing organic matter [54].

Year	**Location**	Organic matter	**ETSAM ($\mu l.O^2.h^{-1}.g^{-1}$)**	EST (μg of fluorescein. $h^{-1}.g^{-1}$)	Pb (ppm)	Ni (ppm)	Cu (ppm)	Zn (ppm)	Cr (ppm)	**Reference**
2003	Boa Viagem Beach	1.764 $g.g^{-1}$ of sediment	7.48		28	23	8	24	16	[54]
2005	Jurujuba Sound	-	3.02-3.38	3.14-3.63	84	63	241	299	116	[70]
2005	Guanabara Bay	5.62 %	1.25-4.69	0.047-0.366	-	-	-	-	-	[72,73]

Table 1. Bacterial metabolic activity and metal concentrations at Guanabara Bay, Rio de Janeiro State, Brazil.

Two years later, 15 samples were collected at Jurujuba Sound, located at east margin of Guanabara Bay (area 4), including Boa Viagem Beach. Jurujuba Sound is a harbor, and the high metal levels found over there were already expected. Both ETSA and EST evaluations were lower than the previous study at Boa Viagem (Table 1). Such results indicated that, despite the high levels of metals within sediments, bacterial DHA activity decreased but was not inhibited, and the microbial community may show some tolerance or resistance mechanism against metals [70].

In order to investigate the tolerance and resistance through metal retention by bacterial biofilm, bioassays and X-ray fluorescence analysis were performed, and results showed that even under elevated metal concentrations (Cu and Zn; 50 ppm), bacteria consortia isolated from polluted mangrove sediments were able to form biofilm, which, in its turns, was able to retain the metals (Figure 2) [71].

Once bacterial biofilm has the capability to adsorb metal through chemical interactions with its constituents [32], resistant bacteria consortia from metal contaminated mangroves are able to use this adaptation to bind metals and consequently to avoid, or at least to hinder, the metal negative effects on their metabolisms, allowing them to oxidize organic matter. Furthermore, the enzymatic analysis showed that the dehydrogenase activity was not affected in the presence of zinc (50 mg L^{-1}), and a dense biofilm with zinc superposing on it was observed.

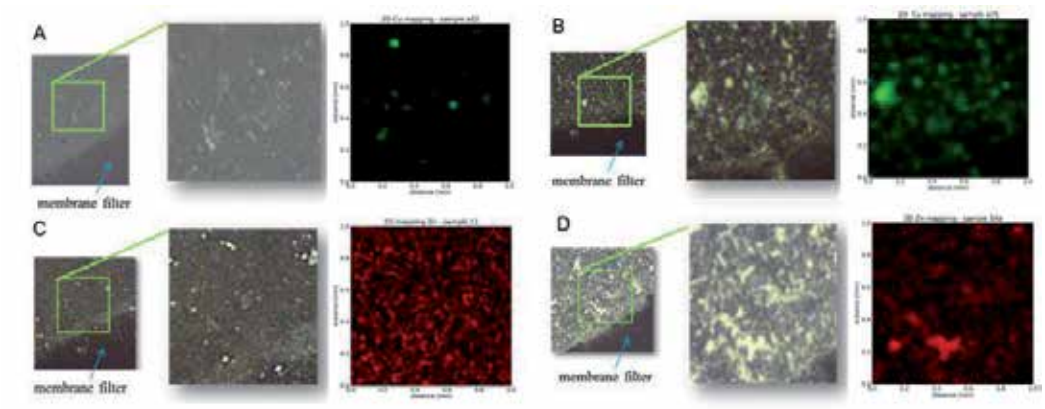


Figure 2. Biofilm formation and metal retention by bacterial consortia resistant to Zn and Cu. from contaminated mangrove sediments.

By contrast, although the presence of copper hindered the activity of dehydrogenases during the bacteria growth's lag phase, the enzyme activity increased afterward, following the exponential growth phase, but with lower magnitude when compared with the control bioassay (without copper). The presence of biofilm was also observed with superposition of copper, however, in less amounts than zinc bioassays. Without a dense biofilm as a protection against metal encounter, dehydrogenases activity decreases. However, as biofilm grows, dehydrogenase activity increases at higher rates during exponential growth, similar to the event observed for zinc bioassay. In this sense, biofilm may be acting both as metal sequestering as protector of bacterial cells embedded in the exopolymeric matrix at mangrove polluted sediments [71].

In 2005, 30 sampling stations were distributed all over the Guanabara Bay, including sampling stations near to Guapimirim mangrove preservation area. The highest levels of organic matter were found in area 1, while the high population concentration was observed at area 2 close to the Guapimirim mangrove. Both EST and ETSA were evaluated at 1.25 to 4.69 μg of fluorescein h^{-1} g^{-1} and 0.047 μL O_2 h^{-1} g^{-1} to 0.366 μL g^{-1} h^{-1}, respectively. The microbial community was characterized through the following anaerobic mechanisms: fermentation, denitrification, and sulfate reduction. The bacterial consortia formed by sulfate-reducing and sulfate-denitrifying bacteria sustain the benthic trophic food web in Guanabara Bay, mainly in the regions near to mangrove areas (2 and 4). The anaerobic bacterial metabolism, besides producing organic acids and sulfate and releasing nitrogen to the atmosphere, usually produces less intracellular energy than aerobic organisms, generating, on a macroscale, a low carbon and nutrient cycle in the anoxic sediment [72,73].

Regarding the carbon sources available for microorganisms, the organic matter quality means discriminating between labile and refractory organic compounds [78]. The labile portion of organic matter mainly consists of biopolymers and includes carbohydrates, lipids, and proteins, which is available to feeding strategies and the distribution of benthic organisms [75,76].

http://dx.doi.org/10.5772/60980

In this context, in order to investigate the carbon sources present at the Guanabara Bay, studies performed in 2005 found highest values of organic matter and biopolymers (proteins, carbohydrates, and lipids) near the Guapimirim protected mangrove (area 2) (Figure 1, Table 2) [75,76].

Year	Location	Organic matter (%)	Carbohydrate ($mg.g^{-1}$)	Lipid ($mg.g^{-1}$)	Protein ($mg.g^{-1}$)	Biopolymeric carbon ($mg.g^{-1}$)	Bioavailable carbon (%)	Reference
2005	Guanabara Bay area 1 (sewage input)	5.83-7.06	0.83-1.5	0.6-1.7	0.04-0.06	0.84-1.70	56.8-62.3	[75,76]
2005	Guanabara Bay area 2 (Guapimirim mangrove)	0.59-6.68	0.210-1.20	0.07-0.50	0.02-0.11	0.19-0.80	46.6-50.3	[75,76]
2005	Guanabara Bay area 3	0.73-6.13	0.55-1.13	0.17-1.23	0.03-0.11	0.30-1.43	49.6-60.4	[75,76]
2005	Guanabara Bay area 4	2.10-6.2	0.61-1.11	0.33-0.62	0.02-0.06	0.52-1.00	52.10-52.9	[75,76]
2008	Guanabara Bay (Suruí mangrove)	1.3-4.0	0.3		0.40	0.13-0.27		[13]
2011	Guanabara Bay area 2 (Guapimirim mangrove)	-	0.12-12.20	0.04-12.78	0.02-0.89	-	31-75	[78]

Table 2. Biopolymer concentrations at Guanabara Bay, Rio de Janeiro State, Brazil.

In 2008, investigations at the mangrove located at northwest of Guanabara Bay (Suruí mangrove) showed similar characteristics from surrounding polluted area (Table 2). The organic matter varied from 1.3% to 4%, with the largest values top layers of cores and highest values for biopolymeric organic carbon (0.13–0.27 g g^{-1}). The higher biopolymer concentration was found in the upper layer of sediments. The carbohydrates showed relative highest level (0.3 g g^{-1}) near Guapimirim mangrove (site 2). Proteins spotted the second highest level with the highest concentration at site 2, with 0.40 g g^{-1}. The microbial metabolism was anaerobic (fermentation, denitrification, and sulfate reduction), with high bacterial population on up sediment layer hydrolyzing organic matter biopolymers, with high esterase enzyme activity and less ETSA. Due these characteristics, this mangrove promotes the formation of pyrite and metal retention, which in it turns contributes decreasing the metal availability to microorganisms and therefore to the other organisms [13,77].

Most recently, in 2011, metal and biopolymers concentrations were evaluated at Guanabara Bay. Comparing with the earlier studies, biopolymers concentration increased in ten times at the bay. High biopolymers values were found again at area 2, near to Guapimirim mangrove. Proteins concentrations showed also lowest values (Table 2). Highest reactive metal concentrations were found associated with finest sediments, high organic content from sludge on areas 1 and 3, and near entrance of the bay, on Jurujuba Sound. The mean bioavailable carbon was evaluated on 53% ± 22%. High metal levels were found in areas with high organic matter content and fine sediments [78].

The anthropogenic activities, interacting with the complexity of the natural environment, may undermine the distribution of nutrients [79], microbial physiology 54,72,78,80], physical parameters [81], and trace elements [82] in marine environments, as well as biological resistance to the pollution [83,84].

5. Conclusions

The chronic dominance of lipids and carbohydrates, mainly due to sewage discharge at estuaries and mangroves systems, may change the bacterial trophic state from aerobic to anaerobic metabolism. This alteration may reflect on decreasing both bacterial efficiency of organic matter degradation and the bacterial productivity. Although the high levels of metals at the sediment, bacteria consortia are able to produce EPS as defense mechanisms. However, although the use of defense mechanisms against metals, the multimetal contamination may still hinder bacterial organic matter oxidation. Thus, the decrease in organic matter biodegradation due to toxic metal levels summed to the less efficient anaerobic metabolisms contributes to degenerating the diversity of microbial benthic communities.

Acknowledgements

The authors would like to acknowledge CAPES-Ministry of Education/Brazil for scholarship, CNPq and LNLS—Ministry of Science and Technology/Brazil for research funding.

Author details

Simone Pennafirme[1*], Inaya Lima[2], José Bitencourt[1], Mirian Crapez[1] and Ricardo Lopes[1]

*Address all correspondence to: spennafirme@id.uff.br

1 Marine Biology Department, Federal Fluminense University, RJ, Brazil

2 Nuclear Engineering Department, Federal University of Rio de Janeiro, RJ, Brazil

References

[1] Crapez MAC. Bactérias marinhas In: Pereira RC, Soares-Gomes A, editors. Biologia Marinha. 2nd ed. Rio de Janeiro: Interciência, 2002. pp. 81–101.

[2] Madigan MT, Martinko JM, Parker J, Fernández MG, Pérez MS. Biología de los microorganismos. Brock, Prentice Hall; 2003. p. 1012.

[3] Pelczar MJ, Chang ECS, Krieg NR. Microbiologia: conceitos e aplicações. São Paulo: Makron Books, 1996. p. 556.

[4] Whitman WB, Coleman DC, Wiebe WJ. Prokaryotes: the unseen majority. Proc Nat Acad Sci. 1998;95:6578–6583.

[5] Zölner E. Structuring effects of mesozooplankton on freshwater and marine microbial food webs. PhD thesis. Christian-Albrechts-Universität. 2004. p. 351.

[6] Rosenkranz F, Cabrol L, Carballa M, Donoso-Bravo A, Cruz L, Ruiz-Filippi G, Chamy R, Lema JM. Relationship between phenol degradation efficiency and microbial community structure in an anaerobic SBR. Wat Res. 2013;47:6739–6749.

[7] Ramos C, Buitrón G, Moreno-Andrade I, Chamy R. Effect of the initial total solids concentration and initial pH on the bio-hydrogen production from cafeteria food waste. Int J Hydrogen Energy. 2012;37:13288–13295.

[8] Azam T, Fenchel T, Field J, Graf L, Meyer-Reil LA, Thingstad F. The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser. 1983;10:257–263.

[9] Evans GG, Furlong J. Environmental Biotechnology: Theory and Application. Chichester: Wiley; 2003. p. 290.

[10] Kreft JU. Conflicts of interest in biofilms. Biofilms. 2004;1:265–276.

[11] Keller L, Surette MG. Communication in bacteria: an ecological and evolutionary perspective. Nat Rev Microbiol. 2006:249–258.

[12] Premuzic ET, Benkovitz CM, Gaffney JS, Walsh JJ. The nature and distribution of organic matter in the surface sediments of world oceans and seas. Organ Geochem. 1982;4:63–77.

[13] Fontana LF, Mendonça Filho JG, Pereira Netto AD, Sabadini-Santos E, de Figueiredo Jr AG, Crapez MAC. Geomicrobiology of cores from Suruí Mangrove—Guanabara Bay—Brazil. Mar Poll Bull. 2010;60:1674–1681.

[14] Beguin P, Aubert JP. The biological degradation of cellulose. FEMS Microbiol Rev. 1994;13:25–58.

[15] Azam F, Long RA. Oceanography: sea snow microcosms. Nature. 2001;414:495–498.

[16] Davidson T, Qingdong KE, Costa M. Selected molecular mechanisms of metal toxicity and carcinogenicity. In: Nordberg GF, Fowler BA, Nordberg M, Friberg LT, editors. Handbook on the Toxicology of Metals. Burlington: Academic Press; 2007. pp. 79–85.

[17] Nordberg GF, Fowler BA, Nordberg M, Friberg L. Handbook on the Toxicology of Metals. Burlington: Academic Press; 2007b. p. 1024.

[18] Herrera A, Cockell CS, Self S, Blaxter M, Reitner J, Arp G, Dröse W, Thorsteinsson T, Tindle AG. Bacterial colonization and weathering of terrestrial obsidian in Iceland. Geomicrobiol J. 2008;25:25–37.

[19] Decho AW. Overview of biopolymer-induced mineralization: what goes on in biofilms? Ecol Eng. 2010;36:137–144.

[20] Madsen EL. Environmental Microbiology: From Genomes to Biogeochemistry. Chichester: Wiley 2011; p. 496.

[21] Arndt S, Jorgensen BB, LaRowe DE, Middelburg JJ, Pancost RD, Regnier P. Quantifying the degradation of organic matter in marine sediments: a review and synthesis. Earth-Sci Rev. 2013;123:53–86.

[22] Ridgwell A, Zeebe RE. The role of the global carbonate cycle in the regulation and evolution of the Earth system. Earth Planet Sci Lett. 2005;234:299–315.

[23] Ridgwell A, Hargreaves JC. Regulation of atmospheric CO_2 by deep-sea sediments in an Earth system model. Global Biogeochem Cycles. 2007;21; doi: 10.1029/2006GB002764.

[24] Nielsen, LP, Risgaard-Petersen N, Fossing H, Christensen PB, Sayama M. Electric currents couple spatially separated biogeochemical processes in marine sediment. Nature. 2010;463:1071–1074.

[25] Weiss M, Abele U, Weckesser J, Welte W, Schiltz E, Schulz GE. Molecular architecture and electrostatic properties of a bacterial porin. Science. 1991;254:1627–1630.

[26] Prescott LM, Harley JP, Klein DA. Microbiology. McGraw-Hill;2002a; p. 1147.

[27] Sigee D. Freshwater Microbiology: Biodiversity and Dynamic Interactions of Microorganisms in the Aquatic Environment. West Sussex: John Wiley & Sons; 2005. p. 544.

[28] Pizarro C, Donoso-Bravo A, Jeison D, Ruiz-Filippi G, Chamy R. Biofilm formation for organic matter and sulphate removal in gas-lift reactors. Electron J Biotechnol. 2011;14:3–3.

[29] Kaiser K, Benner R. Major bacterial contribution to the ocean reservoir of detrital organic carbon and nitrogen. Limnol Oceanogr. 2008;53: 99–112.

[30] Johnson, KS. Manganese Redox Chemistry Revisited. Science. 2006; 313:1896–1897.

[31] Scala DJ, Hacherl EL, Cowan R, Young LY, Kosson DS. Characterization of Fe(III)-reducing enrichment cultures and isolation of Fe(III)-reducing bacteria. Res Microbiol. 2006;157: 772–783.

[32] Harrison JJ, Ceri H, Turner RJ. Multimetal resistance and tolerance in microbial biofilms. Nat Rev Microbiol. 2007;5:928–938.

[33] Nordberg G, Bruce A, Monica N, Friberg L. Introduction—general considerations and international perspectives. In: Nordberg GF, Fowler BA, Nordberg M, Friberg

LT, editors. Handbook on Toxicological of Metals. Burlington: Academic Press; 2007a. pp. 1–9.

[34] Nelson DL, Lehninger AL, Cox MM. Lehninger Principles of Biochemistry. 5th ed. New York: W. H. Freeman; 2008. p. 1100.

[35] Choudhury R, Srivastava S. Zinc resistance mechanisms in bacteria. Curr Sci. 2001;81(7): 768–775.

[36] Rogers EJ, Li SW. Effect of metals and other inorganic ions on soil microbial activity: soil dehydrogenase assay as simple toxicity test. Bull Environ Contam Toxicol. 1985; 34: 858–865.

[37] Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010; 8:623–633.

[38] González AG, Shirokova LS, Pokrovsky OS, Emnova EE, Martínez RE, Santana-Casiano JM, González-Dávila M, Pokrovski GS. Adsorption of copper on *Pseudomonas aureofaciens*: protective role of surface exopolysaccharides. J Colloid Interface Sci. 2010;350:305–314.

[39] Ueshima M, Ginn BR, Haack EA, Szymanowski JES, Fein JB. Cd adsorption onto *Pseudomonas putida* in the presence and absence of extracellular polymeric substances. Geochim Cosmochim Acta. 2008;72:5885–5895.

[40] Morillo Pérez JA, García-Ribera R, Quesada T, Aguilera M, Ramos-Cormenzana A, Monteoliva-Sánchez M. Biosorption of heavy metals by the exopolysaccharide produced by *Paenibacillus jamilae*. World J Microbiol Biotechnol. 2008;24:2699–2704.

[41] Stams AJM, Plugge CM. Electron transfer in syntrophic communities of anaerobic bacteria and archaea. Nat Rev Microbiol. 2009;7:568–577.

[42] Matz C. Competition, communication, cooperation: molecular crosstalk in multi-species biofilms. In: Flemming H-C, Wingender J, Szewzyk U, editors. Biofilm Highlights. London: Springer-Verlag Berlin Heidelberg; 2011. pp. 29–40.

[43] Pomeroy LR, Williams PJI, Azam F, Hobbie JE. The microbial loop. Oceanography. 2007;20:28–33.

[44] Sprocati AR, Alisi C, Segre L, Tasso F, Galleti M, Cremisini C. Investigating heavy metal resistance, bioaccumulation and metabolic profile of a metallophile microbial consortium native to an abandoned mine. Sci Total Environ. 2006;366: 649–658.

[45] Amador EdS. Bacia da Baía de Guanabara: Características Geoambientais e Ecossistemas. Rio de Janeiro: Interciência; 2012. p. 432.

[46] Ribeiro CH, Kjerfve B. Anthropogenic influence on the water quality in Guanabara Bay, Rio de Janeiro, Brazil. Reg Environ Change. 2002;3:13–19.

[47] JICA. The Study on Recuperation of the Guanabara Bay Ecosystem. Japan: International Cooperation Agency. Tokyo: Kokusai Kogyo Co. Ltd; 1994. 67 p.

[48] Carreira RS, Wagener ALR, Readman JW, Fileman TW, Macko SA, Veiga Á. Changes in the sedimentary organic carbon pool of a fertilized tropical estuary, Guanabara Bay, Brazil: an elemental, isotopic and molecular marker approach. Mar Chem. 2002;79:207–227.

[49] Baptista Neto JA, Peixoto TC, Smith BJ, McAlister JJ, Patchineelam SM, Patchineelam SR, Fonseca EM. Geochronology and heavy metal flux to Guanabara Bay, Rio de Janeiro state: a preliminary study. An Acad Bras Ciê. 2013;85:1317–1327.

[50] Kjerfve B, Ribeiro CHA, Dias GTM, Filippo AM, Da Silva Quaresma V. Oceanographic characteristics of an impacted coastal bay: Baía de Guanabara, Rio de Janeiro, Brazil. Cont Shelf Res. 1997;17:1609–1643.

[51] FEEMA. Fundação Estadual de Engenharia do Meio Ambiente Projeto de recuperação gradual da Baía de Guanabara. Fundação Estadual de Engenharia do Meio Ambiente, Rio de Janeiro: FEEMA; 1990. p. 128.

[52] Byers S, Mills E, Stewart P. Comparison of methods of determining organic carbon in marine sediments, with suggestions for a standard method. Hydrobiologia. 1978;58:43–47.

[53] Baptista Neto JA, Smith BJ, McAlister JJ. Heavy metal concentrations in surface sediments in a nearshore environment, Jurujuba Sound, Southeast Brazil. Environ Poll. 2000;109:1–9.

[54] Crapez M, Baptista Neto JA, Bispo MGS. Bacterial enzymatic activity and bioavailability of heavy metals in sediments from Boa Viagem Beach (Guanabara Bay). Anuário do Instituto de Geociências. 2003;26:60–68.

[55] Dubois M, Gilles K, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1958;28:350–356.

[56] Gerchacov SM, Hatcher PG. Improved technique for analysis of carbohydrates in sediment. Limnol Oceanogr. 1972;17:938–943.

[57] Marsh JB, Weinstein DB. Simple charring method for determination of lipids. Jour Lipid Res. 1966;7:574–576.

[58] Hartree EF. Determination of protein: a modification of the Lowry method that gives a linear photometric response. Anal Biochem. 1972;48:422–427.

[59] Rice DL. The detritus nitrogen problem: new observations and perspectives from organic geochemistry. Mar Ecol Prog Ser 1982;9:153–162.

[60] Fabiano M, Danovaro R, Fraschetti S. A three-year time series of elemental and biochemical composition of organic matter in subtidal sediments of the Ligurian Sea (northwestern Mediterranean). Contin Shelf Res. 1995;15:1453–1469.

[61] Stubberfield LCF, Shaw PJA. A comparison of tetrazolium reduction and FDA hydrolysis with other measures of microbial activity. J Microbiol Methods. 1990;12:151–162.

[62] Trevors JT. Effect of substrate concentration, inorganic nitrogen, O_2 concentration, temperature and pH on dehydrogenase activity in soil. Plant Soil. 1984; 77:285–293.

[63] Houri-Davignon C, Relexans JC. Measurement of actual electron transport system (ETS). Activity in marine sediments by incubation with INT. Environ Technol Lett. 1989;10:91–100.

[64] Alef K, Nannipieri P. Enrichment, isolation and counting of soil microorganism. In: Methods in Applied Soil Microbiology and Biochemistry. Michigan: Academic Press; 1995. pp. 23–186.

[65] Kepner RL, Pratt JR. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. Microbiol Rev. 1994;58:603–615.

[66] McAlister JJ, Smith BJ, Curran JA. The use of sequential extraction to examine iron and trace metal mobilisation and the case hardening of building sandstone: a preliminary investigation. Microchem J. 2003;74:5–18.

[67] Baruah NK, Kotoky P, Bhattacharrya KG, Borah GH. Metal speciation in Jhanji River sediments. Sci Total Environ. 1996;193:1–12.

[68] Bousmans, P, Klockenkämper R. Total reflection X-ray fluorescence spectrometry, Spectrochim Acta Part B. 1989;44:433–549.

[69] Nweke, CO, Okpokwasili GC. Kinetics of dose-response relationship of heavy metals with dehydrogenase activity in wastewater bacteria. J Res Biol. 2012;2:392–402.

[70] Baptista Neto, JA, Crapez, MAC, McAlister JJ, Vilela, CG. Concentration and bioavailability of heavy metals in sediments from Niterói harbour (Guanabara Bay/S.E. Brazil). J Coast Res. 2005; 20: 1–7.

[71] Pennafirme S, Lima I, Bittencourt JA, Crapez MAC, Lopes RT. Microbial biofilm study by synchrotron X-ray microscopy. Radiat Phys Chem. doi:10.1016/j.radphyschem. 2015.05.04.

[72] Silva FS, Pereira DC, Sanchez-Nunez L, Krepsky N, Fontana LF, Baptista-Neto JA, Crapez MAC. Bacteriological study in the superficial sediments of the Guanabara Bay, RJ, Brazil. Braz J Oceanogr. 2008;56(1): 13–22.

[73] Sobrinho da Silva F, Bitencourt JAP, Savergnini F, Guerra LV, Baptista Neto JA, Crapez MAC. Bioavailability of organic matter in the superficial sediment of Guanabara Bay. Anu Inst Geocienc—UFRJ. 2011;34:52-63.

[74] Mayer LM, Schick LL, Sawyer T, Plante CJ, Jumars PA, Self RL. Bioavailable amino acids in sediments: a biomimetic, kinetics-based approach. Limnol Oceanogr. 1995;40:511–520.

[75] Jumars PA, Penry DL. Digestion theory applied to deposit feeding. In: Lopez G, Taghon G, Levinton J, editors. Ecology of Marine Deposit-Feeders. Lecture Notes on Coastal and Estuarine Studies. New York: Springer-Verlag; 1989. pp. 114–128.

[76] Graf G. Benthic-pelagic coupling: a benthic view. Oceanogr Mar Biol Ann Rev. 1992;30:149–190.

[77] Machado W, Borrelli NL, Ferreira TO, Marques AGB, Osterrieth M, Guizan C. Trace metal pyritization variability in response to mangrove soil aerobic and anaerobic oxidation processes. Mar Poll Bull. 2014;79:365–370.

[78] Sabadini-Santos E, da Silva TS, Lopes-Rosa TD, Mendonça-Filho JG, Santelli RE, Crapez MAC. Microbial activities and bioavailable concentrations of Cu, Zn, and Pb in sediments from a tropic and Eutrothicated Bay. Water Air Soil Pollut. 2014; 225:1–11.

[79] Wagener ALR. Constraints to the implementation of effective environmental management in coastal areas of developing countries. An Acad Bras Ciê. 2005;77:613–623.

[80] Fonseca EM, Baptista Neto JA, Crapez MC, McAllister JJ, Fernandez MA, Bispo MGS. Bioavailability of heavy metals in Guanabara Bay, Rio de Janeiro (Brazil). J Coast Res. 2009;56:802—806.

[81] Nixon SW. Eutrophication and the macroscope. In: Andersen JH, Conley DJ, editors. Eutrophication in Coastal Ecosystems: Towards better Understanding and Management Strategies. Lund: Springer; 2010. pp. 5–19.

[82] Adriano DC. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability, and Risks of Metals. New York: Springer; 2001. p. 533.

[83] Nevo E, Noy R, Lavie B, Beiles A, Muchtar S. Genetic diversity and resistance to marine pollution. Biol J Linn Soc. 1986;29:139–144.

[84] Bitencourt J, Pereira D, da Silva Neto I, Crapez MC. Evaluation of the sensitivity to zinc of ciliates *Euplotes vannus* and *Euplotes crassus* and their naturally associated bacteria isolated from a polluted tropical bay. Environ Sci Pollut Res. 2014; doi:10.1007/s11356-014-3828-1.

Chapter 5

Biodegradation of Paclobutrazol — A Plant Growth Regulator Used in Irrigated Mango Orchard Soil

Fernanda Vaz, Ednaldo Santos-Filho, Suzyane Silva, Silvany Araújo, Thatiana Stamford-Arnaud, Andrea Bandeira, Ana Cristina Brasileiro-Vidal, Newton Pereira Stamford, Maria Aparecida Mouco and Ester Gouveia

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60818

Abstract

Paclobutrazol (PBZ), [2RS,3RS]-1-[4-chlorophenyl]-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol, consists of a triazole ring and a benzene ring-chloro linked to a carbon chain open. It is a plant growth regulator widely used in many crops in order to produce fruit throughout the year by inhibiting gibberellin synthesis, a hormone responsible for the vegetative plant growth. Actually, studies are showing that paclobutrazol remains active in the soil for a long time, affecting the growth and development of subsequent crops by reducing plant vigor. Biodegradation is an effective and cheap process that can to degrade or transform contaminants to less toxic or nontoxic. In this work, the biodegradation of paclobutrazol was studied using in submersed culture and saturated and unsaturated soils. In these conditions, experiments with biostimulation and bioaugmentation were performed. In the experiments carried out in submersed culture, with biostimulation by addition of glycerol, the PBZ biodegradation was higher than that with PBZ as sole carbon source. The biodegradation of PBZ in unsaturated soils was more efficient when soil samples with a history of application of PBZ were used. The highest number of applications of PBZ favored biodegradation. The biodiversity of the microbiota in the soil favored the biodegra-

dation of PBZ aromatic rings. PBZ was not seen to be phytotoxic and the biodegraded products increased the germination index.

Keywords: paclobutrazol, semi arid region of Brazyl, models, Pseudomonas

1. Introduction

Brazil has a great potential for fruit production, as it has area, climate, and enough water for production throughout the year. The production of mango can be developed under different climatic conditions, but it is commercially viable only within a well-defined range of temperature, rainfall, altitude, insulation, relative humidity, and winds. The fruit is native to tropical climates, but it can be grown in subtropical regions of the planet. Mango (*Mangifera indica* L.) plantations in the country occupy about 74,000 ha, generating a production of over 1.1 million tons. Mango is produced in all regions of Brazil; however, the southeast and northeast regions account for 94% of the total [1]. The area cultivated with mango in the northeast region has increased by 20,000 ha in 10 years. In 2012, that amount alone was responsible for producing more than 85% of the total exported by Brazil [2]. To foster exportation, however, it is necessary to guarantee production whenever the market is receptive and to ensure that the quality of the fruit corresponds to international food safety requirements [3].

The production of mango in Brazil can be divided into two different phases: the first one characterized by extensive cultivation of local varieties with little or no use of technology; the second one characterized by a high level of technology, such as irrigation, floral induction, and improved varieties [4]. Mangos from the Brazilian semiarid region stand out in the national scenario due to high yields and fruit quality, and also to the possibility of year-round production taking advantage of the climatic conditions as well as management techniques (irrigation, pruning, and the use of growth regulators) for plant growth and blossom control [5].

The growth of the mango tree, as well as other tropical fruit trees, is not continuous but comes in vegetative flushes of the terminal and axillary shoots of the branches, before the period of dormancy. For the vegetative or floral growth to happen, two different processes occur in the plant: the growth of the buds and the initiation of the sprouting. The bud starts to grow, which includes the end of the dormancy and a quick development of the shoot. Along with the shoot initiation, the induction happens, and it will define the vegetative type, floral or mixed [6].

