Oil Biodegradation

Bhagwan Rekadwad and Chandrahasya Khobragade

Abstract This chapter reports the sources, impacts of oil spills, and tar pollution in the marine environment. It also highlights the adverse effects of crude oil and tar pollution on the entire marine ecosystem and national economy. Effective policies measures (mechanical, biological, and societal) for recovering and managing oil spills and tar pollution in Open Oceans and on beaches, harbors, and ports are required to be in place to restore their beauty. Public awareness, government, and private participation and polices are necessary to protect, save, and preserve the pretty and precious marine/coastal biodiversity.

Keywords Crude oil pollution • Marine biodiversity • Oxygenase • *Pseudomonas*

1 Introduction

Oil and tar are disturbing the marine ecosystem. This threat is persisting for a long time. Though in recent decades, evidence has shown decreased impacts. These oil spill insistences in open ocean or near sea coast occur on a daily basis through oil exploitation process, transportation, production, filling, etc. The oil spill which occurred in the polar environments gradually cooled and resulted in the formation of ball-like structures ranging from a few centimeters to 300 cm or larger. These have higher density and sink at the bottom of the sea. It affects the deep-sea benthic meiofauna. Also, these oil balls come to seashore by sea current and giant waves in ocean (Freije 2015; Main et al. 2015). These again cause extensive damage to the coastal ecosystem. It limits the availability of various natural resources and affects the livelihood and the national economy. Therefore, it is necessary to find out the various sources of oil spills and tar ball, their impacts on the human being and society, and their control by adopting the necessary control measures. Once oil spills over an area, it is subjected to a variety of weathering processes (both abiotic and biotic). Most of the light chain hydrocarbons will be removed by environmental

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weathering process such as evaporation, dissolution (air–oil suspension), and photochemical oxidation. The heavy chain hydrocarbons are not removed by environmental weathering process. It is very difficult to remove oil slick, deeply percolated oil in sand, and oil-stained sand. Only oil-eating microorganisms have the ability to degrade heavy chain hydrocarbon.

This chapter describes the sources, impacts, and possible ways to reduce/clean the oil spills, tar, and xenobiotic pollution using eco-friendly poly-extremophilic microorganisms.

2 Sources and Impacts of Oil Spills Pollution

India has a coastline of 8118 km. The coastline of important states of India is coming in contact with seashore such as Gujarat, Maharashtra, Goa, Kerala, Tamil Nadu, Andhra Pradesh, Orissa, West Bengal, and Tripura. Marine ecosystems, especially in the Arabian Sea, are stressed by destructive overfishing practices, toxic pollution, and climate change. These are now threatened by additional artificial problems of oil spills and tar pollution (Figs. 1 and 2). The oil spill and tar-ball pollution have become a global problem particularly in industrialized and developing countries such as India. Every day hundreds of liters of oil contaminate the oceans and natural water bodies by curbing and hazardous activities in Open Oceans and on ports, harbors and beaches. This adversely affected the marine environment (Sukhdhane et al. 2013; Rekadwad and Khobragade 2015).

The oil reduces the penetration of light in thermocline by forming a layer on Sea surface. As a result of these, the Ocean productivity in polluted areas decreased. Ultimately, the chlorophyll production in Open Ocean is inhibited. On the other hand, the microorganisms utilize every form of available oxygen in the water. This

Fig. 1 Dark streaks caused by tar balls lying on a south Goa beach during the last week of July 2005 (NIO, Goa, www.nio.org)

