Chapter 9

Role of Biosurfactants on Microbial Degradation of Oil-Contaminated Soils

Sandamali Wijesekara, Mihiri Seneviratne, and Meththika Vithanage

Abstract Hydrocarbon contamination of soil is one of the major environmental problems today due to activities related to the petrochemical industry. Mechanical and chemical remediation and restoration to remove hydrocarbons from contaminated sites have limited effectiveness and costly. Bioremediation is the promising technology for the treatment of these contaminated sites since it is cost-effective and will lead to complete mineralization. Fungi and bacteria have been considered as highly effective in oil degradation. Several bacteria are even known to feed exclusively on hydrocarbons; Arthrobacter, Burkholderia, Mycobacterium, Pseudomonas, Sphingomonas, and Rhodococcus. Fungal genera, namely, Amorphoteca, Neosartorya, Talaromyces, and Graphium are proved to be the potential organisms for hydrocarbon degradation. Although laboratory experiments have indicated that the bacteria can ubiquitously degrade oil constituents, to date there is little convincing evidence that bioaugmentation (addition of more bacteria) significantly enhances the extent of oil biodegradation in soil. The potential benefits of using genetically modified bacteria represent a research frontier with significant results. However, many concerns are often raising due to the effectiveness of indigenous species, limited understanding of various phytoremediation mechanisms, including the regulation of enzyme systems that degrade pollutants. Thus, this chapter presents an updated overview of petroleum hydrocarbon degradation by microorganisms focusing biosurfactants and their mechanisms.

Keywords Oil pollution • Biodegradation • Petroleum hydrocarbons • Bioaugmentation • Phytoremediation

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S. Wijesekara • M. Seneviratne
Department of Botany, Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka

M. Vithanage (✉)
Chemical and Environmental Systems Modeling Research Group,
National Institute of Fundamental Studies, Kandy, Sri Lanka
e-mail: meththikavithanage@gmail.com

J.S. Singh, G. Seneviratne (eds.), *Agro-Environmental Sustainability*,
DOI 10.1007/978-3-319-49727-3_9

9.1 Introduction

Oil hydrocarbon pollution originates from both natural and anthropogenic activities. Contamination due to exploration, draining off from drill sites, accidental spillage, refinery, and transportation has led to global environmental problems (Das and Chandran 2010). The physical and biological effects of oil in the aquatic environment include reduction of light transmission, reduction of dissolved oxygen, damage to water birds, and smothering of intertidal organisms. The toxic effects are exhibited mostly by the light portion (low boiling) of the oil due to the presence of poly aromatic hydrocarbon (PAH) (Atlas and Bartha 1992). The effects include cell damages and death of sensitive organisms and larval stages. The mitigation of oil pollution in sea, soil, and aquatic environment is a difficult task, and various efforts have been taken by the international community to curtail this problem.

In large-scale accidents, oil release into the environment harms the biological system due to the biomagnification of toxic compounds with toxic elements via food webs and food chains (Dillard et al. 1997; Head et al. 2006). The degradation of oil hydrocarbon in aquatic environment, sludge, soil, sediment, and costal ecosystem with natural phenomenon is great deal to overcome these types of problems arising due to the oil contamination. The xenobiotic nature of the oil products and its derivatives are high-risk factors of the environmental degradation due to the bioaccumulation (Head et al. 2006). Oil consists of different compositional ingredients with different chemical and physical properties (Hasanuzzaman et al. 2007). Once petroleum oil is spilled and leaked over the aquatic masses, it will disperse through the surface while releasing volatile compounds to atmosphere (Garrett et al. 2003).

Some hydrocarbons, such as PAH, are known as potent mutagens and carcinogens that pose serious human and environmental health risks. Therefore, the removal of hydrocarbons is essential to improve environmental health. Many different treatment techniques have been studied in order to restore and rehabilitate the soil. The main remediation methods practiced are containment, thermal desorption, incineration, and microbial degradation (Henner et al. 1997).