Flowering in mango is a process that may occur during an extensive period (up to several months) and can have its beginning altered, naturally or artificially, due to climatic conditions, yield of the former harvest, or use of specific crop management techniques, including plant growth regulators [7]. Most of the plant growth regulators inhibit the gibberellin synthesis and can therefore be used for plant growth and flowering management [8]. Among the plant growth regulators used in fruit production, paclobutrazol (PBZ) has shown efficiency in mango flowering management [9]. PBZ (Figure 1) must be applied directly to the soil due to its low solubility, long residual activity, and lack of efficient foliar

http://dx.doi.org/10.5772/60818

uptake [8]. The recommended doses range between 1.0 and 1.5 g, measured by tree crown diameter, and dependent on the cultivar, climate, soil type, and plant nutrition. Paclobutrazol is absorbed by the roots, conducted by the xylem to the leaves and buds, without mobility by phloem [10]. It is persistent in the plant and soil, highly stable in the soil, and its slow degradation lowers plant metabolism [8]. PBZ applied as a soil drench reduces internode lengths and causes earlier and enhanced flowering in mango trees. These results have been confirmed in different locations in the tropics [11].

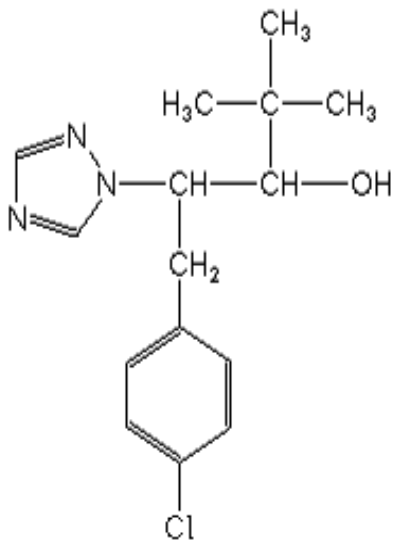


Figure 1. Chemical structure of paclobutrazol.

Paclobutrazol doses applied, each year, are not always adequate because they do not take into account the residue from previous applications. Paclobutrazol increases the compaction of inflorescence in the 'Tommy Atkins' mango proportionate to the applied dose [12]. High dosage, which tends to reduce the panicle length of the treated plants (33% as compared to control), results in the formation of very compact inflorescences, creating appropriate conditions for the incidence of diseases and pests as well as making phytosanitary control difficult [9]. In addition to the phytosanitary problems, excessive doses of PBZ can inhibit vegetative and floral growth longer than desirable, requiring more nitrate sprays to stimulate flowering. The high cost of crop production, for all the reasons that have been mentioned, is only one of the problems, as there is also the question of the accumulation of a chemical in the soil and plant without knowing the consequences over the years, both for the production system and the environment.

Soil application rather than foliar application of paclobutrazol has been found to be more responsive in suppressing the vegetative growth and enhancing the reproductive growth in mango trees [12, 13]. Studies have shown that paclobutrazol needs to be applied annually to increase mango fruit yields [5]. However, the paclobutrazol treatments to the tree basins (soil

under the canopy drip area within a radius of 1.5 m of the tree trunk) may result in its uptake into the trees and thereby result in the persistence of its residues in the mango fruit and also in the soil at the tree basin [13]. Such persistence of paclobutrazol residues in mango fruit may lead to adverse effects on human health. The persistence of paclobutrazol residues in soil may influence the soil microbial activity too. Soil microbial count of a mango orchard soil where paclobutrazol was frequently applied has been shown to be reduced by up to 58% [14].

Soils are becoming polluted by pesticides because of the wide and, often indiscriminate, use of these xenobiotic molecules in agricultural practice. In the soil, pesticides may be involved in several stages, such as retention, transformation, and transport, the intensity of which will affect the potential activity of agrochemicals [15]. Bioremediation is an effective and cheap process that can degrade or transform contaminants to become less toxic or nontoxic [16–18]. Two processes have been found to increase the activity of microorganisms during bioremediation: biostimulation and bioaugmentation [19]. Biostimulation involves the addition of nutrients and/or a terminal electron acceptor to increase the weak activity of indigenous microbial populations by accelerating the decontamination rate since the addition of one or more rate-limiting nutrients to the system improves the degradation potential of the inhabiting microbial population [20, 21]. Bioaugmentation involves the addition of external microbial strains (indigenous or exogenous) that have the ability to degrade the target toxic molecules [22].

In this work, the biodegradation of paclobutrazol was studied using in submersed culture and saturated and unsaturated soils. In these conditions, experiments with biostimulation and bioaugmentation were performed.

2. Methodology

Soil samples were collected from irrigated mango orchards (*M. indica* L. cv. Tommy Atkins) at the Bebedouro and Mandacaru Experimental Stations of the Brazilian Agricultural Corporation (EMBRAPA Semiárido), in the Municipalities of Petrolina (9°09′ S, 40°22′ W), Pernambuco state, and Juazeiro (9°24′ S, 40°24′ W), Bahia state, both located in the São Francisco river valley (northeast of Brazil). The two representative soils were a Yellow Ultisol (Bebedouro) and a Vertisol (Mandacaru). These regions had been consecutively treated with PBZ, with an average dose of 3.57 g of active ingredient per plant. The soil samples were collected 30 days after the last application. An average of 1.5 kg of soil at depths of 15 and 30 cm was collected from four points around eight plants. These samples were stored in a refrigerator for isolation until the beginning of the experiments. Soil samples without a history of PBZ application were also taken from the same farms.

The bacteria were isolated in a mineral medium [23], containing 0.25 g/L paclobutrazol (Cultar 25 SC, containing 25% of the active compound), which was used as the sole carbon source. 10 g of each soil sample (MS—Mandacaru without historical application of PBZ; MC—Mandacaru with historical; BS—Bebedouro without historical; BC—Bebedouro with historical) were added to 100 mL medium in 500 mL flasks. These flasks were incubated at 30°C in a rotatory

http://dx.doi.org/10.5772/60818

shaker (200 rpm). Evidence for bacterium utilization of paclobutrazol was sought by streaking turbid enrichment broths onto a mineral agar medium (15 g/L), containing paclobutrazol or glucose as the sole carbon source, and then incubating these plates under the same enrichment conditions. Pure cultures of paclobutrazol utilizing bacteria were obtained by streaking distinct colonies present on the mineral agar medium plates onto Tryptone Soy Agar (TSA, Oxoid). The isolates were identified by Gram staining test. The Gram-negative isolates were streaked on three selective media for *Pseudomonas*: agar D4 [24]; agar Cetrimide (Merck) and King [25]. Biodegradation experiments were accomplished in a mineral broth with PBZ (1 g/L) and glycerol (5 g/L) or glucose (10 g/L).

Biodegradation experiments in saturated soils (Yellow Ultisol and Vertisol) were conducted in batch using paclobutrazol and paclobutrazol with added glycerol. The experiments were performed under sterile (by Gamma radiation) conditions using the mixed culture of *Pseudomonas* spp. Two concentrations of PBZ (10 and 25 mg/L) according to solubility in water (<26 mg/L) were used. The experiments were carried out in 60 mL flasks, where 5 g or soil was added to a 25-mL volume of liquid. The microorganisms were added with about 10^7 cells/mL. Control experiments were carried out only for the PBZ concentration of 25 mg/L without the addition of microorganisms. These experiments were placed in a rotary shaker (200 rpm) at 30°C for 35 days. Microbial concentration initial was 1.10^7 CFU/mL. This quantity corresponds to the inoculum of microorganisms added to the experiment.

Biodegradation experiments were conducted with the collected soil samples with and without history. Glycerol was added as the additional carbon source. To each 10 g of soil with (P-G: PBZ and glycerol; P-NG: only PBZ) and without (NP-G: PBZ and glycerol; NP-NG: only PBZ) PBZ application history, 30 μg/g of PBZ was added from a solution prepared with a commercial product (Cultar 25 SC). The experiments were carried out in 125 mL flasks at room temperature, without stirring, for 63 days and in triplicate. Samples were withdrawn at 0, 7, 14, 21, 35, 48, and 66 days for the quantification of native microbial and residual PBZ. In experiments with the addition of glycerol (P-G, NP-G), the concentration of this compound in the soil was 150 μg/g. Microorganisms were not added to the soil.

A 2^4 factorial design to study the biodegradation of paclobutrazol was applied. A two-level factorial design with 16 runs was employed to evaluate the individual and combined effects of the four factors: glycerol, mineral medium, inoculum, and soil (Table 1). The levels of the factorial design were glycerol (X_1), with (+) and without addition (–); mineral medium [28] (X_2), with (+) and without addition (–); inoculum (X_3), with (+) and without addition (–); and region of soil collection(X_4), A (+) and B (–).

Infrared spectra of the samples before (E1: 0 days) and after (E2: 70 days) the biodegradation process using only PBZ or PBZ and glycerol in unsaturated soils were measured with FTIR spectrophotometer (Vertex 70, Bruker). The analysis was done in IR region of 400 and 4000 cm^{-1}.

The determination of the phytotoxicity was carried out with samples of biodegradation experiments (0 and 70 days), using only PBZ (4 μg/g) or glycerol (2.4 mg/g) as additional carbon source. Thirty seeds of *Allium cepa* (cv. Vale Ouro IPA-11) were germinated in individual Petri dishes containing 20 mL for each treatment at room temperature for 72 h. Distilled water was

Run	X_1	X_2	X_3	X_4
1	–	–	–	–
2	+	–	–	–
3	–	+	–	–
4	+	+	–	–
5	–	–	+	–
6	+	–	+	–
7	–	+	+	–
8	+	+	+	–
9	–	–	–	+
10	+	–	–	+
11	–	+	–	+
12	+	+	–	+
13	–	–	+	+
14	+	–	+	+
15	–	+	+	+
16	+	+	+	+

Table 1. Conditions of factorial design 2^4 experiments for the biodegradation of PBZ.

used as negative control, totalizing five treatments. After 24 h of treatment, seed germination (%) and root length were measured per treatment, in order to determine seed germination index (GI), as described in Equation (1):

$$\mathrm{GI}\ (\%) = \frac{S_t}{S_c}\frac{R_t}{R_c} * 100 \tag{1}$$

where S_t is the seed germination of treatment (%), S_c is the seed germination of negative control (%), R_t is the root length of treatment (cm), and R_c is the root length of negative control (cm).

3. Results and discussion

3.1. Isolation of bacteria from soil resistant to paclobutrazol

A total of 37 strains were isolated from the soil samples, in which 89% were Gram-negative bacteria. Eleven of these were identified as *Pseudomonas* (Figure 2). The growth curves of the 11 isolates of *Pseudomonas* showed exponential phase between 4 and 6 h. The maximum specific

http://dx.doi.org/10.5772/60818

rates growth (μ_{max}) were higher than 0.30 h^{-1} (Table 2). These bacteria were selected by their capacity to degrade diverse composites, as for example hydrocarbons [26], 2,4-dichlorophenol [27], naphthalene [28], and organophosphates [29]. *Pseudomonas* also participates in metabolic routes of compound degradation similar to paclobutrazol, as chlorobenzene [30] and atrazine [31, 32]. Jackson et al. [33], in research with paclobutrazol biodegradation, isolated nine *Pseudomonas* spp. with biodegradation capacity.

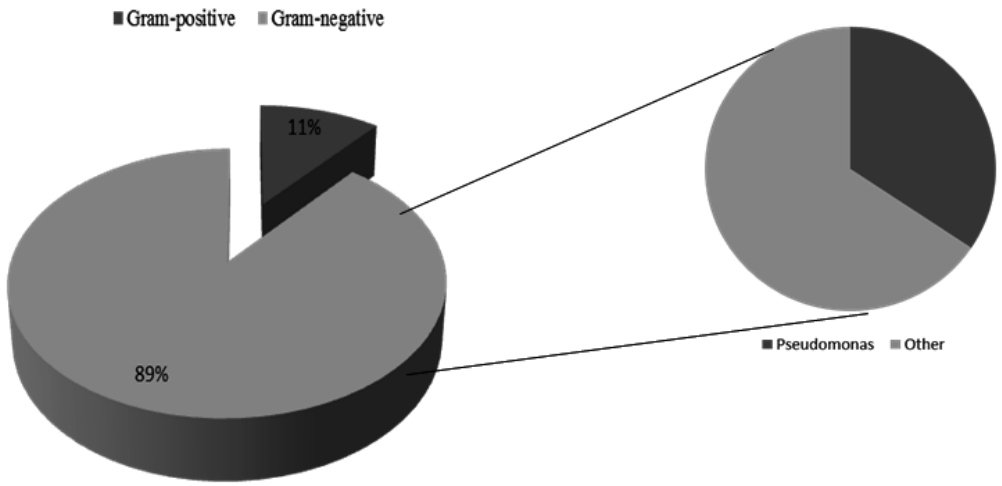


Figure 2. Bacteria isolated by enrichment of soil with paclobutrazol.

Pseudomonas spp.	μ_{max} (h^{-1})
BC8	0.36
MS9	0.45
BS19	0.49
BC20	0.78
BC21	0.96
MS23	0.65
MS26	0.59
MC27	0.68
BS31	0.71
BS32	0.68
BS33	0.68

Table 2. Growth maximum specific rates

Two strains of *Pseudomonas* were identified as *Pseudomonas aeruginosa*; however, these were not used in this work due their pathogenicity [34, 35]. Experiments with mixed cultures of the soil samples MS, MC, BS, and BC were carried out using only PBZ as the carbon source to evaluate PBZ biodegradation. TSA broth was inoculated with the cultures to activate them. After 24 h, at 30°C, bacteria was inoculated into 40 mL of the nutrient broth. After a period of approximately 4 at 6 h of incubation, at 30°C and 200 rpm, mixed cultures were prepared and inoculated into 400 mL of mineral broth, as described by Ridgway et al. [23] and PBZ, 1 g/L. Temperature and agitation conditions of this stage were similar to those for the inoculum. Later, biodegradation experiments were accomplished in mineral broth with PBZ, 1 g/L and glycerol, 5 g/L.

Biodegradation was for MS and BC mixed cultures that had reached the maximum in 20 days of culture, with 47% (MS) and 43% (BC) of PBZ biodegradation. No relation was observed between the PBZ biodegradation and the soil to have a history of application, probably due the isolation to have been for enrichment. Since the results of the experiments with mixed cultures MS and BC were similar, the culture MS was selected to continue with biodegradation experiments.

The experiments carried out with glycerol as an additional carbon source grew and had PBZ biodegradation higher than those with PBZ as sole carbon source. The maximum biodegradation reached about 75% in 10 days of culture. Jackson et al. [33], using *Pseudomonas*, obtained a biodegradation of 79% in 39 days. On the other hand, Silva et al. [14] observed a 56% biodegradation in 90 days, with mixed cultures of *Bacillus*, in an isolated soil sample with a history of application. Table 3 presents PBZ biodegradation in relation to the time, found in experiments using submersed culture. Lee et al. [28] observed that pyruvate can be used as an additional carbon source to stimulate growth and aromatic hydrocarbons biodegradation for *Pseudomonas putida* PG7.

Microorganism	Biodegradation (%)	Time (days)	Additional carbon source	Reference
Pseudomonas	79	39	-	Jackson et al. [33]
Bacillus	56	90	-	Silva et al. [14]
Pseudomonas	47	20	-	Present work
Pseudomonas	75	10	Glycerol	Present work
Pseudomonas	0	-	Glucose	Present work

Table 3. Comparison of the PBZ biodegradation in submersed culture.

When glucose was used as an additional carbon source, the PBZ concentration remained almost constant throughout the observation period (Table 3). PBZ biodegradation did not occur probably due to glucose catabolic repression. The presence of a catabolic repressor, or the presence of a carbon source that represses the expression of certain genes and operons responsible for the utilization of alternative carbon sources, can result in a low concentration inducing specific cometabolic routes [32].

http://dx.doi.org/10.5772/60818

3.2. Paclobutrazol biodegradation in saturated soils

In the experiments with PBZ as the sole carbon source in saturated soils, there was no difference in growth between the two PBZ concentrations used (10 and 25 mg/L). However, in experiments containing glycerol, a higher growth was observed when the concentration of PBZ 25 mg/L was used as a glycerol concentration of 50 mg/L. This can be attributed to an increased amount of glycerol (125 mg/L) compared to experiments with the PBZ concentration of 10 mg/L. The biodegradation of PBZ without an additional carbon source and 10 mg/L of PBZ was approximately 43% after 14 days (Figure 3). However, with glycerol as an additional carbon source, the biodegradation reached 70% in 28 days (Figure 4). The glycerol concentration decreased rapidly and was completely consumed during 4 days, independently of the amount utilized (Figure 5).

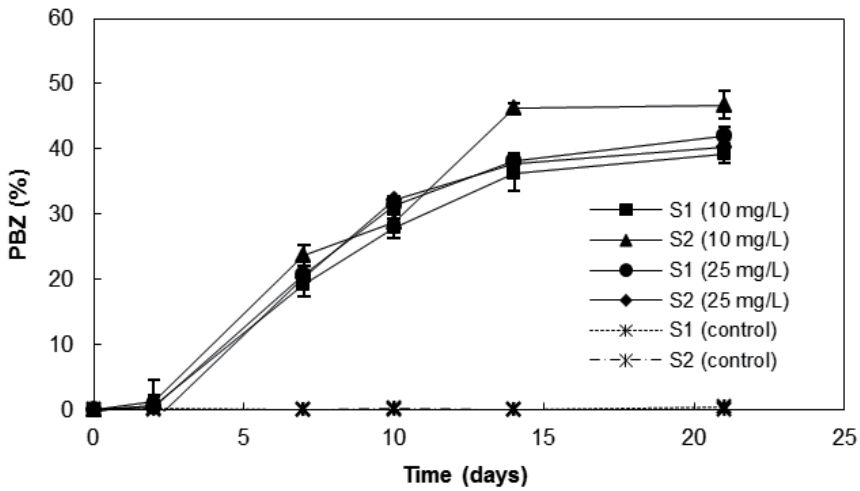


Figure 3. PBZ biodegradation. S1: yellow ultisol; S2: vertisol.

For the two soils and the two PBZ concentrations used (10 and 25 mg/L), there was a lag phase (the period in which there is virtually no biodegradation) of approximately 2 days, when PBZ was used as the sole carbon source. In all the experiments, biodegradation increased after a certain period of time, approximately 28 to 14 days, with only PBZ and PBZ with glycerol, respectively. The addition of a carbon source to the nutrient into the soil is believed to enhance in situ bioremediation by stimulating the growth of microorganisms that are indigenous to the subsurface and are capable of degrading contaminants [32].

3.3. Paclobutrazol biodegradation in unsaturated soils with and without a history of application

Figure 6 shows the PBZ biodegradation kinetics in unsaturated soil without and with a history of application, with and without the addition of glycerol. Experiments in P-G and P-NG soil showed a sustained reduction after the 14th day and only around 1% of the PBZ remaining on

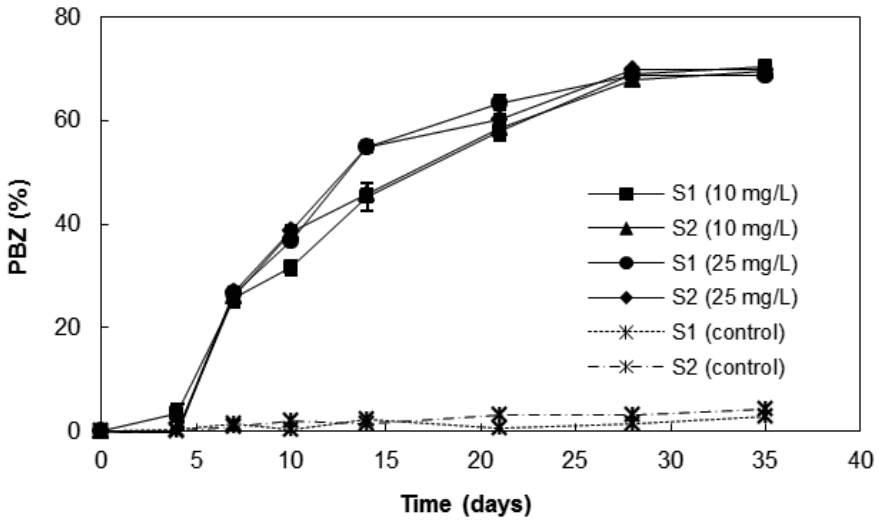


Figure 4. PBZ biodegradation with glycerol as additional carbon source. S1: yellow ultisol; S2: vertisol.

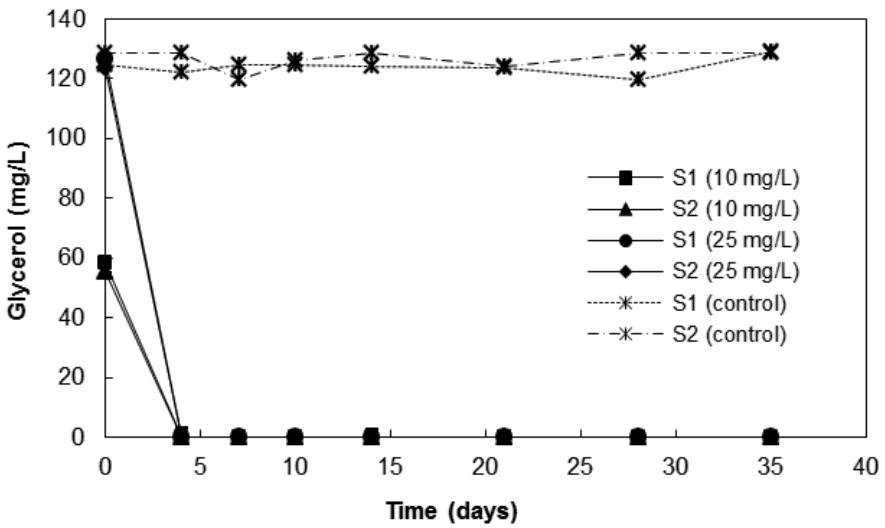


Figure 5. Glycerol consumption. S1: yellow ultisol; S2: vertisol.

the 63th day. This ability of the native microbiota to degrade paclobutrazol was probably due to the historical application. After repeated applications of some pesticides, the native microorganisms in the soil can degrade these compounds as they become suited for agrochemical use as a source of carbon for energy production and growth. Although there are some other factors affecting the persistence of agrochemicals in the soil, such as temperature, pH of the soil, chemical hydrolysis, and water content of the soil, microorganisms seem to play an important role in the degradation of these compounds [34].

http://dx.doi.org/10.5772/60818

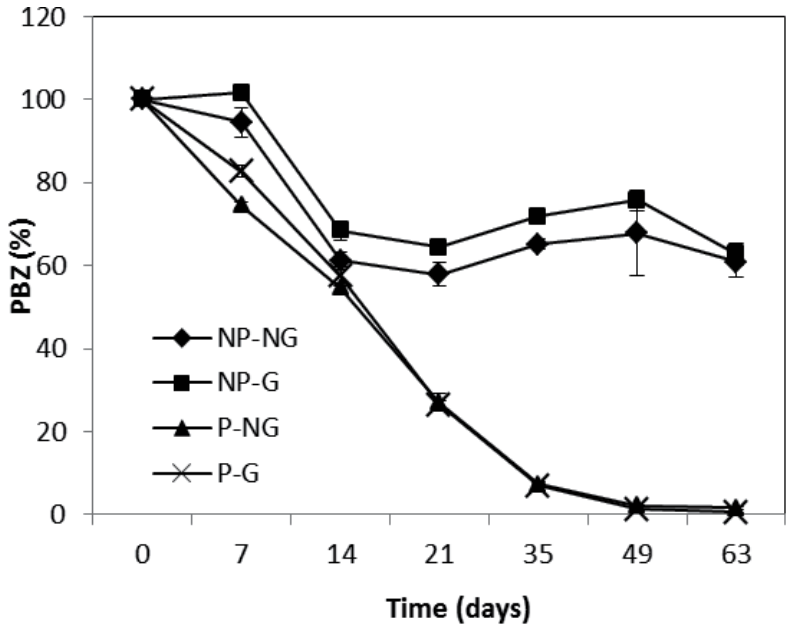


Figure 6. PBZ residual over 63 days.

The PBZ residue in soil without application history of PBZ, containing glycerol (NP-G) or not (NP-NG), was approximately 64% at 14 days. The lower biodegradation rate in NP-G and NP-NG was due to the microbial not being adapted to PBZ since this soil had no history of application.

The biodegradation in NP-G or NP-NG soils was clearly lower than that in P-G or P-NG soils. Biodegradation was not significantly different up to 49 days for experiments in NP-G and NP-NG soils. Similarly, in soil P-G and P-NG soils, biodegradation was not significantly different up to 14 days. Maximum biodegradation occurred in soil with a PBZ application history within 63 days, regardless of the presence of glycerol. This was probably due to the low concentration of glycerol added. Growth was similar; regardless of the addition of glycerol, comparing NP-G with NP-NG soils and P-G- with P-NG soils. However, with respect to the soil with and without history, there was higher growth to soil with history (P-G and P-NG).

The PBZ biodegradation kinetics modeling in the experiments without history (NP), but with (G) and without (NG) glycerol (Figure 7) was similar and presented the highest fit following a double first-order equation. PBZ was consumed at a rate k_1 of 0.0894 and 0.1028 (Table 4), respectively, in experiments with and without glycerol [35].

The kinetic modeling for the experiments with history (P) and with and without glycerol (G, NG) differed greatly from experiments without history (NP) (Figure 8). Both followed a first-order kinetics, where the PBZ was degraded at a constant k rate of 0.0573 and 0.0538 (Table 4), respectively.

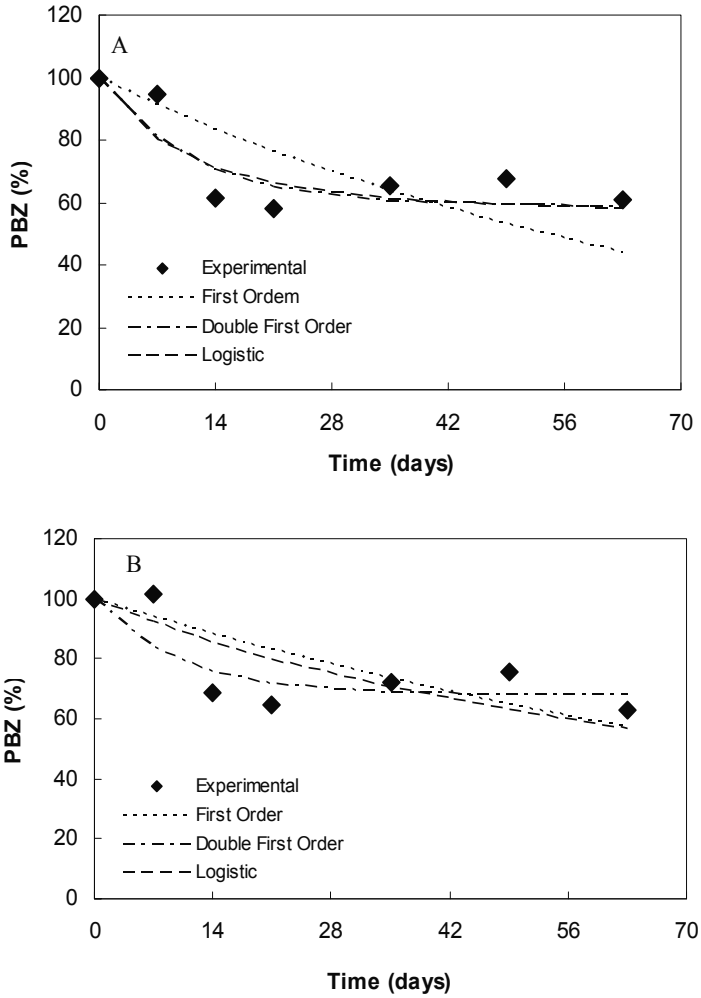


Figure 7. Modeling kinetics of soil without history with and without glycerol. (A) Without glycerol; (B) with glycerol.

Soils	C_0 (%)	First order		Double first order				Logistics		
		K	R	f	k_1	k_2	R	K	k	R
NP-NG	100	0.013	0.62	0.42	0.089	7.12×10^{-11}	0.89	5.76×10^{1}	5.84×10^{-2}	0.88
NP-G	100	0.009	0.51	0.32	0.103	2.69×10^{-11}	0.81	5.00×10^{-4}	6.09×10^{-8}	0.60
P-NG	100	0.057	0.99	1.60	0.057	5.72×10^{-2}	0.99	9.00×10^{-4}	9.44×10^{-7}	0.95
P-G	100	0.054	0.98	1.00	0.054	5.35×10^{-2}	0.98	3.77×10^{-5}	3.72×10^{-8}	0.93

Table 4. Constants and kinetic parameters of the biodegradation of paclobutrazol; NP-NG: soil without history and without glycerol; NP-G: soil without history and with glycerol; P-NG: soil with history and without glycerol; P-G: soil with history and with glycerol.

http://dx.doi.org/10.5772/60818

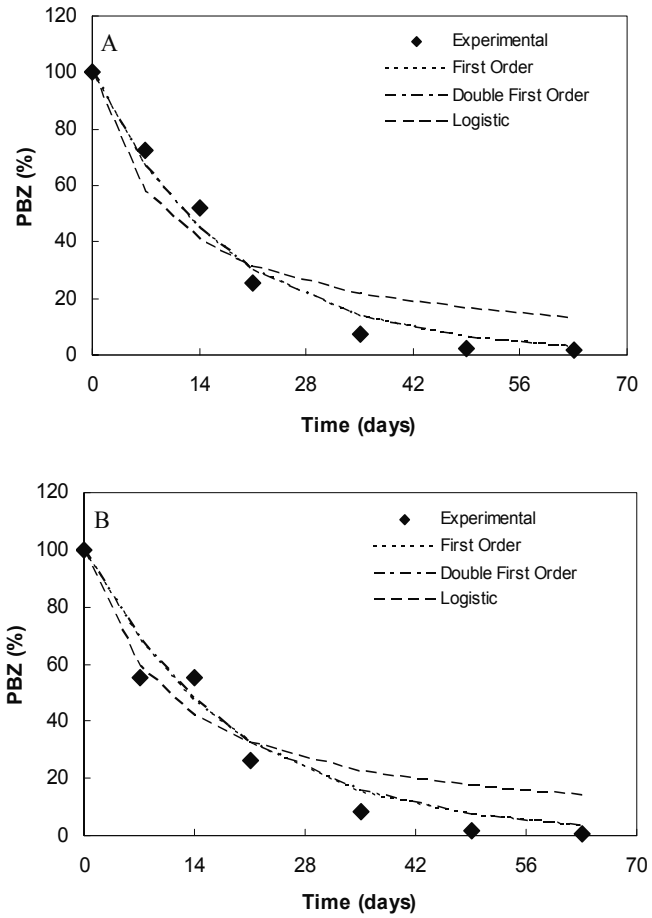


Figure 8. Modeling kinetics of soil with history with and without glycerol. (A) Without glycerol; (B) with glycerol.