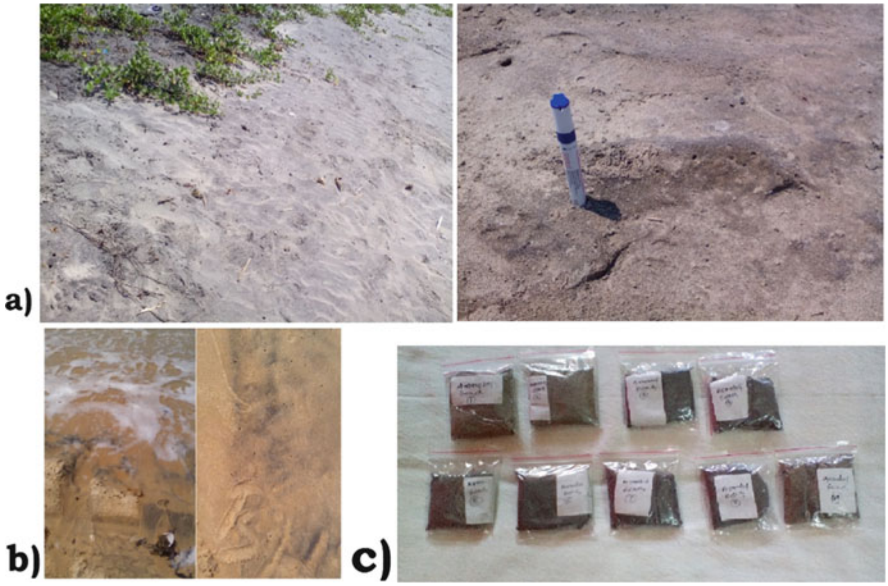

Fig. 2 Field photographs of oil pollution in Goa: (**a**) Arambol beach, Goa; (**b**) Candolim beach, Goa; and (**c**) oil-slicked sand samples collected from Arambol beach

stalled the growth of sensitive species of planktons such as copepods, amphipods, and benthic meiofauna (Kirk et al. 2005; Shi et al. 2005; Chauhan et al. 2008; Lai et al. 2009; Phillips et al. 2009; Sun et al. 2010; Tahhan et al. 2010; Duraisamy and Latha 2011). The remainder of oil slicks, tar-ball residues, and heavy petroleum hydrocarbons formed the emulsion in water and with beach sand. The sun-heated oil spill and tar percolate deep in the sand, and sea current moves it to and fro. The oil covers Sea surface which disallows the mixing of atmospheric oxygen in seawater. It creates the anaerobic environment. The various anaerobic microorganisms include siderophore-producing bacteria flourishing under anaerobic conditions. In the produced siderophore under anaerobic conditions, the free iron present traces dissolved and it is made available for the uptake of phytoplankton (microalgae). It results in the emergence of harmful algal blooms of *Noctiluca scintillans*. It is a free-living nonparasitic species of dinoflagellate. It exhibits bioluminescence when their colonies are disturbed. *Noctiluca scintillans* and *Noctiluca*-like species form *Red tide*. This alga clogs the gills of fishes and marine organisms and their death occurs (Jonker et al. 2006; Riccardi 2010; Tao et al. 2011). The impact of oil pollution is visually seen on the salt marsh plant and mangroves. The crude oil contamination halts the growth of salt marsh plants (i.e., stunted growth of salt marsh plant), reduces the density/thickness of the stem, and lowers percentage of biomass leading to mortality of the plants (Lin and Mendelssohn 2009; Ribeiro et al. 2014). The potential fishing zones (PFZ) are coming under the influence of crude oil and tar contamination. As a result of this, a number of PFZ are reduced. The remainder of the oil slick and tar (residual crude oil) make the Sea coast dirty places. The oil contamination also affected the productivity of fishes in lagoons and ditches. These directly have an impact on the livelihood of fishermen (Kiruri et al. 2013; Rekadwad and Khobragade 2015; Warnock et al. 2015). Moreover, the coral reefs are very sensitive to changes in the surrounding environment. Coral reefs play a key role in the economy of many countries. Coral reefs have hundreds of species in a natural marine ecosystem. The demand for coral reefs is increasing steadily due to the presence of useful natural products. Coral reefs provide coastal protection and supply food and natural products useful in pharmaceutical and cosmetic industries. The major coral reef area is also under the influence of this marine pollution. It directly affects the economy by reducing foreign currency coming to the country through tourism.

