Environmental and Microbial Biotechnology

Inamuddin
Mohd Imran Ahamed
Ram Prasad *Editors*

# Recent Advances in Microbial Degradation

Springer

# Environmental and Microbial Biotechnology

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Ram Prasad
Editors

# Recent Advances in Microbial Degradation

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# Microbial Degradation of Aflatoxin

1

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**Abstract**

Aflatoxins are low-molecular-weight lipophilic compounds widely distributed in nature. Long-term aflatoxin exposure can lead to several consequences, including carcinogenic effect and it can affect all body systems, causing immune system dysfunction and impaired growth in humans. The literature describes 20 types of aflatoxins. Diseases caused by the consumption of aflatoxin are known as aflatoxicosis. Seeds that suffer stress, such as prolonged drought, short irrigation time, damage caused by insects, and use of seeds in areas that do not favor their cultivation, have high rates of contamination by fungi that produce aflatoxins, such as *Fusarium* and *Aspergillus*. Post-harvest contamination occurs worldwide where there are environmental conditions for the proliferation of aflatoxicogenic fungi, such as inadequate storage, improper drying of grains, and plant species not adapted to the climate. An alternative that has been growing in the recent years is

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the use of microorganisms for degradation of aflatoxin, where researches have been showing the use of several bacterial and fungal species in this control. It is noteworthy that the biological degradation of mycotoxins has shown promise because it is effective and environmentally friendly. The aim of this chapter is to understand the microbial degradation of aflatoxin and its effects on human health are addressed such as foods with a higher incidence of aflatoxin contamination and quality control.

**Keywords**

Intoxication · Microbiology · Food safety · Mycotoxins

## 1.1 Introduction

Aflatoxins are defined as low-molecular-weight lipophilic compounds that are widely distributed in nature. When they are ingested, the intestine absorbers them, and soon those toxins can reach the bloodstream, causing risks to human health. When aflatoxin is exposed in long term it can lead to several consequences, including carcinogenic effect, and it can affect all body systems, causing dysfunction of the immune system and impaired growth in humans (Wu et al. 2014; WHO 2018).

From the discoveries of the twentieth century, mycotoxins were reported to be present with greater intensity around the world. Mycotoxins are configured as a serious problem for countries, both economically and for the health of animals and humans. These compounds are detected in contaminated raw materials, food, and feed, causing annual losses of agricultural commodities, food, and livestock industries worldwide (Ismail et al. 2018).

The World Health Organization data estimate that about 25% or more of the world's food crops are destroyed annually due to aflatoxin contamination (WHO 2018). This contamination can occur due to intense cultivation and decreased genetic diversity in cereal crops, which contribute to the formation of fungi that generate as aflatoxins in vegetables (Tola and Kebede 2016). A fungal infection can occur during the stages of cultivation, harvesting, transportation, storage and without processing (Chulze 2010).

Animals can also accumulate aflatoxins in their bodies, for example, ruminants when eating food contaminated with aflatoxin B1. Aflatoxin B1 is absorbed in the gastrointestinal tract and metabolized in the liver by microsomal cytochrome P450, producing aflatoxin M1, a metabolite as toxic as aflatoxin B1, and these metabolites are excreted in the urine and milk of animals (de Roma et al. 2017).

Aflatoxin M1 is considered one of the main xenobiotic compounds in pasteurized and sterilized milk, yogurt, cheese, and other dairy products due to its high stability in various thermal processes, such as pasteurization and treatment in ultra-high temperature (UHT), freezing, or others dairy industry procedures. When present in raw milk, the M1 aflatoxin molecule cannot be inactivated, and it can contaminate the entire milk production chain (de Roma et al. 2017).

For this reason, research aiming to control aflatoxins mitigating their biosynthetic sources, whether in processed foods or their raw materials, has been growing steadily in the recent years. In agricultural commodities, the total absence of mycotoxins cannot be 100% guaranteed due to the complexity of the food chain, so decontamination procedures such as physical treatments can be applied including washing, polishing, mechanical screening, and separation (Patel et al. 2015).

In addition, some strategies can be adopted to prevent aflatoxins in food, such as the use of ultrasound technologies. Gamma irradiation is found to combat food contamination. Another proposal considered effective is the use of fungi and bacteria in this control, eliminating, inactivating, or reducing aflatoxins levels in food products.

The purpose of this chapter is to understand the microbial degradation of aflatoxin. The chapter is structured as follows: First, the main characteristics of aflatoxins and their effects on human health are addressed, followed by foods with a higher incidence of aflatoxin contamination, quality control, and use of microbial degradation as a measure of aflatoxin contamination control.

## 1.2 Aflatoxins and Their Effects on Human Health

Aflatoxins are oxygenated heterocyclic compounds produced by *Aspergillus* genus fungi. The presence of fungi combined with poor conditions of harvest, processing, and environmental factors, such as high relative humidity, trigger favorable conditions for the mycotoxin biosynthesis in food (Patel et al. 2015). The literature describes 20 types of aflatoxins, the most frequent being B1, B2, G1, G2, M1, and M2, as they are detected at significant levels and more often in foods.

The biosynthesis of aflatoxins (Fig. 1.1) is complex and involves a series of enzymes that transform the acetate and malonyl CoA components into hexanoil, the initial unit formed by the synthesis of a fatty acid, which is converted into norsolorinic acid, the first compound stable in the polyketide pathway and metabolite. After successive enzymatic reactions, the formation of versicolor B occurs, from which point the pathway divides (Sweeney and Dobson 1999). A route forms Versicolor A to later produce dimethylesterigmatocystin, then sterigmatocystin, and, finally, *o*-methylesterigmatocystin, generating aflatoxins B1 and G1 (Roze et al. 2013). The other route is responsible for the formation of B2 and G2 aflatoxins from dihydromethylterigmatocystin (Sweeney and Dobson 1999).

The term mycotoxin was used in 1962, after an incident near London, England, which caused the death of approximately 100,000 turkeys. When this mysterious disease was associated with the consumption of peanuts contaminated by secondary metabolites of *Aspergillus flavus*, this incident reinforced the importance of conducting research on fungal metabolites and, especially, the risks involved (Bennett and Klich 2003).

In 1993, the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) classified aflatoxins as carcinogens. The IARC also classified aflatoxin B1, B2, G1, and G2 as “group 1” toxins, causing carcinogenic

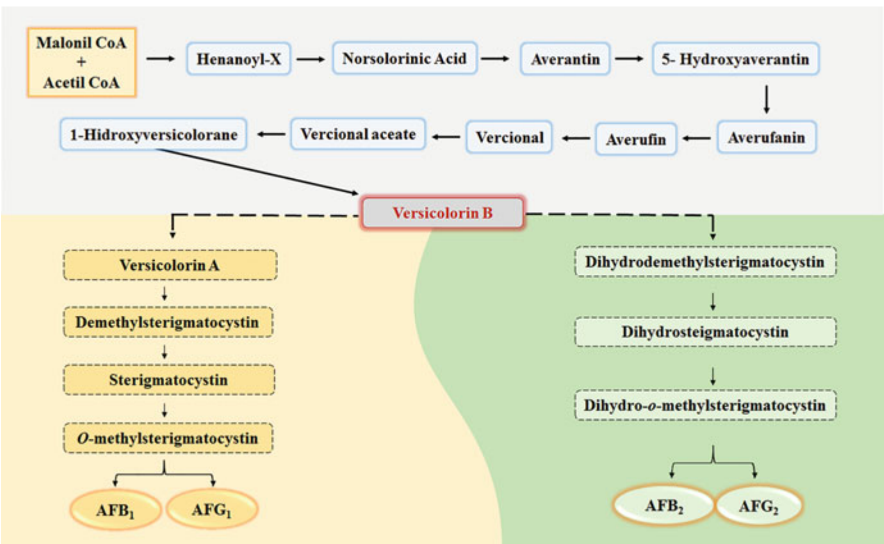


**Fig. 1.1** Aflatoxin biosynthesis

effects in humans (WHO and IARC 1993a, b). Aflatoxin B1 is the most potent natural carcinogen known and is generally the main aflatoxin produced by toxigenic strains. The level of toxicity associated with aflatoxin varies with the types present, with the order of toxicity being the aflatoxins B1 > G1 > B2 > G2 (Jaimez et al. 2000).

Diseases caused by the consumption of aflatoxin are known as aflatoxicosis. Acute aflatoxicosis results in death, and chronic aflatoxicosis results in cancer, immune suppression, and other pathological conditions (Zain 2011). Aflatoxins act at the molecular level, altering important biochemical processes that lead to mutagenicity and carcinogenicity (Van Egmond et al. 2007). However, the veracity of the disease depends on the mechanism of action of each type of aflatoxin, the time of exposure, and the age and nutritional status of the patient (Ajani et al. 2014).

According to Marchese et al. (2018), liver is the main affected organ; the damage caused by aflatoxins is divided into two categories, acute intoxication, resulting from a short exposure, and a large number of toxins, which causes severe damage to the liver, among other problems, including death and chronic sublethal intoxication. Also, it affects the capacity for immunosuppression, causes nutritional disorders, and cancer.

Liu and Wu (2010) have noted the relationship between presence of aflatoxins in the appearance of liver cancer; on the other hand, Gong et al. (2002) identified that aflatoxin impairs growth and weight gain in children. Aflatoxins are also associated with many chronic diseases, including immunosuppression; digestive, blood, and nervous defects; reproductive problems; anemia; and jaundice (Ajani et al. 2014).

Aflatoxin B1, in comparison to the others, is a highly toxic and carcinogenic agent (Patel et al. 2015), and this problem is associated with high thermal stability and a wide possibility of food contamination (Xue et al. 2019). In addition, before forming an aflatoxin B1, the metabolite Versicolor A is formed, which presents a dihydrobisfurane ring (Gauthier et al. 2020), a double bond 2,3, present in the ring, oxide in the body, making the compound highly reactive and carcinogenic (Roze et al. 2013).

According to Caceres et al. (2020), the production group is complex and requires the presence of a series of enzymes. Dohnal et al. (2014) explain that type B1 aflatoxins are produced by means of enzymes and various metabolites, among them cancerous hepatocellular agents, and these metabolites are generated through oxidative, enzymatic, and hydrolytic reductions, unleashing the separation of molecules into more hydrophilic products and conjugation reactions between molecules or their metabolites with other nucleophilic molecules.

The organism contaminated with aflatoxin B1 can generate Aflatoxin M1 (Patel et al. 2015). This conversion takes place in the liver, where the compound undergoes processes involving the cytochrome P450 monooxygenase enzymes for the production of aflatoxin M1, and this metabolite is dangerous for health because it is a potential carcinogenic agent with contamination established mainly by the consumption of contaminated milk (Yabe et al. 2012) and its derivatives. Contamination by aflatoxins is worldwide. The exposure in children is very serious and needs to be studied, since the degree of risk is greater due to biological and exposure factors (Marchese et al. 2018), requiring monitoring of these foods (Er et al. 2014).

## 1.3 Aflatoxins in Food

Every year, millions of people fall victims to the consumption of contaminated food (Martins et al. 2020), with the main contaminant responsible for affecting human health being aflatoxins. These are found in foods such as milk, corn, spices, peanuts, nuts, pistachios, or indirectly through foods of animal origin, such as egg, meat, and dairy products (Kumar et al. 2016). Contamination in agricultural products occurs during growth, harvesting, storage, or transportation (Cotty 2006). Fungal contamination in food results in loss of food products and health problems for consumers (Kedia et al. 2014). Therefore, knowing the main foods exposed to contamination, monitoring and preventing aflatoxin contamination in these foods are important issues worldwide (Chen et al. 2013). The following topic addresses the main foods with an incidence of aflatoxin contamination.

### 1.3.1 Peanut (*Arachis hypogaea* L.)

Peanut (*Arachis hypogaea* L.) is a source of vegetable protein appreciated worldwide (Yang et al. 2020), has high nutritional value, and is rich in essential amino acids, unsaturated fatty acids, and antioxidant vitamins, making this food important

in maintaining human health (de Sousa et al. 2011; Yang et al. 2020). Peanuts are eaten fresh, roasted, and products such as peanut butter, peanut oil, and creams for application in cakes, and they are well accepted in the consumer market (Mohammed et al. 2016; Ayelign and de Saeger 2020).

Although peanuts are nutritionally rich, there are concerns about its safety, as they are legumes that are highly susceptible to aflatoxin contamination, which can occur at all stages of its production chain. The water stresses that the plants go through, due to drought, high soil temperatures before harvest, and damage caused by insects to peanuts in the development period, compatible with oilseeds, provide favorable conditions for the growth of fungi *A. flavus* and *A. parasiticus*, leading to contamination by aflatoxins, mainly type B1 (Gong et al. 2016).

During water stress, the plant's natural defenses are affected, resulting in loss of metabolic activity, reduction of water activity, and imbalance of soil microflora, thereby reducing the growth of competing bacteria and fungi and favoring the growth of the *A. flavus* and *A. parasiticus*, as they grow easily under conditions of low water activity (Pitt et al. 2013).

### 1.3.2 Corn (*Zea mays* L.)

Corn (*Zea mays* L.) is one of the most important cereal crops in the human diet and animal feed (Wu and Guclu 2013). The high level of corn production makes this raw material influential in the national economy, playing a significant role in the industry and food security (Xu et al. 2019). This cereal is rich in nutrients, such as fiber, minerals (Mg, P, and K), vitamins (A, B, E, and K), phenolic acids, ferulic acid, coumaric acid, synergic acid, vanillic acid, serum acid, ferulic acid, coffee, carotenoids, and flavonoids, and its consumption confers health benefits, as it reduces the risk of chronic degenerative diseases (Sheng et al. 2018).

The use of corn in food is diverse and can be used as a basis for industrial products, oil, syrup, starch, animal feed, and ethanol production (Xu et al. 2019). However, corn is highly susceptible to aflatoxin contamination, since the fungi *A. flavus* and *A. parasiticus* are often found in corn and its derivatives (Kebede et al. 2012).

Regions with high temperatures, humidity, and irregular rainfall contribute to the development of aflatoxins in the cultivation of corn. Contamination can occur during cultivation, harvesting, and transportation (Rasheed et al. 2019; Sserumaga et al. 2020). When there is low-quality control in the processing of corn, the fungus colonizes the tissues of the style, grows and reaches the grains, and produces aflatoxins (Accinelli et al. 2014).

Corn-derived products occupy an important position in human and animal nutrition, and it is important to recognize that even in countries with the supervision of these products, in developing countries, the irregular marketing of corn is still common. Therefore, consumers are at high risk of exposure to aflatoxin contamination, and it is necessary to educate consumers and make food entrepreneurs aware of

the damage caused by the consumption of food contaminated by aflatoxins (Cheng et al. 2019).

### 1.3.3 Nuts (*Juglans regia* L)

Nuts contain essential unsaturated and monounsaturated fats, including linoleic and linolenic fatty acids, vitamins, and essential amino acids. Also, they are considered a source of vitamin E, vitamin B2, folate, fibers, and essential minerals, such as magnesium, phosphorus, potassium, copper, and selenium (Sabaté and Wien 2013; Hidalgo-Ruiz et al. 2019). Nuts are eaten as toast, appetizers, and dessert; dried fruits are widely used in traditional bakery and confectionery products; as flavoring agents in drinks and ice cream; and they are also used as soup thickeners (Jubeen et al. 2020).

Fungal contamination of nuts is influenced by factors such as high temperature, humidity, poor storage conditions, and inadequate harvesting practices that cause mechanical damage to nuts and play a vital role in the growth of fungi and production of aflatoxins, especially in the drying stages, storage, and transportation (Nkwonta et al. 2015; Asghar et al. 2017). The resulting effect of this biological and environmental interaction and the deterioration of nuts affects the nutritional and sensory qualities and imposes health risks on those who consume them (Rodrigues et al. 2012).

### 1.3.4 Milk

Milk is a nutrient-rich food, as it is a source of fatty acids, proteins such as casein and albumin, lactose, minerals and vitamins, and vitamins for the growth, development, and maintenance of human health. However, raw milk or dairy products can also be sources of contamination by microorganisms and, consequently, aflatoxins especially for infants and children due to the high consumption of milk and dairy products in their diet (Iqbal et al. 2015a). Aflatoxin M1 is one of the most dangerous metabolites of aflatoxin B1, and this product is excreted in the milk of animals and humans causing the same damage as aflatoxins B1, such as carcinogenicity, teratogenesis, genotoxicity, mutagenesis, and immunosuppression (Min et al. 2020).

When milk is not handled in hygienic sanitary conditions, it loses quality. The main contaminants found in milk and its derivatives include pesticide residues, heavy metals, and M1-type aflatoxins (Awasthi et al. 2012). Contamination of milk by aflatoxins occurs when animals are fed with feed, cereals, harvest by-products, and silage contaminated with aflatoxin B1, which is more abundant in contaminated food and feed. Contamination of livestock feed occurs due to conditions of conservation and adequate storage, mainly in tropical and hot regions with high humidity (El Marnissi et al. 2012).

Moschini et al. (2006) found that in lactating cows fed 5 mg of aflatoxin B1 bolus, plasma concentrations of aflatoxin B1 transformed to aflatoxin M1 are detected

15 min after ingestion. This indicates the rapid absorption and biotransformation of aflatoxin B1 in M1 (Moschini et al. 2006). Several studies in different countries have reported high and low levels of contamination by aflatoxin B1 and aflatoxin M1 in different categories of milk, such as UHT, pasteurized, raw milk, cottage cheese, yogurt, and butter (Xiong et al. 2018; Tadesse et al. 2020 ).

## 1.4 Regulation and Quality Control

Preharvest contamination occurs in temperate and tropical regions. Seeds that suffer stress, such as prolonged drought, short irrigation time, damage caused by insects, and use of seeds in areas that do not favor their cultivation, have high rates of contamination by fungi that cause aflatoxins, such as *Fusarium* and *Aspergillus* (Tola and Kebede 2016). Post-harvest contamination occurs worldwide where there are environmental conditions for the proliferation of aflatoxicogenic fungi, such as inadequate storage, improper drying of grains, and plant species not adapted to the climate (Tola and Kebede 2016).

The species of aflatoxins type B, G, and M have risk classification, as carcinogenic, group 1 (Smoke and Smoking 2004). In case of accidental ingestion of aflatoxins, the liver metabolizes the toxin, forming other types of carcinogenic metabolites, usually causing liver cancer (Wang et al. 2018). In addition to being carcinogenic compounds, they are genotoxic; therefore, the amount of acceptable intake is minimally possible. For example, the maximum levels of aflatoxins established by Codex Alimentarius in nuts, grains, dried figs, and milk are between 0.5 and 15 μg/kg, a fact that made the European Union establish that the level consumed should be as low as possible (Authority 2007).

Codex alimentarius, to prevent and reduce the amount of toxins in cereals, nuts, and other products, has already created several good habits manuals. The creation and good acceptance of the manual developed by Codex designed a uniform orientation for all countries in an attempt to minimize the effects of mycotoxins in food so that it can have an effect, and countries must adapt the manuals according to their climate, culture, and socioeconomic conditions. The recommendations were divided into two: good manufacturing practices and recommended practices based on good agricultural practices (Kabak et al. 2006).

### 1.4.1 Aflatoxin Prevention Strategies in Vegetables

The control in the preharvest of vegetables, in order to avoid contamination by aflatoxins, must take into account agronomic and environmental factors that influence the infection of the plant from the seed to the pod. Such factors can vary considerably from one location to another and even at the same location at different seasons. Particularly, some environments can be favorable to fungal infection and subsequent aflatoxin contamination, and it is necessary to take into account whether the location has climatic and agronomic conditions for the proper development of the

chosen culture. However, for most of these cases, agricultural practices can be used to minimize the effects of aflatoxins (Commission 2004).

Another example of walnut is pistachio, which is a fruit composed of a single grain protected by a thin and showy shell, which in turn is surrounded by a hull. The biggest problem found in pistachios is the immature division of the shell and hull. The husk divides into several parts at least one month before harvest, and usually the husk and the hull remain intact after division. However, a small percentage of pistachios, the peel, and the hull are still divided and adhered to the fruit, thus causing a rupture and a direct access route, from the fungus to the fruit (Commission 2003).

Insects are responsible for the spread of fungal spores, such fungi installed in areas damaged by insects, the flower and silk present in corn, for example, can be the gateway for fungi in the plant, which increases the levels of mycotoxins in the product. However, corn can be recovered in distilleries in the production of alcohol due to the high starch content (Tola and Kebede 2016). Fumigants, essential oils, and alternative solutions, such as the use of ozone, were applied as control measures against insect attacks and, consequently, a decrease in aflatoxins in corn was observed (Chulze 2010).

Before planting, the producer should seek the city hall or agricultural extension service to verify the viability of cultivation in the region and to check the availability of seeds resistant to attacks by insects, fungi, and microbes that can impair the quality of the fruits (Commission 2004).

The use of biological and chemical agents prevents the growth of aflatoxicogenic fungi and, consequently, aflatoxins. The inoculation of non-aflatoxicogenic competitive strains of *A. flavus* and *A. parasiticus* showed a steep drop in aflatoxin contamination in peanut, rice, corn, and cotton seeds, notably due to the production of inhibitory metabolites and competition for substrate (Kabak et al. 2006).

Intensive cultivation of the same species of nuts in the same location can lead to an increase in the population of *A. flavus* and *A. parasiticus* fungi in the soil, which increase the chances of infection in the next plantations. There are reports that different types of soil contribute or not to fungal development. The heavier soil, with more water availability, drastically reduces the amount of fungi due to the decrease in water stress, which can be a partial cause of the infestation. However, in dry and sandy soils, fungi are favored and multiply rapidly in the plant due to the water stress that the plant has suffered (Kabak et al. 2006).

### 1.4.2 Care in Harvesting and Transportation

At harvest, the processing of the products begins, when transporting from the field to the silo or warehouse where there may be a great variation in humidity. For this reason, strict humidity control must be maintained at various points in the load. Another very important factor is the damage and injuries caused in the harvest as well as in the pre-harvest where the broken cereal can be contaminated and in the harvest there can also be damage to the cereal and allow contamination by fungi.

This problem can be maximized with the lack of humidity control, and this whole process must be accompanied by a good strategy for separating healthy grains (Kabak et al. 2006).

### 1.4.3 Postharvest Control

The appearance of mycotoxicogenic fungi also occurs during storage due to the moisture of the grains or the condensation of water in the place close to the cooling sector of the silos. In view of this, it is important to control the aeration of the environment and continuously monitor the silo to minimize the effects of contamination during storage (Kabak et al. 2006). Such attitudes have a high effect on the quality and nutritional value of food, promoting internal and external trade, among other advantages (Udomkun et al. 2017).

High humidity in peanuts and harvested corn increases aflatoxin contamination, adequate drying of peanut grains to $<7\%$ (Waliyar et al. 2015) and corn between 14 and 16% (Chulze 2010), prevents the growth of fungi in general as well as the growth of aflatoxicogenic strains. Drying techniques such as inversion of the line, post-harvest, expose all the faces of the fruits to the sun, and allow the passage of air, which facilitates drying (Waliyar et al. 2015).

In storage, polypropylene bags are used, which provide airtight closure and will reflect a substantial decrease in the contamination by fungi, if the transport and drying have been done correctly (Kabak et al. 2006). Studies show that *Aspergillus* can grow at temperatures from 10–12 °C to 42–43 °C, with an optimal temperature of 32–33 °C (Aflatoxins in peanuts 2002). After the storage of nuts and cereals, factors such as grains and environmental humidity, container or aeration, and storage location play an important role. In the case of open places, they are crucial points for a prolonged storage period without further damage to the product.

### 1.4.4 Strategies for Preventing Aflatoxins in Milk and Dairy Products

The major problem with AFM1 in milk and dairy products is the fact that milk is included in the daily diet of all age groups in many countries and in addition, toxins can contaminate all derived products, such as yogurt and cheese, enhancing the damage caused (Iqbal et al. 2015b).

The presence of aflatoxins in animal milk comes from contaminated food. Currently, cows are fed by the TMR method that means total mixed feed. This method consists of providing fodder with different types of concentrates or by-products, grains, minerals, protein supplements, minerals, and vitamins in exact quantities to form a balanced feed. These products may contain large amounts of mycotoxins from the field, storage, and/or transportation (Rodriguez-Blanco et al. 2020).

Aflatoxin M1 is stable at high temperatures, and some studies indicate that it is stable in cheeses for 60 and 90 days (Oruc et al. 2006). Treatments with ultrahigh temperature (UHT), ionizing radiation, addition of enzymes or additives, and other techniques used to rid food of contaminants are not effective (Matabaro et al. 2017).

### 1.4.5 Detection Methods for Aflatoxins

The objective of qualitative analysis is to direct which method should be chosen, if the objective is only to detect presence or absence, rapid tests can be done, but, if the objective is to quantify the analyte, then quantitative tests should be used. Usually, the analysis procedures follow a sampling sequence, sample preparation (which can count extraction and purification), and analysis (identification or quantification) (Vaz et al. 2020).

There are several developed and validated methods for detecting aflatoxins and fungal contamination in foods, such as thin-layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC), and enzyme-linked immunosorbent assay (ELISA), among others. Optical analyses are also widely used; they are rapid analyses that do not destroy the sample; they are based on fluorescence spectroscopy, near-infrared, and hyperspectral spectroscopy (Tao et al. 2018).

## 1.5 Microbial Degradation of Aflatoxins

Researches have shown the efficient use of technologies, such as ultrasonic (Garg et al. 2013), gamma irradiation (Zhang et al. 2018) against aflatoxin in food and feedstuff. Among the chemical methods, various have been tested including oxidizing and reducing agents, alkalization, reduction, hydrolysis, chlorinating substances, and others. Another category is the detoxifying agents group added in the category of technological feed additives and classified into inorganic (aluminosilicates, hydrated sodium calcium aluminosilicate, bentonites, zeolites, other clays), organic (yeast cell wall, lactic acid bacteria—LAB, micronized fibers and bio-sorbents, activated carbon), and synthetics (organoaluminosilicates or modified clays, polymers) (Vila-Donat et al. 2018).

An alternative that has been growing in recent years is the use of microorganisms, where researches have been showing the use of several bacterial and fungal species in this control. It is noteworthy that the biological degradation of mycotoxins has shown promise because it is effective and environmentally friendly. Vila-Donat et al. (2018) emphasize that, depending on their action, these organisms can act by either binding mycotoxins to their surface (adsorption) or by biotransformation, degrading, or transforming them into less toxic metabolites. Therefore, it is defined at least two main categories: adsorbing and biotransforming agents. More recently, González-Pereyra et al. (2020) highlight to consider three distinct processes: degradation, transformation, or adsorption.

These studies started in the 1960s, where Ciegler et al. (1966) evaluated several organisms (yeasts, molds, bacteria, actinomycetes, algae, and fungal spores) for their ability to degrade aflatoxin. *Flavobacterium* B-184 removed aflatoxin irreversibly from milk, oil, peanut butter, peanuts, and corn. Contaminated soybean was partially detoxified by addition of these bacteria. From that, several studies with LAB were carried out as well as other microorganisms.

LAB are highly studied because of their technological and industrial importance, significant action as food biopreservatives, beneficial effects on human health, desired sensorial effects, and they have proven ability to sequester aflatoxins through all of their growth phases (Peltonen et al. 2001; Khanafari and Karami-Osboo 2007). Haskard et al. (2001) evaluated stability of the AFB1 complexes formed with 12 bacterial strains of LAB, in both viable and nonviable (heat- or acid-treated), and observed binding is of a reversible nature, but the stability of the complexes formed depends on strain, treatment, and environmental conditions. Bueno et al. (2007) have confirmed these results, where the ability of LAB and *Saccharomyces cerevisiae* to remove AFB1 from liquid medium has been tested.

Then, they proposed aflatoxin B1 binding to microorganisms very quickly (no more than 1 min); this process forms a reversible complex between the toxin and microorganism surface, without chemical modification of the toxin; the amount of aflatoxin B1 removed was concentration dependent both on toxin and on bacteria; and similar results were obtained with viable and nonviable (heat-treated) bacteria.

Marrez et al. (2018) evaluated the ability of LAB species in removing or binding AFB1 from whole milk and found great results. *Lactobacillus acidoplilus* achieved 80% reduction in milk within 24 h at 37 °C, whereas *L. plantarum* favored cold storage to reduce 85% of the AFB1 after 1 week. The authors highlight the use LAB bacteria in food and dairy industries as a bioremoval agent of aflatoxins contamination.

Numerous studies have tested the impacts of bacteria *Pseudomonas* sp. (Samuel et al. 2014), *Bacillus* sp. (Gao et al. 2011; Farzaneh et al. 2012; Afsharmanesh et al. 2018; Xu et al. 2019), and *Flavobacterium* sp. (Line et al. 1994; Smiley; Draughon 2020) on aflatoxin B1 degradation. There are also several fungal species known to degrade aflatoxin B1, such as *Pleurotus* sp. (Loi et al. 2016, 2018), *Aspergillus* sp. (Kachouri et al. 2014; Zhang et al. 2014), *Saccharomyces cerevisae* (Shetty and Jespersen 2006), and *Streptomyces* sp. (Harkai et al. 2016).

Wang et al. (2016) developed a thermophilic microbial consortium (TADC7—compost of agricultural wastes such as straws, sawdust, and animal feces) with stable and efficient aflatoxin B1 degradation activity, degrading more than 95% of AFB1 within 72 h and the optimum temperature 55–60 °C. They identified proteins or enzymes in the cell-free supernatant playing a major role in degradation, where *Geobacillus* and *Tepidimicrobium* were the main microorganisms involved in this process.

Taheur et al. (2020) evaluated microorganisms isolated from Kombucha culture (LAB and yeasts) for aflatoxin B1 degradation in the liquid medium and found after 7 days of fermentation that kombucha was able to degrade 97% of toxin in black tea. They have identified three main yeasts: *Pichia occidentalis*, *Candida sorboxylosa*,

and *Hanseniaspora opuntiae,* and the highest aflatoxin B1 degradation capacity was related to *P. occidentalis* (59%). Toxicity studies showed that the biodegraded products were less toxic than pure aflatoxin B1.

Cai et al. (2020) reported efficient aflatoxin B1 degrading by strain of *Stenotrophomonas acidoaminiphila* CW117 (especially to low concentrations), suggesting its potential significance to detoxification on food and feedstuff. The laccase degraded 29.3% of AFB1 within 24 h, and the cell-free supernatant degraded 76.7% of the toxin in the same time, with much lower protein content. Then, these authors concluded CW117 degrades aflatoxin B1 through a combination of enzymes and micromolecule oxides. Zeinvand-Lorestani et al. (2015) also evaluated the effects of laccase from *Trametes versicolor* on the enzymatic detoxification of aflatoxin B1 and found a significant decrease in the prooxidative properties and mutagenicity of the detoxified products with the parent toxin.

Söylemez et al. (2020) determined the optimum aflatoxin B1 degradation conditions by *Panus neostrigosus* reaching degradation rate of 49% in just 1-hour exposure to culture filtrate. It was not possible the identification and characterization of the responsible enzyme(s) for the degradation. However, the authors showed that it was not the laccase and manganese peroxidase (MnP) enzymes.

Several enzymes have been identified as important aflatoxin degraders. Liu et al. (1998) first identified an aflatoxin oxidase (AFO) from the edible mushroom *Armillariella tabescens*. Wang et al. (2018) found an aflatoxin degradation (maximum elimination of 86.0%) treated with MnP obtained from the white-rot fungus *Phanerochaete sordida* YK-624. Analysis suggested that aflatoxin B1 was first oxidized to AFB1-8,9-epoxide by MnP and then hydrolyzed to AFB1-8,9-dihydrodiol, pathway that considerably reduced its mutagenic activity. Xu et al. (2019) identified Bacillus aflatoxin-degrading enzyme (BADE) being the major protein involved in aflatoxin B1 detoxification (reaching reduction levels of AFB1, AFB2, and AFM1 by 92.1, 84.1, and 90.4%, respectively), an isolated enzyme from *Bacillus shackletonii.*

## 1.6 Conclusion

Aflatoxins, when ingested even in young children, cause damage to human health and animals. Thus, the contamination of aflatoxins in food has become a worldwide concern in recent years, resulting in the emergence of several actions aimed at reducing the levels of aflatoxins in food, for example, quality control measures involving good production practices. In addition, the microbial degradation of aflatoxins is an important technology in this control, and it is perceived that it is potentially applicable. However, in addition to the microorganisms already studied and used in the degradation of aflatoxins, the discovery of more selective and sensitive microorganisms should be encouraged in order to make these technologies even more effective and economical for the food industry.

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# Microbial Degradation of Polymers

# 2

Wojciech Stawiński and Katarzyna Wal

**Abstract**

Polymers constitute a wide group of materials used in many industry branches. Some of them have natural origin and can be easily subjected to biodegradation, and others, the plastics, are designed to be resistant and durable; in consequence, enormous amount of plastic finds its way to the environment and generates a severe pollution problem. Among the conventional plastic waste handling methods, such as recycling, incineration, and disposal in landfills, considerable attention has been given to microorganisms consortia forming around plastic waste—the so-called plastisphere. Some of the microorganisms are able to produce enzymes, mostly hydrolases and oxidoreductases, that can depolymerize certain plastics rendering them an accessible carbon source for the growth of microorganisms. The polymer's susceptibility to the microbial action is influenced by physiochemical characteristics of the polymer, such as crystallinity, hydrophobicity, porosity, and presence of additives, as well as abiotic factors such as pH or temperature. Microbial degradation of polymers as a waste management method is promising; however, there is a plethora of challenges that need to be overcome including slow polymer decomposition rate, requirement of stable environment, different condition for decomposition of various

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plastics, and susceptibility of the microorganism to adverse effects of substances that might be released during the decomposition (plastic additives).

**Keywords**

Plastics · Polyolefins · Bacteria · Fungus · Degradation

## 2.1 General Introduction

Polymers are large molecules composed of repeating units. Some of them, the natural polymers, are synthesized by organisms and are essential for the proper functioning of life on Earth; other polymers, the synthetic polymers, have anthropogenic origin and do not naturally occur in the environment. Polymers belonging to this group are commonly called the plastics; they find application mainly in the packaging and building and construction industry (Geyer et al. 2017). Their role in the modern society cannot be underestimated; however, as they are designed to be durable and last for a long time, they cause a serious pollution issue worldwide. Plastic is now omnipresent and tends to accumulate in certain locations. Besides the obvious contamination of land, plastic, generally in its micro or nano form, has been found in food (Peixoto et al. 2019; Rainieri and Barranco 2019), water bodies (Piccardo et al. 2020; Wong et al. 2020; Yang et al. 2020), soil (Kumar et al. 2020; Sarker et al. 2020a), and even in the atmosphere (Chen et al. 2020; Zhang et al. 2020). Some perspective on how extensive plastic pollution is may give the fact that in 2014 geologists discovered a new type of rock and called it the plastiglomerate; it is a combination of plastic and geological sediments glued together. From a geological point of view, plastic will be a diagnostic element of the currently forming geological strata for the later generations (Abbing 2019). It is estimated that by the year 2050, 12 000 Mt of plastic waste will be stored in landfills (Geyer et al. 2017). Another recently introduced term, plastisphere, refers to a film of microorganisms consortia forming around plastic waste, which, in consequence, became a new ecosystem generated by human (Abbing 2019). The organisms inhabiting plastisphere have been attracting interest of the scientific world owing to the potential of finding a solution to the plastic pollution coming right from the nature.

## 2.2 Conventional Way of Handling Plastic Waste

Nowadays, there are three main ways of handling plastic waste, and all of them have some advantages and drawbacks:

– *Landfills*: This is the main plastic waste handling method. Around 80% of the produced plastic ends up accumulated in landfills (Geyer et al. 2017); the waste is disposed of in landfills, covered with soil, and isolated by a layer of clay or plastic lining from below to reduce the exposure to atmospheric conditions and to prevent any contamination of groundwater. This method should be considered a short-term solution, as it does not eliminate the plastic but just stores it in one place; however, it is the simplest and the most economically effective method (Shen et al. 2020).
– *Recycling*: It is the preferred method of handling plastic waste, and approximately 10% of the produced plastic is treated that way (Geyer et al. 2017); however, not all plastics can be easily subjected to recycling. Plastics possess different properties; hence, its recycling also varies. The process requires complex logistics involving collection, sorting, and transportation of the waste; consequently, it might be difficult to keep the process economically viable. Moreover, the presence of additives such as adhesives, colorants, and other substances modifying the plastic' rheology may render the processing and reuse of the materials problematic and technically not feasible (Shen et al. 2020; Thiounn and Smith 2020). There are three types of plastic recycling based on how the material is utilized (Merrington 2017; Thiounn and Smith 2020):
  • primary recycling—direct reuse of discharged, uncontaminated plastic, generally for the same purpose as the original material.
  • secondary recycling—physical reprocessing of plastic with keeping the material's chemical identity, such material usually finds application for different purpose than the original material; plastic also gradually loses its properties after each reprocessing cycle.
  • tertiary recycling—chemical recycling during which the polymer is broken down and the products further utilized for various purposes.
– *Incineration*: this method based on burning the polymers allows to recover part of the energy in the form of heat released during the incineration. This process, as good as it is in reducing the plastic waste mass, generates secondary pollution. Approximately 10% of plastic waste is treated this way (Geyer et al. 2017; Thiounn and Smith 2020).

## 2.3 Plastic Biodegradation

The term biodegradation has evolved together with the development of science. Firstly, perceived simply as degradation of substances by microorganisms leading to mineralization of organic matter, biomass generation and carbon recycling. Nowadays, it is understood as a complex phenomenon influenced by various factors. Biodegradation occurs in several steps (Lucas et al. 2008; Rudnik 2019):

– *Biodeterioration*: the first step in which synergic action of microbial consortia and abiotic factors lead to disintegration of the polymer's structure into smaller fractions. It is a superficial degradation modifying the chemical, physical, and

mechanical properties of the polymer. The intensity of this process can be measured by physical tests (thermal transitions, tensile changes).

- *Biofragmentation (depolymerization)*: occurs due to the action of the enzymes and free radicals secreted by microorganisms, e.g., produced from $H_2O_2$ reacting with ferrous atoms in the Fenton reaction. The polymers are cleaved into oligomers, dimers, and monomers that can be further detected by chromatographic or spectroscopic methods. Enzymes catalyze one or a series of reactions leading to diverse chemical conversions, generally on the way of oxidation, hydrolysis, reduction, molecular inner conversion, or esterification (Harshvardhan and Jha 2013). The most important groups of enzymes acting in this process are:
  - oxidoreductases: mono- and dioxygenases incorporate one or two oxygen atoms to create alcohol or peroxyl functional groups; peroxidases catalyzing reactions between an electron acceptor groups (amino, carboxyl, phenol, phenyl, aliphatic unsaturation or thiol) and a peroxyl molecule; or oxidases, copper-containing metalloproteinases, involved in hydroxylation and oxidation reactions,
  - hydrolases: cellulases, cutinases, amylases or lipases, or esterases and endopeptidases attacking the bond in amide groups or carboxylic linkages. This group of enzymes works on the so-called catalytic triad mechanism involving serine, aspartate, and histidine.
- *Assimilation*: the final step of biodegradation, the depolymerized fragments of the polymers are integrated inside the microorganisms' cells. During this process, the ingredients are used to produce energy, maintain activity, grow the cell biomass, and for reproduction. This can happen on the way of aerobic or anaerobic respiration or fermentation. This step closes the polymer life cycle, ideally, transforming it into environment benign, nontoxic molecules. The intensity of the process can be measured by metabolites production or biomass growth (Lucas et al. 2008; Nair et al. 2017; Poznyak et al. 2019).

### 2.3.1 Susceptibility of Polymers to Biodegradation

There is a strong relation between several physicochemical characteristics of polymers and their susceptibility to microbial degradation. The most relevant factors include:

- *Crystallinity*: the polymer's segments must be mobile enough to be able to get into the enzyme's cavity, where the active site is located in order to be degraded. In the case of highly crystalline polymers, the mobility corresponds mostly to their melting temperatures; consequently, the more crystalline the polymer is, the higher is its melting temperature, and the degradation would begin in higher temperature range. In the case of amorphous polymers, the mobility may be estimated by the glass transition temperature ($T_g$) (Müller et al. 2005)

- *Surface hydrophobicity*: The polymer's surface hydrophobicity influences the microorganism adherence to the surface. Generally, a hydrophobic surface will be adequate for the attachment and growth of hydrophobic bacteria, the opposite being true for a hydrophilic microorganisms. Most of the microorganisms and the enzymes taking part in degradation are active in a humid environment. It is a common way to subject polymer to pretreatment increasing their hydrophilicity that results in the formation of various functional groups such as the carbonyl, carboxyl, or ester groups. Hydrophobicity also restricts penetration of water into eroded polymers surface (Park et al. 2003; Domb et al. 2011; Sangeetha Devi et al. 2015)
- *Porosity*: This parameter determines the access of microorganism to the polymer surface. The higher the porosity and the pores interconnectivity, the higher the surface area available for the microorganisms and more intense the bulk degradation. Porosity also affects the diffusion of the polymer degradation products from the matrix. The increase in the porosity upon the microorganisms action leads to void formation and further loss of the polymer's integrity (Bastioli et al. 2020)
- *Durability*: the biodegradation process is strictly connected with the action of microorganisms; however, the abiotic parameters play a significant role as they can enhance or initiate the process. The ability of a polymer to withstand a mechanical damage, the action of solar radiation, or the resistance to oxidative degradation, all influence the rate and occurrence of the biodegradation process.
- *Polymer backbone*: there are generally five groups of synthetic polymers based on their backbone bonds: the polyolefins with the alkaline bond, the polyesters with the ester bond, the polyamides with the amide bonds, the polyurethanes connected by the urethane bonds, and the polyethers with the ether bond. For the biodegradation to take place, the presence of an adequate microorganism able to secrete a proper enzyme is mandatory: hydrolytic enzymes for the degradation of polyesters and polyamides and oxidative enzymes for the degradation of polyolefines and polyethers (Farachi et al. 2020)
- *Molecular weight*: the attack of microorganisms begins generally from the ends of large molecules; the larger the molecular weight, the fewer molecules end available for the microorganisms (Bastioli and Bettarini 2020)
- *Aromatic parts*: polymers having an aromatic part in their structure are known to be more resistant to biodegradation; furthermore, the longer the aromatic sequence, the less readable the polymer for microbial attack (Bastioli and Bettarini 2020)
- *Additives*: biodegradation can be hindered by the presence of certain polymer additives, intermediate products, or impurities that may exhibit adverse effect on the microorganism (Bastioli and Bettarini 2020).

Another set of factors influencing the biodegradation is related to the environment in which the decomposition process takes place, and include:

- *pH*: diffusion of the polymer degradation products may be affected by the pH of the surrounding medium: the solubility of the products may vary depending on

the pH as in some pH range they can exist in unionized, less soluble forms (Domb et al. 2011). Enzymes also have an optimal pH value at which their activity is the highest; consequently, the intensity and occurrence of the biodegradation process is highly pH-dependent (Liu 2020)

– *Temperature*: the first aspect is related to the polymer's segment mobility; the biodegradation is favored in temperatures above the glass transition temperature (Tg) of an amorphous polymer or the melting temperature of a crystalline material (Müller et al. 2005). Nonetheless, the occurrence of purely crystalline polymers is rather rare; hence, both temperatures should be regarded considering the biodegradation process. The second aspect is related to the working speed of a polymer degrading enzyme and its stability. If the temperature is too low, the kinetic is slow, too high temperatures cause denaturation of the enzyme (Dwicania et al. 2019)
– *Other abiotic factors*: polymers, in outdoor conditions, can degrade being exposed to mechanical, thermal, or chemical factors or to radiation [1]. The more intense each of the factors is, the greater the changes it induces in the polymer structure; the effect is further intensified when more than one of the factors act simultaneously. The resultant changes play a significant synergic role in the biodegradation modulation of its paste; in some cases, the action of abiotic factor may be required for the biodegradation process to commence.
– *Miscellaneous*: other factors tuning the biodegradation process are availability of light, $CO_2$ or $O_2$, presence of contaminants, nutrients availability, and humidity (Dwicania et al. 2019; de Wilde 2020).

### 2.3.2 Biodegradability-Based Polymers Classification

Classification of commercially significant polymers based on their biodegradability (Jiang and Zhang 2017; Bastioli and Bettarini 2020; Thiounn and Smith 2020):

A. *Biodegradable*:

– naturally occurring in nature, obtained directly from nature:
  • polysaccharides: starch, cellulose
  • polyamides: soy protein, wool, silk
  • poly(hydroxyalkanoates) (PHAs): poly(hydroxybutyrate) (PHB), poly (hydroxybutyrate-co-hydroxyvalerate) (PHBV)
– synthetic, obtained from renewable sources:
  • poly(lactic acid) (PLA)
  • poly(glycolic acid) (PGA)
  • modified triglyceride-based polymers: derived from plant oil
– synthetic, derived from nonrenewable sources (petroleum):
  • poly(ε-caprolactone) (PCL)
  • poly(butylene succinate) (PBS)
  • poly(butylene succinate adipate) (PBSA)
  • poly(butylene adipate-*co*-terephthalate) (PBAT)

  - poly(ethylene succinate) (PES)
  - Poly(vinyl alcohol) (PVA)

B. *Degradable polymers*: based on conventional polymer matrixes, such as polyolefines, filled with a biodegradable material, transition metal salts, or metal oxides. The fillers disintegrate the matrix or photocatalyze its degradation. The final degradation product is the fragmented polymer matrix.

C. *Non-biodegradable polymers*:
  - low-density polyethylene (LDPE)
  - high-density polyethylene (HDPE)
  - polypropylene (PP)
  - polystyrene (PS)
  - polyvinyl chloride (PVC)
  - poly(ethylene terephthalate) (PET)
  - polyurethane (PUR)

The C-group polymers, the so-called plastics, are commonly used in everyday life applications. Generally considered nonbiodegradable, designed to be durable, resistant, and to last for long time periods, they are also the ones rising worldwide concern regarding plastic pollution. The chapter describes findings in the field of microbial degradation of plastics of the main industrial importance.

### 2.3.3 Polyethylene

Polyethylene (PE) is the most produced and consumed polymer in the group of synthetic polymers and polyolefins (Danso et al. 2019). It usually occurs in one of the two types, branched low-density polyethylene (LDPE) and high-density polyethylene (HDPE) with linear structure (Gopanna et al. 2019). PE application includes among others the production of rigid containers, packaging films, pipes, drums, and bottles (Patel 2016).

Volke-Seplveda et al. (2002) incubated *Aspergillus niger* and *Penicilium pinophilum* with thermo-oxidized (15 days at 80 °C) LDPE at 30 °C for 31 months in the presence or absence of ethanol. For both strains, greater structural and morphological changes occurred in the presence of ethanol, which promoted the production of LDPE-oxidating enzymes. This kind of phenomenon is called the cometabolic process. However, based on the DSC, FTIR, and XRD analyses, *Penicilium pinophilum* exhibited higher material biodegradation efficiency than *Aspergillus niger*. The difference was attributed to the production of extracellular enzymes characterized by certain level of unspecificity by *Penicilium pinophilum,* which were more suitable for LDPE biodegradation. Bonhomme et al. (2003) tested *Cladosporium cladosporoides* fungus for PE films biodegradation containing TDPA pro-oxidatives. Incubation was carried out at 27 °C for 6 months. The results showed microbial growth on the polymer surface; surface pitting and its erosion were noticed after removal of microorganisms. Manzur et al. (2004) investigated biodegradation

of LDPE after treatment in the aging chamber under UV irradiation at 70 °C or thermal treatment at 105 and 150 °C for 120 h. The polymer samples were incubated with a consortium of fungi, *Aspergillus niger, Gliocladium virens, Penicillium pinophilum,* and *Phanerochaete chrysosporium*, for 9 months at 29 °C. The results indicated that LDPE biodegradation was a two-stage process, first involving the microorganisms attack on the LDPE amorphous phase resulting in the polymer crystallinity decrease and then the attack on polymer crystals. Biotreatment of the polymer caused the formation of cavities on its surface and hyphae penetration into the material; however, the aging treatment resulted in more intense hyphae penetrations. Zahra et al. (2010) reported the ability of fungal strains *Aspergillus fumigatus* and *Aspergillus terreus* isolated from landfill to degrade previously photooxidated LDPE samples; the incubation was carried out during 100 days at 28 °C. Changes in the polymer's surface due to pitting corrosion and erosion were noticed for the *Aspergillus* species; tests with other fungus *Fusarium solani* did not cause significant LDPE surface degradation. Pramila (2011) conducted extended research on LDPE samples using fungi isolated from landfills and Pramila and Ramesh (2011) from marine water. The research confirmed LDPE biodegradation ability of fungi *Aspergillus flavus, Mucor cicinelloides*, and *Aspergillus versicolor*. Sowmya et al. (2012) tested a fungus from the *Chaetomium* genus and *Aspergillus flavus* isolated from a landfill for biodegradation of PE disks subjected to UV-irradiation and incubation with nitric acid for 6 days at 80 °C. The applied pretreatment led to more efficient biodegradation compared to the untreated samples; *Chaetomium* spp. caused 40% and *Aspergillus flavus* 20% mass reduction of the treated polymer after 6 months incubation. Esmaeili et al. (2014) evaluated degradation of photooxidized LDPE in the presence of fungus *Aspergillus niger* isolated from a landfill. The samples were subjected to photooxidation for 25 days and incubation with the microorganism for 56 days; the polymer's surface was colonized by the fungus and its surface underwent modifications. Das and Kumar (2014) reported successful biodegradation of LDPE using four *Aspergillus* spp. and one *Fusarium* spp. strains; the microorganisms colonized the LDPE surface and used it as carbon source which resulted in the surface modification and the polymer mass loss. Two *Bacillus amyloliquefaciens* isolates obtained from soil were tested for biodegradation of LDPE boiled in xylene and washed in ethanol. The bacteria exhibited good growth and adherence to the polymer surface; after 60 days of incubation at 33 °C, several chemical changes in the LDPE surface were observed together with the presence of biodegradation products in the extracellular medium (Das and Kumar 2015). Another three strains from the *Bacillus* genus were tested for biodegradation of LDPE pretreated with bleach for 3 h; after 60 days of incubation, high bacterial content on the polymer surface, formation of new functional groups in the polymer structure, and the initial mass reduction on the level of 1.5% were observed (Kumar Gupta and Devi 2019). *Bacillus amyloliquefaciens* strain obtained from plastic waste was able to attack raw and ɣ-irradiation/thermally pretreated LDPE films. Upon 60 days of incubation at 28 °C, initial mass reduction of 3.2 ± 1.3% was observed. Additionally, a decrease in the FTIR spectra region corresponding to the carbonyl band and flattening in the range of

1300–1100 $cm^{-1}$ was recorded. The pretreatment of the LDPE resulted in the formation of low-molecular-weight oligomers in the structure; the oligomers were further metabolized by the bacteria forming 3-hydroxybutyrate detected in the medium (Novotny et al. 2018). Two species of bacteria from the *Enterobacter* genus and one from the *Pantoea* were isolated from soil samples collected near plastic processing plants. The bacteria were mixed in a consortium and tested for LDPE biodegradation and incubated for 120 days at 30 °C. Such consortium was able to degrade up to 81 ± 4% of the polymer strips and 38 ± 3% of LDPE pellets mass; it was almost two times more than that of a consortium of *Pseudomonas putida*, *Pseudomonas stutzeri*, and *Bacillus subtilis* obtained from the Microbial Culture Collection Centre known for their polyethylene-degrading properties (Skariyachan et al. 2016). Sowmya et al. (2015) reported biodegradation of PE by *Penicilium simplicissimum.* The polymer samples were pretreated with UV-irradiation, surface autoclaving, or sterilization. The results showed weight loss of the material at the level of 38%, 7.7%, and 16%, respectively. The fungal degradation ability was also confirmed by FTIR analyses which showed presence of compounds such as alcohols, aldehydes, and carboxylic acids. The degradation was attributed to the action of enzymes laccase and manganese peroxidase. Sangeetha Devi et al. (2015) found a mixture of marine fungi strains *Aspergillus tubingensis* and *Aspergillus flavus,* secreting extracellular enzymes suitable for HDPE biodegradation after formation of a biofilm on the polymer's surface. Gajendiran et al. (2016) performed PE biodegradability tests in the presence of *Aspergillus clavatus*. The incubation was carried out for 90 days and led to 35% reduction in the polymer initial mass. Moreover, AFM and SEM analyses indicated surface erosion, fractures, and grooves and fungal colonization of the polymer surface. Munir et al. (2017) tested fungi *Trichoderma viride* and *Aspergillus nomius* for degradation of PE. After 45 days of incubation the films' tensile strength was reduced, groves of the surface formed, and the weight loss of 5.13% and 6.63% was noticed for the *Trichoderma* spp. and *Aspergillus* spp., respectively. Cyanobacteria *Phormidium lucidum* and *Oscillatoria subbrevis* isolated from PE plastic bags submerged in sewage were capable of degrading non-pretreated LDPE films reducing their initial weight by 30% in 42 days (Sarmah and Rout 2018). In a study of LDPE biodegradation employing *Aspergillus nomius* and *Streptomyces* spp., the microorganisms were able to degrade 4.9% and 5.2% of the initial polymer mass after 45 days of incubation at 30°C. The *Streptomyces* produced eight different biodegradation products and almost twice the amount of $CO_2$ than the *Aspergillus* which degraded the LDPE to six products identified by GC-MS; carbonyl, ester, and vinyl groups were formed in the polymer due to the action of microorganisms (Montazer et al. 2019). *Alcanivorax borkumensis* is another example of a bacterium able to degrade LDPE. In the study of Delacuvellerie et al. (2019), it was able to degrade 3.5 ± 0.3% of the polymer mass in 80 days of incubation at 30 °C; moreover, the biofilm growth on LDPE was approximately two times faster than on PET or PS. Eleven bacterial strains were isolated from landfill soil rich in plastic waste; 8 bacterial genera were identified in the isolates, and included *Pseudomonas, Citrobacter, Micrococcus, Stentrophomonas, Delftia, Ochrobacterum, Acinetobacter,* and *Sphingobacterium.*

The bacteria were inoculated with LDPE pretreated with UV radiation. The most active strain was the *Acinetobacter pitti* that was able to degrade 27 ± 3% of the polymer mass in 4 weeks at 30 °C. This bacterium had not been known before to degrade PE; the most active strain in terms of biofilm production was the *Sphingobacterium moltivourum* (Montazer et al. 2018). Sáenz et al. (2019) reported PE biodegradation by *Aspergillus niger* and *Aspergillus terreus* after 77 days of incubation. The process began at PE surface by the action of a lactase. The strains exhibited degradation ability toward not only LDPE but also HDPE. Biodegradation of that polymer was also evaluated using bacterium *Microbulbifer hydrolyticus* IRE-31 isolated from waste generated by a marine pulp mill. LDPE spheres of 2 mm radius were cultivated for 30 days at 37 °C in the presence of the bacteria; after that time there were evidences of morphological changes in the polymer surface (appearance of cracks) and formation of carbonyl functional groups (hydroxyl and carbonyl) on the surface as a result of the microbial action (Li et al. 2020). Montazer et al. (2019) evaluated the ability to degrade powdered LDPE of four bacteria strains isolated from a landfill soil in Iran, including *Acinetobacter pittii* IRN19, *Delftia tsuruhatensis* IRN27, *Pseudomonas putida* IRN22, *Micrococcus luteus* IRN20, and of four bacteria known to produce biopolymers polyhydroxyalkanoates (PHA) such as *Pseudomonas chlororaphis* PA23, *Cupriavidus necator* H16, *Pseudomonas monteilii* MO2, *Pseudomonas putida* LS46. After 21 days of incubation at 30°C the mass loss of the polymer ranged from 19% in the case of *Micrococcus luteus* IRN20 to 34% for *Cupriavidus necator* H16. Most importantly, it was proven that conversion of LDPE straight to polyhydroxyalkanoate polymers is feasible; short-chain-length PHA was produced by the *Cupriavidus necator* H16 and medium-chain-length PHA by the *Acinetobacter pittii* IRN19 and *Pseudomonas putida* LS46. *Enterobacter cloacae* AKS7 was also recently reported to be able to degrade LDPE. The bacterium was incubated at 30 °C for 45 days with LDPE films. After that time 9% mass loss of the plastic was recorded; additionally, the hydrophobicity, hence ability to interact with the polymer surface, of the bacterium was reduced subjecting it to UV radiation (Sarker et al. 2020b). A study focused on application of a bacterium *Pseudomonas aeruginosa* ISJ14 strain isolated from a landfill revealed that this microorganism was able to degrade LDPE films. After 60 days of cultivation at 37 °C, 8.7% of the polymer initial mass was degraded, numerous fissures in the polymer's structure appeared along with new functional groups, and increased adhesion of the bacteria to the LDPE surface (Gupta and Devi 2020).

### 2.3.4 Polypropylene

Polypropylene (PP) is a thermoplastic polyolefin and the second most produced synthetic polymer in 2016 (Danso et al. 2019; Fesseha and Abebe 2019). The material possesses outstanding properties such as toughness, strength, processing ease, and high melt temperature which favor its use in many areas including labels, personal hygiene, consumable and medical packaging, and construction films (Calhoun 2016).

Representatives of the *Aspergillus* genus, *Aspergillus niger*, and *Aspergillus flavus* are known from their polypropylene biodegrading action. Pandey and Singh (2001) conducted research of photo-oxidized isotactic polypropylene inoculated with *Aspergillus niger* strains at 28–30 °C. After 6 weeks of incubation, fungal growth was observed on the samples and was strongly correlated with the material pretreatment time—the longer the plastic was exposed to the radiation, the greater was the surface colonized by the fungus. A bacterial species from the *Bacillus* genus, *Bacillus gottheilii,* isolated from a mangrove, after 40 days incubation, exhibited ability to biodegrade 3.6% of the mass of PP samples (Auta et al. 2017). Santacoloma-Londoño et al. (2019) evaluated polypropylene biodegradation in the presence of *Aspergillus flavus*. The strain was incubated in two types of agar, yeast extract glucose chloramphenicol and the Sabouraud Dextrose Agar, for 100 days. The obtained results showed 13.7% and 0.0% reduction of initial mass of the polymer, respectively.

### 2.3.5 Polystyrene

Polystyrene (PS) has linear structure and exhibits outstanding scratch resistance, stiffness, and good transparency; this makes it a widely used polymer applied in the packaging and building industry; it is also used for production of disposable medical ware (Gausepohl and Nießner 2001).

Krueger et al. (2015) conducted extended polystyrene sulfonate biodegradability tests using *Gloeophyllum trabeum* DSM 1398 and DSM 3087, *Gloeophyllum striatum* DSM 9592 and DSM 10335, *Stropharia rugosoannulata* DSM 11372, *Trametes versicolor* DSM 11269, and *Trametes hirsute.* Among all evaluated fungal strains, the *Gloeophyllum* species, described as brown rot-fungi, was found to be suitable for PSS depolymerization. Polystyrene biodegradation was tested by Sekhar et al. (2016). Bacterial strains isolated from a plastic waste were identified as *Brevundimonas diminuta*, *Citrobacter sedlakii*, *Enterobacter* spp., and *Alcaligenes* spp. Upon 30 days of incubation, the *Enterobacter* spp. were able to degrade up to 12% of the plastic mass. Auta et al. (2017) evaluated PS-degrading ability of two species of the *Bacillus* genus, *Bacillus gottheilii*, and *Bacillus cereus,* isolated from a mangrove sites. After 40 days of incubation, a mass loss of the samples on the level of 5.8 and 7.4% was observed for the *B. gottheilii and B. cereus,* respectively. The same author, later, investigated other mangrove isolates, identifying in one *Bacillus cereus* and *Bacillus thuringiensis* and *Rhodococcus ruber* in the other. In similar incubation condition, the *Bacillus* sp. was able to biodegrade 4% of the mass of PP microplastic, while mass reduction of 6.4% was recorded in the case of the other isolate (Auta et al. 2018). Santacoloma-Londoño et al. (2019) incubated polystyrene pieces for 100 days with *Aspergillus flavus* in the Sabouraud Dextrose Agar, which resulted in 0.92% reduction of the polymer initial mass. Additionally, some of the polystyrene derivatives, such as polystyrene sulfonate, were be degraded in the medium. Tourova et al. (2020) analyzed biofilms formed on polystyrene collected from seawater and industrial wastewater. In the first case, bacteria from the genera

*Mycobacterium*, *Erythrobacter*, and *Maribacter*, and in the second case, belonging to the *Mycobacterium*, *Arenimonas*, *Pseudomonas*, and *Acidovorax* genera, exhibited polystyrene-degrading potential. A species from the *Pseudomonas* bacteria was isolated from a gut of worm (*Zophobas morio*), known to be able to digest polystyrene; the bacteria was incubated with PS for 60 days at 25 °C. After that the PS surface became hydrophilic, and new carbonyl groups were formed in the structure. The degradation began from oxidation of the C-H benzylic bonds to alcohols and then to carbonyls (Kim et al. 2019). A polystyrene-degrading bacterium was isolated from the gut of another a plastic-consuming larvae *Tribolium castaneum* by Wang et al. (2020); the bacterium was identified as *Acinetobacter* spp. After incubation for 60 days, a mass reduction of 12.14% was noticed for the pure polystyrene samples.

### 2.3.6 Polyvinyl Chloride

Polyvinyl chloride (PVC) is a relatively cheap, widely used thermoplastic polymer with high chemical resistance. It is widely used as a substrate in production of packaging and building materials, furnitures, and toys (Ali et al. 2014; Begum et al. 2019).

De Campos et al. (2003) investigated the impact of blending PVC with polycaprolactone (PCL), a biodegradable polymer, on the final product's biodegradation. The materials were mixed in 1:1 proportion and incubated with *Phanerochaete chrysosporium/Aspergillus fumigatus* strains at 27 °C. After 4 months of the biotreatment, the UV-spectra indicated structural changes in the blends manifested by increased amount of carbonyl groups compared to the reference, untreated samples. *Achromobacter* spp. and *Pseudomonas aeruginosa* were able to biodegrade in 180 days around 35% of the mass of PVC that was plasticized by vegetable oil; no bacterial growth was observed on the unmodified polymer though (Das et al. 2012). Latorre et al. (2012) conducted a study on PVC biodegradation using bacteria able to use as a carbon source a common PVC-plasticizer the di-(2-ethylhexyl)phthalate (DEHP). The studied objects were pieces of shower curtains, and the bacteria were *Chryseomicrobium imtechense*, *Lysinibacillus fusiformis*, *Acinetobacter calcoaceticus*, and *Stenotrophomonas pavanii*. The bacteria were able to lower the DEHP concentration in the samples in 6 weeks what was determined by thermogravimetric methods. Ali et al. (2014) evaluated the ability of fungal isolates of *Phanerochaete chrysosporium* PV1, *Lentinus tigrinus* PV2, *Aspergillus niger* PV3, and *Aspergillus sydowii* PV4 to degrade PVC thin films. After the experiments conducted at 30 °C, surface deterioration and color changes in the materials were observed on SEM images. Moreover, FTIR, NMR, and GPC analyses showed structural changes in the polymer, therefore proving the strains' ability to PVC biodegradation. Vivi et al. (2019) treated PVC with *Aspergillus brasiliensis* ATCC 9642, *Chaetomium globosum* ATCC 16021, *Penicillium funiculosum* ATCC 11797, *Paecilomyces variotii* ATCC 16023, and *Trichoderma virens* ATCC 9645 during 28 days at 28 °C. The samples exhibited superficial erosion with wrinkles and

the occurrence of *Chaeotomium globosum* fertile structures such as perithecia and hyphae. Furthermore, adhesion of *C. globosum* on the material was observed, which indicated first step of PVC biodegradation. Biodegradation of this plastic was also studied by Kumari et al. (2019) who used for that purpose a marine bacteria consortium formed of the *Bacillus* genus species such as *B. paralicheniformis, B. licheniformis* and *B. gycinifermentans, B.sonorensis, and B. aerius.* After 90 days of incubation, the ability to destabilize plastic was confirmed by FTIR analyses of the materials, the appearance of degradation marks on the surface, $CO_2$ production, and the samples' initial mass loss on the level of 0.32%. *Pseudomonas citronellolis* were the bacteria able to form a biofilm of PVC samples in the study of Giacomucci et al. (2019). After 45 days of incubation, fragmentation of the polymer films was observed; moreover, initial weight loss reached approximately 20% and the average molecular weight decreased by 10%. Denaro et al. (2020) tested PVC films biodegradation by anaerobic marine bacteria consortia composed of species belonging to the genera *Acetobacterium, Dethiosulfovibrio, Erysipelothrix, Fusibacter, Psychromonas, Desulfovibrio, Cupriavidus, Cohaesibacter, Pleomorphochaeta*, and *Sporobacter*. After 7 months of incubation at 20 °C, three out of sixteen isolates developed a biofilm on the PVC surface; after another 24 months, the biofilm was observed in additional three isolates. Degradation of the polymer was confirmed by GPS analysis showing evidence of lowering of the average polymer molecular weight and by thermogravimetric studies that revealed decreased thermal stability of the biotreated PVC.

### 2.3.7 Polyurethane

Polyurethane (PUR) is a material widely used for the production of coatings, adhesives, furniture, fibers, elastomers, paints, synthetic skins, and constructional materials (Mathur and Prasad 2012).

Gautam et al. (2007) reported polyester–polyurethane degradation by a lipase produced by *Candida rugosa* fungus. Shah et al. (2008) conducted research on polyurethane degradation ability of several bacterial strains isolated from PU films after burial in soil for 6 months. The PU was the sole carbon source for the microbes; hydrolysis zones were visible in plate assays around the bacterial colonies; secretion of an esterase was detected in samples containing PU. The identified bacteria were classified into the *Corynebacterium*, *Micrococcus, Bacillus, Arthrobacter,* and *Pseudomonas* genera. Nakkabi et al. (2015) tested PU degradation by a strain *Bacillus subtilis* isolated from rotten cedarwood. After 7 days of incubation at 37 °C, the peak in the FTIR spectrum associated with the ester bond completely disappeared, confirming the polymer degradation. *Acinetobacter gerneri* P7 isolated from decomposed foam was proved to be able to bind by forming complexes and degrade polyurethane in the study of Howard et al. (2012). Loredo-Treviño et al. (2011) conducted extended research concerning polyurethane biodegradation; 22 fungal strains belonging to the genera of *Trichoderma*, *Aspergillus*, *Penicillium*, *Paecelomyces*, *Fusarium*, and *Alternaria* were able to effectively degrade PU after

incubation in sand samples. Furthermore, enzymatic characterization of the PU biodegrading strains showed that most of them exhibited urease activity (95.45%); other enzymes such as protease, esterase, or laccase exhibited activity at the level of 86%, 50%, and 36%, respectively. Among all the tested fungi, the *Trichoderma* strains exhibited the fastest growth. Mathur and Prasad (2012) incubated PU with *Aspergillus flavus* for 30 days, which resulted in 60.6% mass loss of the material; the degradation occurred due to the ester bonds rupture. Shah et al. (2013b) isolated a species of Bacillus from soil samples collected near dumping area in Pakistan. The *Bacillus subtilis* MZA-75 after incubation with PU samples for 4 weeks at 30 °C caused numerous changes in the PU structure: cracks and fissures on the surface, hydrolysis of the ester bond reflected in disappearance of the carbonyl and C-O stretching bonds, and lowering of the polymer average molecular weight. Additionally, adipic acid and 1,4-butanediol, the PU degradation products, were detected in the matrix confirming the biodegradation action of the microbe. The same author reported PU degradation ability for *Pseudomonas aeruginosa* MZA-85 strain (Shah et al. 2013a) and by a consortium of that bacteria and *Bacillus subtilis* MZA-75 (Shah et al. 2016). Ibrahim et al. (2011) tested 35 fungal strains for PU degradation. The selected species were incubated with the polymer using the liquid shaking culture, the Petri dish, and clear zone test techniques; 6 strains exhibited PU degradation ability including *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus flavus*, *Fusarium solani*, *Alternaria solani*, and *Spicaria* spp. The highest activity was obtained for *Fusarium solani* with 100% polymer degradation in the shaking liquid method, while in the case of the Petri dish technique, the most active strain was *Aspergillus flavus* with 94% mass reduction of the PU samples. Susceptibility to biodegradation by bacterium *Alicycliphilus* spp. BQ8 of several commercially available polyester polyurethanes was evaluated by Pérez-Lara et al. (2016) for 15 days incubation at 37 °C. Among all the tested polyurethanes, phthalic anhydride–PU foam was the most altered by the microbes; the analyses revealed that the bacteria attacked the ester and urethane bonds by secreting amidases and esterases. Khan et al. (2017) investigated polyurethane beads' fungal degradation after burial in soil. The results showed that *Aspergillus tubingensis* was the microorganism responsible for the PU degradation, which involved three steps: the first one included spore adhesion to the material surface, the second one concerned hyphal growth on the surface, and the third one was enzymes secretion. The fungal strain caused surface erosion, cracks, and pore formation and decrease in tensile strength. *Pseudomonas aeruginosa* was found to biodegrade a novel aliphatic hyperbranched polyurethanes from modified castor oil and sunflower oil; the tests were carried out during 12 weeks cultivation at 37 °C. The presence of numerous hydrolyzable ester linkages in the hyperbranched structure affiliated the growth of the bacteria (Bayan and Karak 2017). Osman et al. (2018) confirmed *Aspergillus fumigatus* effective biodegradation of methylene diphenyl diisocyanate polyester PU pellets. During the degradation process, holes, cracks, and pits formation on the material's surface was observed. Increase in the material's melting temperature was noticed from 191 to 196°C due to morphological changes. Biodegradation of polyurethane foams formed using algae-based polyols was studied by Gunawan et al. (2020). The foams were

kept in a compost or buried in soil for 12 weeks; after that time prominent changes in their structure, the result of microbial action, were found. Bacterial genera identified on the polymers included *Achromobacter, Brucella, Pseudomonas, Stenotrophomonas, Rhizobium*, *Chryseobacterium*, *Herbaspirillum, Ochrobactrum, Rhodococcus,* and *Stenotrophomonas*.

### 2.3.8 Polyethylene Terephthalate

Polyethylene terephthalate (PET) is a polyester commonly used as fibers, films, and packaging material (Ronkvist et al. 2009). PET is usually described as nonbiodegradable, but there are also known cases of its degradation by hydrolytic enzymes produced by microorganisms (Taniguchi et al. 2019; Kawai et al. 2019), cutinases (Ronkvist et al. 2009; Nimchua et al. 2008), lipases (Carniel et al. 2017), and esterases (Liebminger et al. 2007).

PET depolimerization experiments were conducted using an enzyme produced by an anaerobic bacterium *Thermobifida fusca* on PET circular film of 12 mm in diameter. The degradation rate was within the range of 8–17μm per week at 55 °C. The *Thermobifida fusca* degradation efficiency was improved by the addition of $Ca^{2+}$ and $Mg^{2+}$ to the matrix. This operation increased the enzymes' stability at elevated temperatures (Müller et al. 2005). Nimchua et al. (2007) evaluated the ability to degrade PET of fungi *Fusarium oxysporium* LCH1 and *Fusarium solani* f. sp. pisi. DSM 62420. The tests of the enzyme activity were conducted during 168 h at 30 °C to monitor the release of terephthalic acid from the PET fibers. The *Fusarium oxysporium* LCH1 enzyme exhibited nearly two-fold higher activity toward PET fabrics compared to the *Fusarium solani* enzyme; additionally, the hydrophilicity of the PET samples increased after the incubation compared to the untreated polymer. The same author tested 115 fungal isolates for the ability to degrade PET; 22 of them were able to produce PET-modifying cutinase. *Fusarium solani* PBURU-B5 showed highest esterase activity toward the polymer, almost six times higher than the other investigated fungus after 21 days of incubation. The treatment of the polymer with fungal enzymes resulted in ester bond hydrolysis, moisture, and water adsorption increase and enhancement of the polymers hydrophilicity (Nimchua et al. 2008). The degradation activity of an enzyme cutinase obtained from fungi *Humicola insolens* and *Fusarium solani,* and a bacterium *Pseudomonas mendocina,* was tested on 15 × 15 × 0.250 mm PET films. The enzyme's ability to degrade the polymer decreased with increasing PET's crystallinity; the enzyme from the *Pseudomons* had the highest and from *Fusarium* the lowest affinity. The initial hydrolysis rate of the *Humicola* was 7 times higher than that of the two other organisms owing to the fungus' ability to remain active at the temperature close to the polymer's Tg temperature. After 96 h incubation at 70 °C, this microorganism biodegraded 97 ± 3% of the film. At the same time, *Pseudomonas* at 50 °C and *Fusarium* at 40°C degraded only 5% of the polymer mass. All the organisms decomposed PET to terephthalic acid and ethylene glycol (Ronkvist et al. 2009). Fungi from the *Penicillium* genus are quite commonly used for PET

hydrolysis. Liebminger et al. (2007) conducted experiments of PET model substrate-bis (benzoyloxyethyl) terephthalate (3PET) and PET pellets using *Penicillium citrinum* polyesterase. Degradation of both materials led to the release terephthalic acid, mono-(2-hydroxyethyl)terephthalate, and bis-(2-hydroxyethyl)terephthalate after 7 days incubation. Nowak et al. (2011) compared biodegradation of raw and PET modified with polyester Bionolle by *Penicillium funiculosum* in 84 days incubation at 30 °C. The modification with the polyester did not significantly increase the weight loss of the materials; the fungus action was manifested more in chemical modification of the PET as the FTIR and XPS analysis revealed decreed amount of aromatic rings from terephthalic acid in the samples. The degradation occurred due to the action of hydrolytic and oxidative enzymes. Sepperumal et al. (2013) treated PET flakes and powder with *Penicillium* spp., which resulted in chemical changes in both materials. Additionally, it was found out that the fungal strains used the powdered polymer as a carbon source. A cutinase obtained by cloning a gene from *Saccharomonospora viridis* AHK190 and expressing it in *Escherichia coli* Rosetta-gami B (DE3) exhibited a supreme stability in the presence $Ca^{2+}$; the ions enhanced the enzyme's thermal stability rendering it active in temperature up to 65 °C for 24 h. The PET films upon contact with the enzyme first lost their hydrophobic character and with time degraded up to 27% of their initial mass (20–25 mg) during 3 days. There was a significant difference between incubation at 50 °C, where the degradation was neglectable, 60 °C at which it was low, and at 63 °C with the highest intensity (Kawai et al. 2014). An amount of 250 PET environment samples from sediment, wastewater, soil, and activated sludge from PET recycling facility were screened for PET-degrading microorganisms by Yoshida et al. (2016). In one sample, a consortium of bacteria, yeast-like cells, and protozoa was able to degrade PET at a rate of 0.13 mg $cm^2$ $day^{-1}$ at 30 °C. Using limiting dilution, a bacterium from the genus *Ideonella* was isolated and named *Ideanella sakaiensis* 201-F6. The bacterium produced enzymes capable of degrading PET and mono(2-hydroxyethyl)terephthalic acid, the reaction intermediate. PET was converted into ethylene glycol and terephthalic acid. Carniel et al. (2017) tested combination of *Humicola isolens* cutinase and *Candida antarctica* (CALB) lipase B for PET hydrolysis. The enzymes were mixed at two proportions of 1:1 and 9:1 and incubated with PET for 14 days. A strong synergic effect of the enzymes action was observed; the terephthalic acid release was equal to 1606 and 2247μM, respectively, almost 8 times higher than in the case of the cutinase alone. Other bacteria colonies were isolated from soil polluted with petroleum products near Huston in Texas. The selection was made based on the bacteria's ability to produce lipases. Among 192 colonies, five were able to degrade PET: three bacteria species from the *Psueudomonas* (B10, SWI36, and SWI36), and two from the Bacillus genus (*Bacillus thuringiensis* C15, and *Bacillus albus* PFYN01). The PET-degrading experiments were carried out for 6 weeks at 30 °C, in configurations containing single bacteria specs isolates, and consortia. The polymers were degraded to the greatest extent in the consortium containing all the five strains where 3.15% of the PET was biodegraded (Leon-Zayas et al. 2019). Biodegradation studies of PET films obtained from plastic waste from Bakkhali Bay in India showed that bacteria from

the *Vibria* spp. and a fungus from the *Aspergillus* spp. were able to degrade the polymer. The bacteria were more efficient, and upon 6 weeks 35% polymer weight reduction was observed; for the fungus, the mass loss was on the level of 22% (Sarkhel et al. 2020).

## 2.3.9 Other Polymers

### 2.3.9.1 Polyvinyl Alcohol

Polyvinyl alcohol (PVA) is water-soluble polymer widely used in the paper and textile industries as adhesive (Jecu et al. 2010). PVA solutions biodegradation in the presence of white rot fungi *Phanerochaete chrysosporium* was investigated by Huang et al. (2002). Two material samples with polymerization degree of 550–650 (BP05) and 1700–1800 (BF17) were oxidized with the Fenton reagent before the tests and inoculated with the fungal strain at 37 °C for 7 days. The results indicated PVA degradation on the level of 74.4% and 72.8% for the PB05 and PB17, respectively, using chemical oxygen demand; total organic carbon analysis showed the degradation of 63.7% and 57.7%. It was concluded that PVA biodegradation occurred due to the action of manganese peroxidase. Jecu et al. (2010) evaluated the ability of 11 microorganisms to degrade PVA-based materials containing different amounts of PVA, starch, and glycerol. *Trichoderma* sp. 57 and 83, *Aspergillus oryzae* 100, 107, *Penicillium* sp. 41, *Monillia* sp. 90 and 21, *Aspergillus flavus* 111, *Aspergillus niger* 38, 105 and *Aureobasidium pullulans* 102 were selected as model fungal strains. After 7 days of incubation, action of two strains, the *Aspergillus niger* 105 and *Aspergillus oryzae* 107 resulted in the most advanced fungal growth on one of composition comprised of 50% PVA, 15% starch, and 35% glycerol. In the case of the material treated with *Aspergillus niger,* rapid growth was monitored after 4 days. The SEM analysis of the sample showed the holes formation on the surface followed by erosion after 10 days of incubation, indicating the biodegradation process.

### 2.3.9.2 Polycaprolactone

Polycaprolactone (PCL) is a polyester obtained by ring-opening polymerization of (ε-caprolactone) (Tokiwa et al. 2009). Fungal biodegradation of PCL occurred in the presence of several microorganisms belonging to the genus *Aspergillus, Penicillium, Fusarium, Parengyodontium, Trichoderma,* and *Chaetomium*. Sanchez et al. (2000) investigated the process using *Aspergillus fumigatus* during 7 days of incubation at 30 °C and 50 °C on Two types of PLA films were tested: blown and heat-pressed PCL. After 5 days of the isolation, fungal strains degraded approximately 90% mass of samples. Li et al. (2012) evaluated PCL biodegradability in the form of emulsified polymer and heat-pressed films. Among all evaluated fungal strains, the most promising results were achieved for *Penicillium oxalicum* DSYD05. The analysis showed that the fungus released PCL-degrading exoenzyme acting by chain-end scission. The degradation occurred in three steps: slow weight loss, rapid degradation, and renewed slow weight loss. Primarily, the biodegradation process occurred

in PCL amorphous phase, followed by the formation of crystalline spherulites. Znajewska et al. (2018) confirmed PCL films degradation ability of *Trichoderma viride*. The microbiological tests were carried out in laboratory conditions for 6 days and in soil for 6 months. Antipova et al. (2018) conducted extended research concerning polycaprolactone biodegradation. PCL powder was incubated with fungal strains belonging to the genera *Penicillium*, *Aspergillus*, and *Fusarium* at 25 °C for 30 days. All the tested fungi exhibited degradation ability toward PCL; however, *Fusarium solani* VKM F-4202 caused the highest polymer mass reduction on the level of 90%, while *Fusarium solani* VKM F-2316, *Penicillium chrysogenum* VKM, and *Fusarium verticillioides* VKM F-1980 degraded 24 ± 4%, 34 ± 3% and 40 ± 5%, respectively. PCL film incubation for 45 days with *Aspergillus calidoustus* VKM F-2909 and *Penicillium chrysogenum* VKM F-227 resulted in elasticity decrease, film edge deformation, presence of yellowish plaque, and dark-colored mycelium formation. Complete film decomposition occurred after 6 months of the experiment. Vivi et al. (2019) treated PCL films with *Chaetomium globosum* for 28 days at 28 °C. The incubation with the fungal strain resulted in the polymer surface erosion and spherulitic degradation. Furthermore, a significant mass reduction of PCL films on the level of 75% was noticed after the biotreatment.

#### 2.3.9.3 Polylactide

Polylactide (PLA) also known as poly(lactic acid) is obtained in the process of lactide ring-opening polymerization or in lactic acid condensation. Poly(D-lactide) (D-PLA), poly(L-lactide) (L-PLA) and poly(DL-lactide) (DL-PLA) are polylactide stereoisomers. PLA is a thermoplastic material with biocompatible and biodegradable properties (Tokiwa et al. 2009).

Jarerat and Tokiwa (2001) evaluated *Tritirachium album* strain for poly(L-lactide) film biodegradation. Degradation was achieved only for polymer films incubated with malt and protease production medium containing commercial Proteinase K in the presence of 0.1% gelatin, which acted as biodegradation inducer. The compound addition resulted in 76% PLA degradation after 14 days of incubation at 30 °C in the case of isolation in the malt medium, while for the protease production medium only 7% film degradation was accomplished. It was concluded that enzyme released by *Tritirachium album* in malt medium was probably a protease. Khan et al. (2012) reported PLA biodegradation by *Trichoderma viride*, *Aspergillus niger*, *Aspergillus orhyzae*, *Rhyzopus oligosporus*, and *Rhyzopus orhyzae*. The process was monitored by biomass and pH changes. In the case of DL-lactic acid tests, the most effecting fungal strain was the *Trichoderma viride,* while for polylactic acid-oligomers it was the *Aspergillus niger* after 7 days of incubation at 30 °C. Antipova et al. (2018) incubated PLA with *Penicillium aurantiogriseum*, *Penicillium chrysogenum*, *Aspergillus calidoustus*, and *Parengyodontium album* for 30 days. The results showed that the *Aspergillus calidoustus* and *Parengyodontium album* degraded approximately 30% of the polymer mass, while *P. aurantiogriseum* and *chrysogenum* 9% and 5%, respectively. Ding et al. (2018) evaluated PLA/jute composites biodegradation in the presence of fungal mixture including *Gliocladium virens*, *Aspergillus niger*, *Aureobasidium pullulans*, *Chaetomium globosum*, and *Penicillium pinophilum*.

The composites were prepared on the way of extrusion and injection and consisted of 10 wt.% of jute fibers. During the biodegradation process, the PLA surface was degraded progressively which led to a gradual exposure to the air of jute fiber located in the matrix. After 28 days of incubation at 28 °C, the composite mass reduction at the level of 45.3% was observed. Moreover, bending and tensile properties of the material declined, and the jute fibers remained unchanged during experiments. Polylactic acid-degrading bacteria were isolated from effluent sludge and soil. Two strains, *Pseudomonas geniculate* and *Stenotrophomonas pavanii*, were identified in the isolates; both of them were able to degrade PLA with the *P. geniculate* reaching higher efficiency on the level of 1.5% (Bubpachat et al. 2018). Diary wastewater was used for a search of PLA-degrading microbes; a species from the genus *Actinomadura* was identified in the samples. The waste was mixed with soil in which UV-pretreated PLA films were buried. After 15 days incubation, complete disintegration of the polymer occurred (Pattanasuttichonlakul et al. 2018). Bacteria form the genus *Sphingobacterium* and *Chryseobacterium*, and two strains of *Pseudomonas aeruginosa* were tested for PLA biodegradation in the research of Satti et al. (2017), *Bacillus pumilus* was found to be able to degrade PLA in the study of Bonifer et al. (2019), and *Aeromonas* spp. and *Rhodococcus* spp. in the research of Swiontek Brzezinska et al. (2020).

#### 2.3.9.4 Poly(Ethylene Succinate)

Poly(ethylene succinate) (PES) belongs to the group of biodegradable polyesters. It is obtained in condensation process of succinic acid and ethylene glycol (Tezuka et al. 2004).

Microorganisms isolated from a pond, river, and soil environment were tested for PES biodegradation. Among various identified bacteria and fungi, a bacterium *Bacillus pumilus* exhibited a high PES hydrolyzing activity (Tezuka et al. 2004). Ishii et al. (2007) confirmed PES biodegradation by fungi similar to *Aspergillus clavatus* strain isolated from terrestrial environments. Incubation of the samples was carried out at 30 °C for 20 days and resulted in crates formation on the materials surface. The results indicated the degradation process occurring preferentially on the surface amorphous parts, while the average molecular mass of the material only slightly changed. Tribedi et al. (2012) conducted research on biodegradation of PES by *Pseudomonas* spp. The tests were carried out in the presence and absence of glucose. It was shown that glucose can inhibit the microbial degradation; when it was present the mass reduction was approximately two times lower compared to the samples without the additional sugar.

## 2.4 Challenges of Microbial Plastic Waste Abatement

Application of living organisms for resolving one of the most severe issues of the modern world, the plastic pollution, seems like a golden solution; however, it is not as simple as it may look. First of all, the microbial growth and enzymes secretion, and the enzyme's action itself, have to occur in very narrow condition, and a slight

fluctuation in the temperature or pH may result in cessation or significant reduction of the enzyme activity or upset the microbial homeostasis.

The need for plastic segregation will not be overcome as different microorganism are adequate for decomposing various polymers; it is unknown how would they interact with each other in case when they coexist. There are reports on bacteria strains functioning in consortia that exhibited higher decomposition efficiency; however, it cannot be guaranteed that antagonistic behavior would not take place leading to extinction of one or more strains and deterioration of the decomposition efficiency. It can be especially difficult, if not impossible, to find such conditions that could sustain the existence of different microorganisms at the same time and in their optimal growth conditions, especially when both bacteria and fungi are involved.

In relation to the decomposition rate, in all the reported cases the degradation took a prolonged time period of tens of days; moreover, in most of the cases, the degradation was on the level of couple of percent—promising but still low.

Furthermore, most of the reported cases are studied on previously disinfected materials; in real conditions, the plastisphere may be inhabited by a whole selection of microorganisms, not interested in depolymerizing the plastic, competing with the strain that would decompose the waste. It is hard to imagine that all the previously sorted plastic waste be subjected to disinfection before storing in landfill bioreactors of strictly controlled environment. Plastic waste is generally dirty, and the implemented microorganism might choose a more accessible carbon source than the polymer can provide; hence, if not disinfection, at least a washing step could benefit the whole process.

Another aspect concerns a step on which the plastic waste would be fragmented, preferably ground to powder. This should also be included as the higher the porosity and specific surface, the better the interaction with microorganisms.

Another issue is also related to the microorganisms' stability, but this time, in the context of the substances that are added to plastics to modulate their rheology and performance proprieties. These substances doubtfully would be metabolized by the microbes, they would rather be released from the plastics and most probably exhibited some adverse effects on the plastisphere's biota or contaminate the environment in case they find a way to soil or water bodies. Handling of the degradation products may also be problematic; it is not only water, carbon dioxide, or methane but a soup of diverse organic compounds secreted by the microbes, the polymer decomposition products—monomers and oligomers of different molecular weight—released plastic additives and potential reaction products of the above-mentioned chemicals, mixed with fragmented plastic and the microorganisms. It raises an issue of secondary pollution generated that way and requires further action to safely dispose it.

What is more, there would be a need for a place with ample space to store the waste, in the case of biodegradation, the garbage heap cannot be too tall as it would result in temperate increase inside it and destabilization of the microorganisms; it should also provide an efficient equipment to disperse the microbes and mix them with the waste that can also be problematic given the waste quantity.

Last but not least, there is the whole aspect related to the cost to maintain such complex facility. The amount of money needed to keep it operative could render the technology just too expensive to be economically viable and feasible to widely implement.

## 2.5 Conclusion

Pollution of the environment with polymers is a severe problem; the currently used plastic waste handling methods, such as landfills, incineration, or recycling, are not sufficient to provide a satisfactory, cost-efficient, and long-term solution for the increasing amount of plastic garbage generated by human. Within the last 20 years, a significant amount of microbes that may in future be used for the waste abatement has been identified. The microbes are able to thrive on the surface of plastics, the plastisphere, and by secreting enzymes, mainly hydrolases or oxidoreductases, they are capable of depolymerizing the plastics and to use the products as a carbon source. Several species of bacteria were tested for biodegradation of the most common plastic; the bacterial genera from which species were able to degrade polymers were *Acinetobacter, Acinetobacter, Alcanivorax, Alcaligenes, Arenimonas, Arthrobacter, Bacillus, Brevundimonas, Citrobacter, Cohaesibacter, Chryseomicrobium, Cupriavidus, Delftia, Dethiosulfovibrio, Desulfovibrio, Erysipelothrix, Enterobacter, Fusibacter, Ideonella, Lysinibacillus, Maribacter, Microbulbifer, Micrococcus, Mycobacterium, Ochrobacterum, Oscillatoria, Pantoea, Phormidium, Psueudomonas, Psychromonas, Pleomorphochaeta, Rhodococcus, Saccharomonospora, Sphingobacterium, Sporobacter, Stenotrophomonas, Thermobifida, Vibria*; in the case of fungus, the representatives capable of reducing plastic mass were found among genera such as *Aureobasidium, Alternaria, Aspergillus, Candida, Chaetomium, Cladosporium, Fusarium, Gliocladium, Gloeophyllum, Humicola, Lentinus, Mucor, Monillia, Penicillium, Phanerochaete, Paecelomyces, Parengyodontium, Rhizopus, Trichoderma.*

It has been proven that polymers, even the ones considered non-biodegradable such as PE or PET, can be degraded by bacteria and fungi. The process is slow, requires strictly controlled environment, and poses several logistical and technological challenges; however, it offers an alternative for the conventional and not effective plastic waste handling methods. So far, the research has been focused on isolating new species and testing their degrading abilities, mostly in laboratory environment on specially prepared for this purpose plastic samples, in strictly controlled conditions. In the foreseeable future, the research should be extended to investigation in conditions closer to the real ones to better reflect the applicability of microbial-based plastic waste handling technology.

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# 3 Microbial Degradation in the Biogas Production of Value-Added Compounds

Brenda C. G. Rodrigues, Bruna S. de Mello, Luana C. Grangeiro, Arnaldo Sarti, and Kelly J. Dussán

**Abstract**

In recent years, there has been an increase in studies aimed at the valorization and recycling of materials, such as by-products, wastewater, and residues from industries and agriculture to produce value-added products such as biogas and biofertilizers. Anaerobic digestion (AD) is considered the main biological route to obtain value-added compounds. AD technology allows the action of microorganisms in four steps whose syntrophic activity of microbial communities transform substrates into biogas without needing to add chemicals, involving low investment, and operating costs. Moreover, there is a variety of substrates that can be used to generate high-value products from AD. In order to better conduct the AD process, several anaerobic digestion technologies have been proposed over the years varying from conventional systems to high-rate reactors and recently co-digestion and soli-state processes. However, understanding and knowing the role of microbial populations involved in AD have become extremely important to promote a better control of anaerobic systems. In addition, it ensures nutritional

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and operational conditions to favor the biochemical route for the biomethane generation. Therefore, research opportunities regarding the syntrophic metabolism that occurs throughout each step of the microbial degradation is essential for anaerobic decomposition of many substrates and the stability of biogas production. This type of scientific study can help to improve the sustainability and energy balance from using material that is being discarded in the environment.

**Keywords**

Anaerobic digestion · Add-value bioproducts · Syntrophic activity · Microbial population · Biogas production

## 3.1 Introduction

The global energy matrix is based on using nonrenewable sources such as oil, coal, and natural gas (Pareek et al. 2020). In contrast, these sources applied in conventional energy production processes, such as chemical and thermochemical processes, generate pollutants that contribute to greenhouse gas emissions and consequently unfavorable impacts including global warming and climate change (Ahmed et al. 2019; Hill 2009). Thus, an increase can be observed in studies focusing on the valorization and recycling of materials, such as by-products, wastewater and residues from industries and agriculture to produce value-added products, such as biogas. This is because this type of material known as biomass contains high amounts of proteins, sugars, and lipids that can be considered as cheap and abundant raw materials for the synthesis of value-added chemicals and biomaterials (Ahmed et al. 2019; Murthy and Madhava Naidu 2012).

The main biological process used to obtain value-added compounds is anaerobic digestion (AD). AD is considered a green route to produce bioenergy, especially due to the action of microorganisms without needing to add chemicals, involving low investment and operating costs (Cesaro and Belgiorno 2014). AD has been used for many years, and it comprises a natural four-step process including hydrolyses, acidogenesis, acetogenesis, and methanogenesis in which different microbial groups are syntrophically associated with the conversion of organic compounds primarily into biogas, which has methane as a relevant component (Grangeiro et al. 2019). Moreover, during AD, nutrient-rich solid and liquid residues, also known as digestate, are produced and can be applied as a biofertilizer for soil conditioners (Apergis et al. 2016; Kirk-Davidoff 2018). Therefore, AD is considered a process that involves a broad range of value-added bioproducts which can be obtained from a single type of raw material (Grangeiro et al. 2019). As show in Fig. 3.1, a large range of microorganisms are involved in all four steps of AD of carbohydrates, lipids, and protein.

Several studies worldwide have demonstrated the use of different feedstocks as waste as well as wastewater from industrial and agro-industrial processes to produce biogas. Regarding industrial feedstock, there are many studies such as those on

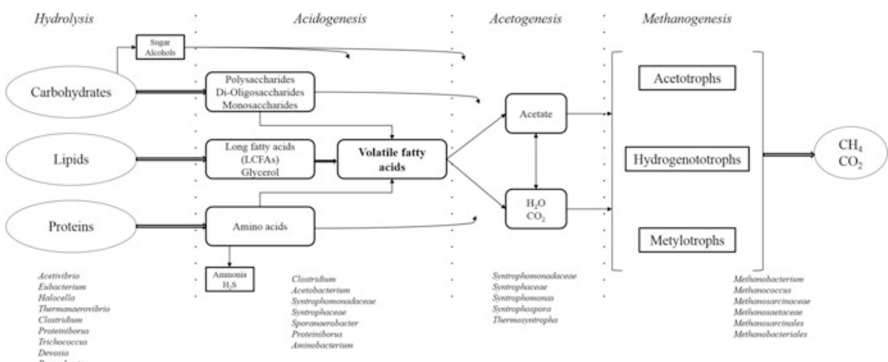


**Fig. 3.1** Metabolic sequences and principal microbial family groups involved in the anaerobic AD of carbohydrates, lipids, and protein. Source: Adapted from Campanaro et al. (2016)

cheese whey (Azbar et al. 2009; Damasceno et al. 2008), cassava wastewater (Jiraprasertwong et al. 2019; Olukanni and Olatunji 2018), whey (Erguder et al. 2001; Luo and Angelidaki 2013), dairy and food processing wastewater (Ahmad et al. 2019; Tikariha and Sahu 2014), breweries (De Araujo et al. 2016), wineries (Basset et al. 2016), soft drinks (Kalyuzhnyi et al. 1997), and others. Some studies reported using glycerin-based wastewater (Amon et al. 2006; Astals et al. 2011) and sulfate-rich wastewater (Sarti et al. 2011). Moreover, different agricultural crops and terrestrial and aquatic plants are used as feedstock for AD (Gunaseelan 1997).

Although biogas production from anaerobic systems is considered a well-established technology for different feedstocks, it is very important to understand the role of microbial populations during the process considering the influence of operational parameters in these systems. Because of that, several studies have proposed innovations for AD performance, including biological strategies and innovating control systems (Tabatabaei et al. 2020).

This chapter provides information about microbial degradation in biogas production from biomass through AD. In the first part, common substrates used to generate products with added value from AD are described. The second part discusses the AD stages and the specific microbial population required in each stage. Afterward, the key factors that influence the microbial community structure during the anaerobic process are investigated. Finally, these anaerobic digestion technologies proposed over the years, as well as new system approaches to enhance biogas production using microbial degradation, are discussed. To sum up, this chapter compiles essential information to better understand the potential of AD as a simple and robust bioprocess for recovery bioenergy.

## 3.2 Substrates Commonly Used in Biogas Production

The substrates used during microbial degradation to generate biogas are primarily organic matter considered as waste, thus resulting in a wide range of raw materials. The composition and physical–chemical structure of the substrate can directly affect the microbiological complex system that favors anaerobiosis, which can greatly affect the stability and biodegradation efficiency of the process (Sharma et al. 1988).

Therefore, substrate characterization is important to ensure microbial nutritional needs, operational conditions, and, consequently, to predict the amount and quality of biogas that can be produced. These factors are intricately linked to the available nutrients and recalcitrant compound content in the substrate. Furthermore, the fractionation of the substrate will depend on the size of its particles, which can benefit or hinder the hydrolysis phase (Aziz et al. 2020; Sharma et al. 1988). Thus, the choice of the process, according to the available raw material, influences the results, which can maximize the production of bioenergy and its quality.

During the anaerobic digestion process, the presence of macro- and micronutrients, as well as trace elements, is necessary for microbial community growth and stability. Carbon, nitrogen, phosphorus, and sulfur must be present in a ratio of about 600:15:5:1 (Sharma et al. 1988; Mata-Alvarez et al. 2014). The carbon–nitrogen (C:N) ratio is the most important process indicator whose ideal range is reported to be ranging from 20 to 35; for instance, substrates rich in proteins can generate high levels of ammonia leading to ammonia accumulation in the medium and can thus irreversibly impair the AD progress (Puñal et al. 2000). The trace elements are especially important when the process is mono-digestion, i.e., only one type of substrate is used during AD. In this case, iron, cobalt, selenium, nickel, molybdenum, and tungsten must be added to the process in case the substrate does not have sufficient amounts for maintaining the microorganisms (Aziz et al. 2020; Bohutskyi and Bouwer 2013; Schattauer et al. 2011).

In fact, biogas and biofertilizer production was not the primary focus of AD processes. This technology was used only to reduce aerobic sludge excess from sewage treatment plants and in the treatment of animal manure, but the biogas generated was not reused for energy purposes. Hence the use of this technology was mainly associated with these two activities, which were the main sources of substrate, currently, for biogas generation (Milanez et al. 2018). However, after the oil crisis in 1980, several studies investigated using different types of biomass with the primary aim of producing good yield and quality of biogas (higher methane content) along with low implementation and operational costs. Currently, agricultural waste, municipal sewage, organic industrial waste, food waste, solid organic municipal waste, aquatic biomass, forest waste, and energy crops have been used in AD processes (Divya et al. 2015; Martínez-Gutiérrez 2018; Pérez-Chávez et al. 2019; Shrestha et al. 2017).

Within this context, choosing the most suitable substrate for biogas production depends on the purpose of the generating unit. If the biogas generated is in plants with main activities in other segments, the substrate will already be defined and the type of application of AD should be chosen according to the characteristics of the

**Table 3.1** Biogas yield and potential to generate biomethane from the most common feedstock used in the AD process

| Feedstock | Biogas yield | Potential to generate biomethane (bcm)[a] |
|---|---|---|
| Livestock manure | 40–80 $m^3$/ton DW[b] | 250 to 370 |
| Sewage | 18–26 L/person-day | 22 to 32 |
| Food waste | 150–180 $m^3$/ton FW[c] | 85 to 100 |
| Crop residues | ~10 $m^3/m^3$day | 300 to 380 |
| Energy crops | 3500–9000 $m^3/CH_4$ hectare | 330 to 490 |

Source: Adapted from Milanez et al. (2018) and Jain (2019)
[a]Billion cubic meters
[b]Dry weight
[c]Fresh weight

substrate generated in that plant. Otherwise, if the intention is a biogas plant, the political and geographical context must be considered, as the type of substrate available in the region for plant feeding, logistics, and tax incentives must be analyzed. For example, in Germany, a country recognized as a world leader in the production of energy from biogas, there is a strong government incentive to generate energy from renewable sources, mainly due to the governmental project to deactivate nuclear power plants. The Könnern plant, in Germany, is one of the largest biogas parks in the world, and the main substrates are derived from agriculture, such as animal waste, agricultural residues, and energy crops (Purkus et al. 2017).

Based on prior knowledge about the type of substrate available for biogas generation, the maximum biogas production can be estimated by the Biogas Generation Chemical Potential, in which the amounts of carbohydrates, proteins, fats, and other compounds refer to the chemical equation involved in the bio-digestion of specific substrates. (Mélo-Schlub et al. 2019; Penteado et al. 2017). In addition to determining the technology to be used, it is possible to accurately calculate the maximum amount of biogas that will be produced. Therefore, it is essential to know the composition of the substrate to be used for biogas production because from this choice the best technological model can be planned for the effective construction of the plant, considering that the size of the bioreactor or digester will depend on the type of substrate and its potential for biogas generation (Li et al. 2017; Weiland 2010).

Table 3.1 shows the participation of the different types of substrates most used in the world for biogas generation and the energy potential of each, if all this material, which is mostly waste, were fully recovered for the bioenergy generation (Jain and Jain 2019; Milanez et al. 2018).

The composition of plant derivatives comprises different polysaccharides. These polysaccharides are formed by carbohydrates linked in straight or branched chains; the structure will vary according to the composition of each biomass. Lignocellulosic biomass, in which the plant cell wall has a predominant presence of cellulose, hemicellulose, and lignin, as plants, are considered of greater complexity for microbial degradation, mainly due to the presence of lignin. The higher the concentration of lignin in the biomass structure, the more difficult and time consuming it will be to

degrade and generate biogas; for instance, forest waste, which has a rigid structure in its largest portion. Cellulose and hemicellulose are relatively easily degradable as well as the simplest polysaccharides (starch and glycogen, pectin, etc.) (Azman et al. 2015; Lynd et al. 2002; Sánchez and Cardona 2008).

The greatest advantage of using plant biomass is related to its abundance and energy potential, which can boost biogas production worldwide. However, the composition of plant biomass can hinder the action of microorganisms; therefore, according to the nature of the biomass, pretreatments as well as long periods of hydraulic retention for digestion are necessary to enable the AD process (Sánchez and Cardona 2008; Wagner et al. 2018). Thus, several studies have been carried out to improve the efficiency of AD involving lignocellulosic materials, for instance the co-digestion of various residues, such as biomass fractionation investigations.

To reuse lignocellulosic biomass in its entirety, many alternatives to improve biogas production have been reported in the literature, such as the use of microalgae, biomass from phytoremediation, residues from insect production, and the substrate of the cultivation of edible fungi (Bulak et al. 2020; Klassen et al. 2016; Kumar et al. 2020; Łochyńska and Frankowski 2018; Ma et al. 2015; Zabed et al. 2020). In addition, to improve the process, co-digestion with commonly used substrates, such as food residues, agricultural residues, and urban pruning residues, among others have been used (Bedoić et al. 2020; Chowdhury et al. 2020; Issah et al. 2020; Parsaee et al. 2019; Vassalle et al. 2020; Wang et al. 2018).

Most substrates of this nature have proteins, which are long chains formed by amino acids form peptide bonds or amides. Several amino acids are considered essential for the cellular maintenance of living beings, and there are more than 20 different types, which when combined can form molecules from simple to complex. These structural characteristics can influence their solubility, as well as influence the release of smaller molecules, which would hinder the microbial degradation of these compounds (Angelidaki et al. 2011). Protein-rich substrates are already used in biogas production, usually from livestock residues, such as animal manure, slaughterhouse waste, and dairy products; in the production of ethanol, residues generated during the distillation stage, such as vinasse, and food industry wastewater (Banks et al. 2012; Luo et al. 2016; Rajagopal et al. 2013; Solli et al. 2014).

The lipid residues, with high levels of fats, are complex molecules of different sizes that can be saturated or unsaturated, and when this type of molecule breaks down, long-chain fatty acids (over 12 carbons in their structure) are generated as well as glycerol (Dasa et al. 2016). The main sources of lipid-rich substrates for biogas production are activities directly linked to the generation of excess fats, such as domestic sewage and industrial effluents, specifically from slaughterhouses, dairy products, wool washing water, processing vegetable oil for human consumption, and grease retention sludges (Affes et al. 2017; Bong et al. 2018; Luostarinen et al. 2009; Sousa et al. 2009; Souza et al. 2009; Xu et al. 2018a). In addition, current studies have demonstrated that algae and microalgae also have lipid-rich composition (Ma et al. 2015). Lipid substrates are commonly considered an excellent source of raw material for biogas generation (Table 3.2), which can produce higher amounts of

**Table 3.2** Biogas production and methane content of feedstocks rich in carbohydrates, proteins, and lipids, respectively

| Component | Biogas (L/g) | $CH_4$ (%) |
|---|---|---|
| Carbohydrates | 0.830 | 50.0 |
| Proteins | 0.921 | 69.8 |
| Lipids | 1.425 | 69.5 |

Source: Adapted from Alves et al. (2009) and Wang et al. (2018)

methane than sources rich in carbohydrates and proteins (Leung and Wang 2016; Li et al. 2017).

Despite the diversity of substrates that can be used to produce biogas rich in methane, attention is needed with the microbiota involved in the system. Protein-rich substrates tend to release high levels of ammonia, which inhibits methanogenic archaea. Substrates rich in carbohydrates can negatively affect AD due to their rapid degradation when compared to protein and lipid materials, leading to an imbalance of the C:N ratio. Consequently, carbohydrate-rich feedstocks can limit the availability of nutrients and cause acidification of the fermentative medium, mainly due to the accumulation of organic acids, which can enhance hydrogen production (De Gioannis et al. 2013; Li et al. 2017; Meng et al. 2015; Paritosh et al. 2017; Xu et al. 2018a).

Some studies have pointed out that substances rich in lipids have a better chemical potential for generating biogas than carbohydrates and proteins, although these are more easily degradable during the AD process. However, during the hydrolysis stage, the release of long-chain fatty acids can lead to inhibition of methanogen, due to the destruction of the cell membrane of microorganisms. Therefore, the hydrolysis of lipids can be the limiting step for AD of substrates rich with this compound (Cirne et al. 2007; Cuetos et al. 2008; Leung and Wang 2016; Sun et al. 2014; Yuan and Zhu 2016; Zhang et al. 2014).

Notably, the individual influence caused by specific compounds in the AD process can be extremely disadvantageous due to its composition. Thus, several studies have been developed to demystify the role of organic composition in the efficiency of AD from mono-digestion and its impacts on the microbiota (Alibardi and Cossu 2016; Li et al. 2016; Rajagopal 2013; Wang et al. 2014; Yin et al. 2014) as in co-digestion, associating substrates rich in different compounds to be complementary (Astals et al. 2011; Koch et al. 2015; Zhang et al. 2013).

It is worth mentioning that regarding substrates from domestic sanitary sewage and food waste, the organic composition varies greatly concerning geographic and cultural location, which are influenced by consumption patterns, food preparation, and seasonal changes (Kobayashi et al. 2009; Meng et al. 2015; Xu et al. 2018b).

## 3.3 Anaerobic Digestion Pathways and Diversity of Microorganisms Involved

As previously presented, there is a variety of substrates that can be used to generate high-value products from AD (biogas and biofertilizers). However, substrates with high-energy potential (organic composition) can also cause stress in the operating system, resulting in a less stable process (Cuetos et al. 2008). When the system is out of balance, the digestion of organic material can become suboptimal, making insufficient use of it leading to a decline in the production and quality of biogas.

In order to better conduct the AD process so that the generation of value-added products can be optimized, the understanding of the syntrophic activity of microbial communities involved in the AD has become extremely important. Moreover, microbial functions as well as the impacts promoted by the different aspects that may occur during bio-digestion have gained notoriety as an essential factor to ensure the efficiency and stability of the process. By knowing and understanding the present microbiota, nutritional and operational conditions can be ensured to favor the biochemical route for biomethane generation (Vanwonterghem et al. 2014).

AD is a biochemical process divided into several stages, and a specific microbial population is required in each stage. Harper and Pohland (1987) reported that there are at least nine different biochemical mechanisms involved in the microbial degradation of organic materials during AD. However, four main stages are considered: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. As the microorganisms are the nuclei of the digesters, researchers have endeavored to identify, within the microbial groups, the main microorganisms responsible for producing methane and their functions according to the stages and operational conditions.

### 3.3.1 Hydrolysis/Acidogenesis

The first phase is coordinated by hydrolytic and acidogenic bacteria and probably fungi. This group of microorganisms is responsible for the conversion of complex organic compounds (hydrolysis)—such as polysaccharides, lipids, proteins—and simpler organic compounds (acidogenesis)—such as long chain fatty acids (LCFA), glycerol, sugars, amino acids, etc.—in volatile fatty acids (acetate, propionate, butyrate, lactate, valerate, caproate, etc.), alcohols, formiate, hydrogen, and carbon dioxide, which further will be used in the following steps (Kandylis et al. 2016).

The degradation of these compounds occurs from extracellular enzymes secreted by bacteria in the fermentation medium or may be present in the cell wall (Azman et al. 2015; Kazda et al. 2014; Schnürer 2016). It is known that in the stages of hydrolysis and acidogenesis there are about 50 bacterial species known and described in different AD processes (Balk et al. 2002; Seon et al. 2014; Yamada et al. 2006).

Normally, hydrolysis of materials rich in carbohydrates is one of the simplest metabolic pathways of AD; it is easily degraded to glucose, which facilitates the

following steps. In lignocellulosic substrates, cellulose is hydrolyzed to glucose, and cellobiose and hemicelluloses are converted into monomers and acetic acid. In this case, the bacteria have an enzymatic complex in their cell wall (celluloses), which has proteins that identify and bind to the cellulose, making the degradation the more efficient. However, the fungi act differently, by adhering to the cellulose surface and penetrating plant cell walls of complex biomass (Ding et al. 2012; Kazda et al. 2014; Schnürer 2016). Hydrolytic microorganisms acting in AD have been investigated to optimize the use of plant biomass entirely from pretreatments which increase substrate readily available (Chowdhury et al. 2020; Issah et al. 2020; Li et al. 2019; Tabatabaei et al. 2020).

Bacteria affiliated to phylum Firmicutes, Bacteroidetes, Fibrobacteres, Spirochaetes, Proteobacteria, Cloacimonetes, Actinomyces, and Thermotogae are the most common microorganisms responsible for degradation of complex molecules into simpler compounds. The most common genera reported are *Acetivibrio, Butyrivibrio, Caldanaerobacter, Caldicellulosiruptor, Clostridium, Eubacteruim, Halocella, Ruminoclostridium, Ruminococcs, Bacteroides, Paludivacter, Fibrobacter, Espiroquetas, Treponema, Fervidobacteria, and Thermotoga* (Azman et al. 2015; Campanaro et al. 2016; Chen et al. 2016; Jia et al. 2018; Lebuhn et al. 2014; Liu et al. 2017, 2018; Seon et al. 2014; Sun et al. 2016). The anaerobic fungi present in hydrolysis are associated with the phylum Neocallimastigomycota. In fact, fungi of this nature are generally found in the digestive system of ruminants, playing an important role in the degradation of lignocellulosic material (Cheng et al. 2018; Dollhofer et al. 2015).

Proteins are hydrolyzed to amino acids by the action of extracellular enzymes, known as proteases. Amino acids can be degraded by the Stickland reaction (a donor amino acid and another electron receptor), which promotes oxidation, producing volatile carboxylic acids that are smaller than the original amino acid (Fig. 3.2a). Alanine, for example, has a three-carbon chain and during this reaction, it is converted to acetate (Ramsay and Pullammanappallil 2001). For example, means containing gelatin are converted to methane through the Stickland reaction, in which alanine (donor) and glycine (recipient), gelatin components, are converted to acetate as shown in Fig. 3.2b (Ramsay and Pullammanappallil 2001; Sangavai et al. 2019).

Amino acids can also suffer from oxidized action (released hydrogens). This route only occurs in symbiosis with hydrogen receptors, such as hydrogenotrophic methanogens, which keep the partial pressure of hydrogen low in the fermentation medium. Despite having two hydrolytic pathways for amino acids, the result of this degradation will always release the amino group in the form of ammonia, and the sulfur from cysteine and methionine is released as hydrogen sulfide (Fig. 3.3) (Schink and Stams 2013).

The main microorganisms involved in the degradation of materials rich in proteins and amino acids are from the phylum Firmicutes, and the most common genera reported are *Anaeromusa, Anaerosphaera, Aminobacterium, Aminomonas, Gelria, Peptoniphilus, Thermanaerovibrio* (Baena et al. 1999, 2000; Plugge et al. 2002; Tomazetto et al. 2014; Ueki et al. 2009), *Clostridium* (Tang et al. 2005), *Proteiniborus* (Hahnke et al. 2018) and *Sporanaerobacter* (Hernandez-Eugenio

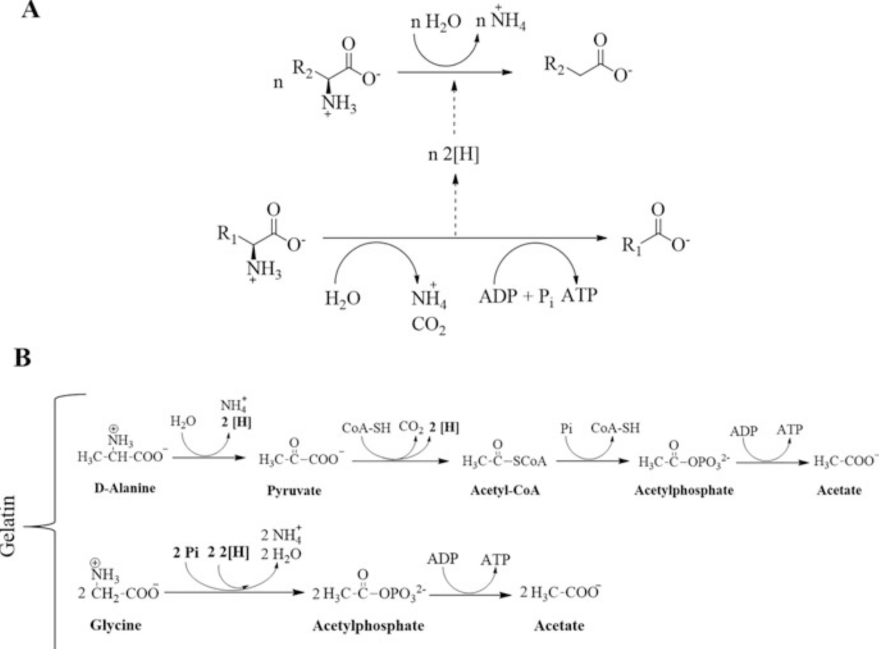


**Fig. 3.2** General Stickland reaction (**a**) Stickland reaction on Alanine and Glycine (**b**). Source: Adapted from Ramsay and Pullammanappallil (2001) and Sangavai et al. (2019)

$$C_3H_6O_2NS\ (Cys) + 2H_2O \longrightarrow C_2H_4O_2\ (acetate) + NH_3 + CO_2 + H_2S + 1/2H_2 + ATP$$

$$C_5H_{11}O_2NS\ (Met) + 2H_2O \longrightarrow C_3H_6O_2\ (propionate) + CO_2 + NH_3 + CH_4S + H_2 + ATP$$

**Fig. 3.3** Cysteine and methionine degradation reactions in the methanogenic pathway

et al. 2002). However, other studies have indicated that bacteria from the phyla Bacteroides, Fusobacteria, and Cloacimonetes can have acidogenic metabolic activity for amino acids during AD (Hahnke et al. 2016; Stolze et al. 2018).