Vaz et al. [36] obtained excellent fits using double first-order kinetic and logistic models in sterile soil and with addition of *Pseudomonas* spp., isolated from soil with no history. Mathematical models can help to identify high levels of toxic substances in soil or fruits of plants treated with pesticides and indicate that such substances are able to be systematically monitored.

Paclobutrazol has been shown to be efficient in treating mango trees in semiarid conditions [9]. Because it needs to be applied directly into the soil, it is inconvenient since it remains and affects future planting. Further, it is difficult to determine the dosage for each future use when only empirical methods are used, as there may remain residue from the previous cycle of application [8].

No quantification is done, nor is it always taken into consideration when deciding the dose. Thus, the amount of paclobutrazol applied to the soil is not always appropriate, and risks of

using doses above the recommended are great. The inflorescences on trees treated with high doses are very compact [5], creating suitable conditions for the incidence of diseases and pests, whose control is also hampered by the format of the panicles. Besides the phytosanitary problems, excessive doses of PBZ can inhibit vegetative and floral sprouting longer than desirable, requiring nitrate sprays to stimulate flowering. Thus, in addition to increasing the cost of crop production, for all the reasons that have been mentioned, there is accumulation of chemicals in the ground making the long-term consequences for the production system unknown.

3.4. Paclobutrazol biodegradation in unsaturated soils with a history of application—Effect of bioaugmentation and biostimulation

PBZ biodegradation under the conditions of 2^4 factorial design is shown in Figure 9, where it is possible to observe that biodegradation occurred under all conditions. Less biodegradation was obtained in runs 1 and 9. In these runs, glycerol, the mineral medium, or inoculum had not been added (control experiments). The biodegradation was possible probably due to action of the native microbiota in the A and B soils since these soils had an application history [35].

Biodegradation was 79% and 60%, when only glycerol was added, in runs 2 and 10, respectively. However, biodegradation was about 85% when glycerol and the mineral medium were added. The combination of bioaugmentation and biostimulation might be another promising way to speed up the biodegradation of recalcitrant compounds. On the other hand, biodegradation reached 94% (runs 3 and 11) with the addition of the mineral medium only. The lack of energy sources or electron acceptors or a lack of stimulation of the metabolic pathways responsible for degradation can inhibit or delay the bioremediation [17, 35].

In runs 5 and 13, biodegradation reached only 38% and 29%, respectively. In these runs, only the bacterial consortium was added. In runs with biostimulation and bioaugmentation simultaneous (runs 6, 7, 8, 14, 15 and 16), a high level of biodegradation was achieved. Values varied between 81% and 96%. Vaz et al. [37] studied the biodegradation of PBZ in two soils under saturation conditions. A maximum value of 70% biodegradation within 28 days was found in experiments where glycerol and the three strains of *Pseudomonas* spp. were used. In the present study, higher values were found probably due to using soils with history of application.

Glycerol (X_1), mineral medium (X_2), and inoculum (X_3) were significant factors in the biodegradation (Figure 10). The addition of glycerol, mineral medium, and inoculum increased the biodegradation, regardless of soil used. However, higher biodegradation values (94% to 96% biodegradation for the runs 11, 14, 15, and 16) were found in Soil B. In these runs, biostimulation was applied by the addition of glycerol and/or the mineral medium. Among the main factors, the most significant was the mineral medium followed by glycerol and inoculum. Both soils (A and B) used in research were different with respect to the percentages of sand, silt, and clay. The factorial design, however, did not differentiate significantly among these soil types.

In relation to the factors of interaction, only those factors involving the mineral medium (X_2) with glycerol (X_1) or the inoculum (X_3): X_1X_2 and X_2X_3, respectively, were significant. These

http://dx.doi.org/10.5772/60818

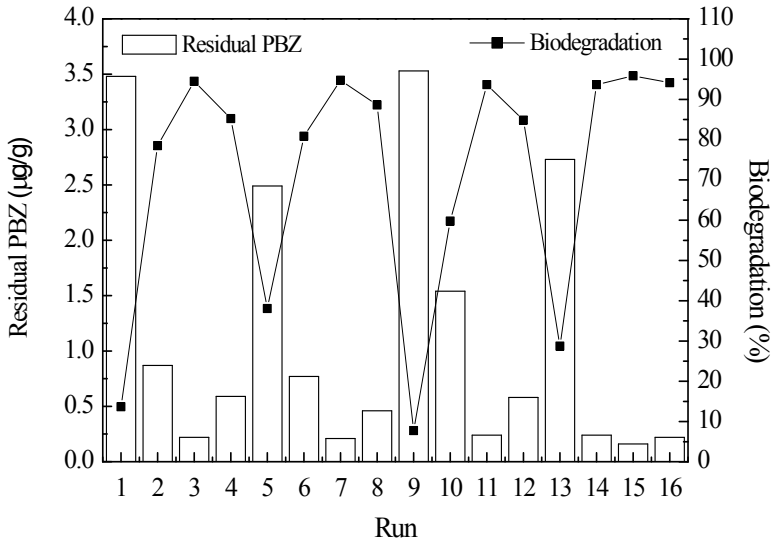


Figure 9. Biodegradation and PBZ residual for each run of factorial design.

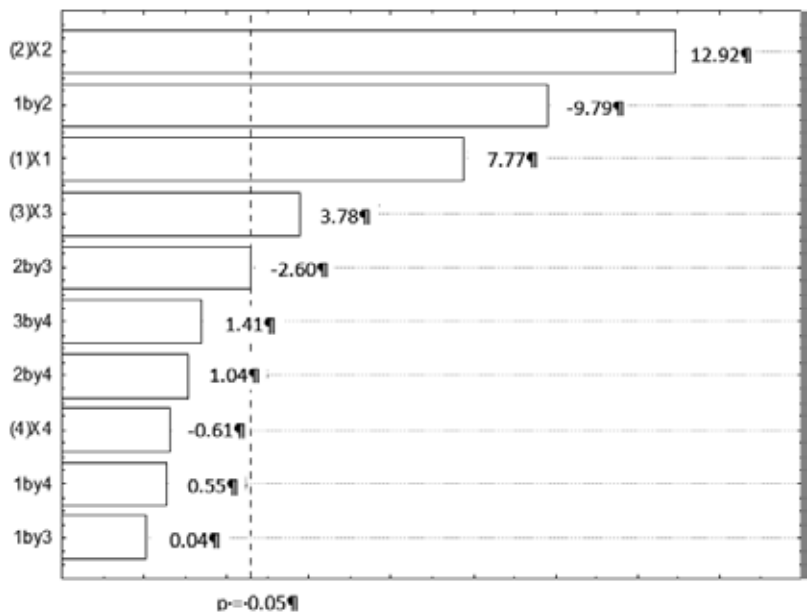


Figure 10. Analysis of significance of independent factors presented as standardized Pareto charts of biodegradation.

effects were negative, indicating that the addition of the mineral medium with glycerol or the inoculum did not favor the biodegradation of paclobutrazol. The addition of glycerol and mineral medium was more significant (–31.31) than the addition of the mineral medium and inoculum (–8.31).

By applying multiple regression analysis on the experimental data, a polynomial model in coded unit explains the role of each factor and its second-order interactions (Equation 2). The negative and positive signs of regression coefficients indicate the antagonistic effect and synergistic effect of each variable, respectively:

$$Y = 70.78 + 12.42X_1 + 20.65X_2 + 6.04X_3 - 15.66X_1X_2 - 4.16X_2X_3 \quad (2)$$

where Y is biodegradation (%) and X_i is level factor in coded unit (+1 or –1), i = 1–4 for four factors. Equation (2) demonstrates that glycerol (X_1), the mineral medium (X_2), and the inoculum (X_3) were responsible for the biodegradation observed. There are only two significant interaction effects (X_1X_2 and X_2X_3). This indicates the additional synergistic effect of these factors. The analysis of variance (ANOVA) was applied to the experimental data and simulated by the empirical model data. The F test was calculated as the ratio between the mean square of regression and the residual mean square. The high value of F (137.63) test and the low p value (0.012405) indicated the significance of the regression.

Figure 11 shows the Log UFCg^{-1} observed in all runs of the factorial design 24 at zero and 40 days. The Log UFCg^{-1} decreased in runs 1 and 9 due to depletion of nutrients in the soils since only sterilized water was added (control runs). In runs 5, 6, 7, 8, 13, 14, 15, and 16, there was also a decrease in the Log UFCg^{-1} since in these runs bioaugmentation was performed and probably the nutrients were not sufficient. On the other hand, in the runs with the addition of glycerol and/or the mineral medium (2, 3, 4, 10, 11, and 12), there was an increase in Log UFCg^{-1}, independent of the soil used.

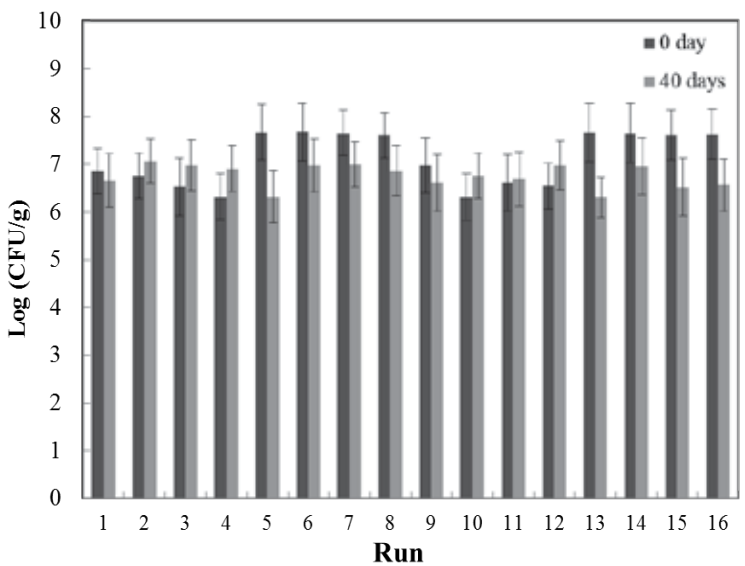


Figure 11. Colony forming unity in each run of factorial design.

http://dx.doi.org/10.5772/60818

3.5. Characterization of samples of biodegradation using FTIR spectroscopy

Figures 12 and 13 show an infrared analysis of PBZ dissolved in distilled water and only methanol, respectively. The solvent used in the preparation of samples was methanol. Comparing the spectra of PBZ and methanol, there is a band at 1650 cm^{-1} only for spectra of PBZ.

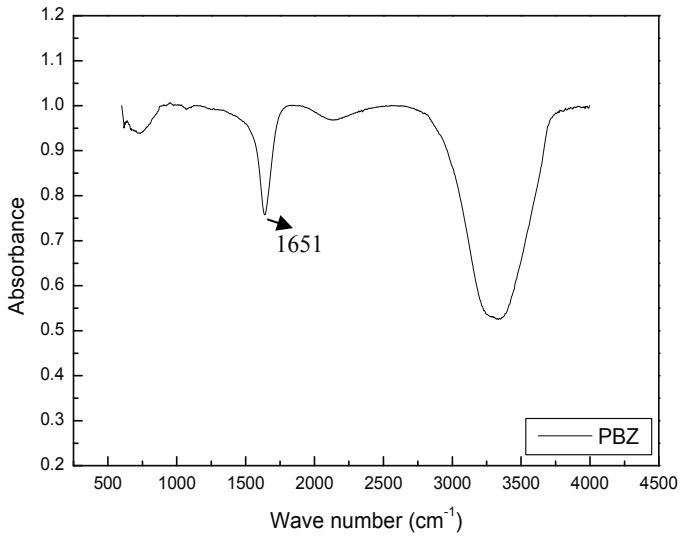


Figure 12. Fourier transformation infrared (FTIR) analysis of PBZ dissolved in distilled water.

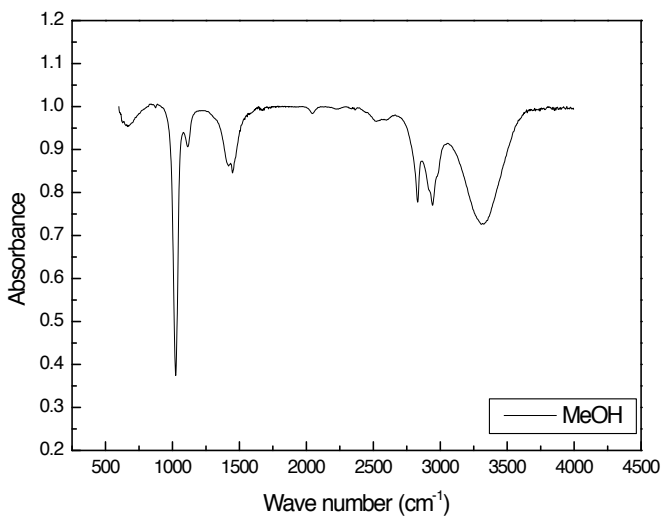


Figure 13. Fourier transformation infrared (FTIR) analysis of methanol.

Samples of soil E1 (only PBZ) and E2 (PBZ and glycerol) 0th day and 70th day of incubation are shown in Figures 14 and 15. A comparison of FTIR spectra of samples after biodegradation (70th day) and before biodegradation (zero day) revealed the lack of the band at 1650 cm^{-1} corresponding to C=C and C=N stretching in the benzene and 1,2,4-triazole rings, respectively, which are observed in the structure of paclobutrazol (Figure 1). Therefore, examination of this particular band confirmed the reduction of paclobutrazol concentration in samples soils after 70 days of biodegradation.

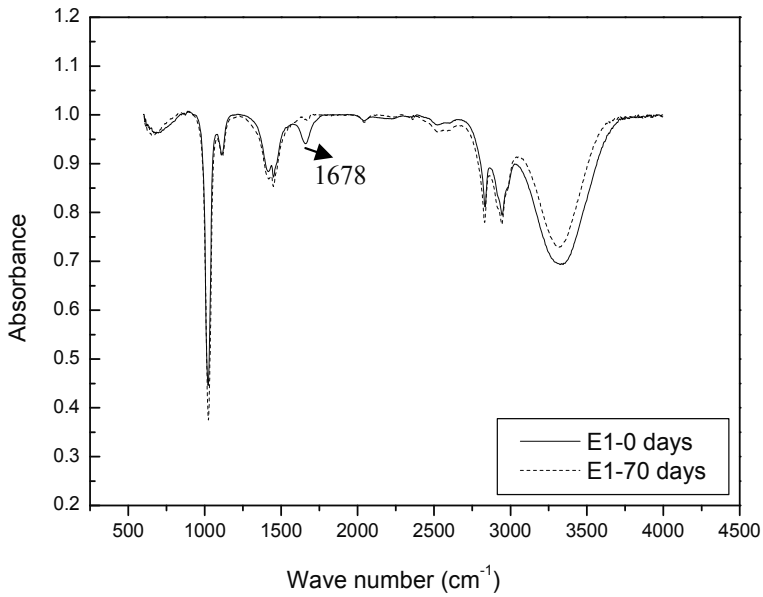


Figure 14. Fourier transformation infrared (FTIR) analysis of metabolites extracted with methanol before biodegradation of PBZ in experiments without (A) glycerol as carbon source additional.

Jackson et al. [33] observed 79% biodegradation after 39 days of incubation, possibly due mineralization of the [^{14}C]-label to CO_2. Since the [^{14}C]-label was located in the chlorobenzene ring of paclobutrazol, the observed loss of labeled carbon indicated some degree of degradation of this functional group by the *Pseudomonas* isolate. These authors concluded that PBZ is at least partly degradable by bacteria (*Pseudomonas* and *Alcaligenes*) in pure culture, with the chlorobenzene ring being catabolized but the 1,2,4-triazole ring was found to be resistant to attack.

3.6. Phytotoxicity studies

Phytoxicity was evaluated based on the germination index, shown in Table 5. According Paradelo et al. [38], phytoxicity between 50% and 80% is considered moderate, while above 80% is absent. These results indicated that the concentration of PBZ (4 μg/g) used is not toxic since index of germination was 83.1%. On the other hand, when was used in PBZ and glycerol, this index decreased at approximately 3%. The metabolites produced during biodegradation

http://dx.doi.org/10.5772/60818

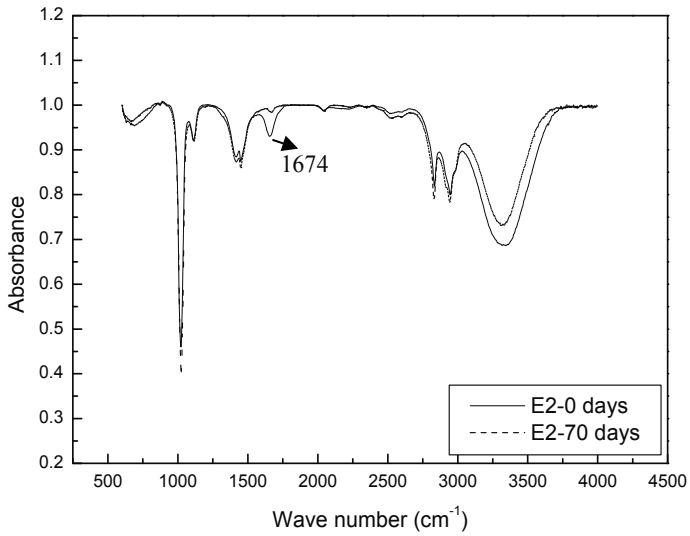


Figure 15. Fourier transformation infrared (FTIR) analysis of metabolites extracted with methanol after the biodegradation of PBZ in experiments with glycerol as carbon source additional.

did not show phytotoxicity and increased index of germination, independently of the addition of glycerol. However, higher increase was observed without addition of glycerol.

Sample	Germination index (%)
Soil before biodegradation experiments (with PBZ 4 μg/g)	83.1
Soil before biodegradation experiments (with PBZ 4 μg/g and glycerol 2.4 mg/g)	80.7
Extracted metabolites after biodegradation experiments in the soil with PBZ	96.6
Extracted metabolites after biodegradation experiments in the soil with PBZ and glycerol	85.2

Table 5. Phytotoxicity studies of PBZ and metabolites produced after biodegradation (70 days) in experiments with unsaturated soil with and without addition of glycerol.

4. Conclusions

The *Pseudomonas* isolated presents a great potential of paclobutrazol biodegradation. The bacterial growth and the paclobutrazol biodegradation were higher in the experiments using paclobutrazol and glycerol as carbon sources. These results indicate that glycerol can be considered a carbon source that stimulates the growth and it does not inhibit the paclobutrazol degradation by *Pseudomonas* spp.

The biodegradation of PBZ in unsaturated soils was more efficient when soil samples with a history of application of PBZ were used. We concluded that this soil bacterium is better adapted

for the degradation of the compound. Mathematical models can help to identify high levels of toxic substances in soil treated with pesticides and indicate that such substances should be systematically monitored.

Soils microorganisms were able to degrade PBZ (control experiments: 1 and 2 of the factorial design), but only with a low increase in biodegradation (<25%). Simultaneous bioaugmentation and biostimulation is not the best strategy. The highest number of applications of PBZ favored biodegradation.

FTIR spectra indicate the biodegradation of PBZ aromatic rings. This probably happens because of the biodiversity of the microbiotics in the soil. This is different from research undertaken with a culture immersed in a mineral medium and a mixed *Pseudonomas* culture, where only benzene chlorate has been degraded. Concentrations of 4 μg/g of PBZ and 2.4 mg/g of glycerol were not phytotoxic, and the biodegradation products increased the germination index.

Acknowledgements

The authors acknowledge the financial support from Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Author details

Fernanda Vaz[1], Ednaldo Santos-Filho[1], Suzyane Silva[1], Silvany Araújo[2], Thatiana Stamford-Arnaud[1], Andrea Bandeira[1], Ana Cristina Brasileiro-Vidal[2], Newton Pereira Stamford[3], Maria Aparecida Mouco[4] and Ester Gouveia[1*]

*Address all correspondence to: estergouveia@gmail.com

1 Department of Antibiotics, Federal University of Pernambuco, Recife-PE, Brazil

2 Department of Genetics, Federal University of Pernambuco, Recife-PE, Brazil

3 Department of Agronomy, Rural Federal University of Pernambuco, Recife-PE, Brazil

4 Brazilian Agricultural Research Corporation, Petrolina-PE, Brazil

References

[1] Agrianual São Paulo: FNP 2014, p. 334.

[2] ANUÁRIO Brasileiro de Fruticultura. Santa Cruz do Sul: Gazeta Santa Cruz; 2013, p. 136.

[3] Lacerda M. P. Manga. Hortifruti Brasil, Piracicaba 2008; 6 (68): 22.

[4] Silva P. C. G., Correia R. C. Sócio economia. Cultivo da Mangueira. Embrapa: Semi-Árido, Petrolina; 2004.

[5] Mouco M. A. do C., Ono E. O., Rodrigues J. D. Plant regulators on vegetative growth of 'Tommy Atkins' mangoes. Acta Horticulturae, Leuven 2013; 992: 187–192.

[6] Tongumpai P., Jutamanee K., Subhadharabanghu S. Effect of paclobutrazol on flowering of mango cv Khiew Sawoey. Acta Horticulturae 1996; 291: 67–70.

[7] Davenport T. L. Reproductive physiology of mango. Brazilian Journal of Plant Physiology 2007; 19(4): 363–376.

[8] Rademacher W., Spinelli F., Costa G. Prohexadione-Ca: modes of action of a multifuncional plant bioregulator for fruit trees. Acta Horticulturae 2006; 727: 97–106.

[9] Mouco M. A. C., Albuquerque J. A. S. Paclobutrazol effect at two mango production cycles. Bragantia 2005; 64: 219–225.

[10] Ferrari D. F., Sergent, E. A. Promoción de la floración y frutificación en mango (*Mangifera indica* L.) cv. Haden, con paclobutrazol. Revista de la Faculdade de Agronomia, Maracay 1996; 22: 9–17.

[11] Yeshitela T., Robbertse P. J., Stassen P. J. C. Effects of various inductive periods and chemicals on flowering and vegetative growth of 'Tommy Atkins' and 'Keitt' mango (*Mangifera indica* L.) cultivars. N Z J Crop Hort Sci 2004; 32: 209–215.

[12] Coelho E. F., Batista L. S., Alves A. A. C. Flowering and fruit set of mango in different doses of paclobutrazol (PBZ). Enciclopédia Biosfera, Centro Científico Conhecer, Goiânia 2014; 10 (19): 1117–1123.

[13] Singh V.K, Bhattacherjee A. K. Genotypic response of mango yield to persistence of paclobutrazol in soil. Scientia Horticulturae 2005; 101: 53–59.

[14] Silva C. M. M. S., Fay E. F., Vieira R. F. Paclobutrazol degradation in tropical soils. Pesquisa Agropecuária Brasileira 2003; 38 (10): 1223–1227.

[15] Ganesh-Kumar S., Solomon R. D. J., Kalimuthu K., Vimalan J. Evidence of aerobic degradation of polychlorinated biphenyl as growth substrate by new bacteria *Stenotrophomonas* sp. Jsg1 and *Cupriavidus taiwanensis* Jsg2. Carpathian Journal of Earth Environmental Science 2010; 5 (2): 169–176.

[16] Kalimuthu K., Solomon R. D. J., Ganesh-Kumar S., Vimalan J. Reductive dechlorination of perchloroethylene by *Bacillus* sp. Jsk1 isolated from dry cleaning industrial sludge. Carpathian Journal of Earth Environmental Science 2011; 6 (1): 165–170.

[17] Kanissery R. G., Sims G. K. Biostimulation for the enhanced degradation of herbicides in soil. Applied and Environmental Soil Science 2011; 1–10.

[18] Li X. J., Lin X., Li P. J., Liu W., Wang L., Ma F., Chukwuka K. S. Biodegradation of the low concentration of polycyclic aromatic hydrocarbons in soil by microbial consortium during incubation. Journal of Hazardous Materials 2009; 172: 601–605.

[19] Lorenzo V. Systems biology approaches to bioremediation. Current Opinion in Biotechnology 2008; 19 (6): 579–589.

[20] Nikolopoulou M., Kalogerakis N. Biostimulation strategies for fresh and chronically polluted marine environment with petroleum hydrocarbons. Journal of Chemical Technology and Biotechnology 2009; 84: 802–807.

[21] Couto M. P. F. S., Monteiro F., Vasconcelos T. S. D. Mesocosm trials of bioremediation of contaminated soil of a petroleum refinery: comparison of natural attenuation, biostimulation and bioaugmentation. Environmental Science and Pollution Research 2010; 17 (7): 1339–1346.

[22] Tyagi M., Fonseca M. M. R., Carvalho C. C. C. R. Bio-augmentation and bio-stimulation strategies to improve the effectiveness of bioremediation processes. Biodegradation 2011; 22: 231–241.

[23] Ridgway H. F., Safarik J., Phipps D., Carl P., Clark D. Identification and catabolic activity of well-derived gasoline-degrading bacteria from a contaminated aquifer. Applied and Environmental Microbiology 1990; 56: 3565–3575.

[24] Kado C. I., Heskett M. G. Selective Media for Isolation of *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas*, and *Xanthomonas*. Phytopathology 1970; 60: 969–976.

[25] King E. O., Ward M. K., Raney D. E. Two simple media for the demonstration of pyocyanin and fluorescein. Journal of Laboratory and Clinical Medicine 1954; 44: 301–307.

[26] Ghazali F. M., Rahman R. N. Z. A., Salleh A. B., Basri M. Biodegradation of hydrocarbons in soil by microbial consortium. International Biodeterioration & Biodegradation 2004; 54: 61–67.

[27] Kargi F., Eker S. Kinetics of 2,4-diclorofenol degradation by *Pseudomonas putida* CPI in batch culture. International Biodeterioration & Biodegradation 2005; 55 (1): 25–28.

[28] Lee K., Park J. W., Ahn I. S., Effect of additional carbon source on naphthalene biodegradation by *Pseudomonas putida* G7. Journal of Hazardous Materials 2003; 157–167.

[29] Foster L. J., Kwan B. H., Vancov T. Microbial degradation of organophosphate pesticide, Ethion. FEMS Microbiology Letters 2004; 240: 49–53.

[30] Mcleish R., Essenberg C. Chlorobenzene Graphical Pathway Map 2004. http://umbbd.ahc.umn.edu/cb/cb_image_map.html (accessed 11 november 2005).

[31] Stephens S., Kotharu P. Atrazine Degradation Graphical Pathway Map 1 2002. http://umbbd.ahc.umn.edu/atr/atr_image_map1.html (accessed 11 November 2005).

[32] Smith D., Alvey S., Crowley D. E. Cooperative catabolic pathways within an atrazine-degrading enrichment culture isolated from soil. FEMS Microbiology Ecology 2005; 53 (2): 265–273.

[33] Jackson M. J., Line M. A., Hasan O. Microbial degradation of a recalcitrant plant growth retardant—paclobutrazol (PP333). Soil Biology & Biochemistry 1996; 28 (9): 1265–1267.

[34] Abdelrahman H. Biodegradation of some pesticides by soil microorganisms. Master thesis. Zagazig University; 2004.

[35] Vaz F. L., Gomes T. A., Rocha C. C. A., Mouco M. A., Gouveia E. R. Paclobutrazol biodegradation in unsaturated soil in the semiarid northeast of Brazil. African Journal of Biotechnology 2015, 14: 298–303.

[36] Vaz F. L., Netto A. M., Antonino A. C. D., Afonso A. C. M, Martins J. M. F., Gouveia E. R. Modeling of the kinetics biodegradation of paclobutrazol in two soils of the semiarid northeast Brazil. Química Nova, 2012, 35: 77–81.

[37] Vaz F. L., Netto A. M., Antonino A. C. D., Gouveia E. R., Martins J. M. F. Biodegradation of paclobutrazol in saturated soil systems by *Pseudomonas* spp. Química Nova 2012; 35: 1090–1096.

[38] Paradelo R., Moldes, A. B., Rodriguez M., Barral M. T. Relationship between heavy metals and phytotoxicity in composts. Food Science and Technology 2008; 6 (2): 143–151.

Chapter 6

Biodegradation of Aromatic Compounds

Mehdi Hassanshahian, Moslem Abarian and Simone Cappello

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60894

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous persistent environmental contaminants generated by natural combustion processes and human activities. PAHs are considered hazardous because of cytotoxic, mutagenic, and carcinogenic effects. Sixteen individual PAH compounds have been identified as priority pollutants by the United States Environmental Protection Agency (U.S. EPA). All substances originated in to the environment by either biogenic or anthropogenic sources. Anthropogenic compounds describe synthetic compounds, and compound classes as well as elements and naturally occurring chemical entities which are mobilized by man's activities. In the marine environment, the fate of pollutants is largely determined by biogeochemical process. Some of these chemical changes enhance the toxicity of the pollutants. Other chemical changes cause the degradation or immobilization of pollutants and, as a result, act to purify the waters. Possible fates for PAHs, released into the environment, include volatilization, photo-oxidation, chemical oxidation, bioaccumulation and adsorption on soil particles, leaching, and microbial degradation. Elevated concentrations of polycyclic aromatic hydrocarbons (PAHs) have been found in mangrove sediments due to anthropogenic compounds.

Pollution and microbial degradation have been suggested as the best way to remove PAHs from contaminated sediments. A significant positive relationship was found between bacterial growth and percentages of aromatic hydrocarbons degradation. The PAH biodegradation ability of the enriched mixed bacterial culture was not related to the degree of PAH contamination in surface sediments. The growth and biodegradation percentages of the enriched mixed culture were not higher that of the individual isolate especially at low salinity (0 and 10 ppt). The principal processes for their successful removal are currently believed to be microbial and bacteria transfor-

mation and degradation. A large variety of bacteria are known, which can utilize PAHs as a sole source of carbon and energy under aerobic and anaerobic conditions. Biodegradation in marine environment was also described attractively. In comparison to their chemically synthesized equivalents, they have many advantages: they are environmentally friendly, biodegradable, less toxic, and nonhazardous. They have better foaming properties and higher selectivity.