Cleanup technologies such as physical and chemical methods, volatilization, photooxidation, chemical oxidation, and bioaccumulation seem to be rarely successful on petroleum hydrocarbons due to the recalcitrant nature and not cost-effective when compared to microbial bioremediation (Prince 1997). Oil pollutants are slow in degradation under normal conditions due to the functional groups present and low water solubility (Desai et al. 2012; Hassanshahian et al. 2012). Therefore, it needs special specific conditions to enhance the degradation of particular oil pollutant at a considerable rate and has to reduce time taken to clean up the contaminated site. Degradation of oil depends on the nature of crude oil, composition, and genes responsible for the secretion of enzymes and ecological and environmental factors. Apart from that, efficiency of oil bioavailability is restricted by poor water solubility of oil contaminant (Banat et al. 2010). However, the mechanical removal of oily substances is very expensive and makes more harmful effects on animals and environment during the period of time (Urum et al. 2006). Recent studies have focused on bioremediation through the indigenous microorganisms in the oil-contaminated surroundings as a potential remediation measure. This chapter provides the background and updated information on microbial degradation of petroleum hydrocarbon contaminants towards the better understanding in bioremediation challenges.

9.2 Bioremediation of Oil Contaminants

Bacteria have long been considered as one of the predominant hydrocarbon-degrading agents found in the environment. The success of bioremediation technologies applied to hydrocarbon-polluted environments depends highly on the biodegrading capabilities of native microbial populations or exogenous microorganisms used as inoculants (Al-Wasify and Hamed 2014; Marchant and Banat 2012). Bioremediation is defined as the use of microorganisms in the biological system to detoxify or remove pollutants using their diverse metabolic capabilities. This process uses an evolving method for the removal and degradation of many environmental pollutants including the products of the petroleum industry (Tang et al. 2011).

Microbial biodegradation is cost-effective, and it serves as highly efficient alternative compared to many types of approaches (Koutinas et al. 2007). Enhancement of crude oil degradation with the use of microorganisms has been identified as a novel approach to overcome oil contamination (Banat et al. 2010). Therefore, many studies have focused on the enhancement of biodegradation of oil contaminants to recover the oil pollution through bioremediation process to clean up the contaminated sites not cleaning up (Soberón-Chávez and Maier 2011). On the other hand, only few studies have concentrated on the generation of the toxic compounds during the bioremediation process by microorganism in some special remediation systems such as salt marshes and estuarine pools (Middaugh et al. 1998; Shelton et al. 1999). The efficiency of microbial degradation has been revealed by with the aerobic degradation (Das and Chandran 2010). According to the studies carried out to reveal the biodegradation of oil, it has aimed to isolate and identify the bacteria and other organisms having highly adapted nature to the contaminated sites to degrade contaminants (Zajic et al. 1977). Scientists have observed and reported isolated organisms from study sites which were chemically and physically stable and their metabolic activities are optimum at the particular environment (Zhang et al. 2010).

Recent researches have paid their attention on natural, safe, cost-effective materials to treat oil-contaminated sites (Van Hamme et al. 2003). Simple oil contaminants with simple structures are processed with different metabolic pathways based on the unique enzyme systems present in microorganisms (Van Hamme et al. 2003). Therefore, they have chosen biosurfactant as the best potential method of remediation of oil spills (Wei et al. 2005). Development of the possible eco-friendly tool to handle the contaminated sites using efficient strategies and an applicable model system is important (Franzetti et al. 2010a). Therefore it has been discovered that the use of naturally occurring bacteria for removal of oil contaminants have built up the new interpretations for the oil recoveries with the production of biosurfactants (Ron and Rosenberg 2002).

Biodegradation of oil can be influenced by the microorganisms present in the natural environment as well as by introducing improved microbial cultures (Table 9.1). Oil spill bioremediation has two main approaches: (a) bioaugmentation, where known oil-degrading bacteria are added to supplement the existing microbial population, and (b) biostimulation, where the growth of indigenous oil degraders are stimulated by the addition of nutrients or other growth-limiting substrates with the supplement of enriching factors of medium (Ojo 2005). Oil-degrading microbial consortia are used as the improved cultures, and it consists with one or more microorganisms showing an ability to degrade oil at a considerable rate (Rosenberg and Ron 1999). Several studies have proven with the evidence that the use of microorganisms to oil recovery is a desirable alternative to remove the oily contaminants from the environment (Banat et al. 2000) (Table 1).

Microorganisms should possess special metabolic activities responsible for the production of metabolites to utilize the oily compounds. Therefore, specific biochemical reactions of microorganisms are needed for the expression of the production of metabolites for the degradation of total petroleum products (Atlas and Bartha 1992). It is very important to know that the reasonable performance of microorganism during biodegradation depends on the enzymatic activity and its mechanism (Obayori et al. 2009).