The degradation of lipid material occurs by extracellular lipases, which catalyze the hydrolysis reaction at the water–lipid interface. After the action of lipolytic bacteria, LCFAs and glycerol are released (Mendes et al. 2012; Stergiou et al. 2013). There is still little knowledge regarding bacteria that degrade lipids in AD. For instance, lipolytic bacteria in AD, such as microorganisms from the phylum Firmicutes, Bacteroidetes, Spirochaetes, and Proteobacteria, have been proposed. Bacteria of the family Caldilineaceae, Bacteroidaceae, and the genera *Trichococcus, Devosia,* and *Psycrobacter* are reported to be the main agents involved in the degradation of fat-rich material (Li et al. 2013; Nakasaki et al. 2020; Petropoulos et al. 2018).

Moreover, the hydrolysis stage requires microorganisms with specific characteristics, according to the substrate. As the first phase of AD, these

microorganisms end up being the most energetically benefited (de Aquino and Chernicharo 2005). After this stage and the release of simpler compounds, the microorganisms found in the subsequent stages can be considered predominant in specific phyla, such as the phylum Firmicutes, in which bacteria from this group are present in the hydrolytic and fermentative stages during AD as well as in different reactors and substrates (Sanz et al. 2017; Wang et al. 2018).

### 3.3.2 Acetogenesis/Methanogenesis

Acetogenic bacteria are microorganisms that convert intermediate organic compounds generated in the acidogenesis and hydrolysis stages. These bacteria have the highest growth rate (~30 min) of the anaerobic community for biogas generation due to the abundance of hydrolysate released, which is directly influenced by the hydrolysis of organic material (de Aquino and Chernicharo 2005; Kandylis et al. 2016). Acetogens convert the hydrolyzed material into acetate, hydrogen, and carbon dioxide as the main products and can use nitrate, sulfate, and even $CO_2$ as electron receptors during the reaction (Ragsdale and Pierce 2008). They can also use hydrolysis products directly to produce acetates, such as carbohydrates and amino acids, and can oxidize pyruvate to acetate, which is a very common by-product generated during AD (Drake et al. 2008; Schink and Stams 2013).

Most reactions carried out by acetogenic bacteria only occur if the partial pressure of hydrogen is low, such as the oxidation reactions of organic acids of short and long chains (Schink 1997). This happens due to thermodynamic reasons, and when the whole microbiological context of AD is analyzed, the energetic condition of the acetogens is less favorable regarding the methanogenic archaea; however, there is a symbiosis between these microorganisms, known as syntropy. The interaction between both groups promotes enough energy for their growth, and therefore acetogens need methanogens and vice versa (Drake et al. 2008; Schink 1997; Schink and Stams 2013). However, there may be an imbalance between these communities causing competition for the substrate and malfunction of the system. The main intermediate products generated in the previous steps and their respective chemical reactions for acetate generation are shown in Table 3.3.

In this case, the main methanogenic pathways (aceticlastic and hydrogenotrophic) are affected by the unbalanced presence of homoacetogens and acetate oxidative bacteria, according to the concentration of acetate and $H_2$ (main methanation substrates), and thermodynamically unfavorable competition may occur. For example, hydrogenotrophic homoacetogens and methanogens (MH) compete for $H_2$ to convert $CO_2$ into acetate and methane, respectively, according to the general equations presented in Table 3.4. Ideally, homoacetogens coexist with aceticlastic methanogens, maintaining sufficient acetate and $H_2$ concentrations for their maintenance (Lee et al. 2015). Table 3.4 shows some homoacetogenic and hydrogenotrophic microorganisms and their respective $H_2$ demands.

Acetogenic bacteria are generally attributed to the genera *Clostridium* and *Acetobacterium* (phylum Firmicutes), but bacteria from the phylum Proteobacteria

**Table 3.3** Acetogenic decomposition of intermediate products

| Intermediate products | Chemical reaction to acetate |
|---|---|
| Propionic acid | $CH_3(CH_2)COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$ |
| Butyric acid | $CH_3(CH_2)_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$ |
| Valeric acid | $CH_3(CH_2)_3COOH + 2H_2O \rightarrow CH_3COOH + CH_3CH_2COOH + 2H_2$ |
| Isovaleric acid | $(CH_3)_2CHCH_2COOH + HCO_2H + 2H_2O \rightarrow 3CH_3COOH + 2H_2$ |
| Caproic acid | $CH_3(CH_2)_4COOH + 4H_2O \rightarrow 3CH_3COOH + 5H_2$ |
| Glycerin | $C_3H_8O_3 + H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$ |
| Lactic acid | $C_3CHOHCOOH + 2H_2O \rightarrow CH_3COOH + HCO_3 + H^+ + 2H_2$ |
| Ethanol | $CH_3(CH_2)OH + H_2O \rightarrow CH_3COOH + 2H_2$ |

Source: Adapted from Chernicharo (2007) and Deublein and Steinhauser (2010)

**Table 3.4** Example of homoacetogens and hydrogenotrophs, chemical reactions and demand for $H_2$

| | Reactions | Species | $H_2$ (ppm) |
|---|---|---|---|
| Homoacetogens | $2CO_2 \xrightarrow{4H_2,\ 2H_2O,\ ADP,\ P_i,\ ATP} CH_3COOH$ | *Sporomusa termitida* | 830 |
| | | *Acetobaeterium woodii* | 520 |
| | | *Aeetobacterium carbinolicum* | 950 |
| Hydrogenotrophic | $2CO_2 \xrightarrow{4H_2,\ 2H_2O} CH_4$ | *Methanospirillum hungatei* | 30 |
| | | *Methanobrevibacter smithii* | 100 |
| | | *Methanobrevibacter arboriphilus* | 90 |
| | | *Methanobaeterium formicum* | 28 |
| | | *Methanococeus vannielii* | 75 |

Source: Adapted from Cord-Ruwisch et al. (1988)

were also mentioned with metabolic activity in these stages (Azman et al. 2015; St-Pierre and Wright 2014).

Regardless of the lipid degradation, glycerol is released after hydrolysis, which is easily converted into volatile fatty acids (VFA). LCFAs demand specific groups of microorganisms to convert them into VFA, as they need to be transported across the cell membrane of acetogenic bacteria, where they are converted to acetate, $CO_2$, and $H_2$ via the β-oxidation pathway (Ma et al. 2015; Nakasaki et al. 2020; Weng and Jeris 1976). The bacteria from the Syntrophomonadaceae and Syntrophaceae families have been identified (Sousa et al. 2009; Ziels et al. 2018), associated with phylum Firmicutes and Proteobacteria, respectively, within the ability to degrade long-chain acids in syntropy with methanogenic archaea.

The degradation of short-chain fatty acids (acetate, propionate, butyrate, etc.) in syntropy with methanogens are well distributed in the phylogenetic scope. The genera *Syntrophomonas, Syntrophospora, Syntrophothermus, Thermosyntropha* and *Pelotomaculum* (phylum Firmicutes), and the genera *Syntrophus, Smithella* and *Syntrophobacter* (phylum Proteobacteria) are described as propionate and butyrate syntrophic bacteria in AD systems (Worm et al. 2014). On the other

hand, microorganisms affiliated to the phylum Cloacimonetes and Chloriflexi are reported to be capable of growing in syntropy with hydrogenotrophic methanogens (Li et al. 2015; Nobu et al. 2015; Pelletier et al. 2008).

The genera *Clostridium, Thermoacetogenium, Syntrophaceticus* and *Tepidanaerobacter*, all from the phylum Firmicutes are the main oxidative bacteria of syntrophic acetate (Westerholm et al. 2016). However, bacteria of the order Clostridiales, Thermoanaerobacterales, Synergistes, the genus *Coprothermobacter*, and the phylum Spirochaetes, Thermotogae, Chloroflexis and Bacteroidetes have been reported as possible oxidants of syntrophic acetate (Bassani et al. 2015; Ho et al. 2014; Ito et al. 2011; Lee et al. 2015; Mosbæk et al. 2016; Müller et al. 2016; Ruiz-Sánchez et al. 2018; Westerholm et al. 2018; Zakrzewski et al. 2012). It was observed that the presence of the genus *Syntrophomonas*, which degrades propionate and butyrate, and the phylum Synergistetes, which are oxidizers of syntrophic acetate, may indicate good acetogenic performance (He et al. 2017; Lienen et al. 2014) and acetotrophic (Schink and Stams 2013), respectively. However, further research is needed to characterize and elucidate the performance of these bacteria during AD.

The last stage of the AD is considered the limiting phase, which comprises methanogenic archaeal. This group of microorganisms is mainly responsible for producing methane and belong to the phylum Euryarchaeota, in which about 65 species are active in the methanogenesis of AD, divided into 4 orders, 8 families and 19 genera (Imachi et al. 2007; Lee et al. 2014; Nielsen et al. 2007; Sousa et al. 2007b; Wang et al. 2018). The methanogenic community normally corresponds to a small portion of the total community (around 2 to 5%) and its growth is considered slow (from 6 hours to 3 days) concerning the other microorganisms involved in AD (Mosey 1983). However, its metabolic activity, with its abundance, is considered high.

The main microorganisms identified in anaerobic reactors are of the order Methanobacteriales, Methanocellales, Methanomicrobiales, Methanosarcinales, Methanococcales, and Methanomassiliicoccales (Enzmann et al. 2018; Lee et al. 2014; Schnürer 2016). The genera *Methanobacterium, Methanococcus, Methanobrevibacter, Methanomicrobium, Methanosarcina, Methanoculleus*, and *Methanosaeta* are considered the main methanogenic arch responsible for the generation of biomethane (Imachi et al. 2007; Nielsen et al. 2007; Sousa et al. 2007b). In thermophilic reactors, the emphasis is given to the genera *Methanoculleus* and *Methanothermobacter* (Carballa et al. 2015; Lee et al. 2014; Sasaki et al. 2011; Ziganshin et al. 2013).

Methanogenic archaeal have three known metabolic pathways for methane release. These routes were divided according to the precursor substrate (Costa and Leigh 2014; Liu and Whitman 2008):

- Aceticlastic route: In this pathway, the acetate is reduced to a methyl group and $CO_2$. The methyl group is reduced to methane after electron donation from the carboxyl group.

- Hydrogenotrophic route: $CO_2$ is reduced to $CH_4$ using $H_2$ or formiate as electron donors.
- Methylotrophic route: Methylated compounds, such as methanol, methylamines, and methyl sulfides, are used to generate $CH_4$. In this case, most methylotrophic methanogens use $CO_2$ as electron donors to oxidize methyl groups.

The main microbial community responsible for biogas production belongs to the group of aceticlastic methanogens since the degradation of organic matter in AD systems depends on the conversion of acetate into methane (VanBriesen 2001). So far, it is known that acetate is used exclusively by microorganisms from the families Methanosarcinaceae and Methanosaetaceae (order Methanosarcinales). Methanosarcinaceae microorganisms stand out due to their ability to grow on different substrates and they can consume hydrogen and methanol, as well as acetate, making them more versatile than the Methanosaetaceae group, which are strictly consumers of acetate (De Vrieze et al. 2012).

Hydrogenotroph microorganisms are present in almost all methanogenic orders, except in the order Methanomassiliicoccales whose metabolic characteristic was not observed (Enzmann et al. 2018). Therefore, this route is not considered as the main route for methane generation due to possible competition with other microorganisms. However, studies have indicated that hydrogenotrophic methanogens are more resistant to stressful conditions than acetoclastic. In this case, when the acetate concentration decreases, the hydrogenotrophic can act in symbiosis with oxidative acetate bacteria and maintain mechanization. This microbial diversity with different metabolic routes makes AD a complex and resilient process (Bialek et al. 2012; Cho et al. 2013; Ma et al. 2013; Madsen et al. 2011; Sousa et al. 2007a; Steinberg and Regan 2011; Werner et al. 2014). Figure 3.4 shows an example of syntrophy between acetogenic bacteria and hydrogenotrophic for the degradation of ethanol to methane.

The *metanosarcina* genus is known to use all known methanogenic metabolic pathways (Fig. 3.5) i.e., the microorganisms of this genus can be hydrogenotrophic, acetoclastic, and methylotrophic. Besides, they are the only known species with this ability and can metabolize at least nine methanogenic substrates (Galagan et al. 2002). However, the production of methane from methylated compounds is also carried out by archaeal of the orders Methanomassiliicoccales, Methanobacteriales and Methanosarcinales (Enzmann et al. 2018), and the WSA2 class was proposed as a methylate reducer restricted to methanogenesis (Nobu et al. 2016).

Notably, the microbial community involved in AD is complex, and the success of the process depends strictly on the syntrophic interactions between them since several environmental factors can intervene in this microbial symbiosis. After identifying the groups and their respective functions, engineering and management techniques can be used, which makes it essential to model development strategies and process optimization (Carballa et al. 2015; Koch et al. 2014). However, these consortia are not fully understood, as most microorganisms are not cultivable at the species level, and metagenome studies indicate a large number of sequences that make it difficult to classify all species present in the system, with taxonomically

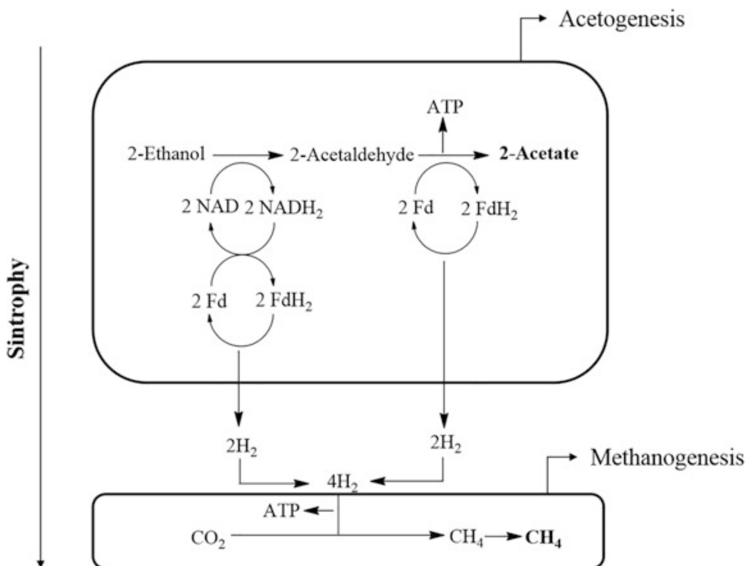


**Fig. 3.4** Syntrophy between acetogenic bacteria and hydrogenotrophic. Source: Adapted from Deublein and Steinhauser (2010)

reported levels higher (such as phylum, order, family, and genus). Therefore, most microbial species are unknown in AD (Campanaro et al. 2016).

Molecular biology techniques, such as PCR and its derivatives, in conjunction with genomic sequencing technologies (16S rRNA) have brought continuous advances for the identification, at the species level, of the microbiota involved in AD. As the analytical techniques become more sophisticated, future studies can be carried out and the list of anaerobic microorganisms will tend to increase. Identification studies of species involved in AD can also elucidate the degradation of different chemical compounds frequently made by members of the same genus, which would enable a better understanding of the metabolic biochemical routes and the syntropy in the anaerobic community for biogas generation. Omics approaches in conjunction with new species isolation technologies can significantly contribute to a better understanding of functions at the cellular level (De Vrieze et al. 2018; Hassa et al. 2018; Maus et al. 2018; Stolze et al. 2016).

## 3.4 Key Factors Imposed on Microorganisms that Affect Biogas Productivity

Stability of biogas production is greatly dependent on the microbial community stability of anaerobic reactors. Temperature, pH and the organic loading rate (OLR) are major factors in shaping the microbial community structure during the AD. The startup of anaerobic reactors is one of the most critical steps in the operation of

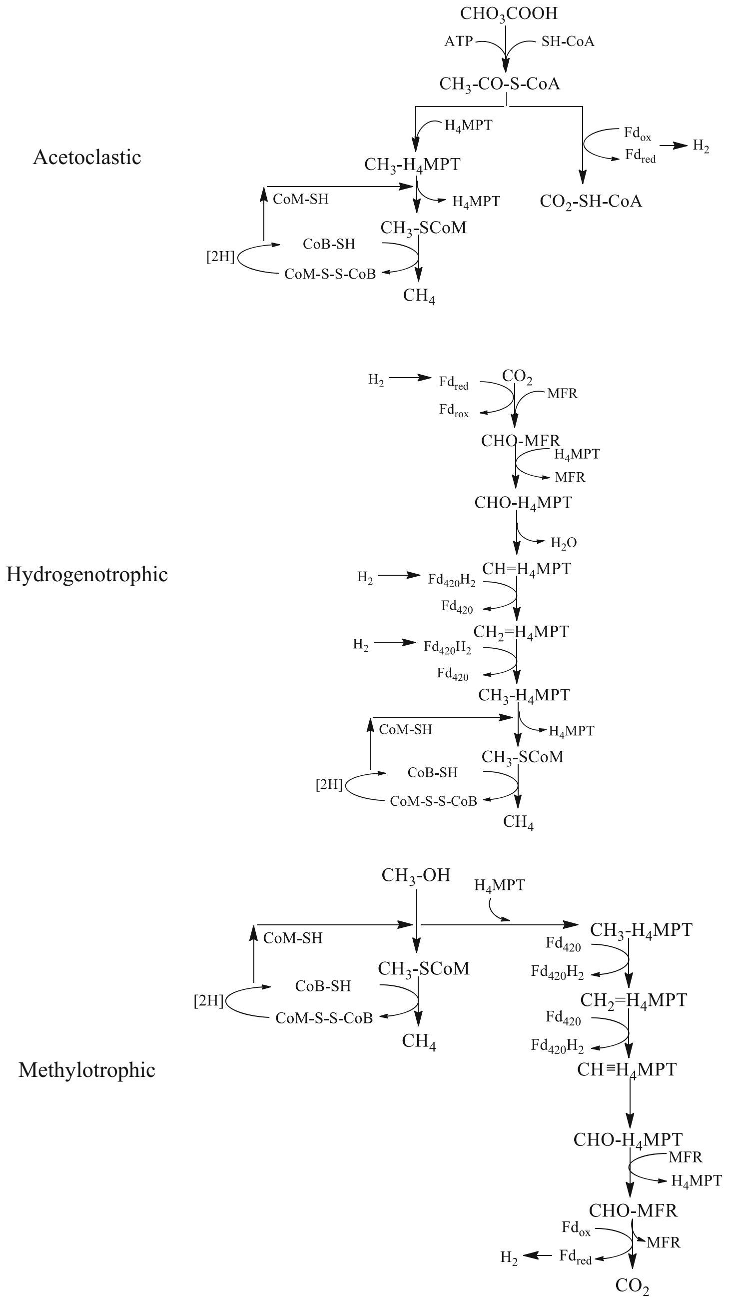


**Fig. 3.5** Biochemical pathways of Methanogenesis. Source: Adapted from Ferry (2011) and Welander and Metcalf (2005)

anaerobic systems, and the conditions applied during this phase will form the structure of the microbial community. Finally, anaerobic co-digestion has been used to improve the anaerobic digestion process with which the combination of different co-substrates is mixed to enhance biogas production.

### 3.4.1 Temperature and pH

Temperature and pH directly affect microbial growth and stability. Limited ranges of pH and temperature are related to the optimal bacterial growth rate, although their survival can occur with larger ranges (Speece 1983).

Variation in temperature during AD affects the stability of all microbial species due to their intracellular proteins that are thermostability dependent, particularly methanogens (Amani et al. 2010). In fact, temperature affects the development of the AD by influencing both the enzymatic reaction and the substrate diffusion rates, and methane formation is strongly temperature dependent (Gerardi 2003).

The ideal temperature levels related to AD are the mesophilic (25–40 °C) and thermophilic range (50–65 °C) in accordance with the optimal temperature range for the groups of microorganisms involved in the entire process (Ahring 1994; Metcalf and Eddy 2004). According to Chernicharo (2007), most of the anaerobic reactors have been designed and are operated in the mesophilic range, although thermophilic processes offer several potential advantages, such as an increase in microbial activity, and thus higher biogas production (Van Lier 2008). At the same time, at higher temperatures, thermodynamics for acetogenic conversions are more favorable, while a general decrease in microbial diversity can be observed, especially the complexity of the methanogenic community (Pap et al. 2015; Zábranská et al. 2000).

Moreover, the thermophilic microbial community is more susceptible to inhibition and sudden environmental changes than the mesophilic one. This inhibition is related to the lower growth yield of thermophilic microorganisms due to their higher decay rate, which is double that of mesophilic anaerobes (Wilson et al. 2008). At thermophilic conditions, the cells of the microorganisms show a tendency to lyse rapidly; also, higher energy is required for their maintenance and specific molecular properties of enzymatic reactions are necessary (Kim et al. 2002).

Several studies have demonstrated that acetogenic degradation of intermediate volatile acids, i.e., propionic and butyric acids, are better at thermophilic temperature due to favorable thermodynamics of syntrophic oxidation; however, methanogenic conversion of acetic acid has been more favorable at mesophilic conditions (Amani et al. 2015; Kim et al. 2002; Pap et al. 2015).

In recent years, due to advances in molecular biology, metagenomic and metabolomic techniques have led to new insights into anaerobic microbial populations in terms of diversity and activity at mesophilic and thermophilic temperatures. Notably, higher diversity of archaeal and bacterial communities is found at mesophilic temperatures, whereas the latter is considerably more diverse and dynamic than archaea at any temperature (Levén et al. 2007; Pycke et al. 2011). In addition, microbial diversity enhances the capability of the community to adapt

and respond to process instabilities, due to its ability of using parallel metabolic pathways (Ferguson et al. 2018).

Community analyses in anaerobic reactors showed the presence of bacteria affiliated with the phyla Chloroflexi, Synergistes, Bacteroidetes, Firmicutes, and Thermotogae in both meso- and thermophilic conditions (Chouari et al. 2005; Levén et al. 2007). Bacteroidetes and Spirochaetes were the dominating fermentative bacteria of the mesophilic processes while Thermotogae was the major group presented in thermophilic process (Hernon et al. 2006). The methanogenic archaeal community was commonly dominated by the phyla Euryarchaeota and Crenarchaeot*;* the acetoclastic methanogens from the genera *Methanosarcina* and *Methanosaeta* were dominant in the mesophilic reactor, and the Methanobacteriales species was abundant at thermophilic temperatures (Lee et al. 2017; Patil et al. 2010; Yu et al. 2014).

The pH is also an important factor in microbial growth and stability, and the ideal range has been reported to be 6.8–7.4 (Speece 1983). In addition, pH is intrinsically related to alkalinity and volatile acids, and these three environmental factors are equally important to the control the anaerobic processes (Chernicharo 2007).

Methanogens are extremely sensitive to pH, and these microorganisms have optimum growth in the pH range between 6.6 and 7.4; values above 8.3 and below 6.0 should be avoided due to their inhibition effect (Gerardi 2003). On the other hand, hydrolytic and acidogenic bacteria are generally less sensitive and can function in a larger range of pH (4.5–8.0); besides, even lower values can be supported (less than 4.5) (Lettinga et al. 1997). Due to the faster growth of acetogenic bacteria, volatile acids can accumulate if enough alkalinity is not provided (1000–3000 mg/L as $CaCO_3$) (Amani et al. 2010; de Aquino and Chernicharo 2005). Therefore, the control of pH aims to mainly reduce the risk of methanogenic inhibition by the low pH values.

### 3.4.2 Reactor Start-up

The properties of the microbial seed community regulate the rate of the reactor start-up whose duration can vary from a few days to even months. If the reactor start-up is not well developed, a long acclimation period can occur which leads to lower biogas production and poor reactor performance (Hickey et al. 1991). Furthermore, a short and successful start-up time is one of the key factors to economically improving the biogas production and increasing the competitiveness of anaerobic reactors (Weiland and Rozzi 1991).

During this initial phase, the hydrolytic and acidogenic bacteria groups dominate the system, especially because of their short generation time, which is highly active while the methanogenic microorganisms are just becoming established due to their low growth rate (Gerardi 2003; Weiland and Rozzi 1991).

The principal microbial factors that influence the start-up of the reactor are the dominating microbial groups, i.e., hydrolytic, acidogenic, acetogenic or methanogenic, growth rate and the shape of methanogenic community, the biomass

yield coefficient, and the rate adaption of the microorganisms to the substrate (Weiland and Rozzi 1991). Hence, start-up strategies should be developed to increase the population of the selected inoculum to improve the reactor performance and biogas production. These strategies include the control of fluctuations of external parameters such as temperature, hydraulic retention time (HRT), pH and OLR (Barber and Stuckey 1998).

The optimum temperature ranges are 33–37 °C and 50–55 °C for mesophilic and thermophilic inoculum, respectively (Kim et al. 2002). The pH of the substrate should be maintained in the range of 6.8–7.2 for the maximum methanogenic growth also to prevent accumulation of intermediate products such as VFA and ammonia (Angelidaki et al. 2006; Gerardi 2003). OLR must be as low as possible, and the optimal value depends on the reactor configuration and substrate composition (Barber and Stuckey 1999). Moreover, a long HRT during the initial period is recommended to avoid biomass washout (Leitão et al. 2006).

The seed inoculum used to start-up an anaerobic reactor is usually taken from another working reactor, especially an up-flow sludge bed reactor (UASB) or waste activated sludge, from a municipal digester or adapted from animal manure (Amani et al. 2010; Chachkhiani et al. 2004; Kobayashi et al. 2009). However, in some cases, start-up can rely on the internal inoculum available in the substrate by cultivating microorganisms throughout the anaerobic self-degradation (Kobayashi et al. 2009). According to De La Rubia et al. (2013), there is a relation between the high levels of *Archaea* microorganisms and the success of the reactor start-up; however, reactors that experienced a difficult start-up period had lower levels of *Archaea*. Therefore, the microbial community seed must contain a high level of methanogen microorganisms to optimize and reduce the start-up time.

### 3.4.3 Retention Time and OLR

Retention time and organic load rate (OLR) are parameters related to each other and to the reactor design and volume, flowrate and organic content of the feed, microbial growth rate and characteristics of the substrate, i.e., organic matter content.

In an anaerobic reactor, there are two retention times to considered: solids retention time (SRT) and hydraulic retention time (HRT). SRT is related to the average time that the microorganisms, i.e., solids, spend in the reactor while HRT is the time the substrates are in the reactor and is related to the reactor volume and influent flowrate (Chernicharo 2007). HRT can be used to control the dominant species in the reactor, whereas SRT is considered as an important factor that ensures the growth and stability of various populations of microorganisms inside the reactor (Kundu et al. 2017; Zhang and Noike 1994). The methanogens, due to their slow microbial growth rate, are the microorganisms that most affect the retention time of the operation.

From an economic point of view, short HRT is preferable due to a smaller reactor volume required, while high SRT maximizes the reactor performance, provides buffering capacity for protection against the shock loads and permits biological

acclimation to toxic compounds (Gerardi 2003; Weiland and Rozzi 1991). However, decreasing HRT usually leads to VFA accumulation which causes inhibition of methanogens and affects biogas production (Kuruti et al. 2017; Mao et al. 2015). Besides, shortening of SRT coupled with increasing OLR can lead to a higher biogas production rate and volumetric methane productivity, which improves the energy balance of the processes and overall degradation efficiency (De La Rubia et al. 2006; Nges and Liu 2010).

According to various recent studies using high-throughput sequencing and advanced molecular techniques, bacteria affiliated with the phyla Firmicutes and methanogens from the order Methanosarcinales, specifically *Methanosarcinaceae* group, were the dominant population at higher HRT, while at low HRT bacteria belonging to the phyla Gammaproteobacteria, Actinobacteria, Bacteroidetes, Deferribacteres, and archaea groups of Methanomicrobiales, Methanobacteriales, and Methanosaetaceae were predominant during the AD (Kundu et al. 2017; Wei et al. 2017; Xu et al. 2018c). At reduced SRT, Vanwonterghem et al. (2015) observed a washout of acetoclastic *Methanosaeta,* while the microbial community was dominated by bacteria from the genera *Alkaliflexus*, *Fibrobacter,* and *Ruminococcus.* By increasing the SRT from 4 to 12 hours, the authors detected the prevalence of populations belonging to the bacterial genera *Ruminococcus, Clostridium, Bacteroides*, and the archaeal genus *Methanosaeta.*

Methane production is particularly affected by the HRT, although HRT has been reported to cause less effects on microorganisms than OLR (Ferguson et al. 2018). During reactor start-up, OLR is preferably kept low and is gradually increased to trigger microbial growth along the operation which can lead to a higher methane production rate (Kundu et al. 2017). The stepwise increase in OLR allows the archaea community to adapt their cell density required for the consumption of VFA rapidly produced by the bacteria; hence, the methanogens are able to produce biogas constantly and attain a new steady state before a new increase in the OLR (Nakasaki et al. 2015).

In a study conducted by Rincón et al. (2008), the effect of OLR was investigated in a CSTR reactor and revealed that at low OLR, firmicutes, mostly represented by the genus *Clostridium,* were the predominant bacteria while the bacterial community which was most abundant at higher OLR were Pseudomonas, Actinobacteria, Bacteroidetes, and Deferribacteres. However, the archaeal community showed a static phylotypic composition mainly consisting of the *Methanosaeta* group as also demonstrated by Nakasaki et al. (2015). On the other hand, other studies showed a significant population shift from *Methanosaeta* to *Methanosarcina* groups by increasing OLR (Boonapatcharoen et al. 2007; Chelliapan et al. 2011).

OLR also influences the formation of anaerobic granular sludge in the high rate reactor, specially the UASB reactors (Hulshoff Pol et al. 2004). Mass transfer between incoming substrate and biomass, microbial proximity for syntrophic reactions, staging, and configuration of phased digesters are the key factors controlling the OLR (Amani et al. 2010). Therefore, the recommended value of OLR is greatly dependent on the type of waste, the operational temperature range, and reactor design.

Hydraulic and organic shock loads are caused by variations in HRT and OLR, respectively, and can result in a significant change in microbial community by altering the balance between the acidogenesis and methanogenesis, particularly affecting the archaeal community (Kundu et al. 2017; Vanwonterghem et al. 2015). The response of each syntrophic group to the change in conditional operations is related to their growth rate, and a proper balance between the different microbial groups is especially important to biogas production stability.

### 3.4.4 Anaerobic Co-digestion

Anaerobic co-digestion (AcoD) utilizes two or more substrates with complementary characteristics to balance the nutrients (C:N ratio), promote dilution of toxic compounds, improve synergism between microorganisms, and for the supplementation of trace elements and/or moisture. By combining co-substrates, AD of complementary residues can be maximized, resulting in higher biogas production, thus improving the economic viability of the AD process, promoting waste recycling, and contributing to environmental conservation (Mata-Alvarez et al. 2014).

Animal manure, sewage sludge, and organic fraction of municipal solid waste (OFMSW) are the most common residues utilized as the main substrate in AcoD (Beneragama et al. 2017; Elalami et al. 2019; Tyagi et al. 2018). Animal manure is usually complemented with agro-industrial wastewater, glycerol (glycerin), and cheese whey, while sewage sludge and OFSW are commonly utilized as co-substrates due to facilitated transportation logistics (Sosnowski et al. 2003). Other co-substrates include food waste, leachate, algae (macro and micro), industrial wastewater, fat, oil and grease (FOG), lignocellulosic biomass (yard waste and crop residues), and sewage (Esposito et al. 2012).

The characteristics and nutritional balance, especially the C:N ratio, of the co-substrates influence the stability between the bacteria and methanogen groups and can promote a more robust microbial community and enhance biogas production (Xu et al. 2018c). In fact, the ideal C:N ratio has been reported in the range of 20 to 35 (Puñal et al. 2000), and the combination of substrates to provide the optimal ratio is necessary to improve the biogas yield from AcoD. For instance, Rahman et al. (2017) reported the highest methane yield when a mixture between poultry wastewater and lignocellulosic biomass had a C:N of 32; (Haider et al. 2015), who co-digested food waste and rice husk, showed that feedstock C:N ratio of 20 promoted the best AcoD performance, while Astals et al. (2011) found an optimum C:N ratio of 23 treating pig manure and glycerin.

Moreover, the microbial community structure is also a major factor in the performance of AcoD. The bacterial community is capable of metabolizing a large variety of substrates although feedstock composition regulates the bacterial consortia diversity, thus influencing the archaeal community (Ros et al. 2013). Bacteria affiliated with the Firmicutes and Chloroflexi groups have been found in a wide range of AcoD processes due to their capacity of degrading a large amount of organic waste (Chouari et al. 2005; Tyagi et al. 2018). Specifically, in AcoD using

animal manure as the main substrate, pyrosequencing results revealed the presence of *Halothermothrix*, *Selenomonas*, and *Halocella* affiliated to Firmicutes and *Methanosarcina* species of the family *Methanosarcinaceae* (Goberna et al. 2010; Riya et al. 2016).

AcoD of sewage sludge with OFMSW, both major urban environmental issues, have been reported to be a sustainable method for disposing of this waste (Edwards et al. 2017). 16S rRNA gene sequences revealed in co-digestion of sewage sludge and urban OFMSW that *Thermonema* was dominant bacteria, while other bacteria belonging to phyla Firmicutes, Bacteroidetes, Synergistes and Thermotoga, particularly the orders *Clostridiales, Bacteroidales, Thermoanaerobacterales, Synergistia* and *Thermotogales* were detected in small amounts; archaeal assigned to the orders Methanobacteriales, Methanomicrobiales, and Methanosarcinales were the predominant methanogens (Fitamo et al. 2017; Martín-González et al. 2011).

Bacteria associated with the phylum Firmicutes and Bacteroidetes, particularly *Clostridia* and *Bacteroidia* species, were reported to be the most abundant microorganisms in AcoD of agricultural waste combined with animal manure (Ziganshin et al. 2013). The facultative acetoclastic *Methanosarcina* genus, the strict acetotrophic genera *Methanosaeta*, the hydrogenotrophic genera *Methanocalculus, Methanobacterium, Methanolobus, Methanoculleus, Methanothermobacter, Methanobrevibacter, Methanocorpusculum, Methanomicrobium,* and *Methanospirillum* and the methanol-consuming genus *Methanosphaera* were detected with the AnaeroChip microarray in the AcoD of cattle manure and olive mill waste, showing the diversity of archaeal during co-digestion (Goberna et al. 2010).

## 3.5 Process Configurations for Biogas Production: From Consolidated Technologies to Innovations

Several anaerobic digestion technologies have been proposed over the years, and the way they are implemented around the world can vary significantly. In developing countries, there are many small-scale household digesters that produce enough biogas to be used in the home, while large-scale agricultural or centralized digesters with large capacities are found in developed countries. Currently, there are 132,000 biogas digesters in operation worldwide, most of them in China, Europe, and United States, where there are over 100,000, 18,202, and 2200 units, respectively (EBA 2019; REN21 2020).

Biogas plants can be separated into three broad categories: micro-digesters using the biogas, scale digesters, which generate electricity and produce upgraded biogas, i.e., biomethane, respectively (World Biogas Association 2019). From an economic point of view, AD systems can be classified into two categories: large-scale AD systems, largely used in sewage plants and wastewater industrial units, and small-scale AD systems, such as households and community scale farms in rural areas (Vasco-Correa et al. 2018).

Likewise, anaerobic systems generally used to treat waste can be separated into two main groups: conventional systems, such as sludge digesters, septic tanks, and anaerobic ponds; and high-rate systems, such as up-flow sludge blanket reactors (UASB), expanded granular bed reactors (EGSB) and an anaerobic sequencing batch reactor (ASBR) (Chernicharo 2007). However, anaerobic processes can be classified according to the solids concentration in the reactor: wet or dry (or solid state) AD processes, whose reactors are operated with solids content below 10% and between 15% and 35%, respectively (Weiland 2010).

Currently, certain reactor configurations are very well established and are considered consolidated technologies, which are used in several industrial and agricultural units. However, new types of reactors have been proposed to enhance biogas production to provide diverse environment for different microbial populations and to control factors of inhibition. Recent research focuses on novel reactor geometry and design as well as two-phase reactors.

### 3.5.1 Consolidated Technologies

Among the consolidated technologies utilized in the AD, the high-rate systems transformed the AD into a commercial and sustainable process which was successfully applied to treat various types of wastewater and residues along with the bioenergy production. Developing high-rate reactors allowed the separation of HRT and SRT, leading to smaller reactor volumes by reducing the HRT applied while high biomass concentration enhances the reactor performance (Lettinga et al. 1997).

High-rate anaerobic reactors can be classified according to the type of biomass growth in the system: suspended (or dispersed) growth, whose microorganisms are present as flocs or granules, and attached growth, which requires a moving or fixed inert support material for the microbial fixation, promoting the formation of biofilms (Chernicharo 2007). Anaerobic filters, expanded/fluidized bed reactor, and the anaerobic membrane reactor are the most commonly known reactors with attached microbial growth while granular sludge is seeded and/or developed in UASB, expanded bed reactors such as EGSB and anaerobic reactor with internal recirculation (IC) (Van Lier 2008).

Among the high-rate reactors, the UASB is certainly the main process configuration in current use. The granulation of anaerobic sludge has been considered the major reason of successfully implementing UASB reactors for AD treatment of several kinds of waste, particularly in domestic sewage treatment (Foresti et al. 2006). The granule is formed by microorganisms that organize themselves into dense and highly structured aggregates, in which a diverse microbial community grows in a syntrophic symbiosis (Liu et al. 2002). The granule structure mainly consists of different layers with the hydrolytic/acidogenic bacteria primarily dominating the outer layer and the inner layer consisting of methanogens (Tartakovsky and Guiot 1997).

Besides years of research, the mechanisms of granule formation are still not fully elucidated; however, it seems that the process is a spontaneous microbial involvement (Show et al. 2020). Theories approaching the physical, microbial, and thermodynamic conditions applied to the anaerobic system have been considered the major factors responsible for granulation. According to Hulshoff Pol et al. (2004), *Methanosaeta concilii* is a key microorganism in anaerobic granule formation. The Cape Town hypothesis stated that granulation depends on *Methanobacterium* strain AZ while the Spaghetti Theory proposed that granule formation can be initiated by the attachment of filamentous *Methanosaeta* on a precursor whose granule microbial structure is further developed by incorporating other microorganisms such as *Methanosarcina* (Hulshoff Pol et al. 2004; Show et al. 2020).

Other consolidated configurations widely used in AD plants are continuously stirred tank reactors (CSTR), in which the microorganisms are suspended inside the reactor through intermittent or continuous mixing; and the expanded bed reactors (EGSB), which are similar to UASBs except that the high hydraulic rate expands the sludge, intensifying the hydraulic mixture providing greater contact between the biomass-substrate. (Mao et al. 2015; Seghezzo et al. 1998).

Regardless of the small-scale AD systems, mostly are household units (2–10 $m^3$), usually located in rural areas in China, India, and other developing countries. There are three types of digesters commonly used: the Chinese fixed dome digester, the Indian floating drum digester, and the tube digester (Surendra et al. 2014; Vasco-Correa et al. 2018). These digesters have a simple design, low-cost installation, and the biogas produced is usually used for cooking and heating.

### 3.5.2 Innovation

Many studies have focused on developing new anaerobic reactor configurations and/or redesigning the existing models to enhance biogas production, yield and quality, and to optimize and economically improve AD. Multi and two-phase AD systems have been used to optimize the AD by separating hydrolysis/acidogenesis and acetogenesis/methanogenesis phases. These systems can utilize two (or more) separated reactors or only one integrated reactor in which the AD phases can be physically separated, such as the anaerobic baffled reactor (ABR) (Ke et al. 2005; Rajendran et al. 2020).

Anaerobic membrane bioreactors (AnMBR) are considered as new-generation technology, whose process can effectively retain key microorganisms such as methanogens allowing high biomass concentration while obtaining a high-quality effluent (Zhen et al. 2019). Various reactor configurations have been developed over the years, such as anaerobic fluidized-bed membrane bioreactors, anaerobic submerged rotating membrane bioreactors (AnSRMBR), anaerobic dynamic membrane bioreactors (AnDMBR), and anaerobic electrochemical membrane bioreactors (AnEMBR) (Maaz et al. 2019). However, there are some challenging aspects that must be overcome to consolidate the use of membrane technology on a large scale,

such as membrane fouling and recovery of dissolved methane (Smith et al. 2012; Vinardell et al. 2020).

The quality of the final effluent of the AD process is a constant concern in wastewater treatment plants. To overcome this issue, the combination of anaerobic and aerobic systems has been used in industrial wastewater treatment for years, particularly the food industry. However, new process configurations are being widely studied to reduce costs and improve the quality of the effluent in order to meet the regulatory regulations for disposal, in addition to increasing bioenergy recovery. The combination of anaerobic and aerobic processes in a single integrated reactor is attractive in the sense of meeting the constraints related to space, odor, and minimal sludge production and at the same time, presenting an excellent cost–benefit ratio. However, most integrated systems reported in the literature lack large-scale implementation (Chan et al. 2009).

### 3.5.3 Solid-state Anaerobic Digestion

Solid-state anaerobic digestion (SS-AD) englobes the biogas production from various solid wastes, such as waste activated sludge, lignocellulosic biomass, and municipal solid waste. The process is usually operated at a total solids (TS) concentration between 15% and 50% and has the potential to provide, besides the biogas, organic fertilizer (soil conditioner) or carrier material for biofertilizers and pelletized fuel (Van Lier et al. 2001).

SS-AD systems have some advantages over liquid AD such as lower energy requirements for heating and mixing, fewer moving parts, absence of floating and stratification of fats and fibers, minimal material handling, and a greater acceptance of inputs containing glass, plastics and grit (Li et al. 2011; Mata-Alvarez et al. 2000). However, AD of solid waste requires longer retention times due to slower mass transportation, which were reported to be up to three times longer than liquid AD (Khalid et al. 2011).

Several reactor configurations, continuous or batch modes, have been utilized for SS-AD. The start-up of SS-AD reactors has been considered as a critical phase, which requires a highly concentrated and active inoculum to improve digestion time and efficacy during the initial phase of operation (Forster-Carneiro et al. 2008). Moreover, the temperature regulates the performance of SS-AD as well as the selection of waste/inoculum and feedstock/effluent ratios and the assessment of anaerobic biodegradability of the waste (Mata-Alvarez et al. 2000).

Nevertheless, there are some issues associated with SS-AD such as lower methane yield and organic matter stabilization and, to overcome them, anaerobic co-digestion has been proposed to enhance biogas production and yield as well as to produce a stabilized compost to be recycled as an organic amendment (Van Lier et al. 2001). Moreover, pretreatment of the substrates can be used to improve the performance of SS-AD by breaking down the complex organic structure into simpler molecules that are easily degraded by the microbial community (Yadvika et al. 2004).

During AD of solid wastes, hydrolysis is considered a major rate-limited phase which influences the conversion efficiency of the process. Regardless of the microbial community in SS-AD, hydrolytic bacteria Firmicutes and *Clostridium* species are most commonly found, while *Clostridiaceae* are microbial community that are responsible for degrading the particulate COD of solid residues (Regueiro et al. 2014). *Thermomonospora, Tepidimicrobium, Pseudomonas, Acinetobacter, Ralstonia, Lactobacillus* and *Shewanella* have been reported to be involved in the degradation of food waste and municipal solid waste (Shi et al. 2020; Wu et al. 2020). The cellulolytic bacteria such as *Ruminococcus Clostridium, Cellulomonas*, *Thermomonospora, Baceriodes, Erwinia, Acetovibrio, Bacillus, and Microbispora* are related to the degradation of lignocellulosic biomass due to their production of cellulases (Heeg et al. 2014; Lo et al. 2009).

A pyrosequencing-based metagenomic study demonstrated that species affiliated to genera *Methanosarcina, Methanosaeta* and *Methanoculleus* of Euryarchaeota, specifically the species *Methanosarcina barkeri fusaro, Methanoculleus marisnigri JR1,* and *Methanosaeta theromphila* consisted of the main methane-producing microbial community in an SS-AD reactor (Li et al. 2013).

Therefore, SS-AD systems may have a high diversity of micro-environments due to the heterogeneous nature of the feedstock. This diversity allows the growth of the microbial groups necessary to complete the first steps of AD, thus providing conditions to establish methanogens.

## 3.6 Conclusion and Future Perspectives

AD is a well-established biological route, commonly used for biogas production and consists of a four-step process that involves the action of different groups of microorganisms. Currently, different materials can be used as feedstock. It is particularly important to study the composition and physical-chemical structure of each substrate. Moreover, the optimal feedstock choice is necessary to plan the adequate technological model for the effective construction of the biogas plant.

Besides the type of substrate, it is necessary to understand the syntrophic activity of the microbial communities involved in the AD. Once knowing the present microbiota, the nutritional and operational conditions can be used to improve the biomethane production. For instance, the major microorganisms presented in the four-steps are: the microbial degradation in the hydrolysis and acidogenesis steps involve the phylum Firmicutes, from genera *Anaeromusa, Anaerosphaera, Aminobacterium, Aminomonas, Gelria, Peptoniphilus, Thermanaerovibrio, Clostridium, Proteiniborus* and *Sporanaerobacter.* The microbial degradation in the acetogenesis step involves acetogenic bacteria from the genera *Clostridium* and *Acetobacterium.* The last step of the AD, considered the limiting phase, involves methanogenic archaeal microorganisms that are the mainly responsible for producing methane and belong to the phylum Euryarchaeota.

The published data for AD indicate that several factors such as temperature, pH and OLR can affect the stability of the microbial community on anaerobic systems,

resulting in high or low biogas rates. Therefore, optimization conditions such as temperature levels (25–40 °C), pH range of 6.8–7.4, short HRT, high SRT maximize reactor performance, provide buffering capacity for protection against the shock loads and permits biological acclimation to toxic compounds. In addition, recent studies claim that advances on molecular biology, metagenomic and metabolomic techniques led to new insights into anaerobic microbial populations in terms of diversity and activity at mesophilic and thermophilic temperatures.

The main microbial factors that influence the start-up of the reactor are the dominating microbial groups, i.e., hydrolytic, acidogenic, acetogenic or methanogenic, growth rate and the dominating shape of the methanogenic community, the biomass yield coefficient, and the rate adaption of the microorganisms to the substrate. Finally, anaerobic co-digestion has been used to improve the anaerobic digestion process in which the combination of different co-substrates is mixed to enhance biogas production mainly due to the equilibration of the nutrients balance in the mixture (C:N ratio).

High-rate systems include reactor configurations, which are well-established and consolidated technologies, which are used in various industrial or agricultural plants. However, novel types of reactors have been studied to provide diverse environment for different microbial populations and to control factors of inhibition. Different solutions in terms of reactor configurations, such as the development of new anaerobic reactor configurations and/or redesign of the existing models are applied to enhance biogas production. For example, two-phase reactors to separate the hydrolysis/acidogenesis and acetogenesis/methanogenesis phases. Besides, the solid-state anaerobic digestion (SS-AD) approach not only englobes the biogas production from various solids waste, but also has the potential to provide organic fertilizer or carrier material for biofertilizers and pelletized fuel.

AD is an efficient and consolidated technology used worldwide to convert different types of wastes, residues, and wastewaters to a highly energetic biogas. The biogas produced in AD processes can be used to generate electricity and heat and be upgraded to biomethane, by removing $CO_2$ and other impurities, rather than being injected to natural gas pipelines or used directly as vehicle fuel. Despite using biogas as a source of renewable energy, the AD process is involved in climate change mitigation; by reducing greenhouse gas emissions and capturing biogas of landfills; improving urban air and water quality by substituting fossil fuel to biomethane and promoting sanitation, respectively. Moreover, biogas generation contributes towards a circular economy, transforming waste and residues into bioenergy, as well as improving food security whose inorganic fertilizers can be replaced by biofertilizers.

Although there are numerous advantages by utilizing bioenergy from AD, the biogas industry is in its initial stages of development. According to the World Biogas Association (2019), only 1.6–2.2% of the full potential of AD is reached and to fulfill the full potential of biogas, policies such as waste management and comprehensive agricultural policies; regulations are also required to encourage the implementation of AD technology. In addition, incentives that favor biogas over oil and fossil fuels,

such as tax exemptions and tax credit, credits for carbon reduction and carbon trading, as well as tax cuts for domestic and small-scale digesters.

However, biogas production has been growing in the European energy market where the number of biogas plants has increased continuously over the past years, especially due to numerous incentives from the government and European Union which includes the obligation certification for energy renewability and taxes policies. Biogas generation and utilization are also representative in China and India although they are restricted to small-scale units and concentrated in rural areas. The United States has the second highest national bio-power capacity and generation; however, the biogas market is stagnant in the country due to the lack of policy drivers and incentives. In Latin American countries such as Brazil, there are over 300 large-scale biogas plants in operation where more than three million cubic meters of biogas are produced daily, to generate electricity and biomethane (CIBIOGÁS - Centro Internacional de Energias Renováveis - Biogás 2019; EBA 2019; REN21 2020).

AD can be considered an industry global growth potential that generated high-value products. The potential of biogas production is high and can be a sustainable option to substitute fossil fuels. Governments must propose new regulations and incentives that favor AD, as well as waste management alternatives. In fact, AD technology allows the action of microorganisms and a key factor to economically improving the AD is to better understand the syntrophic metabolism that occurs throughout in each step of the microbial degradation, as well as the bacteria and archaea microorganisms involved in the processes.

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# Microbial Degradation of Disinfectants

# 4

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**Abstract**

Disinfectants are chemical products that eliminate pathogens and prevent their development. Its use has reduced the risk of infections in different environments such as industry and home. However, due to the synthetic nature of disinfectants, they tend to be toxic to human health, animal and plant life, and tend to bioaccumulate in environments. It is for this reason that various regulatory agencies such as the U.S. Environmental Protection Agency (EPA) have begun to establish protocols to analyze the environmental impact and risks involved in the presence of these pollutants in various settings. The scientific community has not been oblivious to this problem and they have developed bioremediation procedures that have given great results. Thus, it has been decided to take advantage of the ability of microorganisms, found in contaminated areas, to transform complex substances into simple molecules and use them as an energy source. Considering this, this chapter discusses the environmental impact of disinfectants and their toxic effects, but mainly the degradation of these contaminants by microorganisms as one of the promising bioremediation techniques.

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**Keywords**

Pollution · Environment · Microorganisms · Microbial degradation · Bioremediation · Cleavage · Energy source

## 4.1 Introduction

Disinfectants are chemical substances that are applied to inert objects with the objective of destroying or controlling pathogenic microorganisms such as bacteria, viruses, fungi, mold, or mildews that are found inhabiting said object. They work according to various mechanisms of action to achieve this objective, such as cell wall rupture, protein or lipid denaturation of microorganisms, alkylation, or oxidation. Chemical disinfectants can be grouped according to their chemical properties. Disinfectant components must be registered with the Environmental Protection Agency (EPA) (Giagnorio et al. 2017). The "active component" in each disinfectant formula is what kills pathogens through the different mechanisms of action. These active components are commonly aided by other components with several different purposes (Jeffrey 1995). However, being chemical agents, disinfectants are, by nature, potentially dangerous or toxic to living organisms. When compared to other toxic substances, chemical disinfectants can penetrate the body through various modes such as absorption through the nasal mucosa membrane or skin, by ingestion or inhalation. Thus, if chemical disinfectants are used properly with proper precautions, it will be effective. Otherwise, they will be dangerous and hazardous for users and the environment (Oluwasanu 2018).

### 4.1.1 Disinfection

Disinfection is a process by which the microbial forms found in some environments, especially surfaces, are killed through the use of chemical or physical agents. This method does not necessarily destroy all microorganisms but reduces them to adequate levels in which it is not harmful to human and environmental health (Kirmusaoglu 2018). Antimicrobial agents used for this purpose are occasionally used as sterilizing, sanitizing, or antiseptic agents. In general, disinfectants used in human and animal health are relatively toxic and powerful antimicrobial chemicals that are applied to contaminated surfaces, while those used in the agricultural industry are less toxic and less concentrated. Modern disinfectants are based on formulations that include chemical substances, soaps, detergents, and active agents that allow the penetration of the active substances (Kahrs 1995). There is a wide spectrum of disinfectants available that are tailored to the user's needs.

In many definitions of these terms, sanitation products and antiseptics are also referred as products to heal wounds, to clean surgeon's hands and arms before an operation, to disinfect the cow's udders, and in general to all the preparations that are applied directly to the tissues (Kahrs 1995). The semantic distinction among the

terms disinfectant, sanitizing agent, antiseptic, and sterilizer refers to the objective pursued with said products, as well as the composition and degree of concentration of their chemical substances. Thus, it also comes into play the time during which the product must be kept in contact with the surfaces by treatment, the level of waste that can be accepted, and the environment in which it develops in the process (Kahrs 1995; Moore and Payne 2008).

### 4.1.2 Active Ingredients of Disinfectants

Disinfectants owe their action to the active ingredients they contain. These ingredients comprise a wide spectrum of chemicals, ranging from simple inorganic molecules, such as sodium hydroxide, to relatively complicated molecules, such as polymerized quaternary ammonium compounds (QACs) or substituted isothiozolones (Jeffrey 1995). There is a myriad of active ingredients that vary in their physical and chemical properties and limit the choice for a particular application (Denyer and Stewart 1998). For example, it is not reasonable to use a sodium hydroxide solution on surfaces that contain certain metals (tin, aluminum, zinc, etc.), as it would corrode such materials. Likewise, oxidizing agents should not be used in the presence of reducing substances since it would neutralize their effect (Donnell 1999).

Most of the active substances fall into defined and well-established chemical groups. The active substances in disinfectants can be classified into the following groups:

- Quaternary ammonium compounds (QACs)
- Phenols
- Halogenated compounds
- Aldehydes
- Biguanides and polymeric biguanides
- Amphoteric compounds

### 4.1.3 Mode of Action of Disinfectants

In recent years, great efforts have been made to try to elucidate and understand the bactericidal mechanisms of action of disinfectants. In general, whatever the type of microorganism, its process of annihilation by means of disinfectants follows a sequence of steps (Donnell 1999). First, an interaction occurs between the disinfectant and the cell surface followed by penetration into the target cell. It is there when the active ingredients of the disinfectants exert their action. In some occasions, the interactions between the cell surface and the disinfectant can produce irreversible damage in the microorganism, but nevertheless, the majority of the active ingredients act at the intracellular level. This is because many external layers of the bacterial cells can present susceptibility or insusceptibility to these products. The

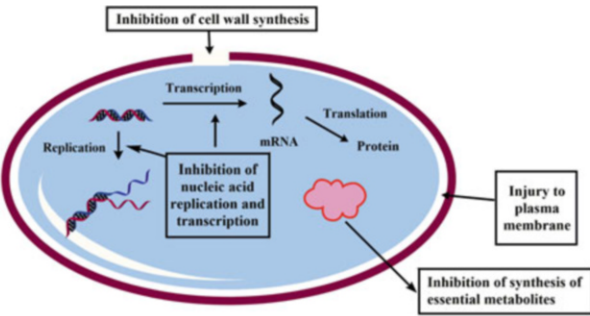


**Fig. 4.1** Mode of action of disinfectants

biocidal action will depend on the type of microorganism that can be summarized in cellular damage produced by inhibition of the synthesis of the cell wall, inhibition of the replication and transcription of nucleic acids, damage to the plasma membrane, and inhibition of protein synthesis or essential metabolites (Fig. 4.1) (Denyer and Stewart 1998).

### 4.1.4 Efficacy of Disinfection

The effectiveness of a disinfectant depends largely on its concentration, contact time, the nature of the disinfected surface, the amount of organic material on the surface, and the type and amount of microorganism present (Mazzola et al. 2009).

The contact time directly affects the disinfection process. Generally, the ideal time is five minutes, since this is the time in which many disinfectants remain on surfaces that are not horizontal (Reyes et al. 2014). This means that disinfectants must be able to ensure their biocidal effect in five minutes or less, depending on the type of application. However, certain biocidal agents can adhere to the surface, extending the contact time (Díaz-Enriquez et al. 2017).

Microorganisms vary their response to disinfectants depending on their cell structure, composition, and physiology. The greatest resistance is presented by bacterial spores, followed by mycobacteria, parasite cysts, small unencapsulated viruses, gram-negative bacteria, gram-positive bacteria, and lipid capsule viruses. Molds are generally more resistant than yeasts and much more resistant than non-sporulated bacteria. Biologically active particles can be viruses, bacteria, yeasts, molds, or spores that interact with the environment to fulfill their purposes (Díaz-Enriquez et al. 2017). Such biological particles sediment, grow, and occasionally form a mass of cells called a biofilm, which defines all contaminating and pathogenic microbiota activity that is formed in cell groups, spatially organized and enclosed in a matrix of organic polymers, on the surface of food or surfaces in contact with them. The presence of these biofilms on surfaces directly affects the efficacy of biocidal agents, either by interaction with the matrix or by the poor diffusion of biocides through it (Kirmusaoglu 2018).

The number of microorganisms affects the action of the disinfectant since a higher microbial load requires higher concentrations of the disinfectant or longer contact times and since adequate surface disinfection is not always achieved with these increases, a combination of these factors is necessary (Díaz-Enriquez et al. 2017). The design of equipment, the selection of materials, and the treatment carried out on them to limit the adherence of microorganisms after cleaning and disinfection are factors that determine the hygiene of surfaces. Extremely porous or rough materials offer a more appropriate surface for adhesion, the presence of these irregularities favors the refuge of organic residues and microorganisms.

## 4.2 Environmental Issues Related to the Use of Disinfectants

A wide variety of chemical products of synthetic origin that are used in disinfection processes are present in the environment, both in aquatic systems and in agricultural soils. Owing to their origin, many of these compounds are toxic or have the potential to become dangerous products in nature. Environmental control agencies around the world have been monitoring and evaluating the environmental impact of these active ingredients. Owing to growing public and government concern about the intriguing problems that have become apparent, environmental scientists, biologists, and chemists have devoted their efforts to determine the behavior and fate of compounds in natural environments. However, the process is undermined by the large number of chemicals used in industry, agriculture, and home (la Farré et al. 2008).

Many of these substances are deliberately or inadvertently released into waters and soils. When disinfectants are used in toilets, sinks, dishwashers, or appliances, the active chemicals are finally flushed down the drain. The water is then routed to the wastewater treatment facilities, where most of the pollutants are removed before the water returns to the rivers and lakes. However, not all pollutants are removed and can accumulate to have a substantial and adverse effect on wildlife, which could lead to dense vegetation that interferes with animal life and eventually decomposes in large quantities (la Farré et al. 2008). When any living beings die as a result of disease control one of the first concerns is related to contaminants. However, the consequences could be dangerous as well. So, there are doses reported that should not exceed, as well as it gets specific for a certain pathogen. The preservation of environmental elements such as soil, water, and air can best be ensured by aiming for efficient disinfectant application (Bruins et al. 1995).

The contamination by disinfecting agents generates not only an environmental problem but also a public health one. These agents are used extensively in homes, hospitals, health centers, and industries for a wide variety of topics and applications being an essential part of cleaning programs (Aktaş 2013). Antiseptics and disinfectants alter the first line of defense to prevent the spread of resistant pathogens. These compounds have the property of damaging the integrity of the cell membrane of microorganisms and causing cancer from the activities of the pathogen. Also, there are several chemicals in cleaning and disinfecting products that can cause or exacerbate asthma because of their sensitizing or irritant properties

(Bruins et al. 1995; Burge and Richardson 1994; Purohit et al. 2000; Savonius et al. 1994; Beaudouin et al. 2004; Gannon et al. 1995; Fujita et al. 2006; Nagy and Orosz 1984; Pechter et al. 2005; Kujala et al. 1995). Owing to all their properties of these compounds and biocides in general, there is a tendency to increase the use of these agents in control measures against infections of bacterial origin, in addition to their daily and habitual use, which has implied the selection of resistant bacterial strains. A pathogen can resist the action of biocides by a natural property (intrinsic resistance), or acquired property, by mutation or by the acquisition of plasmids, transposons, or integrons (Russell 1999). The disinfectant resistance mechanism that is normally encoded in plasmids is mediated by efflux pumps. There are several international studies on clinical symptoms resistant to the most widely used disinfectants, which report bacteria resistance to multiple antibiotics with decreased susceptibility to different disinfectants (Donnell 1999).

### 4.2.1 Disinfectant Effects on the Environment

#### 4.2.1.1 Toxicity

Many compounds used as cleaners can be harmful. They can be found on soil, air, water, and human bodies. EPA categorizes many of these chemicals as "volatile organic compounds", which can be harmful in different ways. These chemicals include phosphorous, nitrogen, and ammonia (Alton 2020). When some of these chemical compounds are used in different ways, they are rinsed down the drain, which will end on a partial elimination by water treatment. However, not all chemicals are drained, getting directly into the environment.

Bleach is one of the most common disinfectants employed during cleaning. It is corrosive and present side effects on human health. From an environmental point of view, bleach is a toxic and, above all, biocidal product. It destroys everything in its path. It kills bad bacteria, but also good bacteria that support biodiversity, flora, and fauna (Surfrider Foundation Europe 2020). The industries and the sewage treatment plants discharge the wastes that contain toxic substances directly into the waterways. These direct pipeline discharges are called the point sources (Soffar 2020).

#### 4.2.1.2 Greenhouse Gases

Many volatiles chemicals have been used as disinfectants. Carbon dioxide gas produces greenhouse effects and affects climate change. Old-school aerosol cans used chlorofluorocarbons (CFCs) as a primary propellant, and those chemicals contributed significantly to ozone depletion and climate change (Alton 2020). CFCs have been used for some decades; however, their use has been restricted by EPA because it cannot be depleted completely from the ozone layer.

### 4.2.2 Principal Disinfectants and Its Side Effects

#### 4.2.2.1 Chlorinated Compounds

The use of disinfectants that are preferred by the cleaning staff has been associated with better hygiene outcomes, particularly where strong chlorine-based disinfectants are the alternative (Friedman et al. 2013). Chlorine is used for its antimicrobial activity, being inexpensive, and faster rate. Chlorine-based disinfectants are usually unstable in both concentrated and diluted forms, as they are affected by heat and light (Bruins et al. 1995). Owing to concerns about disinfection byproducts (DBPs) and chlorine-resistant pathogens, alternatives to chlorine-intensive disinfection have drawn interest; however, most small systems have not been able to comprehensively evaluate alternatives (Cleaners and Disinfectants 2020).

#### 4.2.2.2 Formaldehyde

Formaldehyde may be used either in the liquid or gaseous state (Bruins et al. 1995). It has been used as a disinfectant with a potential capacity to kill viruses and is inexpensive as well. It acts as a preservative and is used as an antimicrobial agent in both skincare products and within the industry, where it is used as a raw material for production at certain workplaces (Pontén and Bruze 2015). In a high concentration, it can be harmful producing health issues. In some cases, it can kill even beneficial microorganisms and plant life.

#### 4.2.2.3 Phenolics

Phenol and its derivate have been studied for a long time. They have been applied as disinfectants that are usually combined with another compound to reduce or enhance their properties. These products are highly toxic to the environment, are generally hard to break down, and are not easily neutralized by organic material. Instructions for use must be strictly followed to avoid prolonged negative consequences for the soil, and animal and plant life (Bruins et al. 1995).

#### 4.2.2.4 Hydrogen Peroxide

Hydrogen peroxide (HP) has long been used as a disinfectant and is effective against viruses, bacteria, yeasts, and bacterial spores in vitro. However, hydrogen peroxide is inactivated in vivo by catalase, which catalyzes the breakdown of hydrogen peroxide into oxygen and water (Akuji and Chambers 2017). It has a potent effect on bacterial and viruses, although at specific conditions it can be corrosive and dangerous for the environment.

#### 4.2.2.5 Peracetic Acid (PAA)

PAA is a strong oxidizing agent usually employed as a disinfectant because of its wide spectrum over microorganisms and fast-acting ability. PAA is even more effective than traditional cleaners as the HP. It is combined with other different disinfectants. PAA used in synergism with HP is a common alternative (Walters et al. 2019). The negative impact produced by PAA is considered small than the other ones.

## 4.3 Bioremediation

Bioremediation is any biotechnological process that uses microorganisms, fungi, plants, or the enzymes derived from them to remove pollutants and toxins from the soil and water (Timian 1996). It is a technology capable of removing pollutants from the environment, allowing the environment to be restored to its original conditions and that prevents further contamination (Azubuike et al. 2016). Bioremediation processes commonly involve oxidation-reduction reactions where the reduced contaminants are oxidized and the oxidized contaminants are reduced (González Rojas and Concepto 2011). There are many different types of contaminants that can be removed with this technique: polycyclic aromatic hydrocarbons, petroleum, disinfecting pesticides, chlorophenols, heavy metals, dyes, sulfates, and so on (Donati et al. 2019). In bioremediation processes, several organisms may be involved, as well as bacteria (bacterial degradation), fungi (mycoremediation), microbes (microbial degradation), algae (phycoremediation), plants (phytoremediation), and molecules derived from these organisms (derivative remediation) (Aktaş 2013). The metabolism of these organisms is used to remove pollutants from contaminated environments.

Bioremediation shows many advantages compared to other remediation alternatives. These advantages include:

- It is a relatively green method that causes less damage to ecosystems.
- This method creates few harmful by-products.
- It is cheaper than most cleaning methods, as it does not require a large amount of equipment.

For the moment, bioremediation has been applied with great success in the degradation of organic chemical compounds and has even been implemented for the removal of waste of nuclear origin.

### 4.3.1 Types of Bioremediation

Bioremediation processes are environmentally friendly techniques for removing environmental contaminants. Two methods based on the removal and nature of the treatment used are distinguished.

#### 4.3.1.1 In Situ Bioremediation

In situ bioremediation consists of stimulating the microorganisms that inhabit the contaminated environment by applying nutrients and oxygen. This means that the method is applied in the place where the source of contamination has occurred (Raja and Kalyanasundaram 2014). With this method, the pollutants are biologically degraded under totally natural conditions to harmless products such as $CO_2$, water, or some attenuated transformation product. Three types of in situ bioremediation processes are distinguished: biostimulation, bioaugmentation, and bioattenuation.

- *Biostimulation* consists of supplementing the environment in which the microorganisms are found with adequate nutrients or conditions: nitrate phosphates, electron acceptors, or pH adjustments. This approach is based on the premise that autochthonous microorganisms are capable of degrading pollutants outside the environment after an acclimatization process. By accumulating scientific evidence, it can be inferred that this method could become the most reliable and safe (Langenhoff 2007).
- *Bioaugmentation* is the addition of specialized and exogenous microorganisms to the medium in order to optimize remediation. It may be appropriate for ex situ bioremediation projects, which will be developed later, with the use of specialized consortia (Vidali 2001).
- *Intrinsic* or *natural attenuation bioremediation* is carried out by the autochthonous microorganisms of the affected environment. These use their enzymatic potential to mineralize (completely biodegrade to $CO_2$) organic compounds or simply degrade them to intermediate products, either in an aerobic or anaerobic environment. It is a widely used method for restoring contaminated soils and waters (Langenhoff 2007).

#### 4.3.1.2 Ex Situ Bioremediation

This process involves excavation procedures or removal and transportation of contaminants from the site where it was found to a place where it will be treated. In general, the treatments applied to the pollutants are enhanced by means of bioreactors (Raja and Kalyanasundaram 2014). Under aerobic conditions, some microorganisms can use organic pollutants such as mixtures of hydrocarbons, phenols, disinfectants, and some pesticides as a source of carbon and energy to degrade them to $CO_2$ and water (Tomei and Daugulis 2013). There are some techniques related to ex situ bioremediation such as landfarming, biopiles, composting, and bioreactors.

- *Landfarming* is a high-tech method used for ex situ bioremediation that calls for the excavation of contaminated soils and their authority over an impervious area. The landfarming technique contains a drainage mechanism for the collection of leachates, which must receive certain treatment in succession. The purification of the soil with this technique is based on the operation of the existing microcircuits on a certain surface (Höckenreiner et al. 2015).
- The *biopile* method consists of stacking the soil that contains contaminants in piles or heaps and facilitating aerobic microbial activity through aeration processes and/or addition of nutrients, minerals, and water. Unlike landfarming, aeration is done by injecting air into a duct system inside the stacks. It is also used for petroleum compounds (Cristorean et al. 2016).
- *Composting* is a biological process consisting of excavating contaminated soils and mixing them with animal and vegetable waste such as fertilizers, manure, straw, pieces of wood, and so on. Under thermophilic conditions, thus stimulating the aerobic or anaerobic microbial biodegradable capacity, which allows

**Table 4.1** Advantages and disadvantages of bioremediation processes

| | Advantages | Disadvantages |
|---|---|---|
| In situ bioremediation | • It is done on-site<br>• It is economical since transportation costs are reduced<br>• Contaminants are permanently removed<br>• It can be applied to dilute and diffuse contaminants<br>• It can treat large tracts of the contaminated area<br>• It is affordable<br>• It may be more difficult to administer than ex situ techniques<br>• In soil remediation, it generates less dust | • It takes longer than the other remediation methods<br>• The microorganisms act only when the pollutant presents a source of energy and nutrient production to carry out the process<br>• When the environmental conditions are not favorable, its degradation capacity is limited |
| Ex situ bioremediation | • They are faster and easier to control techniques.<br>• They are used in a wide range of pollutants.<br>• It is flexible regarding volumes | • It cannot be applied in the removal of heavy metals or chlorinated hydrocarbons<br>• Contaminants must be aerobically biodegradable<br>• Techniques dependent on the material and the environmental conditions such as temperature and climate<br>• Pretreatment of the contaminated area is required and even at the time of bioremediation |

transforming organic pollutants into harmless substances. For the process to be successful, the oxygen level, humidity, temperature, or carbon-nitrogen balance must be controlled (Maitra 2018).

- In the *biological sludge* technique, the soil is excavated, sieved to remove thick elements, and mixed with water and other additives in a controlled bioreactor. Parameters such as dissolved oxygen, temperature, pH, availability of substrates, and humidity are controlled in the bioreactor. The resulting sludge keeps suspended solids and microorganisms (Tomei and Daugulis 2013). The biodegradation rate is usually high.

Some advantages and disadvantages of bioremediation processes are listed in Table 4.1.

### 4.3.2 Limiting Factors of Bioremediation Processes

Bioremediation is a process of vital importance for sustainable development. The applicability of bioremediation processes is determined by various factors such as environmental conditions including temperature, medium pH, microbial population,

oxygen, water, nutrient availability, site microbial density, a fraction of organic matter, capacity field, and the nature of the pollutant to be degraded (Azubuike et al. 2016). In addition, they also influence the processes of co-metabolism and consortia conditions. Co-metabolism refers to the need for certain biodegradators to acquire other substrates to degrade contaminants (Aktaş 2013). This characteristic is important above all to degrade organochlorine compounds.

The physical and chemical heterogeneities of the surfaces affect bioremediation in situ since they control the availability of nutrients and substrates that regulate microbial processes (Langenhoff 2007). If the kinetics of these physicochemical mass transfer processes is slower than the potential rate of biodegradation, the overall rate of bioremediation will be affected and the system will be limited by mass transfer (Vidali 2001). For this reason, the evaluation of the viability of an on-site bioremediation project is dominated by the need to correctly identify and estimate the appropriate speed controlling phenomenon.

Among the factors that determine the efficiency of bioremediation, it is important to highlight the properties of the contaminant (Boopathy 2000). Branched structures are easier to degrade than linear chains, as well as double bonds give stiffness and resistance to the molecule and an increase in the number of benzenic rings in the case of aromatic compounds. Chemical substitutions, such as dicarboxylic acids, nitriles, methylations, halogenations, usually make the molecule more resistant. On the other hand, the biodegradation of compounds that contain nitrogen or sulfur is frequently linked to their use as a source of nutrients (Providenti et al. 1993).

In a contaminated environment, the contaminant can be located in the aqueous phase of the soil, in the organic liquid phase, and/or in the vapor phase. Furthermore, it may be subject to chemical modifications such as hydrolysis, oxidation, or polymerization, processes that influence its availability (Boopathy 2000). For degradation of a pollutant to occur, it must interact with the microorganism's cell in an aqueous medium. Molecules with a significant density of negative charges have difficulty penetrating through cell membranes. However, as the hydrophobicity of the pollutants increases, they tend to be passively transported into the cell, with no energy input. Many organic pollutants are hydrophobic and tend to be absorbed in the soil, specifically in the organic fraction (Vidali 2001). Therefore, a critical aspect for the availability of contaminants is the composition of the environment that will determine the kinetics of absorption and desorption of the contaminants, as well as the mobilization capacity of the microorganisms (Srivastava et al. 2014).

Another aspect that determines the efficiency of bioremediation is the properties of the medium that allow or limit microbial growth and metabolism of the compound. Among them we can mention:

- Permeability, which hinders or allows the existence of gradients and the transfer of the contaminant to microbial populations.
- Temperature, which determines both metabolic rates and the physical state of the pollutant.
- The presence or absence of organic nutrients, oxygen, or other potential aspects of electrons.

- The water retention capacity and the hydraulic characteristics in the case of soils.
- Flow characteristics and pH in the case of groundwater.
- The degree of contamination (Srivastava et al. 2014).

To increase the effectiveness of bioremediation, we must therefore solve these limiting factors, a technique known as biostimulation. As well as the addition of specialized microorganisms to the medium, in order to facilitate the removal of contaminants and enhance and optimize bioremediation.

## 4.4 Microbial Degradation

Microbial degradation has positioned itself as one of the most promising methods in removing contaminants found in various settings. This is due to the ability of microorganisms to accomplish this task in the least dangerous way and in an environmentally friendly way (Singh 1995).

Microbial degradation refers to the decay of all materials of organic origin through microscopic life forms such as bacteria, fungi, protozoa, and other organisms. The microbes that complete the process adapt to the contaminated environment and produce the enzymes necessary for degradation (Bhatt et al. 2019). The intermediary molecules or a degradation product of some organisms become the energy or carbon sources of others to carry out the breakdown of the rest of organic matter (Ratledge 1994). Research in the field of microbes is of vital importance for the reduction of chemical waste and the elimination of most environmental pollutants.

Degradation is described as a physicochemical alteration of the properties of the active components of the pollutants and a redistribution of the nontoxic degraded particles in the environment (Kumar and Sharma 2019). Among the main bacterial families that are involved in pollutant degradation processes are *Pseudomonas, Bacillus, Nocardia, Actinomycetes, Escherichia, Micromonosfora, Mycobacterium, Rhodococcus, Comamonas, Azotobacter, Klebsiella, Thermoactinomycetes, Flavobacterium,* and *Alcalgenes*. The fungi used in degradation are *Candida, Sporotrichum, Thielavia, Paecilomyces, Ganoderma, Geotrichum, Phlebia, Trametes, Thermoascus, Talaromyces, Pnicilium, Aerobasidium,* and *Chaetomium (*Mosratö et al. 2003*)*. Depending on the nature of the microbes, these organisms partially consume the pollutant, destroying the intact parent substance, which is known as primary degradation, or they can wear down completely through a process known as ultimate degradation. The degradation is carried out by means of microorganisms that present different enzymatic activities. Microbial degradation follows three sequential steps (la Farré et al. 2008):

- *Biodeterioration*: Alteration of the physicochemical properties of the pollutant.
- *Assimilation*: Uptake of molecular by microorganisms.
- *Mineralization*: Production of degraded molecules such as $H_2O$ and $CO_2$.

### 4.4.1 Aerobic Degradation

Aerobic degradation is the decomposition of organic pollutants by microorganisms in the presence of oxygen. In this sense, microorganisms can carry out their work under oxidative conditions (Fritsche and Hofrichter 2008). Aerobic microorganisms have an oxygen-based metabolism and are used to oxidizing substrates and obtain an energy source through a cellular respiration process. In the assimilation stage, oxygen is used in chemical reactions that break down small molecules into water and carbon dioxide (Becker 2010).

The aerobic process results in more complete digestion of organic pollutants, reducing accumulation by more than 50% in the vast majority of cases. The aerobic process allows for a better environment for workers and animals as it helps keep pathogens under control (Fritsche and Hofrichter 2008).

### 4.4.2 Anaerobic Degradation

Organic pollutants degrade in the absence of oxygen through an anaerobic process. It is a widely used technique to treat sewage sludge and biodegradable waste because it provides a reduction in volume and mass of the input material in ex situ processes (Young 1995). This process produces various gases (depending on the degraded material) such as $CO_2$ and $CH_4$ and releases them into the environment (Reineke 2005). Degradation is a complex process that involves four steps:

- Hydrolysis of higher molecular weight compounds allows large molecules to be digested into smaller and easier to degrade molecules.
- The second stage consists of the transformation of these small molecules to volatile fatty acids.
- In the third stage, the acetogenic bacteria transform those volatile fatty acids into acetic acid.
- Finally, methanogenic bacteria transform the previous molecules into methane and carbon dioxide, harmless substances for the environment (Ghattas et al. 2017).

### 4.4.3 Role of Microorganism

The complete degradation of an organic pollutant present in waters and soils occurs as a consequence of microbial action. Various relevant abiotic processes in nature can convert organic compounds that cause a certain degree of toxicity and complexity to inorganic substances. As these transformations take place, the microbial populations grow and accept the carbon and nitrogen molecules, product of mineralization, as the energy source necessary for biosynthesis (Alexander 1981).

With many chemicals, a slightly different microbial transformation from mineralization occurs. Generally, this takes place when several contaminants are found

with biological activity and it is not possible to determine any microorganism capable of using them as an energy source. In that case, the evidence for the microbial role in transformation processes is the fact that the compound transforms into non-sterile but non-sterile samples from the natural environment or perhaps transforms more efficiently under non-sterile conditions. Compounds that typically exhibit these conditions include DDT, 2,4,5-T, aldrin, and many chlorinated and non-chlorinated compounds. Microorganisms capable of degrading these compounds have been reported in recent years. The fact that various chemical contaminants are governed by microbial action but, nevertheless, do not control the growth of the populations involved, has led to a growing interest in the phenomenon that has been called co-metabolism (Hazen 2010). The proposal is based on that populations develop on another substrate while generating the transformation (co-metabolism).

From a biochemical point of view, co-metabolism is the transformation of a compound in the forced presence of a substrate during growth or by cells at rest in the absence of the growing substrate is the cornerstone of the biological elimination of chemical compounds from the environment (Hazen 2010). Generally, the populations that act in degradative processes on chemical pollutants are small. This causes a compound that undergoes co-metabolism to change slowly and its speed does not increase over time, in contrast to the actuated substrate in the mineralization process.

The physiological basis that explains the process of co-metabolism is related to the specificity of the enzymes. Some enzymes found in microorganism cells can exert their action on reactions involving chemically similar substrates but slightly different in properties. If the product of the catalytic action of these enzymes does not turn out to be a suitable substrate for another enzyme to continue the degradation process, the contaminants will accumulate (Poursat et al. 2019). This will happen despite the fact that the first enzyme managed to convert the initial substrate into an energy source for the microorganism, however, these products can maintain the toxicity associated with the contaminant.

### 4.4.4 Ecological Consequences

Ecological issues have been of great interest in the study of microbial degradation. When the contaminating products enter an environment, the action exerted by the microorganisms is detoxification, that is, the destruction of functional groups that generate a harmful effect on nature (Alexander 1981). As mentioned earlier, the intermediaries of the degradative processes can be maintained over time and become equally toxic. In the same way, in a process called activation, certain innocuous compounds can be converted enzymatically into dangerous products for the species, in a sequence of mineralization. A clear example of this process is inorganic mercury methylation to produce mono- or dimethylated compounds that present toxicity and are also retained and accumulated by microorganisms (Yuan et al. 2020).

Another important factor to take into account in microbial degradation is that often certain microorganisms transform pollutants that are harmful to them into products that are toxic to others (Nagata 2020). A classic example of this type of situation is the microbial transformation of pentachlorobenzyl alcohol, which undergoes dehalogenation and oxidation. In general, the compound is not toxic when applied to rice crops, but the degradation products (tri- and tetrachlorinated benzoic acids) suppress the normal development of the plants (Tsukano 1986). Also, enzymatic deactivation processes can occur. This deactivation refers to the transformation of a compound that can be activated to a nontoxic (inactivated) compound, that is, that the harmful potential of the molecule is diminished before activation occurs. An example is the transformation of (2,4-dichlorophenoxy) butyrate into a harmless molecule like 2,4-dichlorophenoxyacetate (Macrae et al. 1963).

## 4.5 Microbial Degradation of Active Ingredients of Disinfectants

### 4.5.1 Quaternary Ammonium Compounds (QACs)

These compounds were introduced in 1917, but not until 1935 when was used in the disinfectant industry (Jeffrey 1995). QACs are perhaps the best known cationic surface-active agents. All QACs have a general formula as shown below (Fig. 4.2).

$R_1$ is generally an alkyl group with a C-18 chain. $R_2$ is usually a long or short alkyl chain or an aryl group. Also, $R_3$ and $R_4$ with short alkyl chains. X is a chloride ion, although it can also be a bromide ion.

The efficiency of QACs (Fig. 4.3) decreases in the presence of hard water and they are generally formulated in combination with chelating agents such as EDTA (ethylenediaminetetraacetic acid) or other chemical compounds such as sodium citrate or tripolyphosphate (Gerba 2015). Also, its effectiveness will depend on the environmental conditions, since it works better in alkaline conditions than in acids (Mosratö et al. 2003). QACs are sometimes formulated in combination with other active ingredients, such as chlorhexidine or polymeric biguanides, with the aim of

**Fig. 4.2** General formula of Quaternary Ammonium Compounds (QACs)

tetramethylammonium bromide

benzalkonium chloride

N-decyl-N,N-dimethyldecan-1-aminium chloride

**Fig. 4.3** Common examples of QACs

increasing their efficacy against gram-negative species or, in turn, combining with glutaraldehyde to annihilate a whole range of microorganisms more quickly that glutaraldehyde does alone (Ioannou et al. 2007).

The mode of action of QACs is due to the inactivation of energy-producing enzymes, the denaturation of essential cell proteins, and a disruption of the cell membrane. There is reported literature that supports these and other possibilities (Gerba 2015; Jennings et al. 2016). Thus, it has been proven in several studies that quaternaries as disinfectors act as fungicides, bactericides, and virucides against lipophilic viruses, in addition to low mycobactericidal activity. QACs remove and inactivate contaminants from computer keyboards in just five seconds after application, and without showing damage to the device after repeated applications (Jennings et al. 2016).

The disinfectants based on quaternary ammonium compounds are the most used for this purpose. For this reason, waste is distributed in some environments exposing its potential danger to the ecosystem and human health. The toxicity of these compounds has received significant attention from the scientific community, and studies have focused on the toxicity of QACs in wastewater treatment systems such as receiving waters (Tezel and Pavlostathis 2015).

QACs are toxic to both animal and plant marine life. Its active surface properties allow the solubility of some dangerous micro-pollutants, such as pesticides, so that toxins can easily penetrate living organisms (Zabielska-Matejuk and Czaczyk 2006). Another significant problem is that excessive use of QACs in both household and industrial products can lead to antibiotic resistance. A recent study established that when a microbial community fed with a carbon source was continuously exposed to QACs, its diversity decreased significantly and was dominated by resistant microorganisms such as *Pseudomonas*. This resistance is attributed to the degradation of QACs by *Pseudomonas* (Tandukar et al. 2013). However, another study showed that long-term exposure of household drainage microorganisms to quaternary ammonium-based household disinfectants does not significantly alter antimicrobial susceptibility (McBain et al. 2004). For these reasons, the biodegradation of chemicals found in environments is a very important process because it prevents the progressive accumulation of toxins in organic compounds.

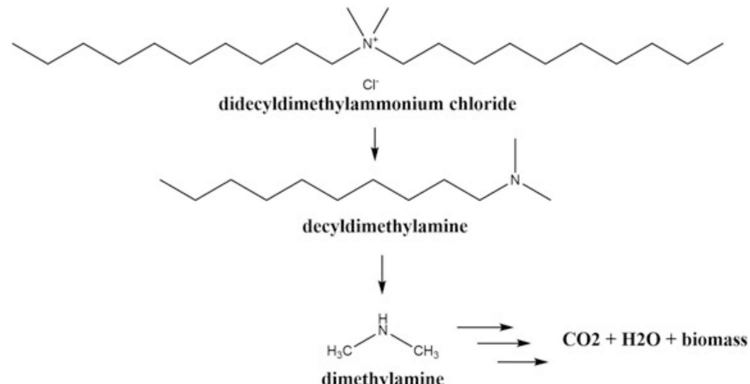


**Fig. 4.4** Degradation pathway of DDAC by *Pseudomonas fluorescens* TN4

QACs are compounds that generally degrade under aerobic conditions. The aerobic degradation of the QACs is attributed to bacterial species of the genus of *Xanthomonas, Aeromonas*, and *Pseudomonas*, among which are: *Pseudomonas putida*, *Pseudomonas nitroreducens*, *Pseudomonas* sp., and *Stenotrophomonas* sp. (Tezel and Pavlostathis 2015). The degradation process can be affected by many factors such as the chemical structure of the active ingredient, its concentration, complexation with anionic surfactants, and adaptation to the microbial community (Zhang et al. 2015). As the length of the alkyl chain grows or a methyl group substituted with a benzyl group occurs, the rate of degradation will be adversely affected (Zhang et al. 2015).

Several studies have reported the microbial degradation of compounds derived from QACs. For example, degradation of didecyldimethylammonium chloride (DDAC) has been reported to be executed through the use of the bacterium *Pseudomonas fluorescens* TN4, which uses the active ingredient as a carbon source. Bacteria was isolated from activated sludge (Nishihara et al. 2000). TN4 degrades the disinfectant through an N-dealkylation process, forming decyldimethylamine and then dimethylamine, as intermediaries (Fig. 4.4). Dimethylamine is easily oxidizable and converts to $CO_2$ and biomass. The effectiveness of the bacterium to degrade other derivatives of QACs was tested, verifying that it also works with monoalkyl- and benzalkonium-QACs, but does not show assimilation in pyridinium-QAC. The degradation process was controlled by means of ion chromatography and NMR (Nishihara et al. 2000).

Degradation of benzyldimethylalkylammonium chloride (BAC) by means of *Aeromonas hydrophila* sp. K has been reported (Patrauchan and Oriel 2003). This organism is capable of using BAC as a source of carbon and energy. In this sense, the bacterial cells were induced in advance to achieve degradation; if this step is not followed, a decrease in the degradation rate is seen. For this study, high-performance liquid chromatography (HPLC) and gas chromatography coupled to a mass spectrometer (GC-MS) were used to analyze the degradation pathway (Fig. 4.5). In the

Fig. 4.5 Degradation pathway of BAC by *Aeromonas hydrophila* sp. K

process of surfactant degradation, the progressive formation of benzyldimethylamine, benzylmethylamine, benzylamine, benzaldehyde, and benzoic acid is observed. The first metabolite formed in the degradative process suggests a cleavage of the C–N bond. In the last stage, benzaldehyde is rapidly converted to benzoic acid, which is subsequently degraded. The authors believe that the toxicity of BAC and its intermediates to the microorganism is responsible for the premature termination of its degradation (Patrauchan and Oriel 2003).

Disinfectants based on benzalkonium chloride are a series of products that are found in the environment either in a mixture with other derivatives of QACs or alone. One study evaluated the microbial degradation of benzyl dimethyl dodecyl ammonium chloride (BDDA) in isolation and the presence of benzyl dimethyl tetradecyl ammonium chloride (BDTA) (Khan et al. 2015). The study was carried out using *Pseudomonas* sp., which were isolated from activated sludge. The process was followed through the use of $^{14}$CNMR and HPLC-MS. BDTA was found to inhibit BDDA degradation as it was more toxic to the growth of the microorganism. When BDDA degradation was performed in isolation, the benzyl ring was fully mineralized. The transformation products obtained were benzyl methylamine and dodecyl dimethylamine (Fig. 4.6). The authors suggest further research in the field of QAC mixtures in wastewater (Khan et al. 2015).

### 4.5.2 Phenols

Substituted phenols in the benzene nucleus are antiseptics that work by denaturing proteins when found at low concentrations (Donnell 1999). The potency of phenol increases as the number of substituents and the length of its molecules increases.

**Fig. 4.6** Degradation pathway of benzalkonium chloride by *Pseudomonas* sp.

**Fig. 4.7** Common phenolic disinfectants

Likewise, the introduction of nitro and halogen groups also increases antiseptic potency. Phenol was one of the first antiseptics described, but it is currently little used due to its high toxicity and irritant power, therefore its use as a disinfectant with limited use is limited (DeBono and Laitung 1997). However, its derivatives such as alkylphenols, chlorinated phenols, nitrophenols, polyphenols, and so on, are used more frequently (Fig. 4.7).

Phenols are recognized for having a broad spectrum of activity against microorganisms such as bacteria, viruses, fungi, and mycobacteria (Goddard and McCue 2001). One of their disadvantages is their low surface activity, which is why they are generally coupled with soapy solutions to increase their penetrating power.

The research reported on the antimicrobial efficiency of phenol and its derivatives show that these compounds are bactericidal, fungicidal, virucidal, and tuberculocidal. At high concentrations, phenol has been shown to act as a powerful protoplasmic poison as it penetrates and breaks the cell wall and inactivates the cell's proteins (Maris 1995). On the other hand, at low concentrations and higher

molecular weight of the phenol derivatives, it causes bacterial death by inactivating the essential systems and by releasing the metabolites found in the cell wall (Mishra and Kumar 2017).

In recent years, the increasing interest in the microbiological degradation of phenol and its derivatives is due to the presence of these compounds in the environment, both as natural and artificial monoaromatic products (Mishra and Kumar 2017). Its presence in wastewater is due to various industrial processes such as the production of explosives, paint, recycling, textiles, pharmaceuticals, disinfection, and the production of cleaning equipment. Since the phenol molecule is heavier than that of water, its dilution becomes a slower and more toxic process. It is for this reason that their negative impact on the environment is evident to the point that these compounds have been recognized as priority pollutants by various organizations, such as the EPA, and there are documents that regulate health standards for their treatment. These compounds are considered to be fatal if swallowed, inhaled, and absorbed through the skin. In addition, human carcinogenic compounds are considered at concentrations ranging from 5 to 25 mg/L. This range is usually lethal for marine life. In the case of plants, at a concentration of 0.1 μg/mL or higher, it can cause the inhibition of photosynthesis (Oluwasanu 2018; Goddard and McCue 2001; DeBono and Laitung 1997). For these reasons, the removal of these monoaromatic compounds from wastewater and terrestrial environments such as maritime is of vital importance, and have been experienced biological, physical, and chemical methods to achieve this goal.

As demonstrated, microorganisms have great potential to transform toxic compounds and use them as an energy and carbon source. However, the process of degradation of phenolic compounds turns out to be a real challenge. This happens due to the number and position of the substituent groups on the aromatic ring. When the compounds present as substitutes nitro, amino, methoxy, and sulfone groups, a negative effect on microbial degradation is observed, however in the presence of carboxyl and hydroxyl groups, an acceleration of the degradative process is observed (Krastanov et al. 2013). Owing to these peculiarities in the structure of these aromatic compounds, the application of prokaryotic microorganisms in degradative processes has been investigated, the most recognized being the bacterial species of the *Pseudomonas* genus (Krastanov et al. 2013).

El-Naas et al. verified that the degradation capacity of a bacteria-denominated *Pseudomona putida* is influenced by environmental conditions such as temperature, pH, initial concentration of the aromatic compound, and the abundance of biomass. High concentrations of phenol inhibit biomass and reduce the rate of degradation. In this study, the researchers immobilized the bacteria on PVA gel pellets. The ideal conditions for degradation to occur were 30 °C, neutral pH, and a phenol concentration of 0.75 mg/L, achieving degradation of the contaminant without being affected by the presence of substitutes in the aromatic ring (El-Naas et al. 2009). Mahiudddin et al. investigated the ability of *Pseudomonas fluorescens* PU1 bacteria to degrade phenols in batch culture. They verified that the microorganism degrades phenols in concentrations above 1000 ppm using a meta cleavage mechanism. The bacteria

Fig. 4.8 Degradation pathway of phenol by *Pseudomonas fluorescens*

Fig. 4.9 Proposed degradation pathway of *p*-nitrophenol by *Pseudomonas aeruginosa*

break down phenol to catechol, and it breaks down in consecutive steps until acetaldehyde and pyruvate are obtained (Fig. 4.8) (Mahiudddin et al. 2012).

Aromatic compounds that have nitro groups as substituents have been shown to be the most toxic and the most difficult to degrade. Its effect has been widely realized with the production of insecticides, herbicides, and explosives (Krastanov et al. 2013). The microbial degradation of p-nitrophenol has been described using various genera of microorganisms. Samuel et al, for example, showed the ability of *Pseudomonas putida* to degrade this compound, with hydroquinone being the greatest degradation product. The mechanism is based on an initial removal of the nitro group and the formation of hydroquinone as an intermediate (Samuel et al. 2014). Zheng et al. also established the effectiveness of *Pseudomonas aeruginosa* to degrade *p*-nitrophenol. Under certain environmental conditions such as 35 °C temperature and a neutral pH, the microorganism grows adequately and plays the role of degrading the contaminant. By HPLC and UV–visible analysis, it was found that the key intermediate was targeting hydroquinone (Fig. 4.9). Furthermore, it is estimated, with this study, that other aromatic compounds such as phenols, naphthalene, aniline, and nitrobenzene can be degraded with this microorganism (Zheng et al. 2008).

Other major pollutants are chlorophenolic compounds. They are widely used in the production of herbicides, fungicides, and insecticides due to their strong biocidal character. Its biodegradation has been an object of interest in this area. The

**Fig. 4.10** Major metabolites produced generated by degradation of 4-chlorophenol using *Candida tropicalis*

**Fig. 4.11** β-ketoadipate pathway for degradation of phenol by *Acinetobacter radioresistens*

microorganisms that have shown effectiveness have been those of the Candida genus. Wen et al. showed that *Candida albicans* is capable of degrading chlorophenols with concentrations above 300 mg/L under anaerobic conditions, with a temperature of 35 °C and an initial pH of 7.0 (Wen et al. 2006). In the same sense, Basak et al. demonstrated that the *Candida tropicalis* bacterium grows in 4-chlorophenol and metabolizes its substrate, degrading the contaminant with the production of three metabolites: 4-chlorocatechol, 4-chloropyrogallol, and 4-carboxymethelenebut-2-en-4-olide identified by HPLC analysis (Fig. 4.10) (Basak et al. 2013).

Bacteria of the *Acinetobacter genus* have shown the ability to grow in aromatic compounds, such as phenol, and catabolize them for energy. For example, Mazzoli et al. showed that *Acinetobacter radioresistens* degrades phenol or benzoate via the β-ketoadipate pathway. By means of genetic analysis, they concluded that the genes responsible for the degradation of the compounds are located in the *A. radioresistens* S13 chromosome (Fig. 4.11) (Mazzoli et al. 2007). In the same genus, *Acinetobacter Calcoaceticus* removed phenolic contaminants from wastewater under aerobic conditions. According to Liu et al, the bacteria efficiently removed 91.6% of the initial 800 mg/L phenol in just 48 h and has a concentration tolerance of as high as 1700 mg/L (Liu et al. 2016).

*Lysinibacillus cresolivorans* bacteria have the ability to degrade *m*-cresol. This result was obtained by Yao et al. who analyzed the degradation rate of the compound at different concentrations under zero-order kinetics (Yao et al. 2011). Optimal degradation conditions were at a pH between 6.8 and 7.3 and a temperature of

**Fig. 4.12** Degradation pathway of *o*-phenylphenol by *Sphingomonas haloaromaticamans*

35 °C, which proves the potential of this bacterium to degrade phenolic compounds (Yao et al. 2011). Pazarlioglu et al. carried out a kinetic study of the degradation of *o*-cresol using *Pseudomonas putida* DSM 548, obtaining the following parameters: maximum specific growth rate ($\mu_{max}$ = 0.519 $h^{-1}$), Monod constant ($K_s$ = 223.84 mg/L), and inhibition constant ($K_i$ = 130.883 mg/L). The batch bioreactor system with the immobilized bacteria allowed the degradation of the contaminant using these kinetic aspects (Pazarlioglu et al. 2012).

A recent study on phenol and *p*-cresol degradation identified microorganisms with the potential to use these pollutants as sources of carbon and energy. Franchi et al. developed successive batch anaerobic digestion of the contaminants using granular or suspended sludge. The results of their study showed that granular sludge has a higher degradation rate for both phenolic compounds (11.3 ± 0.7 vs. 8.1 ± 1.1 mg $L^{-1}$ $day^{-1}$ for phenol and 7.8 ± 0.4 vs. 3.7 ± 1.0 mg $L^{-1}$ $day^{-1}$ for *p*-cresol). Anaerobic processes generated an increase in the population of a bacterium of the *Syntrophorhabdus* genus and the hydrogenotrophic *Aceticlastic archaea* (Franchi et al. 2018).

Through proteomic and genomic analyzes, the activity exerted by the proteins and genes of the microorganism *Sphingomonas haloaromaticamans* in the degradative process of *o*-phenylphenol was identified. Through genomic analyzes, Perruchon et al. found two operons that have an important function in the cleavage of the aromatic ring. In addition, they controlled the formation of key products through chromatographic analysis and proposed a metabolic route for the degradation of the contaminant (Fig. 4.12) (Perruchon et al. 2017).

Triclosan has been widely used as an antimicrobial agent found in wastewater due to its application in the pharmaceutical industry and personal care products. Gangadharan et al. demonstrated that the microbial degradation of triclosan has higher performance under anoxic/anaerobic conditions in a methanogenic system, presenting phenol, catechol, and 2,4-dichlorophenol as metabolites (Gangadharan

**Fig. 4.13** Proposed degradation pathways of triclosan by *Dyella* sp. WW1.

Puthiya Veetil et al. 2012). Its degradation was studied in wastewater using a microorganism identified as *Dyella* sp. WW1, obtaining optimal conditions for the process at 15 °C and pH of 7. In the degradative process, six degradation products were identified using mass spectrometry (Fig. 4.13). Furthermore, it was found that it could degrade another contaminant such as 3,5-dichloro-4-hydrobenzoic acid through co-metabolism in the presence of triclosan, but it could not degrade drugs such as carbamazepine and diclofenac (Wang et al. 2018).

### 4.5.3 Aldehydes

The activity of aldehydes, basically formaldehyde and glutaraldehyde, is linked to the denaturation of proteins and nucleic acids by chemical reduction. aldehydes very well destroy bacteria, microscopic fungi and also have excellent virucidal action. They are used to disinfect appliances, surfaces, and instruments (Cowan et al. 1993).

Formaldehyde has been widely used in liquid preparations for the disinfection of portable toilets. Formaldehyde is a disinfectant with great penetrating power and an irritant action on the skin and mucosa. It is toxic, both in gaseous form (it produces irritation of the ocular and respiratory mucosa) and by ingestion, producing digestive and nervous system disorders. For this reason, it is not usually used locally and is used for the disinfection of instruments and inert surfaces, at this concentration it is used in 40% aqueous solutions, adding methanol to prevent its passage to paraformaldehyde (Jeffrey 1995). Formaldehyde acts on microorganisms through

**Fig. 4.14** Degradation products of formaldehyde by *Methylobacterium* sp.



alkylation of the amino and sulfhydryl groups of proteins and on the atoms of nitrogen found in the ring of purine bases (Karsa 2007).

Formaldehyde is a very common chemical pollutant found in different environments such as water, air, and soil (Kaszycki and Koloczek 2002). Several applications given to formaldehyde lead to the progressive release of the compound in toxic concentrations. This makes their degradation a relevant subject of study. This is why the potential of microorganisms to degrade these toxic compounds has been exploited. Thus, Yamazaki et al. demonstrated the effectiveness of a bacterium of the Bacillus genus called DM-2, which is resistant to formaldehyde, is capable of degrading the pollutant at concentrations above 400 ppm in a medium containing 3% chloride sodium. This study achieved a degradation rate of up to 45 ppm/h when the optical density of the culture at a wavelength of 660 nm was 1.2 (Yamazaki et al. 2001). On the other hand, Kaszycki et al. verified that yeast *Hansenula polymorpha* cooperates with activated sludge in the degradative processes of formaldehyde as well as its derivatives. The degradation was carried out in an acidic medium and a temperature above 20 °C (Kaszycki and Koloczek 2002). Also, Changhong et al isolated a formaldehyde-resistant bacterium with the potential to degrade it. The bacterium was characterized according to Bergey's manual, showing a genetic sequence similar to *Pseudomonas* sp. This bacterium was able to completely degrade 20 mM of the contaminant in 12 h, giving it the potential to degrade higher concentrations under other conditions (Changhong et al. 2012). The degradation of large concentrations of formaldehyde (~2.7 M) was achieved through the use of lyophilized *Methylobacterium sp* cells, which generate formic acid and methanol as degradation products (Fig. 4.14). The study suggests that degradation occurs optimally at a temperature of 40 °C and a pH between 5 and 7, and is caused by the enzyme formaldehyde dismutase. Recently, Thirumarimurugan et al. tested the effectiveness of *Bacillus subtilis* bacteria to degrade formaldehyde under specific conditions of pH, concentration of contaminant, and inoculum size. The degradation was checked by means of Fourier transform infrared spectroscopy (Thirumarimurugan et al. 2017).

Glutaraldehyde is a compound used as biocide with at least three times the activity compared to formaldehyde, but whose stability declines in strong solutions. It is commonly used for the sterilization of sensitive medical instruments. The biocidal effect of glutaraldehyde comes from the alkylation of the sulfhydryl, hydroxyl, carboxyl, and amino groups, allowing the alteration of DNA, RNA, and protein synthesis processes (Donnell 1999). It is a compound that, by direct contact or inhalation, can cause sensitization and irritation of the skin and mucosa. Several cases of glutaraldehyde colitis have been reported in the world literature after colonoscopies, probably caused by glutaraldehyde residues in endoscopes.

**Fig. 4.15** Degradation pathways of glutaraldehyde under aerobic and anaerobic conditions

Glutaraldehyde is considered a toxic chemical compound mainly for aquatic organisms, being more lethal for freshwater fish. Environmental studies suggest that glutaraldehyde is maintained in aquatic environments and tends to bioaccumulate. However, it has also been verified that under aerobic conditions it is degraded to $CO_2$ via glutaric acid as an intermediate. On the other hand, under anaerobic conditions, it is metabolized to 1,5-pentanediol (Fig. 4.15) (Leung 2001).

### 4.5.4 Biguanides and Polymeric Biguanides

Biguanides are active ingredients with a broad-spectrum disinfectant formulation with high bactericidal, fungicidal, and sporicidal efficacy. The efficiency of these compounds depends to a great extent on the pH of the medium, being a pH: 5–7 the optimal for biguanides such as alexidine and chlorhexidine and a pH: 5–10 for polymeric biguanides (Maris 1995).

Chlorhexidine is a strong dicationic base at pH above 3.5 with two positive charges at each end of the hexamethylene bridge (Jenkins et al. 1988). This feature makes it extremely interactive with anions, which makes it relevant for its efficacy, safety, local side effects, and difficulty in formulating it in products. Although it is a base, chlorhexidine remains more stable as a salt and the most common preparation is digluconate salt due to its high solubility in water. In vitro, it has effectiveness against gram-positive and gram-negative bacteria, including aerobes and anaerobes and even fungi and yeasts (Cheung et al. 2012). Compounds that incorporate CPC (cetylpyridinium chloride) to chlorhexidine obtain better results. Chlorhexidine strongly binds to the bacterial cell membrane, which at low concentrations produces an increase in permeability with filtration of intracellular components including potassium (bacteriostatic effect), at higher concentrations it causes precipitation of the bacterial cytoplasm and cell death (bactericidal effect) (Sykes and Weese 2014). On the other hand, alexidine chemically differs from chlorhexidine in the two hydrophobic ethylhexyl groups it presents. This variation gives it faster bactericidal activity and bacterial permeabilization.

Despite very little information on the environmental effects of chlorhexidine, several European regulatory organizations have classified it as a dangerous compound for the environment due to its high toxicity to aquatic organisms. It has been

Fig. 4.16 Direct degradation pathway of chlorhexidine by *Pseudomonas* sp.

detected that it is capable of bioaccumulating in acoustic environments. This is because it is a positively charged hydrophobic molecule which suggests that it can easily adhere to other lipids and fatty tissues of organisms (Castillo et al. 2004). Likewise, the U.S. Environmental Protection Agency has categorized a highly toxic compound for marine invertebrates at potencies higher than 32 μg/L (Lawrence et al. 2008). For these reasons, its degradation has been studied by the scientific community. The first studies that were used to identify microorganisms resistant to the contaminant were reported by Uyeda et al., who demonstrated that *Pseudomonas aeruginosa*, *Alcaligenes fecalis*, *Alcaligenes xylosoxidans*, and *Serratia marcescens* are resistant to high concentrations of chlorhexidine. From these organisms, *S. marcescens* are potential organisms to degrade chlorhexidine (Uyeda et al. 1996). However, new studies have been carried out over the years that would check the efficiency of other microorganisms. This is the case of the research carried out by Tanaka et al, who considered analyzing the biodegradation of chlorhexidine using *Pseudomonas sp*. Using this microbe, two aromatic degradation products were obtained, named (CHADP)-4 and CHADP-6, which are cleavage partners of *p*-chlorophenylurea and *p*-chloroaniline, respectively (Fig. 4.16) (Tanaka et al. 2006).

Polymeric biguanides are active principles that have a wide spectrum of activity and are very effective against *Pseudomonas* sp., therefore, their use is recommended, especially in water packaging industries (Ikeda et al. 1984). PHMB's mechanism of action has been described in numerous articles. The disinfectant has been shown to interact with the surface of the bacteria and is transferred to the cytoplasmic membrane and cytoplasm, where it reacts with phospholipids, causing increased permeability, with the release of lipopolysaccharides, potassium ions, and causing cell death (Ittoop et al. 2015).

PHMB is a chemical compound that has application as a germicide for the disinfection of various devices, the most important being dental utensils and the disinfection of swimming pools (Lucas 2012). Despite being classified as a compound that has a very low risk of causing adverse effects on human health, the presence of the pollutant has been detected on aquatic surfaces and has been found to

**Fig. 4.17** Degradation products of 1,6-diguanidinohexane by *Pseudomonas putida*

have a toxic effect on certain species of marine life (United States Coast Guard Research and Development Center 2004). Its degradation is an important step to avoid accumulation in aquatic environments. Although there is no extensive study in the field of microbial degradation of PHMB, due to, mainly, its structural complexity and heterogeneity of chain length, approaches have been made with model molecules to estimate what a microbial degradation of the polymer would be like. Thus, O'Malley et al. used 1,6-diaminohexane, 1,6-diguanidinohexane, 1,6-di (cyanoguanidine) hexane, and 4-guanidinobutyric acid to assess end-group degradation using microorganisms with the potential to degrade the polymer. The results showed that no bacteria have an affinity for the end-group of cyanoguanidine but one bacterium, *Pseudomonas putida*, was able to use the amine and guanidine end-groups as a nitrogen source (Fig. 4.17). Thus, O'Malley concludes that these end-groups would be the most likely to degrade in the PHMB (O'Malley et al. 2006). Also, there are microorganisms capable of using it as a nitrogen source and they have been isolated. Thus, O'Malley showed that two consortia containing bacteria of the genera *Sphingomonas*, *Azospirillum,* and *Mesorhizobium* were capable of growing based on PHMB. With an isotopic analysis, the percentage degradation of the polymer was verified (O'Malley et al. 2007).

### 4.5.5 Amphoteric Compounds

Amphoteric compounds have both positive and negative charges on their structure, so other cationic or anionic active ingredients can be formulated (Fig. 4.18). In particular, the compounds that have amines in their structure have high efficacy as disinfectants. These compounds have less activity than most quaternary ammonium derived compounds (QACs), however, their work is less affected by the presence of gram-positive organisms (Moore and Payne 2008). They are also easier to remove than quaternaries. Its use has become very frequent in daily life and the pharmaceutical industries. Its activity as a detergent by itself is limited to lipophilic viruses, so it is not effective against solids. To focus their activity in this area, amphoteric compounds are combined with products with a greater spectrum of activity and detergency such as glutaraldehyde and formaldehyde (Donnell 1999).

Although the market, use, and production of amphoteric agents bring advantages to the economy, industrial and sanitary production of the population, there is a primordial transversal theme that is becoming increasingly relevant: the environmental impact related to its use (Brand 2019). Amphoteric agents have mainly become a water pollutant due to their use for laundry and domestic cleaning, that is, when they are used, they will be deposited to treatment plants that are carried by the sewage or in many cases are discharged to soils and surface waters. In the wastewater treatment process, the elimination of large amounts of compounds is carried out through the biodegradation and absorption of particles, however, the metabolites produced in the said process are released in various environmental compartments. The main effects of surfactants on water resources include:

- Amphoteric compounds increase the pH of wastewater and affect the life cycle of aquatic organisms.
- Surfactants cause an increase in nutrients in the river channels that receive the wastewater, producing an increase in algae and bad odors due to the accumulation of phosphorous.
- Heavy metals such as mercury, lead, and chromium can dissolve in water causing alteration of the food chain or genetic damage in aquatic species.
- The oxygen demand to carry out the decomposition of organic compounds originated by detergents produces anoxic conditions causing the death of aquatic flora and fauna.

Hydrophobic Tail

Hydrophilic Tail

Cocamidopropyl betaine (CAPB)

**Fig. 4.18** Chemical structure of an amphoteric surfactant

- Amphoteric compounds have a direct impact on the sedimentation, flocculation, and coagulation processes on the plants that allow and facilitate purification (Brand 2019).

Several studies have been carried out to check the microbe degradation of amphoteric pollutants. In this sense, Merkova et al. showed that the degradation of Cocamidopropyl betaine in activated sludge, at a concentration of 300 mg/L, is produced by gram-negative bacteria of the genus *Pseudomonas* and *Rhizobium*, since they facilitate the complexation of surfactant (Merkova et al. 2017). In another study, Shaw et al. managed to degrade a surfactant called 6:2 fluorotelomer sulfonamidoalkyl betaine using *Gordonia sp*. This microorganism is capable of degrading 70.4% of 60 μM of the pollutant, producing 10 major degradation products (Shaw et al. 2019).

### 4.5.6 Chlorinated Compounds

Chlorine-based compounds are the most widely used active agents as disinfectants, both in industry and at home. It is applied in various scenarios such as drinking water, swimming pool water, and wastewater for disinfection since it is very effective for the elimination of pathogenic microorganisms and relatively cheap. The biocidal action is carried out by chlorine, which is a gas that cannot be used in the formulation of compounds, therefore a means to use it is through reaction with caustic products, which leads to the formation of hypochlorite of sodium, which is the base of many disinfectants (Franklin et al. 1991).

The mode of action of chlorinated compounds is based on an atomic exchange with other compounds such as the enzymes present in the microorganisms. Enzymes in contact with the chloride ion, certain hydrogen atoms in the molecule are replaced by the anion. This produces a structural change in the molecule since if the enzymes do not perform their function properly, the microorganism dies (Marhaba 2009).

Sodium hypochlorite is the basis of various disinfectants. Its disinfecting power comes from its oxidizing properties due to the presence of the $ClO^-$ anion, which attacks the cytoplasmic membrane. NaClO sodium hypochlorite is a salt of hypochlorous acid HClO. In solution, this salt dissociates into $Na^+$ and $ClO^-$ ions (European Union 2017).

The main advantage of chlorinated products is their low cost and that they have a wide range of action against microorganisms. They are effective at low temperatures and generally have no residual activity. Its main disadvantage is its instability, both against environmental conditions and in the presence of organic matter, drawbacks which are minimized in disinfectant formulations (Gallandat et al. 2020).

Due to the wide use that is given to chlorinated compounds (herbicides, fungicides, solvents, fluids, plasticizers, etc.), it is logical to think that they are dispersed in the environment. Indeed, these compounds are defined by the United States Environmental Protection Agency (U.S EPA) as priority pollutants (Oluwasanu 2018). This is due to the ability of chlorine to generate toxicity,

**Fig. 4.19** Initial steps for biodegradation of 1,4-dichlorobenzene by *Pseudomonas* sp. *and Xanthobacter flavus*

accumulate and persist in environments, which has generated a public health problem. It is for this reason that the application of bioremediation mechanisms that reduce these contaminants is necessary, and the best option has been the use of microorganisms that use these compounds as an energy source.

As has been mentioned extensively throughout the chapter, microorganisms are capable of transforming complex compounds into simpler organic molecules, such as $CO_2$. Several microorganisms that use chlorinated compounds for energy synthesis (ATP) have been identified. For example, Spain et al. and Spiess et al, in their research groups verified that *Pseudomonas sp.* and *Xanthobacter flavus*, respectively, are capable of degrading 1,4-dichlorobenzene, by means of two different metabolic pathways that are produced by enzymes induced by cell growth generated by the disinfectant (Fig. 4.19) (Spain and Nishino 1987; Spiess et al. 1995). These microorganisms were also used for the degradation of 1,2-dichlorobenzene by Haigler et al. (Haigler et al. 1988) and Sommer et al. (Sommer and Görisch 1997). In the same sense, under specific conditions, the bacterium *Rhodococcus corallinus* is capable of oxidizing trichloroethene (Saeki et al. 1999; Suttinun et al. 2010).

An interesting degradation is the one that occurs with tetrachloromethane. This molecule is inert due to the fact that it has no C–H bonds, it is nonpolar, and has a tetrahedral geometry, for which radical reactions are necessarily required for its transformation. This mechanism produces two highly reactive entities such as a chlorine atom and a halogenated carbon radical. Due to successive dehalogenations, it is difficult for a microorganism to control the toxicity and a large amount of energy generated by these species. However, studies have been carried out showing that the bacteria *Acetobacterium woodii* and *Moorella thermoacetica* generate various degradation products. This process is produced by a reductive acetyl-CoA pathway that allows the chlorinated pollutant to be assimilated into the biomass under certain conditions (Egli et al. 1988; Hashsham and Freedman 1999; Adamson and Parkin 2001). The degradative process by means of *Acetobacterium woodii* is based on four stages: (1) reductive sequential dehalogenation; (2) substitute transformation; (3) oxidative transformation and, finally, and (4) $CO_2$ assimilation by the acetyl-CoA pathway (Fig. 4.20).

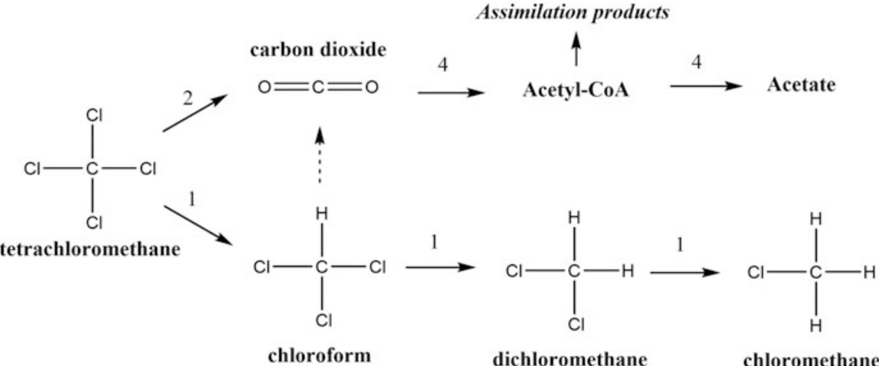


**Fig. 4.20** Degradation pathway of chloromethane by *Acetobacterium woodii*

Based on the experiments compiled in this section, it can be deduced that most of the experiments related to the microbial degradation of chlorinated compounds were made on a laboratory scale, so it is necessary to develop bioreactor systems on an industrial scale in order to treat systems of wastewater and environments contaminated by large concentrations of these compounds.