Keywords: Bacteria, degradation, polycyclic aromatic hydrocarbons (PAHs), marine

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are the major group of marine contaminants that are made of two or more benzene rings. Polycyclic aromatic hydrocarbons (PAHs) are one of the major categories of pollutants entering the marine environment and finally accumulating in the sediments. Their occurrence raises major concerns for human health, especially during coastal activities (bathing waters, aquaculture, etc.), having combined adverse effects still largely [1]. The solubility of these compounds in the seawater is low, and they tend to be bounded to suspended organic matter in the water column and finally accumulate in the marine sediment [2, 3]. Many researchers have experimentally worked on the biodegradation of PAHs, which involves the use of specific microorganisms, specific reducing culture, metabolic pathways, bioavailability and disappearance, growth of the microbes under aerated and unaerated conditions, pH variation, and kinetics of biodegradation [4]. The bacteria were capable of growing on various hydrocarbons like diesel, petrol, lubricating oil, toluene, naphthalene, and kerosene. The tests were conducted to detect the biodegradation of diesel, by amino-oxygenase biodegradation pathway, and it was observed that diesel was degraded within 12 hours [5].

2. Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a group of approximately 10, 000 compounds that are atmospheric, water, and soil pollutants. They are organic contaminants that are formed from the incomplete combustion of a variety of organic compounds. The structure of a PAH consists of molecules containing two or more fused six-carbon atom aromatic rings; only hydrogen and carbon are present in the molecules. There are 18 PAHs considered by the Agency for Toxic Substances and Disease Registry that have adverse health effects: acenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[a]pyrene, benzo[e]-pyrene, benzo[b] fluoranthene, benzo[*ghi*]perylene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, chrysene, coronene, dibenz(a, h)anthracene, fluoranthene, fluorene, indeno(1, 2, 3-cd)pyrene, phenanthrene, and pyrene[6, 7]. Figure [1] shows some PAHs.

http://dx.doi.org/10.5772/60894

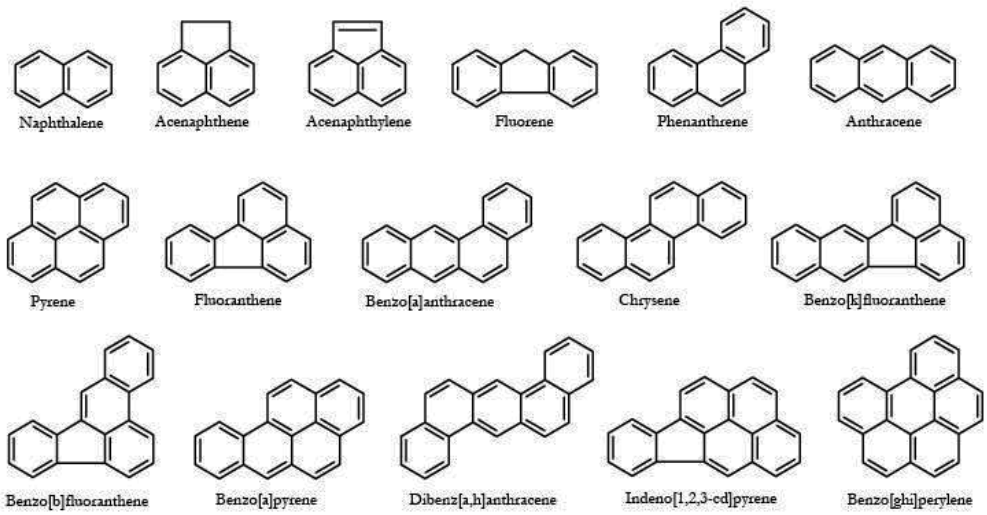


Figure 1. Structures of U.S. EPA's 16 priority pollutant PAH [1].

2.1. Phenanthrene (Phe), pyrene (Pyr), and benzo[e]pyrene (BeP)

The most PAHs in oil products have two and three fused benzene rings with one to four carbon atom alkyl group substitutions. Naphthalene, the two-benzene-ring PAH, often present in significant amounts in petroleum, is relatively volatile, soluble, and degradable. Therefore, naphthalene in weathered oil from an offshore spill may not be available when the contaminated water reaches coastal environments; on the other hand, higher molecular weight species such as phenanthrene (three-benzene-ring PAH), pyrene (four-benzene-ring PAH), and benzo[e]pyrene (five-benzene-ring PAH) can be found in weathered crude oils reaching coastal wetlands. The higher the number of benzene-rings the PAH has, the higher the molecular weight, but the lower the solubility in water [17, 29].

3. Sources of PAHs

The sources of PAHs in a marine environment could include volcanoes, natural fires, fossil fuel fired power generation, combustion engines, municipal discharges, oil pipeline spills, oil fields, offshore drilling platforms, natural oil seeps, and shipping accidents [6]. The sources of PAH are illustrated in Figure 2.

3.1. PAHs in water

The main sources of PAHs in water bodies are atmospheric particulate matter deposition, runoff of polluted ground sources, and pollution of river and lakes by industrial effluents, municipal wastewater discharge, and oil spills [14]. Since PAHs have low solubility and tend

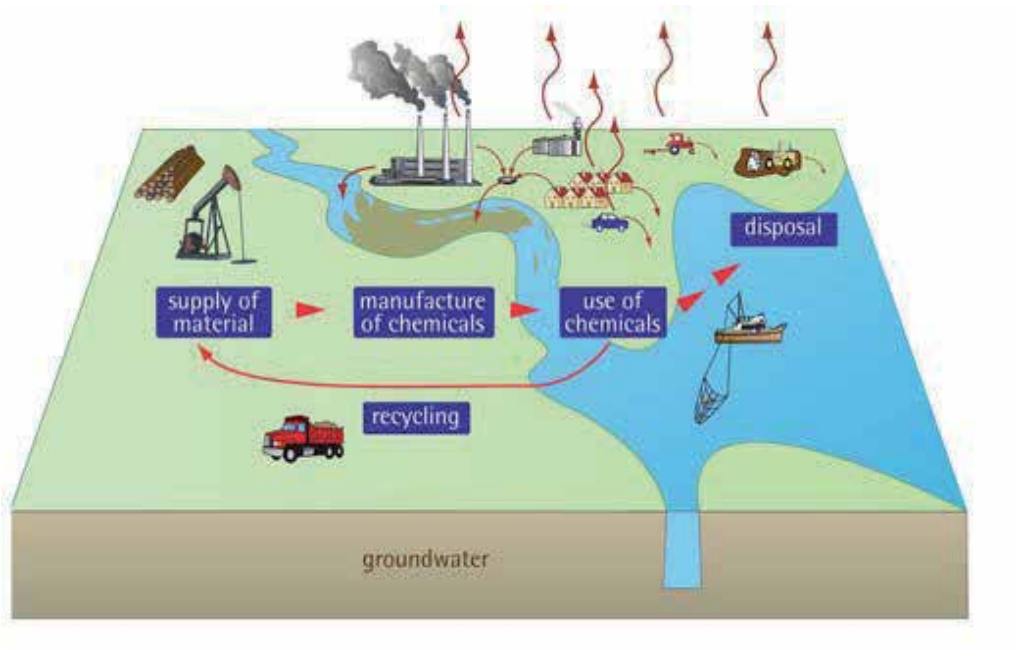


Figure 2. Diagram of the transfer of PAHs in the environment [24].

to adsorb to particulate matter, they are usually found in low concentrations in water bodies. Some PAH concentrations that have been measured in water include marine waters with levels of nondetected to 11 g L^{-1} wastewater in North American and European municipalities with levels of <1 to 625 g L^{-1} and urban runoff in the U.S. with levels of <0.05 to 560 g L^{-1} [14, 15].

3.2. Sources of PAHs in coastal environments and marine

PAHs in coastal environments can come from many sources naturally as well as through anthropogenic activity. They adsorb and tend to stay longer in sediment due to their hydrophobicity (low solubility and high octanol-water partition coefficient). The main sources of PAHs in a marine ecosystem are atmospheric deposition, river runoff, domestic and industrial wastewater, and direct spillage of oil or petroleum products [16]. Figure 3 shows the source of PAH in the marine environment.

4. PAHs biodegradation

PAHs are toxic to marine species and to the environment. As they are absorbed into soil/sediment, many hydrophobic PAHs in soil-water interfaces undergo some physical, chemical, and biological degradation, but biodegradation is the most effective process. Microorganisms utilize only dissolved substrates; and the utilization rates of PAH degradation products are related to sorption/desorption rates of PAHs to and from soil. Since biodegradation is assumed

http://dx.doi.org/10.5772/60894

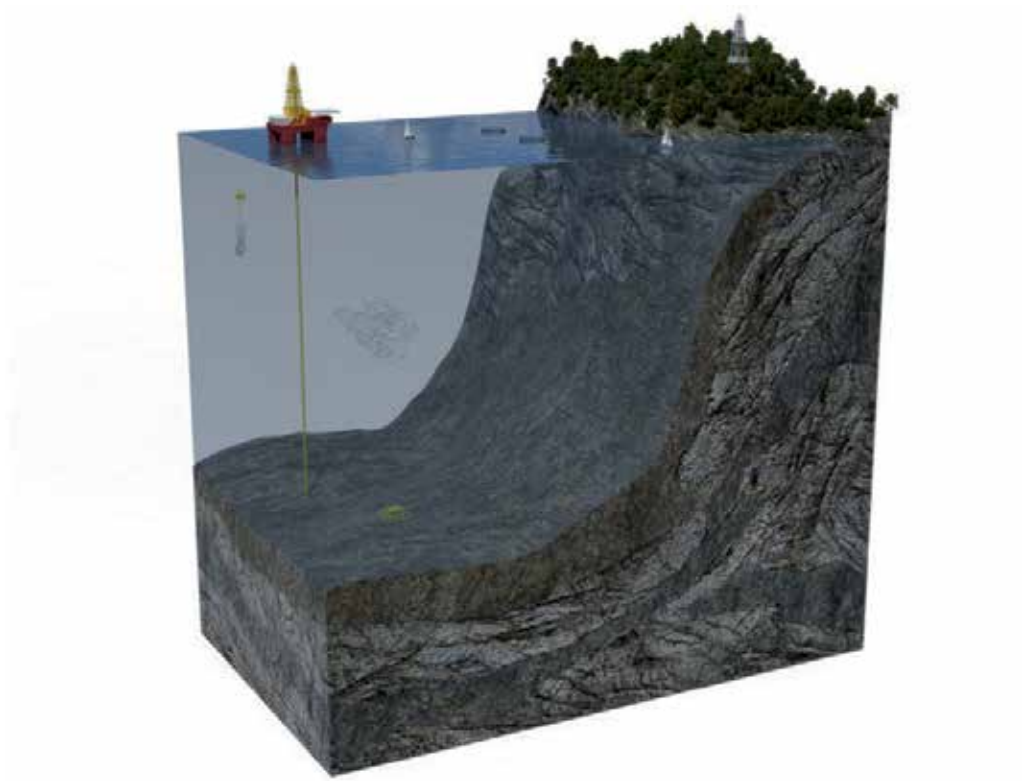


Figure 3. Seeps, oil spills from boats or platforms, and produced water discharge from oil- and gass-producing installations such as the one shown. PAHs in produced water and oil seeps represent a chronic release to the marine enviroments [37]

to be the main reaction in PAH decay in soil, microorganisms require some nutrients to survive and to function. The nutrients in shortest supply are usually nitrogen (N), phosphorus (P), or both, while the supply of K, S, Mg, Ca, Fe, and micronutrient elements is nearly always greater than the demand [16]. Some nutrients can be added to wetland soil and water to enhance the degradation of organic material. The capacity of different wetland soil to retain nutrients may be different. Therefore, it is necessary to investigate PAH degradations in the subtidal and the intertidal wetland soil with the addition of nutrients.

Specific characteristics of coastal wetland ecosystems that make them suitable for PAH biodegradation processes are the large quantity of water, the oxic and anoxic soil for the breakdown of organic matter, and the supporting highly productive, tall emergent vegetation. Some organic compounds degrade favorably under aerobic conditions, such as naphthalene; other compounds degrade favorably under anaerobic conditions, such as DDT; and some others degrade favorably under moderately anaerobic conditions, such as PCBs. Understanding of PAHs degradation pathways and kinetics provides guidance for the selection of effective methods and technology to remediate a contaminated site [18]. The schematic biodegradation of PAHs is described in Figure 4.

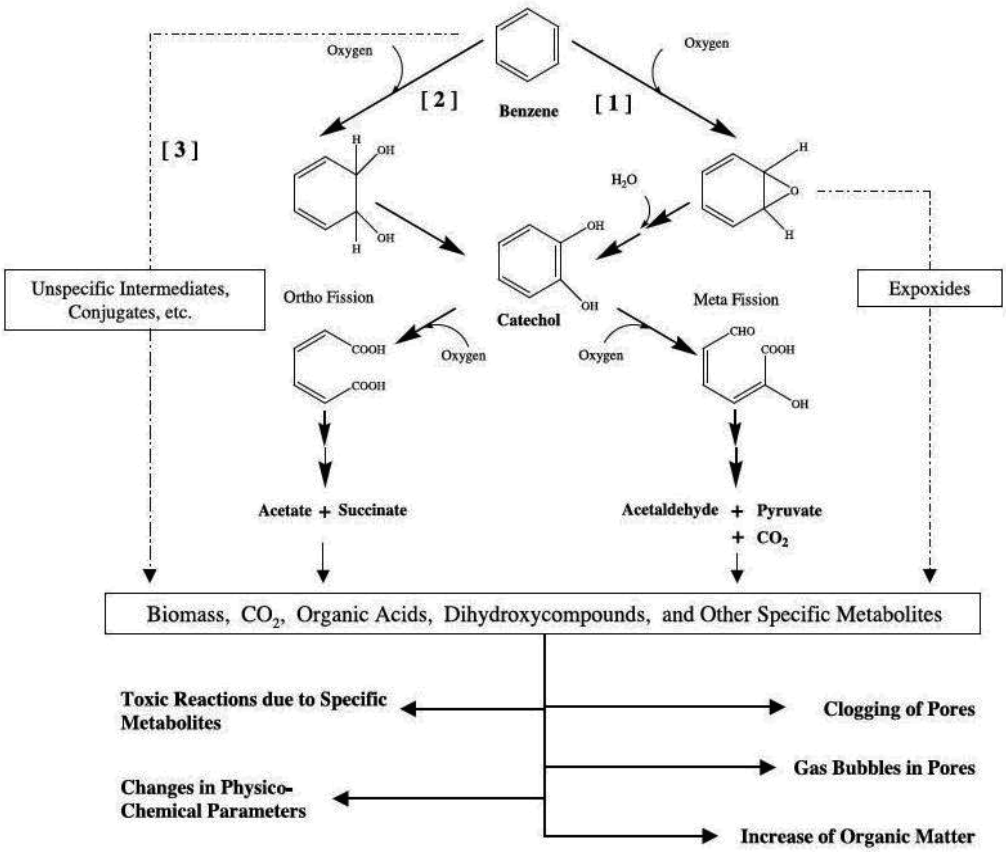


Figure 4. Biodegradation of aromatic hydrocarbons: metabolism begins with the activity of a mono-oxygenase or a di-oxygenase [36].

4.1. Properties PAHs in biodegradation

Pure compounds of PAHs with higher molecular weights are more resistant to biotransformation and pure compounds of PAHs with lower molecular weights transform more rapidly [30]. However, the presence of one PAH can decrease the biodegradation of other PAHs. With the simultaneous presence of phenanthrene, acenaphthene, fluorine, anthracene, pyrene, and benzo[a]pyrene, the biodegradation was decreased for phenanthrene and acenaphtene but was enhanced for fluorine, anthracene, and pyrene (while biodegradation of benzo[a]pyrene did not occur within a 12-day incubation period [31]. There are thousands of PAH compounds in the environment, but in practice, PAH analysis is restricted to the determination of 6 to 16 compounds. Individual PAHs differ substantially in their physical and chemical properties. Generally, the high molecular weight compounds (≥ 4 aromatic rings) are less water soluble, less volatile, and more lipophilic than lower molecular weight ones. The best known model compound from this group is highly

carcinogenic benzo[a]pyrene (BaP). PAHs are listed by the U.S. Environmental Protection Agency and the European Commission as priority pollutants [32].

4.2. PAH-degrading bacteria

PAH degradation has been known for many years to occur in marine sediments. Previous results indicate that culturable hydrocarbon-degrading and PAH-degrading populations are widely distributed and can be enriched from sites of contamination in marine environments [7, 8, 9, 10]. However, other studies indicate that high levels of PAH can be toxic to marine bacteria [11, 12, 13]. Therefore, highly contaminated sites might be inhibitory to PAH-degrading bacteria and other microorganisms.

4.3. Community from the Surface Microlayer (SML) in an estuarine

The sea surface microlayer (SML) represents the interface between the atmosphere and the hydrosphere. Organisms within the SML are known as neuston, and the community of bacteria present within this neuston layer is named bacterioneuston. *Acinetobacter* and *Rhizobium* were also found among the PAH-degrading isolates retrieved from the SML. Many environmental strains of *Acinetobacter* with hydrocarbon-degrading capacities have been isolated in terrestrial and marine environments [19]. The subclass gamma-Proteobacteria was relatively more abundant in bacterioneuston (SML) than in bacterioplankton. The gamma-Proteobacteria subclass includes major PAH-degrading genera, such as *Alcanivorax*, *Cycloclasticus*, *Pseudomonas*, *Oleiphilus*, *Oleispira*, and *Thalassolituus* [20, 21].

4.4. PAH-degrading bacteria in coastal sediment

It is well known that bacterial degradation plays an important role in PAHs removal from the marine environment. Many PAH-degrading bacteria have been found in coastal sediments, including bacteria of *Cycloclasticus*, *Marinobacter*, *Pseudoalteromonas*, *Marinomonas*, *Sphingomonas*, *Vibrio*, and *Halomonas.* However, knowledge about deep-sea environments is relatively less. In previous studies on the deep-sea sediments of Atlantic Ocean and Pacific Ocean, *Cycloclasticus* was the most important bacterium, in addition to *Alteromonas* and *Novosphingobium* [22, 23].

5. Toxicity and the effects of PAHs

PAHs reveal their toxicity following biotransformation to toxic metabolites, which can be bond covalently to cellular macromolecules such as DNA, RNA, and protein. The major PAH-metabolizing enzymes are cytochrome P450 mono-oxygenases, epoxide hydrolase, and several conjugating enzymes [25]. Highly carcinogenic 7, 8- and 9, 10-dihydrodiols are the major PAH metabolites produced by fish microsomes. The metabolites of PAH are mainly conjugated with glucuronic acid. Most conjugates are water-soluble organic anions and are rapidly excreted mostly via the gallbladder or in urine PAHs with high molecular weight, which are not acutely

toxic to fish. However, in the presence of solar ultraviolet radiation, many of them are acutely toxic. Exposure to PAHs causes the suppression of the immune system. A decreased number of melano-macrophage centers in the liver and suppression of proliferative responses of T-lymphocytes [26, 27, 28].

6. Anaerobic biodegradation PAHs

The anaerobic degradation of aromatic hydrocarbons is often presumed to be slow and of minor ecological significance. However, anaerobic biotransformation may play a key role in the transformation of aromatic and PAH compounds when oxygen demand exceeds supply in natural environments. As shown in Figure 5, under such conditions, anoxic or anaerobic degradation mediated by denitrifying or sulfate-reducing bacteria can become a key pathway for the cleanup of contaminated sites [33]. The isolation of denitrifying and sulfate-reducing organisms capable of degrading toluene has led to the elucidation of several biodegradation pathways and phylogenetic relationships between bacterial strains. The isolation of denitrifying and sulfate-reducing organisms capable of degrading toluene has led to the elucidation of several biodegradation pathways and phylogenetic relationships between bacterial strains [34, 35].

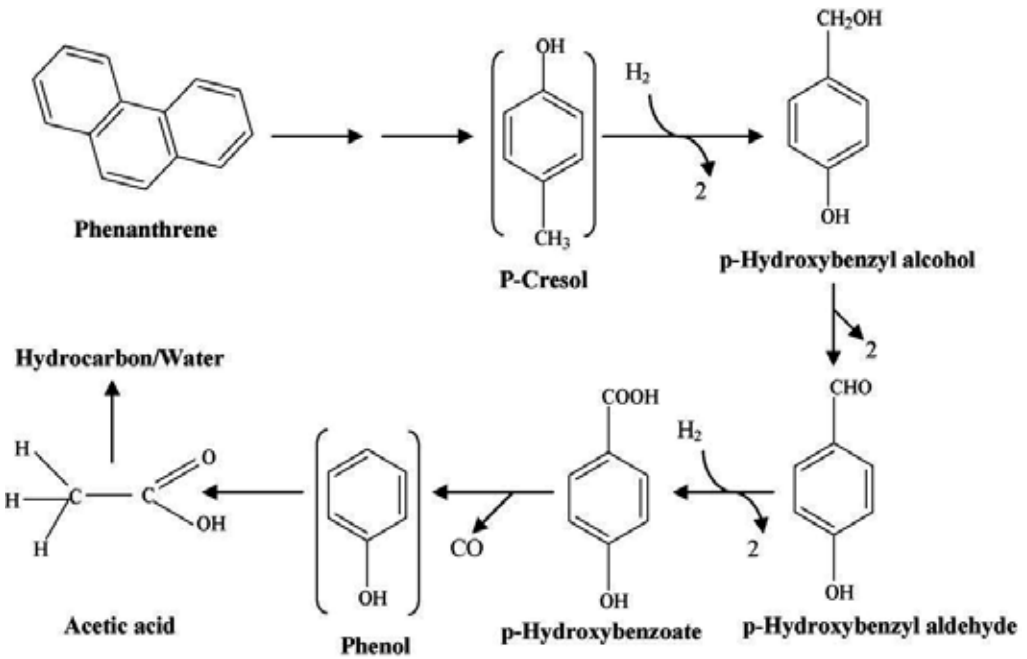


Figure 5. Proposed anaerobic biotransformation pathway of phenanthrene by sulfate-reducing bacteria [38].

http://dx.doi.org/10.5772/60894

7. Aerobic biodegradation

During aerobic degradation by bacteria, PAHs are oxidized to *cis*-dihydrodiols through the incorporation of an oxygen molecule into the PAH. As shown in Figure 6, the *cis*-dihydrodiols are further oxidized to aromatic dihydroxy compounds (catechols), and then PAH rings are cleaved with intracellular dioxygenases. The oxidation of unsubstituted PAHs with very high thermodynamic stability often results in PAH compounds that are less stable than the parent compounds and more susceptible to cleavage [40, 41, 42]. Oxygenase production by bacteria can be increased using biostimulants; for example, salicylic acid is a known inducer of naphthalene dioxygenase. Linoleic acid is a powerful stimulant of pyrene and benzo[a]pyrene degradation by gram-positive bacteria. The addition of humic substances also greatly enhances the microbial degradation of PAHs [43].

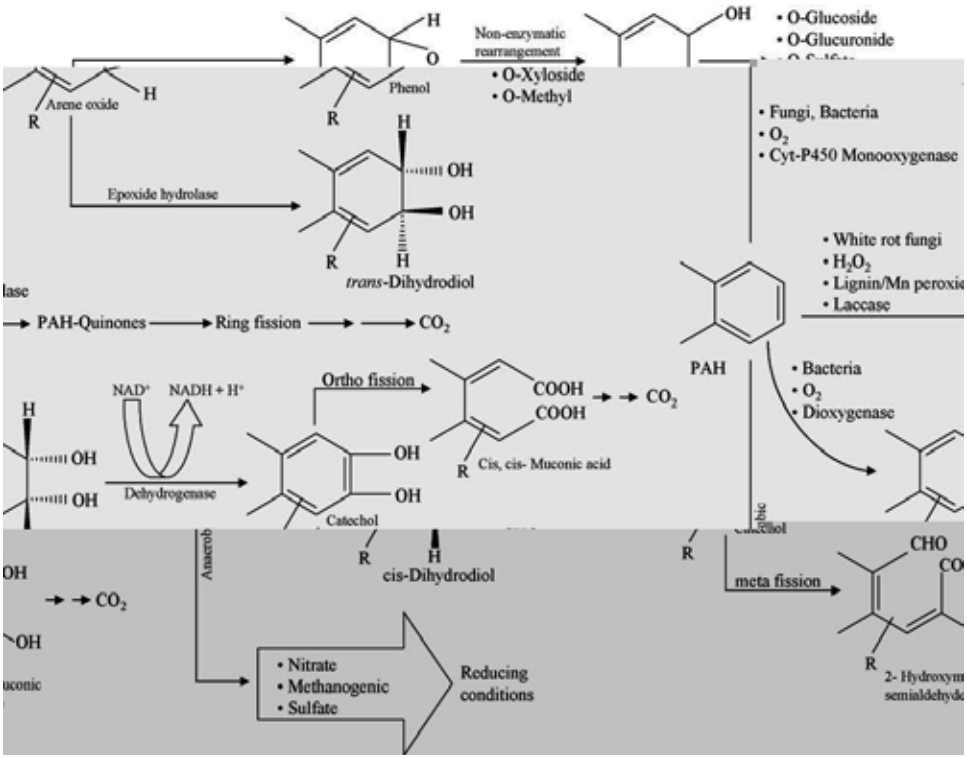


Figure 6. Proposed pathway for microbial catabolism of polycyclic aromatic hydrocarbons [39].

8. Biodegradation of benzo[a]pyrene by marine denitrifying bacteria

Benzo[a]pyrene (BaP) is one of the high molecular weight, 5 ring-PAHs and is listed as one of the priority pollutants classified as a carcinogen by the U.S. Environmental Protection Agency

and the Agency for Toxic Substances and Disease Registry. BaP is ranked number 9 on the priority list of 275 substances. The toxicity of BaP is of high concern because of its ability to accumulate in animal tissues, to cause cancer and hormone disruption, and to affect reproduction. Moreover, BaP was found to depress immune function [44, 45-47]. The principal natural sources of BaP are forest fires, volcanic eruption, pest fires, and burning of crude oil. Anthropogenic sources include the incomplete combustion of coal, oil, gas, wood, rubbish, and other organic substances, incinerator, vehicle exhausts, and cigarette [48]. BaP derived from gasoline automobile accounted for 98% [48, 49]. The natural presence of PAHs in the environment allows many microorganisms to adapt to exploit this naturally occurring as the potential growth substrate. Thus, many bacterial, fungal, and algal strains have been shown to utilize wide varieties of PAHs containing from three to five aromatic rings [50]. The application of microorganisms for bioremediation of PAH-contaminated environment seems to be an attractive technology because they are more specific, effective, economic, and environmental friendly. Biodegradation in marine environment was also described attractively [50, 51]. Denitrification is an important major process; denitrifying bacteria responses to environmental conditions by cycling nitrogen gas back to the atmosphere. These denitrifying bacteria utilize nitrate (NO_3-), nitrite (NO_2-), nitric oxide (NO), and nitrous oxide (N_2O) as terminal electron acceptors in anaerobic respiration. The denitrifying bacteria isolated from marine sediment were *Bacillus subtilis* in majority. *B. subtilis* have the ability to degrade benzo[a]pyrene with a complete degradation efficiency of the initial amount of 200 mg/l supplied over 45 days of incubation at 30°C [52, 53]. *B. subtilis* successfully biodegraded benzo[a]pyrene but failed to increased biomass production [54, 55].

Author details

Mehdi Hassanshahian[1*], Moslem Abarian[1] and Simone Cappello[2]

*Address all correspondence to: mshahi@uk.ac.ir

1 Department of Biology, Faculty of Science, Shahid Bahonar University of Kerman, Kerman, Iran

2 Istituto per l'Ambiente Marino Costiero (IAMC)-CNR of Messina, Messina, Italy

References

[1] Gustafsson, O., N. Nilsson and T.D. Bucheli, 2001. Dynamic colloid-water partitioning of pyrene though a coastal Baltic Spring Bloom. Environmental Science and Technology, 35: 4001-4006.

[2] Baumard, P., H. Buzinski, Q. Michon, P. Garrigues and J. Burgeot Tand Bellocq, 1998. Origin and Bioavailability of PAHs in the Mediterranean Sea from Mussel and Sediment Records. Estuarin Coastal.

[3] Pothuluri, J.V. and C.E. Cerniglia. Current aspects on polycyclic aromatic hydrocarbon degradation processes. In: Sikdar, S.K. and Irvine, R.L., eds. Bioremediation: Principles and Practice Volume III. Lancaster USA: Technomic Publishing Company, Inc., 1998. pp. 461-520.

[4] Masih, A. and A. Taneja, 2006. Polycyclic aromatic hydrocarbons (PAHs) concentrations and related carcinogenic potencies in soil at a semi-arid region of India. Chemosphere, 65: 449-456.

[5] Geetha, S.J., S.J. Joshi and S, Kathrotiya, 2013. Isolation and characterization of hydrocarbon degrading bacterial isolate from oil contaminated sites. APCBEE Procedia, 5: 237-241.

[6] Kennish, M.J., 1996. Practical Handbook of Estuarine and Marine Pollution. CRC Press Inc., Boca Raton, FL, 524 pp.

[7] Foght, J.M., P.M. Fedorak and D.W.S. Westlake, 1990. Mineralization of 14C-hexadecane and 14C-phenanthrene in crude oil: specificity among bacterial isolates. Canadian Journal of Microbiology, 36(3): 169-175.

[8] Roubal, G. and R.M. Atlas, 1978. Distribution of hydrocarbon-utilizing microorganisms and hydrocarbon biodegradation potentials in Alaskan continental shelf areas. Applied and Environmental Microbiology, 35: 897-905.

[9] Shiaris, M.P. and J.J. Cooney, 1983. Replica plating method for estimating phenanthrene-utilizing and phenanthrene-cometabolizing microorganisms. Applied and Environmental Microbiology, 45: 706-710.

[10] West, P.A., G.C. Okpokwasili, P.R. Brayton, D.J. Grimes and R.R. Colwell, 1984. Numerical taxonomy of phenanthrene-degrading bacteria isolated from Chesapeake Bay. Applied and Environmental Microbiology, 48: 988-993.

[11] Calder, J.A. and J.H. Lader, 1976. Effect of dissolved aromatic hydrocarbons on the growth of marine bacteria in batch culture. Applied and Environmental Microbiology, 32: 95-101.

[12] Sikkema, J., J.A.M. de Bont and B. Poolman, 1995. Mechanisms of membrane toxicity of hydrocarbons. Applied and Environmental Microbiology, 59: 201-222.

[13] Tuveson, R.W., J. Kagan, M.A. Shaw, G.M. Moresco, E.M.V. Behne, H. Pu, M. Basin and R. Santus, 1987. Phototoxic effects of fluoranthene, a polycyclic aromatic hydrocarbon, on bacterial species. Environmental and Molecular Mutagenesis, 10: 245-261.