But if an unexpectable oil drainage occurred, the naturally occurring microbial consortium is unable to degrade the spilled oil totally at considerable rate, because an already existing small proportion of microorganism consortium may not be enough to degrade the bulk of oil contaminant and some of them may inactive due to the toxicity nature of oil to them (Shelton et al. 1999). Therefore, it is necessary to use the best biotechnological approach to overcome this type of problems before dispersing oil over the surface. To fulfill this purpose, selection of the best biotechnological solution is very important as an innovative technique.

Table 9.1 Biological systems used to recover the oil contaminations in several countries

Country	Biological system used	References
India, Goan coast	*P. aeruginosa*, *Escherichia fergusonii*	Pasumarthi et al. (2013)
Egypt, Gemsa Bay	*P. xanthomarina*, *P. stutzeri ATCC 17588*	El-Sheshtawy et al. (2014)
China, Nanjing Refinery sludge	*Pseudomonas* sp.	Gao et al. (2000)
Northeast India	*Bacillus subtilis P. aeruginosa* sp.	Das and Mukherjee (2007)
Taiwan	*P. aeruginosa EM1*	Wu et al. (2008)
China—oil storage tank	*Pseudomonas* sp.	Yan et al. (2012)

Exxon Valdez and BP Deepwater Horizon oil spill were the two large-scale oil spills (Atlas and Hazen 2011). The experimental evidence suggests that those are the worst oil spills in US history with svere environmental impacts of oil contamination (Bragg et al. 1994). Because petroleum hydrocarbons consist in crude oil with diverse derivatives of both aromatic and aliphatic hydrocarbons, is easily escape into groundwater reservoirs and surrounding environment. Generally petroleum hydrocarbons naturally exist in all marine environments with the compatible nature of naturally occurring microorganisms. Those microorganisms are capable of utilizing those contaminants by using oil as an energy source (Garon et al. 2002).

9.3 Mechanism of Microbial-Mediated Petroleum Hydrocarbon Degradation

Basically bioremediation expresses the mean of transformation and mineralization of toxic compounds via the endogenous or exogenous process of microorganisms (Franzetti et al. 2010b). In endogenous activity, carbon source has to go inside before it undergoes to the transformation event. Compared to endogenous process, exogenous process occurred in external environment. Therefore, it is essential to bind with carbon and the microbial cell before utilizing it (Mulligan and Gibbs 2004). The ability to form biosurfactants is found in numerous bacterial and fungal species (Desai and Banat 1997), for instance, *Pseudomonas* sp., *Rhodococcus* sp., *Acinetobacter* sp., *Bacillus* sp., *Achromobacter* sp., *Brevibacillus* sp., *Lysinibacillus* sp., *Alcaligenes* sp., *Candida* sp., and *Torulopsis* sp. (Desai and Banat 1997). Biosurfactants enables them to grow on hydrophobic substrate. However, due to low solubility of petroleum hydrocarbon, the biodegradation of contaminant by microorganisms is highly restricted (Banat et al. 2010), but with the presence of extracellular biosurfactants, these compounds are solubilized, increasing its bioavailability to microorganisms by providing contaminant as an effective energy source for them (Joshi et al. 2008). Studies on biosurfactants showed the degradation of contaminant depends on the length and nature of arrangement of the carbon chain. Aliphatic moieties are easily degradable than the rest of aromatic carbon structures (Setti et al. 1993), because aromatic fraction is a heavily branched compound and it is difficult to degrade. Hence, aromatic fraction may persist in the environment for a longer period without any change and will pose a significant harmful effect on an environment (Hasanuzzaman et al. 2007).

Most of the recent studies were focused on the degradation of crude oil. Diverse groups of microorganisms are able to synthesize various types of secondary metabolites (Banat 1995), which have an ability to produce biosurfactant effects to degrade pollutants in an efficient way. Especially the biosurfactants are commonly employed in crude oil recovery and have been explained (Banat et al. 2000).