## 4.6 Perspectives and Conclusions

Cleaning is a fundamental lifestyle for every person in the world. However, its care would be affected by side effects unknown already by the people who practice them. Depending on the chemical compound employed the consequences on the effects will produce hazardous concerns. The negative effects not only will affect human health but the natural environment as well. The environmental contamination produced by the excessive use of disinfectants has become a focus of interest on the part of the scientific community. To solve this problem, the ability of microorganisms to generate enzymes capable of progressively degrading complex molecules into harmless substances and use them as an energy source has been taken into account, obtaining quite encouraging results. The fate of each contaminant embedded in the environment will depend on the microorganisms found in that part of the environment and the method used for the remediation of that place. The lack of species capable of fulfilling this task will cause bioaccumulation of pollutants with harmful potential for the environment. The microbial degradation of disinfectants, as detailed in this chapter, can be produced by co-metabolic transformations, by microbial induction, or by transformations that require energy for their development. Undoubtedly, the bacteria with the greatest genetic capacity to carry out this process are those that belong to the genus of Pseudomonas. Of these organisms, countless genes have been characterized and have allowed the modification of many others that fulfill the mission of biodegrading compounds of environmental concerns.

Future studies related to microbial degradation should be directed to basic and applied aspects of the process. Because bioremediation is an important factor in contaminant removal, it is necessary to understand aspects of microbial genetics, biochemistry, chemistry, and physiology. Likewise, efforts should be made to try to reduce the gap between laboratory-scale studies and industrial processes, since, as has been shown, most investigations are flask experiments. These experiments often do not reflect the results that would be obtained in large-scale processes. The reasons to which this problem is attributed lies in the physiological, environmental, structural conditions, concentrations of the pollutant, and other related aspects that, on a large scale, are usually under constant variation. In the same sense, the processing of dangerous pollutants presents the challenge of discovering new unknown degradation products, making it necessary to know these metabolites, metabolic routes, biochemical aspects, and others. Knowing the potential of microorganisms and the rapid advancement of science, it is possible to achieve great results in bioremediation in just a few years, which will give a break to our nature.

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# 5 Application of Microalgae Consortia/ Cocultures in Wastewater Treatment

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**Abstract**

The imminent unsustainability of water resources and ecosystem security is strictly related to anthropogenic action. Eutrophication of water bodies and toxicity given activity of recalcitrant compounds, such as the release of greenhouse gases, are central adversities in the unfolding of the global economy hereinafter. Wastewater standardly contains N, C, and P in high quantities, in addition to, various metals, pathogens, hydrocarbons, xenobiotics, among other contaminants, and needs treatment for later disposal in surface water bodies. Conventional wastewater treatment systems have inefficiency in removing specific pollutants and several limitations. Thus, the improvement of biotechnological operations alternatives to these processes becomes essential. Within the context, and the extensive need for the advances of eco-friendly energy sources, microalgae are considered potential bioremediators of urban and agro-industrial effluents, enabling the integration of biorefineries, use of gaseous effluents, and generation of added value biomass. The microalgae and bacteria consortia when applied to bioremediation, proved to be suitable for organic and inorganic detoxification and in removing nutrients from the effluent concerning to axenic application of microorganisms. Consonant to the promotion of carbon sequestration of the atmosphere, mitigating climate effects on a global scale, it enables the generation of bioenergy and biofuels, nutraceuticals, animal feed, fertilizers, pigments, and other bioactive products. This chapter aims to discuss the bioeconomy opportunities of the application of microalgae coculture, including

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their limitations and advantages, exploring solutions to microalgae-based sustainability.

**Keywords**

Microalgae consortium · Bioremediation · Wastewater treatment · Pollutant removal · Biofuels · Bioactive products

## 5.1 Introduction

Living under the paradigm of modern anthropocentrism, human action offers severe threats to the sustainability of water resources and the integrity of the ecosystem. The large population growth results in the relentless discharge of waste in superficial water bodies and brings severe risks of eutrophication. Thereby, they threaten water purity and the diverse forms of life that inhabit it. These reflexes, when summed to the greenhouse gases released beyond the natural limit, represent the central obstacles for the hereinafter economy.

Wastewater usually detains substantial concentrations of N, C, and P, (nitrogen, carbon, and phosphorus), as well as several toxic metals, pathogens, hydrocarbons, xenobiotics, among other contaminants and need adequate treatment (Olguín and Sánchez-Galván 2012). The repletion of surface waters with nutrients, mainly N and P, favors the phenomenon of eutrophication and precludes to use it as a clean water supply. Other effects associated with eutrophication include a decrease in vulnerability, toxicity by ammonia ($NH_3$), a bad odor under activity sulfuric acid ($H_2S$), and desertion of animals that use this water source to quench their thirst. The implications of the imbalance on the ecosystem are the rapid detriment of habitat. The deterioration of aquatic environments is considered a barrier to public health, both for the peripheral population and for those who have this water for consumption. For the reintegration of water quality, treatment based on activated sludge is widespread. The sludge generated in the stages of organic carbon bioconversion and withdraw of P and N carries chemical residues prone to additional pollution and lack of treatment. Also, it accumulates toxic agents and various pathogens, having no commercial value, and bringing hazards to society safety and health (Herschy 2012). Moreover, the necessary aeration facilitates the evolution of volatile substances in the environment (Jia and Yuan 2016). These systems have inefficiency in removing specific pollutants, occupy large physical areas, generate secondary pollution, and have a higher cost. Furthermore, not allowing the reutilization of useful elements encountered in the effluent (Qian et al. 2007; Abdel-Raouf et al. 2012; Renuka et al. 2013; Lu et al. 2017).

In this scenario, microalgae stand out for the diversity of species and the multiple possibilities for biotechnological use of their biomass (Deprá et al. 2018). The microalgae consortium presents itself as an broaching option for the treatment and handling of wastewater. The synergic interaction of these microorganisms in the elapse of their growth assimilate inorganic and organic compounds and have the

competence to recycle them from the effluent with the restoration of water quality (Hernández et al. 2016). Likewise, they can remove toxic metal ions, pathogens, hydrocarbons, xenobiotics, among other contaminants (Ma et al. 2014). The perspective of integrating biorefineries for the generation of biofuels with an emphasis on the microalgae–bacteria binary system in the resumption of effluents, promises to solve the barriers necessary to advances on an upscaling (Zhan et al. 2013; Salama et al. 2017).

The purpose of this chapter is to assist in the comprehension of the application of systems based on microalgae, with an assessment of their life cycle and the sustainability of processes. The microalgae consortium is highlighted and promises to overcome current limitations and still produce biomass with high added value subsidies for the industrial sector.

## 5.2 Microalgae and Wastewater: A Biotechnological Initiative

### 5.2.1 Microalgae

Having diverse characteristics, microalgae make up a tremendous biodiversity group. They are unicellular, eukaryotic, or prokaryotic beings, relevantly represented, under the biotechnological optics, by cyanobacteria (*Cyanophyta*), green algae (*Chlorophyta*), diatoms (*Ochrophyta*), and red algae (*Rhodophyta*) (Jacob-Lopes et al. 2018). Cyanobacteria often reported as blue algae and microalgae, are organisms with wide structural and metabolic versatility, being the only class of $O_2$-producing bacteria. As they perform photosynthesis with oxygen release, microalgae have a direct connection in the cycling of C and $O_2$ (oxygen) in the atmosphere. Acting in the foremost trophic level, they are the foundation of the food web in ecosystems. In contrast to terrestrial plants, they can double cell density around 24 h because of the prominent $CO_2$ biofixation and utilization of solar radiation (Lam et al. 2012), besides, not competing with useful soil for food production. They develop from the photoautotrophic pathway using inorganic carbon and heterotrophic metabolism, with the use of organic carbon. Still, they can be mixotrophic, using optionally either carbon sources for nutrition (Mata et al. 2010). Marine, freshwater, and extremophilic microalgae have remarkable characteristics that must be studied and respected regarding their application. Predominantly found in aquatic environments, they can also develop in places of extreme conditions, for instance in glacial regions, in the desert, places with oil, and polluted sites among others. Apt to produce a large gamma of biomolecules belonging to its metabolism, after cultivation, it is still possible to derive microalgae biomass in value-added bioproducts, such as cosmetics, nutraceuticals, biofuels, fertilizers, biopolymers, animal feed, pigments, and among other bioactive compounds (Zhang et al. 2020).

### 5.2.2 Microalgae-Based Sustainability

The choice of potential microalgae species leads to a typical profile, about biomass produced, the operational parameters, cultivation arrangements, and the scale increase (Chang et al. 2017). The local geography and the dexterity to adapt to the desired cultivation system must also be rated. Currently, the application axis of microalgal biotechnology is targeted at the reuse of effluents, bioremediation, and generation of bioproducts (Rashid et al. 2018).

The microalgae purpose can be a pollution control strategy matched with the treatment and reintegration of agro-industrial and urban wastewater, which carries out ecological work. The synergistic approach of the microalgae/cyanobacteria and bacteria consortium allows the bioremoval of N, P, and organic matter from the effluent by multiple metabolic routes, making it more attractive, in some cases than axenic cultivation. Conversely, if the ambition is to obtain biomass of known composition and high purity, axenic cultivation in the effluent is not indicated for being a complex matrix (Chang et al. 2017).

As municipal and agro-industrial effluents are rich in micro and macronutrients, mainly P and N, it is advantageous to apply microalgae cultivation (Benemann et al. 2018). Thus, by simultaneously subtracting nutrients, performing the conversion to specific biomolecules and biomass generation, the treatment plant operating costs decrease (Choix et al. 2018). Studies show that the level of phosphorus bio removal by microalgae can hit total removal and nitrogen by 97% (Queiroz et al. 2013; Koreiviene et al. 2014).

The C:N (carbon/nitrogen) and N:P (nitrogen/phosphorus) ratio is another central factor because depending on each species of microalgae/bacteria, there is an ideal ratio for their biomagnification. Inhibition due to excess nutrients or scarcity of them can be overtaken by mixing different effluents. The characterization regarding the bioavailability of nutrients and potentials growth cycle inhibitors, like hydrocarbons, insecticides, pesticides, drugs, antibiotics, toxic metals, among other substances in wastewater is primordial (Posadas et al. 2017; Butler et al. 2017). Also, the high load of microorganisms in the contaminated water significantly influences the preservation and augmentation of the colony. To solve this drawback, sterilization and filtration techniques are commonly used, but they tend to make the process more expensive (Dixon and Wilken 2018). Thus, all these variables impact the synthesis of proteins, lipids, and cell accumulation (Seyhaneyildiz et al. 2017).

The bioaugmentation of microalgae cells supplemented by the integration of $CO_2$ produced by the industrial sector is an imposing promising alternative (Wang et al. 2016). The key component of flues gas, the $CO_2$, is a form of inorganic carbon disperse in the atmosphere about 1% (Cheng et al. 2006) that can be assimilated by microalgae. Participating in the Calvin cycle, it is responsible for breathing and generating energy in microalgae, its supply complies an indispensable function in the development of the colony. Sobczuk et al. (2000) project to create 1 kg of biomass wherein almost 2 kg of carbon dioxide is absorbed. Already, the injection of this gas in the process accelerates the development of photoautotrophic/mixotrophic cultures, curbing the current launch of greenhouse gases (Russel et al. 2018).

Investigations by Jacob-Lopes and Franco (2013) indicate that adequate content for use, evaluating limits of inhibition, and the existence of toxic agents in the gaseous industrial mixture, this range varies from 5% to 25%. About microalgae biotechnology in exercise, it prevents $CO_2$ from being emitted by bacterial intercommunity in traditional treatment and still results in greater capture of this gas in the atmosphere face to land plants.

The articulation of microalgae-based systems in the reclaim of effluents with $CO_2$ supplementation can subtract the present nutrients, suppressing the costs of biomass generation with its subsequent enrichment (Ramanan et al. 2010; Ebrahimian et al. 2014; Jacob-Lopes et al. 2015; Naresh and Prabhakar 2018; Jain et al. 2019).

### 5.2.3 Operating Cultivation Systems

In open cultivation systems, high rate algal ponds, native water bodies, and raceway ponds are frequently used. It is observed in these models, the intense influence of external agents, intangible temperature control, water loss, and unavoidable invasions of contaminants, such as protozoa, bacteria, microalgae, and fungi that can compete for nutrients with selected species, inhibit their growth and even prey on them. It is also compelling to have a lower rate of $CO_2$ utilization and broad land use for its construction (Muñoz and Guieysse 2006; Chisti 2007). Seasonal intervention, especially in winter, affects the index of P and N bio removal, tending to be slower in open bioreactors (Voltolina et al. 2005). Also, geographically speaking, solar radiation, periods of rain, the incidence of winds, and local humidity must be contemplated (Bux and Yusuf 2016; Pruvost et al. 2016). Despite eventual disadvantages, the small cost of implementation, and the poor requirement for specialized labor for operation, it a widely diffused technique.

The most notorious factor in closed systems is the talent to control the expansion and reproducibility of microalgae, enabling greater use of $CO_2$, reduced risk of contamination, and mastery of global variables (Saharan et al. 2013). The homogenization of the liquid content is indispensable for this system, and is aimed to avoid gradients of cultivation, sedimentation of biomass. To allow the diffusion and exchange of suitable gases it must be fitted with a good mixture. Contiguous to the superior use of solar irradiation, the closed system converges to notable biomass yield in discrimination to the other systems, however, it demands sizable initial capital for implementation and operation. It is commonplace used for the scope of obtaining pure and homogeneous biomass. They are presented in tubular form, bubble column, and panels (Huang et al. 2017).

The great productivity of biomass in contrast to photoautotrophic cultures correlates with the practice of heterotrophic metabolism, usually administered in a fermentation tank. The bottleneck of this system consists of a high risk of contamination and competition for nutrients besides the fact that the selected microalgae species must be capable of cell division in the privation of light. High supplementation costs are incited by carbon supply (starch, ethanoate, dextrose, glycerol, etc.) and may even dispute with the food sphere (Liang et al. 2009). In the search for the

supply of low-value organic carbon, agro-industrial and urban effluents can be incorporated in the context of microalgae bioremediation (Benemann et al. 2018).

Emerging cultivation strategies, such as microalgae biofilm cultivation and membrane photobioreactors, have the talent to transpose current limitations regarding biomass productivity. However, these techniques demand exploration triggered by the scarcity of studies. Unlike the usual means of microalgae cells in suspension, biofilm cultivation of microalgae brings a new perspective of cultivation, promising abatement in production expenses, especially with reference to biomass harvesting (Blanken et al. 2014; Gross et al. 2015). The technique is the foundation on the restrain of the biomass on a solid substrate, which can be gathered by physical removal (Johnson and Wen 2010). Dense layers of packaged biomass can be removed, avoiding the formation of gradients that prevent nutrients and light from reaching algae. The employment of vertical biofilms can be an advantage in urban regions, usually with space limitations (Gross et al. 2015).

The membrane photobioreactor technique allows the percolation of the effluent through a membrane fixed on a substrate, with retention of particulate material and also of biomass. This strategy results in clear water with low nutrient content, solids, and disinfected. The adroitness to operate incessantly enables excellent biomass productivity, conversely, this project has limited applicability. The obstruction and encrustation of the membranes and filters is a central parameter, hindering the enforceability of the system (Chang et al. 2017).

Finally, hybrid bioreactors combine sequential steps to deepen the strengths of open and closed photobioreactors to overcome current limitations in both of us. Initially, cell division is favored by the employment of the closed, aerobic, and photoautotrophic system, minimizing contaminants. Subsequently, the system starts to include the open stage, providing mass exposure to nutrients, $CO_2$, and light diffusion (Brennan and Owende 2010; Nagappan et al. 2019).

## 5.3 Microalgal Bacterial Synergy: Wastewater Treatment

Naturally, microalgae can develop in different native conditions and also in ecological niches, containing bacteria, other species of microalgae, fungi, and cyanobacteria. Thereby, they adopt several harmonic and disharmonious relationships, with mutualism being the ideal ecological relationship for the microalgae consortia (Liu et al. 2017). Initially, the setting of the competitive/complementary mechanism in the coculture by potential species of microalgae/cyanobacteria and bacteria must be evaluated. It can be defined which system is more appropriate, according to the purpose and the respective attributes of the mono or coculture (Table 5.1). The reproduction of spontaneously established consortia in nature and synthetic inoculation are the main mechanisms for erect relationships in the microorganism community. Distinctively from the profiteering of native consortia, there is a tenuous tendency to use the artificial bio-augmentation method, as it promotes rapid growth of the selected strains, without residence of unwanted species (Yu and Mohn 2002). Environmental variations in open system cultivation

**Table 5.1** Aspects of microalgae-based systems

| Monoculture photoautotrophic | Monoculture heterotrophic | Monoculture mixotrophic | Coculture (photoautotrophic microalgae/ cianobactéria + heterotrophic bacteria) |
|---|---|---|---|
| Easy cultivation | Easy cultivation | Easy cultivation | Difficult to build the consortium |
| Difficulty growing in wastewater | Can grow in wastewater | Can grow in wastewater | Ease of growing in wastewater |
| Low biomass productivity | High biomass productivity | High biomass productivity | High biomass productivity |
| Higher risk of contamination | Higher risk of contamination | Higher risk of contamination | Lower risk of contamination |
| Techniques for contamination control | Techniques for contamination control | Techniques for contamination control | Self-regulating contamination control |
| Low EPS productivity | High EPS productivity | High EPS productivity | Very high EPS productivity |
| Less bio flocculation capacity | High bio flocculation capacity | High bio flocculation capacity | Auto flocculation/bio flocculation |
| Various species | Various species | Various species | Various symbiotic combinations |
| Parallel TN and TP bio removal | Simultaneous bio removal of COD, TN, and TP | Simultaneous bio removal of COD, TN, and TP | Simultaneous bio removal of COD, TN, and TP |
| Low nutrient removal | High nutrient removal | High nutrient removal | High nutrient removal |
| Low pollutant removal | Average pollutant removal | Average pollutant removal | High pollutant removal |
| Medium removal and bioconversion of inorganic carbon | High removal and bioconversion of organic carbon | Medium carbon removal and bioconversion | High carbon removal and bioconversion |
| Less resistant to changes in global conditions | Medium resistance to changes in global conditions | Resistant to changes in global conditions | Robust, resistant to changes in global conditions |
| Specific biomass composition | The heterogeneous composition of biomass | The heterogeneous composition of biomass | The heterogeneous composition of biomass |
| Lower lipid content | High lipid content | High lipid content | Higher lipid content |

*TN* total nitrogen, *EPS* extracellular polymeric substances, *TP* total phosphorous, *COD* chemical oxygen demand

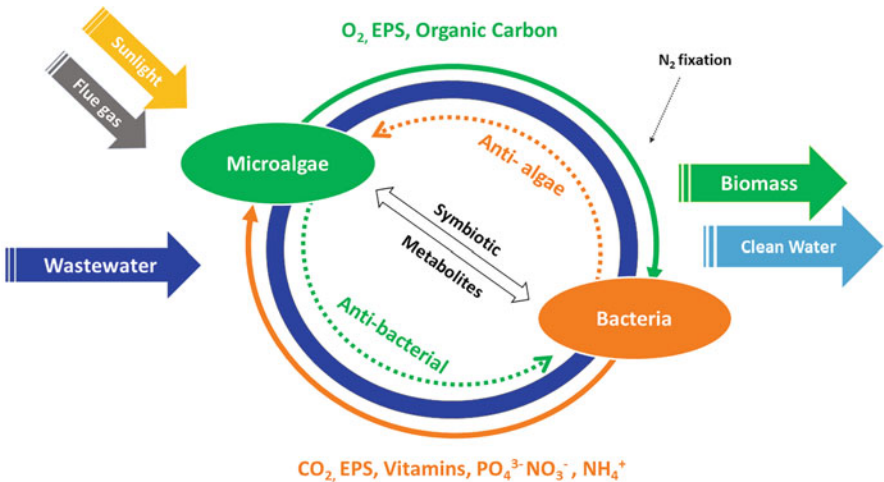


**Fig. 5.1** Simplified mechanism of interaction of the algae–bacteria consortium

significantly influence the consortium stability over the treatment, consequently, there is an inclination to choose sturdy species with tolerance to these variations (Liu et al. 2017).

Analyzing the proposal of a synthetic ecology, it promises to simplify complex relationship systems by understanding the multiplicity of influencing agents. However, the consortium of microorganisms applied in phytoremediation requires balancing. Several aspects act under its physiology and cellular metabolisms, such as environmental conditions, pollutants, the interaction between species, the architecture of the photobioreactor, operating conditions, and sewage profile (Hernández et al. 2016; Pedruzi et al. 2020). In fact, sustaining the lineup of the functional consortium with all its mechanisms working is indispensable to the study of the inoculation phase, the extension of the log phase, and the profile of each component of the binary system (Patel et al. 2017).

The notorious attribute in the microalgae–bacterial symbiosis is the mitigation of supplementation of total oxygen by mechanical means for the heterotrophic bacteria belonging to the consortia (Quijano et al. 2017). Oilgae (2009) project that aeration costs are around 3/4 of total revenue in the treatment station. Besides saving costs, the microalgal–bacterial system reduces the evolution of volatile pollutant compounds in open systems by lessening the use of mechanical aeration.

Since the $CO_2$ produced by bacteria during their cellular activity is reused by microalgae in photosynthesis in their growth and biofixation cycle, it results in the liberation of $O_2$, which is then used by bacteria (Fig. 5.1) (Dao and Beardall 2016). However, homogenization to avoid crop gradients, biomass sedimentation, and promotion of gas diffusion must be provided (Carvalho et al. 2006). Another profit of using binary microalgal–bacterial structure is your sturdiness, generating biomass in the contaminated/polluted water with less pretreatment with monocultures (Rashid et al. 2018).

Extracellular polymeric substances (EPS) are expelled during the metabolic activity of the coculture, performing several functions (Mishra et al. 2011), and are assigned to preserve the web of contact between microorganisms (Nadell et al. 2015). The consortium formation mechanism is anchored on the interaction of microalgae with EPS, bacteria, and suspended particles that start to aggregate. The bacteria reproduce in the phytosphere region of the microalgae, on its outer surface, and the colony's metabolic dynamics are regulated by fragments of biomass that attach/separate from the aggregates formed (Su et al. 2012; Eigemann et al. 2013; Salim et al. 2014; Wang et al. 2016).

It is inferred that bacteria assist in the bioflocculation mechanism, deepening the efficiency of biomass segregation and positively impacting operating costs (Quijano et al. 2017). Equally, by hydrolyzing organic phosphorus in bioavailable states, such as phosphate (Yong et al. 2014), they cooperate in strengthening microalgae cells. The binary microalgal–bacterial system enables a single-step concept, simultaneously performing the bio removal of COD, N, and P (Subashchandrabose et al. 2011). Currently, there is an upward interest in this sphere by the academic community and industry segments with several studies being performed for unialgal and consortium systems.

Typically, the coculture system meets the expected objectives, which are the regeneration of effluent, accrue lipids, biomass yield, and may even overcome certain limitations of axenic cultivation (Devi et al. 2012; Beacham et al. 2017). The genus *Chlorella* and *Scenedesmus,* are sumptuously used in consortia. Its adaptability to various effluents, as well as satisfactory rates of removal of various nutrients and pollutants, establish favoritism in its application. Symbiosis with bacteria that promote the growth of the genera *Azospirillum* and *Bacillus*, bacteria from native consortia of the genus *Brevundimonas* and *Sphingomonas*, and bioconversations of organic material, such as *Pseudomas*, are exposed in the literature (Litchfield et al. 1969; Mayo and Noike 1994; Mouget et al. 1995; Lebsky et al. 2001; Tate et al. 2013; Mujtaba et al. 2015).

However, even with multiple benefits, the application of microalgae cultures and consortia is present only in demonstration/test plants on a pilot or semi-industrial scale. The limitations circulate the scaling-up and economic overview of the processes. The ample surface area for the treatment unit, the refinement of cultivation parameters, and the elevated costs of harvesting microalgal biomass must be addressed (Lam et al. 2012). In contrast, studies appoint that the induction of biomass bioflocculation by microalgae–bacteria interaction can reduce harvesting costs. In the scenario of nutrient bio removal, carbon biofixation, and cell growth, the consortia have advantages compared with axenic cultures, which advocates it as a research subject (Renuka et al. 2013; Rashid et al. 2018). However, it is still needed to research the interaction dynamics of the consortia and to better understand the real bioflocculation mechanism, and it is not possible to affirm the total superiority over monoculture (Gutzeit et al. 2005).

## 5.4 Nutrient and Pollution Removal of Microalgae Consortium

Wastewater usually includes organic matter, nitrogen compounds, phosphorus, among other components, causing eutrophication of the waters, making additional treatment necessary in effluent treatment plants (Liu et al. 2017; Tang et al. 2018). Microalgal–bacterial consortium are already well known for removing these components from wastewater through the use of open and closed bioreactors (Zhang et al. 2020).

Assimilation is the primary mechanism for removing inorganic nitrogen during the growth phases of photoautotrophic and heterotrophic microorganisms (Gonçalves et al. 2017). Nitrogen ($NO_3$ and $NO_2$) is reduced to ammonium and this is absorbed by microalgae for its metabolism (Gonçalves et al. 2017; Liu et al. 2017; Zhang et al. 2020).

Other ways of removing inorganic nitrogen are through ammonia volatilization, nitrification, and denitrification (Daims et al. 2015; Basílico et al. 2016). Ammonia is oxidized to nitrite and then to nitrate from the nitrification process. The denitrification process is the reverse, where nitrate is reduced to nitrite (Courtens et al. 2016). Enzymes, such as Gln Synthase, Glu 2-oxoglutarate aminotransferase, and Glu Dehydrogenase, are secreted from ammonification reactions by microorganisms, and these enzymes have the ability to convert organic nitrogen to ammonia (Simsek et al. 2016).

Inorganic phosphorus, such as $H_2PO_4^-$ and $HPO_4^{2-}$, is essential for the metabolism of bacteria and microalgae since phosphorylation, and can be incorporated into organic compounds. Part of the assimilated phosphorus generates ATP from ADP (Cai et al. 2013). Large amounts of phosphorus are absorbed by microalgae and bacteria, being stored as intracellular polyphosphate (Schmidt et al. 2016). The use of $Ca^{2+}$ and $Mg^{2+}$ions also contribute to the removal of phosphorus in wastewater by the precipitation process. In addition, they can be adsorbed by hydrogen bonds with extracellular polysaccharides secreted by bacteria and microalgae (Li et al. 2013; Lu et al. 2016a, b).

Microalgae technology is a low-cost approach and considered efficient in removing nutrients and other contaminants in effluents (Zhu et al. 2016; Tang et al. 2018). The absorption of nitrogen and phosphorus by microalgae allows the microalgae–bacterium intercropping system to carry out advanced removal of these components from wastewater without the need to add other carbon sources (Tang et al. 2016; Tang et al. 2018).

Biotic interactions occur through cooperation between the consortium of microalgae and bacteria. Microalgae can support bacterial growth by supplying organic carbon, such as carbohydrates and proteins. In parallel, the absorption of $O_2$ and the release of $CO_2$ by bacteria results in an increase in the growth of microalgae (Fig. 5.1) (Gonçalves et al. 2017).

Adequate bioreactors become necessary for the development of the microalgal–bacterium consortium for the effective removal of nutrients from wastewater. Open bioreactors are vulnerable to different factors, such as the invasion of other species of microorganisms, inconsistent temperatures, insufficient homogenization,

evaporation, limited lighting, which impair microalgal growth (Zhang et al. 2020). Closed bioreactors are more attractive to overcome the disadvantages associated with open bioreactors. Different types of photobioreactors are used to a microalgal–bacterium consortium, for example, bubble column, air transport bioreactor, flat, agitated, tubular, conical tank, soft structure, and some modified hybrid photobioreactors, as described earlier (Lee and Lei 2019).

Among the mentioned bioreactors, the most used configurations are the flat plate, tubular, and column reactors. Photobioreactors have their distinct advantages and limitations concerning the complexity of the operation, space required, energy consumption, biomass production and scale-up level, and nutrient removal efficiency (Zhang et al. 2020). Table 5.2 shows the removal of organic carbon, and nutrients from different wastewaters by suspended and immobilized microalgal–bacteria consortium in different bioreactors.

Heavy metals are environmentally dangerous because they are considered toxic and accumulate in soil and water (Priyadarshini et al. 2019). Previous research has confirmed the feasibility of using microalgal–bacterial intercropping to remove various heavy metals. Studies by Orandi et al. (2012), clearly demonstrated the microalgae–bacterial consortia to be explored for the removal of heavy metals from wastewater resulting from mining activity, concluding that the consortia could remove up to 50% of the metals such as copper, nickel, manganese, zinc, antimony, selenium, cobalt, and aluminum.

The absorption of heavy metals by microalgae and bacteria is potentially toxic, but the level of toxicity will depend on the concentration and nature. Metals like Ni, Mn, Cu, and Fe can meet the energy and structural demands of the cell of these microorganisms, as they are considered essential for their biological functioning (Priyadarshini et al. 2019). The removal of heavy metals by these microorganisms is achieved mainly through ion-exchange, physical adsorption, complexation, and bioaccumulation. The removal can also occur from the precipitation process, which occurs with an increase in pH during photosynthesis of microalgae (Cuellar-Bermudez et al. 2017). Some examples of heavy metal removal from wastewater by microalgal–bacterial consortium are shown in Table 5.3.

Microalgal–bacterial consortium also has demonstrated the ability to degrade several dangerous compounds such as organophosphate insecticides, toxic pesticides (Subashchandrabose et al. 2011), hazardous organic substrates including acetonitrile, phenol, and some types of emerging organic contaminants (Muñoz and Guieysse 2006; Matamoros et al. 2015) and hazardous inorganic compounds (Ryu et al. 2015). Microalgal–bacterial consortia present possible mechanisms involved in removing these compounds, such as biodegradation, photodegradation, cell adsorption, bioaccumulation, or volatilization (de Godos et al. 2012; Loos et al. 2013; Cuellar-Bermudez et al. 2017).

However, for the efficient removal of nutrients and polluting compounds from wastewater, some crucial factors in the consortium of microalgae must be taken into account. These factors consider the specific interactions of microalgae and bacterial species, the composition of the cell wall, the stiffness, and cultivation conditions such as light intensity, pH, and temperature (Quijano et al. 2017; Zhang et al. 2020).

**Table 5.2** Efficiency of removal of nutrients and organic carbon from wastewater by suspended and immobilized microalgal–bacterial consortium. Adapted from Gonçalves et al. (2017) and Zhang et al. (2020)

| Microalgal–bacterial consortium | Source of wastewater | Bioreactor used | Carbon (%R) | Nitrogen (%R) | Phosphorus (%R) |
|---|---|---|---|---|---|
| *Suspended microalgal–bacterial consortia* | | | | | |
| *Scenedesmus quadricauda* and nitrifying bacteria | Synthetic wastewater | Tubular photobioreactor, $V = 1$ L | n.a. | 100.0 | n.a. |
| *Chlorella sorokiniana* and activated sludge | Piggery wastewater | Photobioreactor, $V = 3.5$ L | 47.0 | 21.0 | 54.0 |
| *Chlorella*, *Scenedesmus* and *Stigeoclonium* and activated sludge | Municipal wastewater | Photobioreactor, $V = 60$ L | 85.44 | 92.68 | 82.65 |
| Activated sludge bacteria with *Chlorella* sp., *Acutodesmus obliquus*, and *Oscillatoria* sp. | Piggery wastewater | Photobioreactor, $V = 3$ L | 86–87 | 82–85 | 90–92 |
| *Scenedesmus* sp. and activated sludge bacteria | Municipal wastewater | Photobioreactor, $V = 0.8$ L, two-phase photoperiod operation | 92.3 | 95.8 | 98.1 |
| *Chlorella vulgaris* and *Bacillus licheniforms* | Synthetic wastewater | Photobioreactor, $V = 0.15$ L | 86.55 | 88.95 | 80.28 |
| *Chlorella vulgaris* and activated sludge bacteria | Municipal wastewater | Closed suspended system, batchmode, $V = 1.5$ L | n.a | 30.9–100 | 65–98 |
| *Chlorella sorokiniana* and activated sludge bacteria | Potato-processing wastewater | Photobioreactor open, semicontinuous, $V = 5$ L | 86.1 | 95.0 | 80.7 |
| *Chlorella* sp., *Pediastrum* sp., *Phormidium* sp., *Scenedesmus* sp. and activated sludge bacteria | Municipal wastewater | Closed photobioreactor, semicontinuous, $V = 4$ L | n.a. | 61.2 | 30.2–56.8 |
| *Immobilized microalgal–bacterial consortia* | | | | | |
| Cyanobacteria *Phormidium* sp. | Aquaculture wastewater | Erlenmeyer flask, $V = 0.5$ L | 80.2 | 57.9 | 88.6 |
| *Pseudomonas putida* and *Chlorella vulgaris* | Municipal wastewater | Erlenmeyer flask, $V = 0.5$ L | 97.0 | 100.0 | 100.0 |

| *Oscillatoriales*, *Scenedesmaceae*, *Microcoleus,* and *Acutodesmus* | Primary wastewater | Batch photobioreactor without aeration, $V = 1.2$ L | 77.0–82.0 | 41.0–57.0 | 11.0–44.0 |
|---|---|---|---|---|---|
| Microalgae *Chlorella* and *Scenedesmus*, and bacteria *Flavobacterium*, *Micropruina,* and *Comamonadaceae* | Synthetic domestic wastewater | Batch sequencing photobioreactor, $V = 8$ L | 91.4 | 99.2 | 94.8 |
| *Chlorella*, *Scenedesmus*, and activated sludge bacteria | Artificial municipal wastewater | Photobioreactor, $V = 2$ L | n.a. | 93.0 | 35.9 |
| *Chlorella spp*. and *Azospirillum brasilense* | Municipal wastewater | Batch photobioreactor, $V = 0.6$ L | n.a. | 15.0–100.0 | 36.0 |
| *Chlorella sorokiniana* and activated sludge bacteria | Piggery wastewater | Continuous photobioreactor, $V = 7.5$ L | 45.0 | 94.0–100.0 | 70.0–90.0 |
| Bacteria *Rhodocyclaceae* and the microalgae *Chlorophyta* | Synthetic municipal wastewater | Photobioreactor, $V = 2$ L, Light intensity of 225 μmol $m^{-2}$ $s^{-1}$ | 95.0 | 99.0 | 42.0 |

*%R* removal efficiency, *V* working volume, *n.a.* not applicable

**Table 5.3** Heavy metal removal from wastewater by microalgal–bacterial consortium. Adapted from Subashchandrabose et al. (2011)

| Microalgal–bacterial cosortia | Source of wastewater | Bioreactor used | Metal and its removal efficiency (%R) |
|---|---|---|---|
| *Spirulina platensis* and sulfate reducing bacteria | Tannery wastewater | Open photobioreactor | Cu 79.2%; Zn 88.0%; Fe 100% |
| *Chlorella* sp., *Scenedesmus obliquus*, *Stichococcus* sp., *Phormidium* sp., *Rhodococcus* sp., *Kibdelosporangium aridum* | Oil polluted wastewater | Pilot installation | Cu 62%; Ni 62%; Zn 90%; Fe 64% Mn 70% |
| *Chlorella* sorokiniana, and *R. basilensis* | Synthetic Bristol medium with metal | Photobioreactor | Cu 57.5% |

In addition, the consortium between microalgae and bacteria depends on the availability of nitrogen and phosphorus present in wastewater (Liu et al. 2012). Another critical factor that affects these consortia is the stage of growth of microorganisms. Microalgae–bacterial consortia are not static, as they stop being mutualistic and become parasites according to the growth phase (Tang et al. 2010; Seyedsayamdost et al. 2011).

Microalgal–bacterial consortium has a double benefit since, in addition to carrying out wastewater treatment; the biomass produced is useful for biotechnological applications (Quijano et al. 2017; Lee and Lei 2019; Zhang et al. 2020). Thus, conduct research over a circular bioeconomy perspective becomes interesting.

## 5.5 Integrated Microalgae–Bacteria Biorefineries in Circular Bioeconomy

Wastewaters are organic and inorganic nutrients sources in this aspect, the utilizing of microalgal–bacterial consortia in wastewaters bioremediation opens up opportunities for reusing, remanufacturing, and recycling of nutrients possible for the circular bioeconomic model (Nagarajan et al. 2020; Wicker and Bhatnagar 2020).

The circular bioeconomy is based on the combination of two terms, bioeconomy and circular economy, and aims to maximize resources and eco-efficiency, and also, to minimize greenhouse gases, water footprint, the demand for fossil carbon, and generation of waste (Giampietro 2019; Suzanne et al. 2020). Broadly the bioeconomy contemplates the sustainable use of renewable natural sources or biomass (McCormick and Kautto 2013; Bröring et al. 2020). While the circular economy is substantiated on the application of resources in a closed-loop mode in contradiction to the current linear manner economy, maintaining environmental,

economic, and social balance (Homrich et al. 2018; Reike et al. 2018; Suzanne et al. 2020).

Aware of the market opportunities that this economic model can procure, the global transition toward a circular economy has resulted in several nations adopting new environmental regulations. Europe, implemented the Waste Framework Directive in 2008 (EC-Regulation Regulation 2008); in the United States, the concepts were introduced in 1984 from the Resource Conservation and Recovery Act and later amended in 2002 by the Pollution Prevention Act (USA 1984; 2002); while in China, it was implemented in 2008 as the Circular Economy Promotion Law.

Therefore, in the search for complete bio-circular approaches, alternative operations are encouraged with eco-design products, renewable energy resources, waste prevention, recovery, and reuse (Suzanne et al. 2020). In this way, the circular bioeconomy can be obtained through sustainable pollutant management. Hence, it is imperative to seek an economical and sustainable platform for the bioremediation of effluents since conventional systems are energetically costly and are inefficient in removing specific pollutants (Sharma et al. 2020).

In this regard, the innovation in the use of microalgal–bacterial biotechnology in the waste bioremediation aligned with economic and environmental objectives provides an integrated biorefinery model (Senthil and Yaashikaa 2020; Nagarajan et al. 2020). Biorefineries are a key concept for the switchover to the circular economy by the deployment of integration of technologies and processes seeking to optimize the application of biomass toward the output of bioenergy, inputs, and marketable end products (Ubando et al. 2020; Awasthi et al. 2020; Arun et al. 2020).

This approach aims to valorize effluents, adding alternative solutions that are more attractive to microalgal–bacterial biotechnology. Once it excludes the need to explore freshwater resources for obtaining microalgal biomass, it increases microalgal biomass productivity, causes bioflocculation for better harvesting, and lower operating costs with aeration required in the aerobic remediation of pollutants (Olguín 2012; Ismail et al. 2017).

At this juncture, the cocultivation of microalgae associated with bacteria can be easily integrated into a circular process. As it offers dual-purpose, simultaneously removes nutrients by assimilatory means, and nutrients can be recuperated in the biomass and converted into diversified types of bioproducts targeting countless markets under the framework of a circular economy (Stiles et al. 2018; Serrà et al. 2020).

With the appropriate technology, microalgal–bacterial biomass components can be turned into value-added commodities, such as biofuels, one of the most promising applications (Zhang et al. 2020). In this context, Meng et al. (2019) suggested that light intensity has a favorable result on lipid production and the accumulation of monounsaturated fatty acid and polyunsaturated in microalgal–bacterial consortia at synthetic wastewater bioremediation. Similarly, Liu et al. (2018) report that the genera *Rhodobacteraceae*, *Xanthomonadaceae*, and *Scenedesmus* obtained from granular sludge were potential biodiesel candidate production. Arcila and Buitrón (2016) evaluated the methane production by microalgal–bacterial consortia submitted to treating municipal effluent. Also, according to Ismail et al. (2017), the

association of microalgae and bacteria applied in the treatment of simulated pharmaceutical effluents, supplemented the biomass generated with biocompounds including proteins, fatty acids, essential and nonessential amino acids.

Finally, it should be noted that despite various benefits, an in-depth understanding of the biotechnological applications of microalgal–bacterial biomass is still lacking, particularly the life cycle approaches should be conducted to quantify sustainability aiming at economic and social aspects (Nagarajan et al. 2020; Wicker and Bhatnagar 2020; Ubando et al. 2020).

## 5.6 Challenges and Future Prospects

The microbial consortia/cocultures between microalgae and bacteria draws attention because they present advantages such as having a reduced environmental impact, requiring little oxygenation, reducing the cost of the process, the potential for the production of bioproducts of industrial interest (Mishra et al. 2019; Zhang et al. 2020). However, current insights into the mechanisms of symbiosis and the benefits gained from each other are still scarce. The microalgae–bacteria consortium start-up process can take up to 6 weeks. This period can be reduced by using low solar irradiance and temperature control (Liu et al. 2017). This initial delay in the consortium of microalgae–bacterial is a significant limitation of this technology. However, bioactive substances and biostimulant reagents can be added to the photobioreactors. These substances will be slowly released into the culture medium, accelerating the growth and metabolic activities of microalgae and bacteria, reducing the adaptation period of these microorganisms increasing the efficiency of this technology (Yu et al. 2017; Teixeira and Granek 2017).

Many species, photosynthetic and heterotrophic microalgae and bacteria, have not yet been studied, and have become a challenge to illustrate the complete events, such as nutrient cycling and metabolic pathways (Zhang et al. 2020). Studies with genetic and metabolic engineering may present a possibility for the improvement of these microorganisms, designing cultures of bacteria and microalgae with reaction speeds higher than those already existing, to remove a greater number of pollutants (Lindemann et al. 2016; Karig 2017).

As demonstrated in this chapter, the use of microalgal–bacterial consortia is considered to be renewable and sustainable biotechnology. Bacteria contribute to cell lysis, supporting microalgae productivity by increasing biomass production. This bioproduct can be applied on a large scale in biorefineries (Zhang et al. 2020). However, it is a high-cost technology, so an assessment of the availability of technical–economic resources and the productivity of microalgal–bacterial biomass as feedstock for the production of biofuels becomes interesting in order to map a clear perspective of economic viability (Quinn and Davis 2015).

Another important aspect is the life cycle assessment, to determine the environmental impact, the capacity for renewal, and the energetic dynamics of the biotechnological process (Trivedi et al. 2015). Mathematical models can improve the understanding of the principles and mechanisms of pollutants and the microalgae–

bacterium consortium, including mass balance and metabolic functions (Liu et al. 2017). Thus, for a large-scale application, further studies are necessary to assess the sustainability and economic viability of these biotechnological processes.

## 5.7 Final Considerations

The high biodiversity and the immense potential not yet mapped makes microalgae a clever stunt to meet the current demands, be it bioproducts or clean technologies. Integrated biorefineries promise to reintegrate water quality without a strong chemical approach, producing less or zero potential waste to generate additional pollution. Whereas, in addition to carrying out ecological work, the generation of expressive value-added biomass destined for commodities and biofuels. The reclaim of elements presents in the effluent previously lost through conventional treatment, will be reused. Likewise, several volatile compounds derived from microalgae can be collected with appropriate techniques. The industry's appeal is the economic sustainability of the processes involved in upscaling. The continuous and profitable use of the algae–bacterium consortium is a boost for scale-up for industrial applications. However, it requires more research in terms of control of the symbiotic mechanism and dominion of the life cycle.

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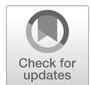

# Microbial Degradation of Food Products

6

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**Abstract**

Contamination and degradation of food products by microorganisms are a major problem for the food industry, with serious damage to economic circulation and public health worldwide. Therefore, the objective of this chapter is to clarify how the contamination of microorganisms occurs, the main damage to food, production of toxic substances, economic losses, and new food preservation technologies, with a focus on developing a sustainable and safe food industry. Most foods contain structural ingredients, such as a high concentration of carbohydrates, perfect conditions for an outbreak of microbial infection. The microorganisms that grow in food alter their chemical composition, appearance, taste, texture, and characteristics such as color, shape, and aroma. In addition, some fungi produce mycotoxins that are highly toxic. Finally, contaminated food undergoes degradation and putrefaction, causing irreparable losses to producers.

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Thus, we address new technologies that may be the key to increasing the shelf life of food and reducing the rate of contamination. In conclusion, microorganisms are a persistent problem and difficult to eliminate, especially considering the possibilities of artificial selection. However, emerging technologies can help preserve food for longer, without increasing the risk of artificial selection of the microorganism or even causing changes in food.

**Keywords**

Pathogens · Food security · Shelf life · Artificial selection · Mycotoxins

## 6.1 Introduction

Microbial contamination and subsequent degradation of food products due to the biological activities of pathogens are a current problem, faced from the beginning of the food production chain to the consumer base. Since ancient times, human beings have faced problems in food preservation and we can say that human survival itself has been through understanding how to preserve food for long periods. Human civilization has managed to survive using various food preservation strategies, for example, man in the ice age period has adapted to preserve food on ice. In the Bronze Age, we have food preservation by cooking and drying. Although food preservation techniques have evolved, contamination problems still persist, now with new aggravating factors (Ganivet 2020).

The idea of eradicating all microorganisms from the food production chain is almost impossible. And we can say that although all modern techniques help to eliminate pathogens, contamination problems still persist. So, how can we adapt to live with microbes? In fact, microorganisms have been on planet Earth longer than any other living organism, and adapt to survive in the most variable conditions, whether adverse or not. During the entire period of evolution of microorganisms, there was no major change in their primordial states, except for several molecular adaptations. Molecular adaptations are the great advance of microorganisms and perhaps their greatest triumph in the evolutionary chain (Chatterjee and Abraham 2018).

Thus, microorganisms can survive, reproduce, and continue to evolve, adapting their molecular arsenal and conquering new territories. Therefore, it is not easy to eliminate pathogens from the entire food production chain, considering all the evolutionary force (molecular evolution) present in microorganisms. Therefore, we have to think about new food preservation strategies, considering deeper aspects, such as evolution, adaptation, and artificial selection (Newell et al. 2010).

Given this scenario, we realize that microbial contamination has been relentless, especially in modern times. Today we realize that although the academic community has made great efforts to develop technologies to eliminate pathogens, we are losing the war with our weapons. Deeper aspects of microorganisms were not considered, that is, their ability to adapt and evolve. Since the past 50 years, our modern society

has selected microorganisms in almost all industrial sectors through artificial selection. Thus, several resistant and better-adapted microorganisms have been the focus of several problems for the food industry, with profound impacts on the health of the population and the financial market (Pittia and Antonello 2016). In this context, this chapter brings to light the reader relevant scientific information on the degradation of food products by microorganisms, the negative effects and forms of preservation, especially new technologies, with a focus on sustainable industry and with high standards of food safety.

## 6.2 Contamination and Microbial Degradation of Food Products

Food contamination and degradation refer to the presence of pathogenic and non-pathogenic microorganisms that can cause changes in the product, such as odor, taste, texture, and appearance during the proliferation of microorganisms due to their natural metabolic activity. In addition to sensory changes, some microbial agents can produce harmful toxins and chemicals that shorten the shelf life of food and can cause various diseases in humans (Lianou et al. 2016).

Contamination can occur at any production stage, from harvesting or slaughtering to processing, transportation, and storage, due to surfaces that come into contact with food. in addition to factors such as the quality of raw materials and inputs; incorrect cleaning of equipment, utensils, and installations, inadequate packaging of waste and by-products, inadequate handling practices, temperature variations, among others (Singh et al. 2018).

The level of quality and safety of food is based on microbiological assessment since most foods represent a rich source of nutrients for microbial development. The increase in population and, consequently, the increase in food consumption, and changes in the eating habits of the population, such as:

1. Consumption of raw, lightly cooked, or exotic foods
2. Higher consumer demand for food products of superior sensory quality
3. Increase in functional, nutritional properties, and demand for organic products with less pesticides and additives, helped a modern revolution in the food industry.

These examples make the industry seek to develop different technologies and treatments applied in all stages of food processing. How to limit the growth rate in the exponential phase, reducing the maximum microbial population density, and thus maintaining the quality of the final product, the nutritional value, and providing consumers with a healthy and safe product (Havelaar et al. 2010; Jacxsens et al. 2010; Chatterjee and Abraham 2018).

### 6.2.1 Major Food Pathogens

Each food has a microbial profile that depends on the flora, the source of the contamination, the physical and chemical factors intrinsic to the food (composition, pH, water activity, presence of natural antimicrobial compounds, etc.), and environmental factors such as temperature (Baron and Gautier 2016).

Microorganisms can be classified as deteriorating and pathogenic. The so-called deteriorants cause changes in the food product, which makes it unacceptable for consumption from a sensory point of view. While pathogens are those that can cause a range of diseases in the consumer, such as botulism, food poisoning, and other enteric infections by the production of harmful toxic metabolites. These microorganisms can be bacteria, fungi, yeasts, and viruses (Wirtanen and Salo 2007). Table 6.1 shows some examples of bacteria and fungi responsible for food contamination, changes suffered by food, and methods of inhibition.

**Table 6.1** Examples of bacteria and fungi responsible for food contamination, their possible changes, and methods of inhibition. Summary based on studies of Newell et al. (2010) and Faille et al. (2018)

| Name | Affected foods | Changes in food | Inhibition methods |
|---|---|---|---|
| *Bacteria* | | | |
| *Clostridium botulinum* | Meat | Acidification, flavor change, protein breakdown, oxidative rancidity of fats | Storage under refrigeration; thermal treatments |
| *Staphylococcus aureus* | Raw milk and dairy products in general | | Storage under refrigeration; thermal treatments |
| *Salmonella* spp. | Eggs | Yolk blackening; unpleasant odor, changes in skin pigmentation | Eggshell protection (cuticle, internal membranes, and pores); inner protection (lysozymes, conalbumin, and avidin) |
| *Escherischia coli* | Meat and animal products | PH increase; production of volatile compost with a strange taste; pigmentation change | Storage under refrigeration; thermal treatments |
| *Fungi* | | | |
| *Aspergillus* spp. | Raw milk and dairy in general | Proteolysis and lipolysis; increase in pH; color change; production of volatile compounds with an unpleasant odor, production of toxin | Thermal treatments |
| *Penicillium* spp. | Cereals | Toxin production and formation of dark spots | Heat treatments and drying |

### 6.2.2 Contamination and Degradation of Food Products by Bacteria

Microorganisms that degrade food are responsible for most foodborne diseases and have been the most investigated cause of diseases related to intestinal infection, reinforcing the resistance of these pathogens, despite the prevention and control techniques applied in the industry (Newell et al. 2010). In general, bacteria need water to thrive, survive in the presence or absence of oxygen, classified as aerobic and anaerobic, respectively, or both (optional anaerobic). Their shapes are bacilli, coconuts and others, like spirochetes. Prefer protein-rich environments and can produce toxins (Lopez et al. 2018).

Bacteria can grow and multiply from the nutrients present in food, causing undesirable changes that vary depending on the type of bacteria, in addition to factors such as pH, osmolarity, temperature, and oxidation, as well as processing methods, which also influence bacterial development (Chatterjee and Abraham 2018). For example, stainless steel surfaces, floors, conveyor belts, or equipment, have been reported with the presence of bacteria (Faille et al. 2018).

Bacteria can evolve, adapt, and become resistant. In addition, a microbial community formed by bacteria can adhere to a surface, distributing its growth to form a solid matrix (biofilm). Microbial biofilms are a major problem for the food industry, as they are the focus of widespread contamination. Therefore, techniques and treatments to prevent biofilm formation must be effective in inhibiting them and, thus, preventing the spread of more bacteria to other environments (Van Houdt and Michiels 2010; Chandki et al. 2011).

Modern and advanced techniques have been developed and applied in the industry for the preservation and safety of food (Alvarez-Ordóñez et al. 2015). However, not every product can be subjected to heat treatments, as they can degrade nutrients or cause unwanted changes, requiring more sophisticated technologies to overcome bacterial resistance in the food industry, such as microwave pasteurization, ultrasound, plasma gas, ultraviolet light technology, and others (Galis et al. 2013; Motarjemi et al. 2014).

### 6.2.3 Contamination and Degradation of Food Products by Fungi

Fungi grow in a wide range of pH, temperatures, and water activity and use various types of substrates such as lipids, proteins, and carbohydrates. They are divided into molds and yeasts and are capable of deteriorating foods with intermediate humidity, bakery products, cherries, drinks, and fermented products causing economic losses (Chalupová et al. 2014).

The presence of fungi is related to the production of harmful toxins and microtoxins in foods, making them of low quality (Njobeh et al. 2009). Toxins are secondary metabolites produced by some types of fungi (Terzi et al. 2014) detected in several products (Njobeh et al. 2010), aflatoxins being considered the most important due to their significant negative impact on health and trade. These

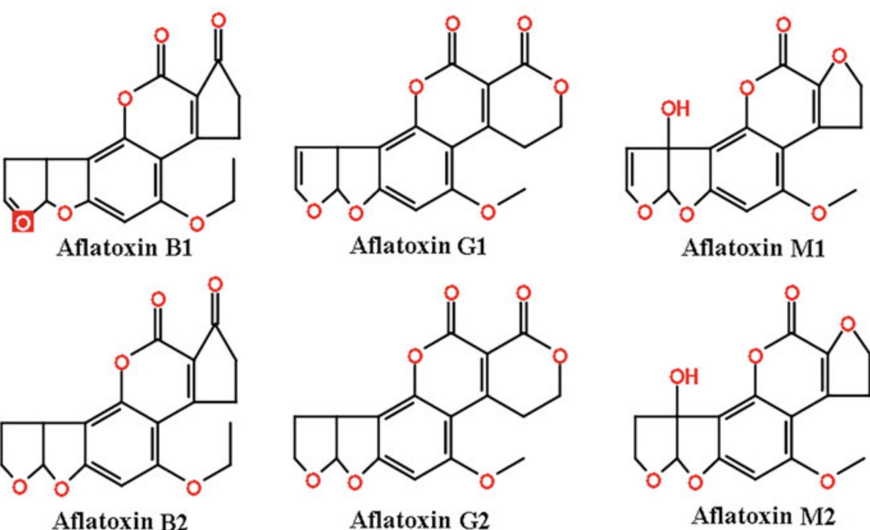


**Fig. 6.1** Main aflatoxins produced by fungi of the genus *Aspergillus* spp. Source: the authors

products resulting from the secondary metabolism of fungi may vary according to the substrate and environmental conditions (pH, temperature, humidity, and the presence or absence of oxygen) (Rocha et al. 2014; Yehia 2014).

The identification of fungi that spoil food is an important step in managing food safety and quality. Prevention or decontamination methods can involve removing contaminated products, inactivating or reducing the level of toxins in food, and can be done by physical, chemical, and biological means, depending on the type of product and the toxin (Halasz et al. 2009).

The toxins produced by fungi (also known as mycotoxins) are a mixture of highly toxic secondary metabolites. There are numerous known mycotoxins and they are all easily produced in natural environments. Fungi like *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp., are known to easily contaminate fruits, processed foods, and vegetables, producing highly toxic toxins. Three groups of mycotoxins are currently recognized, namely aflatoxins, ochratoxin, and fumonisins, both of which will be addressed below.

Aflatoxin is produced by fungi of the genus Aspergillus spp., mainly in foods rich in carbohydrates such as bakery products, peanuts, and industrialized products in general, such as almonds, cheese, fluid beds, among others. Aflatoxin is highly toxic and has mutagenic and carcinogenic properties.

Fungi of the genus *Aspergillus* spp. produce four types of aflatoxin, known as B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2). In addition to these, there is a group of aflatoxins known as M (AFM1 and AFM2), which are highly toxic and have direct effects on cell dysregulation, resulting in programmed cell death (Terzi et al. 2014). The main aflotoxins produced by fungi of the genus *Aspergillus spp* can be seen in (Fig. 6.1).

Fig. 6.2 Examples of Ochratoxin produced by fungi. Source: the authors

Ochratoxin is a secondary metabolite produced by a variety of species of fungi; especially those of the genus *Penicillium* spp. and *Aspergillus spp*. *Aspergillus* are the most common, among them *A. carbonarius*, *A. melleus*, *A. ochraceus, A. sclerotiorum*, and *A. sulphureus*. Among *Penicillium* spp., we have two relevant species, namely, *P. verrucosum* and *P. nordicum*. Ochratoxin is found mainly in cereals, dry beans, wine, coffee, and other food products. Even in small amounts, ochratoxin is highly toxic and is associated with endemic nephropathy in Balças, a degenerative disease of kidney and liver functions, leading to organ failure and death (Ayofemi Olalekan Adeyeye 2020). The main ochratoxins produced by fungi can be seen in (Fig. 6.2).

Finally, fumonisins are mycotoxins produced by fungi of the genus *Fusarium spp*, and like the other mycotoxins already reported, they also have harmful effects if ingested. The main fungi that synthesize fumonisins are *F. oxysporium* and *F. verticillioides*. These mycotoxins are found mainly in foods such as corn and are a major cause of production losses if fungi are not controlled. The concentration of these mycotoxins depends on several factors, however, studies with corn contaminated by the fungus *F. oxysporium* showed that fumonisin B1 is found in a higher concentration than fumonisin B2. The consumption of these toxins can lead to serious effects, especially on organs such as the lung and liver, and even more severe effects such as cerebral necrosis (Ayofemi Olalekan Adeyeye 2020). The main fumonisin produced by fungi can be seen in (Fig. 6.3).

**Fig. 6.3** Examples of Fumonisins produced by fungi. Source: the authors

| | R1 | R2 |
|---|---|---|
| Fumonisin B1 | OH | OH |
| Fumonisin B2 | H | OH |
| Fumonisin B3 | OH | H |

## 6.3 Major Problems Associated with Contamination and Spoilage of Food

Contamination and spoilage of food are problems that can cause very serious damage to human health, and in some situations it can be irreversible, leading to death. For that reason, one of the main purposes of the food industry is to produce foods free of any contamination, ensuring the food safety of the product and its consumers. Problems in the supply and processing chain usually change the environmental conditions of food and promote the growth of microbial agents, instigating contamination and spoilage. The contamination and spoilage of food can be caused by three main agents physical, chemical, and biological. In this chapter, we will only deal with the main problems related to the contamination and spoilage caused by biological agents, which cover most of the reported problems of food contamination.

### 6.3.1 Problems Related to Meats

Meat is known as one of the most perishable foods due to its chemical composition that allows microbial growth at high levels, contributing significantly to the deterioration or deterioration of meat (Doulgeraki et al. 2012). Various problems related to contamination and deterioration of meat is reported by the scientific food community. Most of these problems are linked to the observed effects, which are quite variable, including discoloration, slime formation (visible growth), development of strange odors and flavors, and changes in texture (degradation), among others (Nychas et al. 2008).

The color of the meat is one of the main factors that consumers take into account when purchasing the product and the change in the natural color of the meat is always associated with problems about its quality. Meat discoloration occurs because the microbial agents are capable to destroy meat myoglobin causing the greening/graying of meat by combining with hydrogen sulfide of microbial origin. In

addition, these agents can be broken down to create green or yellow bile pigments by microbial hydrogen peroxide (Pellissery et al. 2020). *L. sakei, Hafnia alvei, S. putrefaciens,* and lactic acid bacterias (LAB) are the main responsible for the mentioned types of coloring (Dušková et al. 2013). The production of microbial pigment is also visually perceptible on meats where Pseudomonads, molds of the genera *Cladosporium, Sporotrichium, and Penicillium* produce a range of black, white, blue, green, and yellow pigments (Pellissery et al. 2020). In some situations, the yellow fluorescent pigment is associated with the action of *P. fluorescens* (Cornelis 2010). In addition to the changes in the food visual aspect, the consumption of contaminated and spoiled meats can cause the development of several food-borne diseases.

Gas production is another problem caused by microorganisms that can affect meat quality. Unwanted gas formation generally occurs in vacuum-packed meat. Hydrogen and carbon dioxide are produced and released by the action of anaerobic bacteria, causing problems such as changes in pH, putrid odors, exudate formation, and color changes (Iulietto et al. 2015). Similarly, ropy slime appearance on the surface of meat due to the presence of *Lactobacillus spp*. and *Leuconostoc spp*. is another visual problem that has a negative impact on consumer purchase decision (Iulietto et al. 2015).

In the same way, off-odors and off-flavors are generally noticeable in meats before any other signs of deterioration. Volatile acids such as formic, butyric, and propionic are responsible for the acidic odor of microbially spoiled meat (Pellissery et al. 2020). Usually, off-odors are perceived when the bacterial population reaches $10^7$ CFU/g (Casaburi et al. 2015). In addition, when the meat is stored at temperatures close to freezing, occurs the formation of a cotton bud mycelium, without sporulation, of white color, called whiskers. The main agents of this deterioration are *Pseudomonas, Moraxella, Alcaligens, Aeromonas, Serratia, Pantoea Thamnidium, Nucor*, and *Rhizopu*s species (Nychas et al. 2008).

### 6.3.2 Problems Related to Milk and Dairy Products

Milk and dairy products can be contaminated by microorganisms at any stage of the processing chain, causing problems to these products. The main forms of contamination that cause problems to milk and dairy products are the inadequate cleaning of milking equipment, cross-contamination by handling, animal feces, oscillation in temperature of refrigeration tank, inadequate pasteurization, and storage (Lopez et al. 2018; Beletsiotis et al. 2011). There are a large number of infectious diseases that can be transmitted to humans through milk. The most important pathogens are *Salmonella sp., Pathogenic Escherichia coli, Listeria monocytogenes, Campylobacter jejuni, Yersinia enterocolitica*, and *Staphylococcus aureus*, which can cause several fatal diseases (Iqbal et al. 2016). However, most of these pathogenic microorganisms are destroyed by pasteurization. That is why the thermal process should be well executed.

Furthermore, under refrigeration temperatures, some psychrotrophic bacteria present a higher growth rate, producing lipolytic and proteolytic enzymes, which cause problems on the quality of milk and dairy products due to their ability to resist thermal treatments (Nsofor and Frank 2013). Milk and dairy products are also susceptible to contamination by yeasts and molds that can cause changes in the color, texture, and odor of these products. The main microorganisms responsible for these contaminations are *Candida, Galactomyces*, and *Yarrowia* for yeasts and *Penicillium, Mucor*, and *Cladosporium* for molds (Garnier et al. 2017)

### 6.3.3 Problems Related to Eggs

Eggs are widely consumed worldwide because it is considered a low-cost food and partially meets the nutritional requirements of a person. However, some problems with the contamination and spoilage of this food can cause damage to consumer. The main form of contamination occurs through the eggshell when the microorganisms penetrate through its membranes and access the egg content, resulting in deterioration (Whiley and Ross 2015). Bacteria, yeasts, and molds can be the cause of undesirable alterations such as the release of gas, formation of visible colonies, changes in the coloration, texture, and odor of the eggs (Techer et al. 2014). *Salmonella Enteritidis*, which do not changes the color, smell, and consistency of the egg content, is a pathogenic bacterium that is considered the main factor for foodborne illnesses in developed countries, and it is important to highlight that eggs are the most common food that causes these infections (Jan et al. 2018).

### 6.3.4 Problems Related to Vegetables and Fruits

Fruits and vegetables are perishable foods due to their high water content (approximately 95%), which can facilitate the growth of both pathogenic and spoilage microorganisms, therefore these foods are more susceptible to contamination (Yousuf et al. 2020). The contamination and spoilage of fruits and vegetables can occur during the growing, harvest, and processing steps. In fruits with low pH, especially citrus fruits, the bacteria do not grow and fail to develop, since the pH changes the metabolism. However, molds and yeast are able to grow on these foods (Carlin 2013). For instance, Basidiomycetous can cause problems such as dark spots, dark lesions, gangrene, sour and soft roots, as well as it can produce cutinase that is related to the cuticle degradation of various fresh vegetal products (Carlin 2013). Another problem that affects some types of fruits and vegetables is the contamination by pathogenic microorganisms, mainly related to ready-to-eat products, which are responsible for several outbreaks caused by *Salmonella* ssp. and *E. Coli*. O157: H7. Infection by these microorganisms can cause serious damage to human health such as gastroenteritis (Amrutha et al. 2017).

### 6.3.5 Problems Related to Canned Foods

The main problem caused by the consumption of canned foods is botulism. Botulism is a rare and serious bacterial disease caused by the ingestion of toxins from *Clostridium botulinum*, which is considered the most potent among the known toxins (Momose et al. 2014). Botulism is characterized as an extremely serious disease, with acute evolution, causes digestive and neurological disorders, as a result of the ingestion of several types of food, including canned foods (Rhodehamel et al. 1992) As *Clostridium botulinum* is a sporogenic bacterium, the spores can remain in the food in case of inadequate sterilization in the heat treatment stage, and start to multiply and produce toxins (Featherstone 2015). Therefore, all low-acid canned foods (pH $>$ 4.5) that are contained in packages completely free of oxygen, such as canned products, are potentially botulogenic and attention needs to be doubled in order to avoid serious health problems (Peck et al. 2020)

## 6.4 New Technologies to Reduce the Rate of Contamination and Degradation of Food Products

The quest for food preservation dates back to prehistoric times through the empirical use of salt, sugar, drying, and smoking. In some places where there were caves, snow and ice were used as a cooling resource. Later, ice was first transported in 1799, and in 1877 to Linde, the first industrial compact refrigerator process. Nicholas Appert, in 1810, took a big step in preserving food by adding heat, developing the tightening process that gave rise to canning and heat sterilization. Over the years, new technologies have been and are being developed in response to historical events such as the Second World War and space exploration, and to the intense population growth that went from 2.5 to 8 billion between 1950 and 2020. In this way, the objective of modern studies is to increase the shelf life of food, safety in consumption without causing damage to health, and the maintenance of sensory qualities and the chemical composition of macro and micronutrients. Next, we will discuss some of these emerging technologies (Ganivet 2020; Jiang et al. 2019; Misra et al. 2017; Pittia and Antonello 2016).

Cold plasma (CP) is a nonthermal technique that uses as a principle the active species contained in ionized gas such as electrons, free radicals, ions, and so on, leading to excitation, de-excitation, and ionization reactions that result in the decontamination and sterilization of food products affected by spores and products of deterioration of pathogenic organisms. It is also used in packaging processing to improve its barrier properties and add antimicrobial activity. The technique can be used by direct CP contact; indirect CP applying plasma-activated water during washes, sprays, or mists; and package CP discharge (Bourke et al. 2018; Ekezie et al. 2017; Fang et al. 2017).

Bosch et al. (2017) studied the effectiveness of using cold plasma (CP) at atmospheric pressure with ambient air in the degradation of mycotoxins produced by species of *Fusarium* spp., *Aspergillus* spp., and *Alternaria alternata*. The study

showed the effectiveness of the method in the degradation of pure mycotoxins, being considered a promising method in the decontamination of food products affected internally or externally as on the surface of cereal grain. Connolly et al. (2013) performed the inactivation of *Escherichia coli* in plastic packaging with helium/air plasma at atmospheric pressure. The authors concluded that the technique was effective in reducing 1.5 logarithmic cycles of specific cell regulatory systems, generating the decontamination of the packaging of fresh products.

The inactivation of microorganisms through sterilization by supercritical carbon dioxide (SC-$CO_2$) is also considered an emerging and promising technique in the treatment of food, as it is a green and environmentally safe technique, preserving the integrity of foods, especially thermosensitive and hydrolytically sensitive. The technique is dependent on variables such as pressure, temperature, $CO_2$ density, treatment time, $CO_2$ flow rate, depressurization rate, and use of additives. The mechanism involved in inactivation has not yet been fully elucidated; however, it is believed that cell rupture may occur due to volume expansion when high-pressure $CO_2$ is suddenly depressurized and/or the physiological deactivation that consists of the simultaneous or isolated occurrence of seven steps:

1. It is derived from acidification of the medium due to the dissociation and formation of carbonic acid from the contact of $CO_2$ with the water present in the medium
2. It results from the change in the cell membrane
3. There is a decrease in intracellular pH
4. The low pH inactivates key enzymes and inhibits cell metabolism
5. There is an inhibitory effect on microbial metabolism through molecular $CO_2$ and $HCO_3$ affecting the reactions of carboxylation and decarboxylation
6. Intracellular electrolyte balance disorder occurs
7. There is the removal of the vital constituents of cells and their membranes (Ribeiro et al. 2020; Soares et al. 2019).

Checinska et al. (2011) validated the sterilization method of biological pathogens using SC-$CO_2$, water (3.3%), and hydrogen peroxide (0.1%), at 80 atm, 50 °C and 30 min. The authors described that the method was effective in sterilizing pathogens present in biofilm structures, fungal spores associated with nosocomial infections, and SAFR-032 endospores of *Bacillus pumilus*. Silva et al. (2013) studied the inactivation of pathogenic *E. coli* through sterilization with SC-$CO_2$, evaluating the effects of pressure, rate of depressurization, and pressure cycling, the authors concluded that the increase in the number of cycles and pressure of the system increased the efficiency of inactivation inferring that the process is useful in non-thermal sterilization of food. Sterilization of palm fruits with SC-$CO_2$ was performed by Omar et al. (2017) to inactivate microorganisms and enzymes responsible for the degradation of palm oil. The authors pointed out that the process was able to completely inactivate lipase-producing microorganisms at 10 MPa, 80 °C and 60 min, avoiding the formation of free fatty acids, moreover, with the process they

managed to drastically reduce the amount of oil from the palm oil plant (POME) generated during steam sterilization.

Active packaging has been used in food preservation in order to isolate them from the external environment and protect them from the action of microbiological, chemical, and physical hazards, maintaining quality and prolonging shelf life. Active packaging interacts with food and is considered more effective compared to the simple addition of active agents on food surfaces by sprays or drips, because in these processes there is a rapid diffusion and the denaturation of the agents by the food may occur, reducing its effectiveness. Agents added to packaging must be safe and have been regulated by competent bodies such as the United States-Food and Drug Administration (U.S.FDA), Brazil National Health Surveillance Agency (ANVISA), and European Commission (Ribeiro-Santos et al. 2017; Sung et al. 2013). Studies have been developed to evaluate, mainly, the antimicrobial and antioxidant effect of active agents from plant extracts as an alternative to the synthetic ones, commonly used.

Ramos et al. (2012) evaluated the antimicrobial activity of polypropylene films incorporated with thymol and carvacrol. The authors pointed out that the thymol impregnated film showed greater inhibition against bacterial strains of *S. aureus* and *E. coli* compared to carvacrol, the compounds also had the potential to be used as agents antioxidants. Peng et al. (2013) investigated the addition of green tea and black tea extracts to chitosan films in the preparation of active packaging film. According to the authors, the addition of the extracts resulted in a decrease in water vapor permeability and increased the antioxidant capacity of the films due to the significant improvement in DPPH radical scavenging activity. Albuquerque et al. (2020) impregnated *Piper divaricatum* essential oil with supercritical $CO_2$ in fish (*Cynoscion acoupa*) skin gelatin films. The authors concluded that the addition of the oil resulted in 41.63% of antioxidant activity index and the film presented greater flexibility and opacity when compared to the control film.

Nanotechnology is a resource that has been used in food science and technology to improve production and application in the areas of processing, packaging, storage, transportation, functionality, and safety. Nanomaterials vary from 1 to 100 nm and are appreciated due to their properties that allow to increase solubility, bioavailability, and protection during the processing and storage of active compounds that are generally chemically unstable, thermosensitive, and photosensitive. Several antimicrobial agents are used in nanoencapsulation such as alkaloids, antioxidants, phytochemicals, essential oils, plant extracts, and so on, reducing microbial contamination and adding functional and sensory properties to foods (Bajpai et al. 2018; Prakash et al. 2018).

Mohammadi et al. (2016) evaluated the antibacterial activity of microparticles prepared with three different molecular weights and nanoparticles of chitosan crab shells against *P. fluorescens*, *E. carotovora*, and *E. coli*. The authors found from the results that there was a positive correlation between the particle size and the molecular weight of chitosan, the maximum antibacterial activity was verified in the nanoparticles and the microparticles, the antibacterial activity was dependent on the application. In the study by Mohanta et al. (2017), the biosynthesis of silver

nanoparticles with the extract of leaves of the *Protium serratum* was performed. The nanoparticles showed antibacterial activity against *P. aeruginosa*, Escherichia coli, and *B. subtilis*. As well as antioxidant activity to DPPH and hydroxyl radicals, and biocompatibility to the L-929 fibroblast cell line, not being harmful to the human body. In addition to oral administration being effective against gastrointestinal diseases and stomach ulcers, the study highlights the potential application in the food industry.

## 6.5 Conclusions

The degradation of food products by microorganisms is almost inevitable, because, like everything in nature, the cycle of the food chain is relentless. However, although the existence of microorganisms predates any other living thing on earth, the evolutionary process has improved these living organisms, especially at the molecular level, with incredible adaptations. The survival of microorganisms in specific conditions and their evolution are the key to a deep understanding of how to intervene in their development, growth, and reproduction and, thus, increase food preservation.

Our modern civilization has had great economic losses due to contamination and subsequent degradation of food. However, modern studies have helped to understand how new technologies can be applied to increase the shelf life of food, or even to reduce contamination rates in products still in the field.

One of the main problems of food contamination by microorganisms is directly related to public health. Foods contaminated with fungi produce mycotoxins, which even in small amounts can be fatal. Therefore, the issue of food security persists as a current issue and one that deserves constant efforts to reduce impacts on human health. Thus, we conclude that in-depth studies are still needed to understand relevant aspects about the evolutionary biology of the main food pathogens and more efforts in the development of green technologies to reduce the contamination rate and increase the shelf life of food.

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# Microbial Degradation of Xenobiotic Compounds

7

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**Abstract**

Xenobiotic compounds are extraneous chemicals accumulated in the environment that are posing a threat to the biosphere. The highly recalcitrant nature of xenobiotic compounds makes them resistant to biological degradation. However, numerous microorganisms have been extensively explored for their competency in the degradation of such compounds. Polycyclic aromatic hydrocarbons, nitroaromatic compounds, aromatic hydrocarbons, as well as halogenated aliphatic, azo compounds, *s*-triazines, and organic sulfonic acids, are essential classes of pollutants with xenobiotic structural characteristics. These compounds must be assessed for their degradation extent employing efficient microbes. In natural environments, the surrounding 'environment's physicochemical attributes may influence and indeed constrain overall biodegradation performance. Moreover, during microbial degradation, several biotic and abiotic factors such as pH,

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temperature, etc., influence the process. The current chapter presents an overview of the microbial degradation of xenobiotics. A systematic approach for efficient degradation and factors affecting the overall process is comprehensively discussed. Further, the current challenges and opportunities concerning the degradation of distinct xenobiotics are also discussed.

**Keywords**

Biodegradation · Xenobiotic · Mineralization · Co-metabolism · Pesticides · Chlorinated hydrocarbons · Haloaromatics · Azo compounds · Nitroaromatic compounds

## 7.1 Introduction

In the last few decades, unprecedented population growth and precipitous industrial development have accelerated the use of new synthetic compounds. These anthropogenic compounds, which are unknown to the living species, are collectively called as xenobiotic. These human-made compounds (inorganic, organic, or metal-containing) with the artificial chemical structure are not a natural component of the biosphere and appended into the environment by artificial means. Hence, synthetic organic compounds (xenobiotics) have become a threat to the environment (Jeffries et al. 2018). In the Greek language, Xenos means "strange" and biotics means "life-related" (Ojo 2007). These strange, exogenous synthetic substances have "unnatural" structural features to which microorganisms have not been exposed during evolution (Greń 2012). Most of the xenobiotic compounds are toxic, and unprecedented release of them in the environment instigates a global concern. The xenobiotic compounds mainly include pesticides, herbicides, fertilizers, pulp/paper bleaching agents, drugs, fuels, solvents, carcinogens compounds, antibiotics, synthetic azo dyes, refrigerants, and other organic compounds (Jha et al. 2015). Xenobiotic compounds are also called recalcitrant, as they resist natural decomposition and remain in the environment for a longer time (Jha et al. 2015). The unusual chemical and physical properties of xenobiotics compounds make them recalcitrant to biodegradation (Godheja et al. 2016). The chemical structural factors, such as the type, number, the relative position of bonds, and the nature of substituents, are mainly accountable for the xenobiotic character that resists microbial enzyme's attack (Anawar et al. 2017). The bond energy of carbon and halogen bond (-C-X) is extremely high and needs a large amount of cleavage energy. Hence, the presence of halogens and other groups, such as nitro, sulfonate, methoxy, amino, etc., as a substitute makes synthetic organic compounds non-degradable (Knapp 2003). The aromatic nature, cyclic structure, and branched linear chains further enhance xenobiotic nature. Furthermore, the high stability, insolubility in water, and considerable molecular weight are the other prime factors for the nondegradability of these compounds (Phale et al. 2019). Based on structural moiety or characteristics, xenobiotic compounds can be classified into six different types. The halocarbons are the primary type of xenobiotic compounds having halogen atoms, namely, CI,

Br, F, or I. The *primary sources* of halocarbons are paints, condenser units of cooling systems (Freons, $CCl_3F$), insecticides (dichloro-diphenyl-trichloroethane (DDT), lindane, etc.), and herbicides (dalapon) and solvents (chloroform, $CHCl_3$). The paper mill effluent also contains different halocarbons, such as pentachlorophenol, tetra-chloro-guaiacols, tetra-chloro-catechol, etc. The *second type* of xenobiotic compounds comprises of two benzene rings covalently linked and substituted with halogens commonly recognized as polychlorinated biphenyls (PCBs). The primary sources of PCBs are mainly plasticizers employed in the synthesis of plastics, coolants used in transformers, and heat exchange fluids (Godheja et al. 2016). The synthetic polymers (polyethylene, polystyrene, polyvinyl chloride, nylons, etc.) used as garments, wrapping materials, etc., comprise the *third type* of xenobiotic compound. The sulfonate ($–SO_3$) group bearing detergents, commonly called alkyl benzyl sulfonates, are the *fourth type* of xenobiotic compound. The oil mixtures, due to their toxicity and insolubility in water, become recalcitrant and constitute the *fifth type* of xenobiotic compounds. Pesticides and herbicides, such as organophosphorous, benzimidazoles, methyl parathion, and morpholine, having an aliphatic or aromatic cyclic ring structure with different groups as a substitute forms the *sixth type of xenobiotic* compounds (Anawar et al. 2017).

The persisting nature of xenobiotics compounds in the environment for long time results in bioaccumulation or biomagnification. The accumulation of harmful synthetic compounds has more prolonged stability in soil that triggers impediments in soil ecosystems. Similarly, the long-lasting occurrence of extensively used aromatic herbicides such as triazines used for constraining the growth of broad-leaved weeds in agricultural fields and urban and recreational areas, alter the soil environmental conditions (Gouma et al. 2014; Cook 1987). Similarly, freshwater and marine ecosystems are also affected by xenobiotic compounds. These toxic compounds are entered into the food chains and accumulated in the high concentration level. The non-degradable pesticide and herbicides straightway influence organisms, soil quality, and also reaches nearby freshwater bodies (Abatenh et al. 2017a). Drugs and antibiotics are foreign to the human body and can be considered as xenobiotics. These compounds can trigger disorder multiple cellular communication pathways directly linked to the growth, development, and normal physiological function (Greń 2012). The crucial components of the cell which interact with xenobiotics are proteins, lipids, and DNA, the latter leading to a mutation that can lead to cancer. Many of the xenobiotic compounds, such as substituted phenolic compounds, phthalates, etc., behave as endocrine disruptors, causing preterm birth, early weaning, and altering the quality of semen, while adversely affecting menstruation cycle and duration of lactation in humans.

The traditional methods employed for the remediation of xenobiotic contaminated sites include chemical treatment, low temperature-induced thermal desorption, incineration, and photocatalytic treatments (Greń 2012). Apart from these, biological methods, such as bioremediation and phytoremediation, are found to be more efficient, safe, economical, and sustainable than thermal and chemical processes (Jha et al. 2015; Anawar et al. 2017). Bioremediation is most widely utilized to remove xenobiotic compounds through degradation, immobilization, or

detoxification of these hazardous materials while employing suitable microorganisms (Greń 2012; Abatenh et al. 2017a). The bioremediation technique encompasses numerous microbial species possessing different degradation mechanisms to eradicate the toxic contaminants from the environment. Microbial degradation of xenobiotics compounds is a natural tactic to confiscate environmental pollution (Poursat et al. 2019). The more comprehensive range of growing conditions and high tolerance to chemical contaminants make microbial degradation an efficient green method for mitigating nondegradable xenobiotics pollutants. The detoxification of xenobiotics compounds through microbial metabolism has been well studied. The comprehensive understanding of microbial transformations of xenobiotic compounds (mainly synthetic organic compounds) with a brief discussion of the removal of different toxic compounds and the factors influencing the processes have been discussed in this chapter.

## 7.2 Microbial Biodegradation

The nutritional requirements of microorganisms are usually organic carbon as a source of energy. This nutritional versatility of microorganisms can be exploited for the degradation of pollutants (Abatenh et al. 2017b). The well-organized detoxification process of contaminants by breaking down to less or non-toxic elements or completely mineralized or transformed into carbon dioxide in the environment employing microorganisms are termed as bioremediation. Usually, bacteria, fungi, and archaea are bioremediators that can be employed as biological agents to carry out bioremediation (Strong and Burgess 2008).

### 7.2.1 Different Microbes for Xenobiotics Degradation

Nearly half of the biomass of earth is microorganisms representing high diversity in the biosphere (Sinha et al. 2009). 'Microbes' vast adaptability propounds a simple, economic, and greener approach to mitigate environmental pollution while facilitating the biodegradation of xenobiotic compounds. Additionally, microorganisms are an integrated part of biogeochemical cycles. Due to high adaptability, microbes can grow at extreme heat, subzero temperatures, dry conditions, and in the absence of oxygen, hence playing an active role in the biosphere's sustainable development (Srivastava et al. 2014). Figure 7.1 depicts the possible ways of biological transformations of xenobiotics in the environment (Greń 2012).

The catabolic activities can be carried out by bacteria-mediated degradation or fungal-mediated degradation and are crucial in converting complex toxic organic compounds into less or completely nontoxic residues (Srivastava et al. 2014). Both aerobic and anaerobic bacterial genera are actively employed to bioremediate a wide range of xenobiotic compounds. Various aerobic bacteria strains like *Escherichia*, *Pandoraea*, *Bacillus*, *Moraxella*, *Pseudomonas*, *Rhodococcus*, *Sphingobium*,

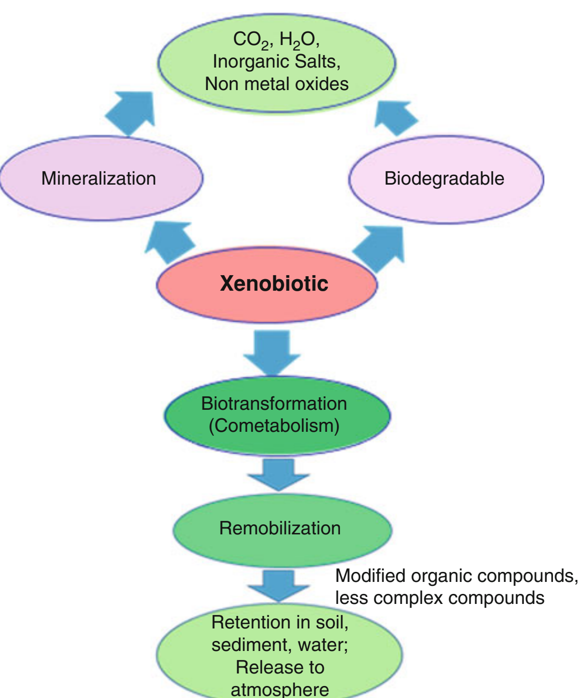


**Fig. 7.1** Possible ways of biological transformations of xenobiotics in the environment (Greń 2012)

*Gordonia*, *Micrococcus*, etc., are employed in the degradation of a wide range of xenobiotic compounds (Van Ginkel 1996; Gangola et al. 2018; Bhatt et al. 2019). Anaerobic bacterial strains, such as *Methanosaeta*, *Pelatomaculum*, *Methanospirillum*, *Desulfotomaculum*, *Syntrophobacter*, *Syntrophus*, *Methanotrophic*, *Methanogenic*, *Cyanobacteria*, etc., are also vastly used in bioremediation (Novotný et al. 2018; Benn and Zitomer 2018). In microbial degradation of organo halogenated compounds such as dichlorodiphenyltrichloroethane, pentachlorophenol, 1,2,3,4,5,6-hexachlorocyclohexane, etc., highly toxic compounds are primarily reduced to less chlorinated intermediates. *Flavobacterium* and *Pseudomonas* strains have been investigated to degrade, specially organo-phosphorous pesticides, aromatic or aliphatic hydrocarbons, phenols, and dyes (Jeffries et al. 2018; Ortiz-Hernández et al. 2003). *Dehalococcoides* sp. has been reported for the decomposition of highly toxic chlorinated hydrocarbons pollutants (Chrast et al. 2019; Saibu et al. 2020). Similarly, polyaromatic hydrocarbons are highly toxic to the ecosystem and also to human health. Various bacterial strains *Pseudomonas*, *Arthrobacter*, *Mycobacterium*, *Sphingomonas*, *Alcaligenes*, etc., are reported as potential agents for the bioremediation of various aromatic hydrocarbons, such as naphthalene, substituted benzene, substituted aniline, xylene, anthracenes, phenolic compounds, etc. (Patil and Yadav 2018; Mpofu et al. 2020; Miyazawa et al. 2020; Tusher et al. 2020). Three enzymes, *esterases*, *permeases*, and *dioxygenases*,

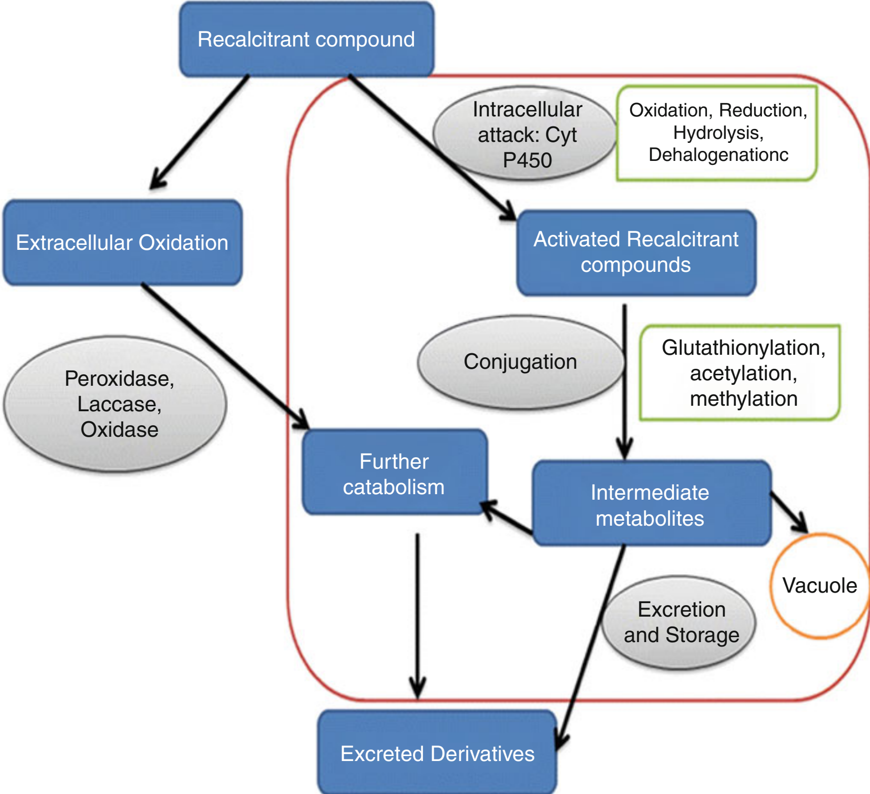


**Fig. 7.2** Fungal degradation of xenobiotic compounds (Copyright© 2016, Springer Nature. All rights reserved, reprinted with permission) (Deshmukh et al. 2016)

consecutively degrade phthalate isomers (Vamsee-Krishna and Phale 2008). Bacillus bacterial species effectively mineralize benzimidazole compounds and oil spills (Xu et al. 2018). The dyes get bioaccumulated in natural environments due to high chemical and protolithic stability and pose a negative impact on the food chain. Synthetic and azo dyes used in textile industries can be degraded by bacteria strains, such as *Anoxybacillus*, *Bacillus*, *Exiguobacterium aurantiacums*, *Sphingomonas* sp. *Xanthomonas* sp., etc. (Takon 2019).

The fungi induced biodegradation (Fig. 7.2) of xenobiotic compounds is termed as mycoremediation (Ceci et al. 2019). The fungal-mediated biodegradation is more robust than the bacterial degrading processes, as the fungi can nurture in the presence of high concentrations of toxic organic pollutants (Akhtar and ul Mannan 2020). The rapid colonization of substrates due to 'fungi's mycelial nature facilitates deep infiltration into the pollutant molecule and completely mineralizes it (Bielčik et al. 2019). The degradation of organic pollutants by fungi from aqueous solution is facilitated through the adsorption process. The fungal species, such as

basidiomycetes and ascomycetes, degrade polycyclic aromatic hydrocarbons (PAHs) through laccases, a copper-containing enzyme (Viswanath et al. 2014; Arregui et al. 2019). The petroleum hydrocarbons are completely mineralized by *Pleurotus pulmonarius* an edible rot fungus, *Morchella conica*, and *Tylospcno fibrilnsa* of *Mycorrhizal* species (Liu et al. 2020). The fungal species, such as *Aspergillus niger*, white-rot fungus, *Phanerochaete chrysosporium*, etc. are widely employed in the bioremediation of fertilizers and pesticides (Deshmukh et al. 2016).

To enhance the efficiency of biodegradation of xenobiotic compounds, isolation of bacterial strains with unique catabolic capabilities and genetically modification of degradative pathways is essential (Tahri et al. 2013). The development of engineered strains with superior biodegradation capability becomes a challenge to the scientific community. Genetically engineered microorganisms (GEMs) can be developed by modifying enzyme specificity and affinity, construction pathway, bioprocess development, monitoring, and control (Tahri et al. 2013). From the past decades, genetically engineered microorganisms emerge as an efficient option for the complete degradation of xenobiotic compounds. Table 7.1 depicts a summary of bacterial and fungal genera efficiently utilized in the biotransformation of xenobiotic compounds.

## 7.2.2 Parameters Influencing the Rate of Biodegradation

The regulation and optimizing of microbial degradation of xenobiotic compounds is a complex process. Its efficiency depends on various factors such as the chemical nature and concentration of pollutants, their availability to microorganisms, 'microorganisms' nature, and environmental factors (Abatenh et al. 2017a). The slow rate of microbial degradation of contaminants is associated with the environmental factors, such as pH, temperature, low moisture, presence of oxygen/other electron acceptors, nutrient contents, and also the chemical nature of pollutants (Knapp 2003).

### 7.2.2.1 Chemical-Specific Factors

#### State/Solubility/Hydrophobicity

The microbial degradation is an enzyme-mediated reaction, generally taking place in an aqueous medium. The most important factors affecting the degradation are the substrate's (pollutant's) solubility, state, and hydrophobicity. The 'substrate's physical condition is crucial for biodegradation as many of the liquids and solids recalcitrant organic compounds have low solubility, which may resist degradation. Even the surface area of pollutants affects the rate of biodegradation. The finely divided small particles with greater surface area enhance the attachment of degrading microbes on pollutants and further enhance the degradation rate (Jain et al. n.d.). The hydrophobic property of some organic compound's steers bioaccumulation in the fatty tissues of higher organisms. A hydrophobic organic compound may cause slight damage at lower trophic levels; however, it gets drastically high toxicity for higher trophic levels in the food chain (Jha et al. 2015).

**Table 7.1** Bacterial and fungal genera efficiently utilized in the biotransformation of xenobiotic compounds

| Compounds | Bacteria | References | Fungus | References |
|---|---|---|---|---|
| *Pesticide* | | | | |
| DDT | *Alcaligenes eutrophus*, *Dehalospirilum multivorans* | Sinha et al. (2009) | *Ph. chrysosporium*, White-rot fungi | Ojo (2007) |
| 2,4-Dichlorophenoxyacetic acid (2,4-D) | *Flavobacterium Arthortbacter*, *Pseudomonas cepacia*, *Alcaligenes eutrophus* | Sinha et al. (2009), Ortiz-Hernández et al. (2003) | *Umbelopsis isabellina* | Nykiel-Szymańska et al. (2018) |
| Atrazine | *Nocardia*, *Pseudomonas*, *Rhodococcus* | Sinha et al. (2009), Ambrosoli et al. (2005) | *T. versicolor* , *Pl. ostreatus*, *Ph. Chrysosporium*, *Pleurotus pulmonaris* | Knapp (2003), Phale et al. (2019) |
| Parathion | *Flavobacterium*, *Pseudomonas dimuta*, *Acenetobactor* sp., *Pseudomonas* sp., *Enterobacter* sp. and *Photobacterium* sp. | Jha et al. (2015), Knapp (2003) | *Aspergilus niger* AN400 | Abo-Amer (2011) |
| Diazinon | *Flavobacterium* | Abo-Amer (2011) | *Apergillus niger* MK640786 | Abo-Amer (2011) |
| Fenthion | *Bacillus* | Gangola et al. (2018) | White-rot fungus *Phanerochaete chrysosporium* | Rani et al. (2011) |
| Carbofuran | *Achromobacter*, *Pseudomonas*, *Flavobacterium* | Jha et al. (2015), Knapp (2003) | Genus *Gliocladium* | Parte et al. (2017) |
| Lindane | *Bacillus* sp., *Chryseobacterium joostei* | Gangola et al. (2018) | *Ph. Chrysosporium*, *Pleurotus florida*, *Phanerochaete eryngi* | Ulčnik et al. (2013) |
| *Halogenated organic compounds* | | | | |
| Vinylchloride | *Dehalococcoides* sp. | Yoshikawa et al. (2017), Saiyari et al. (2018) | *Aureobasidium pullulans* | Webb et al. (2000) |
| PCE (trichloroethylene) | *Dehalococcoides ethenogenes*195 | Novotný et al. (2018) | *Graphium*, *Trametes versicolor*, *Ganoderma lucidum*, and *Irpex lacteus*, | Marco-Urrea et al. (2008) |
| *Aromatic hydrocarbons compounds* | | | | |
| Napthalene | *Pseudomonas putida* | Nwinyi et al. (2016) | *P. chrysosporium* and *T. harzianum* | Ghosal et al. (2016) |

| PCP (pentachlorophenol) | *Psedomonas* sp. | Wald et al. (2015) | *T. versicolor*, *Absidia* and *Cunninghamella* | Patil and Yadav (2018), Bhatt et al. (2019) |
|---|---|---|---|---|
| 3CBA (3-Chloeo benzoic acid) | *Arthrobacter* sp. | Jeffries et al. (2018), Mpofu et al. (2020) | White rot and *Ectomycorrhizal* | Ghosal et al. (2016) |
| 1,4 DCB | *Alcaligenes* sp. | Benn and Zitomer (2018), Kurt and Spain (2013) | *Pl. ostreatus* | Marco-Urrea et al. (2009) |
| 2,3,4-Chloroaniline | *Pseudomonas* sp. | Yoshikawa et al. (2017), Nwinyi et al. (2016) | *Basidiomycetes* | Bielčik et al. (2019) |
| 2,4,5-T (2,4,5-trichlorophenoxy acetic acid) | *Pseudomonas* sp. | Liang et al. (2014) | *Eupenicillium* sp. | Itoh et al. (2013) |
| Fluoranthrene | *Pseudomonas cepacia* AC1100 | Nwinyi et al. (2016), Wald et al. (2015) | *Penicillium janthinellum* VUO 10,201 | Boonchan et al. (2000) |
| Pyrene | *Mycobacterium* PYR-1, *Sphingomonas paucimobilis* | Sinha et al. (2009), Yang et al. (2013) | *Trichoderma harzianum*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Crinipellis stipitaria* | Ghosal et al. (2016) |
| Xylene | *Penicillium chrysogenum*, *Pseudomonas putida* *Phanerochaete chrysosporium*, *Dechloromonas* sp. (RCB) | Takon (2019), Wald et al. (2015) | *Cladophialophora* sp. | Tahri et al. (2013) |
| 4-Chlorophenol | *Alcaligenes* sp. | Miyazawa et al. (2020) | *R. rhodochrous* | Ghosal et al. (2016) |
| Dioxins | *Dehalococcoides* sp. | Sinha et al. (2009), Tusher et al. (2020) | *Basidiomycetous* | Nakamiya et al. (2005) |
| RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) | *Desulfovibrio* sp. | Sinha et al. (2009) | *Cladosporium resinae*, *Cunninghamella echinulata varelegans*, *Cyathus pallidus* and *Phanerochaete chrysosporium* | Ghosal et al. (2016) |

(continued)

**Table 7.1** (continued)

| Compounds | Bacteria | References | Fungus | References |
|---|---|---|---|---|
| Benzene | *Dechloromonas* sp. | Jeffries et al. (2018), Strong and Burgess (2008) | *Cladophialophora* and *Cladosporium* | Tahri et al. (2013) |
| *Phthalate compounds* | | | | |
| Phthalate | *Burkholderiacepacia*DBO1 | Sinha et al. (2009), Vamsee-Krishna and Phale (2008) | *Aspergillus*, sp., *Fusarium* sp., and *Penicillium* sp. | Steliga (2012) |
| *Azo dyes* | | | | |
| Reactive Dark Blue K-R | *Exiguobacterium* sp. | Sarkar et al. (2017) | *Penicillium* sp. QQ | Steliga (2012) |
| Reactive Green 19 | *Proteus vulgaris*, *Micrococcus glutamicus* | Liang et al. (2014) | *Trametes versicolor* U97 | Singh and Singh (2017) |
| Acid Orange 7 | *Aeromonas caviae*, *Protues mirabilis* and *Rhodococcus globerulus* | Singh and Singh (2017) | *Enterococcus faecalis* | Singh and Singh (2017) |
| Sulfonated di-azo dye reactive red HE8B, RNB dye | *Bacillus* sp. ETL-2012, *Pseudomonas aeruginosa*, *Bacillus pumilus* HKG212 | Gangola et al. (2018) | *Pl. ostreatus*, *Micrococcus glutamicus* NCIM-2168 | Ferraz et al. (2011) |
| Acid Orange 7 | *Aeromonas caviae*, *Protues mirabilis* and *Rhodococcus globerulus* | Singh and Singh (2017) | *Enterococcus faecalis* | Singh and Singh (2017) |
| Red HE3B, Remazol black 5B, red HE7B | *Providencia* sp., *Pseudomonas aeuroginosa Alcaligenes faecalis* and *Commamonas acidovorans* | Rieger et al. (2002) | *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergilus terreus* and *Penicillium* sp. | Singh and Singh (2017), Wesenberg et al. (2003) |
| *Organophosphorous compounds* | | | | |
| Chlorpyrifos | *Flavobacterium* sp. ATCC2755,1 *Pseudomonas diminuta*, *Micrococcus* sp, *Enterobacter* sp. | Ortiz-Hernández et al. (2003), Nabil et al. (2011) | *Phanerochaete chrysosporium*, *Pencillium brevicompactum*, *Trichoderma harzianum*, *Aspergillus sydowii*-CBMAI 934, *Penicillium raistrickii* CBMAI 931 | Kumar et al. (2018) |

| Fenitrothion | *Flavobacterium* sp., *Arthrobacter aurescenes* TW17, *Burkholderia* sp. NF100 | Ortiz-Hernández et al. (2003) | *Aspergillus parasiticus*, *Trichoderma viride* | Rani and Devi (2018) |
|---|---|---|---|---|
| Ethoprophos | *Flavobacterium* sp., *Pseudomonas putida*, *Enterobacter* sp | Ortiz-Hernández et al. (2003), Karpouzas et al. (2005), Mardani et al. (2017) | *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium moniliforme*, *Trichothecium* | Kumar et al. (2018), Singh and Walker (2006) |
| Cadusafos | *Flavobacterium* sp. | Karpouzas et al. (2005) | *Gliocladium* | Javaid et al. (2016) |

### Size and Shape

In general, the degradation of pollutants is an enzyme-induced biochemical process. The degradative enzymes are located inside a microbial cell and within the cytoplasmic membrane, acting as a selective permeability barrier to the foreign molecule. Further, the formation of an enzyme–substrate complex is necessary to initiate any enzymatic degradation reaction (Jain et al. n.d.). The inability of most of the recalcitrant compounds in complexing with the enzyme's active site due to their size, and probably their shape becomes a crucial factor in affecting the rate of degradation, and this can be overcome by employing techniques using extracellular/isolated enzymes (Knapp 2003).

### Toxicity

Various xenobiotic compounds are toxic to some or more extent to most of the microorganisms. The chloro or nitro derivatives of phenols and fungicides are highly toxic to many microorganisms, as they kill susceptible microbes or merely inhibit their growth (Knapp 2003). For example, toluene is highly toxic to most of the bacteria and damages their membrane structure. Many nontoxic chemicals kill microbes while inhibiting a specific key enzyme during the degradative pathway.

### Concentration

When the xenobiotic compounds present in less amount in the ecosystem, the lack of biodegradation is observed, it may be due to low concentrations of compounds that do not provoke the formation of the enzymes required to degrade it. Even low organic compound concentrations may not provide a sufficient amount of carbon and energy to microorganisms. It was reported that 75% mineralization of herbicide 2,4-Dichlorophenoxyacetic acid (2,4-D) in stream water took place in just 8 days when the concentration of 2,4-D was between 0.00022 and 0.022 g/L. However, when the concentration was less than 0.0000022 g/L, only 10% mineralization was observed (Knapp 2003).

### Molecular Structure

Although xenobiotic 'compounds' fate depends on the entire molecule properties, the presence of different subunits may affect the degradation rate process to some extent. Microbial enzymes quickly disintegrate the functional 'groups' presence, including carboxylic acids, esters, and amide bonds, as all these structural moieties are vulnerable to hydrolysis and do not require any special coenzymes. Whereas amine compounds are less susceptible to degradation than amides due to the requirement of oxygenase, oxidases, or dehydrogenases enzyme and specific co-factors (Abatenh et al. 2017a). The presence of some structural moiety hinders the degradation process. For instance, the quaternary carbon atom (carbon atom that has attached to four other carbon atoms), impervious to degradation as they resist the formation of a carbon–carbon double bond (Knapp 2003). Moreover, the degree of branching also affects biodegradability due to the formation of quaternary carbon (Jha et al. 2015). The presence of diazo linkage, aromatic sulfonic acids, and polychlorinated aromatic or aliphatic moiety is less vulnerable to biodegradation

due to their xenobiotic nature. Similarly, the presence of several substituents also affects the rate of biodegradation. It is clearly understood with the aerobic degradation of the chlorinated phenoxy-acetate herbicides (such as 2, 4-D and 2,4,5-trichlorophenoxy acetic acid (2,4,5-T)). Many microorganisms are capable of degrading 2,4-D. The presence of one extra chlorine atom in 2,4,5-T makes it more resistant to mineralization. Similarly, the position of substituents also affects the rate of microbial degradation. The ortho and para isomers of disubstituted aromatic compounds showed a similar type of the degradability due to similar electron pattern. In contrast, meta isomers of disubstituted aromatic compounds showed varied degradability.

#### Type of Microbe

In case of bacterial degradation, the Gram-positive and Gram-negative bacteria respond differently to organic, inorganic, and metal-bearing substrate. Hence, the type of bacterial strain also affects the rate of degradation. Due to diverse metabolism, Gram-positive bacteria can adhere to a wide range of aromatic hydrocarbons, metal-bearing substrate, and biopolymers (Narancic et al. 2012). Gram-positive bacteria produce spores as a response when it comes in contact with toxic chemicals and extreme physical conditions. The most utilized bacteria for bioremediation are *Bacillus anthracis* and *Streptomycetes* sp. followed by *Rhodococcus*, *Arthrobacter*, *Gordonia*, and *Nocardia genus* for the disintegration of aromatic organic compounds like benzene and its derivatives, naphthalene, and biphenyls, etc. On the contrary, the presence of lipopolysaccharide outer membrane in Gram-negative bacteria makes them more permissive to toxic compounds (e.g., PAHs).

### 7.2.2.2 Environment-Specific Factor

#### pH

The metabolic processes that occur during microbial degradation of xenobiotic compounds include bio-sorption, a pH-dependent phenomenon. The contaminated site's pH also affects the net negative charge on the microbial cell surface and alters the isoelectric point in the solution. Hence, even a slight change in pH affects the rate of metabolic processes. The acidic pH increases the solubility of metal ions, which further intensifies metal ions' adherence to get attached to the microbial cell surface (Strong and Burgess 2008). The overall efficiency of microbial degradation enhances at lower pH (Rehan 2016). Additionally, ligands' ionic state such as carboxyl residue, phosphoryl residues, S–H groups, and amino acid groups changes with change in pH value, thereby altering the degradation process(Srivastava et al. 2014).

#### Physical Factors

The critical physical factor affecting the rate of bioremediation is *temperature*. It either enhances or slows down the bioremediation process. The survival of microorganisms entirely depends on temperature. In low-temperature environments, such as polar regions, there will only be a constrained and specified microflora,

which is responsible for the decrease in the degradation process"s capability. The microbes metabolically get inactive at a sub-zero temperature in this region due to the ceasing of the transportation phenomenon within the microbial cells leads to even freezing of the entire cytoplasm. The other physical factor affecting the rate of biotransformation is pressure. The environmental conditions, such as high pressure at the abyssal depths of the oceans, constrain the microflora (Abatenh et al. 2017b).

#### Nutrient Availability

For growth and reproduction, microorganism needs various nutrients. The carbon–nitrogen–phosphorus (C:N:P) ratio is a crucial factor in the balancing nutrient and the biodegradation efficiency (Singh and Singh 2017). The addition of specific nutrients in appropriate quantity is the best possible way to increase the efficiency of bioremediation. In a few particular biodegradations, some particular nutrients, such as metals as a cofactor, are required to carry specific enzymatic conversions. The degradation process facilitated by the cyanocobalamin enzyme of microorganisms that contain vitamin B12 as a prosthetic group/cofactor requires cobalt (Liu et al. 2014). Similarly, white-rot fungi require manganese when manganese peroxidase is used in the biodegradation process (Viswanath et al. 2014). Few microorganisms need growth factors, such as specific amino acids, nucleotides, or vitamins/cofactors. Few nonessential cofactors could also enhance the rate of particular degradation. For example, flavin nucleotides (FAD) addition increase the rate of anaerobic reduction of azo dyes (Viswanath et al. 2014).

#### Presence of Oxygen

The microorganisms facilitate the degradation process through aerobic and anaerobic modes. The oxygen requirement diverges from one organism to another based on their degradation route. It is observed that hydrocarbon metabolism gets enhanced in the presence of oxygen. Generally, the aerobic mechanism is the most versatile metabolism. However, various investigations in anaerobic mechanisms have been carried out to mineralize a wide range of xenobiotic compounds, such as chlorinated phenols, PAHs, and PCBs. It is noted that the more highly chlorinated compounds are more vulnerable to anaerobic degradation through the reductive chlorination mechanism. Whereas the less chlorinated compounds are more susceptible to aerobic degradation (Singleton 1994). The microbial degradation of lignin and lingosulfonates in wood pulping is only facilitated in the presence of oxygen (Knapp 2003). In certain biodegradative reactions, degradation of polychlorinated organic compounds, such as dichloro-diphenyl-trichloroethane (DDT) or lindane, is effectively carried out anaerobically. In some cases, the degradation process gets hindered in the presence of $O_2$. For example, in the mineralization of azo dyes, electrons get expended in the reduction of oxygen rather than the cleavage of the target compound's diazo bond resulting in a decrease in the efficiency of the biodegradation process.

**Inhibitory Materials**

Some toxic compounds act as inhibitors, which may either inhibit the degradation process or kill microbes in the process. The xenobiotic compounds such as phenol, toluene, or cyanide hinder a wide range of microbes. Some natural compounds (e.g., phenolic components derived from decomposing plant tissues, or heavy metals from ores) are also encumbering the degradative processes. Phenol and 'toluene's high concentration is highly toxic to microorganisms, whereas, at low concentrations, they usually degrade. Even higher salinity inhibits microbial growth.

On a broader scope, to enhance the understanding of the essence of microbial degradation of toxic xenobiotic contaminants in the environment based on the type of xenobiotic compound, microbes used, aerobic and anaerobic degradation, and utilization of genetically engineered microbes are needed to be considered.

## 7.3 Microbial Degradation of Different Xenobiotics

### 7.3.1 Polycyclic Aromatic Hydrocarbons

The toxicity, low bioavailability, genotoxicity, poor biodegradability, and carcinogenic nature of polycyclic aromatic hydrocarbons (PAHs) exemplify them as organic pollutants. PAHs enter in the environment and accumulate in food chains while emerging as hazardous environmental pollutants posing a threat to public health. PAHs are aromatic compounds consisting two or more fused benzene rings in angular, linear, and cluster arrangements (Cerniglia 1992; Schützendübel et al. 1999). The US Environmental Protection Agency (US EPA) listed 16 PAHs as the most hazardous and human carcinogen pollutants. Chemically, PAHs are non-polar colorless to pale yellow or light greenish solids with a pleasant odor and comprise nitrotoluene and chlorinated organic compounds, such as pentachlorophenol, polychlorinated biphenyl, and chlorinated dioxin. PAHs are broadly spread as pollutants in the air, soil, freshwater reservoirs, sediments, surface water, and groundwater systems (Chang et al. 2014). Anthropogenically, PAHs are emitted into the environment during the incomplete combustion of coal, tar, petrochemical, gaseous fuel, automobile exhaust, etc. during industrial and other human activities or accidental discharge (Megharaj et al. 2011). Naturally, PAHs are formed during forest fires, volcanic eruptions, etc. (Abdel-Shafy and Mansour 2016). PAHs are formed as a waste product during the pyrolysis of organic substances at high temperatures. The type of product formed depends on the starting material's chemical nature and transformation temperature (Megharaj et al. 2011). Among PAHs, phenanthrene compound photosensitized human skin and caused mild allergies. Benzo (pyrene) was identified as highly carcinogenic for human beings. The high covalent binding of PAHs to DNA, RNA, and proteins can be correlated with carcinogenicity (Santarelli et al. 2008). Few transformation products of PAHs are more hazardous than parent PAHs. For example, at the cellular level, enzyme cytochrome P450 monooxygenase oxidizes PAHs into epoxides in the human body. These epoxide products are highly reactive and alter normal cells into a

cancerous one. PAHs also alter the functioning of hormone metabolizing enzymes of the thyroid glands and harm the reproductive and immune system (Rubin 2001). The bioremediation of PAHs using bacterial, fungal, and algal species depends on the surrounding conditions, such as nutrients, number and species of the microorganisms employed, chemical nature of the PAHs, etc. The different bacterial species were explored for the degradation of PAHs, including *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Alcaligenes*, *Nocardia*, *Bacillus*, *Arthrobacter*, and *Acinetobacter*, (Table 7.1). Similarly, various fungal strains employed for the degradation, including *Aureobasidium*, *Candida*, *Rhodotorula*, and *Sporobolomyce*s (found in marine environments), *Trichoderma*, *Mortierella*, etc. (found in marine and soil environments).

There are two bacteria-mediated approaches (*aerobic and anaerobic*) depending on oxygen involvement in PAHs' complete mineralization using microorganisms. The aerobic catabolism of PAHs is carried out by hydroxylation and oxygenolytic mediated aromatic ring cleavage. In this type of degradation, the oxygen acts as a final electron acceptor and a co-substrate. The *aerobic bacterial degradation* of PAHs embracing either monooxygenase or dioxygenase enzymes mostly under aerobic conditions. The detailed mechanism of dioxygenase mediated biodegradation was elaborated by several studies (Müller et al. 1998). Dioxygenase enzyme, a multicomponent enzyme, generally comprises reductase, ferredoxin, and terminal oxygenase subunits (Mallick et al. 2007). The general mechanism of aerobic degradation of PAHs consists of two steps; first, *cis*-dihydrodiol is formed due to hydroxylation of an aromatic ring via dioxygenase. The product *cis*-dihydrodiol further aromatized by dehydrogenase enzyme and forms a diol intermediate. These so formed diols also undergo ortho-cleavage or meta-cleavage by dioxygenases, forming catechols that are further converted as TCA cycle intermediates (Rubin 2001). Mallick et al. proposed a biodegradation mechanism of phenanthrene carried by *Staphylococcus* sp. strain PN/Y isolate from petroleum-contaminated soil (Mallick et al. 2007). The metabolic pathway for phenanthrene initiates with deoxygenation of phenanthrene at 1,2-position followed by meta-cleavage of phenanthrene-1,2-diol break down at meta-position to 2-hydroxy-1-naphthoic acid. The formed acid product then undergoes a series of reactions to yield catechol, which is further metabolized by catechol-2,3-dioxygenase to 2-hydroxymuconaldehyde acid (TCA cycle intermediates). The bacterial degradation of PAHs also proceeds through the *cytochrome P450-mediated pathway*, which involves the production of *trans*-dihydrodiol (Rubin 2001; Harvey 1996). Moody et al. have proposed the mechanism of the degradation of more carcinogenic benzo-[*a*]pyrene by *Cytochrome* P450 using *Mycobacterium vanbaalenii* PYR-1 strain (Fig. 7.3) (Moody et al. 2004). In the degradation of benzo-[*a*]pyrene mediated by *Microsomal cytochrome* P450 enzymes; first 10-oxabenzo[def]chrysen-9-one get formed, which further gets converted into benzo[*a*]pyrene *cis*-9,10-dihydrodiol through deoxygenation at C-9 and C-10. The successive meta cleavage and aromatic-ring closure form the dihydroxy intermediate, i.e., 10-oxabenzo[def]chrysen-9-one. In another route, benzo[*a*]pyrene get oxidized to *cis*- and *trans*-11,12-dihydro-11,12-dihydroxybenzo [*a*]pyrene mediated by *M. vanbaalenii* PYR-1. The *Cytochrome* P450 and *Epoxide*

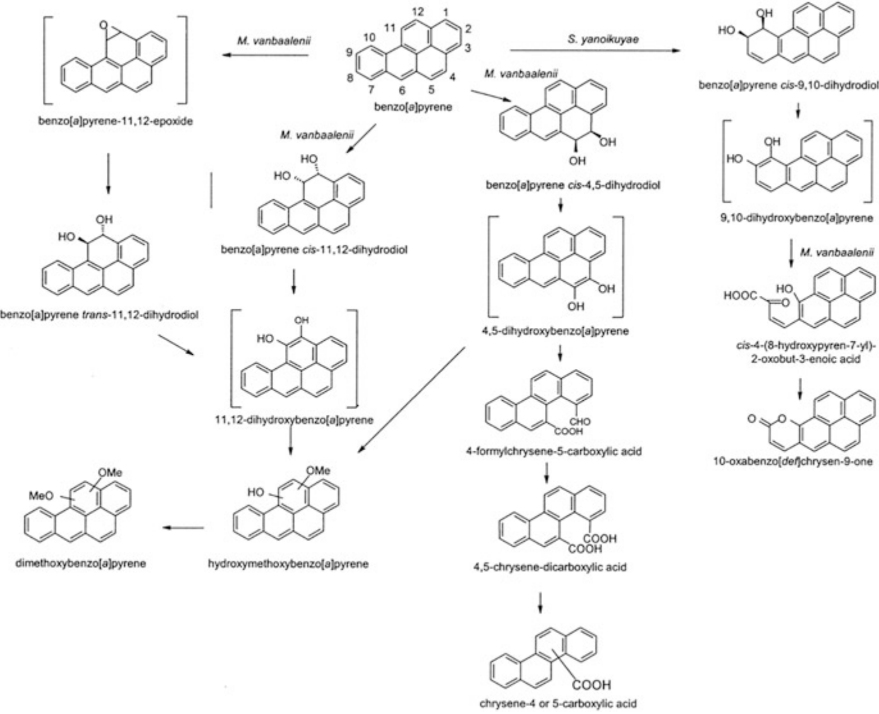


**Fig. 7.3** Pathway for the degradation of benzo[*a*]pyrene by *M. vanbaalenii* PYR-1. Compounds in brackets are hypothetical intermediates (Copyright© 2004, American Society for Microbiology. All rights reserved, reprinted with permission) (Moody et al. 2004).

