

Chapter 9

Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) by Microbes Isolated from the Marine Sponge *Biemna fortis* (Topsent 1897)



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Abstract Industries rely on oil-based products as a significant source of energy. Spillages and accidental leakages are frequent during the extraction, refinement, transportation, and hoarding of oil and their products. The living beings on earth are foremostly contaminated by hazardous polycyclic aromatic hydrocarbons (PAHs); therefore, their degradation is essential. The inadequate use of chemical and mechanical techniques to expel hydrocarbons from the sullied marine ecosystem is not cost-effective. The conversion of complex natural contaminants to other simple natural substances by biodegraders such as microorganisms may allude to absolute mineralization into carbon dioxide, water, and inorganic substances through the mechanism of bioremediation. Previous research works on PAH-degrading bacteria are mainly focused on the utilization of terrestrial microbes; however, the potential use of marine microbes is unexplored. There is an enduring international interest in exploring the application of microbes isolated from marine sponge *Biemna fortis* having high PAH-degrading potential. This book chapter represents an updated overview of the potential application of microbes isolated from marine sponge *B. fortis* for PAH degradation.

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9.1 Introduction

The remediation of surroundings defiled with perilous materials has got a lot of consideration about the potential unfriendly impacts of toxicants on civic well-being. The genotoxic, mutagenic, and carcinogenic properties of polycyclic aromatic hydrocarbons (PAHs) involved in a congregation of natural contaminations are of great concern (WHO 1983; Cerniglia 1992; Mastrangelo et al. 1996; Schützendübel et al. 1999). PAHs have fused aromatic rings in linear, angular, or cluster arrangements which are electrochemically stable, resistant to biodegradation. The carcinogenic index generally tends to increase with an increment in the number of aromatic rings, structural angularity, and hydrophobicity, and an increase in the molecular weight decreases its volatility (Mackay and Callcott 1998; Marston et al. 2001).

Small PAHs include up to six aromatic rings, and large PAHs include more than six aromatic rings. However, PAHs may also contain four (chrysene, naphthacene, pyrene), five (benzo(a)pyrene, pentacene), six (coronene), seven, or more rings (ovalene with ten rings). The igniting of remnant fuels, gas produced by the combustion of engine oil, production of bituminous coal and gas, and the burning of waste generate PAHs which contaminates soil (Harvey 1991; Cai et al. 2007; Das et al. 2008). PAHs are the most extensive organic pollutants of soil, and water bodies (Puglisi et al. 2007), if inappropriately managed and/or fortuitously released to the ecosystem, may endure in soil for a longer duration causing serious damage (Chaineau et al. 2000, 2005).

The US Environmental Protection Agency (USEPA 2002) has documented 16 PAHs as the major environmental pollutants on the basis of profusion and toxicity (Liu et al. 2001; Samanta et al. 2002; Bamforth and Singleton 2005; Puglisi et al. 2007) as depicted in Fig. 9.1. Some of them are carcinogenic, mutagenic, and teratogenic (Adonis et al. 2003; Cai et al. 2007).

PAHs are omnipresent, nonpolar, and extremely hydrophobic, have affinity for fatty tissues (Van der Oost et al. 2003), and are therefore being considered as substances of prospective human health hazards and marine life (Mastrangelo et al. 1996; Hughes et al. 1997; Binkova et al. 2000; Marston et al. 2001; Xue and Warshawsky 2005; Okafor and Opuene 2007; Fagbote and Olaufekum 2010; Lee and Byeon 2010).

The bioaccumulation of PAHs in diverse food chains in the environment is quite frightening (Morehead et al. 1986; Xue and Warshawsky 2005). Awareness of the ecological fate and biodegradation mechanisms of PAHs is incited by their in-destructive impacts on human well-being. The coastal and oceanic sediments are eventual sinks for the readily adsorbed particulate matter by hydrophobic PAHs (Hughes et al. 1997; Yu et al. 2005; Osuji and Ezeburio 2006). PAHs cause significant hazards attributable to their cancer-causing nature in marine life forms, for example, benthic, demersal and pelagic fishes, crustaceans, and shellfish (Peruguni et al. 2007). The destinies of PAHs in the surroundings are linked with both abiotic and biotic events including volatilization, photooxidation, concoction oxidation,