9.4 Structure of the Biosurfactant

Biosurfactants are the naturally occurring structurally diverse groups showing surface-active mechanisms with amphiphilic properties. It consists with both hydrophobic and hydrophilic moieties with surface-active compounds (Fig. 9.1). Most biosurfactants are anionic or neutral, but few are cationic due to the presence of amine group. Biosurfactants are amphipathic molecules which accumulate at interfaces, decrease interfacial tensions, and form aggregates such as micelles. However, biosurfactants show unique biochemical properties which are highly important in biological applications. Biosurfactants are easily accessible and acceptable for the environmental application, especially for both land and sea (Cameotra et al. 2010).

9.5 Classification of Biosurfactants

Biosurfactants are classified based on their chemical structure, composition, molecular weight, physicochemical properties, mode of action, and the microbial origin that produced them (Zinjarde and Pant 2002) (Table 9.2). Its hydrophobic fraction may consist with the saturated or unsaturated fatty acid, and hydrophilic fraction may consist with the amino acids, peptides, and saccharides. Based on its chemical composition and chemical behavior, it can be divided into three major classes as follows (Desai and Banat 1997):

1. Glycolipids (lipopeptides, lipoproteins, and phospholipids)
2. Polymeric biosurfactants
3. Particulate biosurfactants

In some cases, biosurfactants can be categorized based on its molecular weight as low- and high-molecular-weighted polymers (Rosenberg and Ron 1999). Low-molecular-mass biosurfactants include glycolipids, phospholipids, and lipopeptides, whereas high-molecular-mass surfactants include amphipathic polysaccharides, lipopolysaccharides, proteins, lipoproteins, and complex mixtures of

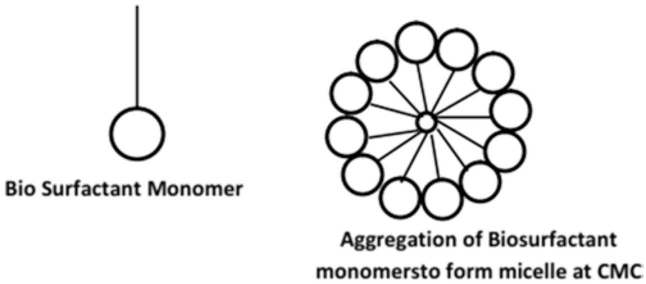

Fig. 9.1 Surfactant structure showing hydrophobic head and hydrophilic tail and formation of micelle at CMC

Table 9.2 Classification of biosurfactants and their use in remediation of oil contaminants

Biosurfactant			
Group	Class	Microorganisms	References
Glycolipids	Rhamnolipids	*Pseudomonas aeruginosa*	Rosa et al. (2010)
	Trehalolipids	*Rhodococcus erythropolis*, *Arthrobacter* sp., *Nocardia* sp.	Franzetti et al. (2010b), Shao (2011)
	Sophorolipids	*Torulopsis* bombicola, *T. petrophilum*	Whang et al. (2008)
Fatty acids phospholipids and neutral lipids	Corynomycolic acid	*Corynebacterium lupus*	Bozo-Hurtado et al. (2012)
	Spiculisporic acid	*Penicillium spiculisporum*	Tabuchi et al. (1977)
	Phosphatidylethanolamine	*Pseudomonas fluorescens*	Appanna et al. (1995)
Lipopeptides	Surfactin	*Bacillus subtilis*	Cooper et al. (1981)

biopolymers. Compared to the high-molecular-mass biosurfactants, low-molecular-weight compounds are efficient in lowering the surface and interfacial tension of contaminant, whereas high-molecular-mass compounds are effective at stabilizing the oil in water emulsion (Calvo et al. 2009).

9.6 Properties of Biosurfactants: Natural Choice for Bioremediation with Biosurfactants

Biosurfactants have been identified as eco-friendly, cost-effective, easily degradable substrate produce by microorganisms as their secondary metabolite, and they are producing mixture of biopolymers (Rosenberg and Ron 1999). It aims to mineralize and/or biotransform toxic contaminants to nontoxic or low-toxic compounds while utilizing it by microorganisms (Sanscartier et al. 2009). Due to high demand for the biosurfactants, the attention has been paid on artificially synthesizing this eco-friendly natural compound (Atlas and Bartha 1992). However, it has shown that synthetic chemicals had severe adverse effects on an environment over the use of natural surfactants. When the biosurfactant is ready to activate on any hydrophobic substrate to degrade it, biosurfactant accumulates at the interface between fluid and solid with the effective reduction of surface and interfacial tension. Hence, it is allowing those two dissimilar phases to mix and interact with each other more easily (Soberón-Chávez and Maier 2011).