*hydrolase* act on benzo[*a*]pyrene *trans*-11,12-dihydrodiol and convert it into benzo [*a*]pyrene 11,12-epoxide. To explore the metabolic ability of 15 different bacterial isolates segregated from petroleum oil-contaminated sites, they were investigated at controlling factors concerning PAHs' degradation rates (Saeed et al. 2011). It was reported that the maximum of PAHs was optimally degraded at 15°C at an oxygen level of 4 ppm. Simarro et al. studied the impact of different parameters such as C/N/P ratio, sources of the nitrogen, carbon, and iron, the iron concentration, and pH on biodegradation on naphthalene, phenanthrene, and anthracene with a degrading bacterial consortium C2PL05 (Simarro et al. 2011). The high biodegradation efficiency was observed when the optimized factors were at the ratio (100:21:16) for C/N/P using 0.1 $mmol^{-1}$ concentration of $NaNO_3$ and $Fe_2(SO_4)_3$ at pH 7 on a mixture of glucose and PAHs (as carbon source).

The unavailability of oxygen in an environment poses a crucial challenge to microorganisms to exploit aromatic compounds as growth substrates (Fuchs et al. 2011). The lack of molecular oxygen in anaerobic conditions bestows redox potential as a critical factor for biodegradation of PAHs in soils, sediments, and aquifer systems (Karthikeyan and Bhandari 2001). Various studies highlighted the efficient

anaerobic degradation of fluorine, anthracene, phenanthrene, naphthalene, acenaphthene, etc. (Carmona et al. 2009; Cui et al. 2008). PAHs" anaerobic degradation usually involves the reductive destruction of the aromatic ring using nitrate, sulfate, or ferric ions as a final electron acceptor (Carmona et al. 2009). The bacterial degradation of PAHs *under anaerobic conditions* becomes possible when PAHs are bioavailable in a sufficient amount, and the presence of particular inorganic electron acceptors (nitrate, sulfur, etc.) in the native microflora encompasses degradative enzymes with genetic setup for encoding. The reduction of nitrate ($NO_3^-$) into different nitrogen oxides and molecular dinitrogen using bacteria is known as *microbial denitrification* in which nitrate assist as a terminal electron acceptor in the oxidation of PAHs (Karthikeyan and Bhandari 2001). Various studies highlighted the PAHs degradation under nitrate-reducing conditions in a different anoxic habitat, such as soils, lakes, rivers, oceans, petrochemicals contaminated site, oil spillage, sewage sludge, etc. (Ambrosoli et al. 2005; Liang et al. 2014; Chang et al. 2002; Dou et al. 2009; Wang et al. 2012). Both low- and high-molecular-weight PAHs are mineralized by nitrate-reducing facultative anaerobes. The microbial mineralization of naphthalene under denitrifying conditions in pristine and oil-contaminated soil slurry was successfully reported by Al-Bashir et al. (Al-Bashir et al. 1990). The complete mineralization of naphthalene and 2-MN in contaminated arctic soil at low temperatures under nitrate-reducing conditions using genera like *Acidovorax*, *Bordetella*, *Pseudomonas*, *Sphingomonas*, and *Variovorax* was investigated (Eriksson et al. 2003). Lu et al. highlighted that the 2- and 3-ring compounds among the 16 priority PAHs are mineralized by sediment enrichment culture under denitrifying conditions rather than sulfate-reducing prerequisites (Lu et al. 2012). The microbial degradation of highly toxic benzo(*a*)pyrene, a class 2A carcinogen identified by the International Agency for research in cancer (IARC), is well studied. The benzo(*a*)pyrene, fluoranthene, and phenanthrene were completely mineralized by using *Pseudomonas* sp. JP1 form river sediment (Yang et al. 2013). Qin et al. has studied *Microbacterium* sp. strain activity under the denitrifying products nitric oxide and nitrous oxide as electron acceptors and disintegrated around 84.2% of benzo(*a*)pyrene at the BaP/nitrate ratio of 1:33 in 10 days (Qin et al. 2017). The sulfate-reducing bacteria are ubiquitous in freshwater, groundwater and marine sediment, volcanic mud, and anaerobic sludge (Muyzer and Stams 2008). The common sulfate-reducing bacteria are *Deltaproteobacteria*, *Clostridia*, *Nitrospirae*, *Thermodesulfobacteria*, and *Thermodesulfobiceae*. During anaerobic degradation of PAHs, the reduction of sulfate occurs with the formation of hydrogen sulfide ($H_2S$) (Meckenstock et al. 2016). The naphthalene and 2-methylnaphthalene were successfully degraded by N47 (derived from the soil of a contaminated aquifer near Stuttgart, Germany) comprised of an unidentified member of *Deltaproteobacteria* in association with 7% of *Spirochaetes* members (Safinowski and Meckenstock 2004). Rothermich et al. successfully revealed the *in situ* mineralization of 14C-naphthalene and 14C-phenanthrene in petroleum-contaminated, anoxic, and sulfidogenic harbor sediments (Rothermich et al. 2002). The biotransformation of fluorene and phenanthrene could be carried through a series of hydration and hydrolysis reactions followed by decarboxylation with

subsequent p-cresol and phenol (Tsai et al. 2009). Himmelberg et al. have reported TRIP1, a new sulfate-reducing enrichment culture belong to the *Desulfobacteraceae* family for phenanthrene degradation (Himmelberg et al. 2018). The initial reaction involves the degradation *via* carboxylation to 2-philanthropic acid as the primary intermediate. The intermediate is then converted into corresponding CoA ester by the enzyme 2-phenanthroate-CoA ligase.

Apart from bacterial degradation, some fungi are also used to degrade PAHs by co-metabolizing PAHs into diverse oxidized products and $CO_2$. The bacterial degradation of PAHs mainly incorporates *Dioxygenase* enzymes and partially monooxygenase-mediated reactions, whereas fungal degradation only involves monooxygenase enzymes. The fungal mineralization of PAHs follows various enzymatic pathways, and the efficiency of this biotransformation mostly depends on fungal species and growth conditions (Cerniglia 1992). Both of the ligninolytic fungi/white-rot fungi and non-ligninolytic fungi are employed for the degradation of PAHs. The metabolic activity of ligninolytic fungi implicates lignin peroxidase (LiP), manganese peroxidase (MnP), and laccases (a phenoloxidase enzyme) are capable of mineralization of xenobiotic compounds. The aromatic ring present in PAHs structure is oxidized by hydroxyl free radical produced by ligninolytic enzymes, and PAH-quinones and acids are formed. The activity of non-ligninolytic fungi such as *Cunninghamella elegans* includes P450 monooxygenase like enzymes that facilitate initial oxidation of PAHs. The ring epoxidation reaction is catalyzed by P450 monooxygenase. This catalytic reaction yields arene oxide, which is unstable and immediately converted into *trans*-dihydrodiol through epoxide-hydrolase catalyzed reaction (Tortella et al. 2005). The arene oxide formed during the metabolic activity of cytochrome P450 can also be reorganized into phenolic derivatives via non-enzymatic reactions in conjugation with sulfate, xylose, glucuronic acid, or glucose (Pothuluri et al. 1996). The other ligninolytic fungi, (brown-rot fungi) such as *Flammulina velutipes* and *Laetiporus sulphureus*, metabolize PAHs like fluoranthene, phenanthrene, and fluorine and mostly produces hydrogen peroxide to degrade hemicelluloses and cellulose (Li et al. 2010). The high degradation of low-molecular-weight (LMW) PAHs (2–3 ring compounds) was perceived in *Aspergillus* sp., *Trichocladium canadense*, and *Fusarium oxysporum(*Silva et al. 2009*)*. The complete degradation of high-molecular-weight (HMW) PAHs (4–7 rings) was reported in, *Acremonium* sp, *Aspergillus* sp., *Verticillium* sp., *and T. canadense*.(Kumar et al. 2018) The total mineralization of PAHs by fungi insinuate that it can be utilized as a valuable candidate for the degradation of PAHs in contaminated sites.

The biodegrading potential of naturally occurring microorganisms can be boosted by enhancing the metabolic activity or broad substrate specificity of certain enzymes associated with PAH-degrading pathways. A modified microorganism (GMM) or *genetically engineered* microorganism (GEM) is produced by altering genetic material using genetic engineering techniques or recombinant DNA technology (gene conversion, gene duplication, plasmids mediated gene delivery, etc.) are employed to enhance mineralization of PAHs pollutants in the environment (Megharaj et al. 2011). The engineered *Pseudomonas putida* developed by molecular techniques

produced catechol 2, 3-dioxygenase (C23O; EC 1.3.11.2), which decomposed polycyclic aromatic hydrocarbons (PAHs) (Mardani et al. 2017; Xia et al. 2005). 2, 3-Dioxygenase is a key enzyme, mainly a nonheme iron dioxygenase for the disintegration of PAHs by altering catechol into 2-hydroxymuconic semialdehyde (Mesquita et al. 2013).

### 7.3.2 Azo Compounds

Around 60–70 % of dyestuffs utilized in textile and other industries, such as paper, food, cosmetics, etc., are azo dyes. Azo dyes have complex aromatic structural moiety comprising one or more (–N=N–) azo bonds that are not found naturally, making them xenobiotics (Lourenço et al. 2006). Almost 3000 different diverse azo dyes are synthesized and utilized for coloring purposes and accountable for the generation of substantial effluent waste (Singh and Singh 2017). The effluent majorly containing dye from various industrial practices directly expulsed into freshwater bodies has become a severe concern. The azo dye loaded effluent impart several ill effects on living systems, which include decreased aquatic photosynthesis, deplete dissolved oxygen, and pose toxic effect (carcinogenic and mutagenic) on flora, fauna, and humans. Azo dyes generally comprise azo linkages, linking phenyl, naphthyl rings substituted with functional groups like nitro, chloro, sulfonate, hydroxyl, triazine amine, methyl etc. (Asad et al. 2006). The azo dyes containing a single azo bond are termed as monoazo dyes (e.g., disperse blue 399, reactive yellow 201, acid orange 52). Diazo dyes comprise of two azo bonds (acid black 1, reactive brown 1, amido black, brown 2). Triazo dyes (direct blue 78, direct black 19) and polyazo dyes (direct red 80) are commonly used. The presence of a functional group, a number of azo bonds, type, and configuration, poses an impact on its degradative capacity (Rani et al. 2011). The degradation of xenobiotic and recalcitrant natured azo dyes is widely carried out to uncover diverse microorganisms" role in azo dyes" bioremediation. Various bacterial strains from various ecological niches such as soil, water, fecal matter, contaminated food materials, etc., are employed for complete mineralization of azo dye. The competence of microbial degradation of azo dyes relies on the adaptability and the activity of the selected microbes. A large number of bacterial and fungal species has been reported for the mineralization of various azo dyes in the last two decades and are listed in Table 7.1. Various studies reported the bioremediation of azo dyes using pure bacterial cultures, such as *Proteus mirabilis*, *Pseudomonas luteola*, *and Pseudomonas* sp., etc. (Pointing and Vrijmoed 2000; Wu and Der Jean 2012; Waghmode et al. 2011). Certain dyes are more hazardous than their degradation products. Recent studies highlighted that the complete mineralization of dye is not achieved as carcinogenic intermediates are produced during degradation (Rieger et al. 2002). The synergistic metabolic activities of consortia (mixed bacterial strains) culture enhanced the degree of mineralization. In a bacterial consortium, the different bacterial strains may react with the dye molecule at different positions or decompose intermediate metabolites (Tony et al. 2009). The consortia comprised of *P. rettgeri* strain HSL1 and *Pseudomonas* sp. SUK1 are

employed to degrade Reactive Black 5, Reactive orange 16, Direct Red 81, and Disperse Red 78 azo dye (Bumpus 1995). In the degradation of an azo dye, the primary step is reductive cleavage of azo bond that results in the formation of aromatic products under an anaerobic environment (Bumpus 1995). The nitro group of azo dyes are mutagenic and forms toxic products, such as 1, 4-phenylenediamine, *o*-toluidine, etc., during degradation, whereas sulfonated azo dyes have low or no mutagenic effect (Ferraz et al. 2011). Similarly, the extensively used Methyl Red is also mutagenic. It can be degraded to form *N*,*N*-dimethyl- phenylenediamine (DMPD), which is toxic and mutagenic in nature (Ayed et al. 2011). The genotoxic metabolites, 40-aminoacetanlide or 5-acetamido-2-amino-1-hydroxy-3,6-naphthalene disulfonic acid, get formed during the breakdown of acid violet 7 by *Pseudomonas putida* (Abdel-Shafy and Mansour 2016). Acid Violet 7 azo dye cause lipid peroxidation, chromosomal abnormalities, and inhibition of enzyme *Acetylcholinesterase*. The other azo dye, Disperse Blue 291, poses genotoxic, mutagenic, cytotoxic effect and is responsible for DNA fragmentation in human hepatoma cells (Tsuboy et al. 2007).

The microbial degradation of azo dyes is facilitated by enzymes such as azoreductase, laccases, lignin peroxidase, manganese peroxidase, and hydroxylases. Some aerobic bacterial strains (e.g., *E. coli* contain flavin reductase) can break the azo bond through the reduction process carried by *Azoreductases* in oxygen-rich environments (Fig. 7.4). Enzyme *Azoreductase* needs electron donors (redox mediator) such as FADH or NADH to carry out redox reaction to break azo bond resulting in toxic intermediate aromatic amine under an aerobic environment. In the next step, the membrane-bound azoreductase enzyme completely mineralizes the intermediate amino acids (Sarkar et al. 2017). The bacteria consume azo dye as a carbon or nitrogen source and also form a product from glucose (Lima et al. 2014). The laccases enzyme (phenol oxidase) comprises multicopper atoms with less substrate susceptibility and is an active enzyme capable of degrading numerous aromatic xenobiotic compounds (Kalyani et al. 2012). Various *bacterial strains*, such as *Pleurotus ostreatus*, *Schizophyllum commune*, *Sclerotium rolfsii*, *Neurospora crassa*, etc., can be employed for the degradation of azo dye. Phenol oxidase (laccase) uses copper ion as a mediator to oxidize the aromatic amine. Laccase follows a free radical-mediated mechanism for mineralization of azo dye and produces phenolic degradative products. *Laccase* oxidizes the phenolic rings using electron, and phenoxy radicals get formed, which immediately oxidized to produce carbonium ion. Simultaneously, the nucleophilic attack of water makes a 4-sulfophenyldiazene and benzoquinone in the process (Camarero et al. 2005). Further, phenyldiazene radical get produced from the oxidation of 4-sulfophenyldiazene with loss of molecular nitrogen producing sulfophenyl hydroperoxide (Singh et al. 2015a). A particular bacterial strain, *Extremophiles* (e.g., *Exiguobacterium*), is used to treat high saline dye effluent, as common bacteria species could not withstand the high temperature and high salinity (Ambrósio et al. 2012; Ng et al. 2017).

The studies revealed that bacteria degradation of azo dyes under aerobic conditions is restricted due to hindrance in the azo bond cleavage (Ola et al.

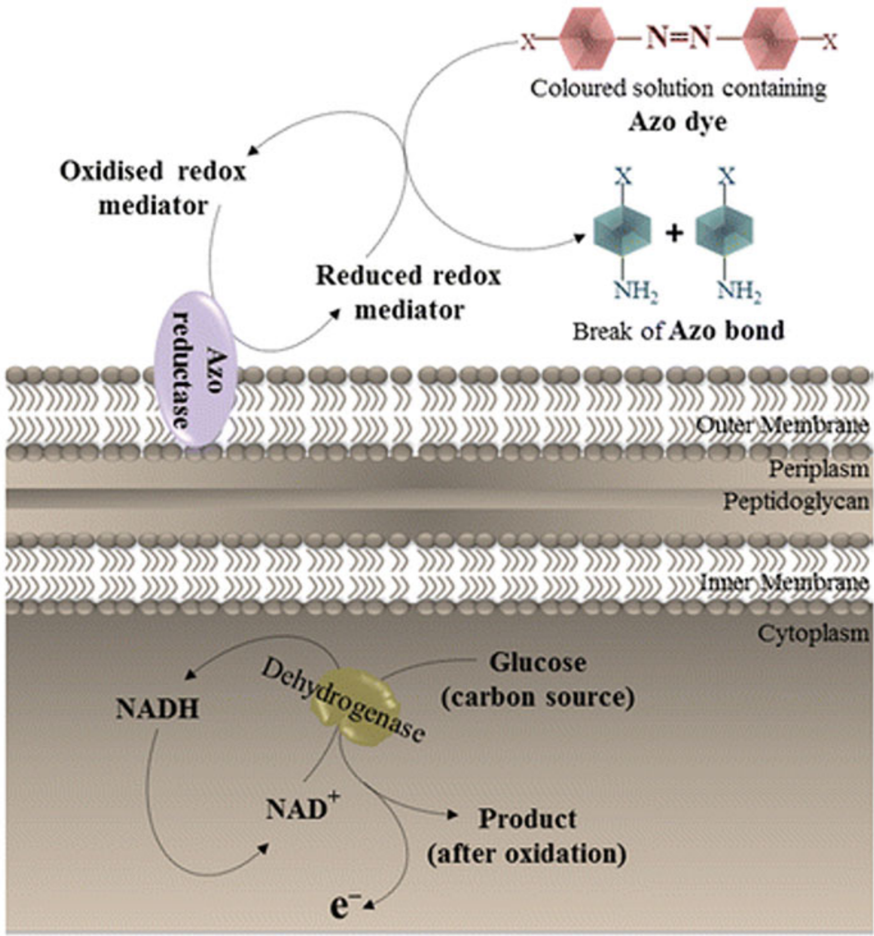


**Fig. 7.4** Mechanism of *Azoreductase* action in the reduction of azo dyes (Copyright© 2017, Springer Nature. All rights reserved, reprinted with permission) (Sarkar et al. 2017).

2010). Besides, azo dyes are disintegrating into intermediate compounds but not completely mineralized in the presence of oxygen. By employing aerobic–anaerobic degradation, azo dyes can be completely mineralized (McMullan et al. 2001). In the coupled process, the first azo bond breaks up with the formation of aromatic amines under anaerobic conditions, and then ring cleavage in amino acids facilitated by nonspecific enzymes occurs under aerobic conditions (Lu and Liu 2010). In this way, coupled anaerobic treatment followed by aerobic treatment completely disintegrate azo dyes (Singh and Singh 2017; Bumpus 1995). It was observed that the high efficiency is obtained using mixed bacterial culture rather than the pure strain.

*Fungal* facilitated degradation is a potential alternative to bacterial degradation of azo dyes. The ligninolytic fungi of class basidiomycetes are extensively used in the

degradation of azo dyes due to their capability to alter metabolic reactions based on varying carbon and nitrogen sources. The white-rot fungi, *Phanerochaete chrysosporium*, is the utmost employed for bioremediation. Instead of it, other fungal species, such as *Trametes (Coriolus) Versicolor*, *Pycnoporus sanguineus*, *Aspergillus flavus*, *Pleurotus*, *Coriolus Versicolor*, *Aspergillus ochraceus*, *Bjerkandera adusta*, *Rhizopus oryzar*, etc. have been highlighted for degradation of azo dyes (Saratale et al. 2009; Fu and Viraraghavan 2001). Generally, white-rot fungi produce lignin peroxidase, manganese-peroxidase, and laccase enzymes competent in mineralizing complex toxic aromatic compounds, such as PAHs, dyes, and steroids compounds (Revankar and Lele 2007). The factors governing the rate of degradation of azo dyes mainly are nutrient availability, time, pH, stirring speed, temperature, oxygen supply, and the number of additives added (Singh and Singh 2017). Therefore, all these parameters should be optimized in order to maximize the rate and extent of degradation.

Thus, biological systems embracing microorganisms such as bacteria and fungi bestow an inexpensive and sustainable method for the degradation of various complex azo dyes. However, the effectiveness of biological treatment for removing azo dyes relies on the adaptability and metabolic activity of selected microorganisms.

### 7.3.3 Organophosphorus Compounds

Organophosphate pesticides (OPs) are the heterogeneous compounds that are extensively used commercial pesticides comprised of phosphoric acid derivatives. Around 140 OP compounds being utilized as growth regulators and pesticides. The commercially available pesticides paraoxon, diazinon, chlorpyrifos, parathion, coumaphos, isofenphos, parathion, dichlorvos, parathion-methyl, profenophos, etc. have been used broadly, and their various degradation tactics have been studied extensively (Ortiz-Hernández and Sánchez-Salinas 2010). Organophosphorus compounds (generally called organophosphate pesticides) are the degradable esters, amides, or thiols derivatives of phosphoric, phosphonic, phosphinic, or thiophosphoric acids. Presently, the widely used organophosphate pesticides are chlorpyrifos, parathion, fenitrothion, ethoprophos, profenofos, etc. (Table 7.1). Chemically, organophosphorus compounds contain two organic groups and cyanide, thiocyanate, or phenoxy groups as side chains (Balali-Mood and Abdollahi 2014). The rate of biodegradation of organophosphorus compounds is affected by various environmental parameters, such as pH, temperature, and sunlight availability. The high solubility makes these compounds more vulnerable to human and animals and instigates severe health hazards. Due to this fact, the removal of organophosphate compounds is extensively studied, and more efficient sustainable treatment technology is being investigated. Typically, the microbial biodegradation mechanism of organophosphates proceeds through the cleavage of P–O alkyl and aryl bonds by a hydrolysis reaction, which is the enzyme-mediated process. The enzymes involved in these hydrolysis reactions are *hydrolase*, *phosphotriesterase*, *phosphatase*, and

*carboxylesterases* (Singh and Walker 2006; Lu et al. 2013). Various bacterial and fungal strains have been isolated from contaminated sites and verified as an efficient biological tool for transforming organophosphates (Table 7.1).

Chlorpyrifos (*O*,*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) pesticides are the most widely applied for the controlling breeding and populace mosquitoes, flies, and other household pests (Kim and Ahn 2009). The removal methods of chlorpyrifos have been largely studied (Wang et al. 2019). The half-life period (an essential property of xenobiotics) of chlorpyrifos in soil and water is 38 and 2118 days (Wang et al. 2019). It is degraded by isolated microbes derived from agricultural soil, industrial sludge, activated sludge, effluents, etc. Chlorpyrifos is degraded co-metabolically and catabolically by bacteria isolated from different kinds of matrices (Chishti et al. 2013). The bacterial strains, such as *P. putida*, *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, *Pseudomonas nitroreducens*, and *Pseudomonas fluorescence*, *Bacillus aryabhattai*, *Stenotrophomonas* sp, *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Lactobacillus plantarum*, and *Lactobacillus sakei*, etc., are used for mineralization of chlorpyrifos (Nabil et al. 2011; Verma et al. 2014). Pailan et al. reported the highest degradation rate of chlorpyrifos and parathion at an optimal concentration of 200 mg $mL^{-1}$ by isolate obtained by West Bengal agricultural soil, India, and was found to be an efficient candidate (Pailan et al. 2015). *O*,*O*-diethyl *O*-[6-methyl-2-(1-methylethyl-4-pyrimidinyl)] ester insecticide commonly called Diazinon used in home gardens and on farms to control a wide variety of leaf-eating insects, cockroaches, ants, and fleas in residential localities (Seo et al. 2007). The high solubility in water and 40 days long half-life period illustrated it as toxic to the biosphere. Abo-Amer demonstrated high metabolic activity of the *Enterobacteriaceae S. marcescens* derived from the Saudi Arabian agricultural field against such compounds (Abo-Amer 2011). Similarly, Seo et al. successfully used *Arthrobacter*, and *Mycobacterium* strains that were isolated from the petroleum-contaminated soil sample of Hawaii (Seo et al. 2007). The organophosphate pesticides are used at tremendous scale as insecticides need more enhanced degradation strategies to reduces the chances of their accumulation and related health hazards. Engineered microorganisms should have been designed to increase the potential ability of microbial degradation processes for organophosphorous compounds.

### 7.3.4 Halogenated Hydrocarbons

Halogenated hydrocarbon or halocarbons are the hydrocarbon compounds where at least one hydrogen is substituted by the Group 17 (Group VIIa—the *halogen* elements are astatine (At), bromine (Br), chlorine (Cl), fluorine (F), iodine (I), and tennessine (Ts)). Some of the halocarbons produced naturally during the halogenation reaction, such as the combustion of biomass during the forest fire. However, most of the halocarbon present is synthetic and intentionally produced by humankind as principle products or by-products. Halocarbons in which hydrogen forms the carbon-hydrogen bond replaced with one of the halogen species shows more

structural and chemical stability. The halogenation process of aliphatic hydrocarbons and aromatic hydrocarbons is extensively used to produce different economically favorable chemicals, used in millions of tons globally per year. Due to structural and chemical stability, these halocarbons have found their extensive use in day-to-day products, mainly in lubricants, fire extinguishers, solvents, insulators, paints, varnishes, plasticizers, pesticides, etc. Halogenated hydrocarbons are part of the synthesis of polyurethanes and polycarbonates as an intermediate. The chemicals, such as herbicides and pharmaceuticals, contain a variety of halogenated hydrocarbons. Also, the chemical in metal-cleaning, fire-extinguishing compounds, rubber, plastic, paint/varnish, healthcare, textile, fungicides, and insecticides comprise of saturated halogenated hydrocarbon (Bhat and Vaidyanathan 1995). One of the major adverse impacts of these halogenated hydrocarbons is their unregulated release into the environment. Most of these compounds are considered carcinogenic and can harm humans and other animals.

#### 7.3.4.1 Biodegradation of Halo-Aliphatic Compounds

Chlorinated aliphatic hydrocarbons are the large group of contaminants that found their way to the environment. It causes several adverse effects on the environment as well as on human health. Due to the toxicity and carcinogenicity of chlorinated hydrocarbons, there is an urgent need for these compounds" extensive remediation process. Dehalogenation is one of the notable routes for the degradation of these compounds. Dehalogenation by the use of microorganisms gives an excellent alternative. There have been extensive studies performed to establish the various methods to identify and isolate a variety of microorganisms capable of degrading chlorinated hydrocarbons (Leisinger 1996). Trichloroethylene (TCE) is mainly used as a solvent in the paper industry, textile industry, paint removers, typewriter correction fluids, and automobile industry. Many years of TCE usage has led to the accumulation of this hazardous chemical in the environment, especially to the groundwater bodies. TCE is a common groundwater contaminant and considered a potential carcinogen and mutagen (Leisinger 1996). Early research for biodegradation of TCE was based on the postulate that if mono-oxygenase enzymes of methanotrophs oxidize and dechlorinate halogenated methane, it might be possible that methanotrophs may also degrade TCE. Wilson et al. have shown a significant decrease in the TCE concentration 2 days after using exposed soil containing methanotrophs. In the study, the unsaturated soil sample was exposed to natural gas to favor methanotrophs' growth and then packed in the glass column, to which water containing TCE was passed through the column (Moriyama et al. 1988). Similarly, Fogel et al. reported TCE degradation into $CO_2$ and water-soluble products by mixed culture of methane utilizing bacteria. These bacteria were isolated from the sediment soil sample. These microbes were exposed to methane to enhance the growth of methane-degrading bacteria (Fogel et al. 1986). Pursuing on the same path, Little and coworkers isolated a pure strain of microorganism Strain 46-1 (Type-1 methanotrophic bacterium) was capable of degrading TCE in the presence of methane or methanol. The isolated strain was able to degrade TCE into $CO_2$ and water-soluble products under anaerobic conditions. Strain 46-1 was found to convert

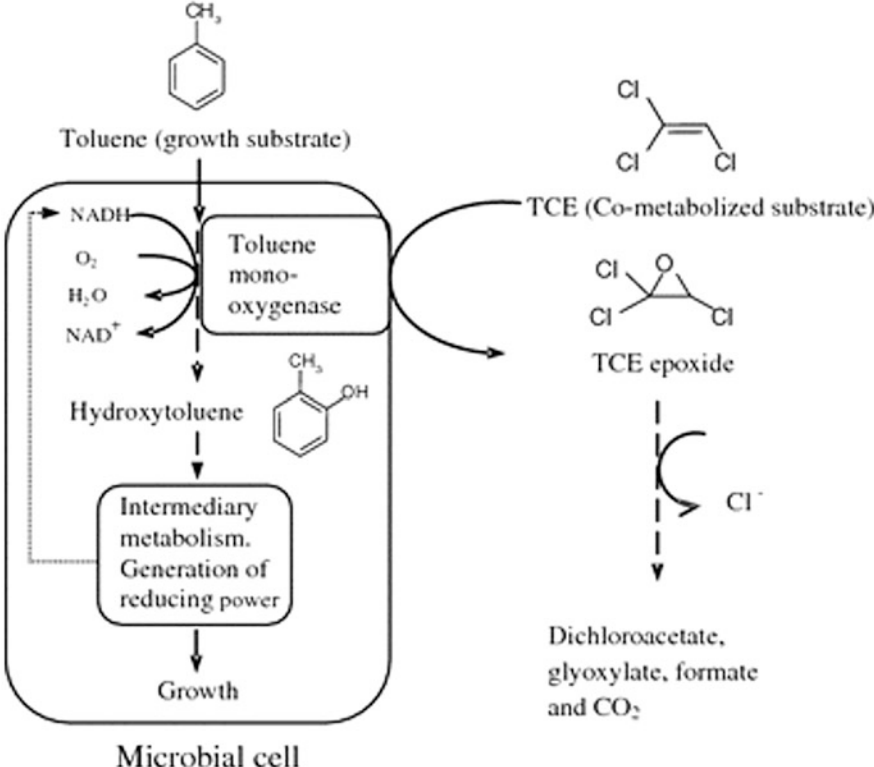


**Fig. 7.5** Proposed mechanism for degradation of TCE by toluene-oxidizing bacteria (Copyright© 2012, Springer Nature. All rights reserved, reprinted with permission) (Suttinun et al. 2013)

about 40% of supplied TCE when incubated for 20 days. Among the converted 40%, 15.1% were the water-soluble products, and 11.4% were $CO_2$ (Little et al. 1988). Apart from methanotrophs, several other studies reported the bacterial degrading TCE using toluene and phenol as a growth substrate (Suttinun et al. 2013; Li et al. 2014). The bacteria produce toluene monooxygenase, which helps in oxidizing TCE in the co-metabolism system (Fig. 7.5). Some of the commonly used TCE-degrading bacteria are *Pseudomonas*, *Burkholderia*, *Methylosinus*, *Nitrosomonas*, *Alcaligenes*, *Acinetobacter*, *Mycobacterium vaccae*, *Nocardioides* sp. CF8 (Arp et al. 2001; Amin et al. 2014; Alpaslan Kocamemi and Çeçen 2006; Halsey et al. 2005). Many monooxygenases and dioxygenases producing microorganisms have been reported, which can facilitate TCE degradation. Some of the examples are *Nitrosomonas europaea* (ammonia monooxygenase), *Pseudomonas* sp. JS150, *Burkholdria cepacia* G4, *Pseudomonas putida* F1,*Pseudomonas fluorescens* CFS215, *Pseudomonas* sp. JS150, *Pseudomonas* sp. W31 (toluene 2-monooxygenase) *Pseudomonas fluorescens* CFS215, *Rhodococcus* sp. L4 (isopropyl benzene/toluene dioxygenase),

*Pseudomonas butanovora Rhodococcus erythropolis* BD2, *Pseudomonas* sp.W31 (toluene dioxygenase), *Mycobacterium vaccae*, *Nocardioides* sp. CF8 (butane monooxygenase), etc. (Alpaslan Kocamemi and Çeçen 2006; Halsey et al. 2005; Suttinun et al. 2009; Leahy et al. 1996; Yang et al. 1999; Lange and Wackett 1997; Morono et al. 2004). Sullivan et al. established *Methylosinus trichosporium* OB3b for the TCE degradation (Sullivan et al. 1998).

#### 7.3.4.2 Biodegradation of Haloaromatic Compounds

Structurally, halogenated aromatic compounds contain one or more atoms of halogens (chloride, fluoride, bromide, and iodide) and a benzene ring. In industry, many organic compounds are used with the modified chlorinated aromatic moiety. Such compounds may be modified with one or more functional groups. Such compounds are vastly used as herbicides, pesticides, pharmaceuticals, and disinfectants (Marier 1982). Chlorinated aromatic hydrocarbons are the PCBs and used as hydraulic fluids, insulating fluids for electricity transformers and capacitors, coolants, cutting oils, adhesives, stabilizing additives in plastics, etc. Most PCBs are toxic compounds and are classified as persistent organic pollutants (Tilson and Kodavanti 1997). Some of the derivatives of chlorobenzene (especially monochlorobenzene and dichlorobenzenes) have been used as chemical intermediates and solvents in the manufacturing industry. Hexachlorobenzene is the raw material for a plasticizer for polyvinyl chloride, synthetic rubber, porosity controlling agent, and military's pyrotechnic compositions in electrodes' manufacture. Benzyl chloride is used to manufacturing quaternary ammonium chlorides, dyes, tanning materials, pharmaceutical, and perfume. Chloronaphthalenes have been used as lubricant additives, heat transfer media, dielectric fluids, solvents, and electric insulating material (Kurt et al. 2012). There are severe health hazards as these halogenated aromatic compounds are accumulated in the environment and come in contact with the human population. Toxicity of the halogenated aromatic hydrocarbons has been related to gastrointestinal and neurological symptoms (nausea, headaches, and central nervous system depression) as well as acute irritation of the eyes, mucous membrane, and lungs, reproductive disorders, liver dysfunction (hepatitis, jaundice, and porphyria), and acne (chloracne) (Moldoveanu 2019). Given the hazardous nature and severe health effect, there is a need to find and assess the methods to remove or degrade these halogenated aromatic hydrocarbons.

Chlorophenol contains at least one chlorine atom and at least one hydroxyl group at the benzene ring and is a highly toxic and has cytotoxicity, carcinogenicity, and mutagenic properties (Arora and Bae 2014a). Depending on the structural varieties, it can be generally grouped in five major classes as mono-chlorophenols (MCPs), poly-chlorophenols (poly-CPs), chloro-nitrophenols (CNPs), chloro-aminophenols (CAPs), and chloro-methyl phenol (CMPs). Chlorophenols are the vital constituent of pesticides, herbicides, solvents, pulp-paper industry, and dyes. Extensive use of these compounds and inadequate waste distribution leads to the accumulation these compounds on a hazardous scale. To address the issue, many researchers have been working on microbial degradation, as microorganisms can completely degrade it. Chlorophenols follows the general degradation mechanism of halogenated

aromatic hydrocarbon. First, monooxygenase catalyzes hydroxylation on either ortho-position or para-position of the benzene ring structure. If hydroxylation occurs at para position, then chlorocatechols is formed and if hydroxylation at ortho-position, chlorohydroquinones. The intermediate formed is further catalyzed by either ortho-cleavage, meta-cleavage, hydroxylation, or dehalogenation, followed by the benzene ring structure disruption. Under anaerobic condition, first chlorine is removed from the benzene ring structure giving partial or fully dechlorinated intermediate. So, in the case of the anaerobic condition, dichlorination is done before the benzene ring disruption. In aerobic conditions, dichlorination can be done before or after ring disruption (Arora and Bae 2014a).

### 7.3.5 Nitroaromatic Compounds

Nitroaromatic compounds are the organic compounds that contain at least one or more nitro group ($-NO_2$) and is the most useful organic compounds in the chemical industry. They have employed a significant part of the synthesis of explosives. Some of the examples of nitroaromatic compounds are nitrobenzene, nitrotoluenes, nitrophenols, etc. Naturally, they are produced as a product of the metabolism of plants, bacteria, and fungi. Also, they are accumulated in the environment by the incomplete combustion of fossil fuels. During the combustion, hydrocarbons released serve as the substrate for the nitroaromatic compounds by nitration. The naturally occurring bacterial genus *Streptomyces*, *antibiotic-producing bacteria*, *produces a wide variety of antibiotics*, *containing* a nitroaromatic component. Chloramphenicol, aureothin, neoaureothin, orinocin, azomycin, dioxapyrrolomycin, thaxtomins, rufomycins are some of the well-known antibiotics produced from the *Streptomyces* sp. (Peres and Agathos 2000; Singh et al. 2015b). *Structurally all these compounds contain one or more nitro groups*, *but a large number of nitroaromatic compounds are produced and used for industrial purposes such as* pesticides, dyes, explosives, polyurethane foams, elastomers, and industrial solvents. The recent exponential growth of industry and substantial use of explosives has given rise to the production of synthetic nitroaromatic compounds. Due to the unregulated use of pesticides and ineffective effluent treatment of chemical industry waste, these harmful nitroaromatic compounds find their ways to nature, such as water bodies, fertile lands, and dumping zones. Studies have proved that some of these compounds have ecotoxicity, immunotoxicity, carcinogenicity, and mutagenicity to humans and microorganisms (Peres and Agathos 2000; Singh et al. 2015b). There is a huge requirement for the degradation study of these compounds to humans' well-being and the safekeeping of nature. In the following section, some of the mechanisms for the degradation of nitroaromatic compounds are briefly explained.

Generally, aerobic degradation of nitroaromatic compounds in bacteria is a three-step procedure (Fig. 7.6). The first step involves changes in the nitroaromatic substrate's substituent group due to monooxygenases or dioxygenases enzyme. Monooxygenase adds one oxygen to the benzene ring, causing the release of the nitro group. While in the case of dioxygenase, the addition of two hydroxyl groups

**Fig. 7.6** The general pathway for the degradation of the nitroaromatic compounds by aerobic bacteria (Creative Commons Attribution License© 2015, Intech Open.) (Singh et al. 2015b)

causes the release of two nitro groups in the form of nitrate from the benzene ring. Hydroxyl group is induced by the enzymes to give dihydroxy aromatic metabolites. Usually, dihydroxybenzenes or catechols (1,2-dihydroxybenzenes) are metabolites produced in the first step and is the substrate for the second step of catabolism. The carbon–carbon bond in the ring structure is disrupted by the dioxygenases enzymes producing an unsaturated aliphatic acid. As there are two types of dioxygenases enzymes, two courses of action can happen during the ring opening. One is the intradiol (or *ortho)* dioxygenases ($Fe^{3+}$ requiring enzyme) produces *cis*, *cis*muconic acid, or a derivative, and second is the extradiol (or *meta)* dioxygenases ($Fe^{2+}$ requiring enzyme) produce 2-hydroxymuconic semialdehyde or a derivative. Finally, the ring cleavage derivatives produced in the second step are converted to small aliphatic compounds directed toward the central metabolism. Ring cleavage and the following metabolic steps for intradiol cleavage are referred to as the/3-ketoadipate (or *ortho)*pathway and for extradiol cleavage as the *meta* pathway (Singh et al. 2015b; Kulkarni and Chaudhari 2007).

Nitrobenzene is one of the essential classes of chemicals in the nitroaromatic compounds family. The substitution of the nitro group and chlorine group gives more structural stability to the compounds. Nitrobenzene and its derivatives are primarily used in the synthesis of aniline, the principal precursor for synthesizing azo dyes, pesticides, urethane polymers, rubber, explosives, and pharmaceutical products. Although compounds have significant advantages for the industrial processes, their extensive and accumulation in the environment, they present a substantial threat to the living organisms. Nitrobenzene and its derivatives are considered to be toxic and easily absorbed through the skin. It can damage the eyes, central nervous system, kidney/liver dysfunction, fatigue, weakness, headache, and acute methemoglobinemia (Kumar et al. 2017; Arora and Bae 2014b). Various

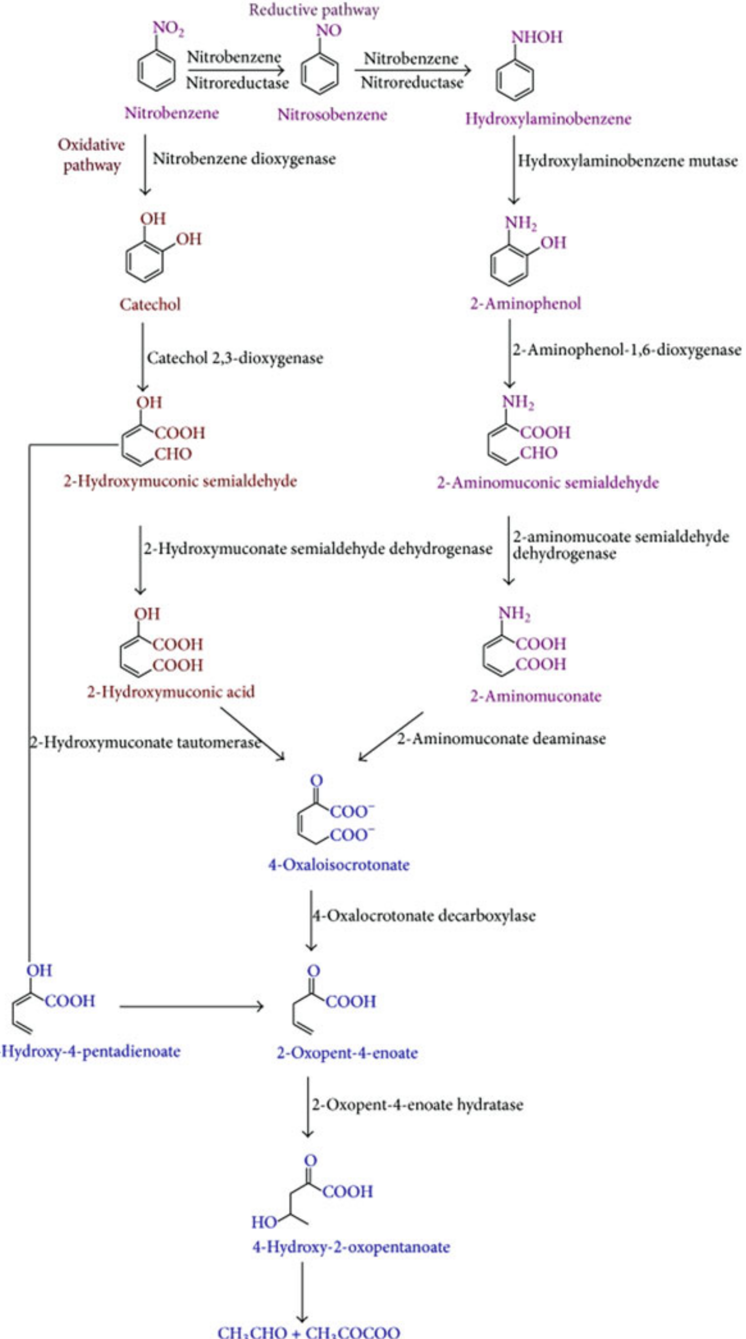


**Fig. 7.7** The reductive pathway or oxygenated pathway for Nitrobenzene (Creative Commons Attribution License© 2014, Hindwi) (Arora and Bae 2014b).

microorganisms are found in nature, which has the capability of using nitrobenzene and its derivatives as the sole carbon source employing the co-metabolism system. Nitrobenzene is degraded by either a reductive pathway or an oxygenated pathway in the microbial system (Fig. 7.7) (Arora and Bae 2014b). In the oxygenase pathway, first nitrobenzene dioxygenase adds one hydroxyl group (-OH) to the benzene ring producing catechol. Further, catechol 2,3-dioxygenase ($Fe^{2+}$ requiring enzyme) disrupts the benzene ring structure to give 2-hydroxymuconic semialdehyde and ultimately reduced to acetaldehyde and pyruvic acid by a series of the enzyme. By following the reductive pathway, initially, nitrobenzene is converted to hydroxylaminobenzene by nitroreductase. Then hydroxyl aminobenzene mutase adds one hydroxyl group (-OH) to a benzene ring, and di-oxygenase opens up the ring structure to give 2-aminomuconic semialdehyde. Gradually 2-aminomuconic semialdehyde is degraded to acetaldehyde and pyruvic acid by series of enzymes (Arora and Bae 2014b). *Pseudomonas pseudoalcaligenes*, *Pseudomonas pseudoalcaligenes* JS45, *Pseudomonas putida* HS12 produce the enzyme required for the benzene degradation. *Pseudomonas pseudoalcaligenes* were found to be using nitrobenzene as the sole carbon source. Studies show that *Pseudomonas mendocina* KR-1 converted nitrobenzene to a mixture of 3- and 4-nitrophenol, *Pseudomonas pickettii* PKO1 degrades nitrobenzene to 3- and 4-nitrocatechol via 3- and 4-nitrophenol. *Pseudomonas stutzeri ZWLR2-1 uses the* 2-bromonitrobenzene as sole carbon source and energy source (Singh et al. 2015b; Somerville et al. 1995). In another investigation, *Comamonas testosteroni* and *Acidovorax delafieldii* strains were isolated from the municipal activated sludge. These microorganisms were found to degrade nitrobenzene by catechol pathway. *Comamonas* sp. strain CNB-1 utilizes 4-chloronitrobenzene (4-CNB) and nitrobenzene as sole carbon sources (Zhao and Ward 1999). ZhiquanLiu et al. reported species of cyanobacteria *Microcystis aeruginosa can absorb nitrobenzene and reduce to* aniline with the help of nitrobenzene reductase (Liu et al. 2014). Chunli et al. isolated strain from the nitrobenzene-contaminated sludge. The isolated strain was completely capable of remineralizing nitrobenzene and using it as sole energy and carbon source. The strain was identified as *Rhodotorula mucilaginosa Z1 (*Zheng et al. 2009*).*

During the anaerobic degradation, nitroaromatic compounds were reduced to aromatic amines through electron transport. The reduction is facilitated by the nitroreductase enzyme, which transforms the nitro group into nitroso derivatives, hydroxylamines, and amines. As microorganisms completely degrading, nitroaromatic compounds are rare; thus, anaerobic microorganism works in symbiotic with aerobic microorganisms (Singh et al. 2015b). 2,4,6-Trinitrotoluene, commonly called as TNT, is one of the primarily used military explosives around the world. Due to wars and war-like activities worldwide, large areas of fertile lands, forest lands, and water bodies get contaminated. After the explosion, only ash, crystalline debris, flakes, and yellowish powder remains, and studies have proved that these contents can be toxic to rodents, fish, plants, and algae. Not only the explosion but also the synthesis of TNT itself is a hazardous and poisonous method. The precursors required for the TNT synthesis are toxic, mutagenic, and

carcinogenic (Singh et al. 2015b). Degradation of TNT through microorganisms is an extensively well-researched area (Rieger et al. 2002; Serrano-González et al. 2018). Various species of *Clostridium*, *Desulfovibrio*, and archaebacteria as *Methanococcus* sp. can completely degrade TNT under anaerobic conditions. Various *Clostridium* sp. are capable of completely degrading TNT under anaerobic conditions. TNT is reduced to 2,4,6-triaminotoluene. *Clostridium acetobutylicum*, *Clostridium bifermentans* CYS-1, *Clostridium bifermentans* LJP-1, Clostridium pasterianum, *Clostridium thermoaceticum* are some of the *Clostridium* sp., which degrades TNT. *Desulfovibrio* sp. was found to use 2,4,6-trinitrotoluene (TNT) as a sole nitrogen source(Rieger et al. 2002). *Methanococcus spand*, *Veillonella alkalescens* are some of the microorganisms that degrade TNT anaerobically (Rieger et al. 2002).

### 7.3.6 *S*-Triazine

*S*-Triazine is an organic compound with a six-membered heterocyclic structure with alternating carbon and nitrogen atoms joined by a double bond and represented by the general formula $(HCN)_3$ $_{\text{(Rehan 2016)}}$. *S*-Triazine is a herbicide and is usually used to control grassy weeds and broadleaf in crops like macadamia nuts, sugarcane, pineapple, sorghum, and corn (Singh et al. 2016). Also, *S*-triazine herbicides are used on the golf course and residential lawns (Rehan 2016). Atrazine (2-chloro-4-ethyl amino-6-isopropyl amino-1,3,5-triazine) is the most widely used s-Triazine–based herbicides. Simazine (6-chloro-*N*,*N*′-diethyl-1, 3, 5-triazine-2, and 4-diamine) has also been used as herbicides to control annual grasses and weeds in the crop field (Jiang et al. 2020). These herbicides are highly persistent in the soil and accumulate due to their prolonged degradation rate (Jiang et al. 2020; Almeida Lage et al. 2019; Fuscaldo et al. 1999). Atrazine causes damage to the immunomodulatory and reproductive systems of reptiles, crustaceans, mammals, and amphibians, while for humans, it is a carcinogen and endocrine disrupter (Jiang et al. 2020; Hayes et al. 2002).

The degradation of s-triazine has been studied using several bacterial species such as *Agrobacterium*, *Nocardiodes*, *Pseudomonas*, *Pseudominobacter*, *Stenptrophomonas*, *Rhodococcus*, *Chelatobacter*, and *Arthrobacter*, etc. The mechanisms of degradation of *s*-triazine are well discussed and reported in several pieces of literature (Phale et al. 2019). The bacterial pathway includes atrazine degradation into carbon dioxide and ammonia via different routes, as noted earlier (Phale et al. 2019; Singh and Jauhari 2017). The first pathway involves the atrazine chlorohydrolase catalyzed dechlorination of atrazine into hydroxyatrazine, followed by cyanuric acid metabolism to yield biuret. Further, the generated biuret is metabolized by biuret hydrolase to get allophante followed by allophanate hydrolase catalyzed conversion to produce $CO_2$ and $NH_3$. Other pathways include the atrazine monooxygenase, or *N*-dealkylase catalyzed synthesis of deisopropylatrazine or deethylatrazine via oxidative removal of isopropyl or ethyl group, respectively. As discussed above, the formed products are further converted into ammonia and

carbon dioxide via cyanuric acid metabolism. Atrazine's biodegradation involves the different individual species or microbial consortia to prevent atrazine's effective degradation. Xu et al. have studied and proposed a 16 different microorganism combination to get effective degradation of atrazine (Xu et al. 2019).

Several fungal strains, such as *Phanerochaete chrysosporium*, *Aspergillus flavipes*, *Hymenoscyphus ericae* 1318, *A. fumigatus*, *Trichoderma viride*, *Pleurotus pulmonarius*, etc., have been used for the degradation of atrazine (Singh and Jauhari 2017; Marco-Urrea and Reddy 2012). Fungal degradation of atrazine involves the formation of deisopropylatraine and deethylatrazine via N-dealkylation. The finished product then can be further converted into the $CO_2$ and $NH_3$ using microbe consortia, as discussed earlier. Recently, Lopes et al. have shown the efficient degradation of atrazine (82%) using the fungal strain *Pleurotus ostreatus* INCQS 40310 over 22 days of fungal incubation (Lopes et al. 2020).

### 7.3.7 Organic Sulfonic Acids

Organic sulfonic acids or organic sulfonates-based xenobiotics compounds have found applications in the various industries, such as a surfactant, hydrotropic, or optical brightener in detergents, dyes industries, personal care, food, wetting agents, dispersants, and pharmaceuticals, etc.(Nicolella et al. 2005; Cook et al. 1998; Merrettig-Bruns and Jelen 2009). Some of the common sulfonates are *p*-toluenesulfonic acid, *m*-nitrobenzenesulfonate, naphthalenesulfonates, alachlor-ethanesulfonate, saccharine, alkylbenzenesulfonate, amino benzene sulfonates, amino naphthyl sulfonates, etc. (Cook et al. 1998). These compounds are derived either by direct sulfonation of starting reactants or as a by-product of chemical and process industries. The microbial degradation of sulfonates depends on their molecular structures as the benzene, and mono substitute naphthalene sulfonates are readily biodegradable, whereas the complex molecules like nitro, amino, and hydroxy group substituted sulfonates are difficult to degrade (Nicolella et al. 2005). Further, these compounds are resistant to biodegradation by bacteria using normal hydrocarbons due to highly specific transport enzymes' requirement to facilitate their entry in the cells (Singh et al. 2012). The degradation of sulfonates mainly occurs via desulfonation reaction using several anaerobic and aerobic microorganisms that can utilize these substrates as a source of carbon and energy (Cook et al. 1998).

The dissimilating anaerobic degradation of sulfonated compounds are well studied using several bacterial strains like *Desulfovibrio* sp. RZACYSA, *Bilophila wadsworthia* RZATAU DSM 11045, *Syntrophomonad* GRZTAU DSM 11270, *Desulfovibrio desulfuricans* IC1, *Desulfovibrio* sp. RZACYSA, *Desulfomicrobium baculatus* DSM 1741, *Desulfovibrio* sp. GRZCYSA DSM 11493, *Desulfobacterium autotrophicans* DSM 3382, *Alcaligenes* sp. NKNTAU DSM 11046,.*Paracoccus denitri¢cans* NKNCYSA, *Desulfovibrio desulfuricans* ATCC 29577, *Alcaligenes* sp. NKNTAU, etc. Similarly, the bacterial strains for assimilating sulfonates" degradation are also studied using *Klebsiella* sp., *Clostridium pasteurianum*,

*Clostridium beijerinckii* EV4, *Clostridium* sp. strain *like* KNNDS, RZES, RZHS, etc. (Cook et al. 1998; Merrettig-Bruns and Jelen 2009; Linder 2018). *Clostridium pasteurianum* DSM 12136 has been used for the assimilating type of degradation for several aromatic sulfonates such as 4-xylene-2-sulfonic acid,1,3-benzene-disulfonic acid, 4- aminobenzenesulfonic acid, 4-toluenesulfonic acid, and 4-xylene-2-sulfonic acid (Chien 2005). The substituted sulfonates are challenging to degrade, and very few reports are published showing a partial degradation of these compounds. González-Gutiérrez et al. have reported partial degradation of amino-naphthyl sulfonates via a series of reactions carried out by enzymes using anaerobic sludge along with dextrose and yeast extract as a source of carbon and nitrogen, respectively (González-Gutiérrez et al. 2009).

Aerobic degradation using bacterial consortia and pure cultures is reported for the degradation of sulfonated compounds, where these organisms utilize the sulfonates as sources of carbon and energy. Several pure and consortial bacterial strains such as *Alcaligenes* sp, *Hydrogenophaga palleroni*, *Agrobacterium radiobacter*, ,*Pseudomonas pseudoalcaligenes*, *Pseudomonas citronellolis*, *Pseudomonas testosterone*, *Sphingomonas* sp. ICX, *Comamonas testosteroni* A3,*Sphingomonas xenophaga* BN6,*Pesudomonas* sp. BN9, *Pseudaminobacter salicylatoxidans* BN12, *Methylosulfonomonas methylovora*, are used for aerobic degradation of sulfonated compounds (Cook et al. 1998; Linder 2018; Ruff et al. 1999). Song et al. have reported the efficient use of *Arthrobacter* sp. 2AC and *Comamonas* sp. 4BC strains for naphthalene-2-sulfonic acid biodegradation (Song et al. 2005).

Several fungal strains, such as *Ganoderma* sp. En3, *Saccharomyces cerevisiae*, *Lipomyces starkeyi*, *R. toruloides*, *Trametes versicolor*, *Coriolopsis gallica*, *Pleurotus ostreatus*, *Cunninghamella polymorpha and Penicillium chrysogenum* have also been reported for the assimilation type desulfonation of sulfonates (Wesenberg 2003). Song and Burns have studied fungus, i.e., *Cunninghamella polymorpha*, *to suppress naphthalene-2-sulfonic acid condensation products efficiently (*Song and Burns 2005*)*. Coasta et al. have shown high efficiency of *Penicillium chrysogenum for biodegradation of linear alkyl sulfonates*, i.e., sodium dodecylbenzene sulfonate (Costa et al. 2020).

## 7.4 Conclusion

Various national and international research efforts have been encouraged in recent years, given the extent of pollution led by xenobiotics in multiple environments. Understanding of the mechanisms of biodegradation of xenobiotic compounds is still in its infancy. Although certain mechanisms have been observed in laboratory conditions, their study in a natural environment remains largely unexplored. The molecular mechanisms of biodegradation and biomineralization of xenobiotics are yet to be fully understood. The diversity of microorganisms associated with these different stages of biodegradation is also in a primitive stage. Understanding these processes is especially important in defining the biodegradation rates of xenobiotics and better predicting the xenobiotic-contaminated environment's future. The

primary site of plastic xenobiotics is the landfills, which ultimately pushes it into the water bodies. Although spreading awareness can limit xenobiotics to some extent, it cannot be wholly relied upon, and a significant step towards excluding it from the environment needs to be taken. Replacing conventional xenobiotics with bio-based and biodegradable alternatives seems to be the only option to tackle this global environmental crisis. Finally, it can be concluded that for environmental safety, we have to restrict or reduce the use of xenobiotics while simultaneously encouraging the development of more efficient biodegradable compounds that can replace the traditional xenobiotics to make the planet earth a sustainable biosphere again.

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# 8 Microbial Degradation for the Production of Value-Added Compounds: Biohydrogen from Dark Fermentation and Microbial Electrolysis Cells

Iván Moreno-Andrade and Bibiana Cercado

**Abstract**

Biotechnological processes for biohydrogen production have been developed in recent years, among which dark fermentation and the use of microbial electrolysis cells are two technologies with economic feasibility for the production of organic acids and gaseous fuels such as hydrogen. One of the advantages of these technologies is the possibility of using organic waste as a substrate for the application of the bioprocess. In this chapter, the background, fundamentals and applications of both technologies are presented. Dark fermentation can produce hydrogen, $CO_2$, and volatile fatty acids as final byproducts of the anaerobic conversion of substrates rich in carbohydrates. This process can be used as an initial step in a biorefinery for the treatment of waste and production of energy and value-added subproducts. Microbial electrolysis cells combine electrochemical cell elements such as electrodes and an external energy source with characteristics of biological reactors to produce energy in the form of electrical current, biogas and hydrogen. Hydrogen gas is formed through the combination of abiotic and biological electrochemical reactions, the latter being catalyzed by electroactive microorganisms. Microbial electrolysis cells represent a flexible technology that provides multiple routes to take advantage of residual biomass for energy production; the focus in this chapter is to describe relevant aspects that characterize this technology.

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**Keywords**

Biohydrogen · Dark fermentation · Microbial electrolysis cell · Organic solid waste

## 8.1 Hydrogen Production by Dark Fermentation

Dark fermentation (DF) is a well-known process by which it is possible to obtain hydrogen ($H_2$) as a final byproduct of the anaerobic conversion of substrates rich in carbohydrates. The use of organic waste is the most promising method for $H_2$ production via sustainable processes in biorefineries for the treatment of waste and production of energy and value-added subproducts. However, currently, the main limitations for the use of organic waste are low production and possible long-term instability. Several investigations have focused on scaling the process, as this is necessary for optimization of $H_2$ production. Several factors affect $H_2$ production: pH, organic loading rate, hydraulic residence time (HRT), solid residence time (SRT), partial pressure, temperature, substrate, and inoculum (Favaro et al. 2013; Liu et al. 2006).

The production of $H_2$ in DF is a natural response to the cellular need to release excess electrons and is carried out in conjunction with the production of different amounts of liquid metabolites such as some organic acids (i.e., acetic, propionic, formic, or butyric acid) and/or solvents (i.e., ethanol and butanol). The most common byproducts in glucose fermentation are acetate and butyrate. The reactions in DF are thermodynamically favorable and spontaneous; however, they are restricted by the biological regulation of microorganisms and by the interactions of microbial communities (Moscoviz et al. 2016).

In practice, the hydrogen yield ($YH_2$) is within the range of 1.2–2.3 $molH_2/mol_{glucose}$ (Agyeman and Wendong 2004; Wong et al. 2014), which is lower than the stoichiometric maximum of 4 mol $H_2$ due to two factors: (i) cell growth and (ii) consumption of substrate without $H_2$ production from different microorganisms present in mixed microbial cultures. $H_2$ is a key intermediate product, and it can be consumed for the production of metabolites (Hallenbeck and Benemann 2002; Saady 2013). The principal reactions for the production and consumption of $H_2$ in DF are presented in Table 8.1. In general, microorganisms that participate in DF are obligate or facultative anaerobes (Mathews and Wang 2009). These two groups of microorganisms possess particular metabolic pathways in which different types of enzymes and coenzymes are involved, leading to differential yields of $H_2$.

Organic waste has complex components, which mainly include polysaccharides, proteins, and lipids. Polysaccharides can be more easily converted to hydrogen and achieve a high hydrogen yield; proteins and lipids have lower energy conversion efficiency, but they are necessary for microbial growth (Wang and Yin 2019). Biochemical pathways are initiated when organic waste is hydrolyzed through the metabolism of microbes; complex polymers are first hydrolyzed to glucose (Fig. 8.1). In general, $H_2$ production begins with the conversion of glucose to

**Table 8.1** Principal reaction of production and consumption of $H_2$ in dark fermentation (Dong et al. 2009; Valdez-Vazquez et al. 2009)

| Reaction | Equation |
|---|---|
| Acetic fermentation | Glucose + $2H_2O$→2Acetate + $2CO_2$ + $4H_2$ |
| Butyric fermentation | Glucose→Butyrate + $2CO_2$ + $2H_2$ |
| Mixed fermentation | 4Glucose→2Acetate + 3Butyrate + $8CO_2$ + $8H_2$ |
| Propionic fermentation | Glucose + $2H_2$→2Propionate + $2H_2O$ |
| Anaerobic oxidation | Butyrate + $2H_2O$→$2H_2$ + 2Acetate + $H^+$ |
| Acetogenesis | $4H_2 + 2HCO_3^- + H^+ \rightarrow$ Acetate $+ 4H_2O$ |
| Sulfate-reduction | $4H_2 + SO_4^{-2} \rightarrow HS^- + 3H_2O + OH^-$ |

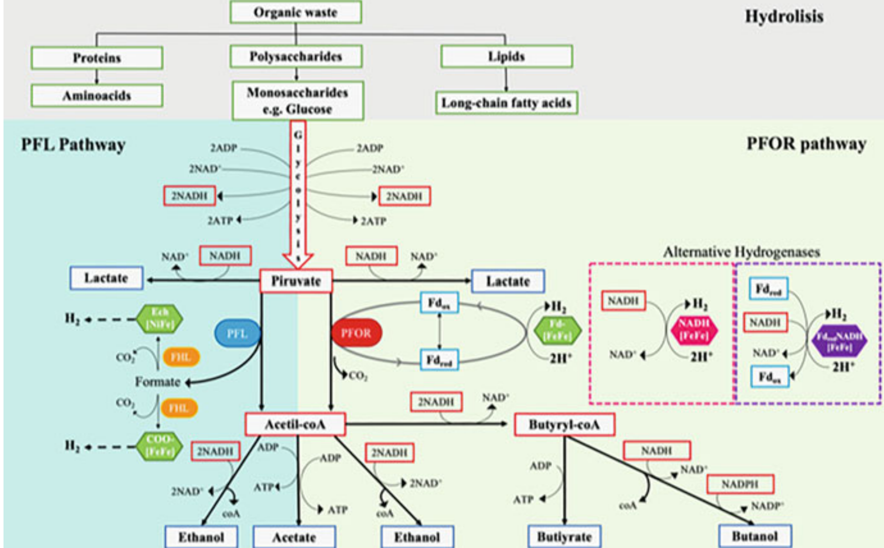



**Fig. 8.1** Biochemical pathways for the hydrogen production via dark fermentation of organic waste. Modified from Hallenbeck et al., (2002), Ramírez-Morales et al. (2015), and Cabrol et al. (2017)

pyruvate through glycolysis. The latter is transformed to acetyl-CoA by two metabolic enzymes: (i) pyruvate ferredoxin oxidoreductase (PFOR), which is associated with obligate anaerobic microorganisms, and (ii) pyruvate formate lyase (PFL), which is common in facultative anaerobes. In both cases, acetyl-CoA is converted to acetate, butyrate, or ethanol, depending on the microorganisms involved and the environmental conditions (Ramírez-Morales et al. 2015; Tapia-Venegas et al. 2015).

In the PFOR pathway, the pyruvate from glycolysis is oxidized to acetyl-CoA by the PFOR enzyme, which also generates a reduced ferredoxin molecule ($Fd_{red}$). This molecule is subsequently oxidized by a ferredoxin-dependent hydrogenase (Fd-[FeFe]), which is responsible transferring the electrons to $H_2$ cations ($H^+$), thus forming molecular hydrogen ($H_2$).

Obligate anaerobes such as species of the genus *Clostridium* can produce additional $H_2$ from nicotine adenine dinucleotide (NADH) molecules generated during glycolysis, which are oxidized by the action of two types of hydrogenases, one dependent on NADH (NADH-[FeFe]) and another dependent on NADH and reduced ferredoxin (NADH-$Fd_{red}$ - [FeFe]), which occurs under conditions of the low partial pressure of $H_2$ (less than $6 \times 10^{-4}$ atm) (Mathews and Wang 2009; Ramírez-Morales et al. 2015).

In the case of PFL, facultative anaerobes completely or partially metabolize pyruvate from glycolysis to formate, which is converted into $H_2$ and $CO_2$ via catalysis by the formate hydrogen lyase (FHL) complex, which, depending on the microbial species involved, has a hydrogenase with nickel and iron ions ([NiFe] hydrogenase) or a formate dependent hydrogenase ([FeFe] hydrogenase) (Tapia-Venegas et al. 2015; Cabrol et al. 2017). The microorganisms that use this metabolic pathway are not capable of producing $H_2$ through the oxidation of NADH, so they are limited to a theoretical yield of 2 mol $H_2/mol_{glucose}$ (Hallenbeck and Benemann 2002).

## 8.2 Microbial Groups Involved in the $H_2$ Production by DF

Hydrogen producing bacteria (HPBs) can be divided into three groups: spore-forming obligate anaerobes, nonspore-forming obligate anaerobes, and facultative anaerobes with fermentative metabolism (Cabrol et al. 2017).

The representative genus of spore-forming $H_2$-producers is *Clostridium* (the most abundant and efficient HPB), reaching yields between 1.5 and 3 $molH_2/mol_{hexose}$ (Laothanachareon et al. 2014). Owing to their ability to form spores, they can withstand heat treatment, facilitating their selection as an inoculum. Additionally, the species of this genus exhibit different metabolic patterns, and their relative abundance varies depending on the substrate used, the operating conditions, and the configuration of the process. For example, *Clostridium acetobutylicum* can switch from acidic metabolism (production of $H_2$ from acetate or butyrate) to solventogenic metabolism (production of acetone and butanol through the consumption of $H_2$), which can occur under conditions of low pH, low growth rate, and high concentrations of carbohydrates (Lütke-Eversloh and Bahl 2011). Finally, *Clostridium* species have other activities of interest, such as the saccharolytic activity of *C. tyrobutylicum* (Jo et al. 2007) or the cellulolytic activity of *C. celerecrescens* (Liang et al. 2010), and *C. acetobutylicum* (Wang et al. 2008).

Nonspore-forming obligate anaerobes are capable of persisting and even dominating despite thermal treatment in mixed cultures when the operating conditions of the reactor are favorable in terms of the source of the inoculum, selection of the substrate and operating parameters, thereby replacing *Clostridium* species. These microorganisms mainly belong to the phyla Firmicutes and Bacteroidetes and can attain acceptable $YH_2$ when *Clostridium* remains inactive (Cabrol et al. 2017).

Facultative fermentative anaerobes can be considered "challengers" in $H_2$ production since, in practice, some of them can reach or even exceed the hydrogen

productivity reported for *Clostridium* cultures (Patel et al. 2014). Despite their lower theoretical yields, facultative anaerobes may be attractive for their lower sensitivity to $O_2$, consuming it during system startup; however, the presence of these microorganisms is limited by drastic treatments of the inoculum or substrate. This group comprises members of the Enterobacteriaceae and Bacillaceae families, including genera such as *Enterobacter* and *Bacillus*, respectively (Cabrol et al. 2017).

In addition to HPB, other microbial groups participate in, favor, or harm the production of $H_2$. In a complex community during DF, positive interactions can be obtained by using microorganisms that are not capable of producing $H_2$ or those that are low-efficiency $H_2$ producers via different mechanisms: (i) regulating the presence of $O_2$ presence (e.g., *Bacillus*, *Klebsiella*) (Cabrol et al. 2017), (ii) regulating the pH by acidification of the medium (e.g., lactic acid bacteria or LAB) and through the oxidation of short-chain fatty acids, preventing their accumulation and achieving a buffering effect (e.g., lactic acid production by LAB such as *Leuconostocaeae* and *Streptococcaceae;* consumption of lactic acids by organisms such as *Megasphaera* or of propionic acid by organisms such as *Syntrophobacter and Syntrophomonas)* (Santiago et al. 2019; Chojnacka et al. 2011); (iii) increasing the hydrolysis of complex substrates, for example, by using some LAB, *Bacillus, Ruminococcus* species (Motte et al. 2014); and (iv) increasing the cell concentration in the reactor through the production of exopolysaccharides (*Bacillus*, *Paenibacillus*) or the formation of granules (*Prevotella, Klebsiella)*, preventing the washing or loss of biomass and offering protection against toxic or hostile environments for HPB (Cabrol et al. 2017; Liang et al. 2010).

On the other hand, a negative interaction can be observed mainly via $H_2$ consumption by four groups of microorganisms: methanogenic, homoacetogenic, sulfate-reducing, and propionate-producing microorganisms, which use $H_2$ as an electron donor in their metabolic processes, or via substrate competition, which inhibits $H_2$ production (Cabrol et al. 2017).

Although LAB species are attributed a positive role in the production of $H_2$ due to their hydrolytic activities and pH regulation activity, these bacteria are associated with negative interactions such as competition with HPB for pyruvate, a substrate involved in the PFL and PFOR routes of $H_2$ production. Additionally, LAB can inhibit HPB by drastically lowering the pH due to the synthesis of lactic, acetic, and propionic acids or via the production of toxic compounds such as bacteriocins or hydrogen peroxide.

## 8.3 Hydrogen Production from Organic Solid Waste

Biohydrogen, as a suitable energy source, requires economically viable conversion methods. The factors affecting economic viability include feedstock issues, such as cost, logistics, and supply, which are the main determinants of the overall economy of biomass-to-hydrogen conversion techniques (Staffell et al. 2019; Kamaraj et al. 2020). Waste materials such as agricultural waste, municipal waste, and industrial

waste represent feasible feedstocks for decreasing the cost of raw material. Another advantage is the ability to use DF as the first step in biorefinery processes (producing $H_2$ as a biofuel and organic acids as subproducts). Organic waste is the most promising waste for bioenergy production due to its current widespread availabilityat low or no cost, distinct biogas ($H_2$ and methane) production potential via digestion alone or codigestion with another feedstock, and lack of utility in other commercial applications. The principal organic wastes for $H_2$ production are the organic fraction of municipal solid waste (OFMSW), food waste, agricultural residual waste and animal-generated waste, due to their high abundance, worldwide availability, and low cost compared to other feedstocks. The OFMSW and food waste contain both macro- and micronutrients, such as carbohydrates, lipids, proteins, minerals, and vitamins, with easy-to-degrade characteristics and low levels of inhibitory compounds, making them the most promising source for hydrogen fermentation (Panigrahi and Dubey 2019; Kamaraj et al. 2020). In the case of food waste, preoperative processing (including storage) can affect the initial characteristics, since the initial production of volatile fatty acids (VFAs) and consumption of carbohydrates (at a low rate) modify the initial pH and content of substrate susceptible totransformation to $H_2$. To obtain higher biohydrogen yields, in addition to the above factors, parameters such as moisture load, volatile solids, nutrient composition, and biodegradability, are of paramount importance (Kamaraj et al. 2020).

The chemical composition of organic waste can vary depending on the source and the time of sampling, and this variation directly influences the production of $H_2$. Alibardi and Cossu (2016) reported that the variation in the concentration of carbohydrates in organic waste directly affects the production of $H_2$. Low $H_2$ yields were attributed to organic waste rich in cellulose and lignin. These results are consistent with the study conducted by Dong et al. (2009). They focused on the production of $H_2$ from different components of the organic waste fraction, finding that the components rich in carbohydrates (potato and rice) attained the highest $H_2$ yields (134 mL/g $SV_{removed}$ and 106 mL/g $SV_{removed}$); these components had the highest amounts of lipids and lignocellulose.

Lipids are not considered suitable substrates for the production of $H_2$ since the long-chain fatty acids (LCFAs) generated by β-oxidation are thermodynamically unfavorable in DF, unless the $H_2$ partial pressure is maintained at extremely low levels, which is not a common situation in DF processes where hydrogen is constantly generated to decrease the reducing equivalents generated. For proteins, all the pathways for degradation to amino acids involve the generation of VFAs and ammonia as secondary metabolites, and approximately 90% of the anaerobic degradation of amino acids is carried out by the Stickland reaction, which does not produce $H_2$. Oxidative deamination is not thermodynamically favorable (unless at very low $H_2$ partial pressure), and reductive deamination consumes $H_2$ (Dong et al. 2009). Additionally, the generation of ammonia by the degradation of amino acids can affect the system's productivity. Regardless of their role as a direct substrate for $H_2$ production, some amino acids, such as methionine, alanine, histidine, cytosine, and lysine, can improve $H_2$ productivity in DF since their bioavailability can

improve the growth and biological activities of fermentative bacteria (Sharma and Melkania 2018).

Differences in the origin and type of organic solid waste (OSW) result in a variation in the solid content. A high solid content can limit the mass transfer between the substrate and the microorganisms; in addition, it is possible to increase the production of fatty acids. The optimal initial concentration of total solids (TS) when using organic waste depends on the composition of the substrate, the reactor configuration, and the active biomass. Valdez-Vazquez and Poggi-Varaldo (2009) studied the effect of ST during $H_2$ production using organic waste, evaluating a range of 23–35% ST, and reported an increase in $H_2$ productivity with decreasing TS content. In this study, a specific maximum $H_2$ production of 463.7 NmL/kg/d and a yield of 54.8 NmL/gTS$_{removed}$ were achieved.

Several studies applying OSW have been performed to determine the kinetic parameters that can estimate the hydrolysis of waste; these kinetic coefficients depend on certain experimental conditions, such as the amount of inoculum and the substrate-to-microorganism ratio (García-Gen et al. 2015). The ranges of the hydrolysis kinetic constant ($K_h$) of carbohydrates (0.5–2 $d^{-1}$), proteins (0.25–0.8 $d^{-1}$), and lipids (0.1–0.7 $d^{-1}$) vary depending on the characteristics of the residue. Lignin and cellulose are degraded slowly and incompletely and could require additional pretreatment to achieve partial degradation.

## 8.4 Parameters Affecting the Dark Fermentation of Organic Waste

Various parameters can influence $H_2$ production, impacting the yields ($YH_2$) or production rates. Among the most relevant physicochemical parameters are the following:

*pH*. pH affects the hydrolysis of complex substrates and the activity of enzymes necessary for the production of $H_2$ (e.g., hydrogenase), the predominant microbial population, and its main metabolic pathways (Anzwar et al. 2014). The optimal pH for $H_2$ production is in the range of 5.5–6 (Van Ginkel et al. 2001), and a wide operating range of 4.5–8.0 has been reported, which is due to the diversity of the inoculum and substrate (Ghimire et al. 2015). However, a pH below 5.0 is not considered optimal since at this value the change from acidogenesis to the production of alcohols is triggered (Oh et al. 2003).

The two main factors that affect pH are carbon dioxide and volatile acids. The pH range of 6.0–7.5 and the buffer capacity are completely dependent on the carbonic gas/alkalinity system, which, in equilibrium with the dissociation of carbonic acid, tends to regulate the hydrogen ion concentration. pH plays a crucial role during the production of volatile fatty acids, as it directly influences the end products of fermentation. Lactate production has been observed during the fermentation of residues (10–20 g/L) at pH 4.0, showing a microbial community composed predominantly of *Lactobacillus,* followed by *Bifidobacterium*, with the latter considered to be a genus of acetate-forming LAB (Wu et al. 2015). At low pH values

(4.0), the enzymatic activity of hydrogenase may be inhibited (De Gioannis et al. 2013).

This parameter also affects the solubility of molecules such as $CO_2$ and the availability of nutrients such as ammonium and phosphate, which are essential for microbial growth. Extreme pH values are not tolerated by microorganisms since, under acidic or basic conditions, the microbial components are hydrolyzed or some enzymes are denatured. The optimal pH levels for the activity of acidogenic microorganisms are between 5.5 and 6.5, with acetate and butyrate production favored between pH 4.5 and 6.0, while neutral pH promotes ethanol and propionate production, decreasing $H_2$ production. pH is also a critical factor for the inhibition of methanogenesis, preventing $H_2$ consumption (Ramos et al. 2012).

In many cases, to maintain an optimum pH in the reactor, it is necessary to increase alkalinity using substances such as sodium bicarbonate, sodium carbonate, ammonium hydroxide, sodium hydroxide, potassium hydroxide, and calcium carbonate. Sodium bicarbonate is used regularly due to its high solubility and low toxicity (Varnero 2011).

*Particle size*. For OSW, it is necessary to reduce the particle size using mechanical treatment (crushing or grinding), thereby increasing the solubility and availability of organic matter. The smaller the particle is, the greater the surface area in which the bacteria act, increasing the $H_2$ yield (Tosuner et al. 2019). Particl of OSW that are less than 0.5 mm in size offer better $H_2$ production rates than larger particles (Moreno-Andrade and Buitron 2015).

*Micronutrients.* Micronutrients are necessary as enzyme cofactors such as magnesium (e.g., hexokinase, phosphofructokinase, and phosphoglycerate kinase for glycolysis), iron (hydrogenases), sodium (increase in $Fd_{ox}$ reduction by NADH), nickel ([NiFe] hydrogenase in the PFL pathway) or calcium (contributing to cellular growth) (Balachandar et al. 2013; Bundhoo and Mohee 2016; Karadag and Puhakka 2010). In the case of OSW, these microelements are usually present and need to be added only when absent. For example, the characterization of food waste from a buffet restaurant (with COD of 1.2 g/gTS and 0.37 gTS/g of waste) showed the presence of Fe (70.5 mg/L), Na (127.0 mg/L), Ni (0.01 mg/L), Ca (7.3 mg/L), Cu (0.01 mg/L), Mn (0.2 mg/L), and Al (1.1 mg/L).

*Hydrogen partial pressure*. Low values of hydrogen partial pressure facilitate the mass transfer of $H_2$ from the liquid to the gaseous phase. In DF, if the concentration of $H_2$ in the liquid phase increases, the oxidation of ferredoxin catalyzed by hydrogenase becomes less favorable, thus reducing $H_2$ production. It has been reported that the increase in metabolic pathways can also shift towards the production of lower-end products, such as lactate, ethanol, acetone, and butanol. The maximum value for this parameter depends on the bacterial species and the substrate. An ideal range is between $9 \times 10^{-4}$ and $9 \times 10^{-6}$ atm (Ostrem 2004).

At the metabolic level, if the partial pressure is low enough (<60 Pa), the NADH produced during glycolysis can also be used to generate $H_2$, but most of the NADH will probably be oxidized through other fermentative routes. To reduce the partial pressure of $H_2$ in the medium, especially in highly concentrated bioprocesses, agitation is the most common technique (Valdez-Vazquez and Poggi-Varaldo

2009). Controlled agitation, in addition to regulating the partial pressure of $H_2$, can also be use to remove the metabolites produced, mix fresh substrate with the bacterial population, distribute the temperature evenly, and prevent the formation of dead spaces without biological activity that could reduce the practical volume of the reactor, preventing the formation of foams and sedimentation (Varnero 2011). A continuous mixing speed of 100 rpm in the reactors improves stability during process start-up. Mixing can be carried out with mechanical agitators, sludge recirculation, or biogas injection, with mechanical agitation being the most effective.

*Temperature.* This is an essential parameter in the growth of microorganisms and, consequently, in the generation of $H_2$. DF can be carried out at different temperatures: mesophilic (25–40 °C) and thermophilic (40–65 °C). Mesophilic conditions turn are more stable than thermophilic conditions due to the wide variety of fermentative microbial species favored in the mesophilic range (Yang et al. 2018).

Shin et al. (2004) evaluated $H_2$ production from organic waste by two reactors at different temperatures (35 and 55 °C); in both experiments, butyric acid was found to be the main metabolite at pH 4.5. The presence of methane was detected only at a mesophilic temperature in a 50 h incubation period, demonstrating the inhibition of methane production and the decrease in propionate production under thermophilic conditions. Microbiological analyses also demonstrated the presence of LAB under thermophilic conditions and bacteria of the genus *Clostridium* at 55 °C. Additionally, temperature affects the metabolic pathways and therefore the proportions of the metabolites obtained (Ghimire et al. 2015). Higher production yields ($YH_2$ values) have been reported under thermophilic conditions than under mesophilic conditions (Ziara et al. 2019; Valdez-Vazquez et al. 2005), suggesting that high temperatures improve the hydrolysis of the substrate. However, thermophilic systems tend to be more unstable when faced with changes in temperature. In these systems, a sudden increase in temperature can affect the fermentation process, decreasing the production of $H_2$. The principal drawback is the cost of increasing the temperature to maintain a thermophilic system. Therefore, $H_2$ production processes have mainly used mesophilic temperatures. Regardless of the temperature at which the system is operated, the condition needs to be maintained once the steady-state has been reached (although it can vary up to ±0.5 °C), if constant production of biogas is desired. Small fluctuations (±3 °C) can be tolerated, especially if the process remains stable in terms of other important parameters, such as pH and alkalinity.

*Hydraulic retention time (HRT).* The time necessary for $H_2$ production depends on the substrate characteristics, degradability, and organic loading rate of the system (in TS or COD). The HRT needs to be long enoughfor organic waste hydrolysis and $H_2$ production but short enough to avoid the growth of methanogens (with a slow growth rate of 0.0167–0.02 $h^{-1}$), which would consume the $H_2$ produced or displace the $H_2$ producers (high growth rate of 0.172 $h^{-1}$). For easy-to-degrade waste, the HRT will be short. Methanogenic archaea grow slower (in HRT>1 d) than $H_2$-producing microorganisms (Ramírez-Morales et al. 2015). HRT is the main parameter determining the community composition in the process (Santiago et al. 2020). Finally, a decrease in the HRT reduces the size of the reactor and, thus, the investment costs. The typical HRT for $H_2$ production ranges between 6 and 36 h.

*Sludge retention time (SRT).* The SRT is the average time that organic matter or volatile solids are in the system. In continuous stirred tank reactor (CSTR) and plug flow reactor (PFR), reactors without recirculation, the HRT and SRT are the same (Varnero 2011); however, if there is recirculation of solids, the SRT and HRT vary significantly between the two types of reactors. In the case of sequencing batch reactors (SBRs), the HRT and the SRT are decoupled due to the settling phase in this process.

The SRT is the most important factor to control the conversion of solids to gas and to maintain the stability of the system. It has been reported that high SRT values can allow adequate hydrolysis of the particulate substrate (increasing the soluble COD) and degradation of the substrate. However, this promotes the growth of microorganisms that affect the process, such as $H_2$ consumers (e.g., homoacetogenic, sulfate-reducing bacteria and methanogenic archaea) (Moreno-Andrade et al. 2015; Castillo-Hernandez et al. 2015; Castelló et al. 2009; Santiago et al. 2020). On the other hand, the low values of this parameter can limit the growth of microbial populations that are harmful to the process.

It is necessary attain a balance between substrate degradation and biomass retention to maximize the production of $H_2$.

Control of the SRT and HRT is a key factor to maximize $H_2$ production. Studies have documented the use of various HRT and SRT values to maximize $H_2$ production using organic waste. Table 8.2 shows that HRT and SRT depend on the type of substrate used during $H_2$ production. Short HRTs reflect the use of easily assimilated substrates for microorganisms, and in combination with long SRTs, they increase substrate hydrolysis.

The levels of fatty acids increase or decrease according to the different metabolic pathways favored by the operating conditions. Kim et al. (2008) evaluated different HRTs and SRTs in an SBR using organic waste, reporting SRT values of up to 126 h without the presence of methane. The highest production of $H_2$ was attained at an HRT of 30 h and an SRT of 126 h (2.73 L $H_2/L_{reactor}$/d), due to improved hydrolysis in the process. Santiago et al. (2020), using food waste in an SBR, reported the maximization of $H_2$ production, achieving high substrate hydrolysis and yield (127.26 $mLH_2/gCOD_{removed}$) at an SRT of 60 h and an HRT of 16 h. The use of different SRTs and HRTs also influenced the subproducts of the process, since with an SRT of 20 h and an HRT of 16 h, acetic acid-like fatty acids were mainly obtained, while with a long SRT (60 h), the obtained fatty acid was butyrate. A short SRT (<20 h) affects $H_2$ production (up to 90%) when OSW is used as the substrate.

## 8.5 Bioreactors for Hydrogen Production

Different configurations of bioreactors have been applied for hydrogen production from organic waste, including systems operated in batches (including SBRs), continuously and semicontinuously. CSTRs have been widely used for the production of $H_2$ due to their simple construction, ease of operation, effective homogeneous

**Table 8.2** SRT and HRT used for hydrogen production from different organic wastes

| Reactor type | T (°C) | SRT (h (h) | HRT (h) | Substrate | Yield $H_2$ | $H_2$ production ($LH_2$/L·d) | Reference |
|---|---|---|---|---|---|---|---|
| SBR | 35 | 54 | 6 | Starch | 2.44 mmol $H_2$/g $glucose_{added}$ | 4.12 | Arooj et al. (2008) |
| SBR | 35 | NA | 36 | Food waste | 2.77 mmol $H_2$/g glucose | 1.16 | Kim et al. (2010) |
| SBR | 35 | NA | 24 | Food waste | 3.83 mmol $H_2$/$gVS_{added}$ | 0.75 | Moreno-Andrade et al. (2014) |
| SBR | 37 | 264 | 72 | Palm oil mill effluent | 13.45 mmol $H_2$/$gCOD_{added}$ | 6.7 | Badiei et al. (2011) |
| SBR | 30 | NA | 24 | Sorghum waste | 3.77 mmol $H_2$/g glucose | 0.93 | Saraphirom and Reungsang (2011) |
| SBR | 35 | 126 | 30 | Food waste | 3.20 mmol $H_2$/g $SV_{adicionado}$ | 2.73 | Kim et al. (2008) |
| SBR | 35 | 55 | 16 | Food waste | 5.0 mmol $H_2$/$gCOD_{added}$ | 1.8 | Santiago et al. (2020) |
| CSTR | 35 | 12 | 12 | Starch | 4.99 mmol $H_2$/g glucose | 4.29 | Arooj et al. (2008) |
| CSTR | 35 | 12 | 12 | Sorghum waste | 0.4115 mmol $H_2$/g sorgo | 1.74 | Antonopoulou et al. (2008) |
| CSTR | 35 | 12 | 12 | Food waste | 0.5 mmol $H_2$/$gVS_{aded}$ | 0.5 | Castillo-Hernandez et al. (2015) |

*NA* not available

mixing, and ability to maintain a fixed HRT. However, in this type of reactor, the SRT is equal to the HRT, so when operating at low HRT, active biomass washing may occur (Hafez et al. 2010). Methane production has been reported to be responsible for instability in CSTRs a few times under specific conditions, such as HRT and pH values higher than 24 h and 6, respectively, at least under conditions that allow the growth of methanogenic organisms (Castelló et al. 2020).

Castillo-Hernandez et al. (2015) studied the volumetric production of $H_2$ by evaluating different HRTs (24, 12, and 8 h) and observed that the increase in HRT resulted in a decrease in COD removal, proteins, and $H_2$ yield. The highest production of $H_2$ (19.8 mmol $H_2$/L/d) was attained at 12 h, while at an HRT of 8 h, the production of $H_2$ decreased by up to 40%, with propionic acid as the main VFA produced.

Arooj et al. 2008 reported the washing of active $H_2$-producing biomass by decreasing the HRT to 3 h in a CSTR using starch as substrate; this was reflected in the decrease in $H_2$ production. However, when evaluating residence times between 9 and 6 h, an increase in acetate production was observed due to the consumption of $H_2$ (homoacetogenesis). The above was estimated according to the mass balance for each HRT. This fermentative route can be eliminated by appropriate control of the residence time. To avoid biomass washing, it is necessary to provide more significant contact between the substrate and the microorganisms and avoid changes in the microbial population. It is necessary to apply an adequate SRT when complex substrates are used, such as OSW.

Some studies have focused on decoupling SRT and HRT. Hafez et al. 2010 studied the production of $H_2$ using glucose as a substrate in two CSTR systems for $H_2$ production (one including a settling tank) operated at an SRT of 2.2 d and an HRT of 8 h. The highest $H_2$ production stability was obtained when the SRT was controlled by the settling tank (increasing the yield of $H_2$ by 38%). The lack of a settling tank results in a failure due to biomass washing (in an HRT of 8 h). Reactor configurations that allow the decoupling of HRT and SRT (such as the SBRs) can improve $H_2$ production and yield.

SBRs are an alternative system for the production of $H_2$ using organic waste; these reactors are characterized by a periodic system of well-defined phases: filling, reaction, settling, and drawing. These reactors have the advantage of operating in a flexible manner, and all the stages are carried out in the same reactor. Piemonte et al. (2014) studied the reaction time as a key factor for $H_2$ production in an SBR, and found that a reaction time greater than 3.43 h ensures a stable $H_2$ production efficiency. Strict control of each of the stages will allow guiding of the metabolic route towards the production of the desired products.

The settling phase showed separation into three layers: (i) high accumulation of lipids, (ii) retention of nonsettled solids in the supernatant, and (iii) retention of solids in the bottom of the reactor (Kim et al. 2010). By decanting the first layer, it is possible to eliminate the lipids accumulated in the reactor and avoid possible failures during the production of $H_2$ (Castillo-Hernandez et al. 2015). For OSW, the third layer contains the microorganisms and solid waste combined; for this reason, in the typical measurement of total solids or volatile solids, the sum of both is considered.

Accurate measurement of the distinction between active biomass and organic residues can be performed by molecular techniques.

Moreno-Andrade et al. (2015) studied the production of $H_2$ in an SBR using organic waste from a cafeteria, and the maximum $H_2$ production was 0.715 L/L/d at an HRT of 24 h. The main fatty acids produced were acetic acid followed by butyric acid. The microbial analyses showed a similarity of the microbial communities among the different HRTs evaluated. The stability of the composition among HRTs could be related to the use of the same substrate throughout operation. The genus *Megasphaera* represented 85% of the total in all the analyses performed, followed by the genus *Selenomonas*; it is possible that this type of microflora may be present in the organic waste and is constantly fed to the reactor (Castelló et al. 2009).

CSTR and SBRs are usually operated with suspended biomass; however, the use of biomass retention in granules, as in upflow anaerobic sludge blanket reactors (UASBs), expanded granular sludge bed reactors (EGSBs), or inert supports such as fixed bed reactors (FBRs), has been proposed mainly for semisolids and liquid substrates (cheese whey or wastewater). These types of reactors exhibited a higher microbial diversity than reactors with suspended biomass; however, currently, it is still not clear whether it is more convenient to have a high diversity or to have highly specialized communities that could ensure high productivity (Castelló et al. 2020).

## 8.6 Fundamentals of Microbial Electrolysis Cells

The catalytic activity of electrochemically active microorganisms during organic matter oxidation supports bioelectrochemical technology known as microbial electrolysis cells (MECs). MECs combine elements of electrochemical cells, such as electrodes, a membrane, and an external power source, with characteristics of biological reactors that include the feeding of biodegradable matter and the presence of microbial communities at favorable temperature and pH values (Liu and Hu 2012). This set of components results in a hybrid system for the generation of products of commercial value; the products obtained from MECs are mainly organic acids and gaseous fuels such as hydrogen and methane (Fig. 8.2).

A conventional MEC is made up of two compartments separated by a membrane that is selective to the passage of ions. The anode compartment is fed with biodegradable matter that supplies basic nutrients (C, H, O, N, S, P) to the microorganisms that are suspended and forms a biofilm on the anode electrode. Electroactive microorganisms degrade organic matter via oxidation reactions in which electrons, protons, and carbon dioxide are produced. A part of the electron flow is directed to the solid material of the electrode, and they circulate through an external circuit towards the cathode electrode, thus generating an electric current. The protons produced during the oxidation of matter ideally migrate to the cathode chamber through the selective membrane. The external energy source makes it possible to impose a potential difference between the anode and the cathode, and this condition causes the occurrence of reduction reactions on the cathode. The oxidation on the anode and reduction on the cathode are linked to electron flow, which adds to the

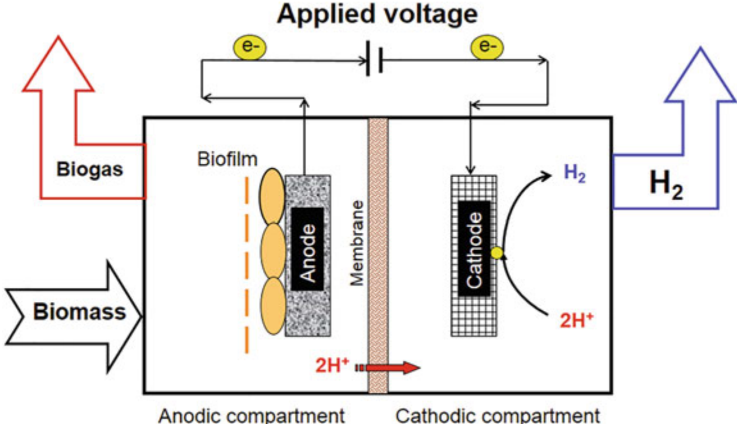



**Fig. 8.2** Schematization of the components of a microbial electrolysis cell. Modified from Rosales-Sierra et al. (2017)

flow of electrons generated by microbial activity (Rozendal et al. 2006). The total electric charge reaching the cathode results in an electrode reaction for the formation of products of interest in the cathodic chamber; the electric circuit is closed by protons present in the form of ionized water molecules ($H_3O^+$). Thus, the electrons or electric charge are combined with protons on the cathode to form hydrogen molecules (Rabaey and Rozendal 2010).

The oxidation reaction at the anode is represented in Eq. (8.1).

$$C_aH_bO_cN_d + H_2O + \text{inoculum} \rightarrow CO_2 + 2e^- + 2H^+ + \text{biomass} \qquad (8.1)$$

The microorganisms or inoculum act as pseudocatalysts for the oxidation reaction of the organic matter, and ideally, all the matter is mineralized to carbon dioxide. This reaction occurs with carbonaceous matter such as acetate at a standard reduction potential of −0.280 V/SHE for acetate and −0.430 V/SHE for gluclose.

In the cathodic chamber, the reduction reaction in a neutral medium is represented in Eq. (8.2).

$$3H_2O + 2e^- \rightarrow H_{2(g)} + 2OH^- + H_2O \quad E^0 = 0.83\ \text{V/SHE} \qquad (8.2)$$

The number of moles of hydrogen and electrons produced in the oxidation reaction is proportional to the number of moles of hydrogen gas produced in the reduction reaction; thus, the overall reaction in the MEC can be presented as Eq. ((8.3).

$$C_aH_bO_cN_d + \text{inoculum} \rightarrow H_{2(g)} + CO_2 + \text{biomass} \qquad (8.3)$$

The theoretical thermodynamic potential of the cell is given by the positive potential of the anode minus the negative potential of the cathode. In this way, the

potential of the cell represents the theoretical energy necessary to carry out the global reaction.

When the MEC is fed with acetate, the theoretical thermodynamic potential of the hydrogen-producing cell is $E_{cell} = -1.10$ V, while in the case of glucose-fed MECs, the potential is $E_{cell} = -1.26$ V.

In addition to the possible variations in anodic reactions, the hydrogen reduction reaction can be modified by the concentration of protons in the medium, which is indicated by the pH of the electrolyte (Zeng and Zhang 2010). The reduction reaction of ionized water in acidic pH is presented in Eq. (8.4).

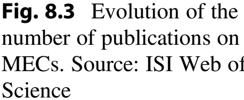
$$2H_3O^+ + 2e^- \rightarrow H_{2(g)} + 2H_2O \qquad E^{0'} = 0.0\ \text{V/SHE} \tag{8.4}$$

The MEC potential changes to $E_{cell} = -0.28$ V with acetate and to $E_{cell} = -0.43$ V with glucose.

These calculations with model substrates and a theoretical cathode potential are an indication of the wide variety of conditions in which MECs operate to produce hydrogen. The estimation of the actual cell potentials is influenced by interferences caused by compounds in complex substrates and by energy losses at the solid-liquid interfaces and in external connections; therefore it is necessary to evaluate the cell potential in each particular MEC system.

## 8.7 History of the Development of Bioelectrochemical Systems

The development of MECs was preceded by microbial fuel cells (MFCs) and can be attributed to knowledge generated from the latter; the initial research on MECs increased rapidly. In the 1990s, MFCs were studied with pure cultures of microorganisms fed simple synthetic substrates, and external mediators were used to favor the transfer of charge at the cathode (Allen and Bennetto 1993). At the beginning of the 2000s, Lovley's research group reported charge transfer between various *Geobacter* species and solid electrode material (Lovley 2008), and this knowledge was used for the application of the electrical current produced in marine sediments to power ocean-monitoring devices (Donovan et al. 2008). From these first reports, MFCs were developed without the use of external mediators, complex substrates such as chitin and starch were investigated, and studies on different electrode materials began (Rezaei et al. 2007; Dumas et al. 2007). Circa 2005, Logan and his research group used wastewater to feed MFCs, and they proposed a new system for the treatment of water simultaneously with the production of energy that could be used to reduce energy consumption for wastewater treatment (Bruce E. Logan and Rabaey 2012). Based on this perspective, new cell designs were proposed; in microbiological research, new genera, families, and species of electroactive microorganisms were identified (Logan and Regan 2006; Rabaey et al. 2007). At the end of the first decade of the 2000s, two mechanisms of electron transfer were established: direct transfer through the membrane of the microbial cell and pili, and transfer via redox mediators produced by microorganisms (Schröder



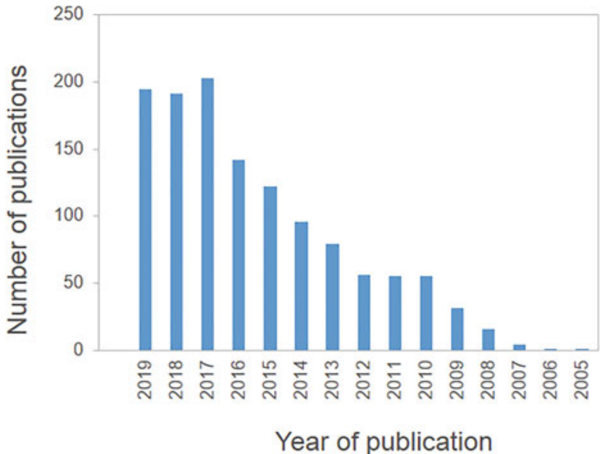



**Fig. 8.3** Evolution of the number of publications on MECs. Source: ISI Web of Science

2007). The increased understanding of charge transfer mechanisms led to the development of models that included microbial species with different functions and different charge transfer mechanisms (Marcus et al. 2007). In the same period, efforts to scale up electrochemical cells from ten milliliters to several liters were made (Rabaey et al. 2005), in addition to scaling down to microsized electrochemical cells. For the former, the results did not allow continuing with the scaling since it was frequently observed that the power of the cell decreased with increasing dimensions; microcells, however, exhibited adequate performance for use as biosensors for organic matter, toxic compounds, and biofilm growth (Su et al. 2011). Starting in 2010, the applications of MFCs were diversified, with which the terminology was expanded to include electrolysis, desalination, and electrosynthesis cells. The terminology for microorganisms, cells, and operation was compiled in a review, and efforts are continually being made to standardize the nomenclature at the international level (Schröder et al. 2015).

The first mention of the possible electrolysis operation of an MEC was provided by Allen and Benetto in 1993 (Allen and Bennetto 1993). Later, Liu et al. (Liu et al. 2005) presented the first operating results of MECs. Since then, the number of publications on MECs has increased exponentially, with an unexpected plateau in 2017–2019. The observed plateau was possibly due to the limitations observed during the scaling of MECs and because research on real applications is scarce (Fig. 8.3).

## 8.8 Electroactive Microorganisms and Electron Transfer Mechanisms

Microorganisms with the ability to transfer and receive electrons or electrical charge from a solid electrode material have been called electroactive, electrogenic, exoelectric, and anode-respiring bacteria (for those that transfer charge to the anode).

In the jargon of electrochemistry, an electroactive species is one that generates a current signal at a particular potential value, due to which it seems convenient to use the term electroactive in a generalized manner, while exoelectrogenic or endoelectrogenic can be used to distinguish the function of the microorganism on the electrode; however, these terms cannot be used for classification since there are species that perform both functions. For example, *Geobacter* spp. are frequently reported in anodic biofilms but have also been identified in cathodic biofilms.

The species of microorganisms studied in depth are those of *Geobacte*r and *Shewanella*. The genome sequence of *Geobacter metallireducens* has been investigated for molecular modification of charge exchange structures that include pili and flagella (Dantas et al. 2015). In *Shewanella* species, the production of the charge-transporting metabolites and changes in cytochromes activity have been studied as a function of the electrochemical potential to which the microorganisms are subjected (Brutinel and Gralnick 2012).

In addition to the study of pure cultures, mixed cultures made up of nonelectroactive and electroactive species have been investigated. Nonelectroactive species are used to degrade complex organic molecules, and once compounds with short carbon chains are obtained, the electroactive species consume mainly acetate, as observed with a mixed culture consisting of *Clostridium cellulyticum-Geobacter sulfurreducens* (Ren et al. 2008).

Thus, basic research on charge transfer mechanisms is based on the study of pure cultures of known species, while applied research exploits consortia of natural media and effluents to inoculate MECs. Consortia exhibit greater resistance to changes in substrate, pH, and temperature; sources of electroactive communities are mainly marine sediments, sewage, aerobic and anaerobic sludge, granular sludge, soil, compost leachate, and effluents from other MECs that have been in operation for several months or years.

The use of consortia is frequently accompanied by a process of acclimatization to the environment and enrichment of electroactive species through successive cultures. Differences between the community in biofilms and the community in suspensions have been reported (Flayac et al. 2018; Cardena et al. 2018). This finding is relevant because it indicates that different biological processes could occur to maintain a balance in the MEC. The ecological relationships of suspended communities and electroactive biofilms continue to be a focus of research.

Consortia in long-term operated reactors seem to evolve from high to low diversity, thereby enriching electroactive species, which results in the formation of high-performance bioelectrodes (Cardena et al. 2018).

Charge transfer mechanisms have been classified into direct transfer and mediated transfer. Direct charge transfer involves cytochromes in the cell membrane, pili, and flagella, while mediated transfer is carried out by redox mediators, which are either produced by the microorganisms or added to the medium (Kumar et al. 2017).

The cytochromes involved in the electroactive properties are a function of the microbial species. For instance, multiheme cytochromes and multicopper proteins are found in *Geobacter* spp., while a series of multiheme cytochrome complexes are present in *Shewanella* spp. Cytochromes are also present in pilus-like structures,

which favor electron transfer over relatively long distances and also favor contact between microbial cells; moreover, the conductivity in pili is also attributed to the truncated PilA monomer (Costa et al. 2018).

The mediated electron transfer involves molecules that act as electron shuttles that have mostly negative reduction potentials. Flavins are natural mediators involved in two-electron reduction; flavin adenine dinucleotide (FAD) has a standard reduction potential of −0.219 V but varies with the type of flavo-protein and the active site (riboflavin, flavin mononucleotide). Phenazine is produced by *Pseudomonas* spp., this mediator has a standard reduction potential of −0.323 V/Ag/AgCl, while pyocyanine has a potential of −0.247 V/Ag/AgCl. The reduction and oxidation of mediators occur in the periplasmatic membrane, thus facilitating the transfer of electrons to the electrode (Marsili et al. 2008).

## 8.9 Substrates: Types and Limitations for Current Production

Substrates for electroactive microorganisms can be simple or complex and are supplied at low or high concentrations; however, simple and low-concentration substrates are favorable for electricity production and therefore for hydrogen gas formation on the cathode. Simple substrates include short-chain organic acids such as acetate, propionate, and butyrate, as well as fermentable substrates such as glucose and lactose.

Acetate is the model substrate for microbial electrochemical cells because it leads to the greatest production of electrical current. Acids with 1–5 carbon chains undergo conversions that can increase the acetate concentration in the medium, thus favoring the charge transfer process by electroactive microorganisms (Rosales-Sierra et al. 2017).

Fermentable substrates undergo partial oxidations in which the demand for electrons is greater than that for the transformations of short-chain organic acids because the fermentation reactions are more diverse in fermentation pathways. In addition, the degree of reduction also influences the preference or affinity of electroactive microorganisms for certain organic compounds. The compounds favoring bioelectrochemical processes are in the order: acetate, short-chain organic acids, and simple fermentable substrates.

Proteins present in wastewater have also been proven to act as substrates in bioelectrochemical systems; however, they are used very rarely due to the low production of hydrogen observed (Lu et al. 2010).

Complex substrates of known composition that have also been tested include cellulose, starch, and chitin. Other complex substrates of unknown composition are used in research aimed at water treatment and waste recovery. Wastewater from domestic, industrial, and other bioprocesses is rich in organic matter and essential nutrients for microbial activity, but effluents with toxic, inorganic and recalcitrant compounds such as metals and drugs have also been tested in MECs (Paz-Mireles et al. 2019). The use of effluents from other bioprocesses is of great interest for the integration of biorefineries, in which maximum energy efficiency is desirable.

The concentration of the substrate fed to the cells is a major factor in the electron transfer process. Simple substrates of known composition have made it possible to identify the limiting concentration for batch fed systems through behavior that follows the Monod model. The substrate concentration value that corresponds to half the maximum current value has been defined as the substrate saturation constant (Torres et al. 2010).

The saturation constants for complex substrates are difficult to determine because the transformation of high molecular weight molecules into simpler molecules causes a diauxic-type current response in which a current plateau is not observed but peaks or consecutive periods of increase and stabilization of electrical current are observed (Cercado-Quezada et al. 2010).

High concentrations of substrates of unknown composition are the least favorable condition for charge transfer; however, it is closer to actual conditions during the application of the cells. MECs fed complex and high-concentration substrates are being investigated for optimization of both hydrogen production and organics removal.

Based on studies on different types and concentrations of substrates, it is possible to confirm that, although they present limitations for electrical current production, it is necessary to continue with tests of complex substrates at high concentrations since this condition will enable exploitation of actual industrial effluents.

## 8.10 MEC Voltage and Electrode Potential

Charge transfer follows a chain from an oxidized compound to the anode through the microbial cell. The potential of the compound that acts as a substrate tends to have negative values; for example, for acetate the standard reduction potential is −280 V/SHE. Then, the compound is metabolized inside the cell, and a fraction of the charge generated is transferred via the cytochromes towards the electrode, which is subjected to a fixed positive potential.

The variation in potential between a negative value and a positive value represents energy production according to Eq. (8.5).

$$\Delta G^0 = -nF\left(E^0_{\text{substrate}} - E^0_{\text{anode}}\right) \tag{8.5}$$

At the cell membrane-electrode interface, the standard reduction potential of cytochromes is difficult to control due to the diversity of microbial species that can colonize the electrode, the variety of cytochromes that occur in the membranes, and the possible variations in the potential of cytochromes as a function of their position on the membrane. On the other hand, the potential of the electrode can be controlled using a potentiostat/galvanostat.

Owing to the uncertainty in establishing the standard reduction potentials of the cytochromes involved in the transfer of charge to the electrode, the potential of the known substrate (either acetate or glucose) is usually used for theoretical calculations. Table 8.3 lists the reduction potentials of some key elements in the

**Table 8.3** Standard reduction potential of compounds of biological interest (Rabaey and Rozendal 2010; Torres et al. 2010)

| Redox couple | $E°$, V/SHE |
|---|---|
| $CO_2$/glucose | −0.430 |
| $2H^+/H_2$ | −0.420 |
| $CO_2$/acetate | −0.280 |
| $2H_3O^+ + 2e^- \rightarrow H_{2(g)} + 2H_2O$ | 0.0 |
| Cytochrome c ($Fe^{3+}$)/cytochrome c ($Fe^{2+}$) | +0.254 |
| $3H_2O + 2e^- \rightarrow H_{2(g)} + 2OH^- + H_2O$ | +0.830 |
| $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$ | +0.840 |

transfer of microorganisms to the electrode and to other compounds that might be found in the medium.

The difference in potential between glucose or acetate and the reported cytochromes is positive, resulting in energy production. When the final charge acceptor is a compound with a reduction potential greater than the potential of cytochromes, for example, oxygen with water as a product of the reaction, charge transfer is favorable because it results in a large amount of energy production. On the contrary, when the final acceptor is hydrogen ion, which forms hydrogen molecules, the potential difference is negative and there is no spontaneous energy production.

As previously mentioned, the cell voltage is proportional to the difference in potential between the reaction at the anode and the reaction at the cathode; thus, the theoretical potential for hydrogen production from acetate is −1.10 V/SHE. However, due to energy losses that include the resistance to the transfer of electrical charge in the connections and ionic charge in the electrolytic medium, the voltage required is greater, as shown in Eq. (8.6).

$$E_{\text{cell}} = \left(E_{\text{anode}} - \text{IR}_{\text{activation,anode}} - \text{IR}_{\text{concentration,anode}}\right) - \left(E_{\text{cathode}} - \text{IR}_{\text{activation,cathode}} - \text{IR}_{\text{concentration,cathode}}\right) - \text{IR}_{\text{ohmic}} \qquad (8.6)$$

The relationship between the flow of charge in the electrode-electrolyte interface and the reduction potential of the compounds is represented by the Nernst-Monod equation (Eq. 8.7). The charge transfer between the substrate and the electrode is explained by the Butler-Volmer equation (Eq. 8.8) (Torres et al. 2010).

$$j = j_{\max}\left(\frac{1}{1 + \exp\left[\frac{-F}{RT}\left(E_{\text{OM}} - E_{\text{K}}\right)\right]}\right) \qquad (8.7)$$

where $j$ is the current density

$F$ is the Faraday constant

$R$ is the ideal gas constant

$T$ is the operation temperature

$E_{\text{OM}}$ is the potential of the outer membrane cytochromes

$E_{\text{K}}$ is the anode potential at a maximum half current

$$j = j_0 \exp\left[\frac{nF}{\mathrm{RT}}(1-\propto)\left(E_{\mathrm{anode}} - E^0\right)\right] \tag{8.8}$$

where

$J_0$ is the exchange current

$\alpha$ is the charge transfer coefficient

$E^0$ is the reduction potential for the species on the electrode

The anode potential is an element of experimental control, and the effect of this potential on the growth and activity of microorganisms has been evaluated in diverse studies. The results for different species and electrode materials seem to agree on the following aspects:

- The species forming the biofilm are influenced by the potential of the electrode.
- Microbial activity increases with electrode potential.
- The mechanism of electron transfer, direct or mediated, is affected by the electrode potential.
- There is an optimal electrode potential for the current production.

The anode potential was investigated in the range of −0.3 V to 2.0 V/SHE, whereas the cathode potential was investigated in the range of −0.4 V to −1.1 V/SHE (Jafary et al. 2015). The effects of the cathode potential on the activity of microorganisms capable of receiving charge are similar to those of the anode potential.

The potential of the electrodes is controlled in the laboratory via a potentiostat; however, for real applications, it is envisaged that a power source would be needed. Control by using a power source is not necessarily precise for each electrode, the anode and cathode suffer fluctuations along with both biofilm development on the carbonaceous material and fouling or corrosion of the metallic electrode.

A comparison between the use of a potentiostat and a power source showed superior overall performance using the potentiostat (Nam et al. 2011); however, the power source could be a more viable option for a high-scale industrial application.

## 8.11 Parameters Affecting the MEC Performance

The physical-chemical parameters used for the evaluation of MECs are the pH and conductivity. The concentration of hydrogen ions in the electroactive biofilm on the anode can limit oxidation reactions. The pH of the electrolyte that surrounds the cathode directs the hydrogen evolution reaction; as mentioned previously, at neutral pH, the production of hydroxyl ions increases the pH of the medium (Eq. 8.2).

The conductivity of the anolyte is controlled by the addition of salts compatible with microbial activity, such as sodium chloride and potassium chloride, while the conductivity in the catholyte is adjusted by changes in the composition and

concentration of buffer solutions. Research on electrolytic media is aimed at reducing the concentration of salts and their cost (Nam and Logan 2012).

One of the biological parameters that characterize the cells is the amount of sessile and planktonic microbial biomass. Biofilm development is monitored via scanning electron microscopy; transmission electron microscopy is also used to evaluate the roughness of biofilms. Another tool is scanning electrochemical microscopy, which can be applied for monitoring electroactive biofilms.

Electrochemical performance is determined by open circuit potential, cyclic voltammetry, linear sweep voltammetry, electrochemical impedance spectroscopy, and chronoamperometry. The open circuit potential shows the equilibrium state of the electrode-electrolyte systems when there is no current flow, and there is a distinction between the open circuit potential for an electrode and the open circuit voltage for the cell. The latter is referred to as the difference in potentials between the anode and cathode, while the former refers to only one electrode with respect to a reference electrode (SHE, Ag/AgCl, SCE, etc.). The equilibrium in a microbial electrochemical system is pseudostationary since the biofilm is a dynamic system; therefore, the open-circuit potential can be reached in a few hours for previously colonized electrodes but may require tens of days for noncolonized electrodes.

The potential of electrodes at zero current is positive for the anode and negative for the cathode, however, the biofilm on the electrode material causes the evolution of the potential in the anode. Figure 8.4 schematizes the current profile against potential in each of the electrodes in an electrolysis cell. The change in potential that occurs due to biofilm development at the anode causes the cell voltage to decrease, so the external energy consumption also decreases.

The cyclic voltammetry technique involves scanning the potential of a stationary working electrode using a triangular potential waveform (Fig. 8.5a). During the potential sweep, the potentiostat measures the current resulting from electrochemical reactions occurring at the electrode interface and triggers the applied potential. Voltamperograms indicate the location of redox potentials for cytochromes, mediators, and other electroactive species (Fig. 8.5b).

The scanning speed and the presence or absence of substrate during voltammetry allows obtaining the kinetic parameter (α) to estimate the current density in a redox system (Rousseau et al. 2014).

Chronoamperometry consists of applying a fixed potential to the electrode during a period in which the current generated is recorded during the reactions occurring at the potential value. Changes in current occur due to changes in the concentration of reactants in the vicinity of the electrode or modifications of the surface, such as biofilm growth.

The area under the curve that forms the chronoamperogram represents the experimental electric charge generated in the electrode-electrolyte system at a given potential and serves as an element of comparison between MECs.