[14] Latimer, J. and J. Zheng, 2003. The sources, transport, and fate of PAH in the marine environment. pp. 9-31. In: P.E.T. Douben, ed. PAHs: An Ecotoxicological Perspective.

[15] Dabestani, R. and I. Ivanov, 1999. A compilation of physical, spectroscopic and photophysical properties of poly aromatic hydrocarbons. Photochemistry and Photobiology, 70: 10-34.

[16] Alexander, M., 1999. Biodegradation and Bioremediation, 2nd ed. Ithaca, NY: Academic Press, 453 pp.

[17] ATSDR, 2005. Toxic substances portal—polycyclic aromatic hydrocarbons (PAHs). Agency for Toxic Substances and Disease Registry U.S. Department of Health and Human Services, Public Health Service. Atlanta, GA.

[18] Reddy, K.R. and R.D. DeLaune, 2008. Biogeochemistry of Wetlands: Science and Applications. Taylor & Francis Group, 774 pp.

[19] Karolien, V., R. Annemie, W. Pierre, M. René and S. Dirk, 2004. *Acinetobacter* diversity in environmental samples assessed by 16S rRNA gene PCR-DGGE fingerprinting. FEMS Microbiology Ecology, 50: 37-50.

[20] Watanabe, K., 2001. Microorganisms relevant to bioremediation. Current Opinion in Biotechnology, 12: 237-241.

[21] Franklin, M.P., I.R. McDonald, D.G. Bourne, N.J. Owens, R.C. Upstill-Goddard and J.C. Murrell, 2005. Bacterial diversity in the bacterioneuston (sea surface microlayer): the bacterioneuston through the looking glass. Environmental Microbiology, 7: 723-736.

[22] Dong, C., X. Bai, H. Sheng, L. Jiao, H. Zhou and Z. Shao, 2014. Distribution of PAHs and the PAH-degrading bacteria in the deep-sea sediments of the high-latitude Arctic Ocean. Biogeosciences Discussion, 11: 13985-14021.

[23] Cui, Z., Lai, Q., Dong, C., and Shao, Z, 2008. Biodiversity of polycyclic aromatic hydrocarbondegrading bacteria from deep sea sediments of the Middle Atlantic Ridge, Environmental Microbiology, 30 10: 2138-2149,.

[24] Abbey, F., Wick Nicholas, W., Haus Beshr, F., Sukkariyah, Kathryn, C and Haering Lee Daniels, W.2011. Remediation of PAH contaminated soils and sediments a literature review. Virginia Polytechnic Institute and State University Department of Crop and Soil Environmental Sciences. 1-102.

[25] Masfaraud, J.F., Pfohl-Leszkowic, A., Malaveille, C., Keith, G., and Monod, G, 1992. 7-Ethylresorufin O-Deethylase Activity and level of DNA-Adduct in Trout Treated with Benzo(a)pyren. Marine Environmental Research. 34:351-354.

[26] Faisal, M., Weeks, B.A., Vogelbien, W.K and Huggett, R.J, 1991. Evidence of aberration of the natural cytotoxic cell activity in *Fandalus heteroclitus* (Pisces:Cyprinodontidae) from the Elizabeth River, Virginia. Veterinary Immunology and Immunopathology, 29(3-4): 339-351.

[27] Myers, M.S., Olson, O.P., Johnson, L.L, Stehr, C.S, Hom, T. and Varanasi, U.1992. Hepatic lesion other than neoplasms subadult flatfish from Puget Sound Vashington:

Relationship with indices of contaminated exposure. Marine Environmental Research, 34: 45-51.

[28] Maccubbin, A.E, 1994. DNA adduct analysis in fish: laboratory and field studies. In: Malins, D.C. and Ostrander, G.K., eds. Aquatic Toxicology: Molecular, Biochemical, and Cellular Perspective. USA: CRC press, pp. 267-294.

[29] Overton, E.B., W.D. Sharp and P. Roberts, 1994. Toxicity of petroleum. In: Cockerham, L.G. and Shane, B.S., eds. Basic Environmental Toxicology. Boca Raton, FL: CRC Press, pp. 133-156.

[30] Park, K.S., R.C. Sims and R. Dupont, 1990. Transformation of PAHs in soil systems. Journal of Environmental Engineering (ASCE), 116: 623-640.

[31] Yuan, S.Y., J.S. Chang, J.H. Yen, B.V. Chang, 2001. Biodegradation of phenanthrene in river sediment. Chemosphere, 43: 273-278.

[32] Bakowski, W. and D. Bodzek, 1988. WielopierScieniowe weglowodory aromatyczne w naturalnym srodowisku czlowieka—pochodzenie, wystepowanie, toksycznosc, oszacowanie emisjiw Polsce. Arch Ochr Srodow, 3-4: 195.

[33] Edwards, E.A., L.E. Wills and D. Grbic-Galic, 1991. Anaerobic degradation of toluene and xylene—evidence for sulfate as the terminal electron acceptor. In: Hinchee, R.E. and Olfenbuttel, R., eds. In Situ Bioreclamation: Applications and Investigations for Hydrocarbon and Contaminated Site Remediation. Stoneham, MS: Butterworth-Heinemann, 463-471 pp.

[34] Biegert, T., G. Fuchs and J. Heider, 1996. Evidence that anaerobic oxidation of toluene in the denitrifying bacterium *Thauera aromatica* is initiated by formation of benzylsuccinate from toluene and fumerate. European Journal of Biochemistry, 238: 661-668.

[35] Rockne, K.J. and S.E. Strand, 1998. Biodegradation of bicyclic and polycyclic aromatic hydrocarbons in anaerobic enrichments. Environmental Science and Technology, 32: 3962-3967.

[36] Alloway, B.J. and D.C. Ayres, 1993.Organic pollutants. In: Chemical Principles of Environmental Pollution, 1st ed. India: Chapman and Hall.

[37] Pampanin, D.M. and O. Sydnes, 2013. Polycyclic aromatic hydrocarbons a constituent of petroleum: presence and influence in the aquatic environment. Intech, 1-36.

[38] Tsai, J.-C., M. Kumar and J.-G. Lin, 2009. Anaerobic biotransformation of fluorene and phenanthrene by sulfate-reducing bacteria and identification of biotransformation pathway. Journal of Hazardous Materials, 164 (2-3): 847-855.

[39] C.E. Cerniglia, 1992. Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation, 3: 351-368.

[40] Alexander, M., 1999. Biodegradation and Bioremediation, 2nd ed. San Diego, CA: Academic Press.

[41] Ghanavati, H., G. Emtiazi and M. Hassanshahian, 2008. Synergism effects of phenol degrading yeast and ammonia oxidizing bacteria for nitrification in coke wastewater of Esfahan steel company. Waste Management and Research, 26 (2): 203-208.

[42] Hasanshahian, M. and G. Emtiazi, 2008. Investigation of alkane biodegradation using the microtiter plate method and correlation between biofilm formation, biosurfactant production and crude oil biodegradation. International Biodeterioration and Biodegradation, 62: 170-178.

[43] Hassanshahian, M., G. Emtiazi, R. Kermanshahi and S. Cappello, 2010. Comparison of oil degrading microbial communities in sediments from the Persian Gulf and Caspian Sea. Soil and Sediment Contamination, 19 (3): 277-291.

[44] Hassanshahian, M., H. Tebyanian and S. Cappello, 2012a. Isolation and characterization of two crude-oil degrading yeast strains, Yarrowia lipolytica PG-20 and PG-32 from Persian Gulf. Marine Pollution Bulletin, 64: 1386-1391.

[45] Hassanshahian, M., G. Emtiazi and S. Cappello, 2012b. Isolation and characterization of crude-oil-degrading bacteria from the Persian Gulf and the Caspian Sea. Marine Pollution Bulletin, 64: 7-12.

[46] Hassanshahian, M., M. Ahmadinejad, H. Tebyanian and A. Kariminik, 2013. Isolation and characterization of alkane degrading bacteria from petroleum reservoir waste water in Iran (Kerman and Tehran provenances). Marine Pollution Bulletin, 73: 300-305.

[47] Hassanshahian, M., G. Emtiazi, G. Caruso, S. Cappello, 2014a. Bioremediation (bioaugmentation/biostimulation) trials of oil polluted seawater: a mesocosm simulation study. Marine Environmental Research, 95: 28-38.

[48] Hassanshahian, M., M.S. Zeynalipour and F.Hosseinzadeh Musa, 2014b. Isolation and characterization of crude oil degrading bacteria from the Persian Gulf (Khorramshahr provenance). Marine Pollution Bulletin, 82: 39-44.

[49] Hassanshahian, M., M.M. Yakimov, R. Denaro, M. Genovese and S. Cappello, 2014c. Using real-time PCR to assess changes in the crude oil degrading microbial community in contaminated seawater mesocosms. International Biodeterioration and Biodegradation, 93: 241-248.

[50] Tebyanian, H., M. Hassanshahian and A. Kariminik, 2013. hexadecane-degradation by Teskumurella and Stenotrophomonas strains isolated from hydrocarbon contaminated soils. Jundishapur Journal of Microbiology, 26 (7): e9182.

[51] Hassanshahian, M. and M.M. Yaghoobi, 2014. Cloning and gene expression of cytochrome P450 gene from *Alcanivorax borkumensis* Bacterium. International Journal of Advanced Biological and Biomedical Research, 2(1): 76-85.

[52] Amini Boroujeni, N., M. Hassanshahian and M.R. Khoshrou, 2014. Isolation and characterization of phenol degrading bacteria from Persian Gulf. International Journal of Advanced Biological and Biomedical Research, 2(2): 408-416.

[53] Hassanshahian, M., 2014. The effects of crude oil on marine microbial communities in sediments from the Persian Gulf and the Caspian Sea: a microcosm experiment. International Journal of Advanced Biological and Biomedical Research, 2(1): 1-17.

[54] Hassanshahian, M. and S. Cappello, 2013. Crude oil biodegradation in the marine environments. In: Biodegradation—Engineering and Technology (Chapter 5). InTech Open Science, 101-135.

[55] R.D. Hunter, S.I.N. Ekunwe, D.E Dodor, H-M Hwang and Ekunwe, 2005. *Bacillus subtilis* is a potential degrader of pyrene and benzo(a)pyrene. International Journal of Environmental Research and Public Health, 2(2): 267-271.

Section 3

Novel Technologies in Biodegradation and Bioremedation

Chapter 7

Advantages and Limitations of Using FTIR Spectroscopy for Assessing the Maturity of Sewage Sludge and Olive Oil Waste Co-composts

Loubna El Fels, Mohamed Zamama and Mohamed Hafidi

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60943

Abstract

Composts prepared using different solid and liquid organic wastes from various sources can be used as growing media when these materials present adequate proprieties for plant development. The stability and maturity are among the main characteristics of composts. The purpose of this study is to recommend specific bands of the IR spectrum recorded on different composts to enable qualitative and rapid monitoring of the stages of biodegradation during composting. At the beginning of humification, the significant decrease in the intensity of the band located at 1735 cm^{-1} shows that lignin is affected at the first stage of the composting process. At the end of the humification, the band located toward 3450–3420 cm^{-1} at the beginning of the process undergoes a systematic shift ($\Delta\nu$ of the order of 10 cm^{-1}) toward lower wave numbers. The band located at 1660–1650 cm^{-1} on the Fourier transform infrared spectroscopy (FTIR) spectra before composting shifts systematically toward 1640 cm^{-1} at the end of humification. This phenomenon can be used as index of compost maturity. Measuring the band at 1035 cm^{-1} as an internal standard, it is possible to quantify the degradation rate of organic matter.

Keywords: FTIR, composting, humic substance, maturity, humification, shift bands

1. Introduction

Olive oil production in Morocco amounts to approximately 60,000 tons year^{-1}. Olive mills produce large amounts of solid waste, around 70,000 tons year^{-1}, which serves for combustion, and olive mill wastewater reaching about 640,000 tons year^{-1}. However, the liquid is sent directly into the sewer systems, stored in evaporation lagoons, or illegally spread on the land [1]. In addition, the purification system used in the treatment of wastewater generates considerable quantities of sludge, estimated in 2010 at 40,000 tons year^{-1}, and the forecasts are for 300,000 tons year^{-1} in 2025 [2]. The problems raised by the elimination of this sludge are generally underestimated or poorly taken into account when wastewater plants are designed. The possibilities of sludge evacuation are limited and are subjected to various technical, economic, legal, and environmental constraints.

The by-products of wastewater treatment and of olive oil wastes can become environmental pollutants owing to their high organic loads and the presence of molecules such as phenolics [3]. Some of the sugars and polyols can be used as sources of carbon and energy for the growth of microorganisms. The high levels of phenol monomers and polymers lead to chemical oxygen demand (COD) and biological oxygen demand (BOD) about 200–400 times higher than those of typical municipal sewage. However, these organic wastes are known to contain fertilizing elements, encouraging their recycling in agriculture. The need to preserve natural resources and especially nonrenewable sources implies organic waste recycling [4]. Sustainable reuse of waste in agriculture as compost or substitute peat is advantageous because of its environmental and economic benefits. However, the direct use, especially of sewage sludge, in agriculture is limited by the presence of pathogens, including parasites, viruses, bacteria, and fungi, as well as organic pollutants (phthalates, HAPs, PCBs, etc.) and trace metals (Cr, Cd, Hg, Zn, Cu, Pb, etc). To overcome these risks, treatment is necessary to reduce and eliminate adverse effects and to maximize the effectiveness of the materials once applied to the soil. The composting of organic waste seems to be one of the best ways to reduce potentially dangerous harmful residues especially olive residues when combined with organic waste such as animal manure.

For several years, our own research area focused mainly on the recycling of olive mill waste (pomace, liquid effluent) and the sludge generated by the treatment of wastewater from sewage treatment plants. Both these types of organic waste are major sources of phosphorus and organic nitrogen [2, 5, 6, 7], and their recycling involves a composting process, which produces humic substances (HSs) that can be used to improve soil quality. During this last decade, works in our laboratory focused on the humification process occurring in (i) the detoxification of liquid effluent from oil mills in order to change its phenolic composition [7]; (ii) the co-composting of solid olive mill waste, straw, and household waste with the liquid effluents from oil mills [5, 8]; and (iii) the composting of moistened municipal waste and co-composting of sewage sludge with green waste (straw, fresh green plants, palm waste, household waste) [2, 9].

Various techniques and spectroscopic methods have been used for the analysis and characterization of HS in our work. The studies were conducted on humic substances (HSs) and their essential fractions (fulvic and humic acids). Our research is consistent with literature data and

http://dx.doi.org/10.5772/60943

allows the atomic composition of HS to be determined. Despite their heterogeneity, similarities in composition are observed between the different humic fractions in particular segments of the isolated molecules, obtained by destructive techniques. The major differences and some characteristic functions of HS can be detected by nondestructive spectroscopic methods such as Fourier transform infrared spectroscopy (FTIR) and ^{13}C-NMR [2, 5]. The relative proportions of functional groups and their degree of branching affect the characteristics of humic and fulvic (HA and FA) fractions directly. As shown by El Fels et al. [10], the differences in the atomic and molecular composition (molecular fragments obtained after Pyr-GC-MS) could be interpreted by determining a humification rate. However, these techniques combined with the physicochemical parameters cannot predict the chemical/biological reactivity.

The extreme variability in the molecular features of HSs relates back to precursor compounds and the environmental conditions under which HS formed such as origin of starting material and processing [11]. Despite that the interest of HS and their structural characterization have been published for many different substrates [12, 13], their molecular structure is still under discussion. The reason is that HS have been shown to contain a wide variety of associated molecular components such as polysaccharides, polypeptides, lignins, hemicellulose, esters, phenols, ethers, carbonyls, quinines, lipids, peroxides, various combinations of benzene, acetal, ketal, and lactol, and furan ringed and aliphatic compounds [11]. Some studies suggest the predominance of aromatic units in HSs, whereas others have shown that many humic extracts contain largely aliphatic structures. Many factors, such as the origin of humic material, the extraction technique, and the purification methods are responsible for the discrepancies found [14]. The hypothesis of by Schnitzer and Khan [12] is that humic acids (HAs) can be assumed to be highly cross-linked aromatic polymers of high molecular weight with covalent carbon–carbon, ether, and ester linkages connecting the substituted aromatic moieties. Other studies support HSs as groups of similar particles associated by weak covalent and noncovalent bonds to "homogeneous" aggregates, which in unfractionated HSs form large mixed aggregates [15, 16]. Therefore, purification is needed to eliminate the weakly bound long alkyl chains from the raw humic material. It removes the extractable monomers or fragments that interfere with the analysis of the main macromolecular structure of the HSs. Purification permits the isolation of the more homogeneous structures and provides better representative information about the real changes of humic macromolecular structure when using various chemical and spectroscopic techniques (elemental analysis, Fourier transform infrared analysis, and ^{13}C-NMR spectroscopy). Gonzalez-Vila et al. [17] suggest that a systematic study of the organic material in composts should include the characterization of both the colloidal organic fraction (which conforms to the operationally defined compost HSs) and the "extractibles" (lipids and water-soluble products).

The extraction and structural determination of a pure fraction of HS is not as yet feasible owing to their complexity and heterogeneity. Only the evolution of HS during their successive transformations made it possible to identify a series of groups and functions present. FTIR and NMR analyses of humic structure sequences indicated that during co-composting process, the HS results from the biodegradation of cellulose, lignin, and hemicellulose and consist of molecules generally containing functional groups such as O-H, C-O, C-O-C, and O-CH_3. These

groups give rise to characteristic bands in the IR spectrum, which have been the subject of various assignments.

Analysis of FTIR spectra in the case of chemical/biochemical reactions generating specific end products is done by following the appearance/disappearance of characteristic bands of some functional groups in the products formed, or by following the band shifts in the case of reactions that involve structural changes. In contrast, during incomplete transformations as in composts, the FTIR spectra recorded at different stages of humification contain both characteristic bands of the products formed and these of the starting reagents which are not completely consumed. Studies conducted by Wershaw et al. [18] on peat soil, agricultural soil, and lake sediment found that many of the chemical structural features of the original plant material were incorporated into the humic acid structure, including lignin, carbohydrate, and long-chain aliphatic structural groups. It is necessary to know the nature, composition, and the starting raw material structure to distinguish the bands of the HS formed.

This review examines the FTIR results obtained from our earlier works on the different composting processes to characterize and quantify the HSs. An appreciation of the analytical methodology depends on an understanding of aspects of the chemistry of HS. The FTIR spectrum of a given co-compost is the result of the contribution of different components of the raw material that constitutes it, such as cellulose, hemicellulose, and lignin. In order to highlight the contribution of each component to the spectrum, we performed FTIR spectrum analysis, taking into account the bands of the starting raw material that may be incorporated and not degraded at the end of the process. This procedure allows the identification of FTIR spectrum bands that can reliably track the evolution of the degradation of organic matter in a given compost.

2. Using FTIR as rapid tool to analyze organic composts

Fourier transform infrared spectroscopy (FTIR) is currently one of the most important analytical techniques available to analyze various substrates. One of the greatest advantages of this technique is that virtually any sample in any state may be analyzed. For example, liquids, solutions, pastes, powders, films, fibres, gases, and surfaces can all be examined with a judicious choice of sampling technique. FTIR has facilitated many different IR sampling techniques, including attenuated total reflection (ATR) and diffuses reflectance infrared Fourier transform (DRIFT) spectroscopy [19].

FTIR spectroscopy is used to study different composts without any previous chemical treatment likely to cause inappropriate reactions. This technique is widely used to characterize the evolution of organic substrates, mainly when these HSs are extracted from the soil and from composted waste, such as sewage sludge and olive oil mill waste [6, 20, 21]; HA was extracted from sewage sludge and bottom sediments [22, 23, 24]. In addition to identifying the functional groups by their characteristic frequencies, it is possible to follow the HS composition by comparing the relative intensities of certain absorption bands [22, 23, 25, 26]. The disappearance or the appearance of new bands in the FTIR spectrum provides information about

http://dx.doi.org/10.5772/60943

the matter evolution and its interaction with heavy metals when they are present in sewage sludge [24, 27]. However, as reported by Haberhauer et al. [28], Ellerbrock et al. [29], and Kaiser and Ellerbrock [30], in the case of HS, HA, and FA extracted from composts, even after purification, there often remains a relatively large portion of organic compounds such as cellulose, lignin, hemicellulose [15], or mineral (silicates or clays) presenting bands that overlap with those of HS compounds, i.e., in the 1200–1000-cm^{-1} region [28, 30]. Also, the indexing of FTIR spectra of entire compost remains complicated considering the overlap of mineral phase bands with those of recalcitrant organic matter and those of HSs formed during the humification process. In this case, it is difficult to accurately attribute the FTIR spectrum absorption bands in the 3500–3280-cm^{-1} and 1200–1000-cm^{-1} regions. Observed wave numbers and intensities of bands at these regions of spectrum vary from one bibliographic reference to another, and various attributions have been put forward:

- The broad and intense asymmetric bands appearing at 3450–3280 cm^{-1} were attributed to the elongation of vibrations of OH linked by hydrogen bonding and NH groups of HA extracted from a soil or sewage sludge [24, 31, 32, 33].
- On the IR spectrum of HA extracted from composts of sludge, a band in the region 1200–1000 cm^{-1} has been attributed to aromatic ethers and to Si-O by [24], and to C-O stretching vibration of C-carbohydrates, aromatic ethers, and polysaccharides by [27, 34, 35, 36].

Smikovic et al. [37] attribute the band at 1035 cm^{-1} in the IR spectrum to mineral compounds. Although different wastes can be distinguished by their fingerprint region (1500–900 cm^{-1}), this region also reveals fresh and undecomposed materials [38]. In our research, the infrared spectra (FTIR) recorded on different composts and their HA and FA fractions show the changes occurring toward the end of the humification. Small differences in band positions that we found on the FTIR spectra of several samples from different composts require careful examination of the FTIR spectra in order to relate the spectral changes to structural changes in HS during their humification and to provide a more accurate attribution of the vibration wave numbers corresponding to the groups in HS and determine the noncharacteristic bands, which give limited information.

3. Methodology

3.1. Composting trials

Different composting trials were conducted in a heap on a purpose-built platform as follows:

- Activated sludge from Marrakech treatment plan (50%) mixed with (50%) of palm waste for 180 days [2].
- Olive mill water (15%) mixed with pomace (75%) and (10%) of municipal solid waste for 150 days [8].
- Activated sludge taken from the wastewater aerobic treatment plant of Khouribga (Morocco) (66%) mixed with (34%) of fresh plant matter for 130 days [39]

- Lagooning sludge from Marrakech treatment plant (90%) with straw (10%) for 180 days [11].
- Olive mill water (25%) mixed with pomace (70%) and straw (5%) [5].

The mixtures were prepared so as to optimize the co-composting parameters, i.e., 60% humidity and a C/N ratio of about 30. To provide aerobic conditions, the mixtures were mechanically turned each week. The sampling was carried out at different stages during composting. These were at the initial mixing stage, intermediate stage, and final co-composting stage.

3.2. Humic substance extraction

The humic substance (HS) from each composting sample was extracted. The samples were treated three times with 40 mL of distilled water so as to extract the water-soluble non-HSs (sugars, proteins, etc.). Then the HSs were extracted with 40 mL of NaOH (0.1 M). This was repeated several times until the extract obtained was colorless. The supernatant obtained was centrifuged at 4000*g* for 15 min. After filtration, the supernatants were pooled to determine the level of total soluble carbon.

The HA were separated from the total HSs by acidification with H_2SO_4 to reach a pH of 1. At this pH, the HA form a precipitate, while the FA remain in solution. After being left to settle for 24 h at 4°C, centrifugation at 4000*g* for 20 min left the FA in solution while the HA were recovered in the pellet. The content of each fraction was determined by the $KMnO_4$ oxidation method.

3.3. Lipid removal

Free lipids were removed before extraction of HSs using a 2:1 chloroform–methanol mixture as mentioned by Amir et al. [16]. Lipid extraction was carried out three times at 4°C using 15 g fresh samples with 120 mL solvent mixture. These pretreated samples were then subjected to evaporation to remove remaining solvent, and they were then washed three times with water to remove other nonhumic water-soluble molecules, such as sugars and proteins, which might interfere with the analysis of the HSs. Humic substance assay was performed as mentioned above.

3.4. FTIR of extracted humic acid

Potassium bromide pellets of each freeze-drying of the various complete samples and each of the extracted fractions were prepared by pressing under vacuum a mixture of 250 mg of dried KBr and 1 mg of sample (composts, humic acid, and separated fractions). The spectra were obtained using an FTIR Perkin Elmer 1600 [6, 7, 8, 9, 11] and the Bruker Vertex 70 FTIR [2] spectrometers (128 scans at a resolution of 2 cm^{-1} were carried out). Infrared spectral analyses were carried out over a 4000–400-cm^{-1} range. The instrument was carefully calibrated using water vapour. The uncertainty in band frequencies is estimated to ±1 cm^{-1}.

http://dx.doi.org/10.5772/60943

4. Spectroscopic characterization of composts

The spectral data obtained from the analysis of FTIR of FA, HA, and HSs extracted from several composts at different stages of humification [20, 22, 23, 39] are presented in (Tables 1 and 2). In the absence of an internal reference, it is difficult to relate the evolution of band intensities to the appearance/disappearance of new product. The general pattern of bands on the compost spectra shows no dramatic intensity changes. However, the wave numbers measured on the FTIR spectra of whole compost [2] and extracted HS [39] compared to frequencies of bands during composting show modifications (Figure 1). Different mechanisms and structures have been proposed ranging from basic conceptual structures [13], to more detailed structures on the basis of degradation products using pyrolysis and NMR as shown by [2, 22, 23]. However, it is difficult to obtain structural information on HS from the composting process by using FTIR spectra without the input data obtained by other techniques. The variation of the band intensities provides information on the evolution of the humification process [9], but the intensity ratios of the bands cannot give quantitative information on the degradation rate. In addition, these intensity ratios vary from one bibliographic reference to another [8, 40]. However, with regard to the structural complexity of the HS, only their functionality should be used, which involves the characterization of the functional groups present in the HS [41].

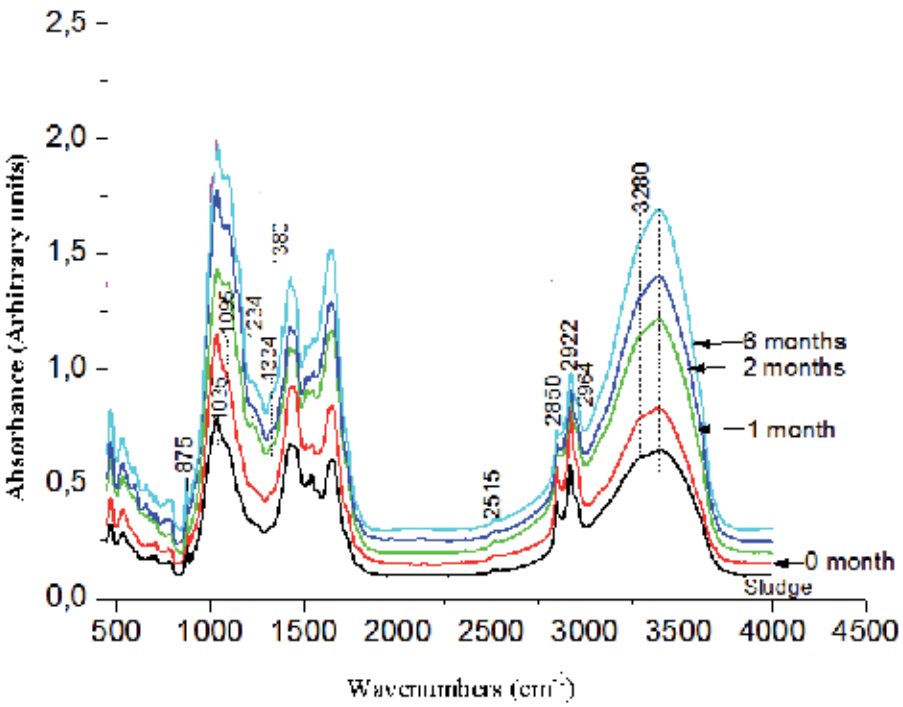


Figure 1. FTIR characteristic of whole composts versus time of composting of sludge with lignocellulosic waste [2].

Fulvic acid				Humic substance				
RM	**60 d**	**135 d**	**Δν (cm^{-1})**	**RM**	**15 d**	**60 d**	**135 d**	**Δν (cm^{-1})**
3412	3405		**18**	**3426**	3426	3423	**3400**	**26**
		3394						
2931	2934	2936		2927	2926	2925	2927	
				2853	2856	2856	2854	
1652	**1644**	**1637**	**15**	**1651**	**1652**	**1651**	**1651**	**0**
1561	1561	1561		1540	1533	1533	1533	
				1420	1430	1428	1428	
1408	1408	1405						
1385	1384	1386		1386	1386	–	1386	
1239	1221	1221	17	1226	1244	1244	1244	18
1143	1143	1143						
1124	1121	1121						
1072	1074	1080						
1037	**1035**	**1037**	**0**	**1035**	**1032**	**1040**	**1036**	**1**
				874	**874**	**875**	**874**	
620	622	624						

$\Delta\nu = (\nu_{RM} - \nu_{Final\ phase})$; RM = raw material; d = day.

Table 1. FTIR characteristic of extracted humic substance and fulvic acid at versus times of composting of sludge/green waste [6, 39].

Fulvic acid*				Humic acid**					Lyophilized samples***				
RM	**90 d**	**180 d**	**Δν (cm^{-1})**	**RM**	**15 d**	**60 d**	**135 d**	**Δν (cm^{-1})**	**RM**	**E1**	**E2**	**E3**	**E4**
3418	3400	**3400**	**18**	**3420**	3418	3418	**3408**	**12**	**3430**		**3426**	**3405**	
				3385			3385						**3394**
2920	2922	2911		2924	2922	2925	2925		2922	2928	2917	2928	2929
1656	1647	**1640**	**16**	**1652**	1643	1643	**1642**	**10**	**1633**	**1634**		**1619**	**1622**
1544	1550	1550		1543	1554	1544	1543						
1411	**1400**	**1408**	3						1377				
				1385		**1386**	**1387**	2		1385			
											1408	1408	1408
									1260	**1256**	**1261**	**1267**	**1258**
				1235	**1236**	**1236**	**1235**						
1198	**1198**	**1198**											
1040	**1038**	**1038**	**0**	**1033**	**1035**	**1033**	**1035**	**0**	**1038**	**1034**	**1040**	**1040**	**1034**
625	617	617							544	536	597	619	591

$\Delta\nu = (\nu_{RM} - \nu_{Final\ phase})$; d = day; E = different treatment.