Measurement of biosurfactant activity has been done by the measurement of surface and interfacial tension, stabilization of emulsion, and hydrophilic-lipophilic

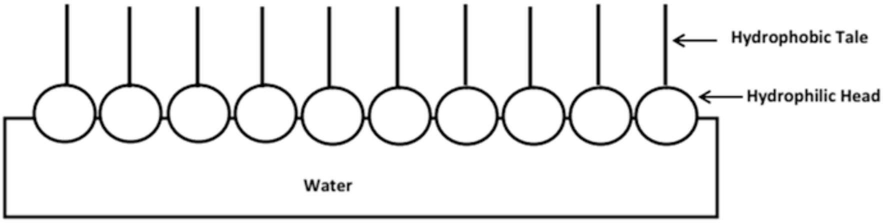

Fig. 9.2 Accumulation of biosurfactants at the interface

balance (HLB) of the surfactants and contaminant (Oberbremer et al. 1990). Surface tension was measured at the air-water and oil/water interface with the use of tensiometer (Salamanca et al. 2001). The high molecular weight surfactants are less effective in reducing interfacial tension, but are important at coating the oil droplets and preventing their coalescence (Fig. 9.2). These are highly efficient emulsifiers that work at low concentrations (Ron and Rosenberg 2002). Emulsification activity is measured by emulsification assay. It is based on the ability of the surfactant to regenerate droplets in particular aqueous assay system (Kim et al. 2000). Biosurfactants can increase the bound substrates by desorbing them from surfaces or by increasing their apparent water solubility.

9.7 Relationship Between Surface Tension and Critical Micelle Concentration (CMC)

The efficiency and effectiveness of biosurfactant depends on the formation of critical micelle concentration of the surface-active compound with the collection of sufficient surface-active molecules on the substrate. At the concentration above CMC, biosurfactant molecules gathered to form micelles, vesicles, and lamellae on the substrate as a continuous bilayer (Whang et al. 2008). Formation of micelle enables to increase the solubility and bioavailability on hydrophobic contaminant to make easy degradation while reducing the surface tension (Desai and Banat 1997). However, an efficient biosurfactant has the lower CMC value; therefore, less amount of biosurfactant is required to decrease the surface tension (Nguyen et al. 2008) (Fig. 9.3). It has direct relationship with the formation of microemulsion layer (Desai and Banat 1997). That allows the contaminant to adjust to the hydrophobic environment leading the formation of stable liquid mixture of water and oily particles by the formation of droplets dispersed on the liquid phase (Soberón-Chávez and Maier 2011).

Relationship between surface tension and CMC is expressed in the semilogarithmic plot of the surface tension of a solution against the surfactant concentration as mentioned below (Fig. 9.4). HLB indicates the promotion of surfactant activity in water-in-oil or oil-in-water to emulsify the contaminant easier. If HLB value is less