The electrochemical impedance spectroscopy technique is based on the excitation of the electrochemical system by a sinusoidal signal around a fixed potential. This technique provides information on the distribution of resistances including the

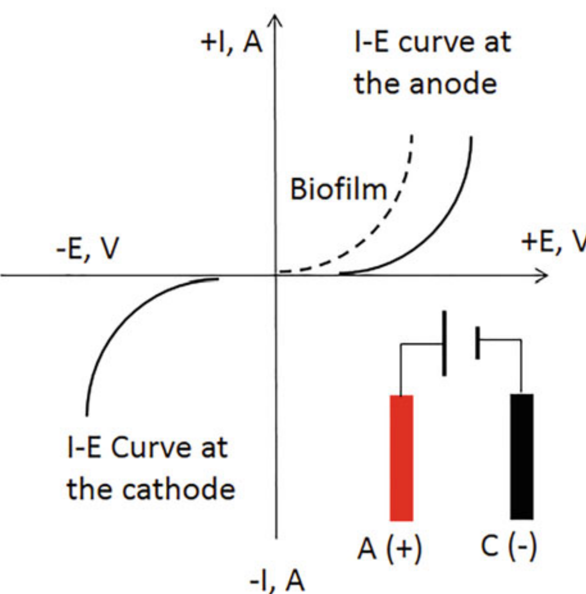



**Fig. 8.4** Diagram of the evolution of current with changes in anode, bio-anode and cathode potentials in an electrolysis cell. Modified from Bockris (1998)

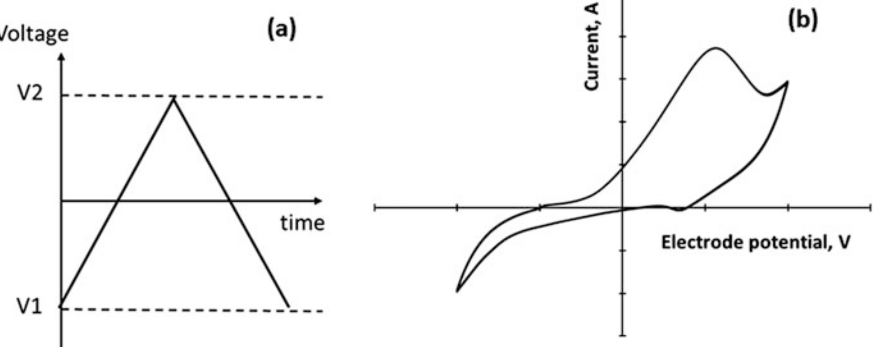



**Fig. 8.5** Cyclic voltammetry. (**a**) Representation of changes in potential over time. (**b**) Representation of changes in current with the electrode potential. Modified from Bockris (1998)

ohmic resistance and the charge transfer resistance. The impedance data are represented in the form of Nyquist spectra and the form of Bode plots (Fig. 8.6).

The analysis of the Nyquist spectra is preceded by the establishment of an electronic circuit that is equivalent to the elements that constitute the electrode–

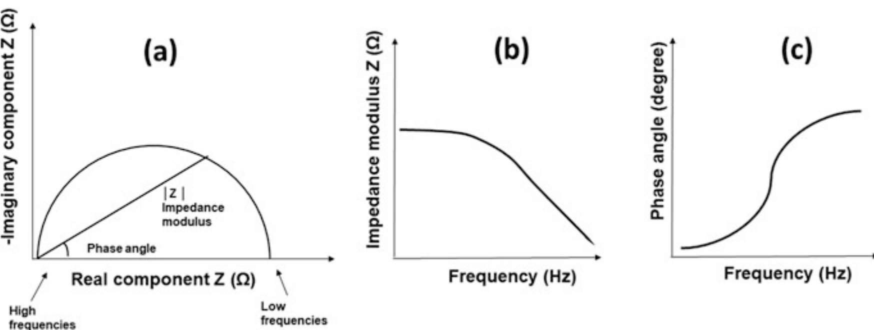



**Fig. 8.6** Graphic representation of impedance data. (**a**) Nyquist spectrum. (**b**) Bode-impedance module. (**c**) Bode-phase angle. Modified from Bockris (1998)

**Table 8.4** Electrochemical techniques used for the evaluation of MECs (Bockris 1998)

| Analytical technique | Information provided |
|---|---|
| Open circuit potential | Evolution and stability of bioelectrodes |
| Voltammetry | Evidence of redox chemical species<br>Electrochemical potential at the maximum electroactivity<br>Kinetic parameters |
| Chronoamperometry | Maximum current density at a certain substrate concentration<br>Experimental charge |
| Electrochemical impedance spectroscopy | Ohmic resistance<br>Charge transfer resistance |

electrolyte interface. A mathematical fit to the semicircle that is regularly observed in bioelectrodes allows semiquantitative estimation of the ohmic resistance. The ohmic resistance is determined by the first intersection of the semicircle with the *X*-axis, the real impedance component, and the resistance to charge transfer is indicated by the diameter using the second intersection of the semicircle with the *X*-axis. Table 8.4 summarizes the usefulness of the electrochemical techniques for the evaluation of MECs.

## 8.12 Hydrogen Production Rate in MECs

The production of hydrogen from residual organic matter using MEC technology is of great interest due to the low cost of the raw material, the use of microorganisms as self-regenerating catalysts, and the diversity of products derived from the cell operation. Depolluted water, electrical current, and hydrogen gas have value in different industrial areas; therefore, it is of great importance to achieve efficient high-scale MEC operation with high productivity.

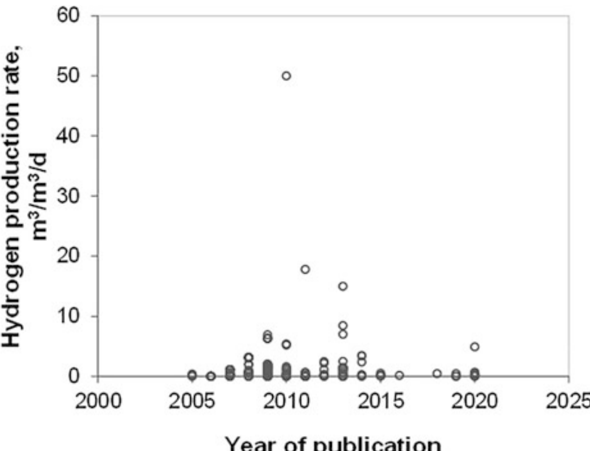



**Fig. 8.7** Maximum hydrogen production rate reached in 170 publications in the period 2005–2020. Source: ISI Web of Science

In the state of the art, hydrogen production is mostly in the range of 0.01–2.0 $m^3/m^3/d$, and only a few studies have exceeded a production value of 5 $m^3/m^3/d$ (Fig. 8.7).

The causes of low hydrogen production reported are diverse. Initially, investigations focused on the optimization of electrode materials, the charge transfer mechanisms in biocathodes, and the relevance of the use of complex substrates. In almost all of these studies, the design of the cell was not considered; therefore, the optimal volumetric production was not reached.

Some of the highest hydrogen production rates are summarized in Table 8.5. The most commonly used substrate is acetate; however, some studies with complex effluents also reported high rates. Sasaki et al. (2012) used a complex mixture simulating a garbage slurry in an MEC operated at 1.0 V to produce up to 2.45 $m^3/m^3/d$, Li et al. (2014) used corn stalk fermentation effluents in a cell inoculated with cow dung compost and operated the cell at 0.8 V to produce 3.43 $m^3/m^3/d$.

Pure cultures are scarcely used as inocula in MECs that show the highest hydrogen production rate; instead, microbial consortia are preferable. An inoculation strategy involves the use of inoculum from a bioelectrochemical device (MFC or MEC) in operation; in this manner the presence of electroactive microorganisms is enriched (Jeremiasse et al. 2012; Croese et al. 2014; Marshall et al. 2013).

The potential applied to the anode varies from 0.6 V to 1.1 V; however, there is no clear relationship between the applied voltage and the hydrogen production rate. Studies on biological cathodic reactions are more recent (Jafary et al. 2019). The cathode potential for a higher hydrogen production rate is approximately −0.65 V (Croese et al. 2014; Jeremiasse et al. 2012; Marshall et al. 2013).

Higher hydrogen production rates should be viewed with caution because normalization to cell volume can result in an arithmetic effect that greatly increases the production rate. A production rate close to 5.5 $m^3/m^3/d$ was obtained in 50-mL cells (Tartakovsky et al. 2009; Hrapovic et al. 2010), while a rate of 18 $m^3/m^3/d$ was

**Table 8.5** Summary of MECs showing high-rate of hydrogen production. Source: ISI Web of Science

| Substrate | Inoculum | Applied potential (V) | Hydrogen production rate ($m^3/m^3/d$) | Reference |
|---|---|---|---|---|
| Acetate | *Shewanella oneidensis* MR-1 | 0.6 | 2.00 | Hu et al. (2009) |
| Glycerol | Anolyte from an MFC running over 1 year | 0.9 | 2.00 | Selembo et al. (2009) |
| Acetate | Effluent from a biocathode chamber | −0.7 | 2.20 | Jeremiasse et al. (2012) |
| Effluent from biocathode chamber | – | −0.7 | 2.40 | Croese et al. (2014) |
| Synthetic garbage slurry | Methanogenic sludge | 1 | 2.45 | Sasaki et al. (2012) |
| Mineral solution with sodium bicarbonate | Supernatant from an MEC | −0.59 | 2.50 | Marshall et al. (2013) |
| Acetate | Domestic wastewater | 0.8 | 3.12 | Call and Logan (2008) |
| Cornstalk fermentation effluent | Cow dung compost | 0.8 | 3.43 | Li et al. (2014) |
| Acetate | Biofilm from anodes from an MEC | 1 | 4.90 | Wang et al. (2020) |
| Acetate | Anaerobic mesophilic sludge | 1 | 5.40 | Hrapovic et al. (2010) |
| Acetate | Anaerobic sludge | 1 | 6.30 | Tartakovsky et al. (2009) |
| Acetate | Activated sludge | 0.9 | 8.44 | Liang et al. (2014) |
| Acetate | Domestic wastewater | 1 | 17.80 | Cheng and Logan (2011) |
| Acetate | Effluent from an MEC | 1 | 50.00 | Jeremiasse et al. (2010) |

reached in a 10-mL cell (Cheng and Logan 2011), and the maximum rate of 50 $m^3/m^3/d$ was obtained in a 4-mL cell (Jeremiasse et al. 2010). Recently, a 10-L MEC provided a high hydrogen production rate, on the order of 4.9 $m^3/m^3/d$. This remarkable performance was achieved by using chloroform to inhibit hydrogen-consuming bacteria (Wang et al. 2020).

These data show the need to maintain high hydrogen productivity in high-volume MECs fed complex substrates to bring microbial electrochemical technology closer to real applications.

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# 9 Microbial Degradation of Lipids

Moises Bustamante-Torres, David Romero-Fierro, Jocelyne Estrella-Nuñez, and Emilio Bucio

**Abstract**

Lipids are biomolecules essential for the life. They play different important roles in the nature. Therefore, its degradation has been influenced by internal or external factors. Although lipids have shown be fundamental for life, its accumulation can be hazardous for the health. Recent anecdotal studies provide information about the degradation of lipids by enzymes produced by different microorganisms. Besides, microorganism as bacteria and fungi encompasses a wide range of species that can produce positive or negative effects on the environment. Some report highlighted the better benefits of enzymes derived from microorganisms than those derived from other species such as animals and vegetables. Additionally, external factors such as pH, temperatures, substrate, and metallic ions generate important roles during the lipolitic degradation. This chapter describes the biochemistry degradation of the lipids (fatty acids) and some examples related to microorganism species and their source producing

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fundamental enzymes as well as a wide range of applications approached into industrial, biotechnological, and medicine.

**Keywords**

Lipids · Biomolecules · Lipolitic degradation · Enzymes · Microorganisms

## 9.1 Introduction

### 9.1.1 Overview of Lipids

Biomolecules are fundamental organic molecules of life. Several macromolecules (protein, carbohydrates, nucleic acids, and enzymes) and small molecules (amino acids, vitamins, fatty acids, neurotransmitters, and hormones) fall under the category of biomolecules (Suresh et al. 2019). They provide an essential structure, storage, and function supporting the living being's life.

Lipids are usually referred to as fats solid at ambient temperature, and oils are those liquid at ambient temperature (Jambrak and Škevin 2017). Mainly, lipids are considered as water-insoluble organic substances, usually classified as oils and fats. They are key components of the plasma membrane and other cellular compartments, including the nuclear membrane, the endoplasmic reticulum, the Golgi apparatus, and trafficking vesicles such as endosomes and lysosomes (Muro et al. 2014).

Moreover, lipids are fundamental for living beings as energy storage and heat retention. They can be considered as the major class of biomolecules even either potential health risks or important benefits for the life. They have generally been classified into fatty acids (FA), waxes, triaglycerols, glycerophospholipids, sphingolipids, steroid, and terpenes (abundant in plants) (Lipids 2000).

FA is a lipid biomolecule. FA possess a long hydrocarbon chain (a compound consisting entirely of carbons and hydrogens) and a terminal carboxyl group (Aldred et al. 2009). FA can be saturated fatty acids (SFAs), which are highly flexible because the single bonds presented into the structure. Meanwhile, unsaturated fatty acids (UFAs) present one or more double bonds that can be (*cis* and *trans*). Based on that double bonds, the geometry can get two different configurations: *cis* and *trans*. In the *cis* form, the two hydrogen substituents are on the same side of the molecule, while in the *trans* form they are on opposite sides (Gurr and Harwood 1991) (Fig. 9.1). Importantly, most of the naturally occurring FAs in humans are in *cis* configuration (Menaa et al. 2016).

**Fig. 9.1** Configuration of FA *cis* and *trans*

The degradation of FA will not only depend on the configuration but also the size of the carbon chain. Mainly, the FA are subjected to B-oxidation into the mitochondria. However, based on the chain size, it can be undergoing on peroxisomes and glioxisomes (20–22 carbons), with a minimum change during the biochemistry process. This process is carried out by different lipid enzymes, which are synthetized by different microorganisms, which in turn will generate a great number of extracellular lipases.

The lipolytic activity of microbial enzymes has been studied long time ago. There are a wide variety of microbial enzyme sources. Different species as bacteria and fungi produce enzymes with lipolytic activity. However, different external factors such as pH, temperatures, substrate, and metallic ions play an important role during the isolation of these enzymes as well as the sources. There are a wide variety of microbial enzyme sources, which have shown specific properties with potential application on different areas.

The advance in technology has enhanced the knowledge about lipids. This biomolecule presents either positive or negative effects on the health. So, a great number of considerations get to be approved prior to ingestion. The different studies made by scientists and researcher to determine properties of lipids on benefit to the human's beings changing different parameters. Here, we review the lipid degradation.

## 9.2 Biochemistry

Lipids are a set of biomolecules depicting important functions. Its strongly reduced state and its hydrophobicity get it to accumulate anhydrously, without bound water, and therefore without any extra weight of solvation water. On the other hand, its hydrophobicity also allows its accumulation in practically inert droplets due to the low chemical reactivity they present (Becker 2010).

### 9.2.1 Lipids Functions

The major roles of lipids can be described conveniently as structural, storage, and metabolic, although individual lipids may have several different roles at different times or even at one and the same time (Gurr and Harwood 1991).

Carbohydrates provide a great source of energy, meanwhile the energy reserve for humans and plants are based on certain lipids called triglycerides. The function of lipids as energy reserves of high free energy content per unit weight (9 kcal/g) is one of the most general and significant functions of these compounds in animals (Allen 1976), regulating, signaling, controlling, and maintaining the temperature.

It is now recognized that cholesterol, fatty acids, and other dietary lipids serve as precursors for ligands that bind nuclear receptors and participate in signal transduction (Kennedy 2007; Chawla et al. 2001).

In recent years, evidence has emerged showing that lipid signaling is a vital part of cell signaling (Malinauskas et al. 2011; Malinauskas 2008; Wang 2004; Dinasarapu et al. 2011). Biological membranes are made up of glycerophospholipids, sphingolipids, and sterols (Thompson 2021). Membrane lipids are made of polyunsaturated fatty acids (Mandal 2019). Generally, their shape is elongated, with a hydrophilic end or head attached to a hydrophobic moiety by a short intervening region of intermediate polarity (Thompson 2021).

## 9.3 The Process of Lipid Degradation

### 9.3.1 Lipid Degradation

Since lipids are not water soluble, they must be solubilized for digestion. This is the role of bile acids secreted from the liver via the gall bladder (Kennedy 2007). Unsaturated FA undergo autoxidation much more readily than saturated FA (Chiofalo and Lo Presti 2012).

Lipids are aliphatic biomolecules fundamental for life. The degradation process converts a long-chain aliphatic molecule, such as a fatty acid, to a set of activated acetyl-coenzyme A (Acetyl-CoA) unit, which can be processed by the citric acid cycle. It starts from an activated FA molecule, which oxidizes, hydrates, oxidizes, and finally splits, obtaining Acetyl-CoA and an activated FA again but with two fewer carbons (Becker 2010). This process is carried out inside the mitochondria, in the mitochondrial matrix, by means of a metabolic pathway called β-oxidation (Mannaerts et al. 2000).

To carry out the degradation, the first step is the activation and entry of FA into the mitochondria. The free FA found in the cytoplasm are energetically activated by an enzyme located in the outer mitochondrial membrane, acyl-CoA synthetase, which performs the following reaction:

$$\text{Fatty acid} + \text{CoA} + \text{ATP} \rightleftharpoons \text{acylC}\tilde{\text{o}}\text{A} + \text{AMP} + \text{PP}_\text{i}$$

The process proceeds in two stages: First, the FA reacts with adenosine triphosphate (ATP) to form an acyladenylate or acyl~adenosine monophosphate (AMP), and second, the phosphate bond of the energy is transferred to a thioester bond, also of high energy, reacting CoA with acyl-AMP to give acyl~CoA and AMP. Activation of an FA involves an energy cost of two high-energy bonds per molecule (Becker 2010).

The next obstacle to activate FA, or acyl-CoA, lies in the impermeability of the inner mitochondrial membrane. Long-chain acyl-CoA molecules need a transport system that allows them to pass into the mitochondria (Melser et al. 2015). This transport system is made up of a molecule, derived from the amino acid lysine, called carnitine, which binds to acyl-CoA to form a derivative, acyl-carnitine. This transport system is called a carnitine shuttle and constitutes one of the transport modalities at the level of the internal mitochondrial membrane (Melser et al. 2015).

β-oxidation constitutes a stage II catabolic route, in which the degradation of the FA will take place until a common intermediary that is the acetyl-CoA molecule. This route consists of four reactions in a row. At the end of this sequence, the FA has two fewer carbon atoms, which have become independent as acetyl-CoA (Jones and Bennett 2017). The sequence of reactions is depicted at Fig. 9.2.

The resulting acyl-CoA would restart in reaction 1 to undergo another β-oxidation cycle and continue to separate units of two carbon atoms (Melser et al. 2015).

Total oxidation of FA to $CO_2$ and $H_2O$ requires the use of other metabolic pathways. Acetyl-CoA continues its oxidative process through the citric acid cycle in which reduced coenzymes are produced. As mentioned previously, the β-oxidation process is carried out for the most part in the mitochondrial matrix, although it is also possible in other cytoplasmic organelles such as peroxisomes (Mannaerts et al. 2000). Peroxisomal β-oxidation works on very long chain FA, in a range between 20 and 26 carbons, which is developed in the next section.

In addition to SFAs, those with double bonds are also degraded (UFAs). This type of molecule requires the action of other enzymes, in addition to those of β-oxidation. Without the double bond being located in odd positions, like the one that forms between carbon atoms in β-oxidation, the added reaction is reduced to the change in position of the substituents in the double bond, going from the *cis* configuration to *trans* position by the isomerase (Kresge et al. 2010).

If the double bond is located in even positions, two enzymes are used, a reductase that changes the location of the double bond by reducing the molecule, and the isomerase that changes the *cis* to *trans* configuration of the bond (Melser et al. 2015).

The existence of double bonds eliminates the first reaction of the β-oxidation; therefore, there is less oxidation, and consequently, a lower number of reduced coenzymes will be generated, which will translate into lower energy efficiency.

Finally, although they are found in a minority, the oxidation of chain FA with an odd number of carbons is carried out by the same process as even chain FA, with the only difference that the last two products of the reaction. Instead of being two units of two carbon atoms, they are one molecule of two atoms, acetyl-CoA, and another molecule of three atoms, propionyl-CoA (Melser et al. 2015).

### 9.3.2 Degradation of Long-Chain Fatty Acids

Mitochondria are not the only organelle in which FA oxidation can take place. Animal and plant peroxisomes and glioxisomes from germinating seeds are other possible locations of B-oxidation. Unlike the enzymes of mitochondrial B-oxidation, the corresponding enzymes of peroxisomes and glyoxysomes form a multienzyme complex in which, in addition, enoyl-CoA hydratase and hydroxyacyl-CoA dehydrogenase constitute a bifunctional enzyme (Le Borgne and Demarquoy 2012).

The oxidation of very long chain fatty acids (above 20–22 carbons), branched or dicarboxylic acids, takes place in peroxisomes. In these organs, the oxidative process of FA is not aimed at producing energy because they lack an electronic transport chain.

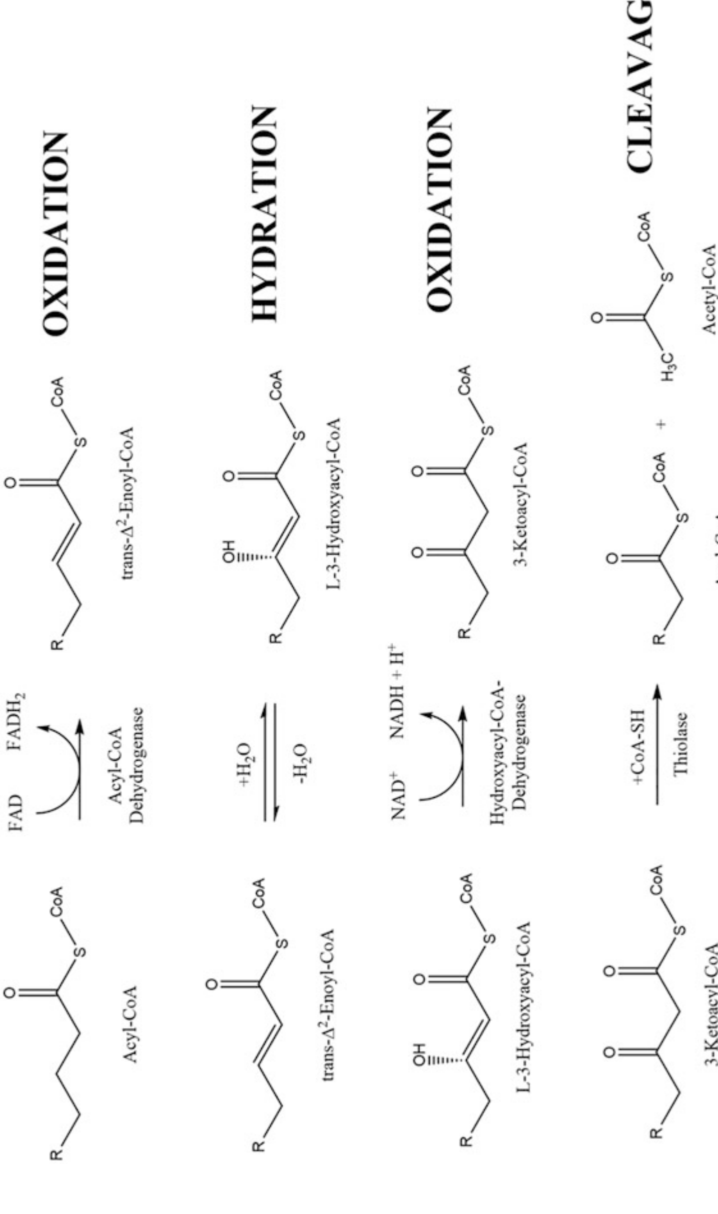


**Fig. 9.2** Degradation process of short-chain lipids

The activation of FA for their peroxisomal oxidation takes place in the peroxisome itself. Then, the oxidation process is similar to that of mitochondrial B-oxidation with the exception that the first oxidation does not have FAD as an electron acceptor, but directly to oxygen, so it generates $H_2O_2$ instead of $FADH_2$ and energy (ATP) that would derive from the subsequent transfer of electrons from $FADH_2$ to the electron transport chain dissipates as heat. Since peroxisomal thiolase is not active with FA of less than eight carbons, shortened fatty acids in the peroxisome will continue their oxidation in the mitochondria.

The hydrogen peroxide ($H_2O_2$) generated is a strong and potentially harmful oxidant, which is why it is eliminated by the cell through catalase, through the following reaction:

Acetyl-CoA is exported to the cytosol, and from there it can enter the mitochondria to be oxidized. Figure 9.3 shows the mechanisms of that fatty acids shortened in length by peroxisomal oxidation can be conjugated with carnitine to pass from peroxisome to mitochondria and continue B-oxidation there.

### 9.3.3 Production, Secretion, and Activation of the Lipolytic Enzymes (Lipases)

Lipases or lipolytic enzymes are responsible for the degradation of lipids, molecules insoluble in water. Lipases have been studied for 300 years; however, its ability to catalyze hydrolysis and synthesize esters has been recognized just 70 years before (Casas-Godoy et al. 2018). This type of enzymes is of great importance in the industry due to their multiple applications because they carry out the degradation of substrates with a high fat content as well as in esterification reactions in the food, pharmaceutical, and cosmetic industries (Becker 2010). Lipases have been found in many species of animals, plants, and microorganisms. However, microbial lipases are much more versatile and have high substrate specificity, as well as regio- and enantioselectivity (Gupta et al. 2004).

Lipases are synthesized by microorganisms, preferably in the presence of inducers such as lipids (Casas-Godoy et al. 2018). These molecules and other substances present in agro-industrial waste allow the growth of microorganisms and act as inducing substances for the production of microbial lipases (Pratush and Gupta 2016). Certain natural oils such as soybean oil, fatty acids, and esters such as soaps, sterols such as cholesterol, bile salts and detergents behave as inducers and therefore are widely used for the production of microbial lipases (Alford et al. 1964).

Microorganisms with a high potential to produce lipases can be found in different habitats. However, they are mainly found in vegetable oil waste or residues used during the production of frying, dairy products industries, soils contaminated with oils, and spoiled food. From these points, bacteria, filamentous fungi, yeasts, and actinomycetes have been isolated, among which the genus *Pseudomons*, *Bacillus*, *Rhodococcus*, *Staphylococcus*, *Rhizopus*, *Candida*, and *Geotrichum sp* stand out for their great capacity to generate extracellular lipases, thus facilitating, the recovery of these enzymes from the culture medium (Celligoi et al. 2017).

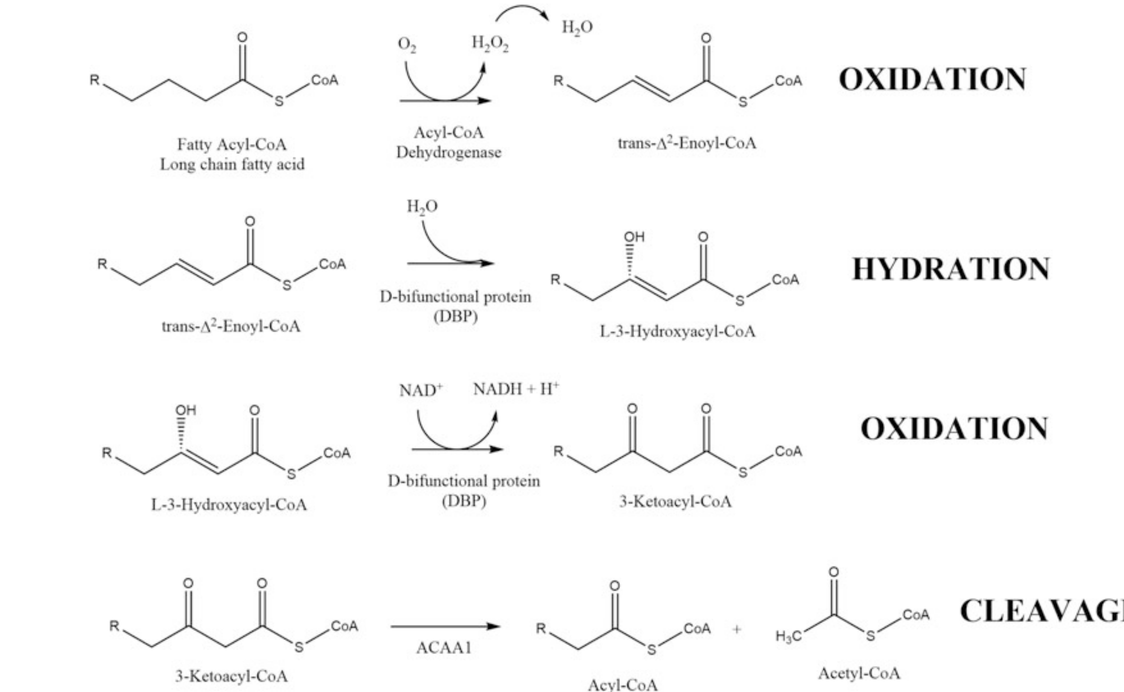


**Fig. 9.3** Degradation process of long-chain lipids

Gene expression of bacterial lipases is highly regulated, with cell density–dependent regulation being the most general mechanism. However, there are additional activators and repressors that modulate the gene expression of these enzymes in response to a wide variety of environmental signals (Pratush and Gupta 2016).

After protein synthesis, most of these enzymes fold by themselves in the cytoplasm or during its secretion process, although in some cases their folding requires the intervention of intramolecular or intermolecular chaperones, or proteins responsible for establishing disulfide bonds (Alford et al. 1964).

Regarding their enzymatic properties, bacterial lipases are active on a wide range of substrates in which, they carry out different hydrolysis, synthesis, or group exchange reactions. Furthermore, these enzymes are highly stable in a wide range of temperatures, pH (Becker 2010), and organic solvents. Bacterial lipases are also stable against detergent differentials, ions, and chemical agents and generally do not require cofactors (Gupta et al. 2004). For these reasons, and due to the existing knowledge about these enzymes, their availability, and the fact that the processes in which they intervene are generally less expensive and less polluting, bacterial lipases have received the attention of the scientific community.

## 9.4 Microorganisms with Lipolytic Activity

Lipolytic enzymes have a wide field of investigation by acting on reactions such as synthesis, hydrolysis, esterification, transesterification, alcoholysis, exchange of functional groups, and by its functional stability at different conditions (Samoylova et al. 2019; Gopinath et al. 2013).

### 9.4.1 Species

#### 9.4.1.1 Bacterial Species

There are a large number of bacterial species between Gram-positive and Gram-negative showing the presence of diverse enzymes with lipolytic activity. Lipases, esterases, and laccases were studied under different conditions of pH and temperature. A large number of bacterial species were isolated and characterized in previous research and are summarized in Table 9.1. Hydrolases (lipases and esterases) have shown great lipolytic activity and have been found in many bacterial microorganisms. Certain microorganisms are found from different sources. Figure 9.4 shows the most common species with a preponderant role in the industry and some examples of them.

#### 9.4.1.2 Fungi Species

Fungi are microorganisms preferred as producer's industrial lipases because they usually produce extracellular enzymes, which facilitate the extraction from fermentation medium. Also, fungal enzymes are more stable and have more diverse properties compared to enzymes from other sources. Fungal microorganisms have

**Table 9.1** Bacterial species with lipolytic activity

| Phyla | Gender | Specie | Source |
|---|---|---|---|
| Actinobacteria | *Micrococcus* | *Micrococcus luteus* | Wood (Ramnath et al. 2017)<br>Oils (Haba et al. 2000)<br>Deep-sea sediments (Scholante Delabary et al. 2020) |
| | *Streptomyces* | *Streptomyces costaricanus* | Wood (Ramnath et al. 2017) |
| | | *Streptomyces lavendule* | Water sources (Haba et al. 2000)<br>Contaminated oils (Haba et al. 2000) |
| | | *Streptomyces coelicolor* | Glacier soil (Borda-Molina et al. 2017) |
| | | *Streptomyces cinnamoneus* | Soills (Jaeger and Eggert 2002) |
| | *Cellulosimicrobium* | *Cellulosimicrobium cellulans* | Wood (Ramnath et al. 2017) |
| | *Nocardia* | *Nocardia pneumonia* | Wood (Ramnath et al. 2017) |
| | *Prauserella* | *Prauserella sp.* | Wood (Ramnath et al. 2017) |
| | *Saccharomonospora* | *Saccharomonospora sp* | Wood (Ramnath et al. 2017) |
| Fruit | *Bacillus* | *Bacillus firmus* | Wood (Ramnath et al. 2017) |
| | | *Bacillus ginsengihumi* | Wood (Ramnath et al. 2017) |
| | | *Bacillus thuringiensis* | Wood (Ramnath et al. 2017) |
| | | *Bacillus cereus* | Water sources (Haba et al. 2000)<br>Contaminated oils (Haba et al. 2000) Wood (Ramnath et al. 2017) |
| | | *Bacillus subtilis* | Oils (Haba et al. 2000)<br>Soils (Jaeger and Eggert 2002)<br>Water sources (Haba et al. 2000)<br>Contaminated oils (Haba et al. 2000; Carrazco-Palafox et al. 2018)<br>Glacier soil (Borda-Molina et al. 2017) |
| | | *Bacillus glumae* | Oils (Prasad 2014) |
| | | *Bacillus niacini* | Palm oil mill effluent (Oyedele et al. 2019) |
| | | *Bacillus safensis* | Olive oil-contaminated soil (Abdelli et al. 2019) |

(continued)

**Table 9.1** (continued)

| Phyla | Gender | Specie | Source |
|---|---|---|---|
| | | *Bacillus pumilus* | Oils (Haba et al. 2000)<br>Soils (Jaeger and Eggert 2002)<br>Water sources (Haba et al. 2000)<br>Contaminated oils (Haba et al. 2000)<br>Oilseed (Sandi et al. 2020)<br>Glacier soil (Borda-Molina et al. 2017) |
| | *Straphylococcus* | *Straphylococcus aureus* | Soils contaminated (Haba et al. 2000; Carrazco-Palafox et al. 2018)<br>Soils (Jaeger and Eggert 2002)<br>Water sources (Haba et al. 2000) |
| | | *Straphylococcus haemolyticus* | Soils (Jaeger and Eggert 2002) |
| | | *Straphylococcus sp.* | Contaminated soil (Furini et al. 2018)<br>Deep-sea sediments (Scholante Delabary et al. 2020) |
| | | *Straphylococcus epidermidis* | Soils (Jaeger and Eggert 2002; Qiao et al. 2018) |
| | | *Staphylococcus arlettae* | Olive oil |
| Proteobacteria | *Pseudomonas* | *Pseudomonas aeruginosa* | Wood (Ramnath et al. 2017)<br>Soils contaminated (Haba et al. 2000; Carrazco-Palafox et al. 2018)<br>Soils (Jaeger and Eggert 2002; Kenthorai Raman et al. 2008)<br>Oils (Haba et al. 2000)<br>Glacier soil (Borda-Molina et al. 2017) |
| | | *Pseudomonas stutzeri* | Wood (Ramnath et al. 2017) |
| | | *Pseudomonas cepacian* | Oils (Prasad 2014; Kenthorai Raman et al. 2008) |
| | | *Pseudomonas mendocina* | Oilseeds (Sandi et al. 2020) |
| | | *Pseudomonas luteola* | Glacier soil (Borda-Molina et al. 2017) |
| | | *Pseudomonas glumae* | Oils (Prasad 2014) |

(continued)

**Table 9.1** (continued)

| Phyla | Gender | Specie | Source |
|---|---|---|---|
| | | *Pseudomonas fluorescens* | Oils (Prasad 2014; Kenthorai Raman et al. 2008)<br>Contaminated oils (Haba et al. 2000)<br>Soils (Jaeger and Eggert 2002)<br>Water sources (Haba et al. 2000)<br>Glacier soil (Borda-Molina et al. 2017) |
| | | *Pseudomonas fragi* | Soils (Jaeger and Eggert 2002) |
| | | *Pseudomonas vulgaris* | Soils (Jaeger and Eggert 2002) |
| | *Inquilinus* | *Inquilinus limosus* | Wood (Ramnath et al. 2017) |
| | *Pantoea* | *Pantoea sp.* | Wood (Ramnath et al. 2017) |
| | | *Pantoea ananatis* | Wood (Ramnath et al. 2017) |
| | *Aeromona* | *Aeronoma sobria* | Raw milk (Lotrakul and Dharmsthiti 1997) |
| | | *Aeromona hydrophila* | Glacier soil (Borda-Molina et al. 2017) |
| | *Klebsiella* | *Klebsiella sp.* | Wood (Ramnath et al. 2017) |
| | *Leclercia* | *Leclercia sp.* | Wood (Ramnath et al. 2017) |
| | *Serratia* | *Serratia marcescens* | Industrial effluents (Peil et al. 2016a)<br>Soils (Qiao et al. 2018)<br>Oilseeds (Sandi et al. 2020) |
| | *Klebsiella* | *Klebsiella pneumoniae* | Wood (Ramnath et al. 2017) |
| | *Erwinia* | *Erwinia sp.* | Wood (Ramnath et al. 2017) |

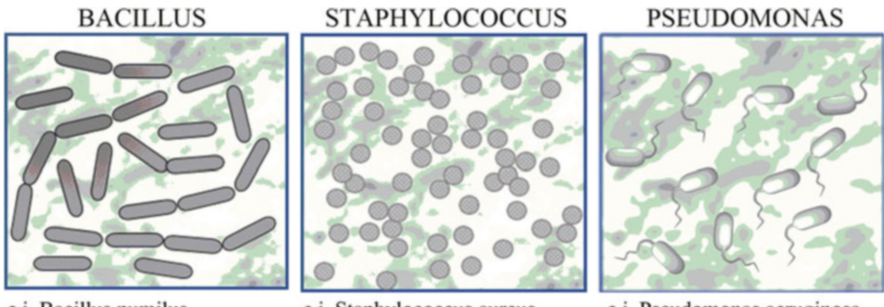


**Fig. 9.4** Bacterial species with highest presence in different microorganisms

been studied by their role in the degradation of undesirable materials or compounds, including sewage waste from domestic and industrial complexes plant, animal, and agricultural wastes, oil spills, and dairy waste (Gopinath et al. 2013). Based on previous reports, there are numerous species from fungal microorganisms that are detailed in Table 9.2.

### 9.4.2 Sources

Microbial enzymes with prominent lipolytic activity could be obtained from numerous sources. The most common are vegetable oils such as neem oil, sunflower oil, cottonseed oil, groundnut oil, pongamia oil, gingelly oil, olive oil, palm oil, and cod liver oil (Prasad 2014). So, based on environmental approach, many types of researcher's report lipolytic enzymes obtained from industrial effluents, contaminated soils, and agro-industrial residues (Gopinath et al. 2013; Oyedele et al. 2019; Qiao et al. 2018; Peil et al. 2016a; Pinotti et al. 2017; Cardenas et al. 2001; Abbas et al. 2002). On the other hand, the grown of microorganism with lipolytic activity at extreme conditions of temperature and pH have been studied. These conditions were reported from glacier soils and deep-sea sediments (Scholante Delabary et al. 2020; Borda-Molina et al. 2017). Moreover, lipolytic enzymes have been reported from wood species (i.e. Eucalyptus sp.) or from oleaginous seeds (Ramnath et al. 2017; Sandi et al. 2020; Lucretia and Tamara 2014).

## 9.5 Factors Influencing Lipolytic Degradation

### 9.5.1 pH

pH effect on the enzyme activity was studied under a range of pH depending on the substrate used. The stability was determined by measuring the residual activity after the incubation (Ramnath et al. 2017). The change in the pH could affect the intracellular and extracellular fractions, getting a different influence between them. Existing enzymes have an optimal pH in the alkaline or acidic range, which has an influence on better activity (Sayali and Satpute 2013).

### 9.5.2 Temperature

Temperature is a factor with potential effects either on the activity and stability of enzymes. There is an optimal temperature range in which the lipolytic effect increase and it is related to the incubation period. For example, lipases and esterases were reported at temperatures from 30 to 35°C, respectively, meanwhile for laccase at range temperature from 25 to 80°C (Snajdr 2007). Thermostable enzymes are determinant objective in industrial procedures related to working and storing temperatures (Sayali and Satpute 2013).

**Table 9.2** Fungi species with lipolytic activity

| Phyla | Gender | Specie | Source |
|---|---|---|---|
| Ascomycota | *Paecilomyces* | *Paecilomyces sp.* | Wood (Ramnath et al. 2017) |
| | | *Paecilomyces variotii* | Oil mill wastes (Gopinath et al. 2005)<br>Wood (Ramnath et al. 2017) |
| | | *Paecilomyces formosus* | Wood (Ramnath et al. 2017) |
| | Aspergillus | *Aspergillus fumigatus* | Wood (Ramnath et al. 2017)<br>Oil mill wastes (Gopinath et al. 2005) |
| | | *Aspergillus niger* | Groundnut oil, olive oil, sunflower oil, soya oil, palm kernel oil (Popoola and Onilude 2017; Almyasheva et al. 2018)<br>Oil mill wastes (Gopinath et al. 2005) |
| | | *Aspergillus flavus* | Oil mill wastes (Gopinath et al. 2005) |
| | | *Aspergillus versicolor* | Oil mill wastes (Gopinath et al. 2005) |
| | | *Aspergillus sp.* | Oilseeds (Sandi et al. 2020) |
| | | *Aspergillus terreus* | Cottonseed oil, palm kernel oil (Popoola and Onilude 2017)<br>Oil mill wastes (Gopinath et al. 2005) |
| | Geosmithia | *Geosmithia arqillacea* | Wood (Ramnath et al. 2017) |
| | Penicillium | *Penicillium verruculosum* | Wood (Ramnath et al. 2017) |
| | | *Penicillium expansum* | Olive oil, palmkernel oil (Popoola and Onilude 2017) |
| | | *Penicillium herquei* | Sunflower oil, soya oil (Popoola and Onilude 2017) |
| | | *Penicillium funiculosum* | Cottonseed oil (Popoola and Onilude 2017)<br>Oil mill wastes (Gopinath et al. 2005) |
| | | *Penicillium oxalicum* | Oil mill wastes |
| | | *Penicillium spinulosum* | Wood |
| | | *Penicillium sp.* | Agroindustrial residues (Pinotti et al. 2017) |
| | Acremonium | *Acremonium implicatum* | Wood |
| | | *Acremonium strictum* | Oil mill wastes (Gopinath et al. 2005) |
| | Phialophora | *Phialophora alba* | Wood (Ramnath et al. 2017) |
| | Curvularia | *Curvularia lunata* | Oil mill wastes (Gopinath et al. 2005) |
| | | *Curvularia sp.* | Wood (Ramnath et al. 2017) |

(continued)

**Table 9.2** (continued)

| Phyla | Gender | Specie | Source |
|---|---|---|---|
| | Aspicilia | *Aspicilia cinerea* | Wood (Ramnath et al. 2017) |
| | Torrendiella | *Torrendiella eucalypti* | Wood (Ramnath et al. 2017) |
| | Brachyalara | *Brachyalara straminea* | Wood (Ramnath et al. 2017) |
| Basidiomycota | Phanerochaete | *Phanerochaete chrysosporium* | Wood (Ramnath et al. 2017) |
| | Basidiomycota | *Basidiomycota sp* | Wood (Ramnath et al. 2017) |

### 9.5.3 Substrate

It is known from previous researches that when microorganisms are cultivated on fat-type substrates, lipase production occurs as a physiological response to the presence of fatty materials into the growth medium (Fickers et al. 2005). The substrate's concentration employed to isolate enzymes influences directly on their lipolytic activity. Likewise, it was reported a promising result from low concentrations to high concentration depending on the bacterial species, the medium, and the method used to measure (Ramnath et al. 2017). Moreover, the molecular properties of the enzyme, the structure of the substrate related to the digestion procedure, and some binding factors influence the interaction between the enzyme and the substrate altering the specificity of the reaction (Jensen and DeJong 1983). Most of the studies test the substrate when an optimal temperature and pH are determined (Prasad 2014).

### 9.5.4 Metallic Ions

Metallic ions could have an inhibitory or a stimulating effect on the enzyme, based on their concentration and binding affinity between the ion and the binding sites (Oyedele et al. 2019). In previous reports, the lipolytic activity efficiency was evaluated depending on the addition of ions such as calcium, copper, mercury, iron, zinc, magnesium, potassium, among others, which influence in the active site of the enzymes (Carrazco-Palafox et al. 2018; Oyedele et al. 2019; Arpingny and Jaeger 1999; Saraswat et al. 2017).

## 9.6 Applications

### 9.6.1 Beneficial Properties

The use of enzyme obtained from microorganism has been feasible due to different factors, such as action over wide range of pH, catalyze a wide range of reactions, do not require cofactors, high substrate specificity and a wide application by its stereo, regio and enantiospecific behaviors (Gunasekaran and Das 2005; Hasan et al. 2006; Saxena et al. 2003). Enzymes obtained from microorganism are often more useful than enzymes derived from animal or vegetable species due to wide catalytic activities, the high yields and stability, fast growth, and they are genetically manipulable (Hasan et al. 2006; Wiseman 1977).

Based on that properties and characteristics, the use of these enzymes has been tremendous interest among scientists and industrialists.

### 9.6.2 Industrial Applications

The most common industrial application was focus on detergents, cosmetics, textile, dairy food, fat and oils, fuel, cleaning, paper, and polymer.

In the area of detergents, lipase enzymes have been used for a long time. The main interest in this industry is focused on obtaining an enzyme that can improve the washing capacity of detergents and minimize the use of phosphate-based chemicals in the typical detergent formulation (Saraswat et al. 2017; Chauhan et al. 2013). Previous research has shown that the use of enzyme improves washing capacity in a high percentage, considering a wide variety of detergents that include trademarks (Hemachander and Puvanakrishnan 2000).

In the dairy industry, bioactive compounds based on microbial enzymes are used in a variety of food such as milk, milk powder, butter, cheese, and meat among others (Gunasekaran and Das 2005; Hasan et al. 2006; El Soda et al. 1995). These were used for beneficial purposes in food processing, flavor development, and improving quality (Hasan et al. 2006). For the food industry, there are some reactions of interest as interesterification, hydrogenation, and fermentation, which are related to the development of functional and healthy foods (Shelley et al. 1987; Ur Rahman et al. 2016).

One prominent research was based on biofuel production through enzymatic transesterification. *Pseudomonas cepacian, Chromobacterium viscosum, Mucor miehei, Rhizopus oryze, Pseudomona fluorescens, Aspergelius penicillium*, and more species were reported for the use in the production of biodiesel fuel (Almyasheva et al. 2018; Man et al. 2009). The *Pseudomona fluorescens* was identified as one specie with the highest activity on the reaction (Tsouko et al. 2016; Iso et al. 2001). Biodiesel based on vegetable oils does not produce sulfur oxide and minimize the soot emission in comparison with petroleum (Hasan et al. 2006). Nowadays, the production of biodiesel is mainly dependent on residual

vegetable oils, and the studies are focused on the optimization of reactions (Almyasheva et al. 2018; Man et al. 2009; Darnoko and Cheryan 2000).

The synthesis of polymers has been taken attention in the industry because they are biodegradable and have a great diversity of renewable natural sources. Enzymes such as lipases and esterases are usually used as catalysts for polymeric synthesis due to their high selectivity under mild reaction conditions (Menaa et al. 2016; Gross et al. 2001).

### 9.6.3 Biotechnological Applications

#### 9.6.3.1 Bioremediation

One of the most important applications was based on environment treatment with the purpose of eliminating pollutants from diverse sources. One source of interest is effluent samples from industries of different kinds. It was reported that there are microorganisms with the potential of enzymes production that can survive to industrial and environmental conditions, and the objective of these studies is to improve the production of certain enzymes with wide applicability and efficiency for bioremediation processes (Gopinath et al. 2013; Peil et al. 2016b; Stuer et al. 1986). The capacity to catalyze the hydrolysis of triglycerides plays an important role in the degradation of fats and oils in contaminated soils (Gopinath et al. 2013; Gunasekaran and Das 2005). Another relevant application is related to plastic degradation, where bacterial and fungi microorganism could degrade polymers like polyethylene, polyurethane using it as a substrate (Glass and Swift 1989). In this process, there are some factors such as kind of polymers, organism characteristics, and type of treatment required, which influence degradation activity (Gu et al. 2000). Figure 9.5 shows many fungi and bacterial enzymes have been discovered and evaluated as plastic degradant process. Some species reported are *Pseudomonas, Comamonas*, *Bacillus, Aspergillus, Caldimonas, Aureobacterium*, among others (Sayali and Satpute 2013*;* Urbanek et al. 2019).

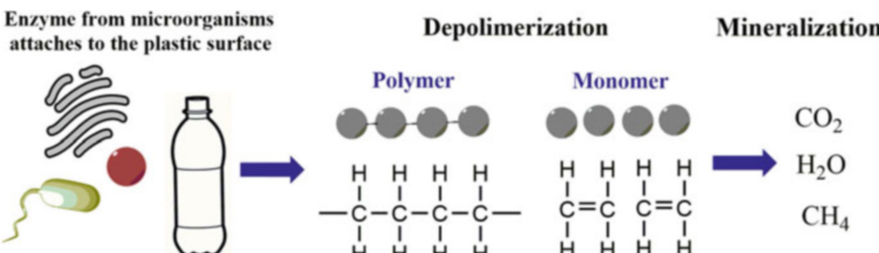


**Fig. 9.5** Plastic degradation by microorganism's enzymes by lipolytic activity

### 9.6.4 Medicine

The use of lipolytic enzymes has been important in the pharmaceutical area due to its enantioselectivity in synthesis reactions (Jaeger and Eggert 2002). In case of lipase enzymes, these were studied as a drug targets and enzymatic markers that were used as diagnostic tools for different diseases (Hasan et al. 2006; Rameshwaram et al. 2018). Moreover, another species was reported by their capacity to produce biosurfactants, which could be useful for its antibacterial, anti-adherent, and cytotoxic activities (Abdelli et al. 2019).

## 9.7 Conclusions

In summary, lipids are biomolecules that fulfill several functions fundamental for life. They are spread into nature in different ways. Lipids are degraded by different enzymes known as lipases, which are synthesized by microorganisms with lipolytic activity. However, external factors are involved during the degradation of this biomolecule. Lipids are water insoluble. The overall process consists of the degradation of lipids in Acetyl-CoA units by the citric acid cycle. This process takes place in the mitochondria. However, in animals and plants, FA oxidation takes place on peroxisomes and glioxisomes.

Lipases are synthesized by microorganisms, mainly those from vegetable oil waste or residues. Lipases are highly stable at different temperatures and pH. Bacteria gram-positive and gram-negative show lipolytic activity. Fungi species are the major source of lipases, producing extracellular enzymes and even with more stables properties. Bacteria, fungi, and even yeast have been studied and isolated, such as the genus *Pseudomonas*, *Bacillus*, *Rhodococcus*, *Staphylococcus*, *Rhizopus*, *Candida*, and *Geotrichum* sp. present a great ability to produce lipases.

Microorganisms with lipolytic activity have been applied to industry (detergents, polymers, food, oils, fuel, etc.). Another potent application is based on the biotechnology with an approach to bioremediation of the environment like plastic degradation and hydrolysis of triglycerides. Finally, lipid functions have been applied to medicine field as drug targets, enzymatic markers, and biosurfactants.

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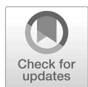

# 10 Microbial Degradation of Steroids

Shashi Kiran Misra, Kamla Pathak, and Devender Pathak

**Abstract**

Steroids are naturally found in humans, plants, fungi, and arthropods to implement several developmental functions. Steroids are also extensively used in the healthcare systems to design a myriad of therapeutics for the management of bronchial asthma, anaphylaxis, rheumatic fever, meningitis, etc. The widespread applications of steroids and their environmental discharges in soil/aquatic systems have posed serious health concerns such as infertility, premature birth, polycystic ovary syndrome, and reduces hatching rate in fish and birds. Being hydrophobic, these stable chemicals and their conjugate forms do not mix well with the water system and reside in the biosphere for prolonged periods. The situation poses an ecological risk and aquatic hazards for all terrestrial and aquatic fauna until these are decomposed completely. Specific microbial genome, biochemical reactions, the key intermediates, and distinct catabolic enzymes participate in the steroidal degradation. The chapter highlights significant aerobic and anaerobic microbes that efficiently catabolize complex steroids into nonsteroidal organic compounds.

**Keywords**

Steroids · Environmental discharge · Biodegradation · Aerobic degradation · Anaerobic degradation

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## 10.1 Introduction

Abundantly found steroids encompass a class of cyclic organic compounds, specified with 17 carbon atoms that are organized as four cycloalkane rings. Steroids are triterpenoids and characterized by a planar and firm carbon skeleton. These ubiquitous chemicals are found in mammals in the form of corticosteroids, sex hormones, sterols, bile salts or bile acids, and mineralocorticoids. These chemicals are derived from a common precursor (cholesterol) through the process of stereogenesis. Stereogenic glands such as gonads (testes and ovaries), adrenal glands (adrenal cortex), and placenta are the main production sites of steroids. Lipid soluble cholesterol is found in all cells of the body and regulates body hormones and vitamin D synthesis. It also participates in managing cell membrane fluidity, thus modulates cell structure (Edwards et al. 1996). Corticosteroids (cortisone-like biochemical) alleviate inflammatory responses from the body, i.e., swelling, itching, erythema, or allergy. Therapeutically these steroidal compounds find their place in the treatment of allergic disorders such as asthma, arthritis, or skin problems. On the other hand, sex hormones play a key role in the development of male and female reproductive systems, and increasing muscle and bone mass. Figure 10.1 displays the development of various steroids from cholesterol.

Generally, steroidal compounds are lipophilic sterols and perform various physiological functions in plants, animals, and humans. Lanosterol and cycloartenol are major sterols found in animals and plants respectively. They control growth, development, and reproduction in the organisms. Plant steroid (brassinosteroid), first isolated from the pollen of *Brassicus napus* is reported to control seed germination, vascular differentiation, apical dominance, and stem/root proliferation in plants (Halliday 2004). Tren, Winstrol V, and Finalex are steroid-based veterinary steroids that relate to muscle power and growth in food production (Steinman and Trainor

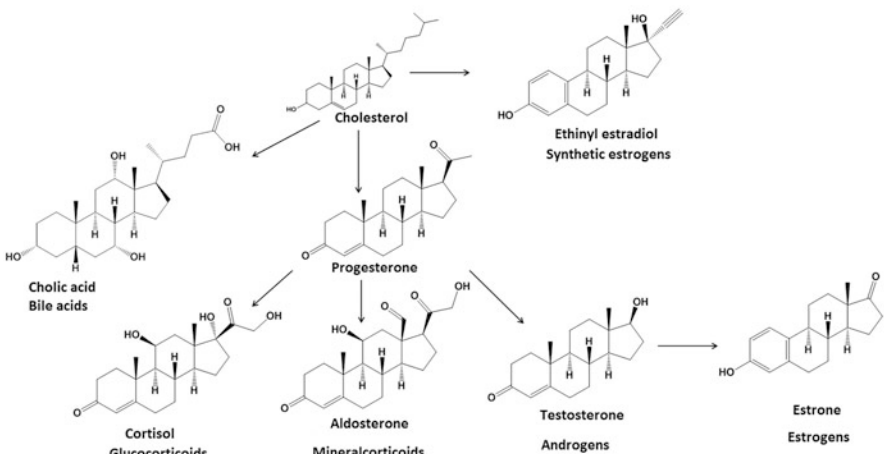


**Fig. 10.1** Development of various steroids from cholesterol (precursor)

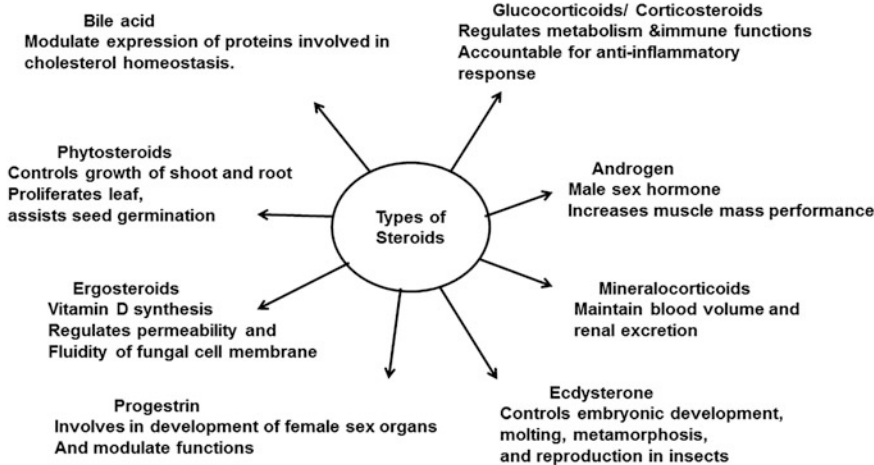


**Fig. 10.2** Differentiation of steroids based on their functions

2010). Ecdysterone found in insect controls molting and metamorphosis of arthropods (Isenmann et al. 2019). Similarly, ergosterol located in the fungal cell membrane controls the permeation of electrolytes and small nutrients across the membrane, thus maintains the cell integrity. Figure 10.2 illustrates the functional classification of naturally occurring steroids.

Hormones (cortisone, androgens, and estrogens) are essential steroidal chemicals that perform a variety of physiological functions in mammals, i.e., regulation of metabolism, salt and water balance, immunological responses, control of inflammation, modulation of sexual characteristics, and capability to endure in injury and illness. These chemicals execute their physiological functions through binding with intracellular receptors (ligand-dependent) and nonclassical membrane receptors. These receptors are modular proteins in which several domains coexist. These are N-terminal (A/B domain) that performs transcriptional activation, C domain or middle region contains DNA binding region, D domain or hinge region maintains receptor inactive, E region consists ligand-binding region and the F domain is C terminal of the protein receptor (Wierman 2007).

Steroids are extensively utilized in pharmaceutical industries and healthcare systems to design countless therapeutic agents namely immunosuppressants, contraceptives, anti-inflammatory agents, diuretics, hormonal therapies, etc. (Fernandes et al. 2003). Administered via oral (betamethasone, prednisolone, fludrocortisone, prednisone), parenteral (dexamethasone, triamcinolone, prednisolone, hydrocortisone), topical (betamethasone, clobetasol, mometasone, fluocinolone), and as inhalants (beclomethasone, budesonide, flunisolide); steroids find applications in various autoimmune disorders, inflammatory responses, hormonal replacement therapy (Addison's disease) and for non-endocrine usage (bronchial asthma, anaphylaxis, rheumatic fever, meningitis, etc.). Additionally, athletes use synthetic androgens or anabolic steroids to accelerate muscle power. Continuous

usage of these anabolic steroids may provoke cardiovascular disease, immune disorders, violent behavior, and sexual impairment. Moreover, the combinations of synthetic estrogens and progesterone are prescribed as oral contraceptive pills for preventing pregnancy and to control unpleasant symptoms of menopause (Cabrera-Munoz et al. 2012). Thus, steroid use is widespread.

## 10.2 Potential Sources of Steroidal Release in the Ecosystem

One of the major concerns that have been consistently ignored is the release of steroids in the biosphere. Living organisms constantly release these chemicals via excreting urine and fecal matter that requires immediate disposal or degradation in a simpler form to manage this new class of pollutants. Agriculture (fertilizers/manure), industries (endocrine-disrupting chemicals), and other household wastes including disposal of pharmaceuticals are the primary determinants of steroid discharge in environmental soil and water systems (Hanselman et al. 2003). Maier et al. (2000) discussed the release of huge livestock excreta containing sex steroids in the environment. The amount of natural estrogen was detected high (approximately 1.3 μg/l) in the marine sediments due to livestock feed and release components, whereas abundant glucocorticoids and mineralocorticoids were observed in urban surface water (Maier et al. 2000; Chang et al. 2009). National sewage sludge survey of the United States has reported 1,014,724,000 tons of animal excreta containing approximately 76 tons of estrogen. The released animal manure containing estrogens gets cycled from the water stream to the soil system to be used as fertilizers for agricultural lands (Andaluri et al. 2012). Reports reveal that the excreta of a pregnant woman may contain more than 250 μg estradiol per day (Johnson et al. 2000).

Urbanization across the globe has led to an ecosystem enriched with steroidal loads released from various sources. This has led to the generation of a novel kind of biogenic and anthropogenic steroidal pollutants for soil and aquatic systems. Being hydrophobic, these stable chemicals and their conjugates do not mix well in the water system, and thus, reside there for prolonged times. The situation poses an ecological risk and aquatic hazards for marine fauna until they are decomposed completely.

## 10.3 Impact of Steroids in the Ecosystem

Human beings are accidentally consuming released steroids through intake of polluted food, water, and inhaled air, and consequently diverse health hazards such as infertility, altered testosterone level, premature birth, breast cancer, polycystic ovary syndrome, early puberty, and irregular menstruation cycle (Fig. 10.3) have become rampant. Reports state that steroidal wastes significantly affect in vitro fertilization in women and do escalate the risk of genital anomalies as well (Fernandez et al. 2007). A series of investigations was performed on the endocrine-disrupting chemicals (natural, and synthetic estrogens, androgens, and

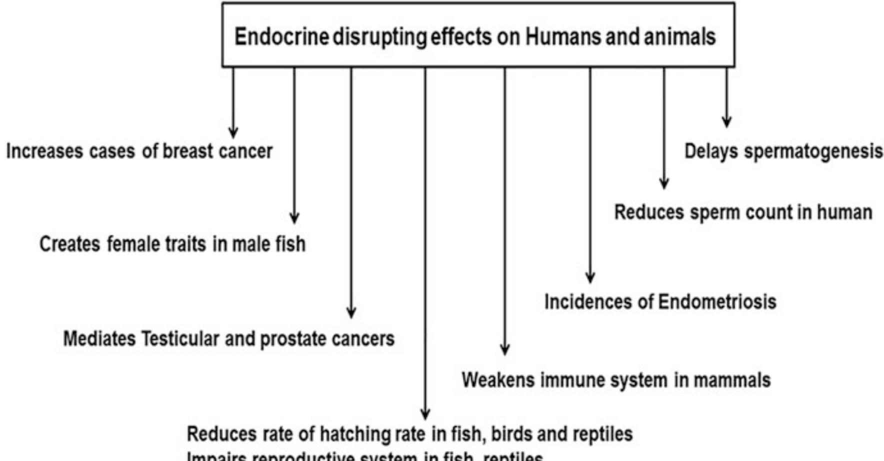


**Fig. 10.3** Ecological impact of steroids on biosphere components

progestin) released from lactating cows injected with steroidal hormones for high milk productivity. The elevated discharge of steroids in agricultural watershed influenced the life cycle of aquatic fauna (Kolodziej and Sedlak 2007).

Animal pheromones are steroid-based chemical signals that elicit communication to other members of the same species in fish and amphibians. Research by Bazaes and Schmachtenberg in 2012 demonstrated the role and significance of steroids in the physiology of the olfactory system of fish. The study suggested that fish uses sulfated steroids for sending their communications to others (Bazaes and Schmachtenberg 2012). Its major derivative, 17-β-estradiol disulphate is a potent olfactory pheromone that participates in larval development (Houck 2009). Other pheromones such as androstenone and estratetraenol are significant for sexual performance in pigs and mice. Direct and indirect exposure to steroids disturbs and dysfunctions the physiology of marine and terrestrial animals. Oestrogen even in minimum concentration can disrupt the endocrine system in fishes, amphibians, and other invertebrates. Evidence stated that the feminization of male fish in an aquatic system that was surcharged with human waste loaded with estrogen. The findings explain the direct impact of estrogens (present in soil and water) in biological communities, thus affecting the ecosystem (Doyle and Meeks 2018).

The University of Iowa laid down a case report on the ecological threat of trenbolone acetate (an anabolic steroid), popularly used for bodybuilding by weightlifting sportspersons. Field experiments based on environmental survey revealed that this anabolic steroid did not completely break down in waterways, existed stable in the system, and posed risk to aquatic animals (Science daily report 2013). US geological survey on the biological activity of glucocorticoids and androgens in water streams was conducted to identify their traces in sewage effluents collected from 100 different sources of 14 states. The outcomes notified a significant presence of glucocorticoids (27%) and androgens (30%) in testing samples. The

survey suggested wide usage of stress hormones, and anabolic chemicals by humans and reported their potential adverse effects in endocrine systems of other organisms (Stavreva et al. 2012). Burton and Wells (2002) reported the adverse effect of 17-β-E2 analog isoflavone on domestic animals. The animal feed was enriched with a sufficient concentration of estrogen that resulted in developmental abnormalities in their genital organs (Burton and Wells 2002). The report emphasized clover disease in cattle (sheep) after grazing progesterone-rich feed that developed permanent infertility and impaired vision due to increased intraocular pressure (Hotchkiss et al. 2008).

Aforementioned critical impressions of steroidal components in the ecosystem have aroused alarming situations that need to be resolved through advanced biotechnology approaches and microbial engineering. Environmental decomposers (bacteria and fungi) competently consume steroidal waste (pollute) as their nutritional and energy sources. These tiny living organisms have been isolated and screened out through microbial bioprocess and recombinant DNA technology. The microbes produce desired steroidal key intermediates for further synthesis of sex hormones, sterols, bioactive agents, etc. Various C-19 (4-androstene 3, 17-dione; 1, 4-androstadiene-3, 17-dione) and C-22 steroidal key intermediates (9a-hydroxy-4-androstene-3, 17-dione; 22-hydroxy-23, 24-bisnorchol-4-ene-3-one) have been industrialized from developed microbial cell factories. Recently, enormous investigations are piloted in pharmaceutical industries that explore catabolic metabolites of degraded steroids in the formulation of newer therapeutic agents, i.e., prednisone, testolactone, dexamethasone, and boldenone (Fernandez-Cabezon et al. 2018).

## 10.4 Microbial Degradation of Steroids

Microbial degradation of such steroids is a functional tool for simplifying the complex structures of these chemicals in simpler degradable forms. This process is considered economical, easy, and flexible compared to the conventional synthetic chemical routes. Microorganisms such as bacteria and fungi both participate in the bioconversion of steroids in soil and water systems. The microbial decomposition process involves multifaceted reactions to simplify steroids. Various chemical reactions such as oxidation, reduction, hydrolysis, isomerization, and amination occur during microbial degradation. They transform the complexity of steroidal molecular configuration through introducing a new hydroxyl group, cleavage of attached side chains, hydration of double bonds, and the development of ketones, aldehydes, and epoxides. Interesting research by Orrego et al. (2009) has demonstrated the active role of soil microbes for bioconversion of phytosterol released from the pulp and paper industry, into androgens.

### 10.4.1 Aerobic Degradation of Cholesterol

Mycolic acid containing gram-negative bacteria (*Mycobacteria tuberculosis*) degrades the last side rings of steroids and consumes them as growth nutrients. Biomimetic studies revealed that the cholesterol catabolism process supports their virulence and is essential for bacterial survival in macrophages. Under aerobic conditions, *Mycobacterium* executes two consecutive steps for the degradation of cholesterol. First, is the elimination of the alkyl side chain at the C17 position, and the second one follows the cleavage of the C/D ring from the parent structure (Capyk et al. 2009). Figure 10.4 displays the segregation of cholesterol C and D ring from original cholesterol during the aerobic metabolism of bacteria (Fig. 10.4). This cleavage is a target site for the development of new therapeutics to mitigate tuberculosis diseases. Numerous CoA thioesters identified as pathway intermediates initiate hydrolytic ring degradation. Enzymes encoded with Tet R, and KstR2 regulate HIP catabolism of the C/D ring. The alkyl chain is degraded by β-oxidation whereas ring A/B is catalyzed through oxygenase to mineralize into carbon dioxide (Crowe et al. 2017).

Dresen et al. (2010) also described cholesterol catabolic processes mediated by *Rhodococcus jostii* and *Mycobacterium tuberculosis*. Both microbes require sterol precursors for their survival, pathogenicity, and virulence. Thus, the release of steroid hormones in the ecosystem empowers microorganisms inside the host microphage. The study also suggested an active participation of flavin-dependent monooxygenase and p-hydroxyphenyl acetate hydroxylase enzymes for bacterial catabolism of cholesterol (Dresen et al. 2010). *Pseudomonas testosteroni* was reported to biotransform 19-Nortestosterone into female hormone estrogens (estrone and 17-β-estradiol) and displayed ring A aromatization of estrogens (Fig. 10.5).

Zhang et al. isolated marine gram-negative bacteria (S19-1) of Enterobacteriaceae family from the Baltic Sea, Germany, which used steroids (testosterone, estradiol, and cholesterol) as growth nutrient. This bacterium could best grow in saltwater (approximately 4% NaCl) at a cool temperature (20 °C). The pk18 plasmids were successfully transformed through chemical transformation into the cells of S19-1

**Fig. 10.4** Aerobic metabolism of cholesterol by gram-negative *M. tuberculosis*

**Fig. 10.5** Bioconversion of 19-Nortestosterone into estrogens by aerobic *P. testosterroni*

**Table 10.1** Comparative half-lives and stability of different estrogens in diverse sample systems

| Sample system | Estrone | 17-β-estradiol | Ethinyl estradiol |
|---|---|---|---|
| Soil/water/sediment | Half-life | Half-life | Half-life |
| Aerobic soil | ~ 0.5 days | ~ 1.7 days | ~ 3.2 days |
| Aerobic water | ~ 1.7 days | ~ 1.8 days | ~ 7 days |
| Aerobic sediment (marine) | ~ 4.4 days | Data not obtained | ~ 20 days |
| Anaerobic sediment (marine) | ~ 70 days | Data not obtained | Not degraded |
| Anaerobic sediment (river~) | ~ 0.7 days | ~ 14 days | Data not obtained |
| Anaerobic lake | ~ 21 days | Interconversion to estrone | Not degraded |

bacteria to generate their numerous replicas for the management of sea steroidal pollution (Zhang et al. 2011).

### 10.4.2 Aerobic Degradation of Estrogens

Minimization or removal of estrogens, androgens, and progesterone from the environment is a challenge and the researchers are investigating aggressively. Research efforts report efficient removal of androgens and progesterone (up to 91%) compared to a relatively low degradation of estrogens (approximately 80%) from soils, river water, aquifers, and marine sediments through microbes (Fernandes et al. 2003). Abundant carbon atoms and several double bonds (reduced compounds) offer a favorable environment for the nutrition and cultivation of various microbes (bacteria, microalgae, yeast, and fungi). Estrogens are the main endocrine-disrupting chemicals that are predominant in groundwater, sewage waste, and soil across the globe.

The average concentration of estrogens: estrone (E1), 17β-estradiol (E2), estriol (E3), and synthetic ethinyl estradiol (EE2) in the water streams is estimated up to 70 ng/L, 19 ng/L, 320 ng/L, and 7 ng/L respectively depending on their water solubility (Ojoghoro et al. 2017; Peterson et al. 1952). The half-life of estrogen is dependent on the rate of degradation estimated through first-order regression curve kinetics. It focuses on the duration of adherence to steroid pollutants on the ecosystem. The estrogens contained in human excreta (E1, E2, and E3) have a

short half-life (2–6 days) owing to their hydrophobic nature in sediments and water systems (Table 10.1).

The biodegradation of E1 and E2 is mediated through redox reactions under biotic conditions.

The synthetic estrogen EE2 has a prolonged half-life (81 days) in aquifer sediments under aerobic conditions (Ying et al. 2002).

The microorganisms present in aquatic and soil systems decompose estrogenic components into non-estrogenic/harmless products. *Rhodococcus sp*., *Narcadia sp*. (strain E110*), Nitrosomonas europaes*, *Novoshingobium tardaugens (strain ARI 1), Phingomonas sp. (strain D12)*, etc., are the estrogen degrading aerobic microorganisms. Aerobic bacteria of the classes *Actinobacteria* and *Proteobacteria* play a significant role in the complete mineralization of estrogens in an atmosphere of carbon dioxide. The FAD-dependent enzymes (3-ketosteroid-Δ1-dehydrogenases) are prominent in a variety of microbes belonging to the Actinobacteria phylum. At the preliminary stage, these enzymes breakdown the double bond between C1 and C2 of A ring of the steroidal substrate through 1(2)-dehydrogenation reaction (Rohman and Dijkstra 2019).

The literature emphasizes that the presence of benzene (A ring) in the parent C18 estrogen restricts microbial attack and damage of structure integrity steadily. Additionally, the structural configuration of estrogen is composed of quarternary C-atoms at 5, 10, and 13 positions that make it stable and resist easy breakdown. The presence of ethinyl group in the estradiol ring prevents its oxidation under aerobic conditions.

Fujii et al. (2001) discussed the isolation and characterization of E2 degradation by gram-negative bacteria *Novosphingobium tardaugens,* ARI 1. Complete conversion of E2 into E1 was reported by species of *Rhodococcus* and *Sphingomonas*. Further E1 was degraded into nonpolluted organic acid through mixed bacteria found in wet soil. Estrogen sulfates, i.e., estradiol sulfate and estriol sulfate both are urine excreta of livestock, and are reported as endocrine-disrupting chemicals. Aerobic microbial conversion occurs by arylsulfatase enzyme present in soil microorganism that displayed first-order temperature-dependent degradation kinetics. Furthermore, Rho and Chu demonstrated the aerobic estrogen bioremediation process by *Sphingomonas sp.* (strain KC8) from a sludge sample. They isolated three crucial metabolites during the process, i.e., meta cleavage product, 4-hydroxyestrone and pyridinestrone, the end product. Gene clusters I and II were involved in the degradation of the ring (Roh and Chu 2010). Moreover, phylogenetically diverse bacteria of genera including *Aminobacter, Mycobacterium, Escherichia, Flavobacterium* have also been identified for the degradation of natural and synthetic estrogens from activated sludge. These bacteria were capable of biotransforming E2 to E1 by the virtue of the inherent monooxygenase enzyme. However, among them only *Aminobacter, Sphingomonas*, and *Rhodococcus* were discussed further in context to the degradation of estrone (Yu et al. 2007).

Hamid et al. identified that the gram-negative *Rhodococcus* completely degraded E1 in sewage within 24 h. However, the rate of steroid degradation relied on the concentration of soil organic components, high pH, moisture content, temperature, and covalent bonding between microbes and steroids (Hamid and Eskicioglu 2012).

**Fig. 10.6** Microbial degradation and interconversion of estrogens under aerobic condition

Various strains of *Rhodococcus equi* (Y 50155, Y 50156, Y50157), and *Rhodococcus zopfii* (Y50158) were reported for significant degradation of all estrogens (E1, E2, and E3) from activated sludge of wastewater system (Fig. 10.6). Yoshimoto et al. investigated selective 17β-estradiol (E2) degradation into non-estrogenic components through *R. zopfii* (strain Y 50158) within 24 h (Yoshimoto et al. 2004). On the contrary, the half-life of EE2 was estimated very high (108 days) in the water stream and was further prolonged if not biodegraded by microbes there (Petrie et al. 2015). Maximum fractions of both the steroids were susceptible to photocatalysis also. The presence of organic matter, depth of water systems (river and lake), and bankside vegetation were the affecting parameters for E2 and EE2 biodegradation. Under anaerobic conditions, both are not degraded and reside for prolonged periods in the soil as the oxygen environment is essential for their microbial degradation (Adeel et al. 2017).

An alphaproteobacterium, *Novosphingobium tardaugens* (NBRC 16725) isolated from the sewage treatment plant in Japan is a gram-negative, rod-shaped aerobic bacteria involved in the degradation of estrogens to fulfill its carbon requirements for growth. Investigations revealed the involvement of numerous clusters of genes namely, the oecA, oec B, and oecC for estradiol degradation, and sal2, scb2AB, and stdA2 genes for denaturation of cholic acid (bile acid steroid). Under aerobic conditions, initially, 17β-estradiol (E2) was oxidized into estrone (E1) by oestradiol dehydrogenase enzyme (OecA). Furthermore, E1 was hydroxylated to produce catecholic A ring by monooxygenase estrone-4-hydroxylase enzyme (OecB). Lastly, A ring was meta cleavaged in the presence of meta-cleavage enzyme

4-hydroxyestrone 4, 5-dioxygenase, Oec C (Ibero et al. 2019). Fan et al. (2007) emphasized that under aerobic conditions estrogens (17-β-estradiol, 6%) and androgens (testosterone, 63%) were mineralized into inorganic matter and carbon dioxide. However, this bioremediation process was restricted in the absence of an oxygen environment, and only 0.9% of 17-β-estradiol and 46% of testosterone were degraded in the agricultural soil system.

An exhaustive survey on the biotic system of wastewater sludge revealed that the strains of *Bacillus amyloliquefacience*, *Bacillus subtilis,* and *Bacillus cereus* actively participated in the conversion of estradiol into estrone. The microbes did not simplify estrone into other organic matter. Additionally, the microbial strains of *Aminobacter*, *Flavobacterium*, *Brevundimonas*, and *Microbacterium* are also involved in making the marine environment estrogen free (Yu et al. 2007). In this context, *Stenotrophomonas maltophilia* is known to cause cleavage of the saturated ring of estrone whereby estrone is biotransformed into tyrosine amino acid and further consumed during bacterial protein biosynthesis. Additionally, *Sphingomonas KC8* and *Sphingomonas EDB-LI1* of Sphingomonadaceae were also identified as estrone transformers (Garcia-Gomez et al. 2013).

### 10.4.3 Aerobic Degradation of Progesterone

Females naturally secrete progesterone (C-21 steroidal ketone) during pregnancy (21–200 ng/ml). Its synthetic form progestin is widely used as an oral contraceptive and for hormonal replacement therapy (EMEA 2004). Studies have reported a high level (~ 200 ng/ml) of excreted hormone and its active metabolite in the water system (Liu et al. 2015). The endocrine-disrupting effect (EDE) of progesterone concentration in aquifer biota is less than 1 ng/ml and thus the excreted level in the water system is alarming. Furthermore, cyanobacteria (*Cynobacterium microchaeta tenera*) and few freshwater microalgae (*Chlorella pyrrenoidosa* and *Scenedesmus obliquus*) through diverse reaction pathways mediate the biotransformation of progesterone into potent androgens (Peng et al. 2014). Ojoghoro et al. (2017) demonstrated putative transportation products of bioremediated progesterone obtained from EAWAG (Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz) biocatalysis process (a study performed by Swiss Federal Institute of Aquatic Science). Their report highlighted biotransformation of several potentially active androgens, i.e., 1, 4 pregnadiene-3,20-dione, androstenedione-3,20-allpregnanedione transformed by microalgae on the water surface (Fig. 10.7).

### 10.4.4 Aerobic Degradation of Androgens

Microbial degradation of androgen (testosterone) was first reported by Talalay et al. (1952). The researchers isolated gram-negative bacteria *Comamonas testosteroni*, a betaproteobacteria from soil and sewage. *Comamonas sp* grows in an aerobic

Progesterone

1,4-Pregnadiene-3,20-dione

3,20-Allopregnanedione

3b-Hydroxy-5a-pregnan-20-one

Androstenedione

**Fig. 10.7** Biotransformation products via aerobic microbial degradation of progesterone

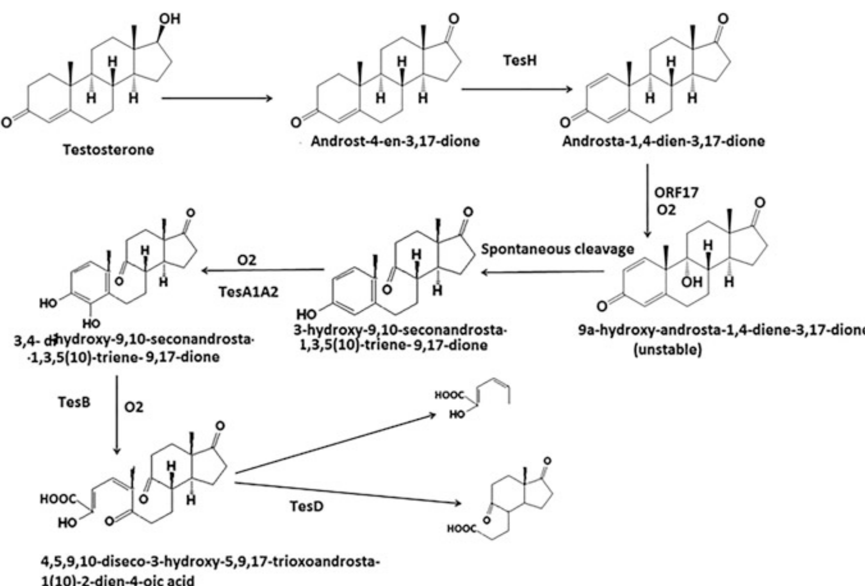


**Fig. 10.8** Schematic aerobic degradation of testosterone by *C. testosteroni*

environment and utilizes androgens as the carbon source nutrients. Studies performed through PCR revealed that an amplified sequence of dioxygenase gene "tesH, B, D" and 16S rRNA are accountable for the catabolism of testosterone (Fig. 10.8). A testosterone inducible regulator "teiR" that mediated the transcription

of genome engaged in initiation of testosterone mineralization was identified in *C. testosteroni* (Chen et al. 2016). The teiR is a repressor protein and is accountable for the expression of gene "3β, 17β-hydroxysteroid dehydrogenase" in this microbe (Wu et al. 2015). Another research performed by Pruneda-Paz and team in 2004, revealed that the teiR deficit mutants of *C. testosteroni* were unable to utilize testosterone as the sole energy source and nutrition. A series of oxidation and dehydrogenase reactions were enduring for aerobic catabolism of testosterone. The key intermediates were androst-4-en-3,17-dione, androst-3,4-dien-3,17-dione, 3-hydroxy-9,10-secon, androsta-1,3,5(10)-trien-9,17-dione and 3,4-dihydroxy-9,10-secon, androsta-1,3,5(10)-trien-9,17-dione. The 9, 10-seco pathway is opted for the microbial conversion of testosterone into several key intermediates (Pruneda-Paz et al. 2004).

Payne and Talalay (1985) investigated another proteobacteria *Alcaligens strain M21* (present in the soil) for catabolism of both testosterone and estradiol. Nicotinamide adenine dinucleotide linked enzymes (3β- and 17α-hydroxysteroid dehydrogenase) were identified in the isolated strains of *Alcaligens* bacteria that actively participated in stereospecific oxidation and reduction of steroids. 17-α-hydroxysteroid dehydrogenase has a higher affinity toward the oxidation of testosterone. This virtue has also been explored for microestimation of steroidal enzymatic cycling and degradation (Payne and Talalay 1985).

Glucocorticoid dexamethasone is a widely used synthetic steroid owing to its multifaceted clinical applications, i.e., allergy, asthma, and autoimmune disorders. An environmental survey has reported the discharge of dexamethasone residues through hospital waste systems that pollute the ecosystem extensively and affect the living organisms through the food chain (Liu et al. 2009). *Pseudomonas alkaligens* was reported for the decomposition of dexamethasone owing to the presence of protease enzymes found in its intracellular liquid. The 41 kDa protease has more than 95% capability for degradation of dexamethasone sodium phosphate sampled from hospital wastewater (Zhu et al. 2015).

Another aerobic bacterium, *Endozoicomonas* found in the marine microbiota (corals and sponges) displays an interesting ecological role in the degradation of testosterone. *E. montiporae CL-33*$^{T}$, a gammaproteobacteria was first isolated from the southern coast offshore of Taiwan. It is a facultative symbiont and its distinctive genomic feature (transposase coded most active IS elements) lessens host environmental stress through eliminating sea slug pollutants. Other species such as *E. elysicola* and *E. numazuensis* can cause complete degradation of testosterone into propionyl Co A and pyruvate (Ding et al. 2016). The microbes of phylum *Actinobacteria* were also capable of complete degradation of androgens (testosterone), progestogens (progesterone), and sterols (cholesterol). Hydroxylation results in the substitution of hydroxyl groups at the α/β site of hydrogen in the steroidal moiety (Petrusma et al. 2014). The hydroxyl groups were derived from gaseous oxygen rather than water molecules hence always preserve the stereochemical configuration for the replaced hydrogen of the parent compound. Several species of *Rhodococcus* such as *R. ruber, R. rhodochrous, R. zoffi, R. equi, and R. erythropolis* can breakdown the complex steroidal structure into simpler ones

**Table 10.2** List of microbial strains isolated from several biotic systems for degradation of steroids

| Class | Microorganism | Strain | Precursor/steroid | Isolated system |
|---|---|---|---|---|
| Actinobacteria | *Rhodococcus ruber M1* | DSM 43338 | Cholesterol, testosterone, progesterone | Activated sludge |
| | *Rhodococcus erythropolis* | ATCC 4277 | Estriol, estradiol | Soil |
| | *Rhodococcus zoffi* | Y 50158 | Oestrogens | Soil |
| | *Rhodococcus equi* | Y50155, Y50156, Y50157 | Partial degradation of synthetic ethinyl estradiol | Soil |
| | *Thermomonospora curvata* | ATCC 19995 | Bile steroid, testosterone | Soil |
| | *Salinispora arenicola* | CNS 205 | Cholesterol, testosterone | Soil |
| | *Amycolicicoccus subflavus* | DQS 3-9-A1T | Cholesterol | Soil |
| | *Nocardia sp.* | E110 | Estrogens | Soil |
| | *Amycolatopsis sp. 75iv2* | ATCC 39116 | Cholesterol, progesterone | Soil |
| | *Actinoplanes missouriensis* | 431 | Cholesterol, testosterone | Soil |
| Proteobacteria | *Comamonas testosterone* | ATCC 11996 | Testosterone | Soil |
| | *Cupriavidus necator* | ATCC 17699 | Testosterone | Activated sludge |
| | *Pseudomonas resinovorans* | NBRC 106553 | Testosterone | Sludge |
| | *Endozoicomona montiporae* | BCRC 17933 | Testosterone | Coral |
| | *Novosphingobium tardaugens* | NBRC 16725 | Estriol, estrone and 17-β-estradiol | Activated sludge |
| | *Sphingomonas wittchii* | RW1 | Testosterone | River |
| | *Shewanella pealeana* | ATCC 700345 | Bile steroid | Sludge |

by catabolism of sterols (cholesterol), androgens (testosterone), and estrogens (estradiol). Sang et al. (2011) demonstrated degradation of steroids present in seawater through marine bacteria *Vibrio H5*. This strain could potentially biotransform androgens (testosterone) and estrogens. Their research identified two estradiol inducible genomes: carboxylesterase and 3-ketosteroid-Δ-1-dehydrogenase accountable for mineralization into carbon dioxide (Sang et al. 2011). Table 10.2 compiles the aerobic microbes isolated from several biotic systems for the degradation of steroids.

## 10.5 Fungal Degradation of Steroids

Literature evidences that few fungi and molds biotransform steroidal components present in the environment through several chemical reactions and side-chain cleavage (Fernandes et al. 2003). *Curvularia lunata*, *Fusarium solani*, *Rhizopus sps., Penicillium sps., Aspergillus sps., Gliocladium sps,* and yeasts have been investigated for efficient degradation and simplification of the steroidal nucleus. The fungal degradation pathway does not require the addition of antibiotics, organic acids, solvents as these components get self-synthesized in the medium during fungal growth and metabolism. Fungal cells are ideal viable components for the degradation of steroids owing to their enormous surface volume ratio provided by hyphae and spores. Furthermore, higher growth rate efficiency enables these viable cells to accelerate the degradation process. The most important virtue of eukaryotic fungi is that they are regiospecific and stereospecific that govern the cleavage of the steroid side chain in one single step.

In the year 1952, Peterson and Murray investigated the transformational activity of *Rhizopus nigricans* conversion of progesterone to hydrocortisone and cortisone successively through several steps (Peterson et al. 1952).

Some fungal species of Fusarium (*F. solani* and *F. caucasicum*) were reported for degradation of steroidal metabolites namely, Δ4-pregnene-3, 20-dione and Δ5-3-β-hydroxy steroids into 1, 4-androstadiene-3, 17-dione (ADD) and 4-androstene-3, 17-dione (AD) by the process of dehydrogenation. *Cylindrocarpon radicicola* is also utilized as an efficient active fungal strain in biofermenter for the conversion of progesterone into ADD (Rohman and Dijkstra 2019). Later, Ojoghoro et al. (2017) reported the environmental transformation of progesterone samples collected from wastewater systems by a group of fungi in alkaline pH (~ 8.5) at cool temperature (Fig. 10.9).



**Fig. 10.9** Fungal degradation of progesterone into cortisone and derivative

Fig. 10.10 Fungal degradation of cortexolone into hydrocortisone

These fungal bioconversion processes led to a new perspective for the development of other adrenocorticoids and glucocorticoids commercially. Several filamentous fungi like *Aspergillus, Mucor,* and *Rhizopus* are involved in the process of hydroxylation of steroids at alpha and beta carbon 11 positions. The genome of filamentous fungi is composed of approximately 200 cytochrome P450 genes that are essential monooxygenase enzymes for the degradation process (Kelly and Kelly 2013). A recombinant strain of *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* are used to biotransform mammalian steroids hydroxylation through xenobiotic metabolism with the help of CYP3A4 (Mehmood et al. 1995). Additionally, certain fungi such as *Cunninghamella blakesleeane* and *Curvularia lunata* both bioremediate glucocorticoid 'cortexolone' into hydrocortisone by substituting hydroxyl group at 11 position of parent steroid (Fig. 10.10).

## 10.6 Anaerobic Degradation of Steroids

### 10.6.1 Anaerobic Degradation of Cholesterol

Anaerobic microorganisms are often located in deep, compacted soil (microsites) where oxygen concentration is restricted. Generally, anaerobic or anoxic conditions are not a satisfactory and efficient way for steroidal degradation by microbes, hence, these sediments and soils are steroid reservoirs (Hanselman et al. 2003). However, betaproteobacteria and gammaproteobacteria can degrade sterol derivatives as these microbes utilize sulfate, nitrate/oxygen as an electron acceptor. Such microbes bioremediate biotic steroidal waste or effluents disposed on soil or sewage waste systems. Relatively limited literature is available on the anaerobic degradation of steroids compared to the aerobic one.

Taylor and Smith first investigated the complete mineralization of steroids through anaerobes under denitrifying conditions in the year 1981. Denitrifying bacteria, i.e., *Rhodocyclus, Azoarcus,* and *Thauera* consume cholesterol as a carbon source (Taylor et al. 1981). During their growth phase, they reduce nitrate into nitrite and again into nitrogen gas thus completing oxidation of cholesterol into carbon dioxide (Harder and Probian 1997). These microbes exhibit common phylogenetic and physiological features as the aerobic ones. The microbes are rod-shaped,

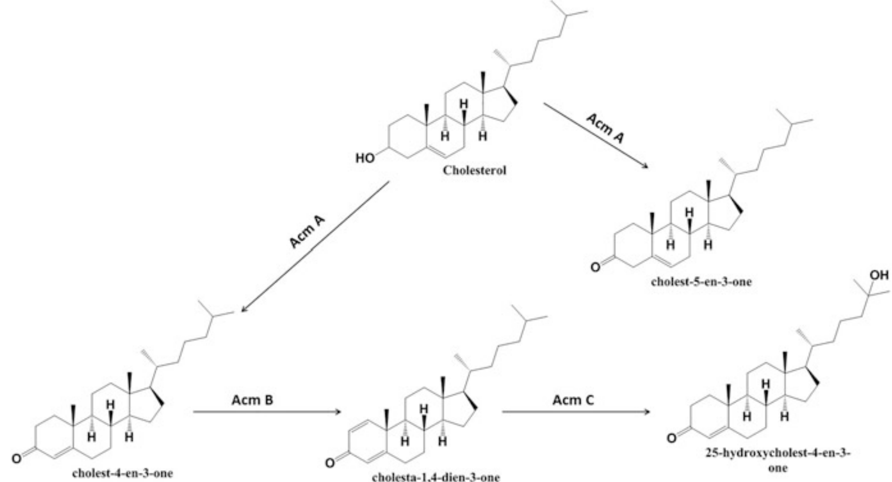


**Fig. 10.11** Anaerobic degradation of common sterol precursor cholesterol by denitrifying bacteria

gram-negative, and mesophilic (cultured in 28–32 °C). The genetic 16S rDNA sequence of β-proteobacteria, i.e., strain *72Chol* and *Sterolibacterium denitrificans* is similar to the denitrifying bacteria and can biotransform sterols and fatty acids. Chiang et al. (2008) reported that 3-ketosteroid Δ1-dehydrogenase (Δ1-KSTD) enzymes present in *Sterolibacterium denitrifican* were accountable for cholesterol ring breakdown through dehydrogenation reaction. Further study presented a detailed report on the anoxic decomposition of cholesterol by *Sterolibacterium denitrificans* (Chol 1ST DSMZ 13999).

Several genes (Acm A, Acm B, and Acm C) and metabolic intermediates/ enzymes were involved in the complete anaerobic catabolism of cholesterol. Initially, oxidation of A ring started at C3 and C5 positions leading to the generation of active intermediates, i.e., cholest-5-en-3-one and cholest-4-en-3-one, respectively in the presence of Acm A protein. Furthermore, dehydrogenation initiated by flavoprotein Acm B at C1, and the key intermediate, cholesta-1,4-dien-3-one was produced. Subsequently, the formation of 25-hydroxycholest-4-en-3-one was mediated with Acm C protein. Figure 10.11 illustrates the sequential anaerobic catabolism of cholesterol by the denitrifying bacteria *Sterolibacterium denitrificans*. The catabolism is mediated by ethylbenzene dehydrogenase, an anaerobic hydroxylase enzyme.

Freier et al. (1994) introduced *E. coprostanoligenes*, an aerobic *Eubacterium* genus bacteria found in the gut contents of the human. This bacterium has the potential to transform cholesterol into coprostanol with the help of enzyme cholesterol reductase (Fig. 10.12). Coprostanol is poorly absorbed in the gut mucosa of humans; hence, it constitutes more than 50% of total fecal steroidal contents.

**Fig. 10.12** Biotransformation of cholesterol into poorly absorbable corprostanol

### 10.6.2 Anaerobic Degradation of Testosterone

Gram-negative, rod-shaped, motile, and non-spore forming γ-proteobacteria *Steroidobacter dentrificans* has been reported for oxidative catalysis of testosterone at 28 °C and neutral pH. *Sdo. dentrificans* (DSMZ 18526) consume steroidal organic substrate as a nutritional source in a nitrate environment. Several dehydrogenations and hydrogenation reactions occur during the A and D ring biotransformation of testosterone (Fahrbach et al. 2008). The conversion process involves some common steps as in aerobic schemes, such as first ring cleavage product (17-hydroxyl-1-oxo-2, 3-seco, androstan-3-oic acid or 2,3 SAOA). Initially, 1-testosterone gets oxidized at C1/C2 via dehydrogenation reaction into 3-ketosteroid $\Delta^1$ dehydrogenase in the presence of molybdoenzyme. Furthermore, the hydroxyl group is introduced at the C17 site via oxidation. The second step involves reduction that results in the generation of androst-1-en-3, 17-dione followed by hydrolase mediated transformation, i.e., 17-hydroxyl-androstan-1, 3-dione (Fig. 10.13). A series of xanthine oxidase enzymes (molybdopterin), iron-sulfur cluster (Fe-S), actABCgene cluster (atcA, atcB, and atcC) participate during catabolism of testosterone. Apart from *Sdo dentrificans* other betaproteobacteria *Azoarcus toluclasticus*, *Thauera terpenia,* and *Sterolibacterium denitrificans* also have the potential for efficient degradation of androgens under denitrifying environment (Chaing et al. 2010).

### 10.6.3 Anaerobic Degradation of Estrogen

Denitrifying bacteria of class gammaproteobacteria and betaproteobacteria (*Sdo. dentrificans*, and *Denitratisoma oestradiolicum*) have been isolated from anoxic river sediments and sludge soil samples. These microbes are capable of causing partial mineralization of natural estrogens (17β-estradiol and estrone) into dinitrogen monoxide by the nitrate reduction process. Estrogens are difficult to biodegrade anaerobically due to the presence of stable phenolic-A ring in their chemical structure. The precise mechanisms, key intermediates, and the enzymes/genes

**Fig. 10.13** Schematic anoxic degradation of testosterone by *Steroidobacter dentrificans*

**Table 10.3** Anaerobic degradation of steroids

| Phylum | Microorganisms | Strains | Electron acceptor | Steroids |
|---|---|---|---|---|
| Betaproteobacteria | *Sterolibacterium dentrificans* | Chol-1S (DSM13999) | Oxygen/ nitrate | Cholesterol, testosterone, and androst-4-en-3,17-dione |
| | *Sterolibacterium dentrificans* | 72 Chol (DSM12783) | Oxygen/ nitrate | Cholesterol |
| | *Denitratisoma oestradiolicum* | $AcBE_{2\text{-}1}$ (DSM16959) | Nitrate | 17-βEstradiol (E2), estrone (E1) |
| | *Thauera terpenica* | 58Eu (DSM12139) | Nitrate | Testosterone |
| Gammaproteo-bacteria | *Steroidobacter denitrificans* | FS (DSM18526) | Nitrate/ oxygen | 17β-estradiol (E2), estrone (E1) and androst-4-en-3,17-dione |

involved in the anaerobic estrogen degradation have not been well investigated. Few studies shed light on the adaptation of the oxygen-independent pathway for degradation of phenolic A ring of estrogens that is different from the aerobic one (Yu et al. 2013). Table 10.3 compiles few anaerobic microbes involved in steroid degradation.

Research on denitrifying bacteria *Sterolibacterium dentrificans* and *Steroidobacter denitrificans (facultative anaerobes) revealed an interesting bioconversion property to modify their metabolism both in oxic and anoxic circumstances. These microbes have the potential to survive in an oxygen-tensed niche that endorses the consumption of steroidal waste as their nutrition sources. This inherent*

*property offers a newer biochemical study as a post-genomic tool for bioconversion of substrates variable environmental conditions.*

## 10.7 Conclusion

For decades, various aerobic and anaerobic microbes are being investigated for the degradation of natural and synthetic steroids into nontoxic organic substrates. These microorganisms assist in preserving the environment by eliminating steroidal pollutants and their undesirable effects from the ecosystem. Several microbes of phylum *Actinobacteria* and *Proteobacteria* are active biodegraders of cholesterol, androgens, and estrogens from diverse soil and aquatic sources. Still, the study needs pursuance on genetic and metabolic engineering that enable innovative development of bioconversion of resistant steroids. There is also a need of cost-effective industrial cell practices for collection of suitable microbial strain that has potential for proficient breakdown of steroids under feasible conditions. Appropriate biotechnological approaches are required for the improvement of steroid solubility and removal of generated toxic substances.

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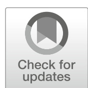

# Microbial Degradation of Phenol and Phenolic Compounds

11

Milan Malhotra, Divya Gupta, Jeetendra Sahani, and Sanjay Singh

**Abstract**

Phenol and phenolic compounds play an essential role in various modern-day industries. However, due to the toxic and recalcitrant nature of phenolic compounds, these compounds have a detrimental effect on the environment and aquatic life. In the recent past, different physicochemical methods have been employed by various researchers for phenol remediation. However, higher cost and generation of secondary pollutants are primary concerns associated with physiochemical treatment. Alternately recent advances in biological treatment have improved our understanding of bioremediation. Biological treatments such as microbial fuel cells and microalgae are gaining popularity for the treatment of phenolic compounds. These processes are less energy and chemical-intensive and can also achieve higher removal efficiency under optimum conditions.

**Keywords**

Phenols · Microbial fuel cell · Biodegradation · Algae

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## 11.1 Introduction

Phenol and phenolic compounds have applications in various industries such as dye, pharmaceuticals, fertilizer, plastics, fiberglass, food industries, and petrochemical (Collins et al. 2005; Krastanov et al. 2013). In some of the sectors, phenol is used directly or converted to derivatives for further application. In the year 2019, global phenol demand was ~11 million metric tons, and this demand is expected to grow in the future. Owing to large-scale industrial applications, these compounds end up in the environment resulting in contamination of river bodies and soil. Phenol and phenolic compounds are toxic and have a major detrimental effect on the environment (Mostafa et al. 2020). Depending on the structure and functional groups, the physicochemical properties of phenolic compound changes.

Several treatment processes are available for phenol degradation from wastewaters. However, the choice of process depends on the concentration of phenol. Processes used for this purpose include distillation, coagulation, adsorption, extraction, chemical and advanced oxidation, and biological degradation. Biological processes use lesser chemicals and energy but can treat wastewater with comparatively lower concentrations as higher contaminant doses are toxic for the microbes. Strict standards have been set up for permissible phenol concentration in surface water with a 1 ppb standard set by EPA (Villegas et al. 2016). It is reported that phenol poses health risks from irregular breathing to tremors and coma at lethal doses. Chronic exposure can even affect the next generation, therefore, phenol removal from aqueous streams is essential. The most common phenol compounds are Bisphenol A (BPA), which is a precursor of plastic, chlorophenols (found in pulp and paper mill wastewater), phenolic resins, and endocrine-disrupting compounds (Villegas et al. 2016).

Biological treatment is suggested as cheaper than other chemical methods for wastewater treatment along with the generation of nontoxic by-products. At concentration up to 400 mg/L, aerobic treatment using acclimatized sewage sludge has shown complete removal at pH 7 while at 1500 mg/L initial concentration, toxicity increased. Another biological method is use of membrane bioreactors. The microbes are trapped or coated on a support such as polyurethane and polypropylene. Membrane fouling limits the use of this technique, however, it can be mitigated using new technologies (Villegas et al. 2016).

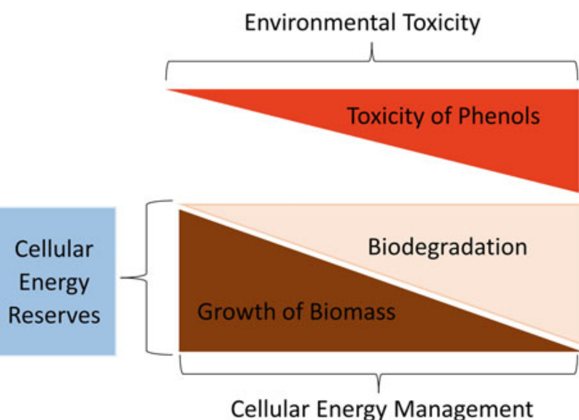
In the recent past, many researchers have used various physicochemical techniques (such as adsorption, advanced oxidation processes such as Fenton's, ozonation, wet oxidation) for the removal of different organic pollutants (Lal and Garg 2015; Malhotra et al. 2018; Karim et al. 2020). Physicochemical processes are efficient in the degradation and removal of organics from wastewater, however, they are energy and chemical-intensive that limits the large-scale application. Besides, physiochemical treatment may result in the generation of secondary pollution such as sludge, spent adsorbent, or intermediate compounds which further require treatment. To overcome these limitations, microbial processes/bioremediation are gaining popularity. Microbial processes such as anaerobic digestion, microbial fuel cell, and aerobic degradation use microorganisms for the degradation of pollutants.

These microbes use pollutants as a carbon source for the growth and in-process result in the degradation of pollutants to simpler and less toxic compounds. In the present chapter, detailed literature is compiled on the recent advancements in the treatment of phenols and phenolic compounds by various microbial methods.

## 11.2 Phenol Removal Using Microalgae

Microalgae have been used for the treatment of various wastewater streams. Microalgae based treatment systems provide several advantages such as they oxygenate the water, which reduces the biochemical oxygen demand (BOD) of the wastewater; they uptake nutrients, thus preventing eutrophication in downstream aquatic systems; and they can fluctuate pH, which helps in inactivating pathogens (Larsen et al. 2019). Also, unlike bacteria, algae use $CO_2$ as a carbon source. Hence, the application of additional carbon sources into wastewater is not required. In general, algae-based treatment is applied primarily to remove nutrients like nitrogen and phosphorus municipal wastewater. However, due to their potential to remove heavy metals and persistent organic pollutants (POPs), microalgae have been used to treat industrial effluents. The phenol removal capacity of algae depends upon the type of substrate, contact time, aqueous pH of the system, and illumination.

A recent study by Papazi et al. (2019) tries to assess the comparative removal of phenols by green alga *Scenedesmus obliquus*. The study reported that after 5 days of contact time, microalga could remove approximately 9% of the less toxic phenol and 90% of the higher toxic pentachlorophenol. Furthermore, it was reported that the amount of algal biomass was higher in the case of phenols with lower toxicity. The results indicated that algae use their energy reserves for biomass production or pollutant degradation, rationally as per the substrate's toxicity. Figure 11.1 represents the schematic of cell bioenergetic strategy, where the environmental toxicity of phenol adjusts the distribution of cellular energy between growth and biodegradation of phenol.



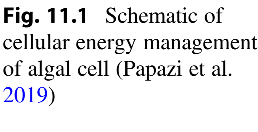



**Fig. 11.1** Schematic of cellular energy management of algal cell (Papazi et al. 2019)

**Table 11.1** Removal (%) of various phenols under light and dark condition using C. *fusca* and A. *variabilis* (Hirooka et al. 2003)

| Phenolic compound | Removal % | | | |
|---|---|---|---|---|
| | C. *fusca* | | A. *variabilis* | |
| | Light | Dark | Light | Dark |
| o-nitrophenol | 100 | 95 ± 10 | 100 | 95 ± 5 |
| m-nitrophenol | 60 ± 1 | 0 ± 1 | 100 ± 0 | 84 ± 5 |
| p-nitrophenol | 77 ± 1 | 10 ± 6 | 4 ± 1 | 4 ± 0 |
| 2,4-Dinitrophenol | 90 ± 9 | 68 ± 2 | 95 ± 4 | 81 ± 14 |
| 2,4,6-Trinitrophenol | 0 ± 0 | 0 ± 0 | 51 ± 3 | 0 ± 0 |
| Bisphenol-A | 85 ± 7 | 22 ± 3 | 23 ± 6 | 0 ± 0 |

Light illumination is an essential factor for the degradation of organic pollutants using microalgae. Hirooka et al. (2003) investigated the effect of light on the removal of various phenolic compounds using two different algal strains (C. *fusca* and A. *variabilis*). Table 11.1 shows the percentage degradation of phenol under light and dark conditions. Results shown in Table 11.1 indicated that under light conditions higher degradation was achieved by both C. *fusca* and A. *variabilis*. Klekner and Kosaric (1992) also reported the importance of illumination for the degradation of phenols by algae. The study reported that phenols were efficiently degraded by living cells of Chlorella in the presence of light. However, under dark conditions, no degradation of phenols was observed even by living cells of algae (Chlorella). Results revealed that after spending 23 days in the presence of phenol under dark conditions. The chlorella cells started degrading phenol after moving to light conditions.

Lima et al. (2004) recovered an aquatic community from the waste discharge container having several aromatic compounds. The recovered microalgae species were then acclimatized with p-nitrophenol and p-chlorophenol for 3 months. After 3 months of enrichment with phenols, two microalgae species, *Chlorella Vulgaris* and *Coenochloris pyrenoidosa* were tested for the degradation of p-chlorophenol. Results revealed that species grown under 24 h light conditions were capable of degrading 50 mg/L of p-chlorophenol within 5 days. The addition of zeolite as adsorbing material did not improve the degradation. Ellis (1977) studied the complete mineralization of phenol and catechol using six species of freshwater algae. For the experiments, *Chlamydomonas ulvaensis, Chlorella pyrenoidosa, Euglena gracilis bacillaris, and Scenedesmus basiliensis* were cultivated. It was found that only four species could degrade phenol and all six were able to degrade catechol. Except for E. gracilis, the degree of degradation of catechol was higher than phenol for all species. This is because all oxidative degradation for aromatic compounds requires two hydroxyls on the aromatic ring, which is present in catechol, not in phenol. Also, prior exposure to 0.1 mM phenol for 90 h resulted in inhibition of subsequent phenol catabolism. The authors explain that pre-exposure for a longer duration and lower phenol concentration may enhance the rate of substrate catabolism.

Studies have shown that both bacteria and algae can remove bisphenol individually. However, algal–bacterial symbiotic relation provides synergistic degradation of bisphenol and gives stability to the treatment process. Eio et al. (2015) attempted bisphenol degradation in the algal–bacterial system. The study observed that the monoculture of *Chlorella sorokiniana* could remove around 50% of bisphenol. However, complete degradation of bisphenol was observed in the algal–bacterial system regardless of initial bisphenol concentration. Furthermore, it was observed that bisphenol concentration above 20 mg/L inhibited the growth of *C. sorokiniana* in the algal system. On the other hand, such a phenomenon was not observed in the algal–bacterial system.

Peng et al. (2006) showed that irradiating algae with near UV light or heat irradiation could enhance the photo-biodegradation of bisphenol. The study reported that irradiation algae secretions might produce OH radicals in the wastewater, which results in enhanced removal. The study also reported that higher removal of bisphenol was obtained in the presence of $Fe^{3+}$ and humic acid. Dayana Priyadharshini and Bakthavatsalam (2016) used statistical optimization design to maximize phenol degradation using *Chlorella pyrenoidosa.* In the study, two-level Plackett–Burman design (PBD) was used to identify significant parameters affecting phenol degradation. PBD identified three parameters as important for phenol degradation, algal cell concentration, contact time, and pollutant concentration. The predicted results from the model revealed that around 97% phenol (initial concentration 800 mg/L) degradation could be achieved after 4 days contact time, with a biomass concentration of 4 g/L.

Adsorption, due to its simple design and production of fewer byproducts, is a preferred option for pollutant remediation. Zheng et al. (2017) studied paranitrophenol (PNP) adsorption on the biochar derived from algal strains. The results indicated that algae-derived biochar's adsorption capacity was higher than that of raw algal biomass and powdered activated carbon. The presence of higher polarizable O-containing functional groups could be the possible reason for increased adsorption capacity in algae-derived biochar. In another study using green macroalgae as a bio-sorbent, a maximum phenol uptake capacity of 20 mg/g was achieved for an initial phenol concentration of 150 mg/L (Aravindhan et al. 2009). The pH is an important parameter for pollutant removal using adsorption, as pH not only affects the surface morphology of the adsorbent but also the degree of ionization and speciation of the phenolic compounds (Li et al. 2012). In a study done by Li et al. (2012) the optimum pH for phenol uptake was found to be 4.0–6.6. At higher pH ($>$10), phenol dissociates to form a phenolate ion, which is negatively charged. The adsorbent surface is also negative at higher pH. Hence, electrostatic repulsion between the adsorbent's surface and phenolate ion results in reduced uptake capacity. In another study using spirulina sp. LEB 18 algal strain, pH 6.0, was found to be the optimum pH for phenol removal (Dotto et al. 2013). The study also reported that the phenol biosorption on spirulina was a spontaneous, favorable, and exothermic process. Although sufficient removal can be achieved through adsorption, adsorbed pollutants do not undergo complete mineralization, making it difficult to handle saturated adsorbent further.

The major limitation of algal treatment is the higher time requirement compared to physicochemical processes and other microorganisms such as bacteria and fungi (Zheng et al. 2017). Other than that, treatment efficiency depends on several factors like illumination conditions, nutrient availability, and external environmental stress. The toxicity of the phenolic compounds is an important parameter for phenol degradation. Highly toxic phenolic compounds may hamper the growth of microalgae, even at low concentrations. Sometimes it is difficult to identify an appropriate microalgal strain for a particular phenolic compound. As every strain behaves differently for each compound, a mixed consortium can overcome this limitation. Adaption to harsh pollutant environment could be another limitation for phenol biodegradation using algae. The time of pre-exposure and concentration of the phenolic compounds is a grey area that needs detailed experimentation.

## 11.3 Bacterial Degradation of Phenols

Owing to their complicated structure, toxic and hazardous xenobiotics are hard to decompose. However, a vast variety of microorganisms utilize these xenobiotics for their growth (Al-Khalid and El-Naas 2012). Such microorganisms can produce enzymes and metabolize those hazardous and toxic wastes. This property can be utilized in disposing of phenol and phenolic derivatives also. Intensive studies have been done to isolate and culture microbes with high-degradation activity (Agarry et al. 2010). The enzymes utilized in the phenol degradation process are hydrolases, catechol dioxygenase, and cis, cis-muconate cyclase among others (Krastanov et al. 2013). For example, fungal degradation of pentachlorophenol (PCP) is done by ligninolytic enzymes and cytochrome P450. *Phlebia acanthocystis* TMIC34875 is a white-rot fungi, which causes the decaying of wood. They are more tolerant to PCP than other Phlebia species in low-nitrogen potato dextrose agar medium and can efficiently degrade the compound to pentachloroanisole and p-tetrachlorohydroquinone, which are then further transformed into tetrachloro-4-methoxyphenol and tetrachloro-1,4-dimethoxybenzene (Xiao and Kondo 2020). The microbes used in the phenol degradation process can either be bacteria, fungus or others and a consortium of different species sometimes works better than single species. For example, Li et al. (2020) studied a collaborative mechanism of Stenotrophomonas sp. N5 and Advenella sp. B9 (9:1) coculture for phenol microbial degradation, where their interplay reduced the inhibition caused by phenol and made them stronger to degrade up to 1200 mg/L phenol completely within 72 h. The key expressed genes based on RNA-Seq results indicated an existing division of labor between N5 and B9 during phenol degradation. Gene ontology results suggested that differential gene expression for three transcriptional factors (LysR, two-component system response regulator, and TetR families) are responsible for microbial cellular constituents' formation, their growth, and metabolism, and enhancing phenol degradation catalytic activity in the coculture.

Several scientists have applied various models to understand the degradation mechanism and kinetics of the reaction, such as substrate inhibition, specific growth

rate, etc. (Al-Khalid and El-Naas 2012). For example, the Haldane model was chosen as the best fit among Aiba et al., Yano and Koga, Teissier, and Webb models while studying substrate inhibition and specific phenol consumption rates by *Pseudomonas fluorescence* in batch culture with 100–500 mg/L phenol as a representative limiting substrate (Agarry et al. 2010). This caused the lag phase to increase from 0 to 66 h and the process of phenol digestion to subsequently delay from 84 to 354 h, revealing a good potential of the microbe in phenol biodegradation. Excess substrate inhibits the microbial phenol degradation, which is temperature-dependent. Substrate inhibition is higher at lower temperatures. That is why the self-cleaning ability of polluted habitations, such as soils, sediments, and groundwater aquifers is reduced when high inhibitory pollutant concentration and low temperatures exist during aerobic and anaerobic phenol decomposition (Eismann et al. 1997).

Digestion of phenols and their derivatives can be performed either aerobically or anaerobically.

Aerobic biodegradation of phenols has been reviewed by Al-Khalid and El-Naas (2012) for treating the wastewater in an ecologically favorable manner. Koch et al. (1991) studied aerobic biodegradation of a concoction of nitrogen-containing aromatic compounds and 22 phenols as a primary carbon source in sample wastewater. A blended culture from soil was adjusted to continuous flow and immobilized on fluidized activated carbon and sand particles. Dissolved organic carbon removal was found to be constant in the sand (59–69%) but with activated carbon, it reduced to 39%. The reason can be increased adsorption of organic matter and inhibitory effect of by-product. The phenolic doses were almost degraded in both systems, but dead-end products were formed. The effect of phenol on aerobic granular sludge containing microbial consortium and extracellular polymeric substances (EPS) was studied by He et al. (2020) for low salinity and strength wastewater treatment. Increased phenol above 20 mg/L activated almost whole phosphorus degradation, but accumulated significant nitrate, around 4 mg/L. Owing to amplified EPS formation but declined bacterial diversity, aerobic granules conserved functional and structural firmness. Halophiles: Stappia, Luteococcus, and Formosa removed simultaneous nutrients and phenol under low-saline conditions.