Table 2. FTIR characteristics of extracted humic substance and fulvic acid for different times of composting of sludge, green waste, and olive oil mill waste [11*, 9**, 20***].

http://dx.doi.org/10.5772/60943

Results of infrared analysis of co-composts of different types of organic matter at different stages, with or without an HS extraction process, showed that only five regions of the FTIR spectrum seem to provide information on the evolution and stability of the humification process. The band located at around 3430 cm^{-1} in the FTIR of composts at the initial stage shift during composting to stabilize at lower wave numbers (3402 cm^{-1}) at the end of the humification process. The increase in the relative intensity of this band during composting shows that the compost has a better water retention capacity, which is likely related to the increase of the cation exchange capacity that changes during composting. Water retention in the soil is correlated to the CEC that is linked to the hydration properties of organic matter and clays [42].

The 3000–2800-cm^{-1} region reflects the hydrophobic properties of the aliphatic organic matter. The bands in this region are better resolved than those in the spectra of composts. We noted a decrease of these bands during the first 8 weeks of humification consistent with the microbial oxidation of the carbon chains of aliphatic and peptidic compounds, which provides information on the maturity of the co-composting process. Moreover, peaks that characterize both methyl and methylene groups in lignin and in compost remain centered at 2855 cm^{-1} and around 2920 cm^{-1}, but the other characteristic peak of the methyl groups is located at 2958 cm^{-1} after the humification process (Figure 1). This frequency change reflects that the structure of the aliphatic groups generated after composting is different from that of the lignin and the cellulose characterized by C-H bands situated at wave numbers below 2930 cm^{-1} (Table 3).

Holocellulose	Cellulose			Hemicellulose			Lignin	
3425[a]	**3417**[c]	–	3200[e]	**3425**[a]	3436[b]	–	**3410**[f]	3410–3460[d]
2928[a]	2924[c]	–		2928	2932	–	2933[f]	2937[e]
–	2916[c]	–	2900[e]			–		2917[d]
					2850			2845[e]
–		–	2800[e]			–		
1736[a]	–	–	–	–	–	–	**1705**[f]	**1717**[d]
1634[a]	1634[c]	–	1630[e]	1646[a]	1634	1606[d]	–1605[f]	1610[d]
	–	–	–	–	–			
1537[a]	–	–	–	–	–	–		1514[d]
1463[a]	1460	–	–	–	–	1460	1460[f]	1462[d]
	1433	1431[d]	1430[e]	1413[a]	1421	–	1424[f]	
1382[a]	1378	1373[d]	1367[e]		1378			
		1338[d]						
1320[a]	–	1319[d]	1315[e]	1324[a]	1324	–	1327[f]	1328[d]
								1276[d]
1258[a]				1242[a]	1262	1251		
	1208	1203[d]	1220[e]	–	–	1212	1213[f]	1220[d]
1161[a]	1161	–	1160[e]	–	–			
1110[a]	**1114**	–	**1110**	**1130**[a]	**1130**	–	1113[f]	
1064[a]	**1064**	–	**1055**[e]	–	**1044**	**1049**		1038[d]

Holocellulose	Cellulose			Hemicellulose		Lignin	
904 [a]				908			
–	897	–	878 [e]	873[a]			854 [d]
–				621[a]		590[f]	

[a] Xu et al. [53].

[b] Xu et al. [45].

[c] Liu et al. [54].

[d] Boeriu et al. [44].

[e] Méndez et al. [55].

[f] Eyheraguibel [56].

Table 3. Usual wave numbers from FTIR spectra.

In the region 1750–1700 cm^{-1}, FTIR spectra (Figure 1) show a significant reduction in the intensity of the band located at 1735 cm^{-1} at the beginning of composting. Lignin is known to contain carboxyl groups represented by carboxyl vibrations between 1750 and 1550 cm^{-1} in the FTIR spectra, and a significant difference was found in the finger print region (1830–730 cm^{-1}) [43, 44]. The FTIR spectra of flax, hemp, and straw lignin show three peaks, whereas the peak at 1647 cm^{-1} seems to be absent in Alcell™ lignin (Figure 2), the band at 1705 cm^{-1} increases in oxidized lignin (flax-ox), in solvent-extracted hardwood lignin and in sulfur-free softwood lignin, but is absent in softwood lignosulfonates and Kraft lignin. From the analysis of the FTIR spectra of plant material, the 1750–1700-cm^{-1} region is characteristic of lignin. In the case of the cellulose and holocellulose extracts, the presence of bands around 1700–1740 cm^{-1}, in the FTIR spectrum of the holocellulosic fraction and its absence in the spectra of cellulosic and hemicellulosic extracts (Table 3), was interpreted by the presence of a fraction of lignin which joins the holocellulose during extraction [45]. On the FTIR spectra of purchased cellulose and hemicellulose, no band was observed in at this region [46]. Our FTIR spectra (Figure 1) show a marked decrease in the band at 1735 cm^{-1} at the beginning of composting. This decrease indicates that structural changes occur in the lignin at the beginning of humification. This is supported by the decrease in the intensity of the band around 1514 cm^{-1}, which characterizes the aromatic skeletal vibration in lignin [47].

In the region 1650–1600 cm^{-1}, FTIR spectra show an increase in the intensity of the band at 1647 cm^{-1} during co-composting. This band, characteristic of $\nu_{C=O}$ of the ionized COO- function, shifts toward 1640 cm^{-1} at the end of humification. However, the band located at 1155 cm^{-1} is attributed to the ν_{C-O} increase. The change in the intensity of bands observed around 1647 cm^{-1} and 1155 cm^{-1} indicates the presence of a carboxyl function which characterizes an ester form.

In the region 1000–1040 cm^{-1}, the band located at 1035 cm^{-1} persists during the humification process and even after calcination at 650°C (Figure 3). It is due to the mineral phase provided by the sludge. The presence of similar bands in this region of the IR spectrum is common when the IR spectrum is recorded from soil that has undergone a specific extraction process [28, 29].

http://dx.doi.org/10.5772/60943

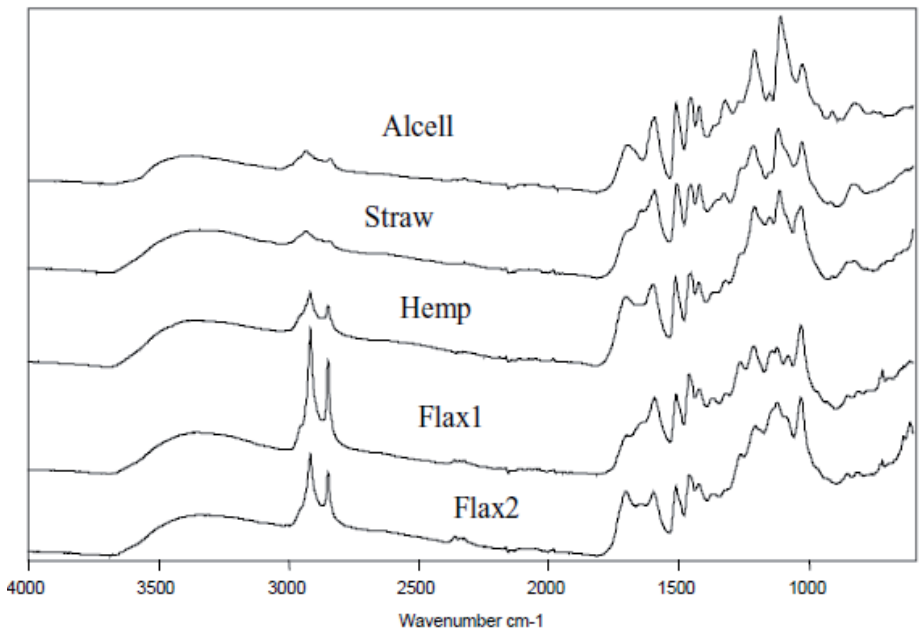


Figure 2. FTIR spectra of lignin from different substrates [52].

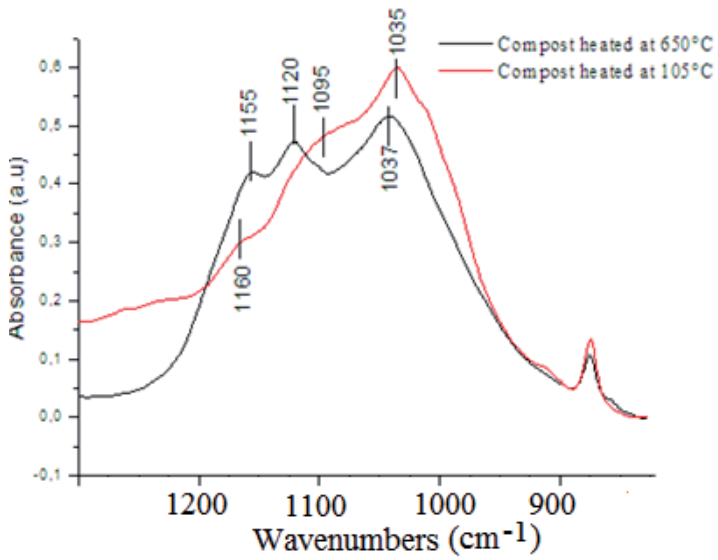


Figure 3. FTIR spectra before and after calcination of compost.

The remaining part of the recalcitrant organic matter such as lignin hemicellulose could contribute to the FTIR spectrum. As shown by Faix et al. [48], the lignin compounds are characterized by the frequencies of the guaiacyl unit (1269 cm^{-1}, G-ring and C=O stretch; 1140

cm^{-1}, CH in-plane deformation; 854 and 817 cm^{-1}). On the other hand, the hemicellulose fraction contains noticeable amounts of polysaccharides and can contribute to the FTIR spectrum by bands identified at 1413 and 1242 cm^{-1}, which designate the methyl C–H wagging vibrations and carbonyl absorbance in pectic polysaccharide substances [45]. Bands around 1161 and 897 cm^{-1} were attributed to C–O–C stretching at the β-(1-4)-glycosidic linkages [49]. It seems that the assignment of bands appearing at wave numbers below 1600 cm^{-1} remains difficult because this region contains vibrations characteristic of different compounds present in recalcitrant organic matter.

The comparison of the FTIR spectrum obtained from HA extracted from uncomposted sludge after lipid removal, lipid-free HA (LFHA), or without lipid removal (HA0) shows the same profiles with shift frequencies (Table 4). The bands located at 3414 and 1652 cm^{-1} shift to 3404 and 1646 cm^{-1}, respectively, at the end of the humification process. However, the wave numbers from FTIR of HA0 are lower (3397 and 1644 cm^{-1}). Piccolo et al. [50] and Preston and Schnitzer [51] suggested that fatty acids occur in HSs partly as phenolic esters and partly adsorbed by weaker forces such as H-bonding and van der Waals forces. Lipids are present as an admixture, or held by noncovalent bonds to humic macromolecules. Our spectra show that the presence of the lipid fraction provokes band shifts toward lower frequencies. This type of shift is encountered in the case of hydrogen bonding interactions. Nevertheless, our spectral data show that even in the presence of the lipid fraction, the FTIR spectra provide information on changes that occur during composting on condition that the appropriate spectral regions are considered.

HA0	**LFHA**			**Δν (cm^{-1})**
	0 d	30 d	180 d	
	3414	**3413**	**3404**	**7**
3397				
2921	2930	2933	2930	
2854	2854	2855		
1644	**1652**	**1647**	**1646**	**2**
1544	1540	1511	1510	
1453	1456	1458	1458	
1414	1420	1420	1419	
	1387	**1387**	**1387**	0
1264	1264	1264	1263	
1231	1230	1229	1227	4
1120	1126	1126	1125	
1076	1076	1076	1076	
1032	**1036**	**1036**	**1037**	**5**

http://dx.doi.org/10.5772/60943

HA0	LFHA			Δν (cm^{-1})
	0 d	30 d	180 d	
987	**987**	**987**	**987**	**0**
611	611			

$\Delta\nu = (\nu_{RM} - \nu_{Final\ phase})$; LFHA = lipid-free humic acid; HA 0 = without lipid removal; d = day.

Table 4. FTIR spectral characteristics versus composting time [11].

It appears from this study that FTIR spectroscopy can be used as a tool to monitor organic matter degradation and stability during composting. The comparison of the relative intensities of the aliphatic chain bands (region 3000–2800 cm^{-1}) reflects the evolution of the compost during the process of humification. The band at 1035 cm^{-1} attributed to mineral compounds could be used as internal reference. FTIR spectra show that whatever the organic substrate used, humification is accompanied by the disappearance of the band at 1750–1700 cm^{-1} whose intensity decreases at the beginning of composting. In parallel, we found that the bands located at 3420 and 1655 cm^{-1} gradually shift to around 3400 and 1640 cm^{-1}, respectively, at the end of composting. This shift toward lower wave numbers can be used as an index of the stability of compost.

5. Conclusion

The detailed analysis of FTIR spectra recorded on different composts led to the identification of five important regions of the spectrum, which can reflect structural changes during composting. From the specific frequency bands, it is possible to follow qualitatively and rapidly the biodegradation steps during composting:

- At the first stage of humification, the significant decrease in the intensity of the band located at 1735 cm^{-1}, which is found only on the FTIR spectrum of lignin, shows that lignin is assigned to the first stage of the composting process.
- At the end of the humification process, the band located around 3450–3420 cm^{-1} is shifted to lower wave numbers by around $\Delta\nu = 10$ cm^{-1}.
- The band located at 1660–1650 cm^{-1}, on the FTIR spectra before composting, is shifted to lower wave numbers by around a $\Delta\nu = 7–8$ cm^{-1} at the end of humification. These shifts can be used as a maturity index of the compost.
- The band at 1040–1035 cm^{-1} is not affected by the process of humification and may be due to the mineral phase in the sludge.
- The 3000–2800-cm^{-1} region reflects the hydrophobic properties of the aliphatic organic matter. The decrease of these bands during humification is consistent with the degradation of aliphatic carbon chains and peptidic compounds, which provides information on the

maturity of co-composting process. Using the band at 1035 cm^{-1} as an internal standard, it is possible to quantify the degradation rate of organic matter in the compost during the humification process, thereby making the FTIR technique quantitative as well as qualitative for assessment of compost maturity.

Author details

Loubna El Fels[1], Mohamed Zamama[2] and Mohamed Hafidi[1]

1 Laboratory of Ecology and Environment (L2E) (Unit Associated with the CNRST, UR-AC32), Faculty of Science Semlalia, Cadi Ayyad University, Marrakesh, Morocco

2 Laboratory of Physico-Chemical of Materials and Environment (LPCME), Faculty of Science Semlalia, Cadi Ayyad University, Marrakesh, Morocco

References

[1] Ait Baddi G, Hafidi M: Traitement des déchets des huileries par un bioprocédé aérobie et production de substances humiques à haute valeur agronomique. Annales de Chimie, Sciences des Matériaux. 2001;26:S459–S464.

[2] El Fels L, Zamama M, El Asli A, Hafidi M: Assessment of bio-transformation of organic matter during co-composting of sewage sludge-lignocelullosic waste by chemical, FTIR analyses, and phytotoxicity tests. International Biodeterioration and Biodegradation. 2014;87:128–137.

[3] Borja R, Martin A, Maestro R, Alba J, Fiestas JA: Enhancement of the anaerobic digestion of olive mill wastwater by removal of phenolic inhibitors. Process Biochemestry. 1992;27:231–237.

[4] ADEME, 1994. La collecte et le traitement des déchets, Guide pratique pour les élus de Midi—Pyrénées.

[5] Ait Baddi G, Hafidi M, Cegarra J, Alburquerque JA, González J, Gilard V, Revel JC: Characterization of fulvic acids by elemental and spectroscopic (FTIR and 13C-NMR) during composting of olive mill wastes plus straw. Bioresource Technology. 2004;93:285–290.

[6] Jouraiphy A, Amir S, El Gharous M, Revel JC, Hafidi M: Chemical and spectroscopic analysis of organic matter transformation during composting of sewage sludge and green plant waste. International Biodeterioration and Biodegradation. 2005;56:101–108.

[7] El Hajjouji H, Fakharedine N, Ait Baddi G, Winterton P, Bailly JR, Revel JC, Hafidi M: Treatment of olive mill waste-water by aerobic biodegradation: an analytical

study using gel permeation chromatography, ultraviolet–visible and FTIR spectroscopy. Bioresource Technology. 2007;98:3513–3520.

[8] Barje F, El Fels L, El Hajjouji H, Amir S, Winterton P, Hafidi M: Molecular behaviour of humic acid-like substances during co-composting of olive mill waste and the organic part of municipal solid waste. International Biodeterioration and Biodegradation. 2012;74:17–23.

[9] Amir S, Jouraiphy A, Meddich A, El Gharous M, Winterton P, Hafidi M: Structural study of humic acids during composting of activated sludge-greenwaste: elemental analysis, FTIR and 13C NMR. Journal of Hazardous Materials. 2010;177:524–529.

[10] El Fels L, Lemee L, Ambles A, Hafidi M: Identification and biotransformation of lignin compounds during cocomposting of sewage sludge-palm tree waste using pyrolysis-GC/MS. International Biodeterioration and Biodegradation. 2014;92:26–35.

[11] Amir S, Hafidi M, Merlina G, Revel, JC: Structural changes in lipid-free humic acids during composting of sewage sludge. International Biodeterioration and Biodegradation. 2005;55(4):239–246.

[12] Schnitzer M, Khan SU. Humic substances in the environment. New York: Marcel Dekker, 1972.

[13] Stevenson FJ. In: Humus Chemistry: Genesis, Composition, Reactions, vol. 443. New York: John Wiley & Sons, Inc., 1982.

[14] Gonzalez-Vila FJ, Ludemann HD, Martin F: 13C-NMR structural features of soil humic acids and their methylated, hydrolyzed and extracted derivatives. Geoderma. 1983.31:3–15.

[15] Wershaw RL, Pinckney DJ, Booker SE: Chemical structure of humic acids, I. A generalized structural model. Journal of Research of the U.S. Geological Survey. 1977;5:565–569.

[16] González-Villa F. J, Almendros G, del Rio J. C, Martin F, Gutiérrez A, Romero J: Ease of delignification assessment of wood from different eucalyptus species by pyrolysis (TMAH)-GC/MS and CP/MAS 13C-NMR spectrometry. Journal of Analytical and Applied Pyrolysis. 1999;49:295–305.

[17] Wershaw RL, Pinckney DJ, Llanguno EC, Vicente-Beckett V: Analytica Chimica Acta. 1990;232:31.

[18] Fan M, Dai D, Huang B: Fourier transform infrared spectroscopy for natural fibres. Fourier transform–materials analysis. IntTech. Dr Salih Salih (Ed.), ISBN: 2012, 978-953-51-0594-7.

[19] Hafidi M, Amir S, Revel JC: Structural characterization of olive mill wastewater after aerobic digestion using elemental analysis, FTIR and 13 C NMR. Process Biochemistry. 2005;40(8):2615–2622.

[20] Higashikawa FS, Silva CA, Nunes CA, Mondero MAS: Fourier transform infrared spectroscopy and partial least square regression of the prediction of substrate maturity index. Science of the Total Environment. 2014;470:536–542.

[21] Amir S, Hafidi M, Lemee L, Merlina G, Guiresse M, Pinelli E, Revel JC, Bailly JR, Amblès A: Structural characterization of humic acids, extracted from sewage sludge during composting, by thermochemolysis–gas chromatography–mass spectrometry. Process Biochemistry. 2006;41(2):410–422.

[22] Amir S, Hafidi M, Lemee L, Bailly JR, Merlina G, Kaemmerer M, Revel JC, Amblès A: Structural characterization of fulvic acids, extracted from sewage sludge during composting, by thermochemolysis–gas chromatography–mass spectrometry. Journal of Analytical and Applied Pyrolysis. 2006;77(2):149–158.

[23] Polak J, Bartoszek M, Sułkowski WW: Comparison of some spectroscopic and physico-chemical properties of humic acids extracted from sewage sludge and bottom sediments. Journal of Molecular Structure. 2009;924–926:309–312.

[24] Chefetz B, Ilani T, Schulz E, Chorover J: Wastewater dissolved organic matter: characteristics and sorptive capabilities. Water Science and Technology. 2006;53(7):51–57.

[25] Fakharedine N, El Hajjouji H, Ait Baddi G, Revel JC, Hafidi M: Chemical and spectroscopic analysis of organic matter transformation during aerobic digestion of olive-mill waste-waters. Process Biochemistry. 2006;41:398–404.

[26] Sawalha MF, Peralta-Videa JR, Saupe GB, Dokken KM, Gardea-Torresdey JL: Using FTIR to corroborate the identity of functional groups involved in the binding of Cd and Cr to saltbush (*Atriplex canescens*) biomass. Chemosphere. 2007;66:1424–1430.

[27] Haberhauer G, Rafferty B, Strebl F, Gerzabek MH: Comparison of the composition of forest soil litter derived from three different sites at various decompositional stages using FTIR spectroscopy. Geoderma. 1998;83 (3):331–342.

[28] Ellerbrock RH, Gerke HH, Bachmann J, Goebel MO: Composition of organic matter fractions for explaining wettability of three forest soils. Soil Science Society of America Journal. 2005;69 (1):57–66.

[29] Kaiser M, Ellerbrock RH: Functional characterization of soil organic matter fractions different in solubility originating from a long-term field experiment. Geoderma. 2005;127:196– 206.

[30] Senesi N, D'Orazio V, Ricca G: Humic acids in the first generation of eurosoils. Geoderma. 2003;116:325–344.

[31] Pérez MG, Martin-Neto L, Saab SC, Novotny EH, Milori DM, Bagnato VS, Knicker H: Characterization of humic acids from a Brazilian Oxisol under different tillage systems by EPR, 13 C NMR, FTIR and fluorescence spectroscopy. Geoderma. 2004;118(3):181–190.

http://dx.doi.org/10.5772/60943

[32] Pajączkowska J, Sułkowska A, Sułkowski WW, Jedrzejczyk M: Spectroscopic study of the humification process during sewage sludge treatment. Journal of Molecular Structure. 2003;651–653:141–149.

[33] Niemeyer J, Chen Y, Bollag JM: Characterization of humic acids, composts, and peat by diffuse reflectance Fourier-transform infrared spectroscopy. Soil Science Society of America Journal. 1992;(56):135–140.

[34] Lguirati A, Ait Baddi G, El Mousadik A, Gilard V, Revel JC, Hafidi M: Analysis of humic acids from aerated and non-aerated urban landfill composts. International Biodeterioration and Biodegradation. 2005;56:8–16.

[35] Madari BE, Reeves III JB, Machado PLOA, Guimarães CM, Torres E, McCarty GW: Mid- and near-infrared spectroscopic assessment of soil compositional parameters and structural indices in two Ferralsols. Geoderma. 2006;136(1):245–259.

[36] Simkovic I, Dlapa P, Doerr SH, Mataix-Solera J, Sasinkova V: Thermal destruction of soil water repellency and associated changes to soil organic matter as observed by FTIR spectroscopy. Catena. 2008;74(3):205–211.

[37] Smidt E, Lechner P, Schwanninger M, Haberhauer G: Characterization of waste organic matter by FT-IR spectroscopy: application in waste science. Applied Spectroscopy. 2002;56(9):1170–1175.

[38] Jouraiphy A, Amir S, Winterton P, El Gharous M, Revel JC, Hafidi M: Chemical and spectroscopic analysis of organic matter transformation during composting of sewage sludge and green plant waste. International Biodeterioration and Biodegradation. 2005;56:101–108.

[39] Grube MT, Lin JG, Lee PH, Kokorevicha S: Evaluation of sewage sludge-based compost by FT-IR spectroscopy. Geoderma. 2006;130:324–333.

[40] Hessen DO, Tranvik LJ (Eds.). Aquatic humic substances: ecology and biogeochemistry (Vol. 133). 1998. Springer Science+Business Media.

[41] Bigorre F, Tessier D, Pédro G: Contribution des argiles et des matières organiques à la rétention de l'eau dans les sols. Signification et rôle fondamental de la capacité d'échange en cations. Comptes Rendus de l'Académie des Sciences-Series IIA-Earth and Planetary Science. 2000;330(4):245–250.

[42] Tejado A, Peňa C, Labidi J, Echeverria JM, Mondragon I: Physico-chemical characterization of lignins from different sources for use in phenol-formaldehyde resin synthesis. Bioresource Technology. 2007;98:1655–1633.

[43] Boeriu CG, Bravo D, Gosselink RJ, van Dam JE: Characterisation of structure-dependent functional properties of lignin with infrared spectroscopy. Industrial Crops and Products. 2004;20(2):205–218.

[44] Xu F, Geng ZC, Sun JX, Liu CF, Ren JL, Sun RC, Fowlerd P, Baird MS: Fractional and structural characterization of hemicelluloses from perennial ryegrass (*Lolium perenne*)

and cocksfoot grass (*Dactylis glomerata*). Carbohydrate Research. 2006;341(12):2073–2082.

[45] Yang H, Yan R, Chen H, Lee DH, Zheng C: Characteristics of hemicellulose, cellulose and lignin pyrolysis. Fuel. 2007;86:1781–1788

[46] Zhao J, Xiuwen W, Hu J, Liu Q, Shen D, Xiao R: Thermal degradation of softwood lignin and hardwood lignin by TG-FTIR and Py-GC/MS. Polymer Degradation and Stability. 2014;108:133–138.

[47] Faix O, Argyropoulos DS, Robert D, Neirinck V: Determination of hydroxyl groups in lignins evaluation of 1H-, 13C-, 31P-NMR, FTIR and wet chemical methods. Holzforschung—International Journal of the Biology, Chemistry Physics and Technology of Wood. 1994;48(5):387–394.

[48] Sun R, Lawther JM, Banks WB: Fractional and structural characterization of wheat straw hemicelluloses. Carbohydrate Polymers. 1996;29(4):325–331.

[49] Piccolo A, Campanella L, Petronio BM: Carbon-13 nuclear magnetic resonance spectra of soil humic substances extracted by different mechanisms. Soil Science Society of America Journal. 1990;54:750–756.

[50] Preston C, and Schnitzer M: Effects of chemical modifications and extractants on the carbon-13 NMR spectra of humic materials. Soil Science Society of America Journal. 1984;48(2):305–311.

[51] Gosselink RJA, Abächerli A, Semke H, Malherbe R, Käuper P, Nadif A, van Dam JEG: Analytical protocols for characterisation of sulphur-free lignin. Industrial Crops and Products. 2004;19:271–281

[52] Xu F, Sun JX, Geng ZC, Liu CF, Ren JL, Sun RC, Fowlerd P, Baird M. S: Comparative study of water-soluble and alkali-soluble hemicelluloses from perennial ryegrass leaves (*Lolium peree*). Carbohydrate Polymers. 2007;67(1):56–65.

[53] Liu CF, Xu F, Sun JX, Ren JL, Curling S, Sun RC, Fowlere P, Baird MS: Physico-chemical characterization of cellulose from perennial ryegrass leaves (*Lolium perenne*). Carbohydrate Research. 2006;341:2677–2687.

[54] Méndez A, Fidalgo JM, Guerrero F, Gascoó G: Characterization and pyrolysis behaviour of different paper mill waste materials. Journal of Analytical and Applied Pyrolysis. 2009;86:66–73.

[55] Eyheraguibel B: Caractérisation des substances humiques biomimetiques—effets sur les végétaux, thèse de doctorat de l'institut national polytechnique de Toulouse. 2004. page 86.

Chapter 8

Biodegradation of Petroleum-Polluted Soils Using CNB-Tech – The Nigerian Experience

Iheoma M. Adekunle, Nedo Osayande and Temitope T. Alawode

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62116

Abstract

Remediation of petroleum-hydrocarbon-polluted soil via biodegradation process is viewed globally as an environmentally friendly process. In this study, an overview of past and present field-scale petroleum hydrocarbon biodegradation techniques utilized in Nigeria was conducted using the tools of literature review and field survey. Pilot-scale biodegradation of hydrocarbons in petroleum-impacted clay soil of up to 42-year-long contamination using novel and eco-safe CNB-Tech was carried out. This was followed by a comparative evaluation of crop growth performance on crude-oil-polluted soil remediated using a biodegradation technique adopted by a reputable oil company in Nigeria and the innovative CNB-Tech. The study revealed that CNB-Tech is an innovative, time-effective, cost-effective and eco-friendly bioremediation technique and has the potential to excel over some existing biodegradation procedures employed by many oil industries especially in the developing countries.

Keywords: Petroleum pollution, Biodegradation, Environment, CNB-Tech, Nigeria

1. Introduction

Nigeria is a constitutional federal republic, the most populous country in Africa with over 170 million people of divergent cultural values, inhabited by over 300 ethnic groups. The country comprises thirty-six states and the capital territory (Abuja) out of which nine (Abia, Akwa Ibom, Bayelsa, Cross River, Delta, Edo, Ondo, Imo and Rivers States) fall within the Niger Delta Region. The Niger Delta region is reputed for oil industry operations that commenced in 1956. The first oil well (Fig. 1) was discovered in Oloibiri, Bayelsa State, after which many oil wells were found in the other states of the Niger Delta Region. The advent of oil mining brought financial boom but afterward came trails of petroleum-based pollution. Environmental degradation due to crude oil spill on land, into the swamps and water bodies with attendant

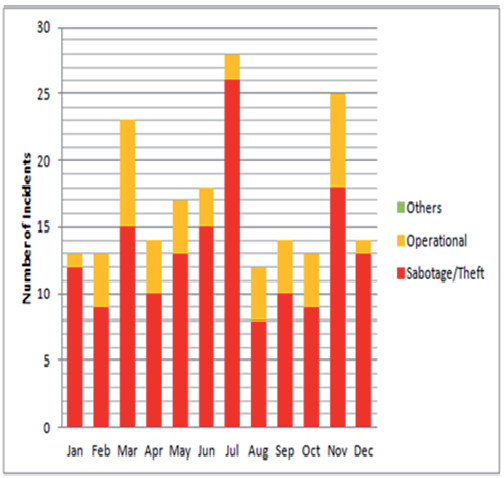
consequences on the ecosystem and public health became topical issue both at the national and international levels. Factors influencing petroleum-based environmental pollutions in the country were identified as: (i) operational failures (corrosion of pipeline, human error and equipment failure); (ii) accidental discharge; (iii) acts of sabotage (oil theft, pipeline bunkering and artisanal refining) and (iv) inappropriate handling and disposal of petroleum wastes.