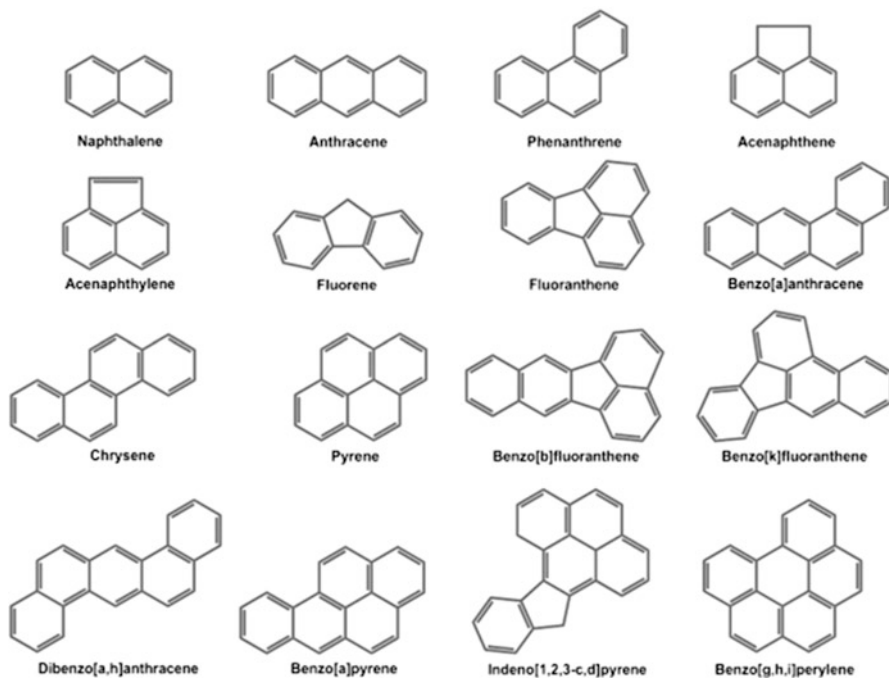


Fig. 9.1 Structure of the 16 PAHs enlisted as priority pollutants. (Bamforth and Singleton 2005)

bioaccumulation, and microbial change. The microbial association with other organisms has been viewed as the most powerful and noteworthy reason for PAH degradation (Cerniglia 1993; Nwuche and Ugoji 2008; Haghghat et al. 2008; Agbozu and Opuene 2009; Atlas and Bragg 2009).

Research on the organic disparagement of PAHs has revealed that microorganisms, fungi, and algae have catabolic capacities that might be used for the remediation of PAH-sullied soil and water (Albert et al. 2005). There has been an increasing concern in the bioremediation of terrestrial and marine surroundings contaminated with PAH and have lethal effects on human well-being. The utilization of microorganisms for the bioremediation of PAH-contaminated environments accounts to be an appealing innovation for reclamation of polluted sites (Mrozik et al. 2003).

The man-made aromatic substances which are incorporated into pesticides, cleansers, oils, solvents, paints, and explosives can be naturally biodegraded enzymatically by the microorganisms (Dagley 1975). The presence of chlorine substituent in these compounds increases their stubbornness to enzymatic degradation leading to their decreased solvency and chemical reactivity (Reineke and Knackmuss 1988). Therefore, as compared to the parent hydrocarbons, chlorinated aromatic compounds are harder to degrade.

The degradation of numerous ecological pollutants like oil and other hydrocarbons through bioremediation process which utilize microorganisms to detoxify or expel pollutants inferable from their diverse metabolic capacities are an advancing

strategy assumed to be noninvasive and relatively less expensive (Nilanjana and Preethy 2011). It is intricate to investigate the biodegradation of PAHs in indigenous habitats. The rate of biodegradation and the degree of bacterial digestion can be attributed to various environmental components, viz., temperature, pH, oxygen concentration, saltiness, light intensity, co-substrates, and season. Besides, in the presence of other nutrients, the level of degradation of polycyclic aromatic hydrocarbons essentially increases (Mrozik et al. 2003).

The sea has been considered as a rich wellspring of compounds having novel structures and biological activities (Archana et al. 2005). The bioremediation of marine environment is significant since seas and estuaries have been the major site of the oil spillage, which happens amid routine tasks of raw petroleum extraction, refining, and circulation and due to the intense mishaps (Anupama and Padma 2009).

Mrozik et al. (2003) demonstrated that the pure and mixed cultures of microorganisms extracted from water bodies can metabolize anthracene and phenanthrene as the sole carbon source. Consequently, another population of marine hydrocarbon-degrading microorganisms has been characterized with a significant role in the biodegradation of hydrocarbons and other related compounds. These microorganisms are isolated from different aquatic sources, for example, sponges, ocean weeds, and so on.

9.2 Marine Sponges

Marine sponges are sedentary organisms which are inhabitants of ocean bed and represent a significant component of the marine benthic environment. Sponges are composed of layers of cells with no clear tissues or organs and appropriate nervous system. Sponges, being ciliates, are filter feeders and feed on planktons and small marine organisms through minute body pores by pumping the circulating water using specialized paddles and tails. They have been living in aquatic habitat for more than 600 million years. More than 10,000 species of sponges have been identified around the world. Numerous sponges harbor microorganisms with bioactive properties, of which a few can be potentially used as pharmaceutical leads (Hill 2004).