There can be different types of reactor arrangement while degrading phenolic compounds, such as continuous flow, batch reaction, fluidized bed, or biofilm. A few of them are discussed here. Shivaraman et al. (1985) studied the microbial decomposition of cyanide, thiocyanate, and phenol in an absolutely mixed aerated system. Thiocyanate biodegradation was not affected at cyanide and phenol concentrations of $22.40 + -1.34$ mg/L and $1251 + -53$ mg/L or less, respectively, but over these respective concentrations thiocyanate degradation was lessened. Cyanide and phenol decompositions were around 96.5–99.9% and 99.9%, respectively. Thiocyanate sulfur oxidized to sulfate. Juang and Wu (2007) used *Pseudomonas putida* BCRC 14365 in a bioreactor of microporous polypropylene (PP) hollow fiber membrane contactor to degrade aqueous phenol and observe the consequences of additional chances in pH (3–8) and NaCl concentration (0–1.78 M). The cells in suspension grew well at 100 mg/L of phenol but only at NaCl concentrations below 0.44 M, while those cells in fiber could not endure a large pH range. However, fiber cells

completely decomposed 500 mg/L of phenol at NaCl concentration up to 1.52 M because of augmented endurance limit to salinity effect by the membrane-attached biofilms and the adequately slow mass transfer of NaCl through the membrane pores. Mostafa et al. (2020) studied phenol degradation by direct interspecies electron transfer (DIET)-promoting condition in upflow of anaerobic sludge blanket reactors (UASB). Application of electrical energy input increased the tolerance to phenol and constant decomposition was attained up to 11 kg COD/$m^3$/d with maintaining a CH4 formation yield of 0.3 m3 $CH_4$/g $COD_{added}$. Higher energy recovery (68%) was attained at 0.3 V than at 0.6 V (49%) but the performance remained similar. Physicochemical properties of granules were changed favorably, for example, settling velocity, increasing diameter, porosity, hydrophobicity, permeability, and integrity coefficient changed from 1.4, 2.2, 0.7, 23, 0.0025, and 87 to 1.9–2.0 cm/s, 2.7–2.9 mm, 0.9, 36–60%, 0.0039–0.0053 $cm^2$, and 94–95%, respectively and also influenced the microorganism consortium, enhancing the supremacy of DIET-related microbes including Syntrophorhabdus, Methanobacterium, and Methanothrix.

Anaerobic degradation is an economical way for phenol removal from wastewater but its reaction kinetics is limited by its toxicity. Adding conducting material, such as carbon nanotube and magnetite nanoparticles can increase the process one-fold by altering extracellular polymeric substances, e.g., protein and humic substances which act as an electron shuttle in extracellular electron transfer and increase the resilience of phenol degradative microbial community—Syntrophorhabdus, Syntrophus, Treponema and Brooklawnia as well as electroactive methanogens—Methanosaeta (Yan et al. 2018). Levén et al. (2012) reviewed the impacts of temperature and microorganisms for phenol decomposition during anaerobic organic waste degradation. During this anaerobic process, biogas and digestate are produced. Decomposition efficiency was observed to be higher at mesophilic temperature than at thermophilic temperature. This may be attributed to distinctions in the diversity of the phenol-consuming bacteria, and/or the existence of temperature-sensitive enzymes. Digestate with high phenol inhibits ammonia oxidizing bacteria in soil when used as fertilizer.

In anaerobic digestion also, different reactor arrangements can be made. For example, the fed-batch culture method was utilized for adaptation and efficient selection of an anaerobic phenol-degrading bacterial consortium by Guieysse et al. (2001). Repeated changes in phenol (up to 200 mg/L) increased biodegradation rates and caused series of structural changes in acclimation of the inoculum, observed by genotypic-fingerprinting techniques (terminal fraction fragment length polymorphism and randomly amplified polymorphic DNA) and analyzed by PCA. Collins et al. (2005) utilized hybrid expanded granular sludge bed-anaerobic filter (EGSB-AF) bioreactors for anaerobic digestion of phenolic wastewater at psychrophilic (<20 °C, in particular, 15–18 °C) temperature. Phenol and COD removal were studied at their respective loading rates of 0.4–1.2 kg phenol $m^{-3}$ $day^{-1}$ (400–1200 mg phenol/[liter wastewater]) and 5 kg COD $m^{-3}$ $day^{-1}$. VFAs were not accumulated, methanogenesis was obtained but the culture remained mesophilic. Rosenkranz et al. (2013) monitored the microbial community structure and

relationship between phenol degradation capacity of an anaerobic sequencing batch reactor (ASBR). The initial degradation rate steadily increased, and the removal capacity stayed comparatively stable. Higher concentrations were still efficient but slower, and adaptation derived structural modifications occurred in community composition. Under the highest feeding concentration, composition change was selected for the Anaerolineaceae family.

Complete degradation of para-chlorophenol (4-CP) is a high energy-demanding process. A sustainable hydrogen ($H_2$)-based membrane biofilm reactor (MBfR) was shown to treat >99% of the surface load up to 10 mg-N/L $NO_3$ and 100 mg/L 4-CP with slightest intermediates amassing at 19.45 e-meq/m2/d in a steady state (Long et al. 2020). $H_2$ acts as the electron donor and activates the dechlorination and denitrification process mediated by heterophilic bacteria, and after that 4-CP products act as electron donors refueling 4-CP decomposition system without a stable supply of $H_2$. $H_2$ created an anaerobic condition for the biofilm and transformed the microbe composition and the feasible degradation pathways of 4-CP. Majorly functioning anaerobic bacteria Thauera converted to aerobic bacteria Xanthobacter when $H_2$ was absent, which further amplified the genes coding the enzymes utilized in phenol aerobic activation and oxidative dechlorination. Overall, denitrification and dechlorination were powered heterotrophically by endogenous organics and autotrophically by exogenous $H_2$ during phenol degradation. This technique provides propitious sight into absolute biotreatment of water containing $NO_3$ and 4-CP leaving no remnants, or soluble chemical oxygen demand (sCOD) piling at the steady state.

Sludge from different sources has different microbial structures despite being exposed to the same xenobiotic. Granular sludge showed elevated dissolution rates than suspended sludge, both for p-cresol ($7.8 \pm 0.4$ vs $3.7 \pm 1.0$ mg $l^{-1}$ day$^{-1}$) and phenol ($11.3 \pm 0.7$ vs $8.1 \pm 1.1$ mg $l^{-1}$ day$^{-1}$) when used in a successive batch of anaerobic digestion (Franchi et al. 2018). This could be due to distinctions in methanogenic pathways and levels of Syntrophorhabdus and bamA gene in the inocula. Elevated starting abundance of bamA gene and Syntrophorhabdus genus, correlates with elevated degradation rates. The genus Syntrophorhabdus along with hydrogenotrophic archaea surge regardless of the sludge origin after re-dosing phenol and p-cresol during the anaerobic process. So, components of bamA marker gene for aromatic degradation and Syntrophorhabdus could serve as a quick screening technique to identify the most adapted sludge in effectively degrading monoaromatic compounds, and thereby, improving the anaerobic process kinetics. Wu et al. (2019) studied the mechanism of polyaromatic hydrocarbons and benzo [a] pyrene removal in an activated sludge of coking wastewater (CWW) where phenol as a co-substrate was studied. Changes in activated sludge bacterial population were observed through high-throughput MiSeq sequencing of 16S rRNA genes of microbes. Tissierella_Soehngenia, Geobacter, and Diaphorobacter were involved in anaerobic digestion, while Rhodanobacter, Thauera, and Dyella prevailed in aerobic. Phenol as a co-substrate engender microbial diversification and enhanced the BaP bioremoval performance from 78.6% to 140%. Wang et al. (2020) assessed the rate of phenol mineralization, methanogenic and electron transport activity,

concentration of coenzyme F420, and sludge of bacterial consortium of five different particle sizes (i.e., <20, 20–50, 50–100, 100–200, and >200 μm) for anaerobic digestion of phenol. Increase sludge floc size improved phenol decomposition rate and bacterial composition. Regardless of the size, the smallest sludge floc complements to form robust syntrophic cooperation in the sludge flocs.

## 11.4 Microbial Fuel Cell for Phenol Removal

The history of electricity from microbes dates back to 1911 by Potter (1911) but it has gained wide attention in the previous two or three decades. Starting with pure organic compounds, now various organic wastes are used as substrates for generating electricity using microbial fuel cells (MFCs). MFCs are gaining attention as the new energy source, which generate electricity by consuming organic compounds present in wastewater. Moreover, MFCs provide the benefit of being an eco-friendly way of treating wastewater without emitting any greenhouse gases as carbon serves as food for the microbes (Rahimnejad et al. 2015). MFCs are seen as a treatment method that may have a net positive energy output. It is a means of bioremediation using electro-active microbes for converting chemical energy in pollutants to electrical energy. It is reported that treatment of wastewater stands as the fourth largest energy consumer in the United Kingdom; therefore, being able to generate electricity during this process can reduce a large amount of energy consumed. Another cell type is microbial electrolysis cell (MEC), which is provided with an external power source, and leads to the formation of $H_2$ from the electrolysis of water at cathode unlike the formation of water from $O_2$ in MFCs.

Currently, there are limitations that have held back the scale-up of MFCs such as low current density, high cost, high mass generation, and use of noble metals as catalysts (Rahimnejad et al. 2015). These issues are being addressed by the continuous efforts of the research community. Although complex wastewater contaminants as substrates pose difficulties in understanding the kinetics, this is a promising way of treating effluents in an eco-friendly manner while generating energy. Pilot plants using MFCs for wastewater treatment have been set up with capacities of hundreds of liters to treat industrial or municipal wastewater (Dong et al. 2015; Ge et al. 2015).

There are usually two chambers for anode and cathode provided with a proton exchange membrane between them. The electrons generated at anode by the activity of microbes using organic substrates are passed through the circuit while the protons pass through the membrane. This proton exchange membrane is also the reason for high internal resistance (Rahimnejad et al. 2015). Oxygen reduction occurring at the cathode is reported to be a rate-limiting step. Anode material chosen should possess certain qualities such as resistance to corrosion and biocompatibility. Carbon and stainless steel are most commonly used as anode materials. The MFCs are operated in anaerobic conditions at the anode and aerobic at the cathode. Recent research also focuses on improving anode material for improving performance by enhancing electron transfer from the bulk phase to the circuit. Carbon nanotubes and even polymers are now used as anode materials (Rahimnejad et al. 2015). Research has

been steadily increasing on aerobic biocathodes, which reduce the formation of $H_2O_2$ at the cathode and eliminate the issue of clogging in porous carbon electrodes (Milner et al. 2016). A list of different microorganisms used in biocathodes is given by Milner et al. (2016) some of which include *Gammaproteobacteria, Xanthomonas, Rhodobacteraceae, Pseudomonas, Nitrospira, Nitrobacter*, and Marinobacter. Activated sludge has been used as inoculum for biocathodes as it contains a range of microorganisms. Gammaproteobacteria were found in maximum abundance in the aerobic biocathode system, which is responsible for the reduction of oxygen. A significant increase in power production has been reported with the use of biocathode along with carbon electrode as compared to only a carbon electrode. The cell potential depends on several factors such as pH, dissolved oxygen, ionic strength of the cathode solution, and proton bridge length (Morris and Jin 2007).

Phenol degradation has been studied by using it as a substrate in MFC. Other wastewaters such as landfill leachate, swine wastewater, domestic wastewater, petroleum compounds in groundwater have been treated using MFCs and shown promising results (Rahimnejad et al. 2015; Morris and Jin 2007). Huang et al. (2012) used aerobic as well as anaerobic conditions for the treatment of pentachlorophenol, which is used in insecticides, resins, and lubricants. They used primary effluent from a domestic wastewater treatment plant as the inoculum. PCP was treated in the cathode chamber performing as an electron acceptor as opposed to treatment of phenol and other contaminants at the anode by various researchers. It was observed that biocathode resulted in maximum degradation rate in the presence of 60:40 $N_2$: $O_2$ gas mixture as compared to pure gases. Degradation of PCP resulted in the formation of tetrachlorohydroquinone (maximum concentration after 48 h), which further dechlorinated to form 2,3,6-tri-chloro-1,4-hydroquinone and 2,6-dichloro-1,4-hydroquinone in aerobic conditions. Additionally, trichlorophenol, dichlorophenol, and phenol were also found accumulating in the system initially but decreased thereafter. Under anaerobic conditions, metabolites are found to accumulate, therefore, a mixture of $N_2$ and $O_2$ provided an atmosphere for further oxidation of the reduced by-products. Furthermore, an increase in temperature to 50 °C, resulted in the improvement in degradation rate, open circuit potential, and power density.

A study using MFC for degradation of phenol includes graphite electrodes for both anode as well as cathode in a two-chamber system consisting of borosilicate serum bottles for treating phenol in anaerobic conditions (Zhang et al. 2017). It was found that *Geobacter* sp. in anode biofilm was the dominant species maintaining phenol degradation by electron transfer. Microbial culture was obtained from paddy soil, which was periodically fed with phenol. Phenol solution (70 μM) was treated in anoxic conditions, which led to current generation until the phenol was degraded. Up to 22.7% of coulombic efficiency, 120 $mA/m^2$ of current density was obtained in the MFC. With every spike of phenol, the current generation peaked and subsequently decreased, however, the power density obtained was low (8.84 $mW/m^2$), which may be due to the use of graphite electrodes rather than platinum as used in other studies and high internal resistance. The presence of fermentative bacteria such as

*Gammaproteobacterium* was also detected in this study, while in aerobic conditions, *Pseudomonas* was found to degrade phenols.

Both fermentative and electro-active bacteria form a film on the anode, which is known as anode microbial community (AMC). Therefore, the complex compounds are first fermented to simpler compounds which are then used for electricity generation, whereas phenols may inhibit power output. More diversity of microbes and acclimation strategy used in a study by Li et al. (2019) resulted in higher power output. The AMC was acclimatized by adding glucose and small amounts of phenolics. Progressively, the number of phenolics was increased, which led to a reduction in voltage, but the degradation rate increased significantly. Moreover, as the reaction proceeds, more bacteria are formed which rapidly convert phenolics to smaller compounds which are then used as electron donors. There is a syntrophic relationship between fermentative and electro-active bacteria, which does not occur in a fermentative system, thus the by-products are accumulated and inhibit the further reactions.

Yang et al. (2013) have reported the use of MFCs for providing electricity to conduct electrosorption of phenol. Electrosorption is usually carried out by supplying external electricity, where the charged and polar molecules migrate toward oppositely charged electrodes and get separated. An activated carbon filter was used as anode and nickel foam as cathode. Multiple MFCs were used either in series or parallel to provide the required current. The effect of electrolyte concentration, pH, and number of MFCs in series and parallel was studied. More number of MFCs resulted in better phenol removal and a series connection gave better results with higher coulombic efficiency (up to 4.5%), COD removal (up to 68%), and average power density (up to 782.6 $mW/m^2$) than the parallel system. Monolayer adsorption was observed in this MFC-sorption system with pseudo second-order showing a better fit. Higher initial phenol concentration increased the adsorption rate.

Studies on the degradation of a mixture of phenolic compounds have also been carried out using MFCs. Hedbavna et al. (2016) treated groundwater containing phenol, cresol, and xylenol (total concentration = 1.4 mM). Acetate formed from the fermentation of phenols was observed as the electron donor thereby improving its removal. Biodegradation of phenol results in the formation of acetate, 4-hydroxybenzoic acid, and 4-hydroxy, 3-methylbenzoic acid (Hedbavna et al. 2016). These by-products were found to migrate through the membrane into the cathode chamber. Acetate was found to be the major carbon source acting as electron donor among different substrates and byproducts used in this study, which was responsible for electricity generation. This suggests that electro-active bacteria use simpler compounds more efficiently rather than the complex contaminants directly.

Extended acclimation, use of mixed microbial cultures, and MFC design are the major factors affecting phenol degradation. A recent study evaluated the performance of MFC for treating phenol in continuous mode which resulted in a better performance with a higher biodegradation rate and power density than batch mode (Moreno et al. 2018). Moreover, they used granular graphite electrodes, which provided an increased surface area for microbial growth. It was found that power density using phenol was much lower in a traditional MFC than that obtained in

MFCs using readily biodegradable substrates such as glucose or butyrate. However, the development of packing type MFC improved the power output and it resulted to be comparable to that with acetate and butyrate, which suggests that higher surface area for growth of microbes is efficient in improving phenol degradation (Luo et al. 2009).

Polyaniline (PANI) is used in many studies for the preparation of metal oxide/polymer electrodes while improving electron transfer because of the benefits it provides, such as electrical conductivity, easily synthesized, and biocompatibility (Khan et al. 2020). Khan et al. (2020) synthesized PANI/$SnO_2$ electrode for the treatment of wastewater containing a mixture of phenol and trichlorophenol. A microbial inoculum obtained from a wastewater treatment plant was utilized. However, in this study, the wastewater was treated in an anodic chamber as well as in a cathodic chamber to test anoxic and oxic conditions, respectively. The results showed that higher voltage could be obtained in oxic conditions with a higher voltage obtained using coated electrodes. Moreover, the treatment efficiency depends on the pollutant concentration, which showed the highest power density and COD removal at 300 mg/L concentration when MFCs were operated with concentrations of 100–500 mg/L phenolic mixture. Coating of PANI/$SnO_2$ led to an improvement in energy production as well as COD removal as it provides higher surface area.

## 11.5 Conclusion

Recent advances in microbial processes for the degradation of phenols and phenolic compounds has improved the overall efficiency of phenol degradation. Processes such as MFC utilize these compounds and also result in electricity generation, which can be a viable solution for pollution remediation. The use of microalgae for phenol degradation can result in simultaneous remediation of pollutants and generation of microbial biomass, which can be utilized for biofuel production. However, these processes are sensitive to shock load and other environmental conditions that need to be studied in detail in future studies.

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# Microbial Degradation of Chlorophenolic Compounds

12

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**Abstract**

Chlorine-Based Aromatic Compounds (CBACs) and its numerous derivatives are referred to as Chlorophenols (CPs) as there consist of complex compounds of chlorine with other aromatics (benzene) and side chains such as nitrogen, amino group, alkyl group, etc. There are a group of environmental pollutants sourced from the released waste of numerous manmade activities. These compounds are recalcitrant in the environment, however, the diverse nature of microorganisms and their flexibility in metabolizing various chemicals has aided their application

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in the degradation of CBACs and derivatives for the generation of energy and carbon sources. This discussion emphasized on the application of various microbial groups including bacterial, fungi, actinomycetes, and yeast in the biodegradation of CBACs, the initial steps that occur before degradation of such aromatic chemicals occurs, the various mode or mechanism of action with the various enzymes required for the breakdown and the future of the application of both microbes and enzymes in the biodegradation of various recalcitrant compounds. The strategy is an implication for future environmental remediation as various chemical recalcitrant and emerging pollutants are being released from expanding human activities.

**Keywords**

Biodegradation · Chlorophenols · Bacteria · Fungi · Actinomycetes · Yeast · Modes of action

## 12.1 Introduction

It is no doubt that the recent advancement and development in agricultural practices, industrialization, and domestic activities (associated with increase population) has increased environmental contaminants and pollution. Most of these activities has released large quantities of wastes/contaminants such as pesticides, herbicides, fungicides, insecticides water disinfectants, chlorine bleach, and synthetic chemical effluents, into the environment which have shown significant ecotoxicological effects or harmful human/animal health impact (Furukawa 2006; Murthy et al. 2008). According to the US Environmental Protection Agency, most of these released contaminants of the environment are chlorine-based aromatic compounds (CBAC), which have their source from the release from hospital effluents, pharmaceutical effluents, industrial effluents, domestic effluents, and municipal effluents. Chlorophenols or CBACs are chlorinated aromatic ring compounds that consists of

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either an aromatic ring/benzene ring, hydroxyl group, and chlorine atom in most cases. It also consists of other possible isomers or chloro-derivatives of methyl- and ethyl-phenols (Ivanciuc et al. 2006). All CBACs or chlorophenols exist in solids state at room temperature except 2-chlorophenol (2-CP), which is a liquid. It has low solubility in the liquid state but its sodium or potassium salts are highly soluble even up to the fourth order of magnitude in aqueous solutions (Ivanciuc et al. 2006). Its acidity rate increases with an increase in the number of chlorine substitutes. The fate of CBACs in the environment has been correlated with the organic carbon partition coefficient (Koc) (Guo et al. 2004). CBAC is widely applied in agriculture, tie/dye and textile companies, pharmaceutical and chemical industries (Arora et al. 2012; Arora and Bae 2014). The substances are priority pollutants that contain chlorine and aromatic components. They are also referred to as chlorophenols (CP) that pose a severe environmental concern because of their abundance in surface waters, domestic water, groundwater, sludge products, and wastewater (Olaniran and Igbinosa 2011).

The diverse applicability of CBAC is reported as a source of harmful toxic effects in human plants and animal health as the compound accumulates in various environments. Exposure to CBAC and its derivatives has also been reported as potential agents of severe health problems including DNA damage, cancer or carcinogenesis, programmed cell death, and organ damage in some cases (Olaniran and Igbinosa 2011). The need to remove these contaminants from the environment became a global concern as their impact continues to increase including mutagenesis, teratogenesis, high toxicity, carcinogenic, low degradability, recalcitrant, and lethal effects.

Various methods applied have been abiotic or physical processes have been employed in the removal of the pollutants, namely activated charcoal usage, advanced oxidation process, and photoelectric approach and UV (Tum and Kariuki 2020). Tum and Kariuki (2020) investigated the rate of degradation among chlorophenol and its numerous derivatives using Titanium oxide as a photocatalyst, which is immobilized on a layer of microscopic glass. Before its usage, it was characterized with diverse photo-differentiating approaches including X-Ray diffraction, electron microscope, and Brunauer Emmett Teller, while degraded units were confirmed with UV–Vis spectroscopy. An appreciable degradation was observed by this approach, however, this is not without specific environmental impacts (Tum and Kariuki 2020).

Other abiotic techniques that ensures degradation and mineralization of chlorinated aromatic compounds are Advanced Oxidation Processes (AOPs), Ultra-violet/$H_2O_2$, Photo-Fenton process, Ultra-violet/catalyst (UV/C), MW/NiO (MWN), Graphite and grapheme oxides, $O_3$/ZnO Ultraviolet/$H_2O_2$/$TiO_2$, Microwave/Ultra-violet and UV/$O_3$/$TiO_2$, etc. (Seo et al. 2017). In addition to other abiotic methods, further application of the chemical removal method was also applied. Such chemical removal methods (such as zero-valent metal reduction, hydrodechlorination, etc.) have been applied for the bioremediation and/or removal but became high-cost dependent. In an abandoned dump-site and various other

environmental milieus, it was observed that some microbial communities degrade diverse chlorine-based aromatic compounds as well as its derivatives.

Numerous studies have affirmed that mixed microbial communities biodegrade CP and other chlorine-based aromatic derivatives because of the degradative potential of these microbes. In addition, some investigators have reported that microbial cultures may be used in a fermenter to biodegrade or biotransform such environmental pollutants and also remove them effectively from the environment or wastewater in some cases, e.g., *Alcaligenes* (Westmeier and Rehm 1987). The microbes are basically diverse in both existence and nature due to the multiplicity of the contaminants and chemical recalcitrant agents. These microbes may be explored, characterized, and used in bioremediation processes or techniques to remove CP and other chlorinated aromatic compounds. Some major examples of such microbes are members of fungi, bacteria, yeast, actinomycetes, etc. These microbes may be grouped into diverse communities including methanogenes, acidophiles, photosynthetic, aerobic, microaerophilic, anaerobic, halophiles, etc. which include *Syntrophaceae*, *Syntrophomonadaceae*, *Methanobacterium* and *Methanosaeta*, *Bacillus* sp., *Pseudomonas* sp., *Stenotrophomonas* sp., *Cupriavidus nantongensis* XT, and *Cupriavidus necator* JMP134 (Pimviriyakul et al. 2020). Employing any of their members in biodegradation or biotransformation also involves the applicability of the various groups it belongs with a view to adequately harnessing their degradative potential. According to Kennes et al. (1996), biodegradation of $^{14}$C-labeled chlorinated aromatic compounds (pentachlorophenol (PCP)) has been determined in an aerated nitrogen bioreactor which is referred to as moist Hagerstown silt-clay-loam compartment while using either cellulose or without using cellulose as an amendments composition aerobically.

During an anaerobic situation, the chlorine-based compound (PCP) would decrease the availability of oxygen, which may result in a reduction in respiration even when cellulose is present. There is also a possibility for the loss of volatile constituents up to about 0.5% of the PCP released into the environmental soil to result in undetectable $^{14}CO_2$; and diverse organic extractable solvent with radioactive potential from all treatments. A chromatographic analysis using both gas and thin-layer types for environmental extracts indicates the presence of derivatives or subunits including pentachloroanisole, while using both aerobic and anaerobic approaches. Other detected derivatives or products from the biodegradation after methylation include 2,3,5,6- and 2,3,4,5-tetrachlorophenols and 2,3,6-trichlorophenol using gas chromatography. The degradation or detoxification of pentachloroanisole was further examined in both aerobic and anaerobic approaches in soils and wastewater (Jannat et al. 2021; Arora and Bae 2014). It is noteworthy that the CBACs of both households, domestic and industrial origin may be subdivided into three different classes with regard to their structural components. The subdivisional class 1 carries aromatic skeleton with components such as chlorobenzene (CB), chloro-phenol (CP) (e.g., 4-monochlorophenol (4-mono-CP), 2,4-dichlorophenol (2,4-Di-CP), 2,4,5-trichlorophenol (2,4,5-Tri-CP), 2,4,6-trichlorophenol (2,4,6-Tri-CP), 2,3,5,6- and 2,3,4,5-tetrachlorophenols (2,3,5,6 or

2,3,4,5-Tetra-CP) and pentachlorophenol (Pent-CP)), chlorinated aniline and their derivatives (e.g., 4-chloroaniline, 4-chloro-2-aminophenol and 2-chloro-4-aminophenol), 4-chlorobenzoic acid and tetrachlorohydroquinone (Tetr-CHQ). The subdivisional class II CBACs are aromatic ring compounds that contain extra moieties attached rings. They are frequently referred to as agents of increased pesticide potency, with high recalcitrance and poor or resistant degradation agents. Notable examples are 2,4-dichlorophenoxyacetic acid (2,4-Di-CP-O-AA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-Tri-CP-O-AA), and other analogs that includes organophosphate herbicides (e.g., chlorpyrifos and profenofos), 2-(4-chloro-2-methylphenoxy) propionic acid (mecoprop or 2(4C-2-MP), 2-(2,4-dichlorophenoxy) propionic acid (dichlorprop), 2-methyl-4-chlorophenoxy acetic acid (MCPA), and 4-(4-chloro-2-methylphenoxy) butanoic acid (MCPB) (Pimviriyakul et al. 2020). The class III CBACs contains greater than a single aromatic ring eg polychlorinated biphenyl (PCBps). Following the above-stated class of CBACs, its biodegradation should provide a sustainable environment, healthy environment using low cost and ecofriendly technologies for the removal of contaminants from the environmental milieu.

Since the biodegradation process may occur either aerobically or anaerobically, the bioconversion or biotransformation of pollutants into utilizable minerals would occur in pathways/mode of action which may be subdivided or group into three steps namely; the upper (initiation), intermediate (middle), and rap-up or lower step/pathways of metabolism. These three aforementioned pathways are successively involved in the bio-conversion of various CBACs and derivatives into metabolisable components for cells. One difficulty encountered during biodegradation is in the intermediate step where various enzymes that share similarities in features are involved but results in the mineralization of CBACs to metabolites used for energy generation of cells. Following the various reports on the microbial degradation of CBACs, its derivatives and pathways, several activities might be involved, which include anaerobic action where there is a complete reduction of CBACs and derivatives (removal of chlorine or dechlorination) in the initiation step. This is followed by an aerobic conversion of monochloride, dichloride, and trichloride aromatic compounds to their corresponding chlorocatachols (CC).

It is important to note that before dechlorination can occur, there must be splitting of the aromatic ring hence the splitting of the aromatic ring occurs first before the formation of CCs. As a continuation of the biodegradation process, polychlorinated phenols in the first step are then subjected to oxygenative dechlorination under an aerobic situation to form (chloro)hydroxyquinol ((chloro)1,2,4-trihydroxybenzene). This would be followed by an ortho-cleavage of the CBACs resulting maleylacetate (Pimviriyakul et al. 2020). It is important to note that the biodegradation of CBACs and other related derivatives may occur either by microbial breakdown of the substrate or by good structure and designed enzymatic activity. The enzymes may be tailored by immobilization on appropriate surfaces and/or application in nano-technology to bring about the needed biotransformation or biodegradation of notable

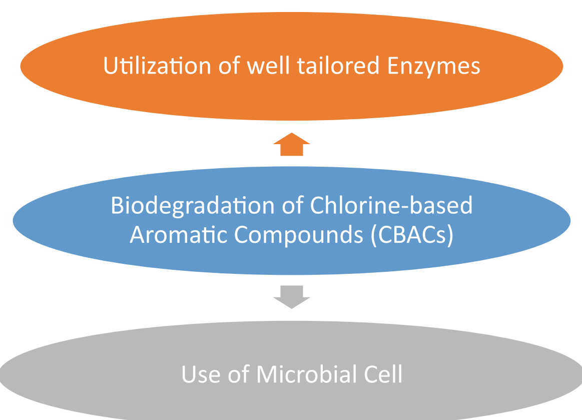


**Fig. 12.1** Approaches of biodegradation of chlorine-based aromatic compounds

environmental pollutants. Figure 12.1 below shows the biodegradation mechanism in both application of microbial cells and the use of enzymes, which are products from microbial biosynthetic production processes. Understanding the mechanism of microbial application in the degradation of CBACs and the use of various enzymes in biodegradation may be a sea of wealth and development in biotechnology and bioengineering of enzymes for sustainable environmental processes.

This chapter intends to review the application of fungi, actinomycetes, and bacteria in the biodegradation of chlorophenols in the environment and health sectors, the modes of action involved in the biodegradation of chlorophenols by microorganisms, and the health and environmental challenges involved with chlorophenols, which could serve as a xenobiotic. Several future recommendations were also highlighted.

## 12.2 General Over View on Biodegradation of Chlorophenols by Microorganisms

Chlorophenols are blends of synthetic inorganic and organic chemicals. They include CP (chlorophenol), DCP (dichlorophenol) TCP (trichlorophenol), TeCP (tetrachlorophenol) and PCP (pentachlorophenol). These chemicals are employed in different commercial and industrial purposes as well as in agriculture (2,4-D, 2,4,5-T, 2,4,5-TCP, and 2,4-DCP) as herbicides in hospitals and at home as an antiseptic (Field and Sierra-Alvarez 2008). Globally, chlorophenols are widely used as dyes, biocides, pesticides, and wood guards. Chlorinated compounds are one of the most dangerous xenobiotic constituents that is characterized by their substituents which make them persistent, non-biodegradable, and recalcitrant. These features make them remarkable as toxic compounds naturally present in the environment (Vallecillo et al. 1999; Arora and Bae 2014). They are classified as first-class

important pollutants by the US EPA and EU (United State Environmental Protection Agency and European Union), respectively (Ruzgas et al. 1995). In addition, similar status was also stated by the World Health Organization and International Agency for Research on Cancers who established that chlorophenols (CPs) have cytotoxic, mutagenic, and carcinogenic properties (Igbinosa et al. 2013). In humans, CPs can be ingested by drinking, eating, and skin contact.

Moreover, different methods such as oxidation by chemicals, extraction of liquid-liquid phase, ion exchange, and adsorption, where the various techniques used in the removal of chemical like CPs from waste effluents (ATSDR 1999; Pera-Titus et al. 2004; Muller and Caillard 2011; Olaniran and Igbinosa 2011; Igbinosa et al. 2013). However, these techniques have been proven ineffective, expensive, non-eco-friendly, and release noxious wastes into the environment. Contrariwise to these, it had been proven recently that a more operative, eco-friendly, cheap, and green method of decontaminating CPs away from the surroundings by the utilization of microorganisms yields a more sustainable result. There is an urgent need to manage and control the environmental and health influences of CPs in the ecosystem. Biodegradation of CPs has been investigated in different media systems (aerobic and aerobic), using the application of microorganisms (Boyd and Shelton 1984; Häggblom 1990; Christiansen et al. 1995; Bae et al. 1996; Armenante et al. 1999; Murialdo et al. 2003; Field and Sierra-Alvarez 2008; Arora and Bae 2014). The degradation of CPs by the actions of microorganisms (fungi and bacteria), is one of the significant factors influencing their concentrations in the environment (Ye and Shen 2004). The capacity of microbial degradation of pollutants anaerobically can be improved by the acclimatization of the potential inoculum, which can be used to detect the consortia degradation at various phases (Buitron et al. 1998). In the aerobic bacterial consortia, there are a multiplicity and selection of specific microorganisms during the acclimatization phase at several daily intervals (Horowitz et al. 1983; Wiggings et al. 1987; Timothy et al. 1989). Owing to the broad and lengthy periods of acclimatization, there is a tendency factor of probable envisaging of exposure of chemicals at different concentrations in the surroundings when compared to the shorter period of degradation by the microorganisms.

CPs can contaminate the water environment easily by migrating into surface and groundwater for the reason that their level of solubility is very high (Smith and Novak 1987). Monochlorophenols can be liberated through the process of the breakdown of aromatic compounds, pesticides, and the chlorination of wastewater, which cannot be effortlessly besmirched and digested by microorganisms (Pritchard et al. 1987). Dichlorophenols, on the other hand, can be easily degraded and metabolized by microorganisms, but the process of aerobic outbreak can be less active in high definition chlorophenols compounds. Several documented works have clearly highlighted the toxicity of CPs in a different environment with a focus on their toxicity and biodegradation, in both anaerobic and aerobic CPs degradation by microorganisms (Solyanikova and Golovleva 2004).

## 12.3 Mode of Action Involved in the Biodegradation of Chlorophenols by Microorganisms

Various investigators have described various pathways and mode of activities involved in the biodegradation of CBAC and its derivatives. The pathway and activity mode are directed by diverse enzymes of different classes and groups including hydroxylase, oxygenase, etc. with some occurring aerobically or anaerobically. The process would include the selection of biotransforming/biodegrading strains of microorganisms, aerobic breakdown of CBACs and derivatives where there will be some peripheral steps for degradation of monochlorinated and polychlorinated phenols and phenol hydroxylases. This will be followed by ortho-pathways for the degradation of (halo)catechol. Such processes would include the 3-chlorocatechol, 4-chlorocatechol and further modification of 3-chlorocatechol by some strains of *Rhodococcus opacus* 1cp, this terminated by the hydroxyquinol pathway. It is pertinent to note that the genetic basis for the broken down of CBACs and other associated derivatives be carefully understood to adequately describe the mode of activity and mechanism of action of the organism or enzymes during biodegradation. As mentioned earlier, the complete degradation of CBACs and derivatives begins with the assimilation and recognition of compounds which is followed by a ring target specific site cleavage (either intradiol or extradiol cleavage), a reaction that is catalyzed by diverse sets of dioxygenases. These well-studied dioxygenases (intradiol and extradiol dioxygenases) catalyze the integration of two atoms of oxygen into the chlorocatechol (CC) bond fission whereby oxygen activation occurs via a coordinated complex of substrate-oxygen-metal (Pornsuwan et al. 2017). The intradiol dioxygenase such as protocatechuate-3,4-dioxygenase and catechol-1,2-dioxygenase have been characterized in both bacteria and fungus of differing types. They are involved in the bond cleavage of two hydroxyl groups of catecholic aromatics resulting in muconate derivatives (b-carboxy-cis,cis-muconate and cis,cis-muconate). The further lactonization of these derivatives by muconate cycloisomerase, decarboxylase, and isomerase result in the formation of b-ketoadipate enol-lactone. It is noteworthy that the reaction pathways of catechol and protocatechuate, which are central intermediates, occurs via the formation of b-ketoadipate enol-lactone step. This step would occur prior to the lactone ring hydrolysis by enol-lactone hydrolase to generate b-ketoadipate (Patel et al. 1975). An additional two-step forward reactions along the pathway catalyzed by b-ketoadipate:succinyl-CoA transferase and b-ketoadipylCoA thiolase would generate acetyl-CoA and succinyl-CoA. The generate acetyl-CoA and succinyl-CoA then enters into the TCA cycle for the generation of energy since both are commonly known intermediates of the TCA cycle (Harwood and Parales 1996). On the other hand, the extradiol cleavage pathway uses both dioxygenases, dehydrolases, decarboxylases, dehydrogenases, tautimerases, hydrolases, hydratases, and aldolase was utilized to catalyze the bond breaking of the two outer hydroxyl groups of catechol and protocatechuate in a catecholic cleavage to form 2-hydroxymuconic semialdehyde. This on further breakdown would yield pyruvate and acetaldehyde in the case of catechol and pyruvate and oxaloacetate or acetyl-CoA in the case of

protocatechuate (Chenprakhon et al. 2019; Arora and Bae 2014). It is important to note that some of the enzymes used for the biodegradation of CBACs as well as other derivatives are not readily produced in the common biosynthetic pathway of diverse microorganisms. Kasai and his colleagues previously observed that *Paenibacillus* sp. harbors the enzyme responsible for the cleavage of protocatechuate-2,3-positions (Kasai et al. 2009). Pimviriyakul and his group also added that simpler pathways are utilized by microorganisms to cleave protocatechol-4,5-position instead of the direct cleavage of protocatechuate-2,3-position since the required gene cluster of the cleavage of 2,3-positions is not readily available in nature (Pimviriyakul et al. 2020).

Although some enzymes applied for the biodegradation of CBACs/derivatives have been extensively studied (e.g., dioxygenase), other necessary enzymes yet remain under-explored, hence exploring and understanding the mechanism of such microbial-derived enzymes, as well as their functions, remains a future prospect in the bioactive molecular discipline. The intermediate pathway involves the transformation of less toxic and more soluble products of the first degradation activity such as catechol, hydroquinone, or protocatechuate via dechlorination. This dechlorination may also occur both by oxidative and nonoxidative process. The oxidative process involves the addition of specific groups, which are catalyzed by oxygenases (for the addition of oxygen) or hydrolases (for the addition of water). Another important note is that the addition of groups (e.g., hydroxylation) in addition to dechlorination must occur first to prepare the CBACs and other derivatives for ring-cleavage dioxygenases before it is converted from aromatics to aliphatics. The nonoxidative dechlorination also occurs in a similar mechanism as reported by Boll and his colleagues (Boll et al. 2014), which is referred to as reductive dechlorination as dehalogenase catalyzes the removal by reduction and hydrolytic activities. The last pathway which is accountable for the elimination of any supplementary moieties that are attached to the CBACs is referred to as the upper or tertiary cleavage pathway where acetyl groups are removed from the CBACs products into easily metabolizable by-products. This pathway is not always necessary for the degradation of lower or smaller CBACs which may be broken down by enzymes of the second or intermediate pathway. However, it involves debranching of aromatics to remove groups or dephosphorylation of chlorinated aromatic organophosphates. Its products are mono-aromatic compounds including chlorine-based benzene, chlorophenol, chloroaniline, and chlorobenzoate based derivatives which act as substrates for enzymes in the intermediate stage of biodegradation (middle or intermediate pathway) (Chenprakhon et al. 2019; Arora and Bae 2014).

## 12.4 Application of Fungi for the Biodegradation of Chlorophenols

Fungi have been known to exhibit the capability to tolerate and breakdown a plethora of hazardous substances (Evans and Hedger 2001). Numerous research on the breakdown capability of fungi has been based on an ecological group known as the white-rot fungi. The spotlight on the degradative capability of white-rot fungi

(WRF) was initiated based on reported findings on the degradative capacity of *Phanerochaete chrysosporium* on a wide assortment of environmental contaminates (Bumpus et al. 1985). WRF are saprophytes that utilize dead organic matter as their carbon source by degrading cellulose, hemicellulose, and lignin portion of the wood cell wall (Thwaites et al. 2006). It has been suggested that this cellulolytic and ligninolytic activity exhibited by WRF is the same employed in the degradation of chlorophenols and other pollutants (Ellouze and Sayadi 2016).

Bioremediation and biotransformation are relevant terms observed in biodegradation especially as some chemical agents have shown recalcitrance in the environment. Although the terms involve the restoration of environmental wellness, its proposed approach must be eco-friendly, cost-effective, and application of traditional alternatives in remediation techniques. Fungi are a group of microbial strains that have shown potential for the dilapidation of recalcitrant compounds such as CBACs and its derivatives. One identified strain of these group of organisms is *Tritirachium* sp. It has shown high levels production of extracellular catechol 1,2-dioxygenase and its action in an in vitro determinative test, which was recently conducted and reported by Nikolaivits and his colleagues (Nikolaivits et al. 2020). This strain has also produced dechlorinated cleavage or chlorine degraded by-products from a starting CBACs which indicates it's potential for the assimilation of xenobiotics and biodegradation. The study of Mileski and his colleagues also reported extensive biodegradation of pentachlorophenol (PCP) by some white fungus referred to as *Phanerochaete chrysosporium*. The fungus was described to possess a lignin-degrading system that aids decline and mineralization of PCP in nitrogen-limited solid or liquid culture. In addition, enzyme products from the organism (e.g., Ligninase) was used to catalyze the initial oxidation of PCP which suggests that in addition to the lignin-degrading capability of the organism, it also harbors the potential for another degradation system, which may also be applied in PCP biodegradation (Mileski et al. 1988). In another related study, the application of the white fungus with silver nanoparticles in a degradation process yielded maximum degradation of PCP and respective mineralization as the compound was first broken into linear chains with an eventual conversion into $CO_2$ and $H_2O$ via reductive dechlorination. Other fungi were also used by various investigators including *Fusarium* sp., *P. chrysorhiza*, *P. sanguine*, *I. circinatus*, *P. filamentosa*, etc. Some investigators utilized the white-rot fungus (*Trametes versicolor*) to influence the production of extracellular laccase as well as its relative activity as a biodegradative agent from the mineralization of 2-chlorophenol (2-CP) by fungal mycelia. A relative formation of 2-chloro-1,4-benzoquinone (2-CIBQ) found was connected with extracellular laccase activity. The primary oxidation step of the biodegradation may therefore be partially attributable to extracellular laccase action which indicates cell-bound degradative processes. Although between 1990 and 2020, about 70 articles were published about studies where fungus was used as the microbial biodegradation of chlorophenols or CBACs and derivatives.

## 12.5 Specific Types of Authors That Have Applied Application Fungi for the Biodegradation of Chlorophenols in the Environment

Yordanova et al. (2013) tested and evaluated the potentials of *Trichosporon cutaneum* (R57) and *Aspergillus awamori* (NRRL3112) on the degradation of phenolics and phenol derivatives. It was discovered that when combined together, their effects on the degradation of phenol was up to 1.5 g/L. On the other hand, when combined individually, their free cells and immobilization capacity did not degrade up to 1.0 g/L. The high synergistic activities and potential capacities of the immobilized systems were shown with these processes of combination and solitary influences on the chemicals. Furthermore, the degradation of various phenolics was also studied. These included chlorophen—ChP (2-benzyl-4-chlorophenol), chloroxylenol—ChX (4-chloro-3,5-dimethylphenol), BPA (bisphenol A), 3-MP (3-methoxyphenol), 4-NP (4-n-nonylphenol), 2,4-DCP (2,4-dichlorophenol), 3-CP (3-chlorophenol) and 2-CP (2-chlorophenol). From the study, it was observed that the use of phenol 2,4-DCP and BPA was very swift at 16 h. 3-MP, CP, 4-CP, 3-CP, and 2-CP utilization was very swift at 40 h between the range of 44–72% and the utilization of ChP and NP were able to degrade very gradually up to 30% in 40 h. Meanwhile, ChX persisted unaffected for 40 h. The investigation of the derivatives was recorded in this order based on the level of the degradation by the microbial strains. The rate of degradation of the phenolics and phenol derivatives at concentrations of 0.3 and 0.1 g/L were also determined. The benefits of the mutual immobilized arrangements for the microbial degradation of the derivatives (phenolics and phenol) when compared to other systems of the two free cells and strains, separated and combined, were also proven to be positive in their degradation actions.

van Leeuwen et al. (1997) tested and isolated the degradation of compounds of Chlorophenolic by *Trichoderma harzianum*. The biological experiment was assessed for their metabolic potential abilities on compounds of CPs; chloroguaiacols and 2,4,6-trichlorophenol by the isolates. The abilities of the isolates sourced 10 m from the point of discharge were evaluated based on the nature of the substrate media. It was observed that there was a corresponding decrease in the absorbable CPs (organic halogens) of 14 carbon IV oxide from 14 carbon labeled CPs. The process of tetrachloroguaiacol dehalogenation was also observed. The potential abilities of other different isolates were temporally evaluated based on the decrease in the levels of tetrachloroguaiacol spiked in the AOX and corresponding culture medium. It was observed that *T. harzianum* mineralized a small percentage between 2–3% of the spiked pentachlorophenol as well as temporary dehalogenated tetrachloroguaiacol at 46% in a saline medium. It was found that each isolate was capable of decreasing the tetrachloroguaiacol concentrations in the respective AOX and the mineralized saline medium. The maximum capacities were gotten from regions closed to the waste discharged location to the lake. The findings from their study showed that the occurrence of the fungi in the water may explain the reduction of the chlorophenolics found therein.

Globally, for about 20 years the amount of pesticide in the environment has dramatically increased. Diez (2010) in a review looked at the biological degradation of some organic pollutants used for pesticide production. The author stated that the residues and by-products transformation of pesticides are found frequently on the surface and ground waters. Special information about CPs (chlorophenols), PCBs (polychlorinated biphenyls), and PAHs (polycyclic aromatic hydrocarbons) on the roles in pesticides, impacts on the environment, and the breakdown action of microbes were highlighted and discussed. Details on the influxes of these chemicals in different aquifer levels in Chile and some foreign nations were also reported. The author recounted that the fungi breakdown of organic xenobiotics is well-thought-out effective technique in the decontamination of the pollutants from the surroundings as a biotechnology procedure called bioremediation. Details on the white-rot fungi degradation of pesticides containing CPs were also discussed. Finally, an effective and simple biobed system was suggested to reduce the environmental impact as well as the manipulation of pesticides when used to fill the spraying device, which is a characteristic point source of contamination. In addition, a traditional fungi consortium and Actinomycetes are needed to protect the technology in order to develop and protect the depletion of the microflora autochthonous inocula.

Kumar et al. (2018) tested and evaluated the biological degradation of free radicals of phenol using strain NPD1401 (*Candida tropicalis*) immobilizer. The result of the biological study showed that the strain was able to break down about 98% concentration of phenol compounds (s1000 mg/L). However, the free cells were able to break down about 63% of a similar concentration in a 9-day incubation period. When the stored beads were reprocessed after about 15 days, it was observed that they were able to break down about 62.1% of the phenol in the saline medium.

Reddy et al. (1997) tested the degrading potential of *Phanerochaete* chrysosporium and *Dichornitus squalens* on chlorophenoxyacetic acids. The influence of $Mn^{2+}$ on the breakdown of CPs by both *Phanerochaete* chrysosporium and *Dichornitus squalens* on chlorophenoxyacetic acids revealed that the strains of fungi were able to catalyzed by mechanistic self-regulating the side-chain cleavage of the chemical. The broken-down of the aromatic ring, relied on the lignin from the fungi via utilizing un-labeled substrates in an alleyway for the breakdown of the chlorophenoxyacetic acids, explains the degrading potential of *Dichornitus squalens*. This also demonstrated that the first product formed was chlorophenol, which was later xylosylated to chlorophenolxyloside. In line with this process, the chlorophenolxyloside was later regenerated to chlrophenol via hydrolysis using intracellular cleavage of *p*-xylosidase. This was later dechlorinated to 2-chloro-*p*-benzoquinone byxylosidase and undergoes subsequent ring-opening and dichlorination reactions by the action of *P. chrysosporium*.

Fritsche and Hofrichter (2005) demonstrated that microfungi with mycorrhiza are also microbial species involved in the environmental biodegradation of pollutants. The authors reported that unlike bacterial, fungi can survive in a very low pH, and moisture environment due to the presence of robust extracellular multi-enzyme complex utilized in breaking down toxicants. Again yeast such as *Trichosporon*

*cutaneum*, *Candida lipolytica*, *Rhodoturularubra*, *C. tropicalis*, *P. anomala*, *C. methanosorbosa* BP-6 *Aureobasidion*(*Trichosporon*) *pullulans*, *Cyberlindnera fabianii*, *Wickerhamomyces anomalus*, *S. cerevisiae*, *Rhodotorula pilimanae*, *Hansenula polymorpha*, *P. guilliermondi*, *Yarrowiali polytica*, *Schizosaccharomyces pombe*, *and C. ernobii* may use aromatic compounds like phenol to grow due to the presence of specific energy-dependent uptake systems.

Abruscia et al. (2007) revealed that filamentous fungi like *Cladophialophora*, *Leptodontium*, *Exophiala*, *Pseudeurotium zonatum*, *Amorphoteca*, *Talaromyces*, *Neosartorya Cunninghamella*, *Fusarium*, *Penicillinum*, *Alternaria alternate*, *Trichoderma longibrachiatum*, *Cephalosporium*, *Mucor Racemosus*, *Rhiloprzs arrhizus*, *Aspergillus* are good biodegraders due to their ability to produce extracellular degradative enzymes referred to as oxido-reductive enzymes when compared to some bacterial species. The authors revealed that the physiological mechanism utilized by these filamentous fungi is of two types, the exo-cellular lignin-degradation made up of lactases, peroxidases, and intracellular cytochromes P450 system.

CPs are environmentally concerned chemicals that have noxious effects on humans, such as cytotoxicity, mutagenotoxicity, and carcinogenotoxicity, as well as the deleterious impact on the environment. However, to curb this menace, several green, eco-friendly, and cheap method of decontaminating these xenobiotic compounds has been recommended for effective ecorestoration as when compared to the long traditional methods. Consequent to this, Hou et al. (2016) tested and evaluated the degradation efficiency of a strain of aniline degrading *Rhodococcus rhodochrous* (DSM6263) on different aqueous phases of chlorophenols. The results of the study revealed that DSM6263 strain was able to decontaminate chlorophenols by hydroxylation at the ortho-region of the chlorophenolic chains. It was observed that when the cells were immobilized with k-carrageenan alongside nanoparticles of $Fe_3O_4$ (9 g/L), they could successfully break down different chlorophenol mixtures, 2,3-dichlorophenol, 4-chlorophenol, and 2-chlorophenol correspondingly that were much higher than the free cells. They recommended the six nanoparticles and the immobilized cells for the degradation of six cycle chlorophenol compounds. It will also serve as a promising tool for the bioremediation of chlorophenols in waste effluents.

One of the major contaminants in the pharmaceutical industrial wastes is a phenol. Of recent, several traditional methods were used to decontaminate this chemical in the effluent, which have proven to be ineffective, expensive, and releases more harmful toxins into the environment, thus resulting in health and environmental harms. Currently, the utilization of microorganisms in the decontamination of the recalcitrant nature of phenol has been proven to effective, cheap, eco-friendly, and green. In line with this, Bernats and Juhna (2015) tested and evaluated the biodegradation of chlorophenols in pharmaceutical waste effluent using white-rot fungi. They used four monocultures of fungi (*Irpex lacteus*, *Gloeophyllum trabeum*, *Phanerochaete chrysosporium* and *Trametes versicolor*). The outcome of the study indicated that *Trametes versicolor* was the most operative strain. The outcome from the optimization of the temperature, pH, and concentration of the biomass, showed that the optimum situations were 25 °C, 5–6, and 10% (v/v)

correspondingly. In optimum situations, the concentration of the CPs was decreased by 93% from 420 ± 12 to 29 ± 1 mg/L with 7 days by *Trametes versicolor*. The findings of this study indicated that the tested biological strain *T. versicolor*, was able to effectively reduce, treat, and remove the concentration of phenol in the pharmaceutical wastewater before improving it with the traditional methods.

Mileski et al. (1988) studied pentachlorophenol biodegradation by the WRF; *Phanerochaete chrysosporium*. The study revealed the complete mineralization of pentachlorophenol by the WRF incubated under nitrogen limiting conditions for 30 days. Toxicity studies of pentachlorophenol on the fungus revealed that the lethal effects of the pollutant could be avoided if the cultures had formed a mycelial mat prior to the addition of the chlorinated phenol. With this procedure, the fungus degraded 500 mg/L of pentachlorophenol during a 24 h incubation period.

Yum and Peirce (1998) demonstrated the 4-chlorophenol and 2,4-dichlorophenol degradation using *Phanerochaete chrysosporium* in immobilized cell reactors with birchwood chips as a carbon source under different growth conditions. The study revealed that the fungus degraded the pollutant at 71% in the course of a 20 day incubation time with no additives. The addition of a nitrogen source increased 4-chlorophenol and 2,4-dichlorophenol degradation rate to 83% over the same incubation period. Based on these results, the authors opined that a more effective treatment system could be developed using the white-rot fungus to degrade chlorophenol under certain nutrient conditions. Valli and Gold (1991) investigated 2,4-dichlorophenol mineralization by *P. chrysosporium* using 2% glucose and 1.2 mM ammonium tartrate as carbon and nitrogen source, respectively, over a 6 day incubation period. The study revealed that under nitrogen limiting settings, *P. chrysosporium* was able to completely mineralize 2,4-dichlorophenol.

Lamar et al. (1993) conducted field studies that evaluated the ability of three ligninolytic fungi: *P. chrysosporium*, *P. sordida*, and *Trametes hirsuta* to remediate the pentachlorophenol (672μg/g) and creosote (4017μg/g) component from soil contaminated with these pollutants. The fungi were applied as pure or mixed cultures to the polluted soils at several inoculum loads. Results of the study revealed an 89% decrease in pentachlorophenol concentration by *P. sordida* (10%). At the same inoculum level, *P. chrysoporium and T. hirsuta* (55%) also degraded the organochlorine at (67–72%) and (55%) respectively within the 56 day incubation period. The mixed cultures of the three fungi did not degrade the pollutant efficiently as did the axenic pure cultures. Based on the previous study, further investigation into the biodegradation capacity of *P. sordida* on pentachlorophenol and Poly-nuclear aromatic creosote in the field was investigated by Lamar et al. (1994). This time, the soil was contaminated with 1058 mg/kg of pentachlorophenol and 1210 mg/kg of creosote. Results indicated a 64% decrease in the concentration of the chlorinated phenol after a 20 week incubation period. Another WRF; *Lentinula edodes* was observed to exhibit significant degradation ability with respect to soil-borne pentachorophenol (Okeke et al. 1997). The authors indicated a 99% depletion rate of the chlorinated phenol in 10 weeks with the monocultures of the fungus. They also reported the production of oxidative enzymes phenol oxidase and manganese peroxidase within 2 weeks of inhibition. Wen et al. (2006) investigated the biodegradation

of 4-chlorophenol under anaerobic conditions with 10% *Candida albicans* PDY-07 cultured from activated sludge. The authors observed that the fungi completely degraded 4-chlorophenol concentrations (200–300 mg/L) within 244 h. Nakagawa et al. (2006) studied 2,4-dichlorophenol metabolism by soil-borne *Mortierella* sp. The fungi reduced 250μM 2,4-dichlorophenol by 55% within 48 h compared with the non-fungal culture, which showed a 26% decrease of the chlorinated phenol within the same period. Rubilar et al. (2011) compared pentachlorophenol metabolism by free cells and immobilized cells of *Anthacophyllum discolor* and *P. chrysoporium* respectively. The cells immobilized with wheat grains showed a higher pentachlorophenol removal rate (64.1–80.4%) compared to that measured with both fungi strains as free mycelium (47.1–50.5%). Wang et al. (2012) examined the prospective of *Candida tropicalis* derived from sludge to degrade phenol and 4-chlorophenol using high-performance liquid chromatography (HPLC) procedure. The authors revealed that the fungi degraded 150 ppm of 4-chlorophenol in 20 h with phenol serving as a carbon source. The authors also opined on the possibility of phenol serving as an inducer of chlorophenol degrading enzymes in fungi with the compound also utilized as a source of carbon. Yordanova et al. (2013) studied the biodegradation of phenol and some of its phenol derivatives using polyamide beaded immobilized cells of *Trichosporon cutaneum* and *Aspergillus awamori*. The observation showed that a combination of the two immobilized organisms degraded 0.1 g/L of each pollutant in the following order: phenol > bisphenol-A > 2,4-dichlorophenol > 3-methoxyphenol > 3-chlorphenol > 2-chlorophenol > 4-chlorophenol > 2-benzyl-4-chlorophenol > chloroxylenol. The rate of degradation of the pollutants except chloroxylenol ranged from 100% to 30% within a 40 h incubation period.

## 12.6 Mechanism of Fungi Degradation of Chlorophenol

The degradative attribute of most fungi with regards to chlorophenol and other environmental pollutants may be attributed to its expressed ligninolytic activity. The Lignin chemical structure of lignin is similar to the chemical structures of many persistent environmental organic pollutants (Yum and Peirce 1998). Ellouze and Sayadi (2016) suggested that the ligninolytic fungi generally degraded xenobiotic substances via an extracellular ligninolytic system, which encompass lignin peroxidase, $H_2O_2$-producing enzymes, manganese peroxidase, and laccase veratryl alcohol (3,4-dimethoxybenzyl alcohol), respectively. It is important to note that both enzyme expression and function is wholly dependent on the fungal species. Some fungal species are known to elicit lignin peroxidase, manganese peroxidase, and laccase together while some species elaborate just one or two enzymes that act simultaneously or separately on the available xenobiotic. Several researchers have reported the degradative activity of these enzymes against xenobiotic and recalcitrant compounds (Cameron et al. 2000; Blanquez and Guieysse 2008).

## 12.7 Usage of Immobilized Fungi Cells over Free Cell System in the Degradation of Chlorophenols

Some authors have demonstrated the degradation potential of chlorophenols by free fungi cells (Field and Sierra-Alvarez 2008; Rubilar et al. 2011). However, usage or application of immobilized fungal cells has proven to have more advantageous than the conventional free cell system as it can enhance the stability of the biocatalyst. Dwyer et al. (1986) opined that the immobilized cells had higher resistance potential to variations in the concentration of the environmental pollutants. Sofer et al. (1990) suggested the option of modifying or adjusting the fungal density in immobilized cell reactors with the aim of generating increased biodegradation output in comparison to free fungal cells.

## 12.8 Application of Actinomycetes for the Biodegradation of Chlorophenols

One of the group members said to be prokaryotes which are simple unicellular but complexly organized organisms. They are grouped into ten major divisions which comprise gram-positive bacteria to which actinobacterial belong. The phylum and the application of 16S rRNA gene analysis have showed that the group which consists of 221 genera is made of 6 classes, 19 orders, and 50 families, with newer taxa continually been revealed (Goodfellow et al. 2012). They are mainly filamentous, gram-positive cocci, and facultative anaerobes or aerobes. In some cases, the gram reaction may appear variable, because of their cellular rigidity and the presence of muramic acid in their cell wall (Goodfellow et al. 2012). They exhibit great diversity in lifestyles and are saprophytically free-living in marine/aquatic environments. Specifically, in soil, they mediate the formation of soil organic matter by enhancing plant-associated commensals and nitrogen-fixing symbionts. They are also gastrointestinal tract inhabitants in animals and pathogens of plants (Goodfellow et al. 2012). Today, the extremobiosphere is covered and colonized by members of the Actinobacteria, which contradicts the traditional perception about the phyla as autochthonous soil and a freshwater group of organisms (Goodfellow et al. 2012). In fact, the *Streptomyces* genus, a member of the Actinomycetales order, is still the richest source of useful microbial metabolites, including clinical antibacterial agents, bioactive metabolites, protein, and tumor inhibiting agents (Olano et al. 2009). Among all 23,000 known bioactive molecules, 45% are known to be secondary metabolites gotten from Actinomycetes, and 80% of them are produced by the actinomycetes and/or *Streptomyces* genus. This diversity in bioactive metabolite manufacture makes them imperative tools for environmental remediation, biomedical companies, medicine, and biotechnological companies who apply them as bio-remediating agents, antibiotic-producing agents, enzyme-producing agents, anticancer agents, and other applications including agro-medicals. Among the existing known bioactive agents isolated, over 90% are produced by terrestrial actinobacterial species. The marine members resident in the marine

environment among the actinobacterial is underexplored as potential sources of novel biological compounds and novel microbial products used for medicine and other commercial purposes. Actinobacteria are an example of a major and most diverse phylum within the bacteria domain, based on 16S rRNA gene studies (Hentschel et al. 2012). They are mainly filamentous, gram-positive cocci, and facultative anaerobes or aerobes. Their cell wall contains muramic acid, with a majority of its members belonging to chemo-organotrophs and high G + C content (Goodfellow et al. 2012).

Recently, attention was given to the members from a multidisciplinary point of view due to the relevance of their bioactive metabolites and chemo-structural characteristics especially in healthcare and remediation of waste or biodegradation (Kalaitzis et al. 2009). CBACs and its derivatives have been observed to be degraded in recent times by these groups of microbes, which possess multiple biosynthetic gene clusters. The various gene clusters can be accessed by genome mining using the antiSMASH software designed for novel biosynthetic pathways. In assessing the novelty of a biosynthetic gene cluster, the gene sequence or a whole genome sequence is obtained from a proficient biodegrading organism and deposited in the Genbank. The deposited sequence is then annotated to obtain a Genbank reference number or reference sequence accession number. With the Genbank reference number, antiSMASH can generate a two-dimensional structure of the various biosynthetic genes and clusters, which is compared with the gene structure of all known similar genes of biodegradation. A novel gene can further be studied for its mechanism of activity and its components while similar gene structures are arranged for the level of similarity and phylogenesis (Tilmann et al. 2015; Blin et al. 2013). Although this group of organisms is not commonly employed in the biodegradation of CBACs and its derivatives, the application has shown promise for future biotransformation or biodegradation of various chloro-aromatic compounds. A mixed culture of actinobacterial members have been observed to biodegrade CBACs as the enzymes applied in the degradation and the intermediate products of biodegradation were observed to be present or produced. In addition, complete biodegradation was indicated as there was a release of chloride from 5-chloro-2-hydroxymuconic semialdehyde after further degradation of such intermediates.

## 12.9 Application of Bacteria for the Biodegradation of Chlorophenols

Globally, more than 400 published articles have reported the application of bacteria in the biodegradation of CBACs between 1990 and 2020 with a majority of the studies in China, the United States, and India. Other countries have applied the use of bacteria but researchers of China appear to have exhaustively applied bacteria and more reports of their usage or their enzymes are currently in the pipeline. Bacterial have shown diversity in degradative potential due to their natural ubiquity and diversity in natural existence. They have been seen to exist in a diverse environment while they develop and advance in the generation of metabolic pathways as well as

in their nutrients/energy/feeding. The degradation of CBACs occur both via aerobic and anaerobic processes making use of the diverse biochemical breaking down potential of bacteria. Some CBACs that have been seen to be degraded by bacteria are monochlorophenols (MCP), polychlorophenols (PCP),chloronitophenols (CNP), chloroaminophenols (CAP), and chloromethylphenols (CMP), etc. Under aerobic condition, CBACs and its derivatives are degraded extensively by bacteria as the organism used the aromatic chlorides as a major carbon source and energy generation. These aforementioned bacteria strains have been applied in various degradation studies with specific attention of CBACs and its derivatives, which has also revealed significant biodegradation of the aromatic compounds. The total number of global published documents on biodegradation of CBACs and its derivatives is shown in Fig. 12.2.

Researchers revealed the degrading ability of some species of *Rhodococcus* and *flavobacterium* utilizing pentachlorophens (Xun et al. 1992; Lee and Xun 1997). *Streptomyces rhochei* strain was found by Golovleva et al. (1992) to degrade the complete spectrum of polychlorinated phenol. The polychlorinated phenol (CPs) is an organochlorine compound used as pesticides and used also as a wood preservative. *Pseudomonas* and *Azotobacter* sp. were found to degrade 2,4,6-trichlorophenol (Takizawa et al. 1995; Li et al. 1991; Wieser et al. 1997; Kiyohara et al. 1992). The initial phases of chlorophenol degradation usually bear three to four composition starting with hydroxylation in substituting position chloroquinol. To achieve great success in the degradation, it is important to know the degrading ability of the microorganisms. Gram-positive and gram-negative bacterial isolates were found to breakdown chlorophenols, while some utilize mono and other dechlorinated phenols.

Apajalahti and Salkinoja-Salonen (1986) together with Golovleva et al. (1992) revealed that *Rhodococcus chlorophenolicus* and *Strochei* 303 degraded a wide range of chlorophenols, from complexes of mono to penta chlorinated phenols. Few strains had not the capabilities of growing on halophenols thereby catalyzing the imputation of hydroxyl group in ortho-end of mono-di- and trihlophenols and producing appropriate halocatechols (Häggblom 1992; Boersma et al. 2001). Hydroxylation, which is known to be a chemical process, that incorporates hydroxyl group into organic complexes is the first stage that aerobic microorganisms utilized during the degradation of chlorophenols (Fetzner and Lingens 1994). Phenol hydroxylases are enzymes that stir up the incorporation of hydroxyl groups into substrates in the direction of single $O_2$ in the cyclic ring, and the next stage is by reduction of water (Hayaishi 1969). *Pseudomonas putida* G131 strain was found to degrade 3-methyl- and 3-chlorocatechol through meta-pathway in the presence of catechol 2,3-dioxgenase as a complex protein (enzyme) (Kaschabek et al. 1998). Initially, some *Pseudomonas* sp. were primarily known for the degradation of 3-chlorobenzoate by first catalyzing the formation of 2-chloromucanate via Chlorocatechol 1,2-dioxygenase this process is possible by using 3-chlorocatechol (Schlömann 1994).

The crystalline element of dienelactone hydrolase synthesized by *Pseudomonas* sp. was collected and the catalytic mode of action was assessed (Pathak and Ollis

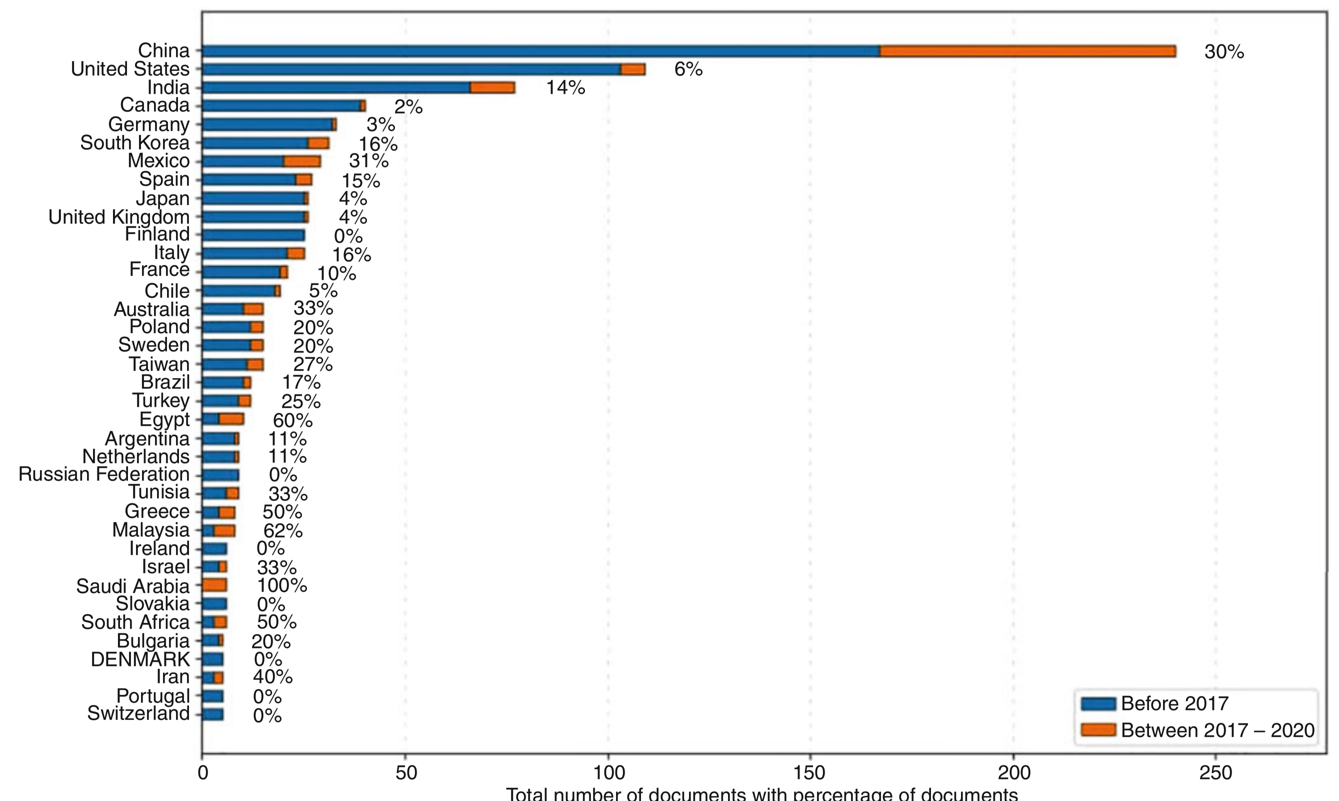


**Fig. 12.2** Global published documents on biodegradation of CBACs and its derivatives

1990; Cheah et al. 1993; Beveridge and Ollis 1995). The degradation of chloroaromatic complexes begins through 4-chlorocatechol branch in the situation where 4C cat serves as a precursor of alteration of the growth substratum and also in the degradation of 3- and/or 4-chlorophenol usually by *Rhodococcus* sp. (Janke et al. 1988a; Janke et al. 1988b).

Field and Sierra-Alvarez (2008) identified various bacteria that were able to breakdown chlorophenols and also discovered techniques in which this could bring about a successful degradation process. Using a method of incorporating mono hydroxyl group into substrates during the process at the *ortho*-ends of the chlorophenols rings thereby producing chlorocatechols, which could be degraded more to produce other complexes (Solyanikova and Golovleva 2011; Mars et al. 1997). They also explained further that the hydroxyl group could be attached to the *para*-ends of the rings bringing about the formation of a benzenediolic compound that comprises hydroquinone containing a mono chloro component. This complex could also be digested by introducing hydroxy group into biological compounds or the removal of the carbon-halogen bond from the complex (Nordin et al. 2005; Xun et al. 1992).

Bacteria degradation of chlorophenols could as well be done by the elimination of ammonium ions via deaminase thereafter, cleavage of the ring (Arora et al. 2013, 2014). The following complexes were gotten from the microbial digestion of chlorophenols they are; 2,3,4-chlorophenols, 2,3,4-chlorocatechol, and chlorosalicylate (Nikodem et al. 2003; Weisshaar et al. 1987; Arensdorf and Focht 1994). Knackmuss and Hellwig (1978); Menke and Rehm (1992) identified and isolated *Pseudomonas knackmussii*, *Alcaligenes xylosxidans* and *Herbasipirillum chlorophenolicum* as microorganism that has the capacity to degrade cholophenols (Konovalova et al. 2009).

## 12.10 Specific Authors That Have Utilized Bacteria for the Biodegradation of Chlorophenols

Diez (2010) briefly reported that across the globe, there has been a drastic increase in the utilization of pesticides in the last decades, thus many of the pesticide residues or by-products are found at an alarming rate in the environment causing unprecedented health challenges. The authors reported that microorganisms are thus necessary and important for the generation of biosurfactants which are eco-friendly and less expensive methods to degrade many of these toxicants. The metabolic machinery and the ability to survive in harsh environments of many microorganisms enables them to be able to biodegrade, although the effectiveness is dependent on several factors such as concentration of the toxicants, nature, microorganisms presence, and physiochemical properties. Thus the authors suggested that these factors can be grouped into biological and environmental characteristics.

Basfar et al. (2017) revealed that generally, chlorophenols (CPs) are environmental contaminant that has been reported to be very toxic, carcinogenic with low biodegradability. Many different compounds or approaches have been identified to

be utilized for the biodegradation of chlorophenols such as gamma radiation, microorganisms. Arora et al. (2014) reported that many examples of CPs and their derivatives such as chloroaminophenols, monochlorophenols, chloronitrophenols, chloromethylphenols, plus polychlorophenols have toxic properties on human health, hence must be effectively removed from the environments through biological and chemical means. The authors revealed that the bacterial method of biodegradation appears to be the most reliable method due to its eco-friendly plus cost-effective approach through the utilization of these CPs as energy and carbon sources. Several pathways have been reported to be involved with the formation of chlorocatechols or (chloro) hydroquinone by the bacteria genes.

Nawaz et al. (2011) highlighted the potential of microorganisms such as rhizospheric bacteria, actinomycetes, or plant-linked endophytic as biological agents in the biodegradation of environmental contaminate as a superior approach compared to chemical or physical methods. Bae et al. (2002) revealed that anaerobic degradation is involved in many microorganism's ways of biodegradation. The main approach recognized to be effective for the removal of xenobiotics has been established through the process which entails biotransformation and biodegradation through the cleavage of the halogen-carbon linkage, reductive dechlorination, *O*-demethylation plus *O*-methylation. Núñez-Gaytán et al. (2010) and Itoh et al. (2000) demonstrated that extracellular laccase of *Coriolus versicolor* is capable of bio-transforming chlorophenols into 2,4,6-trichlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, 2-chlorophenol, 2,3-dichlorophenol. The authors revealed that sinapinic acid derived from sources is capable of biotransforming different chlorophenols but *p*-coumaric and ferulic acids are known to inhibit the process.

Mohanty and Jena (2017) showed that *Pseudomonas* sp. Strain NBM11 and *Acinetobacter calcoaceticus* (NCIM 2286) demonstrated potential capability to biodegrade large phenol entirely in a pH range of 6.8–7.2. It was observed that the degradation capacity increased in many folds within the immobilized cells. Liu et al. (2007) revealed that *Stenotrophomonas* sp. strain can utilize as a source of energy, carbon plus nitrogen *p*-nitrophenol thus degrading 4-chlorophenol through the hydroquinone pathway stimulated through a wide range of temperature in the environment. The authors described the benefit of utilizing microbial biodegradation as a more cost-effective, efficient, and impactful means than the traditional methods. Veenagayathri and Vasudevan (2013) revealed that halophilic bacterial called halophiles can successfully degrade 4-chlorophenol in a minerals salts media and subsequently, the analysis was done utilizing gas chromatography. The authors described the halophiles as microorganisms that thrive and grow well in salinity (high salt) environment referred to as the extremophiles with tremendous potential for biotechnological utilization or applications. They revealed that in the past different bacteria and fungi have been utilized for the biodegradation of chlorophenols such as *Alcaligenes*, *Burkholderia plus Ralstoni*, *Arthrobacter ureafaciens* CPR706, *Arthrobacter chlorophenolicus* A6, and *Arthrobacter*.

Durruty et al. (2011) revealed that a study was undertaken to evaluate the rate at which some microorganisms such as *Achromobacter* sp. nov and *Pseudomonas aeruginosa* can rapidly degrade some notable xenobiotics like 2,4,6-trichlorophenol,

2,3,5,6-tetrachlorophenol plus pentachlorophenol. The authors revealed that during the process, 2,4,6-trichlorophenol demonstrated early degradation and effective biodegradation of these environmental toxicants. Kumar et al. (2008) reported that microorganisms are involved in the generation of biosurfactants which are utilized for the biodegradation of toxicants in the environment with severe health effects. The process of biodegradation is usually activated by surfactants secreted by the microbes through the stimulation by the pollutants. Some of the suggested bacterial are *P. putida*, *Rhodococcus erythropolis*, *P. chlororaphis and Pseudomonas aeruginosa* that are known to produce glycolipid biosurfactants acting as an emulsifier, thus reducing the surface tension and formation of micelles.

Kim et al. (2002) reported that the combination of *P. solanacearum* TCP114 plus *P. testosteroni* CPW301 in a mixture to achieve a higher rate of biodegradation was evaluated in an experiment to degrade 4-chlorophenol and phenol. It was revealed that the microorganisms were able to achieve an excellent rate of degradation. Olaniran and Igbinosa (2011) reported that chlorophenols are found in many pesticides as chlorinated aromatic constituents forming recalcitrant to many agents of biodegradation very toxic and carcinogenic to many living organisms. Thus one of the highlighted means of elimination is through the utilization of microbial biosurfactants with mono, dioxygenases, and oxygenases as notable enzymes involved in the physiochemical process. Many strains of bacteria have been reported to degrade chlorophenols through aerobic conditions such as *Bacillus*, *Norcadia*, *Pseudomonas*, plus *Mycobacterium Coeliacum*.

Jame et al. (2010) briefly described the importance of chlorophenols in many industrial productions and their biodegradation utilizing isolated bacterial with mixture *Pseudomonas* species culture. The authors revealed that the mixed culture showed a significant increase in biodegradation as compared with the single culture. Sandhibigraha et al. (2019) reported that bacterial strain *Bacillus subtilis* significantly degraded 4-chlorophenol in an experiment conducted by the authors in India. They applied different unstructured models to elucidate the kinetics of cell growth or the intrinsic kinetic factors. Thus it was reported that *B. subtilis* MF447840.1 was a potential candidate to be considered for biodegradation of 4-chlorophenol. Kopytko et al. (2002) revealed that the activity and nature of some herbicides can be altered or degraded utilizing bacteria, thus it was evaluated by considering the action of *Pseudomonas putida* on 1,1′-dimethyl-4,4′-bipyridylium and 2,4-dichlorophenoxiacetic acid. The experiment confirmed the possibility of the application of naturally occurring microorganisms in the process of biodegradation of many toxicants in the environments particularly chlorophenols.

Dong et al. (2011a) revealed that *Rhodopseudomonas palustris* PSB-1D bacterial strain could be utilized for the biodegradation of *o*-chlorophenol through a specific enzyme. Pramila et al. (2012) revealed that many bacterial are reported to be useful for the biodegradation of pollutants in the environments due to the fact that many of these bacteria feed on these chemicals as a source of energy, carbon, and nitrogen. The authors reported that these processes can occur in two ways anaerobic or aerobic conditions. *Brevibacillus* sp. and *Pseudomonas* sp. uses anaerobic biodegradation to eliminate pollutants in the environment. In other studies, several bacteria strains have

been utilized for bioderagation process with excellent results obtained such as *Bacillus*, *Corynebacterium*, *Shigella*, *Alcaligenes*, *Staphylococcus*, *Streptococcus*, *Acinetobacter*, *Klebsiella*, *Escherichia*, *Enterobacter*, *Pseudomonas*, *Mycobacterium*, *Rhodococcus*, and *Aeromonas.*

Studies have shown that biodegradation process involves groups of bacteria as single bacteria may not possess the entire capability to biodegrade all the toxicants in the environment. Aerobic pathways for the biodegradation process in bacteria use several catabolic enzymes like dihydrodiol dehydrogenase (BphB), biphenyl dioxygenase (BphA), hydrolase (BphD), and 2,3-dihydroxybihenyl dioxygenase (DHBD) (BphC). Seeger et al. (2010) reported that several pollutants have been degraded by bacteria such as *Providencia stuartii*, *Alcaligenes faecalis*, *Stenotrophomonas*, *Acidithiobacillus ferrooxidans*, *Bacillus*, and *Staphylococcus.* The authors revealed that bacterial possess a reduction mechanism, methylation, or utilizes metals as terminal electron acceptors known to impair metal resistance in aerobic or anaerobic conditions against several pollutants.

Cases and de Lorenzo (2005) reported that biostimulation, bioaugmentation, and biodegradation are approaches utilized to eliminate pollutants from the environment. Recently, the authors showed that bacterial genes such as rhizospheric and endophytic bacteria coding for catabolic enzymes could be genetically engineered to address the issue of biodegradation. The authors described these techniques as recombinant DNA technology, through which microorganisms are reconstructed to exhibit increased degrading ability. It was demonstrated that various risk assessment and quality control processes are involved such as amendment of enzyme affinity and specificity, regulation and construction of pathways, control, monitoring, and development of bioprocess plus application of bioreporter and bioaffinity sensor for chemical sensing, end-point evaluation with toxicity elimination.

Walker et al. (1975) reported that protozoa and algae are very essential members of the microbial family concerning the process of biodegradation. Some isolated algae by the authors such as *Prototheca zopfi*, *Chlorella vulgaris*, *S. quadricauda*, *Lyngbyala gerlerimi*, *C. pyrenoidosa*, *S. capricornutum*, *Scenedesmus platydiscus*, *Oscillatoria rubescens*, *Nostoc lincki*, *Volvox aureus*, *Synechoccus*, *Chlorella*, *S. incrassatulus*, *Westiellopsis prolifica*, *Anabaena inacqualis*, *Stigeoclonium lenue*, *C. sorokiniana* and *Elkatothrix viridis* possess strong biodegradation mechanism like uptake and biotransformation against many environmental toxicants.

Patel and Kumar (2016) demonstrated in their study the potential role of microbial consortium biodegradation ability against chlorophenols as compared to the individual microbes. Spectrophotometric analysis was utilized to evaluate the secondary metabolites during the biodegradation process. It was revealed that the microbial consortium gave a maximum removal rate. Manjarrez Paba et al. (2021) reported that removal of environmental toxicants azo dyes containing chlorophenols utilizing bacterial through biodegradation process. The authors used different bacterial such as *Marinobacter* sp., *Enterococcus faecalis*, *Sphingobacterium* sp., *Enterococcus casseliflavus*, and the findings revealed that all of them displayed a great efficiency for the elimination of the toxicants. Farrell and Quilty (2002) reported that the physiochemical properties of microbial biosurfactants and secondary metabolites

make them useful candidates in biotechnological and industrial applications. Thus, one such biotechnological applications involves the use in the elimination of environmental toxicants through the biodegradation process. *Arthrobacter chlorophenolicus* A6 strain was evaluated by the authors against 4-chlorophenol biodegradation, in the experiment, *luc* and *gfp* biomarker genes were monitored to generate useful information concerning the growth, toxicity, substrate depletion in the metabolic intermediate. The authors concluded that *Arthrobacter chlorophenolicus* A6 possesses the unique ability to degrade effectively 4-chlorophenol in several metabolic pathways.

## 12.11 Application of Actinomycetes for the Biodegradation of Chlorophenols

Chrolophenols are a group of compounds that belong to a group of persistent and toxic chemicals that are also known to be resistant to attack by environmental microorganisms. These compounds are widely employed in the production of various disinfectants and pesticides such as fungicides, herbicides, and insecticides. This has resulted in their conspicuous presence in the environment. They are found in soil, water, and some volatile components in the atmosphere (Olaniran and Igbinosa 2011). These compounds have been reported to be highly carcinogenic and toxic, hence, the United State Environmental Agency (USEPA) identified various chlorophenols as contaminants that are of immense concern (ATSDR 2007). The formation of these compounds occur during various processes such as the chlorination of wastewater, the use of chlorine for the bleaching of pulp during paper production, as well as the breakdown of herbicides containing phenoxy compounds (Bjerketorp et al. 2018).

Various approaches have been put forward for the decontamination of areas polluted by xenobiotics such as chlorophenols, however, most of the approaches are not widely used due to their cost of implementation and technical difficulties associated with them (Conesa et al. 2012). After the fulfillment of their role, chlorinated phenolic compounds usually accumulate in sewage, soil, and water and are resistant to microbial-based degradation. These compounds have also been known to be lethal to different groups of organisms. Numerous industries are associated with the production of chlorophenols. These include industrial activities such as drug production, wood preservation, pesticides, iron integration, and paper production. This compound has been positioned as the 166th most toxic compounds out of 1467 outlined as priority contaminants by USEPA. Guyton et al. (2016) has also documented that these compounds are teratogenic. Compounds of chlorophenols and related compounds derived from them are known to be persistent in the environment and are largely used in the production of dyes and other industrial products. Typical examples of derivatives of chlorophenols that are of serious health concern include chloromethylphenols, polychlorophenols, and chloroaminophenols amongst others. Various chemical and biological means have been utilized for the decontamination of these compounds from the environment. The use of

microorganisms such as bacteria is known as an approach that is relatively economical and environmentally treasured. Different groups of bacteria including other microorganisms such as actinomycetes have been utilized in this regard. The mechanisms for the degradation have been widely explored by different researchers, and documentation have also been made with regards to the enzymes associated with the process of degradation (Arora and Bae 2014).

Bioremediation technology involves the utilization of natural potentials of microbes in the degradation and mineralization of environmental contaminants for the derivation of nutrients and energy. It is a promising alternative in the removal of toxic substances from the environment. The use of actinomycetes for the breakdown of chlorophenols is a sustainable alternative. Some microorganisms commonly employed in the process of remediation of chlorophenols have developed the ability to utilize chlorophenols as their primary carbon and energy source. Degradation through the influence of microorganism is a vital approach for the bioremediation of pesticides and the use of actinomycytes is highly (Fuentes et al. 2010).

## 12.12 Application of Yeast for the Biodegradation of Chlorophenols

Yeast are groups of microorganisms that are rarely used in the biodegradation of CBACs or its derivatives. Recent scientific advancement and development have shown that this group of microorganisms has shown potential for the degradation of diverse chemical agents including chlorine fortified aromatic compounds as their enzymes have shown multidegradative capabilities.

Various investigators have also affirmed the relevance of various enzymes sourced from yeast been applied in the breakdown of diverse chemical compounds including CBACs or other halogenated phenols. According to Katayama-Hirayama and his colleagues, a yeast strain (*Rhodotorula glutinis*) was employed in the biodegradation of Phenol, monochlorophenol, and other CBACs. It was reported that the strain biodegraded 5 mM concentration of phenols while utilizing the substrate as its major source of carbon (Katayama-Hirayama et al. 1994). The biodegradation activity that occurs via the ortho cleavage or ring fission was observed to produce muconolactone into the liquid culture or media where the organism was grown. Other derivatives of CBACs such as 3-Chlorophenol (3-CP) and 4-chlorophenol (4-CP) were completely degraded with a stoichiometric release of chloride ions. However, it was reported that low biodegradation of some monochlorophenol members (2-chlorophenol) was observed with a simultaneous low chloride ion quantity indicating that some low molecular derivatives of CBACs are poorly biodegradaed by yeasts strains. High biodegradation was observed both in 3-CP and 4-CP with an addition of phenol to the culture media indicating enhancement. Other intermediate products such as 4-chlorocatechol and maleylacetic acid were also reported as intermediate metabolites of 3-CP and 4-CP biodegradation using GC/MS analysis. The biodegradation of phenol, monochlorophenols, or CBACs derivatives by yeast strain (*Rhodotorula glutinis*) would release some

chloride ion (dechlorination) at the step between 4-chlorocatechol and maleylacetic acid formation (Katayama-Hirayama et al. 1994).

In another related study, another yeast strain was used by Jiang and his group to biodegrade phenol and 4-chlorophenol (4-CP). Following their study a CTM 2 mutant strain of *Candida tropicalis* was obtained by He–Ne laser irradiation from a wild-type *Candida tropicalis* strain. Their reports revealed that there is high degradability potential among the CTM 2 mutant strain *Candida tropicalis* in biodegrading 4-CP as up to 400 mg/L amount of substrate were degraded within 59.5 h. They further embarked on a dual-substrate biodegradation determination accessing both speed or velocity and capacity to degrade in the presence of phenol using the wide-type and the CTM 2 mutant strain. It was observed that high substrate 4-CP in a total of 4 mg/L was completely degraded within 50.5 h when 300 mg/L of phenol was added to the medium. Their laboratory also described the kinetics using a kinetic model which was proposed by their laboratory (Jiang et al. 2008). In addition to the multiple degradative potentials of yeast strains, its members have been applied in the expression of recombinant enzymes over the years, which investigators and researchers have employed for sustainable environmental protection. Apart from using the whole organism for bioremediation or biotransformation or biodegradation, the bio-products and genes from these groups of microbes are engineered into expression organisms and used for the degradation process. One notable organism that has been applied as an enzyme or gene expressing organism is the methylotrophic yeast, *Pichia pastoris*, which is classified under the family of *Saccharomycetaceae*. The organism was previously used by Philips Petroleum Company in the production of single-cell protein (SCP) from low-cost carbon sources (e.g., methanol) in animal feeds. Philips Petroleum Company and the Salk Institute of Biotechnology collaborated to prepare a heterologous gene expressing strain for industrial and academic or research purposes (Cos et al. 2006). The produced strain was then used for the expression and production of functional heterologous protein. *P. pastoris* is observed to possess several advantages as it is suitable for expressing from milligrams to grams of protein per liter of fermentation broth. Other capabilities of *P. pastoris* include noncomplexity of growth media, friendly culture conditions, ease in genetic manipulation or engineering, ease in controlling laboratory and industrial fermentation process such as aeration, pH, supplied carbon source amount and induction of protein production (Kyzeková et al. 2020).

## 12.13 Mechanism of Degradation of Chlorophenols by Actinomycetes

Several anaerobic bacteria have been reported to be associated with the breakdown of chlorophenols typical of such include pseudomonas sp. and actinomycetes. There are numerous and varying routes for the use of chlorophenolic compounds and their derivatives by various strains of bacteria including actinomycetes. Other groups involved in the process of microbial degradation include azotobacter spp. and

alcaligenes sp. The mechanism of degradation involving actinomycetes has been explored. Basically, the enzymes that are concerned with the breakdown of chlorophenols are the same as those responsible for the conversion of related chloro aromatic compounds (Solyanikova and Golovleva 2004). Meta- and ortho routes modified have been shown to be involved in the catabolic degradation route of chlorophenols derivatives. Based on the mechanism of the reaction through this pathway, the chlorophenol undergoes hydroxylation during which chlorocatechol is formed, which is then subjected to a cleavage involving intradiol before the removal of the chlorine substituent. Some researchers have also reported the breakdown of polychorophenols through the pathway of chlorohydroquinone, however, the chlorosubstiuents have not shown to be removed before the fission reaction of the ring (Bae et al. 1996). One of the most remarkable pathways in the degradation of chlorophenols are the hydroxylquinol route. This pathway has been reported in various groups of microorganisms including actinomycetes, chlorophenolicus, pentachlrophenols, and flavobacterium (Xun et al. 1992). The major enzyme that is responsible for the opening of the ring of the aromatic chlorohydroxylquinol compound in this route is the hydroxyquinol1,2-dioxygenase. During this process, the enzyme brings about the catalysis of the splitting reaction of the aromatic ring resulting in a malevlacetate formation (Li et al. 1991).

Degradation of chlorinated contaminants such as chlorophenols by actinomycetes proceeds through the removal of the chloro group, which is the first major step. However, the conditions of the process are the major determining factor affecting the reaction mechanism. If the condition of the system is aerobic in nature, the degradation process was observed to initiate through the oxygenation of chlorocatechols and then the dechlorination processed only after the breakage of the ring of the chlorocatechols. The degradation of polychlorinated phenols commenced with a hydrolytic step of parahydroxylation giving rise to the formation parahydroquinone containing chlorine substitutes. The degradation process involving anaerobic conditions proceeds through dechlorination in a reduction step, during which chlorine groups are substituted with hydrogen (Wu et al. 2004).

Different studies have attempted to explore other pathways for the degradation of chlorophenols through the action of actinomycetes. Researchers have made significant progress in the investigation of other strains of bacteria or microorganisms that are capable of degrading these compounds apart from actinomycetes. Webb et al. (2001) reported that there are two major routes in the breakdown process. They identify the breakage of the ring resulting in the removal of the chlorine from the aliphatic intermediates. In the second step for the pathway, the metabolite, as well as the initial compound undergoes the process of dechlorination prior to the cleavage of the ring. Nakagawa et al. (2006) reported the use of two possible pathways of degradation by some other microorganisms such as Mortierella sp. The first pathways involve oxidation at the ortho position, which brings about the formation of two dichloroguaiacols compounds; in the next pathway chlorine removal occurs. The degradation of the compounds through a meta cleavage route has been established while some other study using different microorganisms for the degradation of chlorobenzene has been validated (Dong et al. 2011b).

One of the most investigated strains that use pentachlorophenol is of the genus sphingomonas and mycobacterium. Two major patterns have been reported to be used by aerobic bacteria in the biodegradation process of chlorophenols. Monooxygenases, an associated enzyme first attacks the lesser chlorinated phenols giving rise to the formation of chrolocatechols as the first intermediates (Field and Sierra-Alvarez 2008).

Various intermediates are known to be associated with the route of degradation of chlorophenols. The first intermediate in the actinomycetes degradation of is 2,4-DCP. There are different groups of bacteria that make use of 2,4-DCP as their major source of energy and carbon including *Pseudomonas* spp., *Rhodococucus* spp. among others. In a related study, Goswami et al. (2002) showed the possible removal of dichlorophenols using bacillus spp. cells that have been immobilized. The findings from their work showed that lower concentrations of the 2,4-DCP degraded faster, while the suspended and immobilized cells removed the higher contents of the 2,4-DCP at the same rate. The degradation of 2,4 dichlorophenols proceeds via the formation of the intermediate by the enzyme, 2,4-DCP acetate ketoglutarate. The mechanisms for the microbial degradation chlorophenols and their derivative compounds have been reported by various researchers. Some of the microorganism that has been reported includes *Candida* spp., Actinomycetes among others.

Investigated the degradation processes of bromophenol alongside chlorophenols in a batch process using actinomycetes strains. A mixed substrate system was employed in performing testing the process of biodegradation. The findings from the study showed that inhibition of the biodegradation process was not caused by the presence of chlorophenols in low contents. The degradation of bromophenol by actinomycetes was faster than that of chlorophenols. The kinetics studies reveal a strong negative association of chlorophenol biodegradation with that of bromophenol by the organism.

In a study, Bjerketorp et al. (2018) worked on the remediation of soil samples contaminated with dichlorophenols to remove this contaminant through the use of chlorophenolius. They target at formulating a product based on this investigation which could be produced subsequently on a larger scale. They investigated the microbial degrading capacity in both controlled and open environment.

Nowak and Mrozik (2018) carried out a study aimed at the microbial degradation of chlorophenols. They assessed the effect of soil bioaugumentation with *Pseudomonas* spp. and *Stenotrophomonas* spp. with the addition of other sources of carbons such as sodium benzoate. Determination of functional and morphological diversity of various communities of the soil was carried out. It was observed that inoculation with single *Pseudomonas* spp. was most efficient in aiding the biodegradation process. Their results also showed that soil augmentation also enhanced the functionality and diversity of the soil microbial community.

Fuentes et al. (2010) in their study carried out an examination of chlorophenol and organochlorine pesticides in contaminated soil. They did isolation of actinomycetes that were indigenous to the affected soil and carried out an evaluation of the growth of bacteria and the removal of the pesticide. The findings showed that most of the strains of microorganisms were of the group of *Streptomyces* spp. while

one of them was of the group known as micromonospora. The growth of the bacteria was observed to be dependent on the different microorganisms present as well as the nature of the various pesticides (such as lindane and methoxychlor) that were also present. From the microbial remediation of the pesticide using the bacteria, it was observed that the uppermost development and removal efficiency was detected with chlordane. Out of the various strains assessed, a majority (12 from a total of 18) produced chloride, which was released into the supernatants of the culture. These percentages were higher with chlordane than methoxychlor as a source of carbon.

## 12.14 Health Implications of Chlorophenols

Various studies have highlighted the negative health impact of chlorophenols and their by-products such as dioxins in the environment. Generally, there are many industrial applications of chlorophenols, particularly in the manufacturing industries. Igbinosa et al. (2013) revealed that chlorophenols are utilized by many agro, pharmaceutical industries for the manufacturing of biocides, dyes, and vanishes, and found ways into the environment causing harmful effects, thus must be rapidly eliminated from the environments. Many of the chemical and thermal methods utilized are not so effective in the biodegradation of these toxicants resulting in the formation of toxic compounds. The authors demonstrated that these compounds can be mutagenic, genotoxic plus carcinogenic. Adeola (2018) revealed that chlorophenols are ubiquitous toxic compounds released into the environment from different industrial systems. In humans, it is known to cause detrimental effects through its secondary metabolites such as histopathological changes, mutagenicity, genotoxicity, plus carcinogenicity.

Wang et al. (2000a, b) revealed that chlorophenols can generate dilibeterous effects through the production of electrophilic secondary metabolites with pathogenic effects on the genes or DNA resulting in DNA base oxidation or single/double-strand breaks, reduction in antioxidant defense system with increased generation of reactive oxygen species through Fenton reaction. The neurotoxic, teratogenic, immunosuppressive, hepatotoxic plus cytotoxic effects have also been reported. Ge et al. (2017) briefly commented on the role of chlorophenols from various industrial and agro industries on aquatic organisms. It was reported that these compounds are difficult to degrade thus could accumulate in the system causing chronic or acute toxicity in any individual that consumes such aquatic organisms. The authors demonstrated the effects on fish and the potential toxic pathways through reactive oxygen species and DNA damage via lipid peroxidation and suppression of the host antioxidant defense system. Physiologically, it is revealed that the immune system is altered through a couple of macrophages and B cell suppression and downregulating the phagocytotic functions. Other physiological mechanisms involve hormone disruption, alteration in gene expression, downregulation of receptor/hormone system, cell proliferation, mutations, apoptosis induction, epigenetic DNA methylation, and genotoxicity.

## 12.15 Conclusion and Future Recommendations

From the foregoing, microbial degradation of CBACs and its derivatives has shown a particular template of action with little deviation associated with the attached groups (alkyl, amino, nitro, etc.). The specie specific or target specific usage of the bio-products in the form of immobilized enzymes or immobilized biocatalyst has shown few reports probably due to the poor or inappropriate understanding of their mechanism of action and poor applicability. This may be attributed to the activity of some of the enzymes, which are active at either aerobic condition or anaerobic condition. The need for further studies as regards the anaerobic application of the microorganisms and their enzymes in biodegradation is apt. In addition, the application of nanotechnology with whole microbial cells and nano-enzymology remains an under-explored area in global research.

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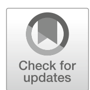

# Microbial Degradation of Proteins

# 13

Moises Bustamante-Torres, David Romero-Fierro, Jocelyne Estrella-Nuñez, and Emilio Bucio

**Abstract**

Proteins are biomolecules fundamental for the life. The 20 amino acid essentials build the essential proteins that encode the genes from the DNA. The peptide bond is formed between the carboxylic acid and amine groups. In contrast, the process by which the proteins are degraded is known as proteolysis by enzymes called peptidases, ubiquitin, and lysosomes. Proteolysis is an irreversible change that affects the functionality of the protein. Specifically, bacteria and fungi have reported a huge source of proteolytic enzymes. Likewise, external factors as pH, temperature, carbon and nitrogen sources and metal ions play important roles during proteolysis. These factors could either enhance or reduce the enzymatic rate (kinetics) and their enzyme yield. This chapter describes the degradation of proteins related to several species of microorganisms as a source of proteolytic enzymes as well as a wide range of applications approached into industrial and biotechnological like pharmaceuticals and bioremediation.

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**Keywords**

Proteins · Biomolecules · Amino acid · Degradation · Proteolysis

## 13.1 Introduction

### 13.1.1 Overview of Proteins

Proteins are made up of a very long chain of amino acids. Proteins play a fundamental function for life in the whole nature. They are the most abundant organic compounds in vertebrates, accounting for approximately 50% of their tissue dry weight (Blanco and Blanco 2017). Proteins are the ultimate players in the processes that allow an organism to function and reproduce (Stephenson 2016). The latest advances have helped to understand the function of several parts of living beings and the process of evolution. Proteins are synthesized according to the information present in the DNA fragment (gene) but for the synthesis of DNA (replication), proteins (DNA polymerase) are required (Saraswathy and Ramalingam 2011).

In 1838, Gerardus Mulder, a Dutch chemist, introduced the term "protein" to scientific literature in his publication "On the composition of some animal substances" where he described the presence of a common complex substance in blood fibrin, serum, egg albumin, gelatin, and the gluten of wheat (Hartley 1951; Vickery 1950; Murray et al. 2017).

The total number of proteins expressed in a cell at a particular time is known as a proteome (Saraswathy and Ramalingam 2011). Proteomics encompasses the study of the proteins expressed in any cell. The human body expresses 25,000–30,000 protein-coding genes approximately. Proteins perform a variety of functions, including enzymatic catalysis, transporting ions and molecules from one organ to another, nutrients, contractile system of muscles, tendons, cartilage, antibodies, and regulating cellular and physiological activities (Gromiha 2009). Besides, proteins can interact with many types of molecules, including with other proteins, with lipids, with carbohydrates, and with DNA (Ardejani et al. 2017; Branden and Tooze 1999; Murray et al. 2006; Mathews and Van Holde 1996).

The proteolytic activity of microbial enzymes has been studied a long time ago. There are a wide variety of species as bacteria and fungi producing proteolytic activity. However, different external factors such as pH, temperatures, substrate, and metallic ions play an important role during the isolation of these enzymes as well as the sources. The long chain of amino acids is released from the proteins as they break down. Free amino acids can be used to form new proteins or to generate energy. Each amino acid is broken down in turn, through a series of chain reactions forming metabolic pathways, so that each amino acid has its own way to form and break down into energy (Goldberg 2013).

### 13.1.2 Amino Acids

There are 20 essential amino acids known. The essential amino acids are as follows: valine, leucine, isoleucine, phenylalanine, tryptophan, threonine, methionine, lysine, arginine, and histidine. Arginine and histidine are essential in certain cases (Litwack 2018). The rest are nonessential because they can be synthesized by the body. Essential amino acids, which generally have a longer half-life than the nonessential ones, are those that are required in the diet since the body cannot synthesize them in adequate amounts to maintain protein biosynthesis (Engelking 2015).

Amino acids are compounds that have an acid or carboxyl (–COOH), and a basic or amine (–$NH_2$) groups (Blanco and Blanco 2017). Amino acids can form covalent bonds between the carboxyl and amine groups, which is known as peptide bond (accompanied by the loss of water) as Fig. 13.1 shows. The R groups of amino acids determine their unique biochemical functions. Of the biochemical reactions of amino acids, the most important is the formation of peptide bonds (Murray et al. 2006).

## 13.2 The Process of Protein Degradation

The process by which amino acids occur from the proteins' breakdown is called proteolysis. The molecules responsible for developing proteolysis are proteolytic enzymes or peptidases, which are present in both bacteria and plants, but mostly in animals (Ehrmann and Clausen 2004).

### 13.2.1 Proteases

Proteases, also known as peptidases, are enzymes that break the peptide bonds of proteins and for this purpose use a molecule of water. Peptidases are found naturally in living organisms, where they are used for molecular digestion and the reduction of

**Fig. 13.1** Peptide bonds formed from carboxylic acids and amine groups

unwanted proteins. Peptidases can break both specific peptide bonds, depending on the amino acid sequence of the protein, and reduce a complete peptide to amino acids (López-Otín and Bond 2008).

Peptidases are present in all organisms and constitute 1–5% of the genome content. These enzymes are involved in a multitude of physiological reactions from the simple digestion of food proteins to highly regulated cascades (López-Otín and Bond 2008).

### 13.2.2 Proteolysis

Proteolysis is a key process in the control of intracellular and extracellular signaling. This control is produced through irreversible changes in the protein structure, which will affect its functionality (Kaiser et al. 2013). Protein levels within cells are determined by the synthesis rate and also by its degradation rate. The average half-life of proteins within cells is in the range of minutes to several days, with degradation rates being an important factor for cell regulation. Degraded proteins can function as regulatory molecules for cellular processes (Pham et al. 2014). In the same sense, the renewal of these proteins is a fundamental aspect for their levels to change in response to external stimuli. On the other hand, there are proteins that degrade in response to specific signals, defining a new mechanism for the regulation of intracellular enzyme activity (Kaiser et al. 2013). Lastly, proteins that show any abnormality or defect are recognized and rapidly degraded within cells. Proteolysis has several functions for the cell, among which are:

- Elimination of methionine at the N-terminus after translation.
- Elimination of signal sequences of peptides after their transport through the membrane.
- Separation of viral proteins that are translated from a monocistronic messenger RNA (mRNA).
- Food protein digestion as a source of amino acids.
- Conversion of inactive proteins into their final functional forms.
- Degradation of cyclins and other proteins required for progression in the cell cycle (Goldberg 2013).

In eukaryotic cells, two main catabolic processes that regulate protein degradation can be distinguished: ubiquitin-proteasome pathway and lysosomal proteolysis (Pham et al. 2014).

#### 13.2.2.1 Ubiquitin-Proteasome Pathway

The ubiquitin-proteasome pathway is involved in the intracellular turnover of proteins and plays an important role in the degradation of short-lived regulatory proteins, involved in a wide range of cellular processes such as regulation of the cell cycle, modulation of surface receptors and ion channels, antigen processing and presentation, and activation of transcription factors (Lecker et al. 2006).

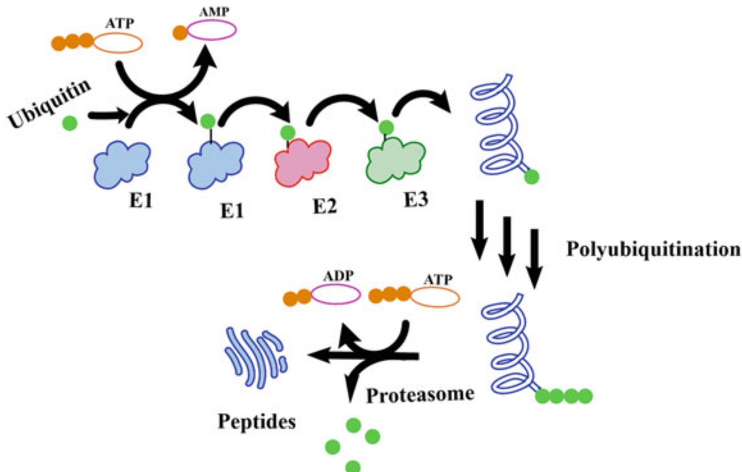


**Fig. 13.2** The ubiquitin–proteasome pathway for degradation of proteins into peptides

Ubiquitin is a small protein found in all organisms and is made up of 76 amino acids. When several ubiquitin molecules in a given conformation bind to the protein that has to be removed, the proteasome (large multiprotein complex responsible for degradation) identifies it as "disposable" and initiates a chain of reactions that end in the destruction of the same (Myung et al. 2001). Ubiquitin is discarded in the process so that it can be used in another. In this sense, it is important to note that both for the binding of ubiquitin and the cleavage of labeled proteins, it requires energy in the form of ATP (Lecker et al. 2006). Labeling of proteins with ubiquitin is, therefore, one of the mechanisms that the cell uses to eliminate molecules (Klein et al. 2018).

As shown in Fig. 13.2, the process begins with the activation of Ubiquitin by the E1 enzyme, a step that requires energy to be carried out. The E1 enzyme activates the carboxyl group of the C-terminus residue (Gly 76), forming a thioester bond between Gly 76 and a Cys residue of the E1 enzyme. Activated ubiquitin is transferred to one of several ubiquitin-conjugating enzymes (E2). E2 accepts ubiquitin bound to the E1 enzyme, forming a thioester bond between Gly 76 and a Cys residue of the enzyme. In most cases, ubiquitin is transferred to a ubiquitin ligase enzyme (E3). This enzyme is capable of recognizing the substrate protein and transfers ubiquitin from the E2 enzyme to the $NH_3$ (amino epsilon) group of the protein to be degraded. In this way, ubiquitinated proteins are formed. The binding of a single ubiquitin to the target protein is not sufficient for the cell to identify it as a protein that needs to degrade. Normally, the binding of 3 or 4 ubiquitins is necessary for the degradation process to take place. This is the reason why the above process is repeated several times, creating a chain of ubiquitins in the target protein. This also prevents the breakdown of some ubiquitin from preventing the labeling of the target protein. Enzyme E3 then releases the already labeled protein (Goldberg 2013; Lecker et al. 2006; Myung et al. 2001).

Once the ubiquitin chain is formed, it supplies a signal to the cell for the removal of the protein, which is handled by the proteasome. It will only recognize proteins that need to be broken down if they are attached to the ubiquitin chain. Protein degradation is an irreversible process. The proteasome will break down proteins to the level of small peptides of 7–9 amino acids and these will be hydrolyzed to amino acids by nonspecific cytosolic proteases (Kaiser et al. 2013).

#### 13.2.2.2 Lysosomal Proteolysis

Lysosomes are membrane-bound cellular organelles that contain digestive enzymes. Lysosomes are involved in various cellular processes. They are responsible for collecting cellular waste remains (Knop et al. 1993). Lysosomal degradation or proteolysis serves to remove intracellular organelles, proteins, or materials to the outside by endocytosis, the peptide fragments that arise from protein maturation of the secretory pathway, and apoptosis (Aits and Jaattela 2013).

Among the many proteins that can be degraded in lysosomes are cell membrane proteins and extracellular proteins. These proteins, once internalized by endosomes, undergo complete degradation after the fusion of these organelles with lysosomes in a process of heterophagy (Zhang et al. 2010). A similar fusion occurs between the lysosomes and the secretory vesicles, which contain proteins synthesized inside the cell and whose ultimate destination resides in the extracellular medium. This degrading process of crinophagy is responsible for regulating the levels of cellular secretion of proteins. The other broad group of lysosomal substrates is cytosolic proteins (Ciechanover 2005). They use various transport mechanisms to reach the interior of the lysosomes. For example, lysosomes encompass portions of cytosol, sometimes whole organs, in a process of macroautophagy or microautophagy, depending on the size of the cytosolic portion encompassed (Ciechanover 2005).

Lysosomal autophagy is characterized mainly by its lack of selectivity. Once inside the lysosome, the embedded proteins are torn together. It is the responsibility of the microautography to maintain the basal levels of intracellular proteolysis (Boya and Kroemer 2008). In addition to these two nonselective processes of degradation of cytosolic proteins in lysosomes, the existence of a direct transport of certain cytosolic proteins through the lysosomal membrane has been confirmed. This process is very similar to the transport of proteins to other cellular organelles (mitochondria, endoplasmic reticles, vacuoles, etc.), cytosolic factors, membrane components, and intralisosomal proteins (Knop et al. 1993).

Although the stimulus that activates this direct degradative pathway has not yet been identified, the existence of a marker signal in the amino acid sequence of the substrate protein (the pentapeptide Lys–Phe–Glu–Arg–Gln or KFERQ) is known, responsible for the interaction with the cytosolic factor involved in this pathway, the 73 kDa heat shock protein (Hsc73) (Agarraberes et al. 1997). This shock protein maintains the substrate protein in a suitable conformation to favor its binding with the lysosomal membrane and its subsequent transport through it.

The first component of this transport system has been identified, a 96 kDa integral lysosomal membrane glycoprotein (lgp96), whose C-terminus region appears exposed on the surface of the lysosome and serves as an anchor point for the

substrate proteins. After binding to the lysosome membrane, the proteins travel to the interior in a transport process that requires the presence in the lysosomal matrix of a 73 kDa heat shock protein, a variant of the cytosolic form (Knop et al. 1993). By comparison with other cellular transporters, lysosomal Hsc73 could act as a driving force, which after anchoring itself to the portion of a protein, that initially reaches the lysosomal matrix, would push the rest of the protein inwards. Once in the matrix and after dissociating from the lysosomal Hsc73, the protein would undergo its complete degradation (Lee et al. 2010).

The description of these selective lysosomal degradative pathways has made it possible to attribute regulatory functions in various physiological processes to lysosomes. On the other hand, it is revealed the existence of a dynamic equilibrium between the different intracellular degradative systems, regulated by their multiple interactions, thus ensuring strict control of this fundamental process, which is protein degradation.

#### 13.2.2.3 Signs for Proteolysis

On all occasions, cells must recognize those proteins that for one reason or another must be degraded. Different proteolytic signals have been found:

- *The amino N-end rule*: Eukaryotic proteins when synthesized always have a methionine residue at their amino end. Later this initial methionine can be removed leaving another amino acid as the head of the amino end. In yeasts, it has been verified that there are a series of destabilizing amino acids (mainly the basic, acidic, and hydrophobic ones) and other stabilizing amino acids. Furthermore, it appears that most mature cytosolic proteins carry the amino terminus blocked by acetylation (Dougan et al. 2012).
- *PEST sequences*: Proteins with a half-life of less than 2 h are rich in regions containing the amino acids proline, glutamate, serine, and threonine (P, E, S, and T, respectively). These regions, between 12 and 60 residues in length, are known as PEST sequences. Very few long-lived proteins are contained in these regions. It seems likely that PEST regions are part of a recognition scheme for enzyme systems that degrade short half-life proteins, possibly including the ubiquitin labeling system (Rechsteiner and Rogers 1996).
- *Cyclins and boxes of destruction*: Cyclins are proteins with control of the eukaryotic cell cycle, which have to be broken down for the cell to continue from metaphase to anaphase. It is a very controlled degradation, dependent on a previous step of labeling cyclin with ubiquitin. In almost all cyclins a signal sequence (destruction box) consisting of nine amino acids (RAALGNISN) has been located that is present between residues 13 and 66 of the protein sequence (Yamano et al. 1998).
- *Pentapeptide KFERQ*: They are peptide sequences that mark cytosolic proteins for their lysosomal proteolysis, being KFERQ one of the signals for proteins to enter lysosomes. This protein signaling is not related to ubiquitin, but there are other heat shock cognate proteins (cytosolic and intralisosomal hsc73) that recognize ubiquitin sequences (Kaiser et al. 2013).

## 13.3 Microorganisms with Proteolytic Activity

Proteolytic enzymes are commonly founded on numerous living organisms and have an important role in cell growth and differentiation (Gupta et al. 2002). Proteolytic enzymes obtained from microorganisms has been given importance by their numerous practical applications, especially in the industry. The proteolytic function is based on the hydrolysis of peptide bonds in proteins, where long chains of proteins are broken into small parts. Bacteria and fungi are been reported as good sources of diverse types of proteases such as alkaline, cysteine, aspartate, and metalloproteases (Banerjee and Ray 2017). The microbial proteases produced could be intracellular or extracellular, and these differ in their properties and applications.

### 13.3.1 Species

#### 13.3.1.1 Bacterial Species

Bacteria are known as the most dominant group of alkaline protease producers. Many studies report the *Bacillus* genera as an important protease producer, which is commonly used in the industry. The interest is based on the possibility of cultivated these enzymes under extreme temperatures and pH (Han and Damodaran 1997). Other bacteria genera with potential use are *Streptomyces* and *Pseudomonas.* Table 13.1 summarized some examples of bacterial species and their sources.

#### 13.3.1.2 Fungi Species

Fungal species have great potential by their versatility in enzyme production. Enzymes with the proteolytic activity of fungal origin have several advantages such as cost, faster production, secrete a large number of enzymes, and easy modification (De Souza et al. 2015). Moreover, they are active over a wide pH range and have a broad substrate specificity (Rao et al. 1998). Table 13.2 shows some examples of fungal species and their sources.