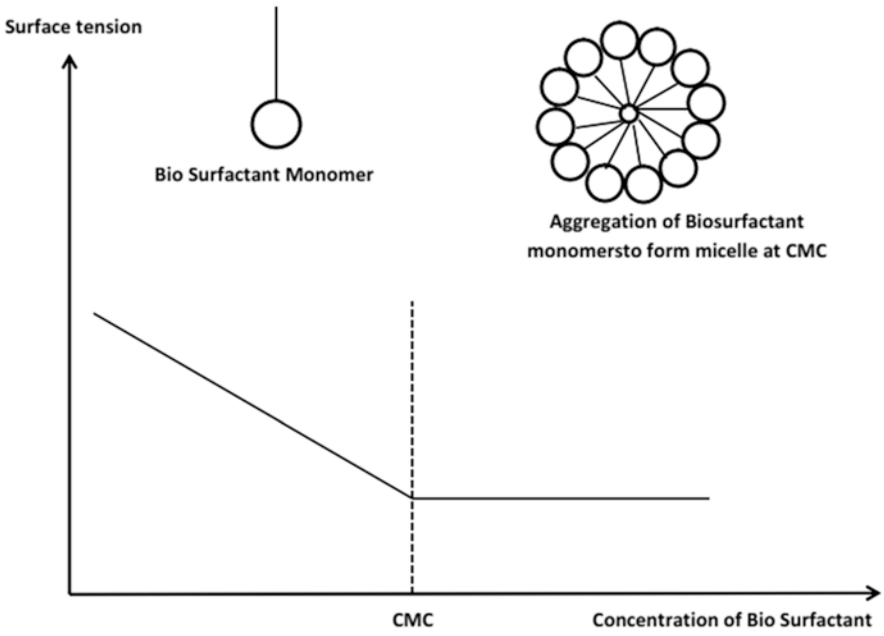

Fig. 9.3 Surface tension vs. concentration of purified biosurfactant

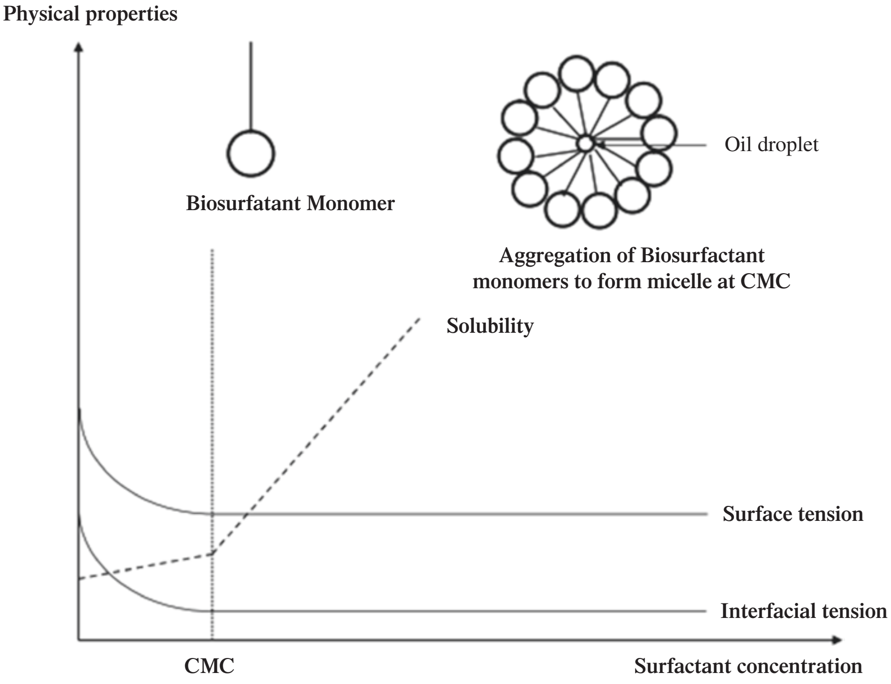

Fig. 9.4 The relationship between biosurfactant concentration, surface tension, and formation of micelles

than 6, it is preferred to emulsify water-in-oil; if the HLB value is in between 10–18, it favors the emulsification of contaminant in oil-in-water (Oberbremer et al. 1990).

In practical approaches, HLB value can be measured as follows:

HLB value = 20 * Mh/M.
Mh—molecular weight of the hydrophilic fraction
M—molecular weight of the whole molecule

HLB value of 0 corresponds to a completely lipophilic/hydrophobic molecule, and a value of 20 corresponds to a completely hydrophilic/lipophobic molecule. Therefore, HLB values of surfactants are useful in predicting the surfactant properties of the surface-active compounds.

If the two moieties of biosurfactant are not arranged in an equal way, this formula does not apply (Vollbrecht et al. 1999).

Ex-HLB value of glycolipid in the system containing water and hydrophobic phase consists with soybean and cyclohexane mixture. For this system, HLB value is measured as follows:

$A = (\mathrm{HLB}_{\mathrm{needed}} - \mathrm{HLB}_{\mathrm{B}})/(\mathrm{HLB}_{\mathrm{A}} - \mathrm{HLB}_{\mathrm{B}})$
A = % of cyclohexane
$\mathrm{HLB}_{\mathrm{A}}$—HLB of cyclohexane (−15)
$\mathrm{HLB}_{\mathrm{B}}$—HLB of soybean (−6)

Using this formula, most stable emulsification index can be determined for the selected system.