Sponges encompass a diverse group of green algae, red algae, cryptophytes, dinoflagellates, diatoms, and a rich variety of microorganisms in their cells (Simone et al. 2005). *Biemna fortis* is one such form of sponge that harbors a large number of bacterial groups, which have the capacity to degrade the high concentrations of polycyclic aromatic hydrocarbons (PAHs) (Farhan and Mahesh 2015). The investigation of biodegradation of aromatic hydrocarbon by microorganism relies on techniques, viz., denaturing gel gradient electrophoresis (DGGE), microbial community fingerprinting by T-RFLP and ARISA, DNA hybridization assay, thin layer chromatography (TLC), and 16S rRNA gene sequencing including fluorescent in

situ hybridization (FISH). Sequencing the DNA of biodegrading microorganisms has opened our scientific outlook experiences into the systems, the event, and the character of dynamic microbes that impact biodegradation of natural ecological contaminations.

9.3 Classification of *Biemna fortis*

Biemna fortis is a marine sponge belonging to the lineage: cellular organisms, Eukaryota, Opisthokonta, Metazoa, Porifera, Demospongiae, Heteroscleromorpha, Biemnida, and Biemnidae. There are several other species belonging to the genus *Biemna* which is diverse in nature (Table 9.1).

Table 9.1 List of species listed under the genera *Biemna*, class Demospongiae

<i>Biemna</i> species	Described by the scientists
<i>B. anisotoxa</i>	Lévi, 1963
<i>B. bihamigera</i>	Dendy, 1922
<i>B. caribea</i>	Pulitzer-Finali, 1986
<i>B. chilensis</i>	Thiele, 1905
<i>B. chujaensis</i>	Sim & Shim, 2006
<i>B. ciocalyptoides</i>	Dendy, 1897
<i>B. cribaria</i>	Alcolado & Gotera, 1986
<i>B. dautzenbergi</i>	Topsent, 1890
<i>B. ehrenbergi</i>	Keller, 1889
<i>B. fistulosa</i>	Topsent, 1897
<i>B. ftabellata</i>	Bergquist, 1970
<i>B. fortis</i>	Topsent, 1897
<i>B. fragilis</i>	Kieschnick, 1900
<i>B. gellioides</i>	Lévi & Lévi, 1989
<i>B. granulosigmata</i>	Lévi, 1993
<i>B. hongdoensis</i>	Jeon & Sim, 2009
<i>B. humilis</i>	Thiele, 1903
<i>B. jeolmyongensis</i>	Sim & Shim, 2006
<i>B. laboutei</i>	Hooper, 1996
<i>B. liposigma</i>	Burton, 1928
<i>B. liposphaera</i>	Hentschel, 1912
<i>B. macrorhaphis</i>	Hentschel, 1914
<i>B. megalosigma</i>	Hentschel, 1912
<i>B. megastyla</i>	Burton, 1959
<i>B. microacanthosigma</i>	Mothes, Hajdu, Lerner & van Soest, 2004
<i>B. microstrongyla</i>	Hentschel, 1912
<i>B. microstyla</i>	de Laubenfels, 1950
<i>B. microxa</i>	Hentschel, 1911

(continued)

Table 9.1 (continued)

<i>Biemna</i> species	Described by the scientists
<i>B.mnioeis</i>	de Laubenfels, 1954
<i>B.novaezealandiae</i>	Dendy, 1924
<i>B.omanensis</i>	van Soest & Beglinger, 2002
<i>B.parthenopea</i>	Pulitzer-Finali, 1978
<i>B.pedunculata</i>	Lévi, 1963
<i>B.peracuta</i>	Topsent, 1927
<i>B.philippensis</i>	Dendy, 1896
<i>B.plicata</i>	Whitelegge, 1907
<i>B.polyphylla</i>	Lévi, 1963
<i>B.rhabderemioides</i>	Bergquist, 1961
<i>B.rhabdostyla</i>	Uriz, 1988
<i>B.rhadia</i>	de Laubenfels, 1930
<i>B.rufescens</i>	Bergquist & Fromont, 1988
<i>B.saucia</i>	Hooper, Capon & Hodder, 1991
<i>B.seychellensis</i>	Thomas, 1973
<i>B.spinomicroxea</i>	Mothes, Campos, Lerner, Carraro & van Soest, 2005
<i>B.strongylota</i>	Rios & Cristobo, 2006
<i>B.tenuisigma</i>	Pulitzer-Finali, 1978
<i>B.tetraphis</i>	Tanita & Hoshino, 1989
<i>B.thielei</i>	Burton, 1930
<i>B.trirhaphis</i>	Topsent, 1897
<i>B.trisigmata</i>	Mothes & Campos, 2004
<i>B.truncata</i>	Hentschel, 1912
<i>B.tubulata</i>	Dendy, 1905
<i>B.variantia</i>	Bowerbank, 1858
<i>B.victoriana</i>	Hallmann, 1916

9.4 Distribution of *Biemna*

The marine sponges *Biemna* are widely distributed and are also known to live in the western and central Indian Ocean Regions.