Figure 1. Status of the first oil well in Nigeria 57 years after discovery, from 1956 to 2013

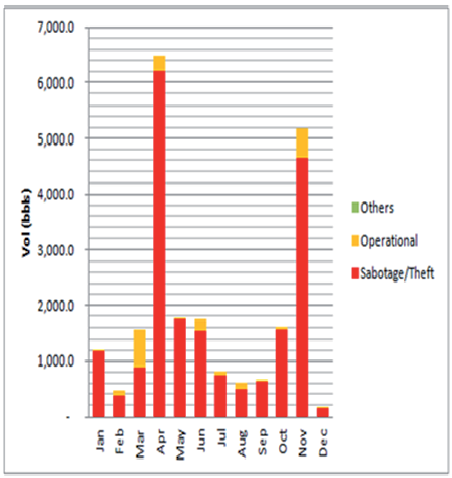
Most of the oil companies claim that acts of sabotage contribute the most to the release of petroleum products into the environment relative to operational failures. This is corroborated by some spill data (Fig. 2) put in the public domain by the Shell Petroleum Development Company, Port Harcourt, Nigeria [24]. These data show that oil spill incidents traceable to operational failure range from 7 to 35%; inferring that acts of sabotage are responsible for 65–93% of oil spill in the Nigerian environment. Secondary data as shown in Fig. 2a bring out the following facts: (i) the number of oil spill incidents and spill volumes are recorded on a monthly basis, (ii) a high spill incident number does not necessarily imply a high spill volume. For instance, the highest number of spill incident (28) was recorded in July 2014, but the largest volume of spill was obtained in April, 2014 with a total number of 14 spill incidents, (iii) acts of sabotage seem to be at the peak three times in a year (at the beginning of the year [92%], midyear [93%], and end of year [93%]) and (iv) the season of the year (wet or dry) does not really play a significant role in the acts of sabotage. These facts, however, require further verification by conducting more detailed analyses using statistical data of previous years.

http://dx.doi.org/10.5772/62116

Irrespective of the oil spill causative factor, petroleum-based pollution endangers the entire environment including the human population [25]. Once petroleum product (crude or refined) is either intentionally or unintentionally released into the environment, the consequences remain the same. In any community impacted by oil spill, the degree of response to such an incident plays an important role in ensuring environmental safety, protection, and sustainability. From the environmental standpoint, the most important issue is that swift, positive and appropriate action aimed at safeguarding the ecosystem be taken once an oil spill occurs.



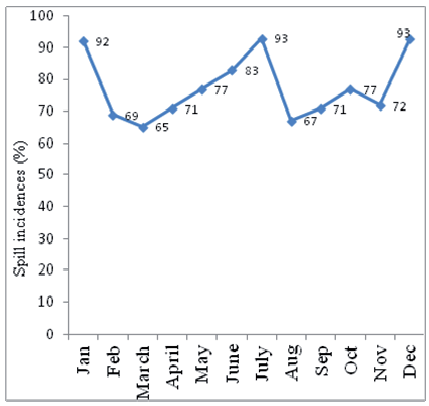
Monthly incidents of oil spill



Volume of spill per month

(a)

S N	Month	Total spill incident	Operational failure		Acts of sabotage	
			No	%	No	%
1	January	13	1	8	12	92
2	February	13	4	31	9	69
3	March	23	8	35	15	65
4	April	14	4	29	10	71
5	May	17	4	24	13	77
6	June	18	3	17	15	83
7	July	28	2	7	26	93
8	August	12	4	33	8	67
9	September	14	4	29	10	71
10	October	13	3	23	10	77
11	November	25	7	28	18	72
12	December	11	1	7	13	93



(b)

Figure 2. a: Oil spill incidents and volume of spill for 2014 in the Niger Delta region of Nigeria as presented by the Shell Petroleum Development Company, Port Harcourt, Nigeria. b: Secondary data showing numerical values of oil spill incidents, volume of spill, and trend in oil spill incident due to sabotage for 2014 in the Niger Delta region of Nigeria

Response actions include site cleanup via recovery of free phase oil, subsequent reduction of the residual petroleum hydrocarbon concentrations to an acceptable value, followed by restoration of the environment to its previous utility status. Options for the reduction of residual petroleum hydrocarbon concentrations are preferably eco-safe techniques. After a cleanup exercise, detoxification of soils polluted with residual petroleum hydrocarbon compounds is necessary. There are different methods by which the concentrations of these pollutants (total petroleum hydrocarbon – TPH, and polynuclear aromatic hydrocarbon – PAH) could be reduced to fall within the acceptable level. The major mechanism involves degradation processes. Degradation generally applies to the breakdown or transformation of complex materials into simpler ones.

Various types of degradation processes include (i) thermal degradation that occurs via the application of heat, (ii) mechanical degradation, which takes place by the application of mechanical force, (iii) photo degradation, which is the transformation of complex compounds by the action of sunlight, (iv) oxidation/chemical degradation that occurs by the addition of chemicals and (v) biodegradation, which proceeds by the action of microorganisms (yeast, fungi, or bacteria). Organic substances that can be broken down by the action of microorganisms are said to be biodegradable. The technique that enables the application of biodegradation to clean up biodegradable organic pollutants from the environment is referred to as bioremediation. An example of a class of organic compounds that can be detoxified via biodegradation is petroleum-derived hydrocarbons. Petroleum-based hydrocarbons generally belong to the normal hydrocarbons known in organic chemistry. Hydrocarbons vary in their degree of susceptibility to microbial degradation. Some high molecular weight polynuclear aromatic hydrocarbons (PAHs) may not be degraded by microorganisms at all. Biodegradation of hydrocarbons proceeds through the major pathways presented in Fig. 3 [19]. A given hydrocarbon is eventually transformed to an acid, which is finally converted to innocuous end product(s).

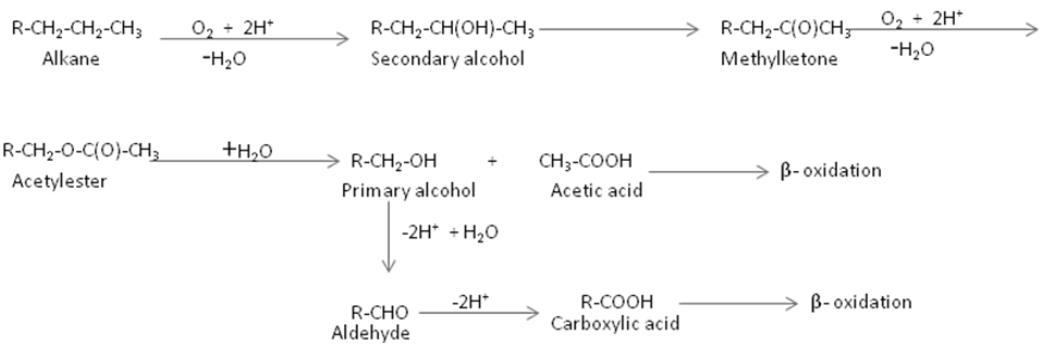


Figure 3. Major processes involved in biodegradation of a typical hydrocarbon compound

For a given biodegradation process, a hydrocarbon compound is generally transformed, through biochemical processes, to more polar organic compounds such as alcohol, ketone, aldehyde and organic acid. Essentially, biodegradation of an organic pollutant depends on the

http://dx.doi.org/10.5772/62116

nature of the target compounds, environmental factors and microorganisms as highlighted in Table 1 [9, 23, 26, 27]. The success of biodegradation of petroleum hydrocarbons at the field-scale platform is highly dependent on effective maneuvering of these three factors. Doing otherwise would endanger the environment.

S/N	Target Compound (Petroleum Hydrocarbon)	Environmental Factor	Microorganism Factor
1.	**Chemical characteristics**: The nature or type of hydrocarbon compounds determines chemical properties that ultimately affect biodegradation. Hydrocarbons could be alkenes or alkanes, cyclic aliphatics or acyclic aliphatics, mononuclear aromatics or polynuclear aromatics in nature. Generally, the extent of biodegradation tends to decrease with increase in number of rings, the degree of condensation and the number of alkyl substituents on the aromatic nucleus. An acceptable sequence for the degree of susceptibility to microbial degradation is: alkanes > branched alkanes > small aromatics > cyclic alkanes	**Temperature**: This affects the viscosity, solubility and bioavailability of hydrocarbons. It also affects the physiology and diversity of microorganisms. Although hydrocarbon biodegradation can occur over a wide range of temperature, the rate of biodegradation generally decreases with decreasing temperature	**Type of microbes**: Microbes involved in biodegradation are fungi, yeast and bacteria. They have their potentials and limitations
2.	**Concentration**: The higher the concentration of a given type of hydrocarbon compound, the slower is the biodegradation	**Nutrients**: Major nutrients required to enhance or stimulate microbial activities are C, N, P. These are important for metabolism. This explains why many stakeholders apply inorganic NPK fertilizers for nutrient amendment. However, the application of NPK fertilizers has inherent negative environmental consequences. In addition, excessive nutrient concentrations can also inhibit the biodegradation activity	**Microbial population**: The population density of microbes is directly related to biodegradation
3.	**Toxicity**: Highly toxic hydrocarbon compound will require more resistant microbes	**Photo degradation**: Photo-oxidation increases the biodegradability of petroleum hydrocarbons by increasing its bioavailability and thus enhancing microbial activities	**Microbial diversity**: The more the variety of microbial population, the more efficient the biodegradation process

S/N	Target Compound (Petroleum Hydrocarbon)	Environmental Factor	Microorganism Factor
4.	**Polarity**: This factor facilitates solubility in polar solvents such as water, hence increases solubility and bioavailability for biodegradation	**Soil properties**: (i) Soil organic matter content: this readily absorbs hydrophobic compounds such as petroleum hydrocarbons. The major binding sites in soil organic matter are the soluble humic substances, in particular, humic and fulvic acids. (ii) Soil moisture: facilitates biodegradation of petroleum compounds because microbes thrive better in moist than in dry environments (iii) Soil pH: is a measure of soil acidity or alkalinity. The acidity (pH) of the soil is an important soil parameter. Soil pH can vary from 2.5 (highly acidic soils) to 11.0 (highly alkaline soils). Soil pH value affects microbial activity with moderate alkaline being the most favorable (iv) Soil aggregate: this increases bioavailability of the pollutant (v) Soil oxygen: little or no hydrocarbon metabolism occurs in strictly anoxic soil condition; hence, oxygen is a very important parameter for biodegradation	**Microbial enzyme activity, adaptability, reproduction potentials, and metabolic capabilities**: The more the enzyme activity, the better and faster the biodegradation process. Microorganisms that can easily adapt to different types of environments are more suited for bioremediation methods. Microbial species with high population turnover is more desirable The availability of microorganisms with appropriate metabolic capabilities is a major requirement for biodegradation of oil sample

Table 1. Description of basic factors that influence the success of a biodegradation process

The objectives of this study are (i) to present an overview of past and present practices in field-scale biodegradation procedures employed in the detoxification of petroleum hydrocarbon polluted soils in Nigeria and (ii) to demonstrate the efficacy of the novel, eco-safe and nano-technology based bioremediation technique (CNB-Tech) in the remediation and restoration of petroleum impacted soils to beneficial end products.

2. Research Methodology

In this study, the research methods used were literature review, field survey, screen house farming, pilot-scale bioremediation and standard laboratory techniques for relevant chemical and biological analyses.

http://dx.doi.org/10.5772/62116

2.1. Assessment of field-scale petroleum hydrocarbon biodegradation techniques utilized in Nigeria: past and present

Research tools used for this study were literature review and field survey. Formal and informal interactions with relevant stakeholders utilizing remediation procedures in petroleum industries and remediation project sites.

2.2. Pilot-scale biodegradation of hydrocarbons in petroleum impacted soil using novel and eco-safe CNB-Tech

Research method employed for this study was a practical pilot-scale remediation using a biodegradation process referred to as CNB-Tech, whose basic procedure has been described in [1]. However, there were modifications specific to the sample matrix used in this study. Permissions to procure petroleum impacted soil material consignments from the Shell Petroleum Development Company's remediation project site and to conduct the pilot-scale project were obtained from the appropriate authorities in the company. The spill site of about 15.6 hectares was situated between latitude 4°N and longitude 7° 7.5'E, in Eleme Local Government Area of Rivers state. This site was impacted by crude oil in 1969 as a result of damage by external device to Bomu-Bonny Trans Niger Pipeline (TNP) at Ejema and was accompanied by fire outburst. The hydrocarbon pollution was therefore up to 42 years long at the time study (ERMS, 2011). With the assistance of project site workers, clay soil sample bulk was collected in 2 x 200 L plastic drums, which were immediately conveyed to the pilot-scale remediation project site in Shell Industrial Area (Shell IA), Port Harcourt.

CNB-Tech biodegradation procedures were then applied to the samples. Untreated clay soil samples served as controls. Both controls and tests were replicated three times. Composite samples, collected under appropriate conditions and methods (before and after treatment) were sent to an ISO certified laboratory in the USA (by courier) and another in Nigeria for the analyses. Quality control and quality assurance protocols were strictly followed and parameters of interest were:

- Hydrocarbon compounds: Total petroleum hydrocarbon (TPH) and 17 polynuclear aromatic hydrocarbons (PAHs)
- Soil fertility parameters: pH, electrical conductivity and nitrogen (N), phosphorus (P), potassium (K)
- Heavy metals: Lead (Pb), mercury (Hg), arsenic (As), barium (Ba), copper (Cu), zinc (Zn), cobalt (Co), and nickel (Ni)
- Soil recovery and restoration indices: Reestablishment of microbial community and ability to sustain plant life investigated via microbial activity assessments at 48 h and 96 h periods (conducted only by the USA-based laboratory) and seed germination potential assessment conducted in Nigeria.

As a demonstration of the beneficial utility of the end product, the CNB-Tech remediated soils were used to grow indicator crops, namely *Zea mays L.*, (corn), *Telfairia Occidentalis* (fluted pumpkin), and *Manihot esculenta Crantz* (cassava) in screen house farming scheme but only the results of the second crop are reported in this presentation.

2.3. Comparative evaluation of growth performances for cassava crop grown in crude-oil-polluted soils remediated using biodegradation technique (RENA) adopted by a reputable oil company in Nigeria and the innovative CNB-Tech

In this study, soil samples from one of the rural communities in Rivers State, Nigeria, called Bomu (K-Dere) in Gokana, Ogoniland (Fig. 4), where crude-oil-impacted farm land area was remediated using RENA technique, were collected and used for this comparative evaluation. The major remediation technique adopted by one of Nigeria's leading international oil companies (the Shell Petroleum Development Company, Port Harcourt, Nigeria) for crude-oil-impacted soil, at the time of study, is referred to as RENA (Remediation by Enhanced Natural Attenuation). Permission to conduct the investigation was obtained from the designated authority of the oil company. Sample collection was supervised by (i) two representative staff of the oil company, (ii) a community relations officer (CRO) and (iii) some representatives of the community youth forum. Due to low literacy level, oral interviews were conducted on the community representatives to elicit information on factors such as (i) type of actions taken during the RENA remediation project, (ii) common utility of the land area prior to spill and (iii) experiences of farmers utilizing the remediated land area. Information was also obtained from the staff of the oil company on the mode of RENA remediation works carried out at the study site.

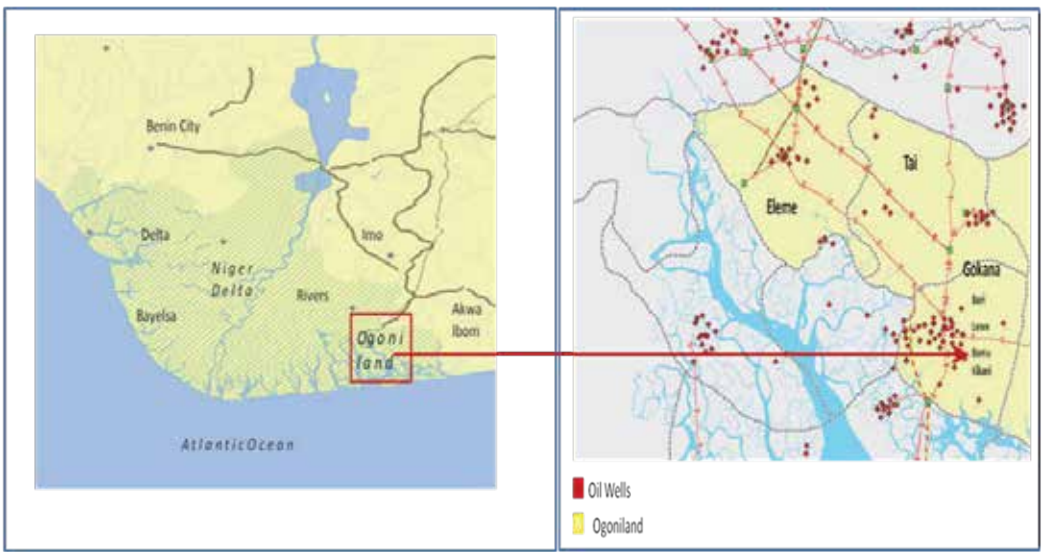


Figure 4. Map showing the location of Bomu in Ogoniland, Nigeria; sourced from [25].

Soil sample collection and analysis: Due to the heterogeneity found at the site, the land area was delineated into three zones (Subsite A, Subsite B, and Subsite C), which are briefly described as follows: (i) Subsite A stood for RENA remediated land area where no food crop was grown. Bulk sample from this site was denoted as IMS. (ii) Subsite B stood for RENA remediated land area used by natives for crop production. Only one type of food crop (Cassava:

http://dx.doi.org/10.5772/62116

Manihot esculenta Crantz) was grown in this subsite. Bulk soil sample collected from this site was denoted as RMS. (iii) Subsite C stood for agricultural area/farm soil, which the locals claimed did not experience crude oil spill. Samples collected from Subsite C were denoted as AGS. This subsite served as control. Following delineation exercise, soil samples were randomly collected from 14 places in a given subsite at depth 0–0.30 m. Samples collected from these places were mixed to give a composite of approximate weight of 56 kg. Bulk soil sample from each subsite was differently stored in sacks and conveyed to project site within 60–90 min.

On arrival at the pilot-scale remediation project site in Port Harcourt, the three different sample bulks of 56 kg each were homogenized, spread out on blue PVC sheets (in order not to contaminate the surrounding environment), air dried in the laboratory and then sieved through a 2 mm mesh size. Grid templates of 12 cells were then created for each sample bulk as shown in Fig. 5. Approximately 2 kg soil was collected from each of the12 subcells, mixed together to give the final composite of 24 Kg soil for a subsite. This was repeated four more times to give five replicate samples for each subsite. All together, 15 samples (n = 15) were obtained for the three subsites in the study area. The 15 soil samples were contained in properly labeled sample bottles, transferred into thermostated, ice-packed boxes and sent to a Chemical laboratory (Laser Engineering and Resources Consultants Limited, Port Harcourt, Nigeria) certified by the National regulatory body. The 15 parameters analyzed for in each soil sample were: temperature, pH, electrical conductivity (EC), total organic carbon (TOC), total nitrogen (N), soil organic matter (SOM), total petroleum hydrocarbons (TPH), potassium (K), sodium (Na), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), nickel (Ni) and zinc (Zn) using standard methods.

Figure 5. The three different sample bulks showing grid template of 12 cells created on each sample, from which samples were collected for physicochemical analyses

Preassessment of RENA remediated soils for crop production: After sample collection for physicochemical analyses, the homogenized and gridded soil samples for each subsite were

remixed and transferred to labeled and designated evaluation pots at 4 Kg per pot and at three replicates per site, giving a total of nine experimental pots in all. All the pots were then arranged in completely randomized block design. The soil in each pot was thereafter watered and allowed 14 days to stabilize. No hole was made in the pots (to avoid loss of matter) but to forestall flooding; soils were watered at about 60% of approximate field capacity, determined against gravity. Cassava (*Manihot esculenta Cranzt*) was selected as indicator crop. A long stem of cassava was obtained from an indigenous farmer who was harvesting from his farm at Subsite C during this study at K-Dere. This was done to ensure utilization of same cassava crop type for (i) the investigation and (ii) the actual farming by the locals. The cassava stem was then professionally cut to uniform pieces of 15 cm (length) for each; to allow for planting in the experimental pots. Complete burial planting method was adopted and stems were planted one per pot. On germination, agronomical parameters monitored during the period of growth were (i) plant height, (ii) stem girth and (iii) plant leaf number. Germinated crops were allowed to grow for 91 days before harvest.

Comparative evaluation of RENA and CNB-Tech remediated soils for crop production: Following the preassessment of RENA remediated soils and based on crop performance, only the soils collected from Subsite B (RMS) and Subsite C (AGS) were used for this comparative evaluation. Procedures previously described for preassessment of RENA remediated soils for crop production were adopted but due to project time constraint, the crops were grown for 37 days.

2.4. Statistical analysis

Data obtained in this study were subjected to relevant statistical analysis using SPSS 17.0 for Windows Evaluation Version. Descriptive statistics were used to obtain means and deviations, Pearson linear correlations were useful for the establishment of relationships and means were compared by Analysis of variance (ANOVA).

3. Results and Discussion

3.1. Review of biodegradation procedures employed for detoxification of petroleum-hydrocarbon-polluted soils in Nigeria

Information from literature review showed that most researchers focused on two major factors: (i) isolation of potential hydrocarbon degrading microbial strains and biostimulation via nutrient augmentation. For instance, [17] isolated about 15 hydrocarbon-degrading bacterial and fungal species from three bitumen deposits believed to be of relevance in biodegradation of petroleum (kerosene and diesel) contaminated systems in Nigeria. [9] carried out an experiment involving biostimulation with agricultural fertilizers to evaluate the biodegradation of hydrocarbon compounds found in a crude-oil-polluted agricultural soil at different levels of soil water. Petroleum pollution of an agricultural soil was simulated on the field by pouring crude oil on the cells from perforated cans. Biostimulation options were (i) introduction of mineral fertilizers and (ii) periodic application of different amounts of water. Results showed an increase in the total heterotrophic bacterial (THB) counts and a corresponding

http://dx.doi.org/10.5772/62116

reduction in soil organic carbon and total hydrocarbon content (THC) at the end of the six-week remediation period. The implication is that by manipulating soil water content and nutrient levels (via inorganic fertilizer application), microbial population and activity were stimulated, suggesting that the level of water in the soil is a major factor that affects biodegradation rate. The use of isolated microbial strains to biodegrade petroleum hydrocarbon has not been successfully applied at the field scale for the remediation and restoration of crude-oil-polluted soils. Most of these works are still at the laboratory scale.

In practice, oil companies in Nigeria contract out bioremediation projects to certified vendors who then apply approved technologies under the supervision of the particular oil company and National Regulatory Agencies. The most commonly practiced bioremediation is land farming, a process believed to utilize indigenous microorganisms to biodegrade petroleum hydrocarbon pollutants under specified conditions.

This is a type of biodegradation by enhanced natural attenuation, which goes by different names for different companies such as RENA for the Shell Petroleum Development Company, Nigeria [25]. Limitations of in situ biodegradation via land farming where environmental controls are not put in place are highlighted in Table 2.

The issues highlighted in Table 2 clearly show that in situ biodegradation via land farming without the necessary environmental control measures, as often practiced, do not achieve legislative compliance and do not meet best management practices locally or internationally and constitute risk to the environment and public health.

S/N	Environmental Issues	Implication
1.	Impact of rainfall/precipitation	When rain falls on the project site, due to lack of critical environmental controls, there will be leaching of hydrocarbons from the windrows and runoffs will be generated
2.	Effect of temperature	This results in evaporation of hydrocarbons with associated occupational hazards to on-site workers and endangered health of neighboring communities
3.	Fate of runoffs	Runoffs emanating from impact of rainfalls on the windrows, constructed during land farming, will endanger nearby farms, communities, swamps, water bodies (ponds, lakes, streams, rivers, and groundwater). Runoffs have the potential to increase polluted land area
4.	Air pollution	Increased temperature such as is experienced in Nigeria will enhance the presence of volatile hydrocarbons in the atmosphere, resulting in air pollution. Most often, air pollution is not monitored during the remediation projects
5.	Vertical infiltration of pollutant	During the in situ biodegradation via land farming, the absence of impervious barriers allows for vertical penetration of oil/pollutants, thus resulting in the pollution of subsoil and groundwater

Table 2. Limitations of in situ biodegradation via land farming

3.2. Results on biodegradation of petroleum hydrocarbons in crude-oil-impacted clay soils using CNB-Tech

Biodegradation of total petroleum hydrocarbons: the initial concentration of total petroleum hydrocarbon (TPH) contained in the crude-oil-impacted clay soil was 33600 ± 245 mg/kg, as provided by the USA-based laboratory. On first dose application of CNB-Tech procedure, the concentration was reduced to 4193 ± 344 mg/kg, corresponding to 87.52% biodegradation. Second dose application further reduced the concentration to 293 ± 20 mg/kg, corresponding to 99% reduction relative to the initial concentration of TPH. The guideline for TPH level in soil stipulated by the Nigeria regulatory body is 5000 mg/kg [11, 21], implying that the CNB-Tech-treated soils contained hydrocarbons within the acceptable range.

Biodegradation of polynuclear aromatic hydrocarbons: Out of the 17 PAH compounds analyzed for in the petroleum-polluted clay soils, only 5 were detected. The individual components of the PAHs found in the petroleum-hydrocarbon-contaminated soil and their respective concentrations in this study were: (i) benzo (k) fluoranthene (8.84 ± 0.71 mg/kg), (ii) benzo (a) pyrene (15.33 ± 3.79 mg/kg), (iii) dibenzo (a,h) anthracene (18.51 ± 9.68 mg/kg), (iv) benzo (g,h,i) perylene (25.02 ± 6.10 mg/kg) and (v) indeno (1,2,3-cd) pyrene (24.69 ± 9.30 mg/kg). Total concentrations summed up to 92.39 ± 26.82 mg/kg. The percentage composition of these PAH compounds relative to the total concentration is presented in Fig. 6.

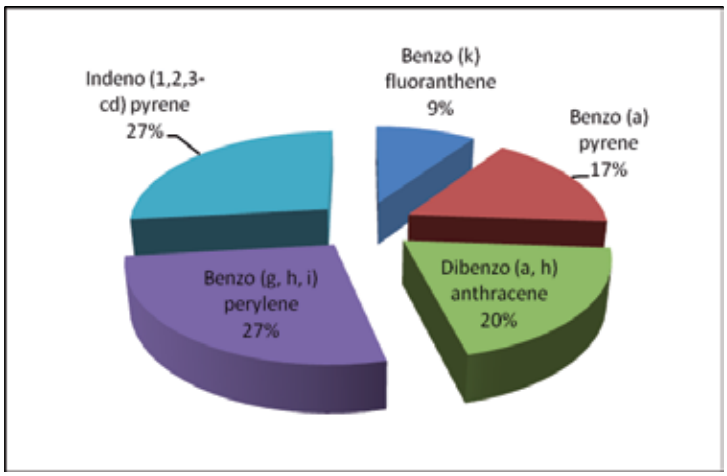


Figure 6. Percent composition of PAH compounds relative to total PAH found in the petroleum-impacted clay soil

Amazingly but very reassuring, none was detected in the CNB-Tech treated samples. Results from the Nigeria-based laboratory showed that by the application of CNB-Tech remediation procedures to the petroleum-hydrocarbon-polluted clay soils, the five PAHs were completely degraded, resulting in 100% reduction in concentration.

Reduction in soil heavy metal concentration: Concentrations of the metals found in the treated soil samples fell below the acceptable levels (Table 3). Relative to the initial concen-

http://dx.doi.org/10.5772/62116

trations in the polluted soils, heavy metal concentrations were reduced by 2.38 to 100% for the different metals presented in Fig. 7.

S/N	Parameter	Mean ± SD (Nigeria)	Mean ± SD (USA)	n	DPR Intervention Value
1.	pH	7.47 ± 0.06 (7.40–7.50)	9.06 ± 0.12 (9.00–9.20)	3	NA
2.	Cd (mg/kg)	7.05 ± 0.60 (6.40–7.65)	ND	3	12
3.	Cu (mg/kg)	9.37 ± 0.53 (7.70–9.85)	12.30 ± 0.69 (11.50–12.70)	3	190
4.	Pb (mg/kg)	BDL	5.79 ± 0.66 (5.10–6.41)	3	530
5.	Ni (mg/kg)	4.55 ± 1.34 (3.10–5.75)	3.39 ± 0.58 (2.96–4.05)	3	210
6.	Zn (mg/kg)	122.86 ± 4.20 (120–128)	51.73 ± 19.50 (12.90–74.40)	3	720
7.	Co (mg/kg)	BDL	ND	3	240
8.	As (mg/kg)	BDL	ND	3	55
9.	Cr (mg/kg)	11.13 ± 1.17 (10.10–12.40)	ND	3	380
10.	Hg (mg/kg)	4.83 ± 0.50 (3.90–5.60)	0.02 ± 0.01 (BDL -0.03)	3	10
11.	Ba (mg/kg)	ND	437.33 ± 66.71 (263–4920	3	625

DPR = Department of Petroleum Resources, BDL = below detection limit, ND = not determined, n = sample population and NA = not available, values in parenthesis stand for minimum–maximum

Table 3. Selected properties (pH and heavy metal levels) of CNB-Tech treated soil samples

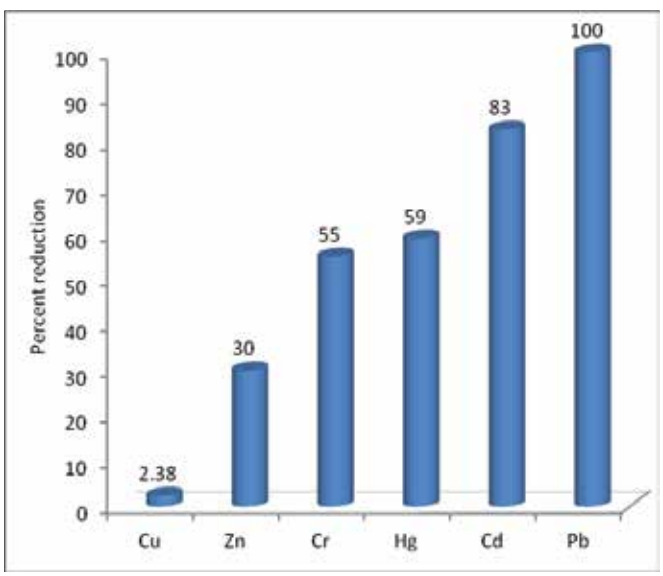


Figure 7. Reduction in soil heavy metal concentrations in CNB-Tech-treated soil samples

Soil material physical appearance: The outlook of the crude-oil-impacted clay soils before and after treatment is presented in Fig. 8. Before treatment, contaminated soil was characterized by strong hydrocarbon odour and was lumpy/pasty in nature. It also exhibited strong oil sheen in soil–water suspension. However, after treatment, there was complete disappearance of hydrocarbon odour and soil texture transformed to nonpasty. The colour changed from dark brown to black, characteristic of humus soil.