9.8 Effect of pH and Temperature on Biosurfactant Stability

The activity of biosurfactant decreases at extreme pH values (Champion et al. 1995). However, biosurfactants are stable at high temperatures (Banat 1995). Biosurfactant was stable during exposure to high salinity (10 % NaCl), elevated temperatures (120 °C for 15 min), and within a wide pH range (4.0–10.0) (Shavandi et al. 2011). The activity of the biosurfactant was enhanced optimally at NaCl concentration of 5 %, pH of 8.0, and temperature of 40 °C. The biosurfactant retained 77 % of its original activity after 120 min of exposure to heat at a temperature of 100 °C (Ilori et al. 2005).

9.9 Mechanisms of Biosurfactants in Biodegradation

The use of biosurfactants to remove oil contaminants is a promising method that can improve the effectiveness of bioremediation in contaminated environments. They can enhance hydrocarbon bioremediation by two mechanisms. The first mechanism includes the induction of substrate bioavailability for microorganisms, while the other involves interaction with the cell surface which increases the hydrophobicity of the surface allowing hydrophobic substrates to associate more easily with bacterial cells (Mulligan and Gibbs 2004). By reducing surface and interfacial tensions, biosurfactants increase the surface areas of insoluble compounds leading to increased mobility and bioavailability of hydrocarbons. Addition of biosurfactants to the polluted site through the process of bioaugmentation is capable of degrading contaminants. This process is expected to enhance the hydrocarbon biodegradation by mobilization, solubilization, or emulsification of contaminants efficiently (Nguyen et al. 2008).

Usually microbial aerobic degradation of alkanes follows intracellular enzymatic pathway (Morgan and Watkinson 1990). Aerobic degradation is the most rapid and complete degradation of organic pollutants by microorganisms (Fig. 9.5). Organic pollutants including various oil pollutants and its derivatives are initially attacked by microorganisms through an oxidative process. Hereafter, activation and incorporation of pollutant into cell is essential before utilizing it. Those two processes are catalyzed by two enzymes named as oxygenase and peroxidase. Then intermediates are formed via the peripheral degradation pathway, Eg–TCA cycle (Fig. 9.6).

Instead of oxygenase degradation pathway being used for the degradation of oil derivatives, it is linked with the many other enzymatic pathways. The activity of cytochrome P450 alkane hydroxylase enzyme is the well-studied enzyme on this event (van Beilen and Funhoff 2007). Degradation depends on the change of length, and the enzyme system efficiency is more important to initiate the degradation (Table 9.3).

Apart from that, another enzyme system has been discussed by van Beilen and Funhoff (2005). The enzyme is di-iron methane monooxygenase showing membrane-bound copper-containing monooxygenase responsible for the degradation of oil with elementary constituent in toxic level (van Beilen and Funhoff 2007).