3 Biodegradation Crude Oil and Tar Using Bacterial Cultures

The crude oil and tar are the petrochemical product that contains both aliphatic and aromatic hydrocarbons. A variety of microorganisms inhabiting the marine as well as terrestrial environment have the ability to degrade hydrocarbons under the oxic and anoxic conditions (Table 1). Halophilic facultative anaerobe *Halobacterium* produce butylated hydroxyl-toluene, 1,2-benzene-di-carboxylic acid, bis-ester, and di-butyl phthalate from these hydrocarbons. *Halobacterium* redistributes the contaminant by conversion of heavy to light hydrocarbons, which are easily degraded by other microorganisms (Hao and Lu 2009). The polycyclic aromatic hydrocarbon (PAH)-degrading bacteria have monooxygenase pathways (MOP). They have the ability to utilize and degrade crude oil, lubricating oil, grease, diesel, petrol, kerosene, naphthalene, and toluene into short-chain hydrocarbons through MOP within a short period of time (12 h) (Geetha et al. 2013). The short chain alkanes are degraded by aerobic microorganisms, mostly bacteria (*Desulfosarcina-Desulfococcus* cluster of Delta-proteobacteria) and fungi. These microorganisms inhabiting coastal and marine environments perform biodegradation through mechanisms such as the sulfate reduction and anaerobic oxidation of methane (CH_4), etc. They consume small alkanes in anoxic conditions. Under anoxic conditions, these bacteria perform homolytic cleavage of C–H bond present in CH_4, propane, n-butane, and similar types of alkanes (Musat 2015). Mixed culture of petrochemical oily sludge-degrading bacteria such as *Bacillus cereus, Bacillus cibi, Bacillus megaterium, Pseudomonas aeruginosa Polaromonas vacuolata*, and *Stenotrophomonas acidaminiphila*; *fungi such as Eupenicillium hirayamae, Aspergillus terreus, Alternaria alternata, Cladosporium sphaerospermum, Rhizopus stolonifer, Fusarium solani*, and *Paecilomyces variotii*; and yeast such as *Candida maltosa, Candida tropicalis, and Candida apicola* favors considerable reduction of aliphatic (90%) and aromatic (51%) hydrocarbons (Jeon et al. 2003; Zheng et al. 2005; Eibes et al. 2006; Wu et al. 2010; Cerqueira et al. 2011; Wiese et al. 2011; Ali et al. 2012; Ameen et al. 2014, 2015; Jagtap et al. 2014, Hasan 2014; Janani

Table 1 Oil spills and tar ball-degrading microorganisms

Isolate	Gene bank Accession no.	Oxic/anoxic condition	
Acinetobacter venetianus	DQ912805	Oxic	Kostka et al. (2011)
Acinetobacter sp.	FJ876296	Oxic	
Alcanivorax dieselolei	AB453732	Anoxic	
Alcanivorax sp.	AB435642	Oxic MPN	
Bacillus sp.	HQ588864	Anoxic	
Halomonas shengliensis	EF121853	Anoxic	
Labrenzia sp.	EU440961	Oxic MPN	
Marinobacter hydrocarbonoclasticus	DQ768638	Anoxic	
Marinobacter hydrocarbonoclasticus	DQ768638	Anoxic	
Marinobacter hydrocarbonoclasticus	DQ768638	Oxic MPN	
Marinobacter vinifirmus	FJ161339	Anoxic	
Marinobacter vinifirmus	FJ161339	Anoxic	
Microbacterium schleiferi	EU440992	Oxic MPN	
Microbulbifer sp.	GQ334398	Oxic MPN	
Pseudidiomarina maritima	EU600203	Anoxic	
Pseudoalteromonas sp.	AY394863	Oxic	
Pseudomonas pachastrellae	EU603457	Anoxic	
Pseudomonas stutzeri	GU396288	Anoxic	
Shewanella algae	GQ372877	Anoxic	
Vibrio alginolyticus	GQ455008	Anoxic	
Vibrio alginolyticus	GQ455008	Anoxic	
Vibrio hepatarius	EU834019	Anoxic	
Vibrio hepatarius	HM584097	Anoxic	
Vibrio sp.	HM640395	Oxic	
Alternaria alternata	KP033203	Oxic	Ameen et al. (2015)
Aspergillus terreus	KP033202	Oxic	
Cladosporium sphaerospermum	KM979605	Oxic	
Eupenicillium hirayamae	KM979606	Oxic	
Paecilomyces variotii	KM979604	Oxic	