A cross section of hydrocarbon contaminated clay soil at site before treatment

Soil after treatment using CNB-tech products

Figure 8. Appearance of the crude-oil-impacted clay soil before and after CNB-Tech treatment

Microbial activity assessment: The digital captures of microbial population in two replicates (RS01 and RS02) of CNB-Tech-treated soil samples and the polluted, untreated crude-oil-impacted clay soil (CTS) are shown in Fig. 9a, while the quantitative counts are presented in Fig. 9b. For ease of presentation, all the values presented in Fig. 9b were raised to 10^7.The mean microbial population for 48 h microbial activity assessment for CNB-Tech-treated samples was 1.98 x 10^7CFU/mL and for 96 h activity assessment, the mean microbial population was 3.56 x 10^7 CFU/mL. The polluted, untreated crude-oil-impacted clay soil samples gave 1.52 x 10^6 CFU/mL and 1.84 x 10^6CFU/mL for 48 h and 96 h activity assessments, respectively.

At 48 h and 96 h assessments, the microbial activity found in the CNB-Tech treated soils exceeded that found in the polluted samples by approximate factors of 13 and 19, respectively. Results indicate that the polluted clay soils did not totally inhibit microbial growth, unlike what was obtainable for polluted oil-based mud [1]. CNB-Tech treatment replenished the microbial community. When soil is fully recovered and administration of treatment terminated, microbial population gradually adjusts back to normal population in the habitat [1].

Utility of CNB-Tech biodegradation end product: The success story of CNB-Tech procedures is not only in the reduction of concentrations of total petroleum hydrocarbons, polynuclear aromatic hydrocarbons and heavy metals but also in the utility of the treated substrates. CNB-Tech biodegradation procedures convert the petroleum impacted soil to "clean" reusable substrate. In this study, the petroleum-impacted clay soil was converted to arable soil suitable

http://dx.doi.org/10.5772/62116

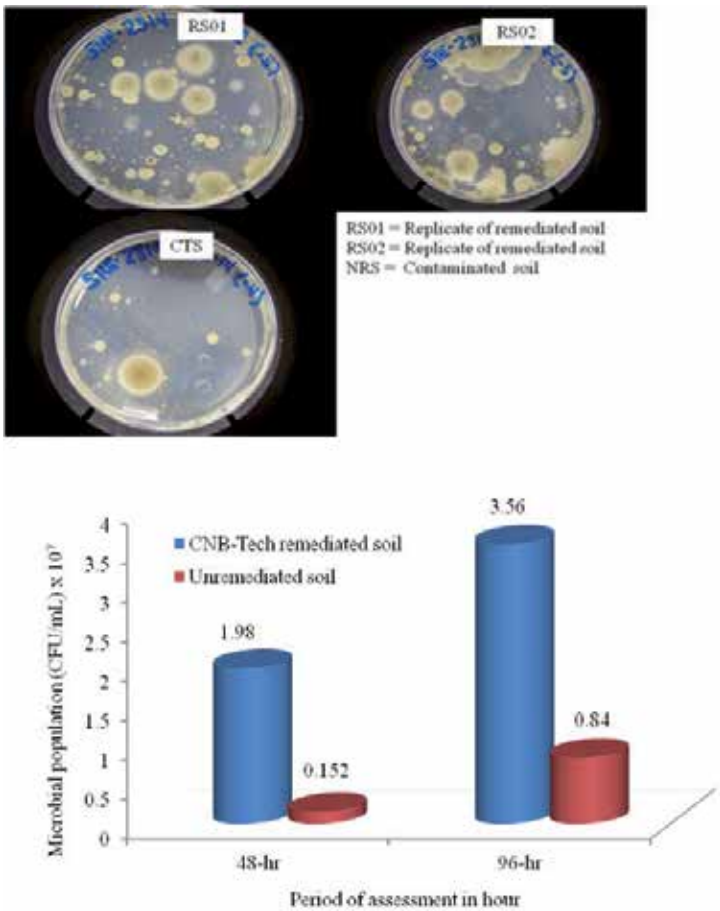


Figure 9. a: Qualitative microbial population in contaminated soil sample (CTS) and samples undergoing remediation (RS01 and RS02) as obtained by the USA-based laboratory. b: Quantitative representation of the microbial population in contaminated soil sample (CTS) and samples undergoing remediation (RS01 and RS02) as obtained by the USA-based laboratory

for crop production. The utility of CNB-Tech-treated soils in crop production was demonstrated in Fig. 10, in which the appearance of green leafy vegetable (Fluted pumpkin: *Telfairia Occidentalis*) grown in CNB-Tech-treated soil [(a) and (b)] was compared with that grown in a real farm (c) in an area not impacted by crude oil within Rivers State, Nigeria.

In terms of crop growth, the CNB-Tech remediated soils gave excellent support to both germination and growth of the vegetable crop. A mean plant height of 207 ± 10 cm was recorded for crops grown in CNB-Tech-treated soils, which excelled over crop performance (171 ± 8 cm) of vegetable crops grown in the control (farm soil) by 21%. On the other hand, petroleum-impacted clay soils used in this study did not support germination or growth of the vegetable, giving 100% inhibition to plant growth. The aim of remediation is to restore polluted site/land area to its previous use or modified beneficial use. The common land use in the Niger Delta region of Nigeria is crop production. Results have shown that CNB-Tech biodegradation

remediation protocol achieved detoxification and restoration of petroleum-hydrocarbon-polluted soil to original land use. Results are in line with the findings reported by [1] for the treatment of polluted-oil-based mud using CNB-Tech. The enhanced crop growth performance of CNB-Tech treated soils could be attributed to increased fertility of the treated soils as supported by data on NPK status obtained in this study. Nitrogen was increased from 0.026% to 0.431%. Phosphorus was raised from mean values of 0.003 to 2.530% and potassium was raised from 0.082% to 0.481% (results from Nigerian laboratory). This is further strengthened by favorable pH status (which has the potential to enhance plant nutrient uptake and soil microbial activity) and reduction of heavy metal concentration (Fig. 7) thereby reducing their potential phytotoxicity.

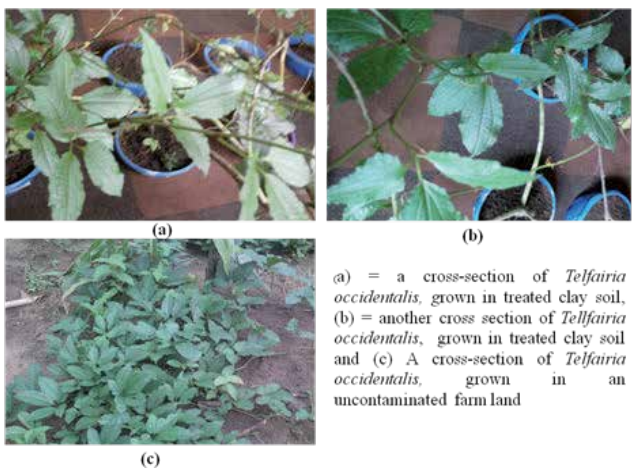


Figure 10. Digital capture, showing cross sections of a green, leafy vegetable crop (Fluted pumpkin: *Telfairia occidentalis*) grown in CNB-Tech-treated clay soils [(a) and (b)] and the same crop grown in a real farm (c); in an area not impacted by crude oil within Rivers State.

The safety of crops grown in CNB-Tech treated soils for animal and human consumption is presently under intensive investigation. The crops are being assessed for hydrocarbon and metal contents in addition to other phytotoxicological parameters. Results of these investigations will soon be published.

3.3. Comparative evaluation of CNB-Tech and RENA remediated soils for crop production

Results on comparative evaluation of CNB-Tech and RENA remediated soils for crop production are presented and discussed. Data are provided on (i) plant height, (ii) stem girth and (iii) leaf number.

3.3.1. Results from preassessment of RENA remediated soils

Plant height: Data obtained for the preassessment of RENA remediated soils' potential to support crop growth are presented in Fig. 11-13. Results showed that the cassava heights for the three subsites A, B, and C increased with growth period. The best height was obtained for

http://dx.doi.org/10.5772/62116

soils collected from the agricultural land area (Subsite C: AGS). Correlations with growth period gave coefficients of 0.897 ($p = 0.001$) for Subsite A, 0.987 ($p < 0.001$) for Subsite B, and 0.963 ($p < 0.001$) for Subsite C. This indicates that crop growth increased with time. After harvest, crop height for Subsite C (unpolluted farm soil: AGS), which served as the control, was 55.73 ± 8.75 cm. Relative to this, the mean height for cassava crop grown in RENA remediated soil/Subsite C (RMS) and Subsite A (IMS) were 30.87 ± 9.07 cm, representing 44.61% reduction and those grown in Subsite A (IMS) gave a mean height of 28.18 ± 3.05 cm, corresponding to 49.43% reduction [relative to the control (Subsite C: AGS)].

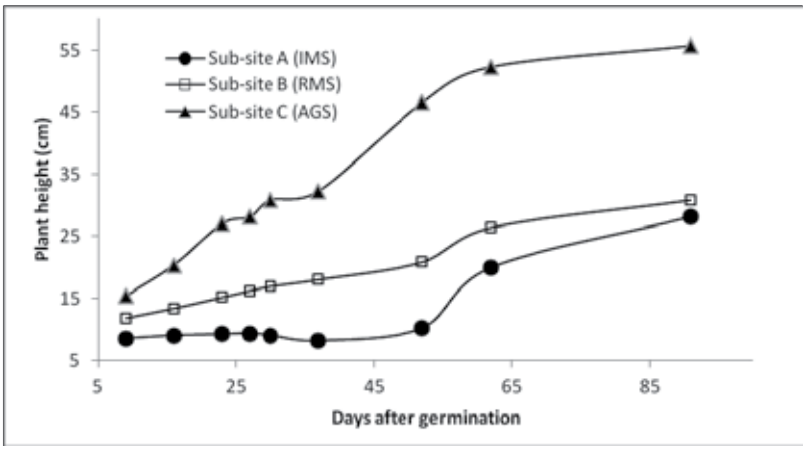


Figure 11. Variance of height of cassava crop grown in certain RENA remediated soils with time

Stem girth and leaf number: Graph of changes in stem girth relative to growth period is shown in Fig. 12. Results also showed that, similar to plant height, stem girth also increased with growth period (Fig. 12). Correlations with the growth period gave coefficients of 0.950 ($p < 0.001$) for Subsite A (IMS), 0.868 ($p = 0.002$) for Subsite B (RMS), and 0.865 ($p = 0.003$) for Subsite C (AGS).

The cassava grown in the control (AGS) produced mean stem girth of 2.20± 0.01. Relative to this, the crop grown in RENA remediated soil (RMS) manifested 48.64% reduction in stem girth, having a mean stem girth of 1.13± 0.06 while that grown in IMS experienced 53.18% reduction; having stem girth of 1.03 ± 0.01. Graph of changes in leaf number relative to growth period is shown in Fig.13. The coefficient of correlation for leaf number versus growth period was 0.871 ($p < 0.002$) for IMS, 0.774 ($p = 0.014$) for RMS, and 0.903 ($p = 0.001$) for AGS. The mean leaf number of cassava grown in the control (AGS) was 55 ± 1. Using the performance of cassava in AGS as reference, cassava crops grown in RENA remediated soils (RMS and IMS) experienced 36.36% and 49.09% reductions in leaf number, respectively; having leaf numbers of 28 ± 6 and 35 ± 6, respectively. Generally, results showed that irrespective of the agronomical parameter, the best performance was obtained in this order: AGS (Subsite C) > RMS (Subsite B) > IMS (Subsite A).

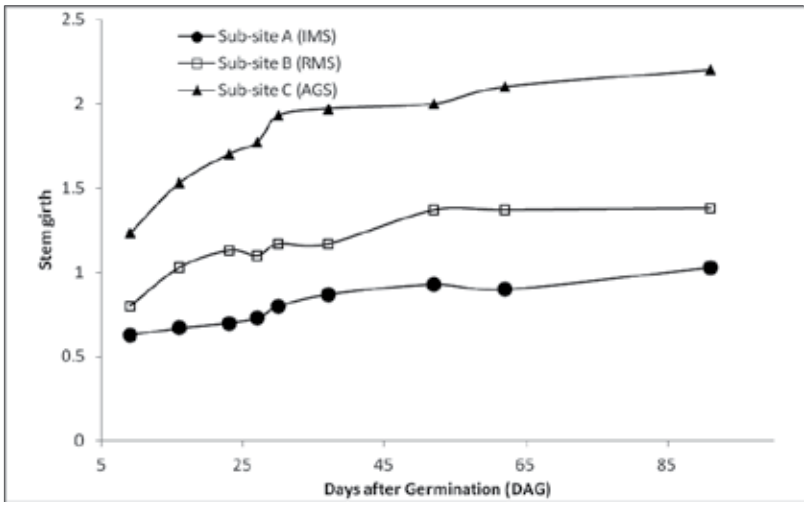


Figure 12. Variance of stem girth of cassava crop grown in certain RENA remediated soils with time

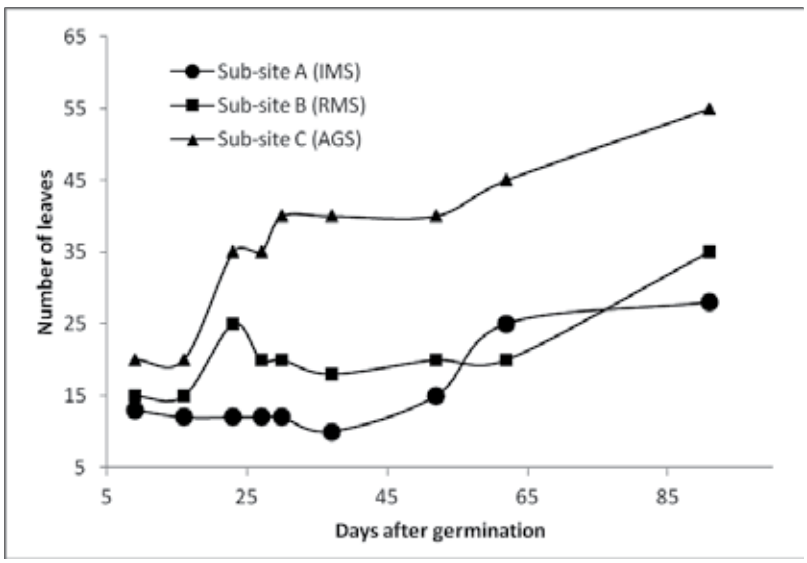


Figure 13. Variance of leaf number of cassava crop grown in certain RENA remediated soils with time

The very poor performance of crops grown in IMS (Subsite A) in comparison to the crops grown in RMS (Subsite B) and AGS (Subsite C) was attributed largely to an observation made at the site. This is briefly explained thus; after a heavy rainfall, the soil surface appeared to be coated with water but underneath was very dry, as illustrated in Fig. 14. This indicates severe soil hydrophobicity; which is a situation where water content of soil is extremely low. By contrast, the soil found at the agricultural site (AGS) after the same rainfall demonstrated

http://dx.doi.org/10.5772/62116

satisfactory water penetration into the soil. The causative factor to this observation is not well-understood but it could have been due to crude oil effect. The release of crude oil into the soil environment often leads to alteration of normal activities of the soil medium. It adversely impacts soil's physical, chemical, and biological characteristics [14]. This perhaps explains why the local farmers did not use Subsite A (IMS) for crop production.

Figure 14. Reduced water infiltration in Subsite B (RMS) relative to the agricultural farm soil (Subsite C: AGS)

3.3.2. CNB-Tech versus RENA remediated soils for crop performance

Highlights of results from comparative analysis between the performances of RENA remediated soil (RMS) and CNB-Tech remediated soils (CRMS) are shown in Fig. 15. ME02 stands for the name of the indicator crop and its replicate number (*Manihot esculenta Crantz*, pot No.2) and DAG stands for day after germination. Fig.15a shows that on the sixth day of growth (DAG-6), the height of cassava crop grown in CNB-Tech remediated soils height was 15.60 cm. The equivalence of this height was found for the crop grown in RENA remediated soil at 37th day of growth (DAG-37). By implication, the performance of the crop grown in CNB-Tech remediated soil excelled that grown in RENA remediated soil by 3.31%. Furthermore, from Fig. 15b, on the 21st day of growth (DAG-21) for cassava grown in CRMS (CNB-Tech remediated soil), the height was 29.20 cm, excelling over the height (19. 60 cm) of cassava in RMS (RENA remediated soil) at DAG-52 by 48.98%.

Keeping day of growth constant (Fig. 15a), and c at DAG-37 (37th day of growth), height of cassava grown in RENA remediated soil was 15.10 cm and that grown CNB-Tech remediated soil (CRMS) was 43.40cm, showing an enhanced performance by CNB-Tech relative to RENA by 187.42%. The growth of crop height per day, presented in Fig. 16, gave 0.31 cm for cassava grown in RENA remediated soil, 0.57 cm per day for that grown in farm soil (AGS), and 0.90 cm for the crop grown in CNB-Tech remediated soil. The improved performance of crops

Figure 15. Digital capture, illustrating growth of cassava crop grown in RENA remediated soil (RMS) and CNB-Tech remediated soil (CRMS), where DAG stands for day after germination, H stands for height, SG stands for stem girth, and ME02 stands for *Manihot esculanta Crantz* (Cassava).

grown in CNB-Tech treated soils over those grown in RENA treated soils was attributed to positive modification of soil properties such as pH, temperature, water dynamics, electrical conductivity, and enhanced plant nutrient bioavailability for easy plant nutrient [2, 3, 6, 7]. CNB-Tech products, which are biodegradable and eco-friendly, are also sources of natural plant and soil-beneficial mixed microbial consortia. CNB-Tech procedures do not involve the use of genetically engineered microorganisms and as a result of in situ generation of microorganisms, eliminates the daunting task of isolating specific microorganisms needed to remove specific contaminant.

According to [15], most remediation/biodegradation guidelines for detoxification of petroleum hydrocarbons are developed mainly for TPH or total mineral oil concentration but the spill of crude oil into the soil could cause varying degrees of toxicity, phytotoxicity, mutagenicity and carcinogenicity actions. Ecotoxicity bioassays should therefore be incorporated as supplementary tools for monitoring treatment effects. In a situation where, for instance, the end-use of the land is farming, using reduction of petroleum hydrocarbon concentrations as the only or major index for closeout of remediation projects without recourse to other ecological and socioenvironmental factors poses some threats to the environment in terms of soil quality, food security, food safety and means of livelihood for the populace. These in turn could stimulate poverty, endanger public health and impact negatively on national security.

In comparison with other works, the result obtained in this study on TPH reduction was higher than 7.42 ±1.02% reduction obtained by [18] when poultry manure alone and in combination

http://dx.doi.org/10.5772/62116

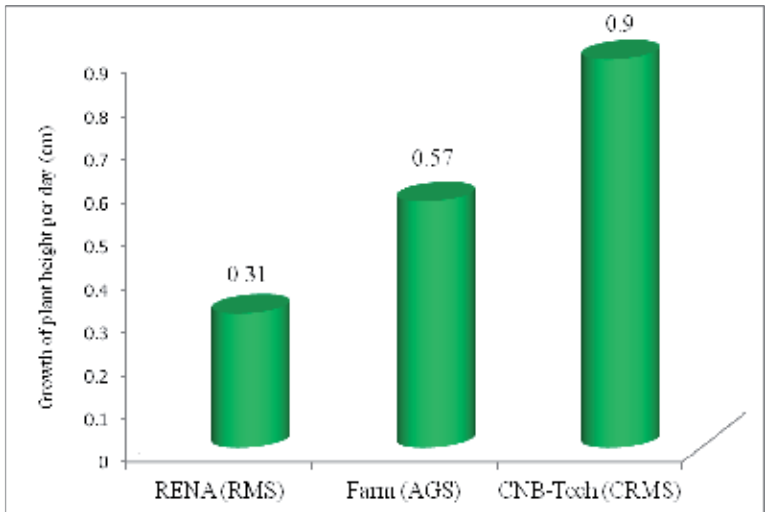


Figure 16. Growth in height of cassava crop per day for RENA remediated soil (RMS), CNB-Tech remediated soil (CRMS), and farm soil (AGS: control)

with glucose was applied to crude-oil-contaminated soil. Comparing the results obtained in this study with related investigations in other parts of the globe, [8] carried out bioremediation on sand samples contaminated with oil spill, which were collected from Pensacola beach (Gulf of Mexico) using isolated fungal diversity associated with beach sands. They investigated the ability of isolated fungi for crude oil biodegradation. Results from their study gave 4.7–7.9% biodegradation. [10] obtained 24.0–57.1% reduction in TPH by applying a biological treatment to crude-oil-contaminated soil in Russia. They used composting system, enhanced by nutrient (NPK fertilizer) addition and inoculation of *Rodococcus*–biosurfactant complexes.

In China, [20] conducted an investigation on two bioremediation technologies (bioremediation by augmentation and conventional composting using crude manure and straw) as treatment options for oily sludge and oil-polluted soil in which the total hydrocarbon content (THC) varied from 327.7 to 371.2 g/kg (327700 to 371200 mg/kg) for dry sludge and 151.0 g/kg (151000 mg/kg) for soil for a period of 56 days; after three times of biopreparation application, THC decreased by 46–53% in the oily sludge and soil. Note that the results (88–99% degradation in TPH) obtained from this present study was from only one dose application of CNB-Tech products. As stated earlier, repeated application of CNB-Tech products by two to three dose applications will achieve 100% degradation of TPH.

[13] carried out bioremediation of petroleum-hydrocarbon-–contaminated soil by composting in biopiles and recorded mineral oil decrease from 2400 to 700 mg/kg, corresponding to 70% reduction after 5 months. Majority of remediation works carried out in other parts of the globe took a period of 3 months to over 12 months to achieve between 75 and 98% reduction in TPH in hydrocarbon-contaminated soils (SGBP, 2007; [16]. CNB-Tech achieves a faster cleanup/TPH reduction, since projects can be completed in days/weeks instead of months/years.

4. Conclusions

CNB-Tech is an innovative, time-effective, cost-effective and eco-friendly remediation technique developed for the detoxification and restoration of crude-oil-impacted environmental matrices polluted with petroleum hydrocarbons, incorporating biodegradation process. This study revealed that it compares and has the potential to excel over some existing biodegradation procedures employed by many oil industries, especially in developing countries. Presently, a mini field-scale project sponsored by National Tertiary Education Trust Fund (TETFUND) is ongoing, focusing on optimization of the CNB-Tech in readiness for field-scale applications for industrial operations and safety assessments of different crops grown in the treated soils.

Acknowledgements

The Shell Petroleum Development Company, Port Harcourt, Nigeria, through its University Liaison and Remediation Units sponsored this project.

Author details

Iheoma M. Adekunle[1*], Nedo Osayande[2] and Temitope T. Alawode[1]

*Address all correspondence to: imkunle@yahoo.com

1 Department of Chemical Sciences, Federal University Otuoke, Bayelsa State, Nigeria

2 The Shell Petroleum Development Company, Port Harcourt, Nigeria

References

[1] Adekunle, I.M., Oguns, O., Shekwolo, P.D., Igbuku, O.O., and Ogunkoya, O.O. (2013). Emerging trend in natural resource utilization for bioremediation of oil-based drilling wastes in Nigeria. In: *Biodegradation – Engineering and Technology*, Eds: R. Chamy and F. Rosenkranz, Intech Publishers, Croatia ISBN 978 -953 -51-1153 -5. Pp. 390-432. http://dx.doi.org/10.5772/56526

[2] Adekunle A.A., Adekunle, I.M., Igba, T. (2012). Assessing the effect of bioremediation agent from local resource materials in Nigeria on soil pH. *J Emerging Trends Engin Appl Sci* (*JETEAS*), UK 3 (3): 526-532. http://jeteas.scholarlinkresearch.org/articles/Assessing%20the%20Effect%20of%20Bioremediation%20Agent.pdf

[3] Adekunle A.A., Adekunle, I.M., and Tobit O. Igba (2012). Impact of bioremediation formulation from Nigeria local resource materials on moisture contents for soils contaminated with petroleum products. *Int J Engin Res Dev*, 2 (4): 40-45 http://www.ijerd.com/paper/vol2-issue4/F02044045.pdf

[4] Adekunle, A.A, Adekunle, I.M., and Igba, T. (2012). Assessing and forecasting the impact of bioremediation product derived from Nigeria local raw materials on electrical conductivity of soils contaminated with petroleum products. *J Appl Technol Environ Sanit*, 2 (1): 57-66. http://www.trisanita.org/jates/atespaper2012/ates09v2n1y2012.pdf

[5] Adekunle A.A., Adekunle, I. M., and Igba T. (2012). Soil temperature dynamics during bioremediation of petroleum products using remediation agent for Nigerian local resource materials. *Int J Engin Sci Technol*, 1 (4): 1-8. http://www.ijert.org/browse/june-2012-edition

[6] Adekunle, I.M. (2011). Bioremediation of soils contaminated with Nigerian petroleum products using composted municipal wastes. *Biorem J*, 15:4, 230-241, DOI: 10.1080/10889868.2011.624137

[7] Adekunle, I.M., Adekunle, A.A., Akintokun, A.K., Akintokun, P., and Arowolo, T.A. (2011). Recycling of organic wastes through composting for land applications: a Nigerian experience. *Waste Manag Res*, 29 (6): 582-–593. DOI: 10.1177/0734242X10387312 Publisher: International Waste Management Association, Netherlands. Weblink: http://wmr.sagepub.com/content/29/6/582.abstract

[8] Al-Nasrawi, H. (2012). Biodegradation of crude oil by fungi isolated from Gulf of Mexico. *J Bioremed Biodegrad*, 3:4

[9] Ayotamuno M.J., Kogbara R.B., and Hart B.A. (2006). The combined effect of oxygen, water and nutrient on the remediation of a petroleum polluted agricultural soil. *J Eng*, 16(2): 119-134.

[10] Christofi, N., Joshua, J.B., Kuyukina, M.S., and Philp, J.C (1998). Biological treatment of crude oil contaminated soil in Russia. Geological society, London, Engineering Geology Special Publications, 14: 45-51.

[11] Department of Petroleum Resources (2002). Environmental Guidelines and Standard for the Petroleum Industry in Nigeria.

[12] Ejama-Ebubuh Remediation Management System: ERMS (2011). First revised remediation execution plan for Ejama-Ebubu project site, UIG/P/SEE, February, 2011, version 2, pp. 5-6.

[13] Jorgensen, K.S., Puutstinen, J., and Suortt, A. –M (2000). Bioremediation of petroleum hydrocarbon- contaminated soil by composting in biopiles. *Environ Poll*, 107: 245-254.

[14] Kingston, P.F. (2002). Long-term environmental impact of oil spills. Review paper. *Spill Sci Technol Bull*, 7 (1-2): 53 – 61.

[15] Liu, W., Luo, Y., and Teng, Y (2010). Bioremediation of oily sludge-contaminated soil by stimulating indigenous microbes. *Environ Geochem Health*, 32: 23-29.

[16] Mandal, A.K., Sarma, P.M., Singh, B., Jeyaseelan, C.P., Channasshettar, V.A., Lal, B., and Datta, J. Bioremediation: an environment friendly sustainable biotechnological solution for remediation of petroleum hydrocarbon contaminated waste. *ARPN J Sci Technol*, 2012: 2, 1-12.

[17] Oboh, B.O., Ilori, M.O., Akinyemi, J.O., and Adebusoye, S.A. (2006) Hydrocarbon degrading potentials of bacteria isolated from a Nigerian bitumen (Tarsand) deposit. *Natur Sci*, 4(3):51-57.

[18] Okolo, J.V., Amadi, E.N., and Odu, C.T.I (2005). Effects of soil treatments containing poultry manure on crude oil degradation in a sandy loam soil. *Appl Ecol Environ Res*, 13(1): 47-53.

[19] Okoh, A.I. (2006): Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. *Biotecnol Mol Biol Rev*, 1(2): 38-50.

[20] Ouyang, W., Liu, H., Murygina, V., Yu, Y., Xiu, Z., and Kalyuzhnyi, S. (2005). Comparison of bio-augmentation and composting for remediation of oily sludge: A field-scale study in China. *Process Biochem*, 40, 3763-3768.

[21] Remediation Management System: RMS (2010), Revision 1, UIG/P/SEE & UIG/P/SEP, SPDC 2009 – 11-00000013. September, pp. 34-35.

[22] Shell Gabon Blackspot Project: SGBP (2007). Land farming in Gabon. NAM.

[23] Sihang, S., Pathak, H., and Jaroll, D.P., (2014). Factors affecting the rate of biodegradation of polyaromatic hydrocarbons. *Int J Pure Ap Biosci*, 2 (3): 185-202.

[24] SPDC (2014) https://www.shell.com.ng/environment-society/environment-tpkg/oil-spills/monthly-data.html Accessed April 16, 2015

[25] United Nations Environmental Programme (UNEP) (2011). Environmental Assessment of Ogoniland. P. 1-262. ISBN:978-92-807-9 Available on line at: http://postconflict. unep.ch/publications/OEA /UNEP_OEA.pdf

[26] Tracy, M.A., Ward, K.L., Firouzabadian, L., Wang, Y., Ding, N., Qian, R., and Zhang, Y. (1999). Factors affecting the degradation rate of poly (lactide-co-glycolide) microspheres in vivo and in vitro. *Biomaterials*, 20 (11): 1057-1062.

[27] Zaidi, B.R. and Iman, S.H. (1999). Factors affecting microbial degradation of polyxyclic hydrocarbon phenanthrene in the Caribbean coastal water. *Marine Poll Bull*, 38 (8): 737-742.

Edited by Rolando Chamy, Francisca Rosenkranz and Lorena Soler

This book contains a collection of research works focused on the biodegradation of different types of pollutants, both in water and solids. The book is divided in three major sections: A) Biodegradation of organic pollutants in solids and wastewater, B) Biodegradation of complex pollutants, and C) Novel technologies in biodegradation and bioremediation.

ISBN 978-953-51-5418-1

Photo by bbbrrn / iStock

IntechOpen