### 13.3.2 Sources

The most common microbial proteolytic enzymes used to come from bacterial and fungal sources. These species have been isolated from various sources like sediments (Kanekar et al. 2002; Venugopal and Saramma 2006), seawater (Joshi et al. 2008), offshore oil fields, contaminated soils (Patil and Chaudhari 2013; Shafee et al. 2005), dairy products (Caldera 2016), poultry compost (Rieger et al. 2017), manufacture of fish meat/oil (Kumaran et al. 2013), and industrial effluents (Boominadhan et al. 2009; Wu et al. 2006; Dhillon et al. 2017). The selection of microorganisms with proteolytic enzymes plays an important role in the activity yield. There is a wide area of sources with prominent applications. Oceanic waters and their derived enzymes have been studied by their novel chemical and stereochemical properties (Homaei et al. 2016; Rosso and Azam 1987). Soils and

**Table 13.1** Bacterial species with proteolytic activity

| Genera | Specie | Source |
|---|---|---|
| *Micrococcus* | *Micrococcus luteus* | Soil (Bach et al. 2002) |
| | *Micrococcus* sp. | Sediments (Kanekar et al. 2002) |
| *Streptomyces* | *Streptomyces anulatus* | Soil (Bach et al. 2002) |
| *Bacillus* | *Bacillus subtilis* | Soil (Bach et al. 2002; Das and Prasad 2010)<br>Waste and industry effluents (Boominadhan et al. 2009) |
| | *Bacillus licheniformis* | Soil (Han and Damodaran 1997; Bach et al. 2002)<br>Marine sediments (Manachini and Fortina 1998)<br>Waste and industry effluents (Boominadhan et al. 2009)<br>Saline soda lakes (Ibrahim et al. 2015) |
| | *Bacillus macerans* | Coffee fruit (Pereira Rodarte et al. 2011) |
| | *Bacillus amyloliquefaciens* | Waste and industry effluents (Boominadhan et al. 2009) |
| | *Bacillus brevis* | Soil (Han and Damodaran 1997) |
| | *Bacillus* sp. | Sediments (Kanekar et al. 2002)<br>Soil (Telagi 2012)<br>Poultry processing (Rieger et al. 2017)<br>Saline soda lakes (Ibrahim et al. 2015)<br>Sea water (Joshi et al. 2008) |
| | *Bacillus pumilus* | Soil (Han and Damodaran 1997)<br>Saline soda lakes (Ibrahim et al. 2015) |
| | *Bacillus circulans* | Contaminated soil (Patil and Chaudhari 2013) |
| | *Bacillus cereus* | Coffee fruit (Boominadhan et al. 2009)<br>Soils (Bach et al. 2002)<br>Contaminated soil of wood factory (Shafee et al. 2005) |
| | *Bacillus cluasii* | Saline soda lakes (Ibrahim et al. 2015) |
| *Staphylococcus* | *Staphylococcus* sp. | Sediments (Kanekar et al. 2002) |
| | *Staphylococcus sciuri* | Soil (Han and Damodaran 1997) |
| | *Staphylococcus intermedius* | Soil (Han and Damodaran 1997) |
| | *Staphylococcus aureus* | Skin (Williams et al. 2017) |
| *Acinetobacter* | *Acinetobacter* sp. | Coffee fruit (Boominadhan et al. 2009) |
| *Arharobacter* | *Arhrobacter* sp. | Coffee fruit (Boominadhan et al. 2009)<br>Sediments (Kanekar et al. 2002) |
| *Enterobacter* | *Enterobacter sakazakii* | Coffee fruit (Boominadhan et al. 2009) |
| | *Enterobacter cloacae* | Coffee fruit (Boominadhan et al. 2009) |
| *Proteus* | *Proteus mirabiliz* | Coffee fruit (Boominadhan et al. 2009) |
| *Pseudomonas* | *Pseudomonas* sp. | Sediments (Kanekar et al. 2002)<br>Soil (Baraniya et al. 2016)<br>Dairy products (Caldera 2016) |
| | *Pseudomonas paucimobilis* | Coffee fruit (Pereira Rodarte et al. 2011) |
| | *Pseudomonas moraviencis* | Soil (Baraniya et al. 2016) |

(continued)

**Table 13.1** (continued)

| Genera | Specie | Source |
|---|---|---|
| | *Pseudomonas aeruginosa* | Vermicompost pit soil (Zambare et al. 2010) |
| | *Pseudomonas fluorescens* | Soil (Bach et al. 2002; Baraniya et al. 2016)<br>Meat waste contaminated soil (Kalaiarasi and Sunitha 2009) |
| | *Pseudomonas putrefaciens* | Coffee fruit (Boominadhan et al. 2009) |
| | *Pseudomonas brassicacearum* | Soil (Baraniya et al. 2016) |
| *Serratia* | *Serratia plymutica* | Coffee fruit (Boominadhan et al. 2009) |
| | *Serratia rubidea* | Coffee fruit (Boominadhan et al. 2009) |
| | *Serratia marcescens* | Soil (Bach et al. 2002) |

**Table 13.2** Fungi species with proteolytic activity

| Genera | Specie | Sources |
|---|---|---|
| Aspergillus | *Aspergillus fumigatus* | Coffee fruit (Boominadhan et al. 2009) |
| | *Aspergillus* sp. | Coffee fruit (Boominadhan et al. 2009)<br>Oil seed (Rajmalwar and Dabholkar 2009) |
| | *Aspergillus parasiticus* | Sediments (Venugopal and Saramma 2006) |
| | *Aspergillus awamori* | Coffee fruit (Boominadhan et al. 2009) |
| | *Aspergillus terreus* | Palm oil mill effluent (Wu et al. 2006)<br>Soil (Sharma et al. 2017) |
| | *Aspergillus flavus* | Coffee fruit (Boominadhan et al. 2009)<br>Oil seed (Kranthi et al. 2012)<br>Soil (Sharma et al. 2017) |
| | *Aspergillus niger* | Soil (Bach et al. 2002) |
| | *Aspergillus ochraceus* | Coffee fruit (Boominadhan et al. 2009) |
| Engyodontium | *Engyodontium album* | Marine sediment (Chellappan et al. 2006) |
| Fusarium | *Fusarium illudens* | Coffee fruit (Boominadhan et al. 2009) |
| | *Fusarium lateritium* | Coffee fruit (Boominadhan et al. 2009) |
| | *Fusarium moniliforme* | Coffee fruit (Boominadhan et al. 2009) |
| Penicillium | *Penicillium* sp. | Soil (Agrawal et al. 2004) |
| | *Peniciliium aurantiogriseum* | Coffee fruit (Boominadhan et al. 2009) |
| | *Penicilium brevicopactum* | Coffee fruit (Boominadhan et al. 2009) |
| | *Penicililum citrinum* | Coffee fruit (Boominadhan et al. 2009) |
| | *Penicililium expansum* | Coffee fruit (Boominadhan et al. 2009) |

sediments are also studied by their diversity of proteolytic genes (Han and Damodaran 1997; Bach et al. 2002; Kanekar et al. 2002; Das and Prasad 2010; Vishwanatha et al. 2010).

## 13.4 Factors Influencing Proteolytic Degradation

### 13.4.1 pH

The proteolytic activity of enzymes is dependent on the pH of the medium used, which is specific to each isolate (Pereira Rodarte et al. 2011). According to pH, the proteolytic enzymes could be classified as neutral, acidic, or alkaline. It was reported that there is a better protease production in the alkaline range as is reported for the species *Bacillus licheniformis* (Boominadhan et al. 2009; Patil and Chaudhari 2013). The change of pH affects directly the reaction rate through changes in the ionic form of the active sites of the enzymes (Bhunia et al. 2013). The pH influences directly metabolic reactions, regulatory mechanisms, and products, which are related to protease production (Patil and Chaudhari 2013). The main proteases used in the industry are neutral and alkaline. The activity of each one depends on an optimum pH which is between 5 and 8 for neutral and around 10 for alkaline (Kuddus and Ramteke 2012).

### 13.4.2 Temperature

Temperature affects directly the reaction kinetics, specifically the hydrolysis rate. By monitoring proteolytic activity based on temperature change, thermal stability at an optimal temperature has been sought (Bhunia et al. 2013). A critical parameter is related to the incubation temperature, which is a determinant of the synthesis and secretion of proteases by microbial species (Patil and Chaudhari 2013).

### 13.4.3 Carbon and Nitrogen Sources

To improve microbial growth, different sources of carbon and nitrogen have been studied. Glucose, galactose, fructose, maltose, lactose, sucrose, xylose, starch, glycogen, mannose, and citric acid are common sources of carbon, which are added to the media (Ibrahim et al. 2015; Joshi et al. 2008). On the other hand, nitrogen sources could be organic or inorganic. The sources reported include peptone, yeast extract, tryptone, casein, insoluble casein, skim milk, gelatin, ammonium nitrate, ammonium sulfate, sodium nitrate, traders protein, and urea (Boominadhan et al. 2009; Ibrahim et al. 2015). Based on previous studies, the results indicate that different carbon sources have different impacts on protease production taken into account factors as the concentration and the organism of origin.

### 13.4.4 Metals Ions

Previous investigations have studied the effect of the addition of mono and divalent metal cations on the proteolytic activity of enzymes obtained from various microorganisms. Among the metal ions studied are $Ca^{2+}$, $Mg^{2+}$, $Mn^{2+}$, $Zn^{2+}$, and $Cu^{2+}$ (Rieger et al. 2017; Bhunia et al. 2013), which are directly related to their concentration (Ibrahim et al. 2015). Depending on the test could be some ions capable of enhancing protease production while other ions inhibit its growth and reduce the enzyme yield. Metal ions have another important role, which is related to the protection of the enzyme against thermal denaturation and keeping its active conformation (Joshi et al. 2008; Mienda et al. 2014). Through a study focused on the proteolytic activity of the *Bacillus mojavensis*, it was evidenced how the addition of divalent cation of $Ca^{2+}$ improves in 56.62% of the protease activity (Hammami et al. 2018). While in other reports, the addition of $Ca^{2+}$ and $Mg^{2+}$ for *Actinomadura keratinilytica* improves the enzyme activity by 120% and 111%, respectively (Habbeche et al. 2014). The same ions were tested at temperatures higher than 50 °C for the species *Bacillus pumilus*, which was obtained at an activity improvement by up to 156% and their thermostability was enhanced (Jaouadi et al. 2008).

## 13.5 Applications

### 13.5.1 Beneficial Properties

In nature, there is a diversity of enzymes with potential proteolytic activity. The production of proteases with bioactive and functional characteristics has had a boom in research, especially for its potential industrial use. The interest in its use has been based on its rapid growth and easy genetic manipulation (Telagi 2012). Within biotechnological processes, the use of enzymes has had a relevant role in the synthesis of products, has several advantages such as higher purity, lower energy consumption, lower production of secondary waste, and high selectivity (Bhunia et al. 2013; Rozzell 1999).

### 13.5.2 Industrial Applications

The use of proteases constitutes two-thirds of the total enzymes in industry and the increasing industrial use has led to obtaining proteolytic enzymes with higher specificity (Pereira Rodarte et al. 2011). Proteases are considered environment-friendly and with a great potential to replace or reduce hazardous chemicals used in industrial processes (Singh and Kumar Bajaj 2017). The use of industrial applications is also influenced by parameters like temperature, incubation period, pH, salt concentration, and high enzymatic production (Bizuye et al. 2014).

The main application of enzymes is concentrated on the detergent industry with approximately 30% of the total world enzyme production (Ibrahim et al. 2015).

Alkaline proteolytic enzymes are particularly used in detergent production with high stability at extreme conditions of temperature and pH. One of the main objectives has been to develop new formulations with energy considerations that are environmentally friendly (Manachini and Fortina 1998). In previous studies, the use of crude enzymes has exhibited high stability and efficiency in removing different strains (Hammami et al. 2018). The enzymes used in detergents are mainly serine proteases which come from the *Bacillus* strain (Mienda et al. 2014). Newer enzymes have to keep the activity and stability under washing conditions and be compatible with detergent ingredients as surfactants and oxidizing agents (Venugopal and Saramma 2006).

In the food industry, microbial proteases are usually exploited during preparation, processing, storage, and consumption. Through previous researchers, it has been sought to enhance functional properties like viscosity, solubility, dispersibility, emulsifying, among others (Benítez et al. 2008). A prominent genus is a *Bacillus,* which has been studied by its high yield to obtain active peptides and process different foods. A significant property is related to the coagulation of proteins by proteases, which is commonly used in dairy products like cheese (Alagarsamy et al. 2006). *Pseudomonas* and *Bacillus* species have been reported by their relation as debittering and ripening agents (Rao et al. 1998), which is even enhanced in combination with lipases (Koka and Weimer 2000; Oscan and Kurdal 2012). Proteases play a relevant role in fermented foods. The application of proteases was observed in cured meat and sausages, having an effect of an increase in nitrogen fraction (Contesini et al. 2017). Another interesting use is related to cold-active microbial proteases, which have high specificity, the low enzyme needed, less undesirable reactions, and easy to inactivate (Kuddus and Ramteke 2012). Microbial proteases, especially alkaline are used for their high nutritional value (Najafi et al. 2005). The hydrolysis of proteins is taken by a nitrogen source, which is used in nutritional supplements. This property is associated with the hydrolysis of polypeptides into smaller peptides and amino acids, which are easily absorbed by the cell (Rao et al. 1998).

There is a wide application in the textile industry, mainly in production, finishing, and washing methods. It has been used to remove sericin from silk, modify the surface of wool and silk, or obtain finishes (Najafi et al. 2005). Pseudomona aeruginosa has been studied for its ability to remove proteins and fabric stains, and having great potential without combining them in detergents (Najafi et al. 2005). Another field of application is associated with the modification of fiber in finishing treatment (Riessen and Antranikian 2001).

### 13.5.3 Biotechnological Application

There is a great potential in the use of microorganisms in the area of biotechnology due to their applicability at a large scale, ease of handling, short time of cultivation, and microbial diversity for enzyme production (Da Silva 2017).

#### 13.5.3.1 Pharmaceuticals

In medicine, proteases are involved in different aspects of physiological processes (Fig. 13.3), and have been related to the production of therapeutic agents against deadly diseases (Rawlings 2004).

Microbial enzymes are used in medicine to treatments against diseases such as thrombosis or cancer, and also are used as digestive aids (Ward et al. 2009). The great diversity and high specificity have been characteristic of microbial proteases, which is why they are commonly used for therapeutic or diagnostic purposes (Singh et al. 2016). Enzymes produced by *Serratia* species are the most effective protease used for anti-inflammatory, anti-endemic, and analgesic purposes (Bhagat et al. 2013; Tiwari 2017). The species *Baciulls subtilis* was reported as effective agents against blood clotting and lipids associated with cardiovascular diseases (Hsia et al. 2009). *Aspergillus oryzae* and *Aspergillus melleus* were reported as an alternative to anti-inflammatory or immune support and as dietary supplements for cardiovascular diseases (Chanalia et al. 2011). *Bacillus pumilus* was investigated for its anti-inflammatory potential, which probes that it can inhibit heat and hypotonicity-induced hemolysis (Sangeetha and Arulpandi 2019).

Different proteolytic species have been used in the cosmetology industry. Streptomyces and Bacillus were studied for their effect on keratin degradation, which could be applied in healthcare by its capacity on epithelial regeneration of skin (De Souza et al. 2015; Chao et al. 2017). This advantage is related to the capacity of proteases to hydrolyze peptide bonds of keratin, collagen, and elastin of the skin (De Souza et al. 2015).

#### 13.5.3.2 Bioremediation

The use of proteolytic enzymes in bioremediation has been an emerging technology, which has an economic and environmental impact, being able to degrade different pollutants, organic and inorganic contaminants. One of the main fields of enzymatic application is related to waste generated by poultry processing industries. The generation of tons of feathers constitutes a considerable source of protein for food additives. *Bacillus subtilis* has been studied as a keratinolytic protease producer, which is used to degrade waste keratinous material (Gupta et al. 2002; Dalev 1994). In another study, the keratinases were used as a potential source for biofuel or fertilizer production (Verma et al. 2017). *Bacillus subtilis* has also been studied in combination with *Bacillus amiloliquefaciens* and *Streptomyces* sp., where the proteolytic degrading capacity is improved (Jacobson et al. 1985). The applicability of *Subtilis* has also been demonstrated in processes that require long incubation periods (Dedavid e Silva et al. 2014). Other functional enzymes are the cold-adapted protease enzymes, which have been studied for wastewater treatment and bioremediation in cold environments contaminated with proteins (Kuddus and Ramteke

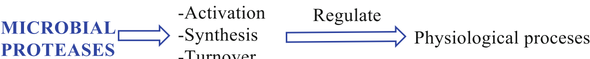


**Fig. 13.3** Microbial proteases and their physiological processes related

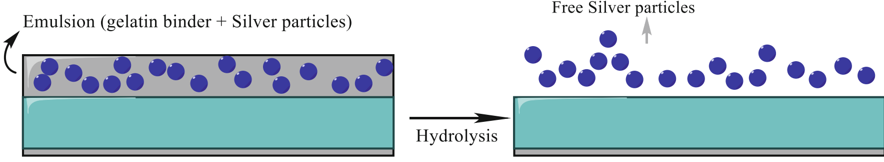


**Fig. 13.4** Hydrolysis for the release of silver from X-ray films

2012). They have been found to have high catalytic efficiency and specificity at low temperatures (Margesin and Schinner 1999). Silver recovery processes usually imply chemical procedures, which are not environmentally friendly. Based on that precedent, the use of proteases plays a degradant role mainly for the silver recovery from X-ray or photographic films. This recovery treatment is based on an enzymatic method (Nakiboglu et al. 2001), which is shown in Fig. 13.4.

It has been reported the high yield of proteases from species as *Bacillus* sp. Obtained from the soil and fish waste (Kumaran et al. 2013; Masui et al. 1999), *Conidioboulus coronatus* (Laxman et al. 2010), *Bacillus sphaericus* from alkaline soils in the Himalaya (Singh et al. 1999), *Bacillus subtilis* from food industry waste (Vanitha et al. 2014), and *Bacillus firmus* from samples from industrial soils (Rao and Narasu 2007).

## 13.6 Conclusions

In summary, proteins are fundamental biomolecules. These biomolecules are undoubtedly a vast interest of biologists to understand life. Protein formation has reach based on the join of amino acids through carboxyl and amine groups, forming the peptide bond.

According to what has been reviewed in the literature, protein breakdown occurs by proteolysis, which uses a proteolytic enzyme known as peptidases. These enzymes are involved in multiple physiological reactions. Also, lysosomes are organelles that contain digestive enzymes and expulse material to the exterior through endocytosis. These proteolytic enzymes are commonly found in different living organisms.

The proteolytic activity can be found on microorganisms like bacteria (alkaline protease producers), and fungi. Besides, external factors play an important role, enhancing or reducing the activity yield during proteolytic degradation like pH, temperature, carbon and nitrogen sources, and metal ions. Moreover, these enzymes have shown important applications in the industry and biotechnological fields. The main application field of these enzymes is an approach to the detergent industry because of the stability in extreme conditions, as well as the fight against different microorganism strains. The bioremediation field has reached news studies because proteolytic enzymes are based on the economic and environmental impact against organic and inorganic pollutants. Likewise, peptidases have been studied in some diseases, showing great diversity and specificity. The understanding of the enzymes,

will determine many functions of the health and the environment. The applications could be almost endless due to the great number of microorganisms that have not been found yet.

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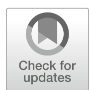


# Microbial Degradation of Microplastics 14

Agnieszka Dąbrowska

**Abstract**

The increasing plastics production and consumption, mainly in the form of disposable products, consists one of the major environmental threats transforming the Blue Planet into the Plastic One. The synthetic materials introduced to the biosphere ends in the global ocean system and gradually degrade. Their constantly growing surface, called Plastisphere, is already considered one of the most important ecological niches. The biofilm on the polymers substrate is investigated. Various bacteria, but also insects and fungus, can decompose those resistant and durable materials. This chapter describes briefly the marine microplastics phenomena, their role and fate in the environment, uniqueness of the Plastisphere and presents the selected recent research on microbial degradation of polymers. Finally, future perspectives and directions are comprehensively outlined.

**Keywords**

Marine microplastic · Microplastics · Nanoplastic · Plastisphere · Biofilm · Biodegradation of polymers

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## 14.1 Introduction to Microplastics

Although the issue of microplastics in the environment, especially marine microplastic, is nowadays one of the most popular topics addressed by specialists, NGOs, media, and citizen scientists, it is worth defining it. Plastic production increased dramatically from 0.5 million tonnes per year in 1960 up to almost 300 in 2013 (Avio et al. 2017). By term microplastic, one intends all fragments of synthetic materials $<5$ mm. Even if the upper limit seems arbitrary and somehow artificial, the size is crucial due to the increasing relative surface, in particular surface to volume ratio, during the fragmentation of material and its numerous consequences. One can underline the area, increasing in time, available for a biofilm.

Although the role of polymer debris as a new ecological niche is still not fully understood and currently investigated, the impact on biota does not present any doubts. Microplastics affect all of the trophic chains and bioaccumulate at higher levels of a pyramid. Although nobody has calculated yet the number of microplastics in the oceans, its mass is estimated to be between 90–150 million tonnes with approximately ~5 trillion pieces floating. However, the sustainability threshold is still unknown and the cascade of effects changing significantly the ecosystem's equilibrium can be triggered easily and develop fast. This risk is often neglected or underrated, one should bear in mind.

Regarding the various and complex impacts of microplastics on the environment, this type of pollution should be included within the list of planetary boundaries (Villarrubia-Gómez et al. 2018). It meets the three requirements being poorly reversible, visible on the global scale and potentially having a disruptive effect on the natural processes. Moreover, the situation changes dynamically as no item stays stable once introduced to the environment. Bing ubiquitous influences all parts of a planet, even pristine and remote regions or the Marine Protected Areas (Barnes et al. 2018). Plastics are already a common part of landscapes, near the urbanized area and densely populated agglomerations, as well as in remote reserves (Fig. 14.1).

Debris can be transferred by wind, water, human activities, fragmented, weathered, float, sink, etc. In consequence, the microplastics localization, physical and chemical properties (roughness, composition, shape, size, etc.), behavior, and changes in time. Finally, this relatively new component of the biosphere is not invisible to the smallest inhabitants of our planet. Bacteria, viruses, fungi, and insects, as well as the phito- and zooplankton in the oceans evolve more rapidly in comparison to the vertebrates and improve their ability to utilize synthetic materials. Among diverse primary and secondary sources of microplastics, one can point out the worn and abandoned fishing nets (the so-called ghost nets) that are densely colonized by different groups of animals and plastered by organic matter (Fig. 14.2).

Within the following paragraphs, that sort of interaction will be described, mainly regarding the various microbial activities, from degradation to the complex Plastisphere system.

**Fig. 14.1** The synthetic materials discarded on a beach: (a) in Gdynia (Poland) and (b) Mykines (Faroe Islands)

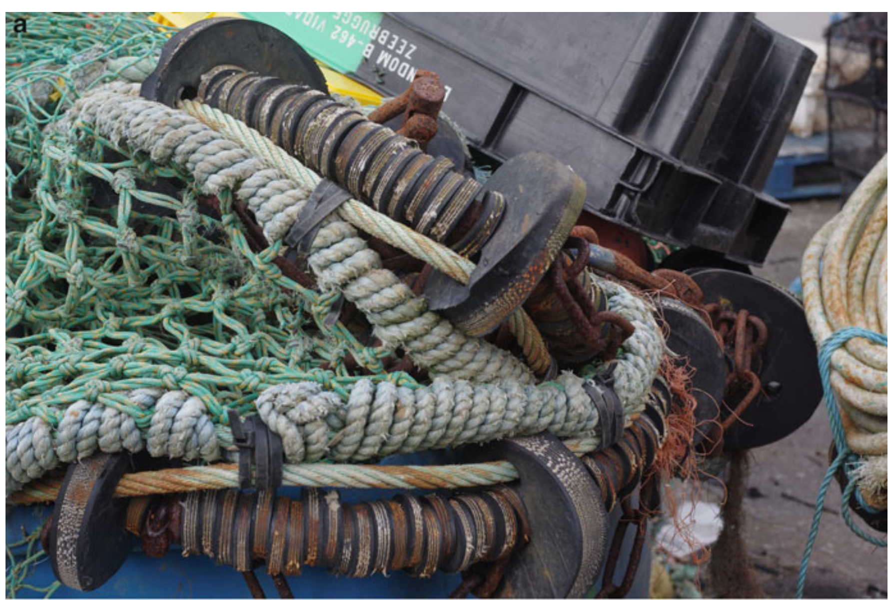


**Fig. 14.2** The fishing nets (a) in the harbour and (b) in the laboratory and their changes during weathering under the UV radiation

## 14.2 Degradation of Polymer Materials

Microplastics in the environment one can divide into the primary ones, already new being <5 mm, or secondary when pristine synthetic materials weather and fragment in time. This gradual fragmentation of microplastics is caused by the weathering triggered both by biological and abiotic conditions. The polymer debris in the marine environment, on one hand, interacts with sea currents, abrasive rocks and sand grinding, UV radiation, chemical compounds, and on the other hand, can be biologically degraded by biofilm growing on its surface. Although the first group of natural conditions is well defined and parametrized, the microbial degradation of apparently durable and no-comestible materials is the domain of recent investigations. The influence of physical and chemical conditions on the biofilm further complicates the modeling of those processes. In particular, the size, concentration, and surface morphology or functionalization (by e.g., –COOH, –OH, $–NH_2$ groups) of the microplastics and nanoplastics determine the potential biofilm on it as it was shown in laboratory research (Miao et al. 2019). The bacterial community will be different on floating debris in respect to the one in sediments.

Regarding the usefulness of synthetic materials, one should care about their durability. The resistance and neglectable degradation over time was also a main goal of the polymer inventors 150 years ago. However, till that time production of nearly half of them switched to single-use items. From that new perspective, easily degraded materials will be more sustainable and green. The concentration on fast production-consumption-wast-recycling is a kind of a vicious circle, but will not be discussed here. One concludes shortly, that to enhance the circular economy the durable but easily biodegradable material will be the aim for the future. Interestingly, not only the bacteria are in the grade of degradation. One can focus also on some species of fungus or insects.

In general, regardless of the species, the microbial degradation of polymers can be divided into the following steps:

- the biofilm formation on the surface in a biodeterioration process
- depolymerization with hydrolysis or oxidation causing fragmentation of chains
- the catabolism of the depolymerization byproducts via bioassimilation
- biomineralization of organic matter and release to the environment of basic compounds and elements ($H_2O$, $CO_2$, $CH_4$, $N_2$).

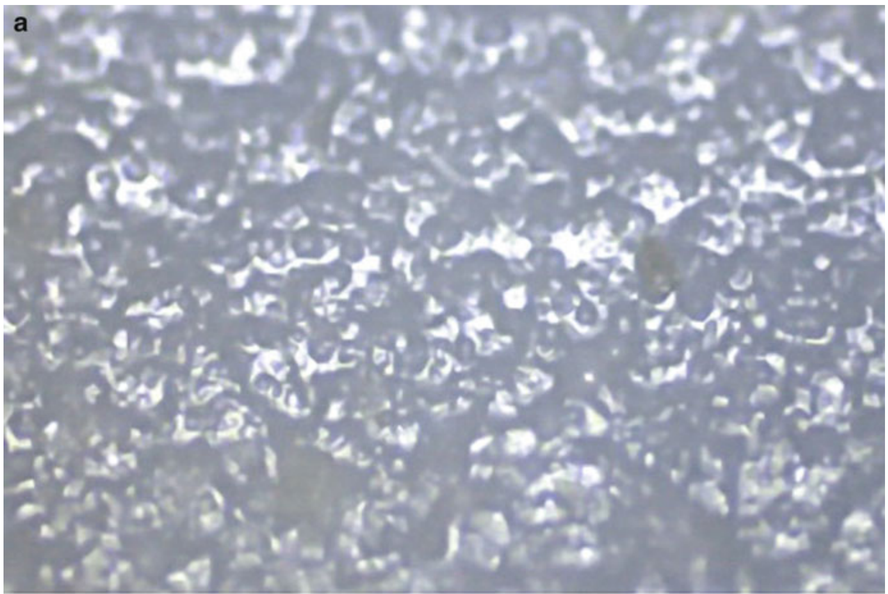
At each step various parameters control the process, such as type of polymer and species in biofilm, physical and chemical conditions of the surface and surroundings of the debris, oxygen availability, temperature, and time. During degradation, a sequence of chemical changes, mostly photooxidation, thermal oxidation, hydrolysis, biodegradation, causes the reduction of the molecular weight and mechanical integrity of the polymer. All processes change the buoyancy of the material and its transport paths. Moreover, the leaching of additives, being frequently the strong contaminants, is observed. One can list as particularly toxic polybrominated diphenyl ethers (which extend resistance to heat), nonylphenol (enhancing oxidative

damage resistance), or triclosan (antimicrobial), phthalates, bisphenol A, alkylphenols. Some of them are strong endocrine-disrupting chemicals. Floating on the surface particles behave differently in respect to those in sea, snow, or sediments. In general, plastics are both the substrate and source of carbon for bacteria. They need to have enzymes dedicated to the decomposition of a particular chemical bond, and the most interesting research can be done by the detailed characterization of the debris partially decomposed. However, it is sometimes not possible due to numerous cracks and irregularities of pristine material, e.g., PS or PET (Fig. 14.3).

One of the most significant problems to initiate the degradation is the hydrophobicity of the surface that makes colonization difficult, if not impossible. Owing to that, the organism can secrete dedicated substances, such as the extracellular enzymes making PE more hydrophilic, biosurfactants, or use the functional groups or already adsorbed compounds, which makes the attachment easier for bacteria. Few examples of laccases or lignin peroxidases can oxidase or/and hydrolyze HDPE. Enzymes take part in terminal oxidation, cleavage of chains, or help the fatty acids metabolization.

Finally, polymer type is crucial in the degradation process as the synthetic materials are numerous and differ significantly. For instance, PET contains aromatic and ester groups, whereas PE is built by stable short carbon chains and PS has a considerable molecular weight. *Marinomonas*, found on PET, were able to degrade cyclic hydrocarbons (phenanthrene, chrysene). *Ideonella sakaiensis* biomineralizes effectively PET, *Pseudomonas putida* PVC, *Rhodococcus ruber* and *Pseudomonas* PS, *Staphylococcus* sp., *Pseudomonas* sp., *Bacillus* sp. decompose PE. The saturated $–CH_2–CH_2–$ chains in PE are resistant to the majority of natural degradations. However, fungi *Aspergillus*, *Acremonium*, *Fusarium*, *Penicillium*, *Phanerochaete* are capable of that. The so-called bioplastics or green plastics are also degraded by biofilm. Microorganism, bacteria, and fungi responsible for the decomposition of PLA are *Actinomadura* sp. T16-1, *Bacillus smithii* strain PL21, *Alcaligenes* sp., *Tritirachium album* ATCC 22563 and *Penicillium oxalicum*, *Pseudomonas* sp., *Candia antarctica*, *Fusrium solani*, *Pseudomonas cepacia*, *Rhizopus delmer*, *Rhizopus arrhizus* for PCL. Polilactids are durable in a marine environment, whereas the poly(ε-caprolactone) are easily fragmented and decomposed in the Baltic Sea. That is why understanding and proper modeling of the microbial polymer degradation is so crucial in establishing the policies about materials used in a maritime industry or any other particular conditions.

Insects are other animals capable of decomposing polymers due to the presence of microbes in their guts, which can be the resource in biodegradation. For instance, the *Bacillus* sp. YP1 and *Enterobacter asburiae* YT1 isolated from the larvae of the Indian meal moth (*Plodia interpunctella*), were capable of PE biodegradation. Similarly, the wax moth chew PE plastic bags due to the presence of *Enterobacter* sp. strain D1 in their guts.



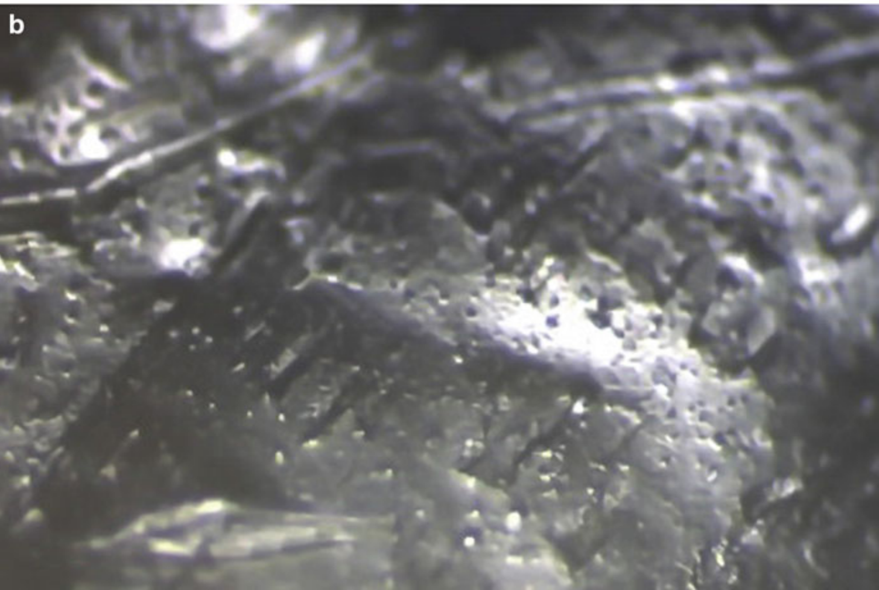


**Fig. 14.3** The microscopic picture (a, b) of the surface of pristine polystyrene

## 14.3 Plastisphere: The Eight Continent

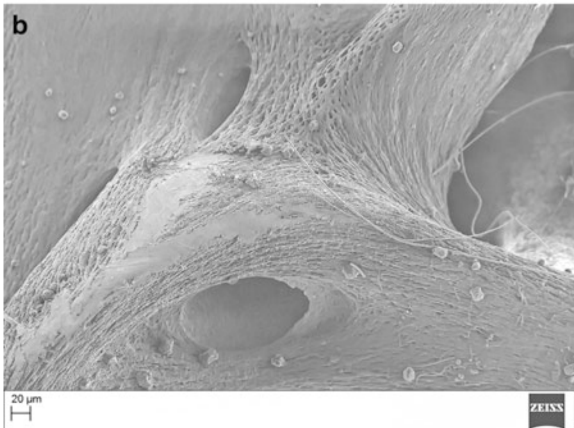
The constantly increasing surface of the polymer debris found in the environment is called a Plastisphere. This special term indicates all the phenomena of the new biological niche available for biota in the Anthropocene Era. As microplastics are ubiquitous, but with different concentrations (for instance 38–234 particles $m^{-3}$ in the Arctic Ocean) (Jiang 2018), so is the Plastisphere. Although nobody knows exactly how this "new eight continent" will change the Earth's self-regulating capacity, for instance, the carbon cycle or the biogeochemical cycling of other elements (phosphorus, nitrogen, etc.), its presence and importance are certain. The substrates from synthetic materials float in the vast and desert oceans and are colonized by bacteria and other species. Usually, the roughness and porosity are favorable in increasing the relative surface and amplifying the available space (Fig. 14.4). As a collateral effect that enhances the proliferation and persistence also of hazardous species (e.g., *Vibrio parahaemolyticus*). Due to that, microplastics should be filtered in wastewater treatment plants (Eckert et al. 2018).

Life on Plastisphere is prolific and abundant, however, it fosters the weathering and decomposition of synthetic material that may cause the leakage of added compounds. As plasticizers, flame retardants, pigments and dyes, pastes, lubricants, etc., are usually present in composite materials and are toxic, the risk of contamination increases. Furthermore, the free surface space is a potential adsorbent for persistent organic pollutants (POPs), heavy metals or viruses and pathogens. That locally compounds their concentration even in a few orders of magnitude.

In many cases, for the inhabitants of a Plastisphere the external conditions, such as the temperature, pressure, concentration of organic and inorganic matter, etc., are less important than the type and fracture level of the microplastic. On the other hand, the environmental conditions and geography, season, time also influences the formation of biofilm on the debris. Moreover, the environmental parameters influence the biofilm itself by changing its density, area, spatial heterogeneity, quorum sensing capability, to name just a few. On the other hand, the microbial communities decompose plastic surfaces, increase the relative gravity thus changing the physical and chemical properties and so the interaction of biofilm-debris is mutual and dynamic (Arias-Andres et al. 2019). Finally, using the transport on the floating debris the previously local bacteria become globally dispersed.

It is confirmed that the species found on the polymer debris, forming Plastisphere, are different (in type or number) than typical representants of the environment around them. They also form islands of pathogenicity, form diverse metabolic pathways, exhibit enhanced horizontal gen transfer and antibiotic resistance.

For instance, at the Mediterranean Sea the *Alcanivorax*, *Marinobacter*, *Arenibacter* are hydrocarbon-degrading bacteria densely populating the polymer debris (PE) or bottles in seawater (polyethylene terephthalate, PET). The *Leptolyngbya* sp., *Pleurocapsa* sp., *Rivularia* sp. are observed in the North Pacific Gyre, and debris from Baltic and the North Sea contained the accumulation of *Vibrio parahaemolyticus* and overall *Vibrio* sp. (e.g., *V. harveyi*, *V. pectinicida*,



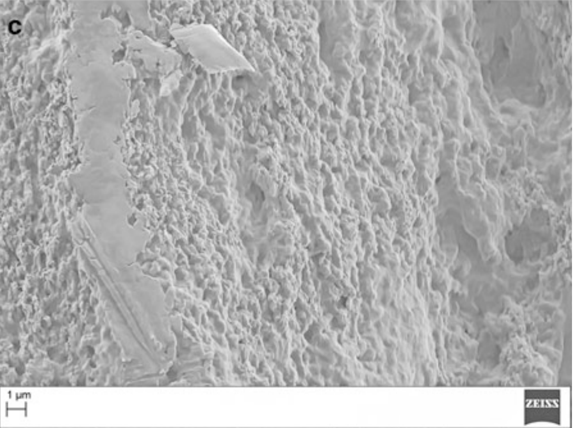




**Fig. 14.4** The SEM microscopic picture (a–c) presenting the developed surface of microplastics found in the fish guts. (Pictures were done by Marianna Gniadek and plastic debris from MIR Gdynia, Poland thanks to the Barbara Urban-Malinga Research Group)

*V. xiamenensis*, *V. anguillarum*) are seven times more numerous on the polymer surface than in a water column.

Furthermore, the total weight of the bacterial population influences the flotage of the debris. For those with a specific gravity lower than saltwater (PP ~ 0.84, PE ~ 0.92–0.96, in respect to the seawater 1.025), the increasing mass could make them end in sediment (as PVC, having 1.38, do naturally).

Plastisphere is not limited to the substrate, with metals and POPs absorbed, for biofilm and vector for viruses and pollutants. Many taxa inhabit the artificial surface: mainly diatoms, coccolithophores, dinoflagellates, hydroids, polychaetes, barnacles, isopod, fungi, bryozoans, and salps. Some usually individual species become the colonial ones.

Finally, one should focus on nanoplastics as a similar phenomenon but with a more intense impact on the environment. For instance, the PS nanoparticles (<100 nm) causes a change in microbial function, carbon and nitrogen cycling, influence the alga growth and photosynthesis or disrupt functional enzyme activities, exhibit toxic effect and induce oxidative stress. Their properties and behavior (e.g., aggregation) depends mainly on the size, buoyancy, morphology, porosity, hydrophobicity and the presence of the surface functional groups or adsorbed organic and inorganic matter (Song et al. 2019).

## 14.4 On the Edge of Science: Selected Recent Studies

The microbial degradation of synthetic materials is currently among the most investigated topics of the 21st century. Among the numerous research, one can distinguish the following directions of studies, the physical and chemical parameters of the Plastisphere, the surface morphology and its detailed qualitative and quantitative characteristics, bacterial species that are particular for microplastics and their geographical distribution, horizontal gen transfer, antibiotic resistance, microbial degradation of the synthetic materials, ecotoxicology and consequences of the debris degradation and their additives leakage, and the global model of phenomena. The methods for analyzing microbial aspects of plastics pollution are diverse, all with their advantages and drawbacks. One can focus on fluorescence electron microscopy, culture-based methods, fluorescence in situ hybridization, illumine amplicon sequencing, fluorescence-activated cell sorting, metagenomes, metatranscriptomes, metaproteome, mesocosm experiments, laboratory incubations, and chemostats. The creation of the operational taxonomic unit (OUT) matrix is a crucial step in all studies.

One of the first evidence of the Plastisphere being an ecological niche for biofilm was obtained during the Tara-Mediterranean expedition (Dussud et al. 2018) that revealed the tree distinguished bacterial communities in the water column, attached to the naturally occurring organic debris and on the microplastics. As particle-attached bacteria play a key role in the degradation of material and its circulation forming phycospheres and deteriospheres, their settlement on the plastic surface may bias the cycle of nitrogen and carbon circulation. Biofilm on the plastisphere differs

from the free-living and sediment communities in many aspects such as cell abundance, morphology, and metabolism. The patchy covering of the microplastic surface dominates. However, the authors state that the question of the existence of specific bacteria typical for plastic debris and different from attached to other particles is still open for discussion. Some preliminary studies show that *Cyanobacteria* might be the predominant residents of the synthetic materials, for instance, the *Calothrix* sp. were found in a mass of *Pleurocapsa* sp. cells. The majority of *Cyanobacteria* found on the Plastisphere belongs to one of the following orders: Oscillatoriales, *Chroococcales*, *Nostocales*, *Pleurocapsales*. The most numerous species are *Synechococcus* sp., *Calothrix* sp., *Scytonema* sp., *Pleurocapsa* sp. Apart from the *Cyanobacteria*, the *Alphaproteobacteria* (mainly *Erythrobacter* sp.), *Gammaproteobacteria* (mainly *Alteromonas* sp.), and *Flavobacteria* are also popular. Hydrocarbon degradation occurs. Finally, the authors report that several hydrocarbonoclastic bacteria were overrepresented and noticed on almost all specimens. They belong to the *Erythrobacteraceae* order and are represented by for instance *Erythrobacter* sp., *Croceicoccus naphtovorans*, *Hyphomonas* sp.

Recent study confirmed that plastics from the Mediterranea Sea host distinctive bacterial communities. *Alcanivorax borkumensis*, having an alkane hydroxylase, seems to be capable of degradation of low-density polyethylene (LDPE) (Delacuvellerie et al. 2019) and exhibits affinity to hydrophobic plastics. Moreover, they contain polyesterases degrading polyesters, both natural and synthetic. Biomineralization of the solid alkane-based structures and their mechanism need to be further studied. In contrast to the surrounding sediments dominated by the *Proteobacteria* (*Gamma-*, *Alpha-*, *Deltaproteobacteria*), plastic-associated communities were composed mainly by *Bacteroidetes* and from *Proteobacteria Alpha-* and *Gamma-* and *Epsilonproteobacteria* or *Vibrio* on floating debris.

In a Bay of Brest, the *Vibrio splendidus* were detected on the microplastics (Frère et al. 2018). Mainly the PE, PP, and PS fragments were detected. The size of particles does not influence the type of biofilm, contrary to the type of polymer. *Pseudomonadales*, *Oceanospirillales*, *Propionispira* were the PE biomarkers, whereas *Alphaproteobacteria* class for PP, *Roseovarius*, and *Nitrosomonas* for PS.

Also in benthic sediments of estuaries typical for a Vitoria Bay (Brasil) the microplastics, predominantly (77%) fibers from fishing nets, were colonized by bacteria and served as a habitat for microorganisms and invertebrates forming the Plastisphere (Baptista Neto et al. 2019).

In research of soil microplastics in Guangdong Province, China (Chai et al. 2020), the family *Hyphomonadaceae* were found responsible for the hydrocarbons degradation and potentially able to reduce the levels of toxic compounds and heavy metals. On the polymer surface, the *Desulfovibrio* and *Lachnospiraceae* were detected, which are not present usually in all soil specimens.

Among interesting directions of research, one can find the considerations about possibly enhanced gen exchange in the biofilms on the Plastisphere (Arias-Andres et al. 2018). According to the recent research, the horizontal gen transfer and the antibiotic resistance are higher in the biofilm on plastics comparing to the free-living communities or natural aggregates. The permissiveness toward plasmids with

antibiotic-resistant genes (*E. coli*) facilitates evolutionary changes at population levels as the microplastics transfer makes them available at the global scale. According to another study, one can claim the microplastics the "reservoirs for antibiotic and metal resistance genes" (ARGs and MRGs) (Yang et al. 2019). The metagenomics analysis was first used to investigate ARGs and MRGs. The size of particles in the North Pacific Gyre does not influence the type of biofilm. ARGs and MRGs abundances, in range $7.07 \times 10^{-4}$–$1.21 \times 10^{-2}$ and $5.51 \times 10^{-3}$–$4.82 \times 10^{-2}$ copies per 16S rRNA, were estimated as ARGs and MRGs are both linked on plasmids thus can be co-transferred. The resistance to aminoglycoside, multidrug, bacitracin, tetracycline, and MLS was confirmed for instance in *Flavobacteriaceae*, which are resistant to antibiotics and heavy metals.

Unfortunately, plastics triggering the development of antibiotic-resistant bacteria are ubiquitous and found also in a pristine and remote area of Earth, such as the Antarctic. A study carried out on polystyrene pieces retrieved in a King George Island (part of a South Shetlands archipelago) (Laganà et al. 2019) isolated 27 bacteria and 7 selected strains were checked for their antibiotic susceptibility profiles. The resistance to cefuroxime and cefazolin, cinoxacin, ampicillin, amoxicillin, clavulanic acid, carbenicillin, mezlocillin was confirmed. Antarctic bacteria were claimed the potential reservoirs for AR gens.

As it was already mentioned, it is not only due to the bacteria biomineralization that the plastics are decomposed. In a recent study (Zhang et al. 2020), the fungus *Aspergillus flavus* (from wax moth *Galleria mellonella*) are capable of PE bio-oxidation and consecutive decomposition due to the presence of strain PEDX3. Hydroxyl groups (3100–3500 $cm^{-1}$), carbonyl groups (1647–1716 $cm^{-1}$), and ether groups (1113 $cm^{-1}$) are present on the FTIR spectra what confirmes the degradation of PE. The laccases exhibit high potential in bio-oxidation.

Although marine microplastics are extensively studied, the terrestrial ecosystems are still poorly understood in terms of the polymer impact on their equilibria. The agricultural soils constitute probably similar sinks for debris as the oceans. In a recent paper (Oliveira et al. 2019) authors focus on the crucial role of insects, being the most diverse and numerous group, in the degradation of deposited on the land synthetic materials. They are famous as bioindicators and some are capable of plastics ingestion and digestion. For instance, termites are the major contributors to synthetic litter decomposition. Larvae of *Tenebrio molito*r eat styrofoam and *Culex pipiensis* larvae of mosquitoes consume microplastics which could be transferred to the adult life stage. However, it probably affects their microbiota.

The same effect of the changes in gut microbiota was observed in mice (Li et al. 2020). Animals subjected to high concentrations of microplastics have increased the number of microbial species (*Staphylococcus*), bacterial abundance, and diversity. On the other hand, one has noted a significant decrease in *Parabacteroides*. Moreover, polyethylene microplastics cause intestinal dysbacteriosis inflammation. The most common in the mice fecal samples are *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Melainabacteria*, *Actinobacteria*, *Deferribacteres*, *Tenericutes*, *Verrucomicrobia*, and *Chloroflexi*. Exposure to the MMs caused statistically

significant change in the number of the most common *Deferribacteres*, *Firmicutes*, *Actinobacteria*. Changes in the gut microbiota influences the natural homeostasis, mainly the immunity system. Regarding all this, the question of human food and health security also remains an important issue to be addressed carefully (Barboza et al. 2018).

## 14.5 Future Perspectives

In the case of a biofilm on the plastics and the phenomena of the Plastisphere the "today's picture is just a past story". Changes and adaptive evolution acts rapidly. The research strategy should focus on the verification of good theoretical models predicting the future state. Among the main questions posed currently, topics to be considered, and interesting area of research, one can find crucial the following ones:

- What are the microbial degradation mechanisms in the case of the polymer materials, especially marine microplastics and nanoplastics?
- What is the theoretical model of the Plastisphere formation and colonization?
- Which parameters are crucial for the development of the biofilm on marine microplastic and how to measure or estimate them?
- Bacteria metabolism in detail.
- Carbon metabolism of microbial communities.
- Functional aspects of biodisintegration of polymers.
- Is there any facilitated gene exchange or increased speciation rate on the polymer debris?
- Creating the global atlas of the microbial species inhabiting the Plastisphere.
- Synthetic material as a vector for bacterial communities' global distribution.
- Horizontal genes transfer in biofilms on polymer particles.
- Complex interdisciplinary studies on ghost netting.
- Are there any new phenomena particular for the Plastisphere?
- Floating debris as a dynamically changing island.
- Quorum sensing in a Plastisphere World.
- Global transport of the biofilm on the polymer particles and creating the invasive species.
- Plastisphere as a reservoir of the antibiotic-resistance,
- The influence of oxidative stress induced by microplastics on the organism's microbiota.

Probably the answers to a few of them and discoveries are just about to be found. It is one's main concern if this knowledge will be used for sustainable development and well-being of the Blue Planet.

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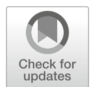

# Microbial Degradation of Antibiotics from Effluents

15

Aditi Singh and Sirjan Saluja

**Abstract**

Antibiotics are the pharmaceutically active compounds, which kill the microbes present in the host bodies and many of these compounds are many times used as nonprescription drugs and after the intake excreted out through urine or feces either as active substances or metabolites. As these antibiotics are biologically active, they may harm the ecosystem as they collect and accumulate in different sites such as groundwater, sewage system, effluents, etc. The antibiotics which are released in the wastewater cannot be removed by conventional wastewater treatment. Worldwide consumption of various active compounds is estimated to be amounting to some 100,000 tons or more per annum. The concentration of antibiotics in wastewater is found to be in the range of less than 1–150 μg/L, which may further increase by nonmedical use of antibiotics, namely in agriculture, animal feeding operations, etc. The antibiotics are broken down into other compounds or transformation products, which are the by-products that are more active than the main compounds. Due to their high polarity, they are less biodegradable and cannot be eliminated efficiently. Since these transformation products are more stable and toxic, they remain in many ecological niches for a longer period of time, when reached in the environment such as groundwater, drinking water, and soil. The prolonged presence of these products in the environment can cause antibiotic-resistant bacteria and antibiotic-resistance genes. Although the resistance genes can occur naturally, but they mostly occur through mutation and resistance from other forms. Various studies in recent times have reported that antibiotics can be degraded by bacteria or by sorption in the sludge

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in wastewater treatment plants. The chapter summarizes the antibiotic contamination in effluents and various approaches for their removal, including bioconversion approaches using microbes.

**Keywords**

Antibiotics · Antibiotic contaminants · Antibiotic resistance · Bioconversion · Bioremediation · Effluents · Metabolites · Multidrug resistance · Pharmaceuticals · Pharmaceutically active compounds · PhACs · Sewage effluents · Wastewater treatment

## 15.1 Introduction

The use of different molds or plant extracts to treat infections has been one of the earliest approaches of mankind. Then with the discovery of compounds like Penicillin by Alexander Fleming has brought a huge change in therapeutics for many dreaded infectious diseases. The word, Antibiotics, for these active metabolites of various fungi having a potential toxic effect on bacteria, was first used by Selman Waksman, a Ukrainian–American microbiologist, who went on to isolate and discover as many as 20 antibiotics. By Second World War, Penicillin became the "wonder drug," saving more lives than ever. Since then antibiotics are one of the most popular types of pharmaceutical product which are being used extensively in medicine, farming, and veterinary clinics. Pharmaceutical companies have had an enormous growth and are developing every day. These pharmaceutically active compounds (PhACs) include all those substances that are used in agriculture, medicine, and biotechnology, in the form of antibiotics, drugs, and hormones. Table 15.1 describes some common antibiotic compounds, their target pathogen, and their mode of action. However, within a span of seven to eight decades, these antibiotics have become one of the biggest challenges for healthcare professionals. The rampant use of antibiotics has resulted in the occurrence of these PhACs in the environment. The chemical substances have lately been detected ubiquitously in various water environments, like surface water (rivers, lakes, stream, sea), groundwater, and water distribution systems as well as in sludge, effluents, and influents of wastewater treatment plants. In the past three decades, antibiotics have been detected as contaminants in all geographical niches, including the pristine polar regions. The reason for this is thought to be their high persistence and low adsorption properties. For example, carbamazepine is detected in groundwaters and diclofenac has been found in tap water. It is now well established that PhACs occurring in the environment constitute a health risk for humans as well as terrestrial and aquatic ecosystems, because of fast-developing antimicrobial resistance (AMR). The overuse and misuse of antibiotics have caused widespread problem of AMR and it is estimated that more than 500,000 people die every year due to AMR complications and this figure is expected to reach as high as 10 million deaths by 2050, causing a worldwide economic burden of $100 trillion. As per a long-term and multicentric study by

**Table 15.1** Some common antibiotics, their target organisms, and their mechanism of action

| S. no. | Antibiotic class | Degradation of microorganisms | Chemical mechanism |
|---|---|---|---|
| 1. | Aminoglycosides—Gentamicin (Garamycin), Tobramycin | Gram-negative bacteria (*Pseudomonas aeruginosa*, *E. coli*) | Inhibit protein synthesis |
| 2. | Cephalosporins—Cefaclor, Cefamandole | *Staphylococcus aureus*, *Streptococcus pneumoniae*, *E. coli*; *Bacteroides fragilis*, *H. influenzae*, *N. meningitidis* | Inhibit cell wall synthesis |
| 3. | Chloramphenicol | *Staphylococcus aureus*, *Streptococcus.* Eye ointment to treat conjunctivitis | Inhibit protein synthesis |
| 4. | Nitrofurantoin | *Citrobacter*, *Enterococcus*, *Serratia*, *Acinetobacter*. | Inactivate essential cell components |
| 5. | Metronidazole—nitroimidazoles | *B. fragilis*, *C. difficile*, Bacterial vaginosis and Pelvic inflammatory disease | Inhibits protein synthesis |
| 6. | Tetracyclines—Chlortetracycline, Oxytetracycline, Doxycycline | *Hemophilus influenzae*, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Chlamydiae*, *Rickettsiae* | Inhibit protein synthesis |
| 7. | Beta Lactams—Amoxicillin, Ampicillin, Aztreonam Carbenicillin, Cloxacillin, Penicillin, Methicillin, Cephalexin, Cefprozil, Cefuroxime | Most consumed class of the antibiotics; given for a wide range of infections, *Staphylococci*, *Streptococci*, *Pseudomonas aeruginosa,* and other gram-negative pathogens—aztreonam | Inhibit cell wall synthesis |
| 8. | Piperacillin (Penicillin group of antibiotics) and Tazobactam (β-lactamase inhibitor) | Gram +ve and −ve bacteria including pathogen producing bacteria like beta-lactamases. | Inhibit cell wall synthesis |
| 9. | Macrolides—Erythromycin, Roxithromycin, Azithromycin, Clarithromycin | Bacteriostatic and are particularly effective against gram-positive bacteria and also efficient against anaerobic microorganisms | Inhibit bacterial protein synthesis |
| 10. | Sulfonamides—Sulfamethoxazole, Sulfamethazine, Sulfapyridine, | Synthetic broad-spectrum antibiotics to treat bacterial and some fungal infections. Also urinary | Inhibiting the conversion of *p*-aminobenzoic to dihydropteroate needed by |

(continued)

**Table 15.1** (continued)

| S. no. | Antibiotic class | Degradation of microorganisms | Chemical mechanism |
|---|---|---|---|
| | Sulfadiazine, Sulfanilamide, Sulfamethizole | tract infections and against *Streptococcus*, *Staphylococcus aureus*, *E. coli*, *Haemophilus influenza*, and oral anaerobes | bacteria for folic acid synthesis |
| 11. | Quinolones—Nalidixic acid Ciprofloxacin, Trimethoprim, Thiamphenicol, Chloramphenicol, Lincomycin, Clindamycin | Used for both gram-negative like *Salmonella* spp., *E. coli*, *P. aeruginosa*. As well as gram-positive bacteria. | Bactericidal activity by deactivating of DNA gyrase and topoisomerase |
| 12. | Fluoroquinolones—Norfloxacin | *Enterococcus faecalis*, *Staphylococcus epidermis*, *Citrobacter freundii* | Interfere with DNA synthesis |

Klein et al. (2018), the usage of antibiotics has increased by 65% from the year 2000 to 2015, along with an alarming rise from 21 billion to almost 35 billion defined daily doses.

Apart from the high use of antibiotics for medical purposes, there are many nonmedical uses of antibiotics, which also become a significant contributor to AMR. As animal feed supplements and other veterinary use—mixing of antibiotics in animal food to promote growth was started by some countries like United States and China. This was subsequently adapted by most countries because this gave a big boost in the animal industry, in terms of increased size of animals, accelerated growth, and reduced loss due to diseases. However, there was no regulation or check on dosage and this leads to suboptimal doses of antibiotics in those animals and thereby reaching the human body or in environment. With the emergence of antibiotic resistance, many countries have reduced their use in animal feed. A large amount of antibiotics produced every year are being used in animal and poultry industry, mainly as growth promoter and not for treating infections. The antibiotics are used as supplements and premixes of animal feed industry. As per a report, as much as 25 million pounds of antibiotics were being used in animal industry in the United States alone till the last decade.

Penicillin and tetracycline are used in poultry feed to improve egg production. Chlortetracycline and sulfamethazine are used in bovine feed to reduce respiratory disease and mortality. However, emergence of antibiotic resistance due to overuse of these substances in animal feed has been reported continuously. Alarming trends have been noticed, like the feed additive Avoparcin which is otherwise not used in humans, but the avoparcin-resistant strains have been found to be resistant to Vancomycin also. European Union has banned all those antibiotic compounds as growth-promoting substances, which have the potential to develop cross-resistance in antibiotics of human health. Denmark has put a complete ban on such

growth-promoting antibiotics in animal feed. But many countries like China and India continue to use it. Also, one antibiotic may be banned in one country; but may still be used widely in another, e.g., vancomycin is banned in Germany, but is still being used in United States (Kumar et al. 2019). Antibiotics are also used many a times as veterinary medicine to treat animals. Some frequently used antibiotics in veterinary practices are tetracyclines, oxytetracyclines, sulfamethazine, penicillin G, lincomycin, etc. Sometimes, suboptimal levels increase the risk of the development of antibiotic-resistant bacteria.

Fish farming is a big industry globally and the use of antibiotics in aquaculture is prevalent. It is given as part of the feed to minimize infections in fish. In aquaculture, commonly used antibiotics are oxytetracycline, florefenicol, sarafloxacin, trimethoprim, ciprofloxacin, and ofloxacin. All these compounds have been detected in water bodies at variable concentrations. In apiculture, antibiotics like Oxytetracycline are sometimes used to inhibit bacterial infection of bee larvae. However, honeybees cannot metabolize these substances, and oxytetracycline is found to be present at very high levels in honey (Meek et al. 2015). In plant and food preservations, antibiotics like streptomycin are used in apple and pear orchard to protect from fire blight infection. Presence of antibiotics has been reported in few vegetables like cabbage, carrot, radish, corn, etc. Bacteriocins are commonly used in canned food and dairy products to limit the growth of *Listeria monocytogenes*. Antibiotics are used in fermentation process during ethanol production to inhibit bacterial infections. Many common domestic products like shampoo, soap, or children's toys contain antibacterial agent, Triclosan. Resistance development toward triclosan in many bacteria has been reported. Similarly, resistance genes for quaternary ammonium compounds (QACs) have also been identified and studied in many bacterial strains; thereby increasing concerns on the prevalent use of these QACs in a variety of common personal care and domestic products.

## 15.2 Presence of Antibiotic Contaminant in Environment and Water Bodies

Antibiotics form one of the highest selling pharmaceutical agents and their production is increasing day by day all over the world. The global consumption of Antibiotics ranges from 10,000 to 20,000 tonnes and thus there has been a tremendous growth of pharma and biotech companies, which have become market leaders in terms of revenue with the sector's annual sales recorded around US $875 billion globally for the year 2010 itself. India is home to a wide range of both large and small pharmaceutical companies, for example, Aurobindo, Alkem, Cipla, or Sun Pharma are some such leading pharmaceutical companies in India. Hindustan Antibiotics (HAL) based in Pimpri, India is a government-run company of India producing recombinant DNA products. The company's main objective was to produce drugs for the healthcare industry, but now it has diversified into different sectors such as veterinary and agriculture as well. India and China together manufacture 90% of the world's antibiotics, which puts them in a top position as a

contributor in spreading AMR. The large production of these compounds has resulted in high concentration of antibiotics or related compounds in pharmaceutical industrial effluents, which reach wastewater treatment plants. During the manufacture of antibiotics, different residues, chemical materials, and ingredients may percolate into the water, which is then discharged into the surrounding water bodies. As per one report, concentrations of the antibiotic moxifloxacin found outside manufacturing units were more than 10,000 times higher than the Indian Government's proposed new limit of 0.05 μg/L. The presence of antibiotics in rivers and potable water in Baghdad City, Iraq was studied by analyzing the potable water from two treatment plants and authors have shown that PhACs like ciprofloxacin, levofloxacin, and amoxicillin were detected in significant amounts in raw water with ciprofloxacin at highest concentration of 1.270 μg/L in the raw water; while ciprofloxacin and levofloxacin were detected in the finished water as well (Mahmood et al. 2019). Such incidents may become a significant contributor to the emergence and spread of AMR, putting these pharmaceutical industries, especially antibiotic manufacturers and related global healthcare industries into regulatory focus. The extensive use of these pharmaceutical compounds is constantly increasing and as per a report in British Medical Journal, one in every seven persons have received antibiotic without any reason at least once in 1-year time span (Chua et al. 2019). The antibiotics or their metabolites are excreted out in urine and feces and thus they are present in significant amounts in household wastewater and then in municipal sewage. Because of their rampant use, PhACs are being released in environment on a regular basis and thus are present in various wastewater sources. Hospital waste and hospital dumps of expired medicines are also another major source of antibiotics reaching in influents and effluents of water treatment plants. Reports have shown that the hospitals in India had many antibiotics like metronidazole, ofloxacin, ceftriaxone in the hospital effluents. The range of these products was from 1.4 to 236.6 UG. Antibiotics like ofloxacin, ciprofloxacin, azithromycin, sulfamethoxazole. Tetracycline and sulfonamide have been found in raw wastewater in China. Many studies have now reported that wastewater treatment that is done presently to remove contaminants is not completely effective in eliminating and removing antibiotics or their related compounds. Therefore, these molecules have been found in hospital effluents, wastewater treatment plant effluents, its solids, soil, and groundwaters. The antibiotics and their metabolites present in surface water get discharged in drains and ultimately reach rivers and seas. This causes further contamination and serious implications on people's health and the environment. Figure 15.1 gives some major sources of PhACs in environment and other water bodies.

## 15.3 Other Environmental Impacts

Apart from that, antibiotics and metabolites are also excreted out by farm and poultry animals, for which these compounds are used as feed supplements. In a report (Pina et al. 2018), it is described that how large volumes of antibiotics which are being used in various agriculture practices are leaching out in the surrounding

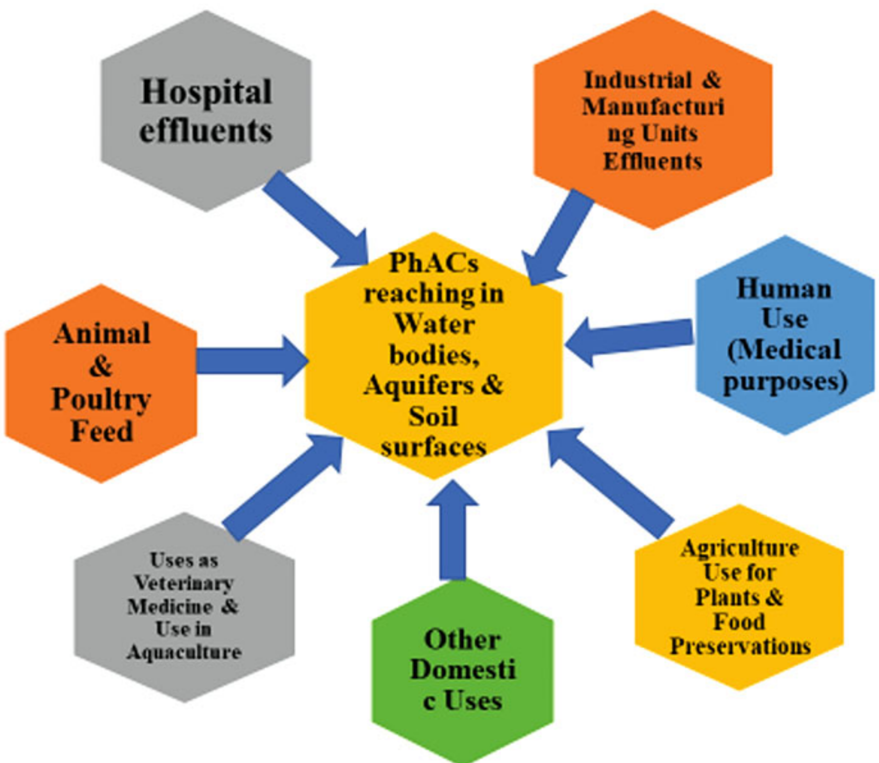


**Fig. 15.1** Major sources of PhACs in environment and other water bodies

groundwaters. The cattle manure is also being frequently used in fields and this may contain significant amounts of antibiotics thus reaching the soil, then in the runoff water, and may also leach to groundwaters. The presence of water-soluble molecules especially less than 1000 Da molecular weight is more frequent in different water bodies and higher concentrations of beta-lactams, aminoglycosides, rifamycins, tetracyclines, quinolones, and fluoroquinolones have been reported (Kumar et al. 2019). It is noted that these chemical compounds are present in highest amounts in wastewater treatment plants and sewage treatment plants; therefore, their effluents frequently contain antibiotics and their derivatives. It becomes thus highly important to be seen where these effluents are mixing in environment. However, the presence of both broad and narrow-spectrum antibiotics is reported from various environmental samples, e.g., hospital effluents are frequently reported to be containing high concentration of common antibiotics, namely fluoroquinolones, trimethoprim, beta-lactams, macrolids, sulfamethoxazole, sulfonamides, lincomycin, etc. (Meena et al. 2015). Another important point to note is that the breakdown rate of one antibiotic may be different from other and that will also have resultant effect on the amount being present in effluents, e.g., Cyclosporin A is a very slow degrading molecule.

The antibiotics erythromycin, roxithromycin, sulfamethoxazole, clarithromycin have been found in seashore water samples from different sea around many countries like Italy, Greece, Germany, Turkey, Belgium, China, and the United States (Nodler et al. 2014). Also, the different category of antibiotics has different breakdown mechanisms; because of their variable physical and chemical properties. Thus, fluoroquinolones and sulfonamides persist longer in environment as compared to macrolides; while aminoglycosides and beta-lactams are reported to persist for shortest time periods. Tetracyclines are degraded in sunlight; but if present in biofilms and in recalcitrant forms, they may persist longer in the environment. Antibiotics found in various ecological niches, get constantly mixed in fresh water and other water bodies through runoff, rainwater, and soil erosion; and thus, interact with microbial communities in various ways. The soil and water microbiota may get severely affected by antibiotic contaminants.

The antibiotics are not only degraded and made present in the environment by feces and urine, but the expired drugs play a role as well. The expired drugs are discarded and drained into the toilet. As the antibiotics are also used in fish farms, some antibiotics such as chloramphenicol and oxytetracycline have been detected in aquaculture ponds and poultry industry. The vegetables taken up by these contaminates also have antibiotics in them. A particular antibiotic may act differently in different places based on their biological and chemical properties. The use of antibiotics in veterinary and poultry industry causes recalcitrant mechanism to survive under the stress of antibiotic which leads to multidrug-resistant bacteria. Several compounds have been shown responsible for disruption of endocrine system of humans as well as animals. These are called endocrine disrupters (EDCs). There are also natural estrogens and contraceptives which relate to diseases of decreased male fertility, testicular and prostate cancer, apart from many other long-term effects.

## 15.4 Impact of Antibiotics in Effluents on Bacterial Communities

Bacteria population gets effected by antibiotics on a large scale which includes not only the targeted bacteria but also the non-targeted populations. Antibiotics act as bactericidal and as mentioned above cause the development of resistance in bacteria even on the nontarget population as a long-term effect, e.g., Fluoroquinolones found in hospital which targets prokaryotes and is toxic to them more than eukaryotes. Antibiotic resistance is the biggest resultant effect of this constant exposure of antibiotics with different microbial communities. Lot of misuse and overuse has led to development of newer and multiple resistant mechanisms among bacteria. As the antibiotics are released with incomplete metabolism in humans and animals, it shows effects against many environmental microorganisms. The increase in the antibiotic's degradation has caused resistance in bacterial species which has irreversible long-term effects. It can survive and multiply by several mechanisms and is resistant to the effectiveness of drugs against particular diseases. Many bacteria such as *Escherichia coli*, *Clostridium*, *Enterococcus,* and *Lactobacillus* show great effect

in their structural composition and biological activity. *Clostridium* showed a reduced growth rate due to toxic effects of sulfamethoxazole, penicillin, erythromycin, ciprofloxacin, and amoxicillin. The *Vibrio fischeri* is also negatively affected by penicillin G, tetracycline.

The survival of bacteria even with antibiotics in sight can cause further harm in nature. The frequent outburst of Cyanobacteria blooms is an all-over phenomenon in freshwater leading to poor water quality, hypoxia, off-flavor in water bodies, and release of cyanotoxins from cyanobacteria strains. Cyanobacteria blooms are also caused naturally by phosphorus to nitrogen ratio, temperature, pH, light intensity; but it has been shown that industrial contaminants can also trigger Cyanobacteria thus producing cyanotoxins. As cyanobacteria are closely related to bacteria, they are more sensitive to antibiotics as compared to algal species. *Microcystis aeruginosa* is a widely distributed toxic Cyanobacteria strains which is affected by antibody contaminants causing an effect on growth and photosynthesis efficiency. The mechanism of interactions remains unclear between antibiotic contaminants and *Microcystis aeruginosa*. It is shown that the two antibiotic components spiramycin and amoxicillin can affect the growth and production of microcystins from algae.

## 15.5 Bacterial Strategies for Antibiotics and Resistance

The antibiotic-resistant mechanisms employed by bacteria have been extensively studied and understood and it is described than any bacteria may exhibit antibiotic resistant by any of these mechanisms—enzymatic inactivation, decreased permeability, efflux mechanism, alteration of target site, or overproduction of target. The capacity to generate these mechanisms may be developed by acquiring the resistant gene. The genetic basis of this resistance can be either chromosomal mutation, which means chromosomally mediated inducible enzymes, and if this is present in any bacteria, then the resistance will be present as an inherent property of that genus. Apart from that, more frequent mechanism is where the resistance gene can also be acquired as plasmid-encoded genetic fragment. In this common genetic basis of resistance, the genetic determinants spread laterally through a population without cell division. All three modes—transformation, transduction, and conjugation can transfer these plasmids encoded new genetic determinants in the bacterial cell. Different antibiotic-resistant genes get activated in an antibiotic-stressed environment and reach normal bacterial populations and finally become part of the normal genome (Cruz-Loya et al. 2019; Kumar et al. 2019). Bacterial species are interchangeable and acquire resistance even with a little overuse, underuse, or misuse. This is mainly caused due to them being in an exposure for a really long time with the antibiotics in low concentration. Antibiotic resistance generally states that a bacterium can survive under stress conditions with the antibiotics present. This occurs due to increased MIC of antibiotics toward the microbe. The microbes escape the drug–target interaction, efflux of antibiotics from cell, or by modification of antibiotics. The antibiotic modified enzymes when added with co-substrates can modify the antibiotics by adenylation, phosphorylation, glycosylation, etc. This can

lead to change in cell surface receptor, redox mechanisms, and hydrolysis. Under heavy antibiotic stress, several bacteria will become resistant even with controlled conditions. The antibiotics resistance is produced mainly due to long-term environmental exposure to low concentration of antibiotics, e.g., the hospital sewage tends to have more resistant bacteria to *oxytetracycline* than the pharmaceutical sewage. Many bacteria like *Pseudomonas fluorescence*, *Staphylococcus* sp. are found to be multiresistant. Natural water also detects antibiotic-resistant bacteria.

High level of density and minimal sensing of microbial consortium leads to formation of biofilm. It prevents the antibiotics from entering the bacteria because the matrix acts as a barrier. Positively charged aminoglycosides antibiotics are prevented to enter into negatively charged biofilm matrix. The high density of bacteria in biofilm consortium increases the chances of selection of resistant bacteria because of antibiotic pressure by increasing the frequency of mutation and rate of horizontal rate transfers. Antibiotics like tetracycline and fosfomycin latter which are used to treat infections against bacteria like *E. coli*, *Staphylococcus aureus*, etc. are used for a long time, and due to mutation in the *gipT* and *UhpT* transporters many strains of bacteria have acquired resistance against them. The resistance acquired by many of these microorganisms demands for the synthesis of artificial antibiotics which are chemically synthesized because AMR is now a global threat and needs urgent attention from regulatory bodies. Centre for Disease Control, United States is constantly monitoring incidents of resistant infections in the community, and other risk factors, which may make spread more difficult to identify and contain. The periodic report from CDC gives out the emergence and spread of new forms of resistance and as per their latest report, there are 18 such AMR pathogens at different levels of concern to human health, namely urgent, serious, and concerning organisms (CDC 2019). Figure 15.2 gives the list of different categories of AMR bacteria or fungi based on the 2019 CDC report.

Detection of antibiotics in environmental samples—many antibiotics, which are mixed in the wastewater, have been extracted by different methods. Tetracycline extraction has been proven difficult as it absorbs strongly in the sludge particles. Ultrasonic assisted extraction method is being used successfully to extract different antibiotics. Other methods such as microwave assisted extraction method, detection of antibiotics by liquid chromatography have also been employed. The structural and functional properties of antibiotics help in identifying them from the environment. Virginiamycin is an antibiotic similar to dalfopristin and is extracted from soil samples by HPLC chromatography technique. The identification of tetracycline is done by adding base such as sodium hydroxide which detects fluorescence on reacting with magnesium ions.

## 15.6 Biodegradation of Antibiotic Contaminants in Effluents

Major sources of PhACs in water bodies are human and veterinary applications and then their excretion and discharge in the environment through sewage treatment plants. More than 3000 different types of PhACs have been detected till now, but it is

**Fig. 15.2** The bacteria and fungi listed as potential antimicrobial threat (CDC's AR Threats Report, 2019, https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf)

the older ones which are more regularly detected, majorly due to extensive use as prescription medicine and generic or over the counter drug (Kookana et al. 2014), e.g., clofibric acid and carbamazepine are two such drugs with high consumption and widespread occurrence in wastewater treatment plants (Marco-Urrea et al. 2009). The removal of PhACs can be done by biotic and antibiotic methods both, of which photodegradation and biodegradation respectively of PhACs, have been identified as the two major sinks; however, conventional treatment of domestic sewage water is usually insufficient for removing PhACs and very low, if any, reduction of antibiotics is achieved. The remaining substances usually bypass treatments and tend to accumulate in water sediments.

### 15.6.1 Biodegradation of Antibiotics by Biotic Methods

The biotic method also called bioremediation or biodegradation is defined as the use of microorganisms to degrade or destroy complex chemical substances present in water or soil sediments into smaller and simpler molecules. Microorganisms from different ecophysiological niches have been reported to degrade and thus detoxify a variety of PhACs like paracetamols, anti-inflammatory drug Ibuprofen as well as antibiotics. Many simple and complex metabolic pathways have been recognized and described which can metabolize a variety of environmental pollutants namely petroleum products and polyaromatic hydrocarbons, pesticides, and insecticides like DDT, endosulfans, carbofuran, etc., plastic contaminants like polyurethane, polycaprolactone, polystyrene, polyhydroxybutyrate, etc., and heavy metals. Many

environmental microbial strains utilize these natural or synthetic polymers as their sole source of energy and carbon. The presence of antimicrobial-resistant gene and capacity for antimicrobial degradation is correlated frequently; however, it is not always true; e.g., presence of antimicrobial-resistant gene had no correlation with the degradation of doxycycline in *Candida* and *E. coli*. The biodegradation of ten PhACs, namely clofibric acid, gemfibrozil, ibuprofen, fenoprofen, ketoprofen, naproxen, diclofenac, indomethacin, propyphenazone, and carbamazepine by nitrifier culture supplemented with ammonia and organic substrates, has been found to be more effective than activated sludge (Tran et al. 2009). Marco-Urrea et al. (2009) have described effective degradation of different PhACs, like ibuprofen, clofibric acid, and carbamazepine by four white-rot fungi, namely *Trametes versicolor*, *Irpex lacteus*, *Ganoderma lucidum,* and *Phanerochaete chrysosporium*. The removal of toxic azodyes, Acid red 183, Direct blue 15, and Direct red 75 was successfully done using *Penicillium oxalicum* SAR-3 isolated from contaminated soil (Saroj et al. 2014). Another frequent contaminant of the environment aquifer is paracetamol, a very common PHACs being highly used consumers and as hospital waste. Regular removal of paracetamols from wastewater is done by electrochemical process, ozonation, UV oxidation, titanium photocatalysis, etc. However, few effective biodegradation processes for paracetamol are also described (Wu et al. 2012). Molds like *Penicillium* and bacterial species like *Pseudomonas* sp., *Stenotrophomonas* sp., *Burrkholderia* sp. have been isolated and identified for the purpose, either individually or as a bacterial consortium. Thus, an exhaustive knowledge on "indigenous microbial communities," frequency to carry antibiotic-resistant genes, redox mechanisms, biodegradation potential, nutritional and carbon requirements, will help in finding out or designing an environment-friendly microbial consortium, which can effectively reduce antibiotics and related contaminants. Many reports have also described that the biodegradation and removal of xenobiotics are effective by augmentation of organic substances as nutrients, especially carbon, nitrogen, and phosphorus sources. Table 15.2 gives out the list of isolated and studied microbial strains characterized by the degradation of PhACs, including antibiotics.

Despite many promising studies on biodegradation of antibiotic PhACs, they have not been found to be effectively removed by biodegradatory processes, may be because of (1) inhibitory nature of antibiotics may hamper microbial metabolic processes which are important for removal; (2) characteristics of antibiotics, operational conditions of wastewater treatment plants, (3) at times relatively low concentration of antibiotics does not initiate enzyme production; (4) sometimes, the breakdown product of an antibiotic is more toxic and persistent than the parent compound, e.g., sulfamethoxazole. Thus different antibiotics may be active against various microbial groups present in wastewater systems and the toxic effects of these antibiotics may limit the growth potential of various microbial community, thereby effectively decreasing the population density in wastewater treatment bioreactors, therefore, toxicity inhibition tests must be conducted for each process (Cetecioglu and Atasoy 2018).

**Table 15.2** Pharmaceutically active compounds, including antibiotics in effluents may be degraded by microbes

| S. no. | Antibiotics | Microbes degrading antibiotics | Mechanisms/isolated from |
|---|---|---|---|
| 1. | Nofralaxcin | *Bacillus subtilis* | Bioreactor scale |
| 2. | Sulfonamides | *Castellaniella* sp., *Brevibacterium epidermidis*. | Artificial coculture |
| 3. | Penicillin G, Penicillin V | β-lactamase enzyme producing bacteria or by chemical hydrolysis | Aerobic and anaerobic processes |
| 4. | Tetracyclines—chlortetracycline, oxytetracycline, and Doxycycline | No effective biodegradation during the wastewater treatment; partially under methanogenic conditions | Removed through adsorption |
| 5. | Paracetamols | *Pseudomonas aeruginosa*, *Pseudomonas* sp., *Stenotrophomonas* sp., *Delftia tsuruhatensis*, *Cupriavidus necator* | Aerobic aggregate, membrane bioreactor biomass, Activated sludge samples |

### 15.6.2 Biodegradation of Antibiotics by Abiotic Methods

The many abiotic mechanisms are equally effective in removing PhACs from wastewater effluents. The sunlight degradation or photolysis of these complex compounds is very much described. The photolytic degradation and photo transformation of ciprofloxacin, penicillin G, meropenem, sulfamethoxazole, have been studied by Lin et al. (2018). Other light-sensitive antibiotics being tetracyclines, quinolones, sulfonamides, nitrofuran, oxolinic acid can undergo sunlight degradation in contrast to monensin, which is resistant to breakdown by direct light. Whereas, fluoroquinolones will be degraded in ultraviolet light. Oxidation is also another frequent chemical process for breakdown of these complex molecules in aerobic environmental niches. Oxytetracyclines and sulfamethoxazole breakdown by ozonation is reported. In soil contaminants, the recalcitration and adsorption of these compounds may limit the degradation process. Metal ions and activated metals have also been utilized for promoting degradation. Cephalosporin and penicillin degradation and using mercury, copper, zinc, cadmium, cobalt metal ions have been reported (Saitoh and Shibayama 2016). Acidic or alkaline conditions also sometimes promote PhACs degradation, namely alkaline degradation of ampicillin. Using microbial enzymes directly for biodegradation is also another approach which has given promising results in some cases e.g. beta-lactamases, cytochrome, $P_{450}$ enzymes.

## 15.7 Conclusion

Though antibiotics have short half-life, but their highly lipophilic, hydrophobic, and nonpolar nature makes them a long persisting substance. Thus, the high concentration of antibiotics in environment is not only responsible for causing severe toxic and carcinogenic effects on human and other terrestrial or aquatic animals; but also aiding in the development of antimicrobial resistance. The actual impact of PhACs contamination may still be not known because the data from developing countries are not always exhaustive. Much less studies are available from resource deficient nations as compared to developed nations. And the alarmingly high AMR is feared to be giving rise to multidrug resistance and superbugs. This has put AMR in a priority position in global agendas. In 2016, United Nations General Assembly came together with WHO to make a very stringent Global Action Plan (GAP) to fight AMR (WHO 2015). It also brings into focus strict legal norms for antibiotic use for medical and nonmedical purposes both, and the need for a constant monitoring. Acting on similar level, Indian Department of Pharmaceuticals has advised pharmaceutical manufacturers to be more cautious about the presence of antibiotics in effluents. Recently, Indian Union Ministry of Environment, Forest and Climate Change is in the process of bringing out new regulatory guidelines for acceptable limits of contamination of all 121 common antibiotics in effluents of wastewater treatment plants (https://www.theweek.in/news/health/2020/02/29/India-leading-the-way-in-averting-antibiotic-apoclypse.html). These regulations will be applied to all pharmaceutical companies in India. With this, India will become one of the first nation to recognize the urgent need of legal regulations for antibiotic use and their effective removal from environment.

Most of the antibiotics are unable to metabolize and thus a large amount is added to the environment by feces and urine which mixes with the receiving water. The hospital effluents play a major role in the contributory source of antibiotics in the environment. The concentration of PhACs in domestic effluents may range between 0.3 and 150 μg/L; whereas effluents from pharmaceutical plants, hospitals, animal feed operations, etc. may be much higher and in the range of 100–500 mg/L. In an exhaustive review by Patel et al. (2019), the emerging concerns of PhACs contamination in aquifers for the past three decades (1990–2018) and their remedial strategies are elaborated. Overall, it is reported that the antibiotics and analgesics top the chart as most frequently detected PhACs in water, out of the eight broad categories of PhACs (Patel et al. 2019). Removal efficiencies of PhACs during wastewater and drinking water treatments are dependent on their physical and chemical properties. It is important to note that removal of pharmaceuticals from water is still not considered to be included in the list of treatments while designing a drinkingwater treatment plant. In India, most of the wastewater treatment plants are based on conventional schemes consisting of aeration, chemical coagulation, flocculation, sedimentation, filtration, and disinfection and all these methods may not be sufficient to remove the PhACs from the effluents. Although it is difficult to completely remove antibiotics from these sites, the removal of PhACs in water and wastewater treatment is essential to prevent environmental contamination and

possible adverse effects, for which bioremediation technique becomes one of the most promising clean technology and this should be explored more.

**Conflict of Interest** The authors declare that there is no conflict of interest.

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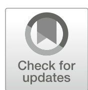

# 16 Microbial Degradation of Oils

Emmanuel Kweinor Tetteh, Mark Opoku-Amankwa, and Sudesh Rathilal

**Abstract**

The recent use of oil as a driving force of the world's economy is being associated with organic pollutants in most of the petrochemical wastes. Notwithstanding, hydrocarbons in oils form part of the widely distributed and most occurring contaminants in the environment, posing serious threats to human health and the ecosystem. Owing to that, a broad spectrum of studies and experiments have been conducted in utilizing oil-degrading microbes for environmental remediation, which has become core to petroleum microbiology and bioremediation. This chapter reviewed crude oil/hydrocarbon compositions and their toxicity in the environment, highlighting microbial degradation of hydrocarbon-contaminated soil and water. It further explored the advancement of different types of microbial community and their dynamics for bioremediation technologies and microbial degradation of oil and hydrocarbons in their contaminated ecosystem. Also, the biological and physiochemical factors affecting microbial degradation of oils are expounded in this chapter. The prospects of genetic engineering of microorganisms for microbial degradation of oils and other applications are presented.

**Keywords**

Bioremediation · Biodegradation · Hydrocarbons · Oily waste · Microbial degradation

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## 16.1 Introduction

Petroleum hydrocarbons (PHC), chiefly petroleum products (such as diesel fuel, gasoline, and oil) are complex substances formed as a result of hydrogen and carbon molecule materializations (Cozzarelli and Baehr 2003; Aminzadeh and Dasgupta 2013). Besides this, it also contains other impurities like nitrogen, sulfur, and carbon, which form part of the widely distributed and most occurring contaminants in the environment (Aminzadeh and Dasgupta 2013). However, crude oil is an essential raw material and energy source for several industries (Varjani et al. 2015; Varjani and Upasani 2016). Thus, the rapid economic growth rate in several economies around the globe has been attributed to the increase in the global consumption of petroleum products (Varjani and Upasani 2016; Ghazali et al. 2004). The rise in consumption level is attributed to the fact that it serves as a raw material for refineries, as a most indispensable energy source, and petrochemical industries for myriad products like petrochemicals, synthetic polymers, and fuel (Varjani et al. 2015). Phylogeny activities like municipal and industrial runoffs, accidental spills, onshore and offshore petroleum industry activities effluent release causes) pollution (Varjani 2017). In effect, it poses both environmental and health risks (directly and indirectly) to all forms of life on the earth (Sajna et al. 2015; Souza et al. 2014; Deppe et al. 2005). PHC pollutants are classified as priority pollutants and recalcitrant compounds, therefore their remediation comes in handy (Costa et al. 2012).

Recently, among the significant wellspring of discharging or releasing dangerous synthetic compounds into the atmosphere and environment has been from the oil refinery industry (Varjani and Upasani 2017) Some of these dangerous chemicals including polycyclic aromatic hydrocarbons (PAHs) are noted to be highly stable in the environment (Varjani 2017; Lamichhane et al. 2016; Abdel-Shafy and Mansour 2016; Zhang et al. 2011). There has been a growing concern about the environmental and human health risk caused by oil industry activities connected with the exploration of crude oil (both intentional and accidental discharge of oil) during the production, transportation, and refining stages (Lamichhane et al. 2016; Zhang et al. 2011), as a result of the continuous pollution of water bodies including streams, rivers, groundwater, and surface water, among others (Abdel-Shafy and Mansour 2016). The marine is said to be the highest and ultimate sink of these pollutants, which is lethal to the marine ecosystem (Costa et al. 2012; Abdel-Shafy and Mansour 2016). In effect, a broad spectrum of experiments and studies have focused on bioremediation, biodegradation, and biotransformation of PHCs (Costa et al. 2012; Lamichhane et al. 2016; Abdel-Shafy and Mansour 2016; McClay et al. 2000).

The concern for applying oil-degrading organisms for remediation of the oil-contaminated environment has become core to petroleum microbiology. Therefore, with a desktop approach, the recent advances in microbial degradation of oils are presented in this chapter. This chapter discusses the composition of crude oil, the toxicity of oil hydrocarbons, the fate of PHCs in the environment, microbial degradation of oil, bioremediation technologies, factors affecting microbial degradation, future works in microbial degradation, which provides conclusion.

## 16.2 Composition of Crude Oil

Crude oil is naturally occurring, extricated from the subsurface, which is conveyed to the refinery for the purpose of distillation to produce diverse products (Varjani 2014; Speight 2014). Petroleum which occurs as a vicious, sticky, and dark liquid is from the Latin word "petra-oleum", which means "rock oil" (Vieira et al. 2007). They contain hydrocarbons, oxygen, sulfur, and nitrogen in varying proportions (Varjani et al. 2015; Chandra et al. 2013). Depending on the quantity of heavy molecular weight components present, oil could be categorized into heavy, medium, or light oils (Varjani 2014). It can also vary depending on the depth, age, and location of the oil well. Therefore, in most cases, constituents of all kinds of oil can be categorized as mixed, paraffin, and asphalt base (Varjani 2014). Furthermore, it is classified into four main fractions which include asphaltenes, resins, aromatics (ringed hydrocarbons), and saturates (aliphatics) (Speight 2014; Chandra et al. 2013).

### 16.2.1 Aliphatic Hydrocarbons

These hydrocarbons do not have double-bonds and constitute the highest proportion of the constituents of petroleum oils in the world. They are classified based on their chemical structures which are cycloalkenes and alkenes (paraffin) (Abbasian et al. 2015). Furthermore, they are branched or linear, unsaturated or saturated open-chain structures including steranes, terpenes, cyclo-alkanes (naphthenes), iso-alkanes, and *n*-alkanes (Rahman et al. 2003). *N*-alkenes are classified into four molecular weight (MW) categories of aliphatic hydrocarbons which include gaseous alkenes, $C_8$–$C_{16}$ (lower), $C_{17}$–$C_{28}$ (average) and more than $C_{28}$ (high) (Abbasian et al. 2015).

### 16.2.2 Aromatic Hydrocarbons (ARH)

These have either one or a myriad of aromatic rings mostly replaced with diverse alkyl groups (Meckenstock et al. 2016). They are also categorized into two main groups which include polycyclic aromatic hydrocarbons (PAHs) (Chandra et al. 2013) and monocyclic aromatic hydrocarbons (MAHs), namely benzene, toluene, ethylbenzene, and xylenes (BTEX) (Costa et al. 2012). PAHs have atleast one benzene ring, and those PAHs with two or three cyclic rings make various hexagon chains with double bonds like anthracene and phenanthrene (three-ringed) and naphthalene (two-ringed), which are also known as light or low molecular weight PAHs (Wilkes et al. 2016). However, PAHs with more than four rings like benzo[a]pyrene and fluoranthene (five-ringed), chrysenes and pyrene (four-ringed) are also known as heavy or high molecular weight PAHs (Costa et al. 2012; Farhadian et al. 2008).

### 16.2.3 Resins

These have several polar functional groups made up of metal traces (Fe, V, and Ni) and N, S, O. They are unstructured solids dissolved in oil (Speight 2014) which are capable of disintegrating in *n*-pentane and *n*-heptane and has aromatic compounds with prolonged alkyl chains (Chandra et al. 2013). More so, resins act as peptizing agents and are similarly structured in oil as surfactant molecules (Chandra et al. 2013).

### 16.2.4 Asphaltenes

These have various polar functional groups like resins and are mostly brown in color, colossal and intricate molecules (Speight 2014). Furthermore, asphaltenes are capable of disintegrating in light ARHs like toluene and benzene (Parra-Barraza et al. 2003). They are also high, and viscous MW compounds made up of polycyclic groups, unsteadily exchanged with alkyl groupings that help to resist biodegradation (Chandra et al. 2013). Resins that are peptizing agents help suspend asphaltenes, thereby aiding crude oil to be stable (Chandra et al. 2013; Parra-Barraza et al. 2003). Asphaltenes and resins have non-hydrocarbon polar compounds as opposed to saturated and aromatic fractions. Furthermore, asphaltenes and resins have more intricate and mostly carbon structures with a lot of oxygen, sulfur, and nitrogen atoms (Chandra et al. 2013). Thus, each constituent has a special synthetic behavior that influences their debasement (Costa et al. 2012). Whereas asphaltenes being a greater molar mass constituent make the central part of oil, saturates constitute the utmost layer in the structural arrangement of the four broad components of hydrocarbons of crude oil (Varjani and Upasani 2017; Speight 2014).

## 16.3 Toxicity of Oil in the Environment

PHC toxins are one of the tenacious natural toxins. They cause permanent and/or extensive damage to the ecosystem due to their bio-magnification (Chandra et al. 2013). Far-reaching release of PHC toxins via leakage and spillages from an abandoned oil refinery, unplugging of oil wells, underground tanks cause contamination of the ocean, groundwater and surface soil (Souza et al. 2014; Prince et al. 2013; Janbandhu and Fulekar 2011; Saeki et al. 2009). Many components of oils are highly toxic and recalcitrant due to the existence of teratogenic, carcinogenic, and hemotoxic constituents like PAHs and BTEX (Souza et al. 2014; Costa et al. 2012; Zhang et al. 2011; Chandra et al. 2013; Meckenstock et al. 2016).

Chronic or acute as well as indirect or direct impact of petroleum pollutants was earlier reported (Chandra et al. 2013; Meckenstock et al. 2016). Hormone imbalance, metabolic reaction disturbances, stunted growth, anoxia, and suffocation in life forms is recognized as indirect or direct effects (Souza et al. 2014; Walker 2012). Short-term effects of PHC pollutants during preening include ingestion, drowning,

smothering, hypothermia, and acute necrosis mortality (Varjani 2014; Desforges et al. 2016). Also, the PHC pollutants have long-term effects that include developmental abnormalities of marine animals like unfused skulls, lack of pigmentation, and jaw reductions (Varjani 2014; Alonso-Alvarez et al. 2007; Van Meter et al. 2006). These impacts create changes in a species community/population, and in this manner, creates changes in the whole ecosystem (Walker 2012). The components of PAHs have the ability to create malevolent tumors, which basically impact other epithelial tissues and skin, as they have a high affinity for the nucleophilic center of macromolecules such as DNA, protein and RNA (Costa et al. 2012; Desforges et al. 2016).

PHCs are further classified into defined scopes of identical carbon fraction(s) or numbers to assess environmental and human health risk. These fraction(s) can further be described and subcategorized to their toxicological, chemical and physical features. First fraction of identical carbon number from $C_6$ to $C_{10}$, represents the most volatile fraction of hydrocarbon mixtures. The second fraction also has a spectrum of an identical number of carbons (C) greater than 10 but less than or equal to 16. The third fraction has a spectrum of an identical number of carbons (C) greater than 16 but less than or equal to 34, and the fourth fraction is more than 35. The fourth fraction of compounds is considered to have the lowest solubility and volatility (Varjani and Upasani 2016).

Furthermore, PHCs pollutants have a negative repercussion on human lives and the ecosystem, and therefore, it is important to extensively discuss the fate of hydrocarbons in the environment which can help combat and control pollution (Walker 2012). Crude is exposed to copious weathering processes like biodegradation, tarball formation, resurfacing, photooxidation, emulsification, dissolution, sinking, dispersion, evaporation, and spreading, which tends to degrade hydrocarbon constituents (Souza et al. 2014). Among all these processes, the biodegradation process is considered to be the most important of all (Varjani and Upasani 2016). For example, the impact of photooxidation in removing the pollutants from the atmosphere is limited because it occurs only in sunlight exposed oil (Widdel and Rabus 2001). Nonetheless, the biodegradation process has intermediate to long-term changes in reducing the pollutants in the environment over a period of time. Regardless of the role of biodegradation in removing PHC from the environment, its microbial degradation also has its limitation as resistant PHCs pollutants in either water or soil would generally increase the number of rings, molecular weight as well as the type in cases of PAHs.

### 16.3.1 Remediation of PHC Pollutants

Many traditional engineering-based physical-chemical cleansing techniques are costly due to the transportation of enormous quantities and cost of excavation of sullied substances synthetic inactivation (utilization of hydrogen peroxide or potassium permanganate as a chemical oxidant to mineralize nonaqueous pollutants such as oil) vis-à-vis ex-situ treatment, incineration and soil washing (Varjani et al. 2015;

Farhadian et al. 2008; Chaudhry et al. 2005). Also, some other physical-cum-chemical methods serve the same purpose as abiotic transformations, volatilization, sorption, dilution and dispersion, among others (Varjani and Upasani 2016; Chandra et al. 2013). The limited efficiency and increasing costs of these conventional physical-chemical treatments have prodded the advancement of other innovative techniques for in situ applications, especially based on the capabilities of microbial degradation of microbes (Farhadian et al. 2008). Microbial degradation is considered to be one of the most auspicious methods, as it utilizes the capacity of microbes for the expulsion of toxins from polluted sites including rivers, marine, soil, etc., in a most environmentally friendly, least hazardous and most effective way (Bhatt et al. 2019; Porto et al. 2011).

## 16.4 Microbial Community Dynamics

Naturally, hydrocarbon-metabolizing microbes are generally disseminated. Problems that arise during attempts to describe natural communities of microbes affected by oil hydrocarbons are aggravated by a bunch of single substrate and metabolic interactions. Notwithstanding the complexities, mechanisms are being created in a bid to readily acknowledge microbial distribution and abundance in a natural system with expectations of linking the structures in the community with their functions in the ecosystem. The justification for tackling this analysis entails depicting the function of microbes at the beginning of oil over geologic time (Magot et al. 2000), assessing the long-lasting impacts of oil pollution, evaluating and developing approaches to waste remediation (Ka et al. 2001; Siciliano et al. 2003), tracing the improvement of pathogenic microbes in the course of remediation, and controlling harmful activities of microbes in the course of producing oil. Perspectives to cataloging communities of microbes and diversifying roles can be extensively categorized into culture-independent and culture-dependent techniques. Both the culture-independent and culture-dependent techniques may involve genetic characterization methods. Conventional culture-based techniques are ultimately natural and depend on distinctive physiologic, metabolic and morphological properties (Van Hamme et al. 2003). They comprise Biolog substrate utilization plates, liquid assays of the most probable number (MPN)-approach and cultivation and isolation on solid media. Culture-independent techniques for analyzing communities started with a first-hand assessment of metabolically dynamic microorganisms with differential stains, for example, phospholipid unsaturated fatty acid analysis, voluminous analysis of aggregated protein banding, fluorescence in situ hybridization, (INT)-formazin, and CTC and 4′,6′-diamidino-2-phenylindole. Table 16.1 shows the utility and limitation of some microbial community techniques for culture-dependent and culture-independent techniques.

**Table 16.1** Limitations and utility of some community analysis techniques (Van Hamme et al. 2003)

| Type | Example | Utility | Limitations |
|---|---|---|---|
| Culture-dependent | Biolog | Overall metabolic activity rapid, detected, and easy to utilize | Sensitive to incubation effects and inoculum size, may not include substrates of interest, selective media may limit the proportion of community detected, no isolates obtained for further studies |
| | MPN | Metabolic function of interest detected | Selective media may limit the proportion of community detected, no isolates obtained for further studies |
| | Plating | Isolates obtained for further studies | Isolates not necessarily, reflective of a specific metabolic function, only a small proportion of the community is detected |
| Culture-independent | Promoter-reporter systems | Monitoring of treatment effects on total cell function | Monitors only those strains with reporter genes inserted, easier to apply when whole genome sequences are available, nature of the promoter must be known |
| | Probes for specific metabolic genes | mRNA detection can reveal information about expression, detect genes with functions of interest | Activity cannot be inferred from the presence of genes alone, limited to known genes |
| | PCR followed by gel electrophoresis | Bulk changes in community structure detected, can identify microorganisms by sequencing resolved bands, no bias from culture media | No isolates for study, no information on activity, differential amplification during PCR, differential RNA or DNA extraction from diverse cells |
| | Reverse sample genome probing (RSGP) | No bias from culture media, quantitative analysis of specific microorganisms in environmental samples | Limited to those microorganisms included in the screen |
| | Staining for active microbes | No bias from culture media, enumerate live microorganisms | Does not differentiate microorganisms with a catabolic activity of interest |
| | Fluorescence in situ hybridization | No bias from culture media, spatially visualize specific microorganism in an environment | Laborious method, not necessarily detecting active microorganisms |

(continued)

**Table 16.1** (continued)

| Type | Example | Utility | Limitations |
|---|---|---|---|
| | Protein banding | No selection pressure if extracted directly | Difficult to link fingerprints to specific microbial groups, no measurement of community function |
| | Phospholipids fatty acid analysis | Changes in fingerprint can show changes in community structure | No isolates were obtained for further study. |

### 16.4.1 Microbial Degradation of Oil

As earlier mentioned, microbial degradation is considered to be one of the most auspicious methods, as it utilizes the capacity of microbes for the expulsion of toxins from polluted sites including rivers, marine, soil, etc., in a most environmentally friendly, least hazardous, and most effective way (Bhatt et al. 2019; Porto et al. 2011). Over the years, it has shown that its accomplishment in debasing xenobiotic compounds differs from physical-cum-chemical methods is ceaselessly advancing due to cost adequacy, simple application, high efficiency, effortlessness, and energy efficiency (Guarino et al. 2017; Khan et al. 2016). In microbial degradation of oil, biodegradation and bioremediation processes could be adopted where biodegradation involves the use of natural microbes for removing pollutants while bioremediation involves the use of laboratory engineered microbes for removing pollutants. The instrumental component for a successful process in bioremediation is the fitting microbial choice which could debase toxins without competing for other autochthonous microorganisms and losing the microbial viability (Mahmoud and Bagy 2018). Xyl (toluene), nah (naphthalene), alk ($C_5$–$C_{12}$) *n*-alkanes were seen as the most destructive pathways of microbes in degrading oil hydrocarbon (Mahmoud and Bagy 2018). Solitary living things can process only a compelled extent of hydrocarbon materials, to such an extent that aggregating of diverse populaces possessing a broad chemical limit is needed to dispose of an intricate blend of hydrocarbons such as oils in marine, freshwater, and soil environments (Priya et al. 2015; Dellagnezze et al. 2014; Tyagi et al. 2011; Cappello et al. 2007).

Microbes are everywhere in the aquatic and terrestrial networks; the most heterotrophic faction involved was fungi and bacteria utilizing-hydrocarbons, which is seen to be widespread and a task of the ecosystem in every single environmental condition, with comprehensive frequencies ranging from 0.003% to 100% of bacterial communities in the marine, 0.13–50% for bacterial communities in soil and from 6% to 82% for soil mycobiota (Foght 2008; Yakimov et al. 2007). Figure 16.1 shows microorganisms in polymer degradation.

The community of bacteria are the predominant potent organisms in degrading oil and is scrutinized as the most elevated petroleum-spillage (can solely degrade hydrocarbons) debasers in the ecosystem (Mahmoud and Bagy 2018). Its particular genes regarding hydrocarbon breakdown assume a fundamental portion in the

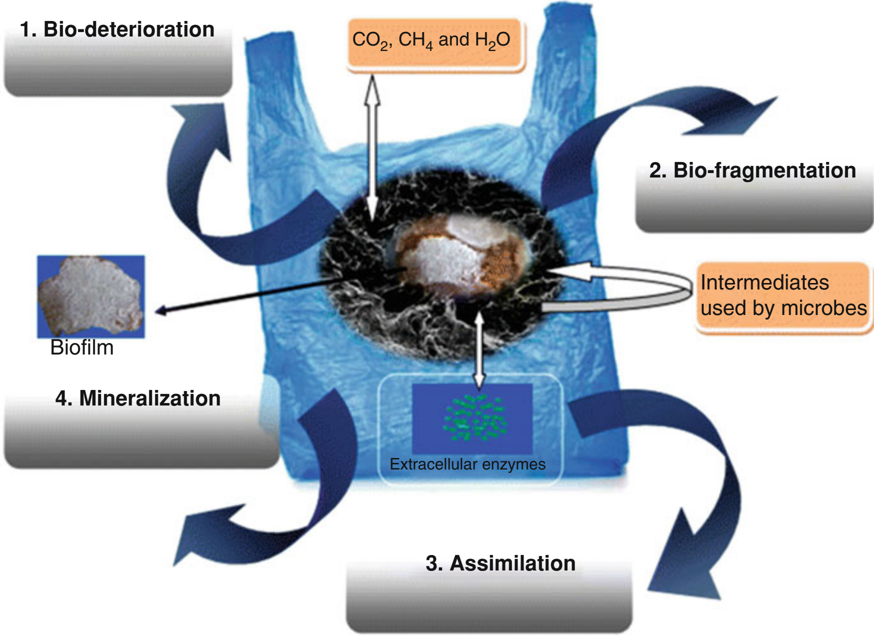


**Fig. 16.1** Microorganisms in polymer degradation (Bhatt et al. 2019)

**Table 16.2** Enzymes used in degrading pollutants in petroleum hydrocarbon. Adapted from Abbasian et al. (2015)

| Enzyme | Petroleum hydrocarbon compound | Name of microorganism |
|---|---|---|
| Dioxygenases | $C_{10}$–$C_{30}$ alkanes | *Acinetobacter* sp. |
| Bacterial P450 oxygenase system (CY153) | Cycloalkanes, $C_5$–$C_{16}$ alkanes | *Mycobacterium*, *Caulobacter*, *Acinetobacter* |
| Eukaryotic P450 (CYP52) | Fatty acids, $C_{10}$–$C_{16}$ alkanes | *Yarrowia lipolytica*, *Candida tropicalis*, *Candida maltose* |
| AlkB-related alkane hydroxylases | Fatty acids, cycloalkanes, alkyl benzenes, $C_5$–$C_{16}$ alkanes | *Mycobacterium*, *Rhodococcus*, *Burkholderia*, *Pseudomonas* |
| Soluble/particulate methane monooxygenases | Cycloalkanes, alkenes, $C_1$–$C_5$ (halogenated) alkanes, $C_1$–$C_8$ alkanes | *Methylomirabilis oxyfera*, *Geobacillus hermodenitrificans*, *Methylobacter*, *Methylococcus*, *Methylocella*, *Methylomonas*, *Methylocystis* |

debasement process of oil pollutants (Yakimov et al. 2007). The associations between the oil-contaminated environment and bacterial cultures are hitched; the bacterial response to oil hydrocarbons makes ground-breaking procedures for improving the degradation capacity (Mahmoud and Bagy 2018). Some of the enzymes used in degrading pollutants in oil hydrocarbons are shown in Table 16.2.

There are copious sequences of genomes in bacteria that can degrade hydrocarbons, for example, *Pseudomonas aeruginosa* N002, *Polymorphum gilvum* SL003B-26A1T, *Desulfatibacillum alkenivorans*, *Geobacillus thermodenitrificans*, and *Alcanivorax borkumensis* (Mahmoud and Bagy 2018). Possible pathways and gene qualities associated with the debasement of hydrocarbons are mostly differentiated by the information from the whole sequencing of the bacterial genome (Hong et al. 2016; Dias et al. 2012; Nie et al. 2014; Callaghan et al. 2012). A handful of bacteria recovered from diverse oil-polluted sites showed their capacity to discard the oil pollutants, e.g., *Vibrio* sp., *P. aeruginosa*, *P. putrefaciens*, *P. putida*, *Pseudomonas alcaligenes*, *Proteus vulgaris*, *Flavobacterium* sp., *Mycobacterium* sp., *Micrococcus* sp., *Moraxella* sp., *Klebsiella aerogenes*, *Klebsiella pneumonia*, *Corynebacterium* sp., *Bacillus marcescens*, *Bacillus licheniformis*, *Alcaligenes faecalis*, *Arthrobacter* sp., *Aeromonas hydrophila* and *Acinetobacter lwoffii* (Ghazali et al. 2004; Jesubunmi 2014; Varjani Sunita et al. 2013; Geetha et al. 2013; Thenmozhi et al. 2011; Khashayar and Mahsa 2010; Hamzah et al. 2010; Mittal and Singh 2009). Olajire and Essien (2014) claim in several ecosystems, there already exists enough indigenous microbial community able to extensively degrade oil hydrocarbons, given that the environmental conditions are favorable. There are diverse merits of relying on autochthonous microbes instead of introducing microbes to debase hydrocarbons. To begin with, the natural populace of microorganisms must have evolved after several years. These microbes can adapt through proliferation and survival in that environment. Furthermore, the capability to use hydrocarbons is spread across several microbial communities. This population occurs in the environment either synergistically or independently and feeds on different hydrocarbons. Commonly, if microbes are adequate in the debased ecosystem, seeding microbes might not be needed. Microbes (fungi and bacteria) have various proportions of utilizing and debasing hydrocarbons in water or soil which is shown in the duplication and colony forming units (cfu) of the segregated microbes. Numerous oil hydrocarbon debasing microbes have been segregated from both marine and soil sources, which are two significant conditions influenced by oil hydrocarbon contamination. Microbes are outfitted with metabolic machinery to utilize products of oils as an energy or carbon source. The metabolic pathways that can be utilized by hydrocarbon-degrading heterotrophs are either anaerobic (uses sulfate and nitrate as a proxy electron acceptor) or aerobic (central electron acceptor like oxygen is utilized) (Olajire and Essien 2014). Aerobic debasement or degradation is considered to be more effective as it occurs more rapidly than anaerobic degradation due to the energy yield per reaction and the less free energy required for commencement. Table 16.3 shows the oil degradation rates under several conditions.

### 16.4.2 Bioremediation Technologies

Bioremediation of soil can be accomplished on-site, over enormous regions (Tomei and Daugulis 2013) without soil disturbance (in situ methods), or can be applied

**Table 16.3** Oil degradation rates under various conditions. Source: Floodgate (1972)

| Bacteria involved | Kind of oil | Experimental conditions | Summary of results |
|---|---|---|---|
| Selected mixed cultures of oil oxidizing organisms | Louisiana crude | Cultivated with oil oxidizing microbes. Ambient temperature of 8–15 °C. Seawater enriched with $(NH_4)SO_2$ 50–100 mL of crude included. Additionally, simulated field studies of enormous tanks (900.1). Around 70 mh oil was added to 200 mL medium, 20 and 30 °C. Shaken flasks with ocean water improved nitrogen, phosphates and inorganic yeast. | In the enormous tanks, the bacteria quickened the loss of oil and changed its physical character. No proof of usage of aromatics was found. Up to around 50% of the crude was lost. The underlying rate was followed by a decrease and afterward another increase. Initial oxidation attributed to breakdown of n-alkenes. |
| Natural marine populations | Atmospheric residue of Kuwait oil | Sands were heavily or lightly oiled at 10 °C. Ocean water permeated through sections of seashore sand (median grain size 250μ) with microfauna and natural environment. | The remaining 90% decayed "immeasurably slowly." Preliminary gas chromatograms proposed the primary loss was of the alkene fraction. These rates applied for several months and accounted for 10% of the oil. Utilizing a "B.O.D." estimation of 5.0, the author calculates a loss of oil from 0.09 g oil/$m^3$.day relying on dosing. Oxygen uptake is utilized as a sign of debasement. |
| Natural seawater population | Crude and several refined products | Batch culture 18 °C, seawater medium reinforced with phosphate and $NH_4Cl$. | The impact of temperature is likewise shown. The presence of effectively debased material "saved" the oil. The presence of phosphate and nitrogen appeared to increase the breakdown of diesel oil in about 2 months. The impact of different chemical and physical factors on oil debasement is illustrated. |
| Mixed culture of oil oxidizing bacteria | American crudes | Oil dispersed on ignited asbestos. About 1 g placed in 100 mL medium. Seawater medium reinforced with 0.01% $(NH_4)_2HPO_2$. Batch | Average around 45%. Between 17.8% and 98.8% by weight of oil removed in 90 days. |

(continued)

**Table 16.3** (continued)

| Bacteria involved | Kind of oil | Experimental conditions | Summary of results |
|---|---|---|---|
| | | culture aerated by shaking 25 °C. | |
| Enriched culture consisting predominantly of marine Pseudomonas | Clear refined mineral oil | Aged seawater plus 0.5% $KNO_3$, probably batch culture 25 °C. | The oxidation of the mineral oil was demonstrated by bacterial growth, $CO_2$ yield, and $O_2$ uptake. The $Q_{10}$ is given as about 3.0 for temperatures in the range of 0 and 40 °C. The average amount of oil debased at 25 °C is given as $1.2 \times 10^{-30}$ mg/day per bacterial cell. Hence it is calculated that if the oil is uniformly distributed in the water and the population is constant at $8 \times 10^6$ organism/mL then the rate of oil degradation will be about 350 g/m$^3$.year at 25 °C and about 36.5 g/m$^3$.year at 5 °C. |
| Soil aerobes | Emba crude and lubricating oils | 23 °C Nitrate or ammonia in mineral salts media. Batch culture. | 1.2 g/m$^2$.day for crude oil (45% of added oil) 0.4 g/m$^2$.day lubricating oil |
| Garden soil aerobes of several genera | Hydrocarbon mixtures in common use | Several temperatures range between 20 and 37 °C. Mineral salts media. Batch culture. | 0.4–0.75 g/m$^2$.day of some materials measured at 28 °C |

off-site following the transfer of sullied material post-excavation (ex-situ methods). As a result of reduced site disturbance and lower costs, in situ methods are generally more desirable contrasted with ex-situ methods (Antizar-Ladislao et al. 2007; Lalevic et al. 2016). The primary in situ bioremediation methods are bioaugmentation, biostimulation, and natural attenuation (Perelo 2010; Suja et al. 2014). Natural attenuation essentially depends on the activity of autochthonous microbial communities to gradually remediate a site, with the main user input being contaminant monitoring (Khade and Srivastava 2017). If contaminant debasement cannot be accomplished due to restrictions in the biochemical capacity of the autochthonous microbial community, then bioaugmentation, the addition of capable microbial populaces to the soil is sometimes conceivable (Simarro et al. 2013). Generally, the best method to improve and accelerate bioremediation processes is to utilize biostimulation, the control of soil characteristics through the addition of restricting nutrients or supplements or electron acceptors (Hamdi et al. 2007; Kauppi et al. 2011). Ex-situ methods include biopile, composting, and landfarming (Dzionek et al.

**Table 16.4** Successful remediation process using bacteria-plant interaction (Lalevic et al. 2016)

| Microorganism | Plant | Microbial characteristic |
|---|---|---|
| *Pseudomonas putida* | *Pisum sativum* | Naphthalene degradation |
| *Burkholderia cepacia* | *Triticum* sp. | Toluene degradation |
| *Pseudomonas putida* | *Populus* sp. | TCE degradation |
| *Pseudomonas* sp. | *Triticum* spp. | Hydrocarbon degradation |
| *Acinetobacteria* sp. | *Oryza sativa* | Hydrocarbon degradation |
| *Sinorhizobium meliloti* | *Sorghum bicolor* | Hydrocarbon degradation |
| *Acinetobacter* sp. | *Lolium multiflorum* | Hydrocarbon degradation |
| *Rhizobium meliloti* | *Medicago sativa* | Hydrocarbon degradation |
| *Pseudomonas* sp. | *Zea mays* | Hydrocarbon degradation |

2016; Georgieva et al. 2010). Landfarming depends on the incitement of microbial activity by aeration and the addition of restricting supplements in open fields of excavated contaminated soil (Khade and Srivastava 2017). This technique is appropriate for the debasement of toxins present at low concentrations (Silva-Castro et al. 2015; Paudyn et al. 2008). Composting is the natural treatment of municipal and agricultural waste, for example, sewage sludge (Tomei and Daugulis 2013) and is suggested for large volumes of waste (Perelo 2010; Suja et al. 2014). Biopile technique has generally been utilized for treating oil hydrocarbon-contaminated soils (Golodyaev et al. 2009), where aerated composted piles are equipped with suitable systems for controlling dissolved oxygen and moisture (Dzionek et al. 2016). Recent advances have been made into phytoremediation as it is considered to be the most cost-effective way of removing hydrocarbons from water and soil. Phytoremediation entails the utilization of plants (and their associated microbial communities) to remove contaminants from air, water and soil (Rezania et al. 2015). The process requires low maintenance and monitoring and capital investments as plants can utilize $CO_2$, water and sunlight for their development (Khan et al. 2016), and improve the soil health and structure as they develop root systems (Nesterenko-Malkovskaya et al. 2012). Table 16.4 shows some successful remediation processes using bacteria-plant interaction. Choosing a bioremediation method generally relies on rate, depth, and pollutant characteristics of contamination, and site characteristics, for example, temperature and soil properties. Table 16.5 then shows the bioremediation technologies in microbial degradation of oils.