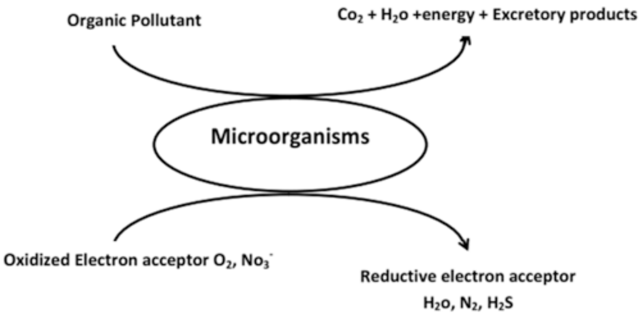

Fig. 9.5 Oxidative biodegradation and its mechanism

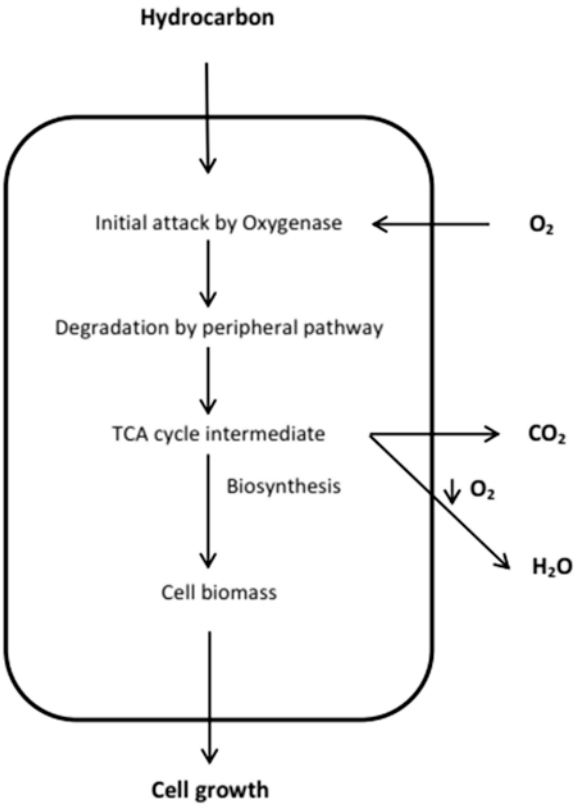

Fig. 9.6 Participation of an enzyme system for the degradation of contaminant

Table 9.3 Enzymes involve in biodegradation of oil contaminants

Enzyme	Substrate	Organisms	References
Methane monooxygenase	Short alkanes	*Methylococcus* sp.	Shigematsu et al. (1999)
	Cyclohexane	*Methylomonas* sp.	Shigematsu et al. (1999)
Eukaryotic P450	Fatty acids, C_{10}–C_{15} alkanes	*Candida bombicola*	Van Bogaert et al. (2009)
Bacterial P450	C_5–C_{16}	*Acinetobacter* sp., *Mycobacterium* sp.	Fujii et al. (2006)
Dioxygenase	C_{10}–C_{30}	*Acinetobacter* sp.	Bundy et al. (1998)
Alkane hydroxylase	C_5–C_{16}	*Pseudomonas* sp., *Rhodococcus* sp.	Whyte et al. (2002)

9.10 Attachment of Microorganism to Substrate as a Mechanism to Obtain the Degradation of Contaminant

In the latest literature, it has explained the interaction between microorganisms and hydrocarbon which is used to utilize as a substrate by microorganisms (Van Hamme et al. 2003). However, by modulating of cell surface, hydrophobicity may increase the surface permeability allowing microorganisms to make direct contact with the oil drops (Patist et al. 2000). However, researchers have reported the low hydrophobicity modulation by microorganism on cell surface permits their adhesion to micelles of emulsified oil. Based on their studies, pseudo-solubilization of substrate by microorganism was used to make direct contact of microbial cells with large oil droplets. Also it has been revealed that at the different growth stages of microorganisms, their capability of producing different secondary metabolites has an ability with changing hydrocarbon accession modes (Singh et al. 2006). An increment of bioavailability of oil to the bacterial has been exhibited by the biosurfactant-coated contaminant (Cameotra and Makkar 2004). This process is involving internalization of hydrocarbon inside the cell for subsequent degradation. It is similar in the process called active pinocytosis (Cameotra and Singh 2009).

9.11 Conclusions and Future Perspectives

Continuous release of oil pollutants through either natural or industrial processes is occurring as one of the leading environmental problems. Hence, the bioremediation as a green technology is an acceptable solution due to the cost and effort involved. Therefore, the use of biology systems is playing an important role in this novel strategy. Hence, currently the demand for biological system in various industries is steadily increasing. In this case, biosurfactants play an important role on oil hydrocarbon degradation. However, the commercial success of biosurfactant is still limited because it has to pay high production cost. It is necessary to find out more economically feasible methods to produce biosurfactant in large scale. Hence, future research must focus on producing low-cost biosurfactants and test. Application of biosurfactant and other microbial degradation methods is still in under experimental level. Therefore, the use of these experimental processes in the field scale is important in order to validate the laboratory results. Most of the studies carried out under in vitro condition are needed to move to field scale evaluating its effectiveness.

The development of microbial degradation of contaminant with improvements in genetic expression via the manipulation of gene expression is very important. Application of this technology benefits in large-scale application of microbial degradation and provides us with a cleaner environment in a safe way. The genetically modified (GM) bacteria give a novel approach in biotechnology. However, still there is a limited understanding on their enzyme regulation systems that degrade pollutants. Studies must focus on degrading both aliphatic and aromatic compounds effectively at the same time, and in this case, the biotechnology may play a rigorous role. Thus, the integration of biological engineering and biotechnology is possible and profoundly needed in order to achieve high yields and low costs on biosurfactant production. High-throughput analysis, predictive computational modeling, or simulation and experimental perturbation can be combined to generate new knowledge in order to design strategies for efficient reactions.

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