Prathiba et al. 2014; Wang et al. 2015). The aliphatic hydrocarbons such as n-hexadecane are one of the main components of the crude oil. Its bioremediation using *Marinobacter* sp., *Mycobacterium*, *Pseudomonas*, and *Pseudomonas*-like species and filamentous fungi could be made more cost-effective by incorporating cyclodextrins (cyclic oligosaccharides). The solubility of these hydrocarbons can be increased through incorporation of suitable hydrophobic molecules into their hydrophobic sites which speed up the process of biodegradation (Li et al. 2008; Sivaraman et al. 2010; Bonin et al. 2015).

4 Phyto-micro-Degradation/Rhizoremediation

The salt marsh plant rhizospheres are one of the habitats for hydrocarbon-degrading microbial community. The process of removal of hydrocarbon using salt marsh plants and their rhizosphere microbial community is known as phyto-micro-degradation. The phyto-micro-degradation has the active contribution in hydrocarbon removal. This process of hydrocarbon removal is remarkable, observed in case of all plants species in salt marshes such as *Carex phacota, Juncus maritimus, Phragmites australis, Spartina patens*, and *Triglochin striata* (Kuiper et al. 2004; Ribeiro et al. 2011, 2012; Wang et al. 2011; Kurzawova et al. 2012; Khan et al. 2013). The rhizospheric microorganisms associated with legumes such as *Rhizobium* sp. and *Bradyrhizobium* sp. have mechanism to transform xenobiotic compound/polychlorinated hydrocarbons (PCB). The incorporation of alkaloids such as flavonoids, naringenin, and apigenin induces enzyme production and enhances PCB degradation by bacteria. This manipulation of rhizosphere community is known as rhizoengineering. It critically enhances the rhizoremediation of xenobiotic compound which is beyond the inherent power of plant–microbe interaction (Xu et al. 2011; Toussaint et al. 2012; Tang et al. 2013; Jha et al. 2015; Jha and Jha 2015).

5 Biodegradation of Crude Oil and Tar Using Immobilized Cells

The use of immobilized microbial culture (pure culture/microbial consortium) is a simple and economic way for the bioremediation of crude oil, tar, and petroleum hydrocarbons. Immobilized microbial cells have increased contact as compared to a single cell with the hydrocarbon droplets. *Pseudomonas* and *Pseudomonas*-like species produce surfactant (rhamnolipid) which enhances the dispersion of water-insoluble crude oil (*n*-alkanes) at a wide range of salinity. Like the immobilized enzymes, immobilized microbial culture has no decline in the rate. Thus, immobilized microbial pure culture and microbial consortium are effective tools in bioremediation of crude oil, tar, petrochemical wastewater, and soil and xenobiotics/recalcitrant compounds (Cunningham et al. 2004; Rahman et al. 2006; Das and Chandran 2011).