### 16.4.3 Factors Affecting Microbial Degradation

Microbial degradation is seen as cost-effective and environmentally friendly as contrasted with other forms of degrading oils. Owing to the capacity of the microbial to adapt to inhospitable environments and their metabolic machinery, they can degrade several organic pollutants in both water and soil. Thus, they play a significant role in site remediation. However, the process is constrained by several factors, including physical and biological factors, among others. Weathering is one of the

**Table 16.5** Bioremediation technologies (Van Hamme et al. 2003)

| Technology | Comment |
|---|---|
| Phytoremediation | Presumably cost-effective, potency to remove oil contaminants being evaluated, utilizes rhizopheric microorganisms and plants in treating contaminated soil |
| Bioaugmentation/ biostimulation | Application of microorganisms and/or mineral surfactants/nutrients to supplement or stimulate natural microbial population at a polluted site. |
| Bioventing | A combination of biodegradation and advective soil venting for in situ treatment of soil, most of the lighter hydrocarbons are volatilized. |
| Biopiling | Uses air, supplemented nutrients and microbial population, year-round operation difficult, slow degradation rates, potentially contaminated surface and groundwater. |
| Landfarming | Cost-effective, uses supplements of mineral nutrients and microbial population, year-round operation difficult, slow degradation rates, potential to contaminate surface and groundwater. |
| Bioreactor | Cost-effective, on-site operation, nonhazardous residues, accommodates a variety of sludges, high biodegradation rates, application of specialized and natural microorganisms in controlled nutritional and environmental conditions. |

factors that can inhibit or speed up microbial degradation in oils. The process consists of emulsion, sedimentation, dispersion, and spreading (Khade and Srivastava 2017). The unpredictable segments of low molecular weight from the hydrocarbons are promptly dissipated like *n*-alkanes and benzene; this helps microorganisms to degrade efficiently (Venosa and Zhu 2003). The oil-in-water emulsion is generally good, which provides bioavailability of hydrocarbons to the microorganisms. Water-in-oil emulsions formation diminishes the surface territory of oil droplets, which for sure reduces bioremediation of oil (Khade and Srivastava 2017). The size of droplets of oil is additionally one of the fundamental components. The smaller droplets of oil are promptly inclined to be debased because of their huge accessibility of the surface area.

More so, microbial degradation on the aromatic and aliphatic hydrocarbons is dependent on oxygen availability. Hydrocarbon pollutants in oil reduce microbial growth by reducing soil air permeability (Meckenstock et al. 2016). Microbial degradation mostly involves aerobic bacteria because oxygenase is the principal enzyme produced during the microbial degradation process by the action of microorganisms which, in most cases, is oxygen-dependent (Das and Chandran 2011). It behaves as an electron acceptor and raises microbial activities hence enhancing the aerobic process of degradation (Abbasian et al. 2015). Notwithstanding, the provision of a sufficient supply of oxygen to empower mineralization of the oil hydrocarbon toxins to occur totally in the soil is costly and challenging (Boopathy 2000). The depletion of oxygen at the subsurface is the restricting factor for microbial degradation of spilt hydrocarbons. In degrading oil hydrocarbon toxins through the anaerobic process, microbes use proxy electron acceptors like carbon dioxide, manganese, iron, nitrate, and sulfate (Meckenstock et al. 2016; Wilkes et al.

2016; Weelink et al. 2009; Boll et al. 2014). Also, numerous microorganisms debase BTEX compounds efficiently under anaerobic conditions (Khade and Srivastava 2017).

Bioavailability is also considered to be a factor that can impact the activities of microorganisms during microbial degradation. Microbial cell rates that could metamorphose toxins during the degradation process are legitimately relative to the pace of contaminant metabolism and uptake, and the rate of transfer or mass exchange to the cell (Singh and Chandra 2014). Thus, increased microbial conversion capacity does not inure to higher biotransformation rates when the mass exchange is a restricting component (Boopathy 2000; Pieper and Reineke 2000).

Temperature also plays an integral role in microbial degradation (Varjani et al. 2014). It affects both the microbes consuming them and the physical condition of the hydrocarbons present in the polluted site (Chandra et al. 2013). It affects the chemical and physical condition of contaminants, metabolism in microbes, soil matrix, gas solubility, and microbial growth rate (Megharaj et al. 2011). There have also been reports that an increase in temperatures augment transferring and diffusing *n*-alkanes of long-chains from the solid state to the liquid state, decreasing viscosity and increasing the solubility of hydrophobic pollutants (Aislabie et al. 2006). Nevertheless, at low temperatures, water solubility is decreased, votalization of noxious alkanes which is short-chain in nature is minimized and viscosity of oil increases which delays the onset of microbial degradation (Varjani and Upasani 2016). Furthermore, on exposure to temperatures that are extremely low, for example, the polar regions, biosurfactants, or bioemulsifiers are significant because they tend to decrease water solubility or increase viscosity of oil hydrocarbons at lower temperatures (Kauppi et al. 2011; Aislabie et al. 2006). Studies again show that 80% of microbial degradation occurred in oil in 27 days when *Bacillus thuringiensis* was utilized (Thamer and Al-Kubaisi 2013). The studies then inferred that this could be because of the production of emulsion materials and environmental factors of availability of ideal temperature or bacterial enzyme, which raised the effectiveness of microorganisms in degrading the oil components.

High temperatures and salinity hinder microbial growth and their products (Chandra et al. 2013). Pressure and salinity have been reported to be specific characteristics of particular ecosystems like deep seas or saline lakes, which constitutes a specific environment that may be hydrocarbon polluted (Varjani and Upasani 2016). Minai-Tehrani et al. (2006) had observed 41% oil debasement (4 months incubation) in soil tests bereft NaCl option, though 12% oil debasement (4 months incubation) was obtained from the same test soil sample with 50 g/L NaCl. Evaluation for microbial degradation mixed hydrocarbon, hexadecane, and tetradecane utilizing blended deep sediment of cultured microscopic organisms was conducted at atmospheric temperature (1 atm) and elevated pressure (495 or 500 atm) (Varjani and Upasani 2016). They presumed that when hydrocarbon toxins reach the deep ocean condition; some oil constituents may contaminate profound benthic areas of the ocean. These toxins in benthic zones of the seas are biodegraded gradually because of the obstinate fragment of oil which could persevere for a long period, might be years or even decades.

Again, oil hydrocarbon mineralization can be influenced by its concentration. With the expanded oil concentration lag stage reduces, while maximum debasing rates and the total degree of mineralization increases (Towell et al. 2011). Oil hydrocarbon degradability likewise relies on its structure. Microbial degradation of oils could occur in descending order which are asphaltenes, polyaromatics, cyclic alkenes, monoaromatics, low-molecular-weight alkyl aromatics, branched alkanes, and linear alkanes (Varjani 2017). Surprisingly, high complete oil hydrocarbon concentrations have demonstrated deadly microbial actions, consequently restricting their biodegradation potential (Varjani and Upasani 2016). Extremely low concentrations of hydrocarbon in oils can also restrict debasement as the supply of carbon might be extremely moderate in aiding microbial growth. Normally, microbial degradation rate rises with decreasing chemical structure complexity and molecular weight of hydrocarbon (Varjani 2014). Same compound(s) in diverse oil are debased to diverse proportions by other or same consortium or organisms, which could be due to the bioavailability of the compound(s) (Varjani 2017). Polluted sites and physical-cum-chemical characteristics of oil are important for successful microbial degradation (Varjani Sunita et al. 2013). These components have unmediated impact on the metabolic activities, type, and number of microflora of any ecosystem (Ghazali et al. 2004). The degraders of hydrocarbons prefer the constituent of the pollutants naturally determines the utilization of less complex compounds (Varjani and Upasani 2016) and their biodegradability. Under appropriate ambience, biodegradation could occur in paraffin as it comprises of alkenes which are medium-chain in nature. In any case, biodegradation can also occur in unrefined oil having significant components as saturates or potentially aromatics, nevertheless, biodegradation might occur in hefty oils having asphaltic-naphthenic components of around 11% within a suitable timespan under ideal growth conditions for microbes (Cai et al. 2007; Ghazali et al. 2004).

Significant microbial community groups, namely viruses, protozoa, algae, fungi, and bacteria are accounted for to have been utilized in hydrocarbon pollutant bioremediation (Varjani and Upasani 2016; Ubani 2016; Waigi et al. 2015; Ishak et al. 2012; Zhao et al. 2011). Microorganisms are the most bountiful groups among which Rhodococcus and Pseudomonas assume a critical role in biodegradation (Varjani Sunita et al. 2013; Zhao et al. 2011; Brooijmans et al. 2009). Towell et al. (2011) examined the impact on the amount of Inoculum on hydrocarbon contaminants and detailed that the ideal absorption of microorganisms in oil hydrocarbon debasement is 106 CFU/g in soil. As microbial communities rise intrinsically, Inoculum degrades and hydrocarbon additions are being utilized, the slack stage also rises. So far, a slack stage of around 170 h at 50 ppm of absorption in oil has been documented for utilizing Inoculum of 107 CFU/g soil (Towell et al. 2011). Also, Varjani et al. (2014) have detailed improvement in the study of hydrocarbon using a bacterial consortium of which they found the following as ideal ambience for microbial growth, 37 °C incubation of microbes with 2% v/v Inoculum at 18 rpm; pH of 7.2; 3% v/v oil. Moreover, lastly, *Bacillus* sp. from animal manures and compost of oil sludge could also be utilized as an agent of bioremediation for cleaning up oil-polluted environments (Ubani 2016).

Nutrients availability assumes a significant role in microbial activities (Boopathy 2000; Varjani et al. 2014). Concentration and type of nitrogen and carbon source utilized in culture medium assume a crucial role in microbial growth (Varjani and Upasani 2016; Dias et al. 2012; Varjani et al. 2014; Zhao et al. 2011). Microbes need phosphorous and nitrogen for amalgamation into biomass, the accessibility of these supplements in similar territory as hydrocarbons is basic. More so, as oxygen is of necessity during the process, nitrogen is also significant in debasing hydrocarbons. Hydrocarbons in oils do not have a noteworthy quantity of some nutrients, for example, phosphorous and nitrogen which are needed for microbial growth. Nevertheless, ammonium, NPK composts, phosphate and urea could be utilized to alter proportions of carbon or nitrogen or phosphorous or potassium (CNPK) (Varjani 2017). Thus, the sufficient proportions of carbon, nitrogen and phosphorous to advance the growth of microbial communities is 100, 10 and 1, respectively (Dias et al. 2012; Zhao et al. 2011; Beškoski et al. 2011). Cultivating with oil debasers has not been proven to be viable; however, adding nitrogenous composts shows expanded rates of degrading oil hydrocarbon. Nitrate is noted to be the principal wellspring of biosurfactant production and nitrogen for microbial growth (Toda and Itoh 2012).

Interestingly, Varjani et al. (2014) reported that no huge impact in degrading oil hydrocarbon was seen in nitrogen source addition. Treating oil-contaminated sites with nitrogen raises the rate of growth in cells and hydrocarbon debasement by maintaining the population of microbes at an increased level of their activities and diminishing their growth at their slack phase (Walworth et al. 2005). Notwithstanding, studies show that an uncontrolled amount of nitrogen could lead to microbial restraint in the soil and therefore nitrogen under 1800 mg N/kg $H_2O$ is ideal hydrocarbon debasement of contaminants (Walworth et al. 2007). Excessive concentration of nutrients, especially increased NPK concentrations represses the debasement of these pollutants (Varjani 2017; Souza et al. 2014; Boopathy 2000). More so, increased levels of toxins concentrations disturb the CNP proportion in polluted sites and limit the functions of oxygen during the process (Sihag et al. 2014). Table 16.6 shows the factors affecting microbial degradation in oil and their conditions for microbial degradation.

## 16.5 Future Works in Microbial Degradation Technologies

At the site of spilt oil, the local microbial community can debase the hydrocarbons, although the rate of microbial degradation can be prolonged. Because of this, oil and hydrocarbons get to the shoreline before being converted or removed in their simpler form, and once they reach the shoreline, the beaches and seashores are severely impacted. In this way, the idea of bioaugmentation is being used. Nevertheless, to accomplish complete bioremediation by bioaugmentation, the microorganisms ought to be capable of adjusting to the high salinity water by debasing numerous hydrocarbons with the production of biosurfactants within a short span. Likewise, the microorganisms must be able to withstand the harmful synthetic chemicals in the

**Table 16.6** Factors affecting microbial degradation in oil (Singh and Chandra 2014; Okoh 2006)

| Factor | Condition |
|---|---|
| Pressure | Degradation of hydrocarbon substrate by a mixed-culture of deep sea-sediment bacteria was monitored at 10 atm and 495 or 500 atm |
| Salinity | The metabolism rate of hydrocarbons reduced with increasing salinity between the range of 3.3–28.4% and the results were attributed to the general reduction in the rates of microbial metabolism. |
| Bioavailability | Several investigations have been conducted for bio-surfactant and bio-surfactant producing bacterial due to their abilities to increase bioavailability.<br>Application of surfactant in areas with contaminated oil may have a neutral, inhibitory, or stimulatory impact on degrading oil compounds by bacteria.<br>The application of external oil spill dispersants or nonsurfactant impacts the rate of alkane degradation. |
| Soil moisture, alkalinity, and acidity (pH) | Water potential of aquatic environment can range from −0.98 and soils from 0.0 to 0.99.<br>Biodegradation of oil sludge in the soil can occur at the optimal rate of 30–90% of saturated water.<br>High variability of pH in soil: alkaline desert −11.0, mine soil −2.5.<br>A pH neutral is favored by most heterotrophic fungi and bacteria. |
| Nutrients | The biodegradation of oil pollution is enhanced by additional nutrients.<br>Several reports have been made on the negative repercussion of excessive use of NPK in the biodegradation of hydrocarbons.<br>Organic fertilizers such as poultry manure have been found to enhance the rate of biodegradation of contaminated soil.<br>Photooxidation expanded the biodegradability of oil hydrocarbons by increasing their bioavailability and consequently improving their microbial activities. |
| Oxygen | The availability of oxygen is dependent on the presence of utilizable substrates, the type of soil, and the rate of $O_2$ consumption by microbes.<br>Oxygen concentration is considered as a rate-limiting variable in the soil during biodegradation of petroleum products.<br>Catabolism of aromatic, cyclic, and aliphatic hydrocarbon by fungi and bacteria in the first stages involves oxidizing substrate by oxygenases, for which molecular oxygen is required. |
| Temperature | Outstanding biodegradation of hydrocarbon was reported in temperate regions in a psychrophilic environment.<br>Highest rate of degradation happens in the range of 15–20 °C in marine, 20–30 °C in freshwater, and 30–40 °C in soil.<br>Reduced temperature decreases enzymatic activities which also decreases the biodegradation rate.<br>A low temperature increases the viscosity of oil and reduces the volatility of toxic low molecular weight hydrocarbons, which in turn, decreases the rate of biodegradation. |

(continued)

**Table 16.6** (continued)

| Factor | Condition |
|---|---|
| Structure and composition of hydrocarbon | Hydrocarbon susceptibility due to microbial attack has been the order of biodegradation rate: Saturates-light aromatics-high molecular weight aromatic-polar compounds or in order of *n*-alkanes-branched alkane-low molecular weight aromatics–cyclic alkanes. |
| Weathering | Photooxidation, evaporation |
| Microorganisms | PHC degraders may be absent or low in numbers |

hydrocarbons. To have such microorganisms that can debase various types of hydrocarbons, biosurfactants producer, and salt resistants, the microorganisms could be genetically changed by utilizing genetically engineering methods of genetic engineered microorganisms (GEM) (Khade and Srivastava 2017). It is the method of control of genetic sequences to improve the adequacy, efficiency, and efficacy of bioremediation. Genetic engineering permits humans to isolate genes from specific organisms and put them into other organisms. A few genetic ways have been created and utilized to improve the organisms, metabolic pathways and enzymes concerning biodegradation (Pieper and Reineke 2000). The fundamental strategy of utilizing genetically engineered organisms in bioremediation is to design such novel strains that have the capacity of debasing high molecular weight polyaromatic compounds. *Pseudomonas fluorescence* HK44 is the principal GEM sanctioned for conducting experiments in the US during bioremediation (Khade and Srivastava 2017). Strain HK44 as a reporter gene contains *lux* amalgamated in a degradative pathway of naphthalene, which consequently permits recombinant microorganisms to exhibit bioluminescence as it is based on special polyaromatic hydrocarbons, for example, naphthalene. The cycle could thus be seen through light detection. Anand Mohan Chakrabarty, an Indian-born American microbiologist, was the pioneer for developing an oil-eating "superbug" that used four strains of Pseudomonas containing the plasmid. Since the isolation of the plasmid from each strain led to the degrading of naphthalene, xylene, camphor, and octane, he combined them to form one strain of *Pseudomonas putida* known as "superbug" that was able to degrade naphthalene, xylene, camphor and octane as depicted in Fig. 16.2 (Renneberg and Berkling 2016). The degradative effectiveness of PAHs can also be upgraded via the GEM. For instance, the rate-restricting steps in already established metabolic pathways could be genetically controlled to produce increased rates of degradation, or new metabolic pathways can be consolidated into strains of bacteria for the debasement of already intractable compounds (Renneberg and Berkling 2016). Also, different strategies utilizing engineered microorganisms can be used for controlling and monitoring stress response assessment, toxicity, and lastly cleaning up hydrocarbons completely (Khade and Srivastava 2017).

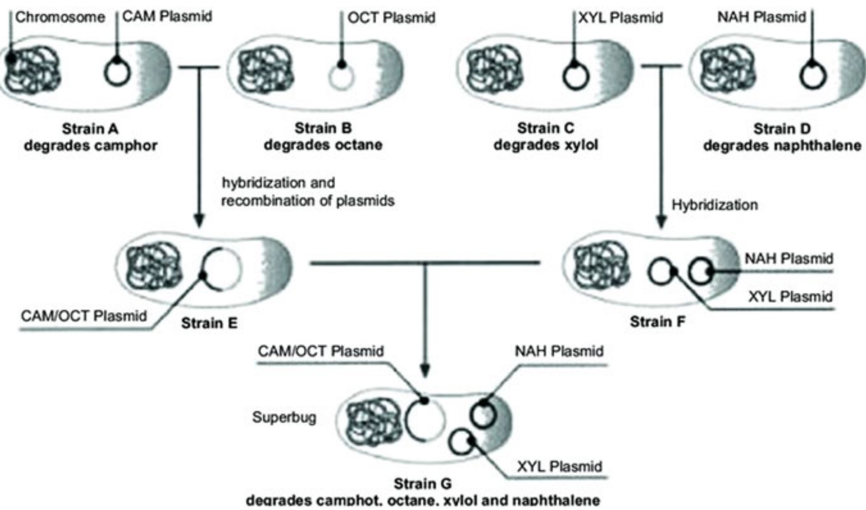


**Fig. 16.2** Genetically modified *Pseudomonas putida* KT2442 (Khade and Srivastava 2017)

## 16.6 Conclusion

In this chapter, microbial degradation of oil wastes is seen as very promising among the bioremediation technologies, which are simultaneously used for environmental conservation and treatment of different waste streams. Despite the growing acceptance of microbial degradation as means to treat aromatic and aliphatic fractions of spilled oils in the marine environment, large-scale application of the process under field conditions and the microbes' stability continue to be a major setback. Therefore, environmental factors such as biological and physiochemical factors need to be examined to enhance the microbial degradation of the oils. Also, microbial degradation is associated with the contaminated environment and their microbial community dynamics. The prospect of genetically engineered microbes emerges a very promising microorganism for microbial degradation of oils and bioremediation technologies for the removal of recalcitrant pollutants and contaminated-hydrocarbons from water and soils, and therefore, requires much attention.

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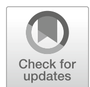

# Microbial Degradation of Biowaste for Hydrogen Production

17

Ouahid El Asri, Soufiane Fadlaoui, Mohamed Ramdani, and Sanae Errochdi

**Abstract**

More than half of municipal waste is biowaste, consisting of the kitchen, food, and garden waste. This type of waste is continuously growing. The biowaste poses a threat to the environment, public health, and the economy of each country if it has not been treated effectively. In order to reduce their daily volumes and their impacts, the microbiological degradation of these wastes using certain well-selected microbes that produce hydrogen is a new global trend that helps achieve previous goals. This chapter presents the different types of microbes and their roles in the conversion of biowaste into hydrogen, as well as the biological and biochemical mechanisms that ensure this conversion. We have also gathered in this chapter a comparison between the three current methods of hydrogen production by microbial degradation (microbial photofermentation, microbial dark fermentation, and two-stage fermentation) and the new discoveries, which make it possible to improve this trend.

**Keywords**

Biowaste · Bioresource · Dark fermentation · Energy · Photofermentation · Microbes

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## 17.1 Introduction

Waste is residual, and is undemanded and unnecessary; it has lost its price and value in the eyes of its proprietor. Urbanization and the consumer economy led to an increase in the amount of waste. This generation of wastes continued to increase without stopping. In 2004, China produced 190 million tons, but in 2030 its production will reach 480 million tons (2030) (Minghua et al. 2009). The European Union generates more than 2000 million tons of nonhazardous and hazardous waste, respectively (Scarlat et al. 2019). Hanc et al. (2011) declared that half of this municipal solid waste is biowaste. Thus, biowaste constitutes one of the types of waste most represented in municipal landfills. In some countries such as the United States and Japan, biowaste production reaches 26 and 20 million tons, respectively (Wang et al. 2018). Some researchers have described the biowaste volume as an alarming and continuous biowaste situation by per capita and municipality (Zorpas and Lasaridi 2013). Garcia-Garcia et al. (2017) declared that one-third of the aliment intentionally grown for citizen's consumption is never consumed, so it is wasted. Currently, several countries worldwide have adopted policies advocating a transition to an economy to reduce, upgrade the volume of biowaste, and minimize dependence on fossil fuels to ensure sustainable economic and eco-friendly.

Biowaste is considered a priority goal for the global transition to a circular economy (Zeller et al. 2020). Among the circular economy, policies are adopted for the microbiological degradation of biowaste. Microbial degradation is the biological decomposition of complicated organic matter into smaller compounds by the microorganisms' community (Tahri et al. 2013). The microbes transform biowaste constituents by enzymatic reactions into suitable products such as methane, compost, hydrogen, and biodiesel. This conversion relies on the microbe's growth on the biowaste as carbon, nitrogen, and energy. Balachandar et al. (2016) declared that biowaste is one of the best favorable organic substrates for hydrogen production because it includes 90% organic matter, promoting microbial degradation. Also, Dugmore et al. (2017) have described that the biowaste is an excellent feedstock for bacterial cultures for several current fermentation processes. Hydrogen-producing fermentation is currently gaining momentum over other fermentations because hydrogen is the dream fuel in the coming years. The microbial degradation for producing hydrogen is the sustainable technology that exploits biowastes as feeds (Arumugam et al. 2014; Nissilä et al. 2014).

Several researchers have confirmed that biowaste is an excellent substrate for hydrogen production (Ghimire et al. 2015; Lay et al. 2003; Ntaikou et al. 2010). The hydrogen ($H_2$) product by microbial degradation of biowaste is a nonpolluting and cleanest fuel renewable fuel. It can produce a high level of energy than any known combustible; it has a critical lower heating value (142 kJ/g) (Møller et al. 2017). On combustion, this gas does not generate any greenhouse gases (Hay et al. 2013). Currently, several works have integrated diverse biotechnologies by using microorganisms' ability to degrade organic components in biowaste with various metabolic ways for performing hydrogen production processes. So, it is an

opportunity to treat the biowaste in order to reduce its impact and produce the gas most of the planet search.

This chapter is a great tool to show the several microbial degradations of biowaste for hydrogen production. This chapter aims to provide recent news concerning hydrogen production using microbial degradation for the past few decades. This chapter is divided into two axes, (a) Firstly, we discussed the definition, world production, and characteristics of biowaste to show the importance of converting this waste into hydrogen, and (b) The Second axis consists of showing the different microbial degradation of biowaste and presenting new microbial applications for hydrogen production.

## 17.2 Biowaste: Definition, Composition, and Production

### 17.2.1 What Is Biowaste?

The definition of biowaste is not easy because it is different for each country. So, there is a wide range of definitions. The biowaste definition can affect the composition, sorting behavior, and treatment process (Boelens et al. 1996). We will study some definitions to choose the most suitable. Bhatia et al. (2018) have described that biowaste is produced in several origins, such as municipal solid waste, forest and agricultural activities, and in the food industry. This definition is extensive; thus, it contains almost all organic waste. The biowaste is collected selectively in some European countries. In Belgium, the biowaste is constituted principally by the kitchen, fruit, and garden waste (Gellens et al. 1995). Boelens et al. (1996) have proposed a straiten definition of biowaste, and it is only biogenic organic waste such as cooking and park waste. Schüch et al. (2016) have described that the biowaste collected according to the European Waste Catalogue is particularly the kitchen, food, and garden waste. Also, Kranert et al. (2020) have confirmed the last definition, and they excluded some organic wastes (manure, agricultural residues, sewage sludge, paper, and processed wood) from the biowaste nomination. Finally, we can define biowaste as biogenic organic material generated by daily household activities such as cooking, kitchen, food, or garden waste.

### 17.2.2 World Production of Biowaste

Austria generated 2 million tons of biowaste per year (Raninger 1996). China generated more than 30 million tons of biowastes every year. In 2014, Germany collected 4.6 million tons of biowaste; out of which, 3.9 million tons of this amount are treated by anaerobic degradation and the rest goes to the composting unit (Fricke et al. 2017). The production of biowaste in the European Union is an estimated 138 million tons per year, as well as each European citizen generates 170 kg of biowaste per year (Razza et al. 2018). Currently, in Lyon of French, the city generated 17,000 tons per year of gardens wastes, the restaurants, and schools

produced between 2500 and 6000 tons per year, biowaste generated by kitchens achieved 45,000 tons per year, and the food biowaste from supermarkets is more than 6000 tons per year (Moretti et al. 2020). Several researchers have confirmed that biowaste's world production increases annually (Zhao et al. 2020). Hanc et al. (2011) declared that the biowaste represented nearly half of the municipal solid waste party. So, the more we produce biowaste, the increasing the rate of municipal waste. This increase of biowaste quantity in each country is linked to several socio-economic and cultural factors of each nation, such as economic growth, the rapid expansion of the cities, and massive migration of population from rural to urban centers (Awasthi et al. 2014; Mian et al. 2017).

### 17.2.3 Biowaste Composition

The composition of biowaste is decisive for choosing a suitable microbial treatment. According to the previous definition, the biowaste is mainly composed of the biodegradable organic fraction (Fig. 17.1). The manual sorting of the biowaste in some European countries showed a mixture of different organic components such as meals, fish, meat, bread, and vegetables (Mahro and Timm 2007).

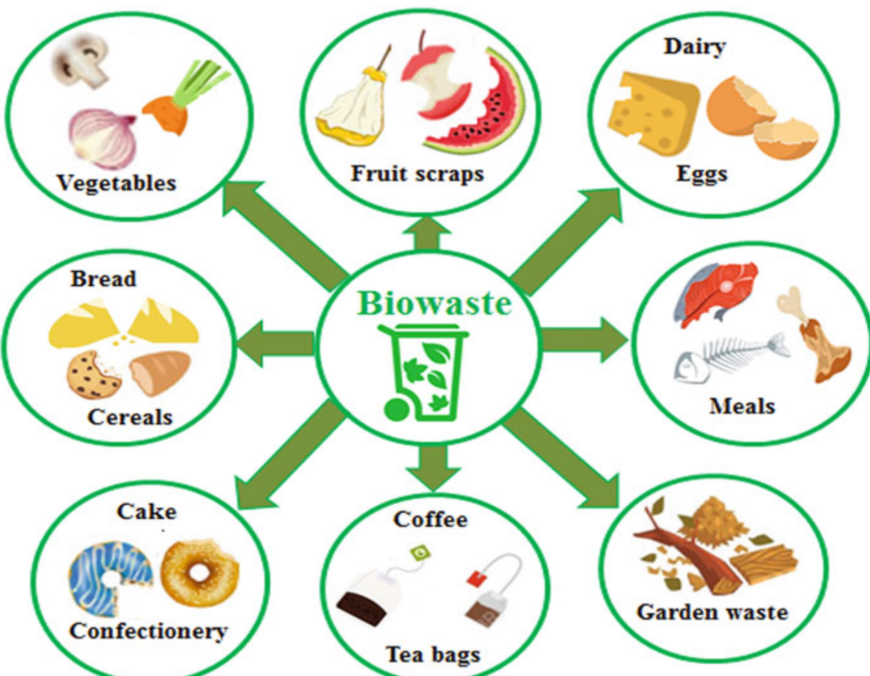


**Fig. 17.1** Different organic components of biowaste

When we compare the composition of biowaste from three countries (UK, Portugal, and Italy), we found that fruits and vegetable debris are the majority (59–69%) (Fig. 17.2). The United Kingdom and Portugal possess waste meals as second components of biowaste, but Italy has pasta and cereals. In Greece, fruit and vegetable constructed 62% of biowaste (Malamis et al. 2015). Hanc et al. (2011) have shown that biowaste's composition is mainly 58.2% of fruit and vegetable debris in the Czech Republic. In the urban municipality, the composition of biowaste is invariable, but in houses, it is influenced by seasonal yard activity (Hanc et al. 2011). Therefore, the biowaste consists mainly of the organic fraction of vegetable origin.

The biowaste is principally composed of cellulose, hemicelluloses, and lignin that are significant plant material components (Ruggeri et al. 2015). The woody and garden waste comprises 70% cellulose and hemicellulose, and 30% lignin, these polysaccharides that are substrate convertible by microbes (Take et al. 2006). In general, this organic vegetable fraction holds two types of fractions, namely, the easily biodegradable organic fraction (starch, simple sugars, lipids, and proteins) and recalcitrant fraction such as lingo-cellulosic polymers (Moretti et al. 2020). Comparing the chemical composition of biowaste between certain countries shows a significant amount of volatile solid that exceeds 90% and a considerable quantity of carbon that can reach 50% (Table 17.1). This carbonic fraction next to the small amount of nitrogen constitutes the necessary nutrient substrate of microbes.

## 17.3 Production of Hydrogen by Microbial Degradation of Biowaste

The appropriate feedstock for hydrogen ($H_2$) production is the biowaste because it is are rich in complex and simple carbohydrates with good biodegradability (Ntaikou et al. 2010). The hydrogen production from biowaste varied between 0.68 and 2.7 mol $H_2$ by hexose molecules (Show et al. 2011). The production of $H_2$ by biowaste-rich carbohydrates, for example, potato and rice are 20 times bigger than the waste contain a large quantity of fat or protein such as egg, fat meat, and chicken skin (Lay et al. 2003). Ghimire et al. (2015) declared that fruit and vegetable wastes are useful feedstocks for $H_2$ production. $H_2$ can be produced by different microbial ways such as direct biophotolysis, indirect biophotolysis, photofermentation, dark fermentation, or microbial electrolysis cells (Nissilä et al. 2014). Among these biotechnologies, dark fermentation and photofermentation are the most suitable methods for producing green hydrogen from biowaste. These biological techniques serve to decrease biowaste volume, greenhouse gas emissions, and nonpolluting energy production like clean hydrogen (Patil et al. 2017).

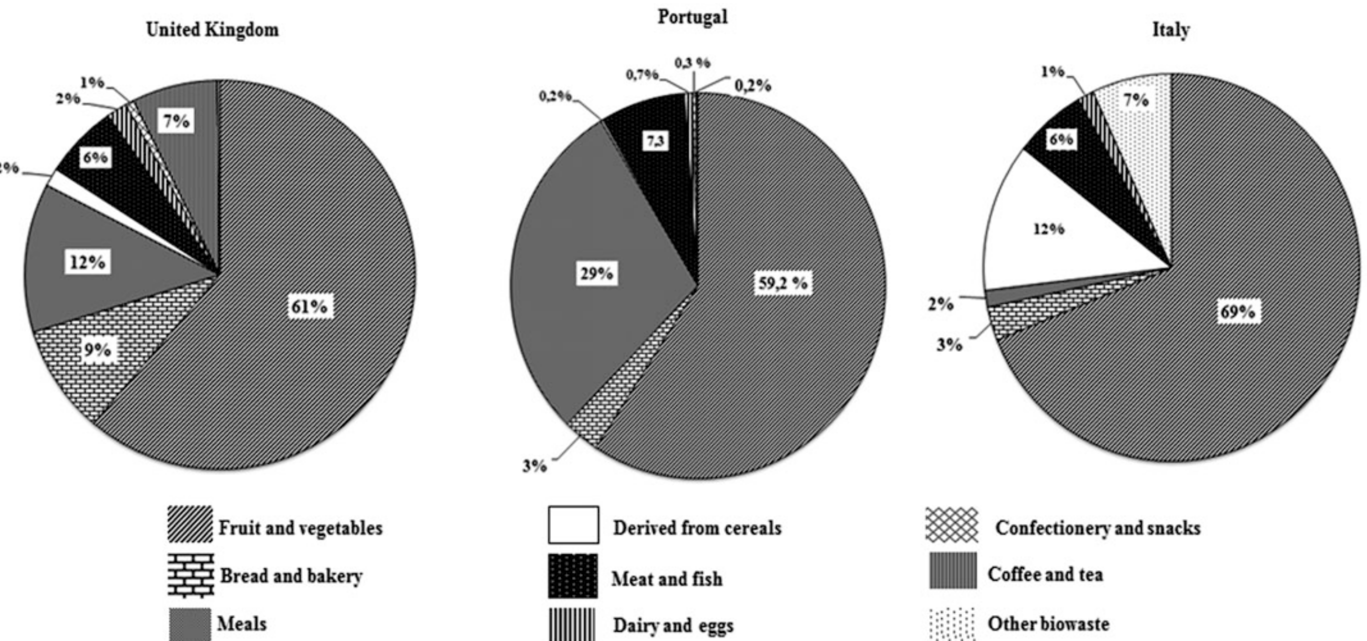


**Fig. 17.2** Biowaste composition of three European countries

**Table 17.1** Chemical composition of biowaste in some countries

| | Dry matter (%) | Volatile solids (%) | Carbone (%) | Nitrogen (%) | References |
|---|---|---|---|---|---|
| French | 19.1–66.2 | 71.7–95.0 | 40.3–52.6 | 1.3–4.1 | Moretti et al. (2020) |
| Czech Republic | 2.3–35.4 | 60–86 | 27.4–55.5 | 1.38–1.78 | Hanc et al. (2011) |
| Greek | 19.3–23.8 | 86.3–88.9 | 53.5–54.5 | 1.88–2.11 | Malamis et al. (2015) |
| Germany | 34.8–44.7 | 59.5–83.0 | 30.6–42.0 | 1.4–1.8 | Mayer et al. (2020) |
| China | 14.9–29.0 | 80.2–92.6 | 46.11 | 3.19 | Li et al. (2016) |
| United Kingdom | 21.7–26.8 | 91.1–94.1 | 48.7–51.0 | 2.9–3.12 | Malamis et al. (2015) |

### 17.3.1 Microbial Photofermentation

Microbial photofermentation is assured by the purple non-sulfur photosynthetic bacterium (PNS) such as *Rhodobacter sphaeroides*, *Rhodobium marinum*, *Rhodospirillum rubrum*, *Rhodobacter sphaeroides*, *Rhodopseudomonas palustris*, *Rhodobacter sulfidophilus*, and *Rhodobacter capsulatus* (Adessi et al. 2017; Anam et al. 2012; Han et al. 2013). Under anaerobic conditions, light energy source, and the presence of organic acids, these bacteria catalyzed the generation of $H_2$ by an enzyme called nitrogenase. In general, during this process, the nitrogenase of *Rhodobacter* sp. can reduce protons to hydrogen and nitrogen to ammonia with electrons and ATP molecules (Ma et al. 2012). We know that biowaste presents an essential fraction of cellulose, lignin, and hemicellulose. It must choose one physical-chemical pretreatment for biowaste to change the intrinsic characteristics of organic matter and convert the carbohydrate polymers such as into fermentable substrates like simple sugar and organic acids (Ruggeri et al. 2015). These simple organic compounds enter the PNS bacterium, and then they are submitted to several catabolism processes such as glycolysis and tricarboxylic acid cycle to reduce $NAD^+$ to NADH (Oh et al. 2013). The electron of NADH produced is transferred to oxidized ferredoxin by a series of membrane-bound in PNS bacteria. So, biowaste's organic acid acts as an electron donor under oxygen-deficient conditions (McKinlay and Harwood 2010). Under the light, the PNS bacterium fixes nitrogen from the atmosphere (Patil et al. 2017). The ATP is also produced by cyclic photophosphorylation with the light source (Adessi et al. 2017). We have the four elements (nitrogenase enzyme, nitrogen fixation, ATP energy, and carbon sources catabolism). Now, the nitrogenase can use three elements to produce $H_2$. Hallenbeck and Liu (2016a) have proposed a global reaction:

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + P_i$$

Under absence or deficient of nitrogen source, nitrogenase can use the proton and ATP directly to produce hydrogen, according to another reaction (Stephen et al. 2017):

$$8H^+ + 8e^- + 16ATP \rightarrow 4H_2 + 16ADP + P_i$$

The *Rhodobacter* species are the most popular microbe in photofermentation, but other microbes used nitrogenase enzymelike *Thiocapsa roseopersicina*, *Allochromatium vinosum*, *Chlorobium vibrioforme*, *Desulfuromonas acetoxidans*, and *Chloroflexus aurantiacus* (Osman et al. 2020). Some researchers have described a global degradation model of microbial photofermentation process from several carbon sources by PNS bacteria that the predicted hydrogen quantity, the specific growth rate of microbes, and measured metabolic fluxes. We cited the model of Gadhamshetty et al. (2008) that was based on 6 of the 17 parameters, then the model made by Golomysova et al. (2010) that is constituted with 287 compounds and 314 biochemical reactions, the model of Hädicke et al. (2011) produced by 143 biochemical reactions, and mathematical models of Akbari and Mahmoodzadeh Vaziri (2017). The models previously can be used as an excellent start-up point to describe biowaste degradation, microbial growth, and hydrogen conversion to accomplish experimental and industrial units.

Bianchi et al. (2010) have used *Rhodopseudomonas palustris* for the degradation of vegetable waste to produce 50.7 ± 2.6 mL $H_2$/L/h. Kapdan et al. (2009) successfully converted wheat starch to hydrogen (46 mL $H_2$/L/h) using *Rhodobacter sphaeroides*. Anam et al. (2012) have recommended using *Rhodobium marinum* for converting sugarcane bagasse and soy sauce waste to 41 and 200 mL $H_2$/L/h, respectively. Photofermentation of the biodiesel industry waste such as glycerol can be achieved to 6.9 mol $H_2$ per molecule of glycerol, which is equivalent to 96% of the theoretical yield (Ghosh et al. 2012).

So, the photofermentation (PF) of biowaste by PNS bacteria has many benefits, (a) These microbes types use a vast diversity of simple molecules issued biowaste pretreatments such as glucose, lactate, malate, acetate, glycerol, succinate, glutamate, propionate, and butyrate, (b) PF can transform these molecules into higher hydrogen output than the dark fermentation process, (c) PNS bacteria have different metabolic ways such as photo-autotrophism, photo-heterotrophism, and chemoheterotrophism, and (d) PF uses low energy consumption; that is, it uses solar energy directly.

### 17.3.2 Microbial Dark Fermentation

Hydrogen production from biowaste by dark fermentation is the best promising method for commercialization between chemical and biological treatment technologies (Rafieenia et al. 2018a). Dark fermentation (DF) is the simplest biotechnology for the production of $H_2$; simultaneously, it generates high ranking yields. It is performed through two groups of microbes, strictly and facultative

anaerobic bacteria. The first group is dominated by *Clostridium* species such as *Clostridium beijerinckii*, *C. butyricum*, *C. thermolacticum*, *C. tyrobutyricum*, *C. thermocellum*, *C. paraputrificum*, *C. acetobutylicum*, *C. saccharobutylicum*, and *C. reinhardtiiand* (Chandrasekhar et al. 2015; Oh et al. 2013). Followed by thermophilic bacteria, let's quote *Caldicellulosiruptor saccharolyticus*, *Thermoanaerobacterium thermosaccharolyticum*, *Thermotoga maritime* (Ntaikou et al. 2010). We can also add to this group rumen bacteria, principally *Firmicutes* sp. like *Ruminococcus albus* (Ntaikou et al. 2009). The second group consists of bacteria optional for oxygen like *Escherichia coli*, *Enterobacter aerogenes*, *E. cloacae*, *Citrobater amalonaticus*, *C. intermedius*, and *Shewanella oneidensis* (Ntaikou et al. 2010; Oh et al. 2013). This last group can grow in a little volume of oxygen but only generate $H_2$ in anaerobic conditions (Singh and Das 2019).

We have already shown that the organic fraction of biowaste is mainly carbohydrates because it is rich in glucose, sucrose, starch, cellulose, hemicellulose, and lignin. These components are considered the most suitable feedstocks for DF because there exists a strong positive correlation between the DF and carbohydrate content of biowaste (Ghimire et al. 2015). The first step of degradation of biowaste for the generation of $H_2$ is the pretreatments. Among the diverse types of pretreatment, Bundhoo et al. (2015) have concluded that heat and acid pretreatments' technologies are popularly used and effective for food waste destined for the dark fermentation process. The second step is the microbial degradation of biowaste by inoculation. This step is different depending on the bacterial species chosen for treatment (Bundhoo et al. 2015; Rafieenia et al. 2018a). In general, this process is based on using the organic molecules of biowaste by the previous microbe as the only origin of energy and electrons. Rafieenia et al. (2018a) have declared that the theoretical amount of $H_2$ is between 4 and 2 mol per glucose molecule. But some researchers such as Woodward et al. (2000) reported that the production of $H_2$ could reach 12 mol $H_2$ per mol glucose. To facilitate the understanding of this biotechnology, we will present for each bacterial group its degradation mechanism:

The first group of strictly anaerobic bacteria mainly the genus of *Clostridium*. These last microorganisms are the predominant microbes in hydrogen production from biomass waste treatment (Chou et al. 2008). Some *Clostridium* sp. can degrade cellulose and plant biomass by cellulosome (it is the multienzyme complex) (Hirano et al. 2016). The glucose produced during the first pretreatment step or released by the biowaste transforms into pyruvate through glycolysis (Singh and Das 2019). *Clostridium* sp. using two enzymes, pyruvate/ferredoxin oxidoreductase, and hydrogenase (Jen et al. 2007; Meinecke et al. 1989; Morra et al. 2016). The first enzyme converted the pyruvate to acetyl-CoA by oxidative decarboxylation. This conversation is accompanied by a transfer of electrons to ferredoxin (Mathews and Wang 2009). So, it is the coenzyme that has an electron receiver role. The electrons present in ferredoxin are then transferred by hydrogenase to protons for the production of hydrogen (Hallenbeck 2005; Nath and Das 2004).

$$\text{Pyruvate} + \text{CoA} + 2\text{Fd}_{\text{ox}} \rightarrow \text{Acetyl} - \text{CoA} + \text{CO}_2 + 2\text{Fd}_{\text{rd}}$$

$$2\text{H}^+ + 2\text{Fd}_{\text{rd}} \rightarrow 2\text{Fd}_{\text{ox}} + \text{H}_2$$

Thermophilic bacteria and some *Clostridium* species have two enzymes, hydrogenase and other enzymes called NADH/ferredoxin oxidoreductase (Ntaikou et al. 2010). During the glycolysis process, the glucose of biowaste is converted into pyruvate by producing two NADH molecules (Hallenbeck 2005). This final product gives up these electrons to ferredoxin by NADH/ferredoxin oxidoreductase (Wang et al. 2010). The ferredoxin will give them in turn to protons by hydrogenase for hydrogen generation (Oh et al. 2013).

The second group of facultative anaerobic bacteria, such as *Escherichia coli* and *Enterobacter* species, has another mechanism. This microbe group has two crucial enzymes (pyruvate/formate lyase and formate/hydrogen lyase) (Hallenbeck 2005; Mathews and Wang 2009). After glycolysis, the pyruvate is converted into two molecules, acetyl-CoA and formate by pyruvate/formate lyase, then the formate molecule is transformed to $H_2$ by formate/hydrogen lyase (Oh et al. 2013).

As we did for the photofermentation, we will present some models for dark fermentation. The microbial degradation models of substrate issued by biowaste in the DK process are useful for estimating hydrogen production, microbial growth, and follow-up of metabolic fluxes. We cited for the strictly anaerobic bacteria, the model of Cai et al. (2010) that allows understanding of the *Clostridium butyricum* degradation of glucose with 34 reactions. The thermophilic bacteria *Thermotoga maritima* is one of the newly interesting bacteria because it is converted into large polysaccharide types, and it has the highest potential production of $H_2$ (Chou et al. 2008). Nogales et al. (2012) presented in silico model with 83 reactions of *Thermotoga maritima*. *Escherichia coli* can generate hydrogen by Kim's model that contains 66 reactions (Kim et al. 2009). Some researchers such as Rafieenia et al. (2018b) have proposed a metabolic network model of culture mixture ($H_2$ generation bacteria, methanogens, and homoacetogenic). This model has 46 reactions propose to study DF by any other mixed microbial inoculum with comparable organic components. We can see that these microbial degradation models in the DF process vary depending on the composition of biowaste, the initial organic substrate, pretreatment technology is chosen, and microbial inoculum type.

The DF of biowaste can be assured by pure and mixed cultures or co-culture. For an example of pure culture, *Clostridium beijerinckii* are generated (108 mL $H_2$/L/h) by converting the food waste (Kim et al. 2008). Perego et al. (1998) have used *Escherichia coli,* and *Enterobacter aerogenes* strains separately for DF of corn starch in order to reproduce respectively 330 and 240 mL $H_2$/L/h. Potato starch is generated 240 mL $H_2$/L/h by using *Enterobacter cloacae* (Kumar and Das 2000). Lee et al. (2011) have grouped in their work more than 30 microbial species of pure strain with their specific substrate transformable into hydrogen by DF. Currently, the mixed-culture is emerging and is an interesting technology for converting biowaste to hydrogen. Some work using bacterial consortia or mixed cultures such as it was done by Patel et al. (2015), have used diverse cultures composed of *Enterobacter*

*aerogenes*, *Proteus mirabilis*, and *Bacillus* spp. for the production of $H_2$ by the degradation of pea-shell waste. Chen et al. (2012) used DF of rice straws by mixed culture (*Clostridium pasteurianum*, *Clostridium stercorarium*, and *Thermoanaerobacterium saccharolyticum*) to produce 7.4 mL $H_2$/g TS. Sharma and Melkania (2017) have used two bacteria, *E. coli* and *Enterobacter aerogenes*, for converting the organic fraction of municipal solid waste to hydrogen with production between 2120 to 1930 mL $H_2$/L of the substrate. In general, the inoculation by mixed cultures in DF of biowaste is an excellent way for industrial applications because it has three reasons, no sterilization process, easy management of the unit, and high conversion of biowaste hydrolysate due to the existence of a large diversity of microbes in the digester unit. Thus, we conclude that a DF of biowaste with a high yield of $H_2$ can be obtained with either pure culture or mixed consortia; this goal depends on the composition of biowaste and the efficiency of microorganisms in the mixed consortia for conversion to hydrogen.

At the end of this part, we noticed that DF is favorable for degradation and microbial valorization of biowaste thanks to certain advantages, (a) This technology is used for various biowaste including the lignocellulosic waste issued by garden residues as suitable feedstocks, (b) It is a low-cost technology because it can produce $H_2$ all day long without light energy, (c) It not only leads to energy recovery but also is one of the cost-reducing waste management methods, and (d) At the end of the process, it produces organic acids such as acetic acid, propionic acid, and butyric acid, which can be used by other biotechnology such as photofermentation, microbial electrolysis cell, cell-free enzymatic system, and anaerobic digestion for increasing the hydrogen yield.

### 17.3.3 Two-Stage Fermentation

The combination of dark fermentation and photofermentation is currently an emerging hybrid technology, and is named a two-stage fermentation. It is based on the significant disadvantage of DF that is the low yield of hydrogen (Das and Basak 2020). This low production in DF is primarily issued due to finals products acetic acid, propionic acid, and butyric acid because the energy is still stored in these products. Photofermentation can use the final organic products of DF to $H_2$ production in lighting conditions, which leads to high additional yield (Pachapur et al. 2019). Therefore, the link between the two processes (DF and PF) is complementary. Hallenbeck and Liu (2016b) have confirmed that this conjunction allows for the nearly complete conversion of carbohydrates to hydrogen. It can produce 12 mol of $H_2$ per 1 mol of glucose:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2$$

Some studies have confirmed that integrating DF to PF generates an immense amount of $H_2$ than separately process from the same quantity of biowaste. Laurinavichene et al. treated the potato waste through three hydrogen production

processes; thus, they obtained 1.4, 3.9, and 5.6 $H_2$ per mol of hexose during DF, PF, and two-stage, respectively. The sugar industry residual, such as beet molasses that contains a high quantity of sucrose (50%), was subject to two-stage fermentation. In the beginning, there was DF by *Caldicellulosiruptor saccharolyticus*, followed by PF with *Rhodobacter capsulates* (Özgür et al. 2010). This technology allows an increase of the production of $H_2$ from 4.2 mol $H_2$ in only DF to 13.7 mol $H_2$ per mol sucrose, which is equivalent to 58% of the theoretical production (Özgür et al. 2010). Yang et al. (2010) described the role of two-stage fermentation in hydrogen generation from corncob. This food waste has undergone an acid pretreatment for the hydrolysis, and then it is transferred to the dark phase that produces 120 mL $H_2$/g-corncob by converting the reducing sugars and oligosaccharides; when the substrate reaches the second illuminated phase, it is converted to 713 mL $H_2$/g-COD from acetic acid, butyric acid, butyl alcohol and ethanol issued by the dark phase (Yang et al. 2010). So, the two-stage fermentation is the high microbial degradation of biowaste into hydrogen, which is not reaching by DF or PF alone.

## 17.4 Conclusion

Every day, the biowaste is produced by daily household activities such as cooking, kitchen, food, or garden waste. Their production continues to increase, leading to the increased risk of destruction of our planet's natural ecosystems. In addition to the lack and exhaustion of sources of energy, the conversion of biowaste into hydrogen by microbial degradation has become a necessity. This chapter has shown three techniques (Microbial photofermentation, Microbial dark fermentation, and Two-stage fermentation) that make it possible to recover this waste by producing clean energy and reducing their volumes and their impact on the environment.

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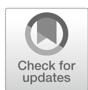

# 18 Microorganisms and Soil Bioremediation: An Environmental Approach

Anuradha and Jagvir Singh

**Abstract**

Organic pollutants, toxic metals, hydrocarbons, such as various sources, have given rise to a serious global problem of soil contamination that has affected human life as well as all the surrounding ecosystems which have created an imbalance in the environment. Our soil, water, air, and forests are all getting polluted. Due to this crisis, floods, droughts, and other natural problems are arising for biodiversity. Now, time is warning that we have to make efforts to maintain the purity of natural sources and their existence.

Microorganisms remediation is a technique that, while being simple, inexpensive, and easy, can prove effective in controlling environmental pollution and saving the life of the earth. Technological and industrial progress has polluted the most agricultural land, which itself is working to take human life towards a great danger. It has inherent capabilities of erosion or alteration of contaminants for hazardous materials. Microorganisms have a major contribution to maintaining the purity of natural sources. The microorganism has been in existence for over 3.5 billion years due to its genetic and physical structure. There are about $10^9$–$10^{12}$ per g microorganisms found in the upper surface of the soil. Bioremediation can be a safe, inexpensive, favorable, sustainable, and beneficial technology in the future.

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**Keywords**

Bioremediation · In situ remediation · Ex-situ remediation · Microorganism · Soil microorganisms · Pollution diagnosis · Biodegradation · Land

## 18.1 Introduction

Pollution is dangerous for our health as well as our environment which has affected every life be it human or wildlife and plant world also on the earth planet. Environmental pollution is a serious problem facing the world where bioremediation has been a useful technology for many decades to reduce it in an effective and easy way (Timmis and Pieper 1999; Cases and de Lorenzo 2005; de Lorenzo et al. 1998). Bacteria, fungi, algae are some microorganisms used in bioremediation. It is capable of reducing environmental waste. Contamination can be reduced significantly by degrading or stabilizing organic and inorganic hazardous materials. With the use of microorganisms in the treatment of contaminated agricultural land with many techniques, its pollution can be reduced (Haro and de Lorenzo 2001).

These microorganisms are characterized by jumping genes, which make them easily resilient even under various adverse conditions and can be toxic at high temperatures, alkalinity, and acidity. Even under these circumstances, they adapt themselves in such a way that they provide sufficient food (Abhilash et al. 2016; Alexandratos and Bruinsma 2003; Anand et al. 2016). Under this process, they are now divided into small molecules such as less harmful or less toxic. Increasing excess nutrients in the soil increases the rate of biodegradation because the extra nutrients stimulate soil microbes (Araujo et al. 2009). As the mycelia plant spreads, fungi are microorganisms that spread like these plants and can store heavy metals in their cytosol. These microorganisms can naturally treat diseases caused by natural pollutants (Jacob et al. 2018; Lima et al. 2005).

Human-caused pollutants, genetically altered microbes, can be used in soil remediation treatment. In its treatment, various types of physical, chemical, and biological approaches are attempted. Biological approaches can be a means of treatment that does not have limitations such as high cost, inefficiency, and changing the natural ecosystem. The technique uses microorganisms to destroy or decompose harmful chemicals from various industries known as bioremediation techniques. The 2012 Olympic park in London can be understood as one of the best examples of brownfields.

After hundreds of years of industrial activity, bioremediation technology succeeded in cleaning up 1.7 million cubic meters of heavily polluted soil to transform this brownfield site into a playground surrounded by 45 ha of wildlife habitats that are by far the greenest and Olympic and the field marked as the most sustainable for Paralympic sports is possible only with bioremediation techniques.

Petroleum components are becoming a problem for contaminated land that results from its exploration, accidents, transportation, and waste disposal or leakage from storage sites or industrial facilities. Iran is a major oil-producing country. According

to one study, around 1,500,000 $m^3$ of soil from its Tehran oil refinery was found to be contaminated with hazardous chemicals found in its crude oil such as benzene and its substitutes which is fatal for any ecosystem. This chapter mostly covers the agricultural land pollution and microbes are the best solution for this pollution (Dong et al. 2013; Fu 2014).

The fungus is a type of microbe that colonizes a wide range in every heterogeneous environment and has the ability to adapt and live in any conditions. Furthermore, they can easily colonize both organic and abiotic surfaces by decomposing chemical substances (Chowdhury et al. 2008; Cunningham and Philip 2000). Fiberglass fungi show certain characteristics that make them more suitable for soil bioremediation than yeast and bacteria. It has the most important types of growth favorable for soil colonization, translocation of nutrients, and water. Mycoremediation represents this biological tool for reducing, altering, or stabilizing environmental pollution.

Saprotrophic basidiomycetes are microbes that use dead organic matter such as wood-decaying fungi. Among them, white-rot fungi are known to have a leading role in biodegradation. White-rot fungi can efficiently degrade both wood impurities and cellulose biopolymers until complete mineralization (Don and Pemberton 1981). While some white-rot fungi which are most capable of degrading pollutants are *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Trametes versicolor*, *Lentina edodes*, *Irpex lacteus*, *Agaricus bisporus*, *Phanerochaete chrysosporium* of these fungi (Ahmad et al. 1997).

*Phanerochaete chrysosporium* has immense potential compared to other fungi in degrading viruses such as carbon dioxide and insoluble compounds.

Metal pollution whether it is water or land has become a common problem. These hazardous metals and heavy metals are used extensively in many industrial processes such as electroplating, textiles, paints, and lither to meet the needs of our lives. Once it has been observed that this type of wastewater is used in agriculture, besides damaging the ecosystem, entering the food chain, and affecting public life (Evans and Furlong 2003).

Plurotus, Aspergillus, Trichoderma are the types of fungi that have proved decisive in extracting heavy metals like lead, cadmium, nickel, chromium, mercury into the ground wastewater.

In the case of polycyclic aromatic hydrocarbons, fungi are highly effective in highly stable, polycyclic aromatic rings and marine environments, with complex organic compounds fused. Other toxins are able to degrade fungal harmless compounds, including petroleum fuels, phenyl in wastewater, polychlorinated biphenyls in contaminated soils using *Pleurotus ostreatus*, two species of the Ecuadorian fungus Pustolus, such as polyurethane and degenerative conditions (Fetzner and Lingens 1994; Finley et al. 2010). On the other hand, pesticide pollution can be long-term and significantly impact decomposition processes, thus making nutrient rotation and degradation costly and difficult (Bennet et al. 2002; Witte and Pfannkuche 2000).

The most commonly used fungus to aid the erosion of such substances is white rot. From a future perspective, not only the insecticide fungi need to isolate and

characterize the newly pesticide mineral but also characterize the chemical, toxicity, and environmental disorders of the metabolites that are generated during the fungicidal biodegradation of the insecticide (Fritsche and Hofrichter 2008). Bioremediation can be a technology used in the future that will help reduce and remove the pollution produced by us to provide clean water, air, and healthy soil for generations to come (Gianfreda and Rao 2008).

## 18.2 Case Study

Abbas Alemzadeh and some of his researchers (2016) were used to isolate bacteria for bioremediation of petroleum polluted soils. This experiment used five soil samples, three of which were contaminated with crude oil and gas oil, and two that were without any external contamination. They then selected seven strains and created a bacterial standard succinate medium (SSM) in which 2.5% v/v kerosene was cultured using it as a carbon source. Another bacterial standard succinate medium (SSM) which was free of C, N, and S. It now took 20% v/v kerosene to test the potential of isolates for utilizing kerosene as their sole source.

In this experiment, he found that four cultures grew more. The analysis showed that one of the two samples containing crude oil contaminated soil, with no external contamination, took 69% of 5% v/v kerosene to degrade 7 days. Whereas in other similar samples, 48% and 42% of 5% v/v kerosene were degraded, with a degradation efficiency of 38% in 7 days without soil contamination second sample. After analysis of the 16S rRNA gene revealed that the first sample revealed the genome structure of *Citrobacter sedlakii*, while the second sample of both experiments was able to display the contaminated *Enterobacter hormaechei* and unpolluted *Enterobacter cloacae* bacterial genome structure (Mojarad et al. 2016; Aghamiri et al. 2011).

## 18.3 Land

It refers to the surface, whose constituent soil, vegetation, and landscape shape are characteristic. It is measured in units of area such as acre, hectare, bigha, or drain. Land is one of the three natural resources, the other two being water and air, which are essential for the existence of life on this earth (Compant et al. 2016). Apart from agriculture, the land has many uses such as forests, pastures, recreational facilities, outdoor structures, roads. Land is the most valuable resource for the sustainable livelihood of the farmer. He plows the land and grows food crops, fruits, vegetables, and other crops on it. Depending on the nature and usage, there are many varieties of land such as agricultural land on which seasonal, annual, or multiyear crops like gardens are planted.

The forest land on which it is built. Barren land is not suitable for agriculture due to any bad luck. Anurvar land that is unproductive such as the Usar land. The moist land which is often waterlogged and remains moist most of the time (Ashmarin et al.

1992). The low land which is in the lower area from which no drainage is carried out and the land is usually waterlogged. Fertile land that is on a high place and has good drainage. Irrigated land with assured means for irrigation. Dry land which does not have irrigation and depends on very little rainfall (less than 500 mm).

### 18.3.1 Soil

The soil keeps the earth covered in a thin cover and, with the appropriate amount of water and air, gives life to the plants. Alluvial soil is found in most (43.4%) area in India and black soil, red soil, and laterite soil are found in other soils. Soil formation is a continuous long natural process. It takes an estimated 3600–6000 years for the construction of surface soil (15 cm deep).

Soil is the upper layer of the earth which provides a natural medium for plant growth. This upper layer of the earth is a conglomerate mixture of mineral particles and organic matter which has been formed in several millions of years and without its life on this earth is impossible to exist. Soil as an integral component of land is a component of the life support system. Soil is a critical resource to support plant growth (Lehmann and Kleber 2015). The soils of different farms may vary in appearance, characteristics, and productivity according to their origin and management, but they all perform equally important tasks in agriculture and food security, forestry, environmental protection, and quality of life (Alloway 1995).

### 18.3.2 Composition of Soil

The mineral matter, organic matter, including living organisms, water, and air are the component of any soil. Dry matter soils have the highest mineral content of about 95–98% (45% by volume) and organic matter is 2–5% (5% by volume) with living organisms. The rest of the solid particles contain water and air in the foliage (50% volume), whose mutual proportions vary.

#### 18.3.2.1 Minerals

The quantity and composition of mineral matter are variable. There are generally pieces of rocks and different types of minerals, some minerals are of large size, but others like dead particles are so small that they cannot be seen even with ordinary microscopes (Ali Elredaisy 2010).

#### 18.3.2.2 Biopsy

There are two components of organic matter present in the soil.

(a) Partially decomposed plant and animal remains.
(b) Stable humus which is black or brown in color and temperate in nature.

Living organisms like microorganisms, earthworms, pests, and others are cultivated with organic matter for their habitat and food. Organism affects the color, physical properties, soil nutrient supply, and adsorption capacity of the soil (Anderson et al. 1993; Armstrong et al. 1992).

The amount of organic matter in most Indian soils is very low, ranging from just 0.2–2% (by weight), but it has a great impact on soil properties and plant growth. Firstly, organic matter acts as a "connector" of mineral material, which develops the fine condition of productive soils. Secondly: organic matter is the major source of two important nutrients: nitrogen and sulfur. Thirdly: Biomass has an important role in maintaining good physical condition of the soil. It holds water capacity and aeration of the soil is controlled. Lastly, the main source of energy for soil microorganisms is bio-organisms, whose activity makes the soil an active organ in the local and global ecosystem (Alexander 1977).

#### 18.3.2.3 Soil Water

Soil water remains in the foliage and is held by solid particles (mineral matter and organic matter) with varying force according to the amount of water. Soil water contains soluble salts which form the so-called soil solution which is important as a means of supplying nutrients to plants. Encourages many physical, chemical, and biological processes in the soil (Atlas 1996).

#### 18.3.2.4 Soil Air

Biological air or oxygen is essential in soil. Its requirement is met with soil air. The gaseous phase of the soil paves the way for oxygen penetration which is absorbed by the roots of microbes or plants in the soil (Backer et al. 1997). At the same time, it also provides a way for the carbon dioxide released by the soil microbes and roots of plants to exit the soil and called soil aeration. Soil aeration becomes critical when the amount of water in the soil is high because air is displaced by water from the pore. The composition of soil air is dynamic (variable) according to the change in activity of soil water and microorganisms (Alexander 1982; Amadi et al. 1996).

## 18.4 Bioremediation Approaches

This technique is very simple and expensive in which microbes are eliminated from the atmosphere. It is mainly useful in breaking down naturally occurring microenvironmental pollutants. The main objective of this technique is to break down complex harmful chemical compounds into less harmful chemicals and reduce toxins. It is divided into two parts to understand the presented technique easily and its utility. In situ bioremediation and ex-situ bioremediation are its types (Nikolopoulou et al. 2013).

### 18.4.1 In Situ Bioremediation

In situ bioremediation desired organisms are carefully familiarized with the environment and their progress is tracked until the site is cleaned, or they can use them off-site. Used to describe 'on-site' in situ. It means local or in position. The term is used to describe various processes in many areas, including biological processes. In biology, in situ refers to the examination at the exact location where the species occurs (Zhao et al. 2018; Bedient and Rifai 1993).

### 18.4.2 Ex-Situ Bioremediation

Ex-situ bioremediation is used to clean contaminated soil and other materials that have been removed and isolated so that they are inert and safe to handle. This technology reduces the buildup of toxins and hazards in landfills and isolation facilities, leaving the earth cleaner for future generations. Examples of ex-situ bioremediation processes including manure, soil bio-piles, landfarming, slurry reactors (Bunge et al. 2003).

In situ bioremediation, participants are broken at the site of origin. Some contaminants are removed from the contamination site. Bioremediation is a biotechnological approach to control environmental pollution. The natural biodegradable potential of biological agents such as bacteria, fungi, plants is discovered in bioremediation (Banerjee and Dey 1992). Some examples of this technique are phytoremediation, bioventing, bioleaching, land-forming, bioreactors, composting, bioaugmentation, and biostimulation (Bashmann and Kinzel 1992).

### 18.4.3 Principles of Bioremediation

In the process of bioremediation, microorganisms use contaminated substances in nature as their food, which has the potential to pollute any environment. Microorganisms are mainly bacteria–microbes that play a major role in the bioremediation process. The reason for this is due to the specific enzymes found in microorganisms and their micro size which make them compatible with bioremediation. This service has been served by microorganisms for billions of years, without which the earth would literally be buried in garbage. We know that microorganisms have specific and unique roles in the detoxification of polluted soil environments and this process has been known by bioremediation or bio-reclamation.

## 18.5 Biodegradation

Microorganisms have the ability to destroy the organic materials present in the environment. They are recycled nutrients in the soil. Almost all biochemical cycles are driven by indigenous microbial populations in soil. Microorganisms degrade

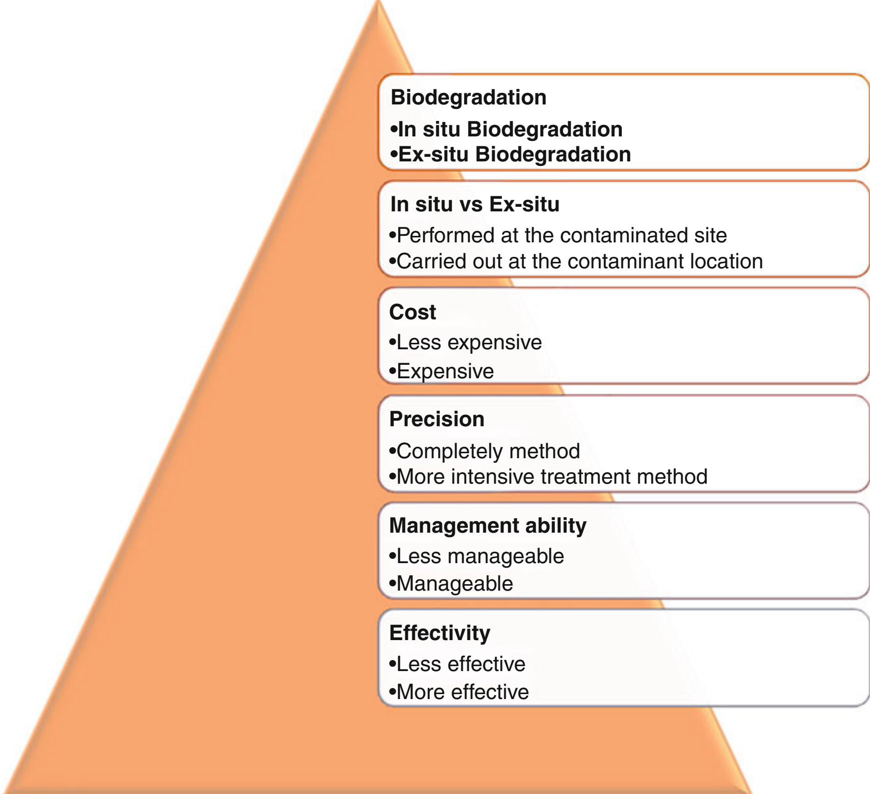


**Fig. 18.1** In situ and ex-situ are types of biodegradation and have certain characteristic properties that can be easily understood on the basis of variation (Haritash and Kaushik 2009)

organic material for their growth and metabolism. Biodegradation and abiotic degradation are the types of degradation. In biodegradation process, the action of microorganisms such as bacteria and earthworms can break down into nontoxic substances. These substances have the unique ability to change their form and structure over time and change into harmless form. These are substances that do not pollute the environment. For example, spicy food, vegetable peels, tea leaves, wood, grass, paper, leather, cotton, cattle dung may be some examples (Ghazali et al. 2004; Haritash and Kaushik 2009).

In abiotic degradation process, it cannot be broken into harmless substances by any biological processes. Nonbiodegradable substances remain unchanged for a long time. Some of them remain dormant and begin to accumulate in our surroundings, while others cannot easily be made less toxic and therefore continue to pollute the environment like glass bottles, metal cans, polythene bags, synthetic fibers, radioactive wastes, plastics, and pesticides such as DDA. As a result, complex organic

**Table 18.1** The comparative study of their character is tabulated (Ghazali et al. 2004)

| S. no. | Character | Impact | |
|---|---|---|---|
| | | Biodegradation | Bioremediation |
| 1. | Biodegradation vs bioremediation | Decompose the process of organic material by microorganisms. | Waste management technique where microbes clean on contaminants site |
| 2. | Nature of process | Instinctive processed without human intervention | An engineered process with human intervention |
| 3. | Motion | Slow | Fast |
| 4. | Management | By nature, | By human |
| 5. | Effect | Both beneficial as well as harmful | Beneficial effect |
| 6. | Duration and place | Occurs everywhere in environment | Occurs at the contaminated site |
| 7. | Expertise required | No need for experts | Required for design and implement |

materials are converted into carbon dioxide and water. Figure 18.1 and Table 18.1 show the character and impact of biodegradation and bioremediation, respectively.

Soil degradation is a serious environmental problem. Generally, degradation of soil quality due to unscientific management and unnecessary use of soil for agriculture, industry, and urbanization is called soil degradation. Soil is the fundamental natural resource and the basis of all surface life. Therefore, soil degradation is necessary to be disposed of in order to be healthy in life.

## 18.6 Microorganism

The number of microorganisms living on and inside our body is ten times more than the total cells present in the body. Microorganisms can be divided into five parts as shown Fig. 18.2.

### 18.6.1 Soil Microorganisms

Retired professor and author of the University of California Veteran Davis is considered a master of this subject. He calls microorganisms “bug sorting.” It is the science of classifying different types of microorganisms. The world of microorganisms is very large. Microorganisms include all bacteria and archaea and almost all protozoa, in addition to some fungi, algae, and chakradhar (rotifer). Microorganisms are ubiquitous. These are found in soil, water, air, inside our bodies, and other types of animals and plants where life is not possible, such as deep, thermal chimneys within geysers where the temperature rises to 100 °C, deep in the soil, several meters below the ice layers, and even in places like the highly acidic environment.

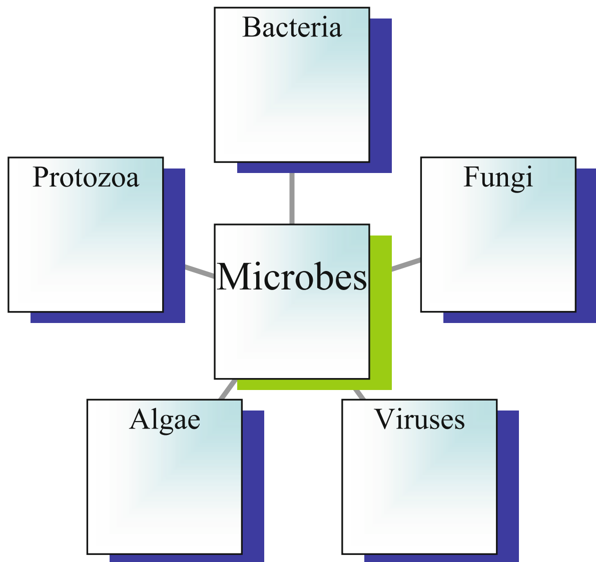


**Fig. 18.2** Classification of microorganisms as five categories

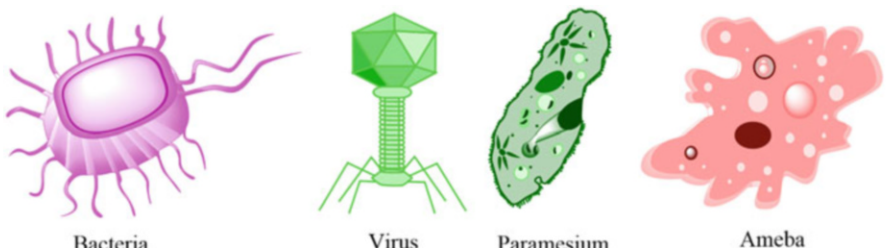


**Fig. 18.3** Different types of soil microbes which are normally found in soil and are useful to plants (Leung 2004; Nies 1999)

Soil is an excellent culture medium for the growth of various types of microorganisms, including bacteria, fungi, algae, protozoa, and viruses, as well as many nematodes and insects are present. The colony of microorganism is usually found in 6-12-in. upper level of soil in the majority which decreases with increasing depth. Number and type of microorganisms depends on the nature of the earth such as depth, humidity, organic matter and temperature. The following types of microorganisms are mainly found in soil (Fig. 18.3):

#### 18.6.1.1 Bacteria

Different types of bacteria are found in soil, some of which are autotrophic. They use Inorganic compounds for their growth and energy. Most bacteria found in soil are heterotrophic which uses organic matters more and more. These bacteria are of the

order Eubacteriales and Actinomycetales. The groups of microorganisms found under Actinomycetales are found in general, Streptomyces, Nocardia, and micro monospora. Bacteria perform important functions like nitrogen binding in the soil. Among them is Clostridium, Agetobacter, Rhizobium, Nitrosomonas, Nitrobacter (Garcia et al. 2015; Glick 2012). Some sulfur bacteria oxidize sulfur-containing gases and convert them into sulfates. It is used by plants or expelled from the land by rainwater. In this process, sulfuric acid is also produced, which solubilizes insoluble soil particles (calcium, phosphate, magnesium, carbonate, calcium silicate, tricalcium phosphate) and make them available for plant use. An important example of sulfur bacteria is Theo Bacillus. In this way, minerals found in the soil like phosphorus also convert bacteria into iron (Goswami et al. 2016).

#### 18.6.1.2 Fungi

Under this, molds and large fleshy fungi like mushrooms come. The development of fungi in soil depends mainly on acidic medium and aerobic conditions on the surface. Fungi in the soil are found in innumerable numbers in both mycelium and spores. These fungi are active in the decomposition of cellulose and lignin present in plant tissues. Mold mycelium penetrates the soil to form a network consisting of soil particles as well as the accumulated water structure so that its importance for agriculture is determined. Along with this, fungi develop the physical condition of the soil. Fungi are found predominantly in the soil of vineyards and orchards. In addition, members of the sugary fungus (members of the Phycomycetes) are humus fungi, parasitic fungi living in the soil, predatory fungi, sting fungi, Deuteromycetes. Some yeasts are also found in many species such as Candida, Crypto caucus, Torula fungi decompose complex organic materials into simple substances, which increases the fertility of the soil. Protein-rich substances are also degraded by fungi that increase the amount of ammonia and simple nitrogen in the soil. The fungus helps in building humus in the soil.

#### 18.6.1.3 Algae

These are generally found on the surface of hydrated land where photosynthetic reactions are possible. They are mostly members of green algae like Chlorophyceae and mainly members of Bacillariophyceae in Diatoms. They are found in large numbers in soil. Nostoc, Anabena, algae have nitrogen stabilization capability. They stabilize the nitrogen of the atmosphere. Blue-green algae are used to transform the weeds into fertile lands. Nostoc is the best example of this. Some algae are used in the manufacture of manure.

#### 18.6.1.4 Protozoa

In terms of size and complexity, protozoa come as the next rung, which was formerly considered to be animals. Now, these different types of one-celled organisms are placed in a separate category called the Kingdom of Protista, how they move. On this basis, they are further divided into categories. Some of these more familiar protozoa are—amoeba, Paramecium, Euglena, Trypanosoma, and Plasmodium. Most

protozoa are not harmful. Some are helpful to humans, but some live on pathogens as pathogenic parasites.

## 18.7 Environmental Application of Microbes

The soil has mainly fungi, which are present and they have an important role in the decomposition of substances. Their main function is to convert harmful substances into nutrients. The best example of this is composting, which is purely an organic process, in which biological decomposition of organic matter in airborne conditions occurs by fungi. Fungi help in making compost by the decomposition of the decomposed substances. He is known as the friend of farmers for keeping his environment pure and making the soil fertile (Leung 2004; Nies 1999). By burning crop residues, the main nutrients present in it are destroyed by burning nitrogen, phosphorus, and potash, and many toxic and poisonous gases like carbon dioxide, mono oxide, methane, benzene, etc. contaminate the environment, causing the crop. The residue should be converted into compost, which leads to the addition of bacterial carbon in the soil (Satyanarayana et al. 2013).

### 18.7.1 Pollution Diagnosis

Large amounts of water are used for irrigation in agricultural work. Except for the part of the water used in agriculture that evaporates or is absorbed by the land, the rest flows back into the water streams. In this way, this water is poured into the natural or chemical fertilizers including pesticides, organic matter, soil, and its residues, and mixes in the water bodies (Silver and Phung 1996).

### 18.7.2 Effect on Water Organic Materials

Sewage or similar contaminated water in which organic matter is present in large quantities, combined with clean water, increases their biologic oxygen demand load, i.e., the water found in the waters of organic materials which are biologically degraded. Also, due to the presence of harmful bacteria in the drinking water, there is a risk of many skin diseases including diarrhea, hepatitis, jaundice (Baker and Herson 1994).

### 18.7.3 Role of Mold

The new idea of making manure after the action of organic decomposition from fungi is very useful in modern agriculture nowadays. For this, the compost culture of lignocellulolytic fungi is made (Gupta et al. 2013). This compost improves the nutrients present in the soil; hence it is used as organic manure.

### 18.7.4 Benefits of Using Compost

Due to the use of compost, a large amount of organic matter is stored in the soil, which is of great benefit. Constant use of compost increases the soil's ability to absorb air and water inside itself. The use of compost improves soil health significantly (Helga and Sergiu 2012). Minerals and microorganisms can be grown simultaneously to increase the quality of compost. A special feature of this compost is that it increases the water absorption capacity of the soil by consuming water four times more than the unweight weight. To keep the environment healthy, farmers should never destroy or burn agricultural waste (Singh and Tripathi 2004). To protect against pests in crops, farmers spray chemicals, pesticides, which are hazardous to the environment and humans. Along with this, the effect of these pesticides is gradually reduced. In such a situation, farmers can undertake microbial management to prevent harmful pests (Kubicek and Druzhinina 2007; Satyanarayana et al. 2012).

## 18.8 Conclusion

Biodegradation is a continuous process that describes the ability of microorganisms present in the environment to decompose. Microorganisms such as bacteria and fungi are known decomposers. Most pollutants are completely degraded by oxygen assisted aerobic biodegradation. Anaerobic biodegradation performs its process in oxygen-absent. It uses the biodegradability of microorganisms to speed up the process of environmental cleanup. This can be done in two ways. In situ bioremediation, contaminants are treated at a single site using a biology system. In ex-situ bioremediation, contaminants are treated at another location from the parent site. This is a significant difference between in situ and ex-situ bioremediation. Bioremediation processes are cost-effective, safe, and nature-based methods over chemical and physical methods.

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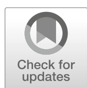

# Applications of Microbes in Bioremediation of Water Pollutants 19

Sinem Diken Gür, Monireh Bakhshpour, and Adil Denizli

**Abstract**

Bioremediation uses microbial metabolism in the presence of optimum environmental conditions to remove toxic pollutants from the environment. Bioremediation of contaminated groundwater or soil is currently the cheapest and the least harmful method to manage the polluted environment and recover contaminated soil. Also, bioremediation is used as a successful and attractive cleaning technique for a polluted environment. Bioremediation and natural attenuation are also seen as a solution for emerging contaminant problems. Microbes are very helpful to remediate the contaminated environment. The number of microbes including aerobic, anaerobic bacteria, and fungi are involved in the bioremediation process. This chapter describes the bioremediation approaches such as bioattenuation, biostimulation, bioaugmentation as well as techniques such as immobilization of microorganisms to improve the microbial degradation capacity. Additionally, the types of pollutants, the mechanisms maintained by microbes removing pollutants, and the applications realized by using various types of microbes are discussed. We focus on these technologies and efforts are directed toward eventual manipulation of the processes of remediation all geared towards making bioremediation technically and economically viable for comprehensive treatment of petroleum hydrocarbon contaminated water source.

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**Keywords**

Bioremediation · Biostimulation · Bioaugmentation · Combined technologies · Microorganisms

## 19.1 Introduction

In the past few decades, environmental pollution caused through the release of hazardous organic and inorganic compounds have been occurred, depending on agricultural processes and industrial growth carried out to meet the increasing needs of humanity. These issues were frequently encountered in developing countries due to the rapid industrialization without a regulatory system to prevent pollution (Dangi et al. 2019). The major pollutants released depending on anthropological activities are heavy metals, pesticides, hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), dyes, pharmaceutical compounds, and industrial solvents such as benzene, toluene, and xylene (Deblonde et al. 2011; Xu et al. 2016). Furthermore, according to technological advances that occurred in recent years, the production processes and the nanomaterials usage for several applications are also constituting a risk for human health and ecosystems by causing a loss of biodiversity (Chen et al. 2017). After the wastes are released to aquatic sources, chemical pollutants are transported to the marine environment via rivers and participate in the water cycle and can affect all parts of the environment (Dangi et al. 2019). Wastewaters produced during industrial processes are harmful for both environment and humans globally. To maintain a clean environment for living systems, the release of toxic compounds from waste streams should be prevented by remediation methods. For several decades, some conventional remediation approaches (solidification, evaporation, reverse osmosis, oxidation–reduction reactions, ion-exchange techniques, and chemical treatment, etc.) have been used despite the limited features of these techniques (Porcelli and Judd 2010; Dasgupta et al. 2015). The main restrictions about these approaches are being expensive, high reagent requirements, and generation of some harmful seconder pollutants during the remediation process. On the other hand, biotechnological processes which utilize microbes (bacteria, fungi, and algae) that have the capability to degrade or neutralize pollutant compounds have been widely applied for treating water pollutants (Zhang et al. 2018; Bouabidi et al. 2019).

## 19.2 Bioremediation

Bioremediation is a cost-effective, eco-friendly, and effective method for the treatment of water sources contaminated with pollutants by utilizing natural biological mechanisms of microorganisms. The bioremediation process realized with microbes relies on enzymes and metabolic pathways of these microorganisms. Microorganisms can use harmful pollutants as an energy and a nutrient source for

their metabolic processes and at the same time convert them into less harmful substances (Abatenh et al. 2017). In effective microbial biodegradation, enzymes belonging to the groups of oxidoreductases, lyases, ligases, isomerases, hydrolases, and transferases which have a wide degradation capacity are involved in removing harmful pollutants or converting them into nonhazardous compounds that are safe for ecosystem (Kumar et al. 2011). However, the success of bioremediation depends on some factors such as the environmental conditions of affected side (pH, temperature, redox potential, $O_2$ content, and the ability of microorganisms that is used). In most aquatic systems, the efficient bioremediation can occur in the pH range of 6.5–8.5. In addition, degradation processes based upon biological enzymes can carry out most effectively at the optimum temperature, so the temperature changes are affecting the rate of microbial activities (Uqab et al. 2016; Liu et al. 2017). As the $O_2$ requirements of microorganisms vary, degradation can occur under aerobic or anaerobic conditions depending on the feature of the utilized microorganism. As effective bioremediation can occur only at environmental conditions that are suitable for microbial growth and activity, sometimes manipulations can be realized on environmental parameters to enhance microbial growth and degradation process.

### 19.2.1 Types of Bioremediation

There are several treatment techniques and technologies applied for achieving bioremediation of toxic compounds. Bioremediation processes are principally performed as in situ or ex situ. While treating the pollutant at the contaminated site is referred to as in situ, ex situ is stated as the transport of polluted material elsewhere before treatment. In the method of in situ bioremediation, pollutants are degraded under natural conditions to $CO_2$, water, or less harmful compounds. Although, both in situ and ex situ methods are based on the metabolic activity of microorganisms, degradation rate, efficiency, and stability of process outcome of the methods are different from each other (Megharaj et al. 2011; Verma and Kuila 2019). The proper bioremediation method performed in order to achieve pollutant removal from the environment is determined by considering various factors like environmental conditions, the number and type of indigenous microorganism population at the site, lastly quantity and type of pollutants. The basic bioremediation approaches are based on (1) bioattenuation that defines as a natural biodegradation method, (2) biostimulation that depends on the addition of some compounds to stimulate the microbial degradation, (3) bioaugmentation involves preadaption of microbial population by exposure to pollutants, and modified by genetically to enhance their degradation capacity and after that inoculate to the contaminated sites (Madsen 1991). In addition to these methods, immobilized microorganisms can be utilized to overcome the limitations of microbial biodegradation such as low operational stability of microorganisms and separation difficulties after-treatment process. The use of cell immobilization techniques in the water treatment provides advantages when compared with the use of free cell by allowing reuse of cells, high resistance capability to environment conditions and toxic compounds, mechanical

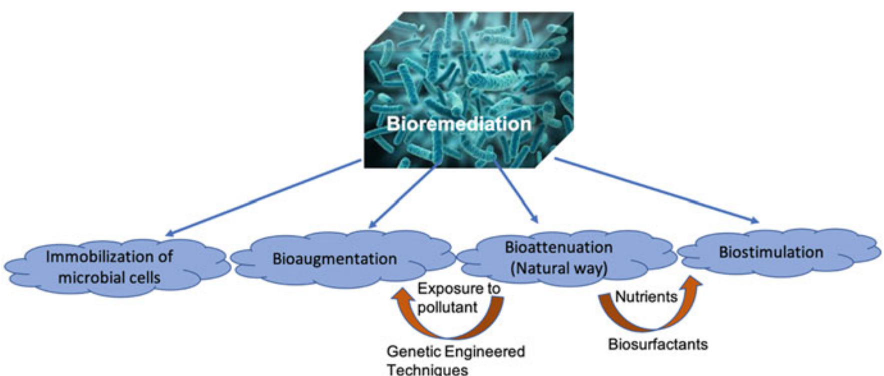


**Fig. 19.1** Types of bioremediations performed by microorganisms

strength, and high stability (Partovinia and Rasekh 2018). The types of given bioremediations are demonstrated in Fig. 19.1.

#### 19.2.1.1 Bioattenuation (Natural Attenuation)

During this process, biotransformation of pollutants to less harmful components and immobilization of pollutants are largely carried out by microorganisms. According to environmental conditions at the contaminated site, the required time for bioattenuation can be varied. Bioattenuation is accepted as an effective method for the bioremediation of many contaminated areas that may not require aggressive methods as well as economical (Smets and Pritchard 2003; Mulligan and Yong 2004). Nevertheless, although microorganisms have even capacity for the degradation of complex organic compounds such as hydrocarbons, improvement of kinetics and rate of degradation is required for efficient bioremediation of these pollutants. In such cases, different bioremediation approaches should be preferred to enhance the biodegradation efficiency.

#### 19.2.1.2 Biostimulation

In this method, while using native microbiota, additional nutrients in the forms of fertilizers, oleophilic, and slow release are used for improving the biodegradation. In order to stimulate the growth and metabolic pathway of naturally existing microbes, besides growth supplements and trace minerals, also environmental conditions like pH, temperature, and oxygen should be adjusted to optimal conditions. Also, small amount of pollutants can be used to enhance the biodegradation process by stimulating the operons of microbial enzymes involved in degradation. The used nutrients such as nitrogen, phosphorous, and carbon are basic requirements of microbes to compose cell biomass, enzyme, and energy. As an example of this method, the use of surfactants, which provide dispersion of the oil and increase bioavailability, as an additional material to achieve complex hydrocarbon degradation can be given (Ortega et al. 2018; Oualha et al. 2019).

#### 19.2.1.3 Bioaugmentation

When natural species of contaminated site are not capable enough for the removal of pollutants, the microorganisms which are adapted to pollutant due to the prior exposure or genetically engineered species that show enhanced capacity to degradation of pollutant need to be added to contaminated aquatic systems (EPA 2006). Additionally, in order to enhance the bioaugmentation efficiency, the consortium of microbes which have different degrading abilities can also be applied to the contaminated sites (Bender and Phillips 2004). On the other hand, species genetically modified through DNA manipulation are much faster than native species and highly competitive to degrade pollutants. Utilization of recombinant DNA technology in order to eliminate the hazardous pollutants is yielding increased degradation rates or new pathways for the degradation of complex compounds that can not break down previously. By using biotechnological tools, a single microorganism which has all required degradative enzymes or pathways can be developed for achieving the toxic compound removal (Dangi et al. 2019). For complete degradation of PCBs, enzymes encoded by two different gene groups need to participate in this process. Unfortunately, due to the inhibitory effects of intermediates produced during process, these two groups of genes cannot be found in a single microbe. After gene transport between *Pseudomonas pseudoalcaligenes* KF707 and *Burkholderia cepacia* LB400 bph strains with the use of recombinant DNA technology, enhancement of the breakdown rate of PCBs, and simultaneous degradation of toluene and benzene have been realized (Seeger et al. 2010). Four main approaches are being considered to develop the genetically engineered microorganism that realized complete degradation of the pollutant; (1) improve the specificity and affinity of enzymes, (2) pathway constitution and regulation, (3) bioprocess development, controlling, and also monitoring, (4) the applications of bioaffinity bioreporter sensor for the use of chemical sensing, toxicity reduction, and end point analysis (Abatenh et al. 2017; Verma and Kuila 2019).

#### 19.2.1.4 Microbial Cell Immobilization for the Bioremediation of Water Pollutants

The immobilized cells demonstrate high potential for the degradation of several water pollutants. Several studies showed that the use of immobilized microorganisms in bioremediation processes could increase the removal efficiency of pollutant by more than 60% (Bouabidi et al. 2019). The success of the microbial cell immobilization method performed to overcome the several limitations of microbial degradation process is dependent on the right carrier or support material selection. Firstly, carrier or support materials need to supply an inert environment to allow microorganisms to keep their enzymes in native form in order to prevent biological activity loss (Qing and Shanfeng 2008). Moreover, cell carriers must be resistant to microbial biodegradation, insoluble in aquatic sites, nonhazardous, and cost-effective. Also, it must allow optimum nutrient diffusion and provide high loading capacity for required cell quantity, high mechanical strength (Bouabidi et al. 2019). In the literature, there were various studies that utilized immobilized microorganisms which proved the success of the method (Table 19.1).

**Table 19.1** Application of immobilized microorganisms for removal of several water pollutants

| Pollutant | Immobilizing matrix | Microorganism | Ref. |
|---|---|---|---|
| Hydrocarbon | Ceramica and cellulose | *Rhodococcus* sp. | Paje et al. (1998) |
| PAHs | Lignocellulosic materials | *Bjerkandera adusta* SM46 | Andriani and Tachibana (2016) |
| Crude oil | Cotton fibers | *Vibrio* sp. HC-3B, *Arthrobacter* sp. HSC8 | Lin et al. (2014) |
| Diesel | Bamboo charcoal | *Acinetobacter venetianus* | Chen et al. (2016) |
| Phenol | PVA–Na alginate/ PVA-Guar gum | *Bacillus* sp. | Ismail and Khudhair (2015) |
| Cr(III) | Alginate gel | *Isochrysis galbana* | Kadimpati et al. (2012) |
| Hg | Silica gel | *Chlorella vulgaris* | Tajes-Martínez et al. (2006) |
| Uranium | Carboxymethyl cellulose beads | *Chlamydomonas reinhardtii* | Erkaya et al. (2014) |
| Phosphourus and amonium | Carrageenan beads | *Spirulina maxima* | Canizares et al. (1993) |
| Acid Black 52 | Na–alginate beads | *Funalia trogi* | Park et al. (2006) |
| Reactive dyes | Na–alginate–polyacrylamide beads | *Pseudomonas putida*, *Bacillus licheniformis* | Suganya and Revathi (2016) |
| Methylene Blue | Ca–alginate beads | *Bacillus subtilis* | Upendar et al. (2016) |

## 19.3 Mechanism of Microbial Bioremediation

Microbes can play an important role in converting pollutants into nonhazardous forms with different mechanisms. Various systems are maintained by microorganisms to remove the pollutant from contaminated sites such as binding, immobilization, totally break down, transformation with oxidation or reduction. For instance, while removing organic pollutants from aquatic environments, degradative enzymes possessed by microorganisms can be used. On the other hand, to convert heavy metals to nontoxic forms, mechanisms such as biosorption, bioadsorption, and immobilization that are utilized by microorganisms to protect themselves from toxic effects of heavy metal ions should be preferred. In order to resolve the heavy metal pollution problems seen in aquatic systems by biosorption, there are three forms of microbial substances used as a biosorbent; (1) exopolysaccharides of microbes, (2) living microbes, (3) and dead mass. In comparison, the metal adsorption capability of dead cells has been found more than living forms (Verma and Kuila 2019). In addition, filamentous fungi like *Aspergillus*, *Mucor*, *Rhizopus,* and filamentous bacteria such as *Actinomycetes* and *Streptomycetes* have shown high metal uptake capacity because of their high surface to volume ratio and involvement of teichoic acids in their cell wall which displayed chemosorption feature (Fosso-Kankeu and Mulaba-Bafubiandi 2014). Especially, the charge density of microbial cells due to

their cell wall structure determines the capacity of metal binding. The ion exchange mechanism caused by the charge density is associated with the groups such as carboxylic acids (of proteins, muramic acids, etc.), nitrogen-based groups (proteins, amino, imidazole etc.), phosphodiester, hydroxyl, and sulfate groups (of serine, threonine, and tyrosine), and glucosamine (presented in chitin) appeared in cell wall structure (Tsezos et al. 2006). The components of the cell wall which act as a ligand to bind heavy metal ions are the compounds of a peptidoglycan layer such as glutamic acid, alanine, teichoic acid, and meso–di-aminopimelic acid in Gram-positive bacteria cell walls (Ayangbenro and Babalola 2017). Likewise, enzymes, lipopolysaccharides, phospholipids, glycoproteins, and lipoproteins are the main components in the cell wall of Gram-negatives. As a result of these features, bacteria are accepted essential biosorbents for the removal of heavy metals from contaminated environments (Gupta et al. 2015). Additionally some microbes including *Bacillus* spp., *Pseudomonas* spp., *Acetobacter xylinum*, *Alcaligenes faecalis*, *Zygomonas mobilis*, *Leuconostoc*, *Agrobacterium* spp., and *Xanthomonas campestris* produce exopolysaccharides (EPS) which are composed of polysaccharides like organic molecules with uronic acid and protein in smaller ratio to protect themselves against environmental stresses such as effects of toxic heavy metals. Biofilm production process which has a crucial role in the biominerilization and biosorption of heavy metal ions is the main source of EPS. EPS is found to be a suitable biosorbent for achieving heavy metal bioremediation (Donot et al. 2012; Yina et al. 2019). When the biosorption capacity of other microbial groups has been evaluated, it was determined that the large biomass of algae gives them the opportunity to remove higher amount of heavy metal. Likewise, fungi can adsorb heavy metals with their mycelium and spores, hence they have the potential for heavy metals bioremediation (Fig. 19.2) (Ayangbenro and Babalola 2017; Yina et al. 2019).

Moreover, some bacterial species have intracellular metal-binding proteins and peptides (metalloproteins) that regulate the number of metal ions inside the cytoplasm by import or export transport of ions. Metal-binding to proteins is site-specific. Hence the occurrence of specific binding sites for the heavy metal that desired to remove from polluted site is the main issue for the higher affinity, selectivity, specificity, and metal-binding capacity. In some situations, for the addition of metal-specific binding sites to microbes, recombinant DNA technology can be utilized (Ma et al. 2009; Ojuederie and Babalola 2017). Also, fungi, yeast, and algae species often consist an example of high ratio of cysteine-consisted metal-binding proteins and low molecular weight which is called as metallothioneins (Mejare and Bulow 2001).

It is also known that bacteria have the capability to convert heavy metal ions to particulate or insoluble forms, which are defined as bioaccumulation. In order to transform heavy metals into nontoxic forms, microbes need energy and they acquire this energy by using heavy metals as an electron acceptor.

Microorganisms also produce extracellular enzymes (laccase, manganese peroxidase, and lignin peroxidase, etc.) that take place in the process of decolorization and degradation of several dyes for the treatment of aquatic systems (Senthilkumar et al.

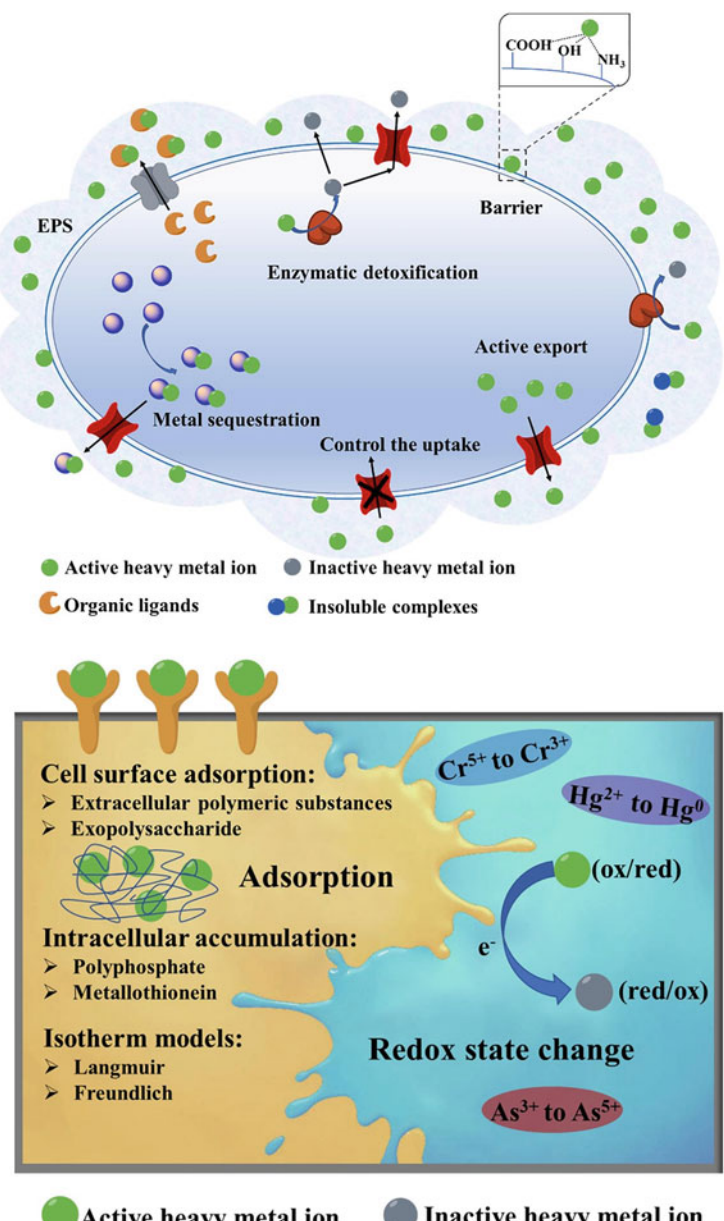


**Fig. 19.2** Proposed detoxification pathways and remediation of microorganisms towards heavy metal ions (Yina et al. 2019)

2014). Some bacteria show capability to oxidize sulfur-based industrial dyes to sulfuric acid. In addition, several bacteria can degrade azo dyes to the colorless amines by their azoreductase enzyme. In this process, amines are consisted of the reduction of azo dyes by the breaking of azo bonds under anaerobic conditions. Then, amines are degraded to nontoxic small molecules under aerobic conditions (Chequer et al. 2011). On the other hand, microbial biomass can be preferred as a biosorbent in order to remove the dyes from wastewaters as with heavy metals (Bhatia et al. 2017).

Microorganisms can utilized as hydrocarbons, which possess highly reduced carbon backbones as an energy source. The first stage of aromatic hydrocarbons and hydrogen-rich alkanes degradation starts with the oxidation of aliphatic hydrocarbons, which results with fatty alcohols formation. Then fatty alcohols are oxidized to form natural lipids compounds such as fatty acids. The final energy gain of this process is based on the hydrocarbon types that degrade, as well as electron acceptors. During the degradation of hydrocarbons and PAHs, increasing bioavailability and bioaccessibility to pollutants can be realized by the assist of biosurfactants/surfactants such as rhamnolipid, Tween 80, and Triton X100 (Ławniczak et al. 2020).

## 19.4 Microbes and Pollutants

### 19.4.1 Microbial Bioremediation of Heavy Metals

Recently, the studies of bioremediation of heavy metals (HMs) via microorganisms have been improved. These studies focus on the species of microorganisms that are used for bioremediation HMs entering into the environment. The bioremediation of HMs is affected by many factors such as bioavailability of pollutants, the activity of microbes, and the bioavailability of the environmental. HMs can cause innumerable hazards to the ecosystem and human health. A small amount of some heavy metal ions (HMIs) such as Pb(II), Cu(II), Cr(VI), Cd(II), and Zn(II) are necessary to plants, but large amount of these HMs turn out to be hazardous for the environment. HMIs show strong electrostatic attractions. The high binding affinity of HMIs to the sites in the several structures of cells causes biomolecule destabilization. Many studies have been published on bioremediation with microorganisms. Some microbial species such as *Pseudomonas*, *Flavobacterium*, *Bacillus*, *Corynebacterium*, *Arthrobacter*, *Rhodococcus*, *Methosinus*, *Stereum hirsutum*, *Mycobacterium*, *Methanogens*, *Nocardia*, *Pleurotus ostreatus*, *Aspergillus niger*, *Azotobacter*, *Rhizopus arrhizus*, *Phormidium valderium*, *Ganoderma applantus,* and *Alcaligens* play an important role in bioremediation of HMs. Table 19.2 shows a summary of the remediation of HMs pollution using bacteria, yeast, and fungi.

Avcioglu et al. used *Klebsiella pneumoniae* for biodegradation of cyanide and optimized biodegradation conditions. They reported 25 °C, pH = 7 and 150 rpm with 0.5 mM potassium cyanide consisted medium for biodegradation condition. They found 87.5% to degrade sodium ferrocyanide decahydrate and 85% potassium

**Table 19.2** Remediation of HMs via microorganisms

| Microorganisms | | Elements | Ref. |
|---|---|---|---|
| Bacteria | *Pseudomonas putida* | Cr(VI) | Balamurugan et al. (2014) |
| | *Bacillus subtilis* | Cr(VI) | Balamurugan et al. (2014) |
| | *Sporosarcina ginsengisoli* | As(III) | François et al. (2012) |
| | *Kocuria flava* | Cu | Coelho et al. (2015) |
| | *Enterobacter cloacae* B2-DHA | Cr(VI) | Rahman et al. (2015) |
| | *Bacillus cereus* XMCr-6 | Cr(VI) | Dong et al. (2013) |
| | *Pseudomonas veronii* | Zn, Cu, Cd | Vullo et al. (2008) |
| | *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* | Fe(II), Zn(II), Pb(II), Mn(II), Cu(II) | Farhan and Khadom (2015) |
| | *Aeromonas* sp., *Pseudomonas aeruginosa* | Ni, Cu, Cr | Paranthaman and Karthikeyan (2015) |
| | *Aspergillus versicolor*, *Cladosporium* sp., *Paecilomyces* sp., *Microsporum* sp., *Paecilomyces* sp., *A. fumigatus*, *Terichoderma* sp. | Cd | Sinha et al. (2011) |
| | *Rhodopseudomonas palustris*, *Aerococcus* sp. | Pb, Cd, Cr | Soleimani et al. (2015) |
| | *Microbacterium profundi* strain Shh49T | Fe | Sinha and Biswas (2014) |
| Yeast | *Saccharomyces cerevisiae* | Cd, Pb | Wu et al. (2015) |
| | *Streptomyces noursei* | Cd, Ag, Pb, Cr, Cu, Ni, Zn, Co | Mattuschka and Straube (1993) |
| Fungi | *Gloeophyllum sepiarium* | Cr(VI) | Achal et al. (2011) |
| | *Aspergillus versicolor* | Ni | Tastan et al. (2010) |

hexacyanoferrate(II) trihydrate with *K. pneumoniae* strain. They also investigated the effect of magnesium, zinc, cobalt, arsenic, chromium, nickel, and iron ions in potassium cyanide degradation (Avcioglu and Seyis 2016).

### 19.4.2 Microbial Bioremediation of Crude Oil, Hydrocarbon, and PAHs

The studies of bioremediation of polycyclic aromatic hydrocarbons (PAHs) via microorganisms from the contaminated environments have been improved in the last decanted. PAHs pollution with benzene rings arranged in a linear, cluster, or angular ways can be hazardous. PAHs have carcinogenic, mutagenic, and teratogenic properties. The incomplete combustion of petroleum, coal, and wood can cause PAHs pollution in industries. Microorganisms can turn the hazardous contaminant into harmless forms. Halophilic archaea widely are used for the bioremediation of hydrocarbons. Temperature, salt concentration, pH, and pressure characterization are utilized for hydrocarbon contamination conditions. The studies on these

characterizations showed the role of extremophilic microorganisms in the bioremediation of hydrocarbons (Oren 2019; Krzmarzick et al. 2018).

Halophilic extremophiles play an important role in the oil-polluted salt marshes bioremediation. Halophilic archaea especially are used for aliphatic, aromatic compounds, and crude oil. Ozyurek et al. developed a method for removing the petroleum products from the contaminated sites with the use of *Klebsiella pneumoniae* ATCC13883 that is isolated from drilling fluid. They also investigated the effect of different physiological conditions and the addition of nitrogen, carbon, and surfactant sources on petroleum biodegradation. They analyzed the hydrocarbon via GC–MS to measure the petroleum biodegradation. They reported a 66.5% biodegradation rate for *Klebsiella pneumoniae*. The effective conditions were showed 1% (v/v) concentration of petroleum at pH 7.0, and 7-day incubation at 150 rpm, 25 °C in their study. With the added Triton X100, glucose increased approximately 68%, 71% the biodegradation rate, and also, yeast extract increased 72.5%. They reported above 90%, 70%, 40% biodegradation rates for hydrocarbons ranging from C10 and C20, from C21 and C22, and C31 and C32, respectively (Ozyurek and Bilkay 2018). Some studies about the bioremediation of hydrocarbons with extreme halophilic archaea are shown in Table 19.3.

**Table 19.3** Remediation of crude oil, hydrocarbon and PAHs via microorganisms

| Microorganisms | Degraded | Refs. |
|---|---|---|
| *Haloferax* sp. D1227 | Benzoate, cinnamate, phenylpropionate, *p*-hydroxybenzoate | Fu and Oriel (1999) |
| *Haloarcula* st. D1 | 4-Hydroxybenzoic acid | Fairley et al. (2002) |
| *Haloferax sulfurifontix* st. CL47<br>*Haloferax alexandrinus* st. B03, B06, AA31, and AA35<br>*Haloferax* sp. HSC4 st. MM27<br>*Haloferax* sp. SC1–9 st. AA41, MM17, and PR13, B07 | Anthracene, naphthalene, pyrene, and/or benz[a] anthracene, Phenanthrene | Bonfá et al. (2011) |
| *Halorubrum ezzemoulense* st. C-41 and C-46<br>*Halobacteriaceae* st. C-50 and C-52<br>*Halobacterium salinarum* st. C-51<br>*Halorubrum* sp. st. C-43 | Phenanthrene, naphthalene, pyrene, and/or *p*-hydroxybenzoate | Erdoğmuş et al. (2013) |
| *Halobacterium noricense* st. SA1<br>*Haloferax elongans* st. WA1, SA3<br>*Halobacterium noricense* st. WA2<br>*Haloferax larsenii* st. SA2, WA3<br>*Halobacterium salinarum* st. WA4 | Oil, benzene, alkanes (C9–C40), Anthracene, biphenyl | Al-Mailem et al. (2017) |
| *Haloferax mediterranei* st. M-11 | Oil | Zvyagintseva et al. (1995) |
| *Haloarcula hispanica*<br>*Halobacterium salinarum*<br>*Halobacterium piscisalsi* | Naphthalene, *p*-hydroxybenzoic acid, Phenanthrene, Pyrene | Erdoğmuş et al. (2013) |

Erdogmus et al. aimed to recognize the halophilic Archaea to degrade the aromatic hydrocarbons. They obtained nine archaeal isolates that used naphthalene, *p*-hydroxybenzoic acid, pyrene, and phenanthrene as energy and sole carbon sources. Identification of the isolates was performed by 16S rRNA gene sequences. The isolates were determined as *Halorubrum ezzemoulense*, *Halobacterium piscisalsi*, *Halobacterium salinarium*, *Halorubrum* sp., *Haloferax* sp., *Haloarcula* sp., and *Haloarcula hispanica.* They reported the demonstration of *H. ezzemoulense* and *Halorubrum* sp. were able to utilize pyrene, *p*-hydroxybenzoic acid, naphthalene, and phenanthrene as the sole carbon sources at 20% (w/v) NaCl concentration for the first time (Erdoğmuş et al. 2013).

### 19.4.3 Microbial Bioremediation of Pesticides

Pesticides are the chemical substances that are used to control the amounts of pests at tolerable levels for the environment. But increased agricultural proceedings to acquire the need of human population have caused the accumulation of these harmful chemicals in environmental sources. The toxicity level of pesticides causes a serious threat not only for human being but also for the biodiversity in the ecosystem. Thus, the toxic effects of these xenobiotics on the environment have forced us to implement several methods to remove xenobiotics from the aquatic environments but some of them have side effects on the ecosystem. In comparison, bioremediation among the present methods is an ecofriendly degradation of pesticides using the microbes (Debarati et al. 2005). Bioremediation of pesticides via microbes is usually reported via *Bacillus* sp., *Pandoraea* sp., *Phanerochaete*, *Chrysosporium*, *Klebsiella* sp., *Pseudomonas* sp., and *Mycobacterium* sp. (Table 19.4).

### 19.4.4 Microbial Bioremediation of Industrial Dyes

Synthetic dyes are widely used in many industries. The large amounts of dyes are released and contaminated in the wastewater during the dying and washing of the industrial textiles. Therefore, dyes in wastewater have become a major environmental menace. Dyes are the chemicals that are consumed highly in several industries such as the textile industry in recent years. The low concentrations of dyes can be seen from their high brilliance and, therefore, undesired in industrial effluents. Dyes are able to generate highly toxic aromatic compounds, which show mutagenic and carcinogenic effects. The solubility of oxygen decreases with dye compounds in the water. Therefore, it can be causing an ecological imbalance for aquatic species. Dyes are classified on the basis of chemical structures, the basis of their origin, and their applications. Dyes widely are anionic, nonionic, and cationic forms (Wang et al. 2017; Rahimi et al. 2016; Xiang et al. 2016).

Many researchers have studied to eliminate these problems in this area. Some of these studies are summarized in Table 19.5.

**Table 19.4** Remediation of pesticides via microorganisms

| Pesticides | Microorganisms | Refs. |
|---|---|---|
| Dichloro diphenyl trichloroethane | *Cyanobacteria* | Megharaj et al. (2002) |
| | *Pseudomonas* sp. | Hay and Focht (2000) |
| Chlorpyrifos | *Serratia marscecens*, *Pseudomonas aeruginosa*, *Klebsiella* sp., *Bacillus cereus* | Lakshmi et al. (2009) |
| | Bacterial consortium | Lakshmi et al. (2008) |
| | Bacterial consortium | Venkata Mohan et al. (2007) |
| | Bacterial consortium | Sasikala et al. (2012) |
| Cypermethrin and diazinon | Bacterial consortium | Katie et al. (2007) |
| 2,4-Dichlorophenoxy acetic acid | Bacterial consortium | Robles Gonzalez et al. (2006) |
| Atrazine | *Ralstonia* sp. M91–3, *Agrobacterium radiobacter* J14a | Park et al. (2003) |
| | *Chelatobacter heintzii* Citl | Rousseaux et al. (2003) |
| Lindane | Mixed Bacterial Consortium | Rajkumar et al. (2004) |
| | *Ganoderma* sp. | Rigas et al. (2007) |
| Endosulfan | Mixed Bacterial Consortium | Kumar et al. (2008) |
| | Algae | Sethunathan et al. (2004) |

**Table 19.5** Remediation of industrial dyes via microorganisms

| Dyes | Type of Dyes | Microorganisms | Mechanism | Ref. |
|---|---|---|---|---|
| Direct blue 6 | Azo dye | *Pseudomonas desmolyticum* NCIM 2112 | Reduction | Kalme et al. (2007) |
| Reactive red 2 | Sulfonated azo dye | *Pseudomonas* sp. SUK1 | Deamination, reduction, desulfonation | Kalyani et al. (2009) |
| Acid orange 7 | Azo dye | Microbes mixture | Reduction, oxidation | Fernando et al. (2014) |
| 4 dyes including reactive yellow 107 | Azo dye | *Klebsiella* sp. VN-31 | Oxidation, reduction | Franciscon et al. (2009) |
| Indigo blue | Miscellaneous | *Cyanobacterium* | Hydrolysis, oxidation, and decarboxylation | Dellamatrice et al. (2017) |
| Malachite green | Triphenyl methane | *Micrococcus* sp. BD15 | OH radical cleavage, hydroxylation | Du et al. (2013) |
| Malachite green | Triphenyl methane | *Pseudomonas pulmonicola* YC32 | Reduction and demethylation | Chen et al. (2009) |

### 19.4.5 Microbial Bioremediation of Other Pollutants

Aracagok et al. used four fungal strain *Aspergillus niger*, *Trametes trogii*, *Phanerochaete chrysosporium,* and *Yarrowia lipolytica* to the investigation of diclofenac degradation potential. The nonsteroidal drug, diclofenac, has been detected in drinking, surface, and groundwater. They reported the most efficient strain for diclofenac with *Trametes trogii*. They suggest that the degradation of the drug can occur via the laccase activity and cytochrome P450 enzyme system (Aracagök et al. 2017).

In another study, they studied four fungal strains *Funalia trogii*, *Phanerochaete chrysosporium*, *Yarrowia lipolytica,* and *Aspergillus niger* for bioremediation of naproxen. The excessive usage of naproxen can be polluted drinking water. They investigated naproxen removal abilities in this study. Also, they reported a 98% removal rate with using *A. niger* and showed two main by-products of fungal transformation, *O*-desmethylnaproxen and 7-hydroxynaproxen via using LC/MS, $^{1}$H NMR, and $^{13}$C NMR (Aracagök et al. 2018).

## 19.5 Future Perspectives and Conclusion

Nowadays, environmental contaminants that depend on anthropogenic activities are a serious trouble for the ecosystem, human health, and biodiversity. The utilization of microbial biomass or metabolic pathways of them for the bioremediation of organic/inorganic pollutants from aquatic environments has many advantages compared with the physicochemical remediation methods. Although there are microbial populations that are environmentally friendly and useful for breaking down most pollutants, there are contaminants that cannot be degraded by previously discovered bacteria. Therefore, enhancing the efficiency of bioremediation processes, novel techniques that improve the degradation capability of microbes to remove toxic compounds safely, should be discovered by genetic engineering (GE), as well as systems biology (SB). SB approaches can be used to obtain valuable information about the biodegradation processes. Then, metabolic engineering (ME) is used to utilize these information for the improvement of metabolic pathways of microbes transforming harmful pollutants into nonhazardous forms. Consequently, it is considered that many detailed and valuable researches have been performed to treat the contaminated aquatic systems with the use of microbes.

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