6 Enzyme Participating Biodegradation Pathways

The rapid and complete degradation of most of the oil pollutants by microorganism under aerobic conditions. It is an oxidative process catalyzed by enzymes such as oxygenases and peroxidases. The peripheral pathway such as TCA cycle converts pollutants into easily degradable forms. The energy required for biosyntheses is

Table 2 Enzymes involved in biodegradation of hydrocarbons

Enzymes	Substrates	Microorganisms	
Soluble Methane Monooxygenases	C_1–C_8 alkanes alkenes and cycloalkanes	*Methylococcus, Methylosinus, Methylocystis, Methylomonas, Methylocella*	Das and Chandran (2011), Rajasekar et al. (2012)
Particulate Methane Monooxygenases	C_1–C_5 (halogenated) alkanes and cycloalkanes	*Methylobacter, Methylococcus, Methylocystis*	
AlkB-related Alkane Hydroxylases	C_5–C_{16} alkanes, fatty acids, alkyl benzenes, cycloalkanes, and so forth	*Pseudomonas, Burkholderia, Rhodococcus, Mycobacterium*	
Eukaryotic P450	C_{10}–C_{16} alkanes, fatty acids	*Candida maltose, Candida tropicalis, Yarrowia lipolytica*	
Bacterial P450 oxygenase system	C_5–C_{16} alkanes, cycloalkanes	*Acinetobacter, Caulobacter, Mycobacterium*	
Dioxygenases	C_{10}–C_{30} alkanes	*Acinetobacter sp.*	
Catechol 2,3-dioxygenase	C6–C_{10} alkanes	*Pseudomonas putida mt-2*	Zhang and Xing (2008)
Cysteine dioxygenase	Cysteine	Genetically engineered *Escherichia coli*	Stipanuk et al. (2008)
Naphthalene dioxygenase	PAH	*Ralstonia*, *Sphingomonas*, *Burkholderia*, *Pseudomonas*, *Comamonas*, *Flavobacterium*, and *Bacillus*	Widada et al. (2002)
Manganese peroxidase	Anthracene, dibenzothiophene, and pyrene	–	Eibes et al. (2006)

obtained through gluconeogenesis pathway. Oxygenases mediate degradation of crude oil, oil spills, and tar through various steps such as attachment of microbial cells to the substrates and production of enzymes and surfactants (Das and Chandran 2011). The crude oil, tar, chlorinated hydrocarbons, fuel additives, and their remainders in the environment-degraded by enzymes. The bacteria, archaea, and yeasts have diverse alkane-oxygenase systems involved actively in the synthesis and production of alkane-degrading enzymes such as cytochrome P-450enzymes, integral-membrane di-iron alkane hydroxylases (alkB, catechol 1,2-diooxygenase, soluble di-iron CH_4 monooxygenases), and membrane-bound copper CH_4 monooxygenases (Table 2) under oxic conditions (Saxena and Thakur 2005; Van Beilen and Funhoff 2005, 2007).

7 Future Perspectives

This chapter keenly explains the factors responsible for oil spills and tar-ball pollution in marine/coastal environment such as beaches and salt marshes. It also highlights the adverse effects of oil spills and tar pollution and their impacts on the pathetic condition of ports and harbors, economy, and dependence of the local population on the Oceans. It is necessary to avoid excessive port and harbor activities, unuseful and poor activities, and bad planning of state/central government in maintaining and preventing damage to the coastline. The policy measures and management are required for the complete restoration and protection of the marine as well as coastal environment and the health of Oceans and regime places especially Arabian Sea. These include the removal of tar balls and other hydrocarbons either manually or treated using modern biological techniques. Microorganisms (*Micrococcus, Rhodococcus*, and *Pseudomonas*-like species) capable of hydrocarbon biodegradation should be used to treat these polluted areas. Strict fulfillment of existing regulation and licensing by the government and use of good practices in marine/coastal environment should be adopted. There will be strict and approved protocols for oil filling and washing of ship at the designated port and harbor. The creation of public awareness and giving information to the public and private participants together through communication media (TV, press, advertisements, etc.) to make sustainable marine/coastal environment, to protect, save, and preserve the precious biodiversity of the coastal region of Goa. This will contribute to improve livelihood of people depending on Sea and enhance national economy.

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