- *Biemna anisotoxa* (LEVI), from South Africa (Levi 1963).
- *Biemna ciocalyptoides* sensu (BURTON), from the Red Sea (Burton 1959) and Seychelles (Van Soest 1994) (homonym of *B.ciocalyptoides* (Dendy 1897)) from southern Australia.
- *Biemna seychellensis* (THOMAS), from the Seychelles Is (Thomas 1973), originally described as a variety of the N Atlantic *B. variantia* (BOWERBANK).
- *Biemna fords* (TOPSENT), from Ambon, Indonesia (Topsent 1897; Desqueyroux-Faundez 1981), Arafura Sea (Hentschel 1912), Straits of Malacca (Sollas 1902), Bay of Bengal (Burton 1930; Burton and Rao 1932), Red Sea (Topsent 1897;

Burton 1959), Mombasa (Pulitzer-Finali 1993) and Sulawesi, Indonesia, Gulf of Thailand, and Truk Atoll, Micronesia.

- *Biemna humilis* (THIELE) from Indonesia (Thiele 1903), Zanzibar and Shimoni (Pulitzer-Finali 1993).
- *Biemna microstrongyla* (HENTSCHEL) from Indonesia (Hentschel 1912) and Mombasa (Pulitzer-Finali 1993).
- *Biemna pedonculata* (LEVI), from South Africa (Levi 1963).
- *Biemna polyphylla* (LEVI), from South Africa (Levi 1963).
- *Biemna sigmodrigma* (LEVI), from South Africa (Levi 1963) (originally described as a subspecies of *B. megalosigma* HENTSCHEL from SE Indonesia).
- *Biemna trirhaphis* (TOPSENT), from Ambon, Indonesia (Topsent 1897; Desqueyroux-Faundez 1981), Red Sea (Burton 1959; Levi 1961), and Mombasa and Zanzibar (Pulitzer-Finali 1993).
- *Biemna tubulata* (DENDY) from Sri Lanka (Dendy 1905), NW India (Dendy 1916), the Mergui Archipelago and Andaman Sea region (Burton and Rao 1932), Providence Reef (Dendy 1922), and Seychelles Is (Thomas 1973).
- *Biemna truncata* (recorded from Aru I., Indonesia (Hentschel 1912), Sri Lanka (Burton 1930), and the Seychelles (Thomas 1973).
- *Biemna bihamigera* (from Providence Reef (Dendy 1922), Aldabra (Levi 1961) and Shimoni, East Africa (Pulitzer-Finall 1993).
- *Biemna democratica* (from the Straits of Malacca; Sollas 1902).
- *Biemna saucia* is a toxic sponge from the NE Indian Ocean (Hooper et al. 1991).

9.5 Description of *Biemna fortis*

Biemna fortis is a massive sponge (15 cm long and 9 cm in diameter), erect with chimney-like projections, often compressed with large fistulose surface processes and terminal oscules; peduncle is masked beneath the sediment with tubular projections noticeable at the surface; the sponge is tough and hispidous; ostia are not obvious, while the oscula (3–8 mm) are terminally situated; surface is woody and cork-like (Fig. 9.2).

The surface is rough, fibrous, compressible, and harsh to touch and has a firm consistency. The ectosomal skeleton has protruding choanosomal megascleres but without any special spicules or structures. The choanosomal skeleton is cavernous, disorganized halichondrid reticulate. Megascleres are exceptionally long and thickest in the basal third of the spicule. Megascleres are styles, smooth, and marginally bended upward (extend: 929–1283 × 16.2–36.5 μm; mean: 1121 × 28.6 μm); microscleres are sigmas, abundant with pointed tips (run: 71–93 × 3.1–5.3 μm; mean: 85 μm × 4.3 μm). Microscleres include sigmas of two sizes, raphides, and microx-eas. The ectosomal skeleton is a mass of extraneously arranged spicules; choano-some rarely contains fiber tracts and is made of bounteous felted spicules scattered with numerous sigmas. They flourish well in territories of saline conditions, found in coral reef and the sandy substrate with coral patches (Belinda et al. 2005).

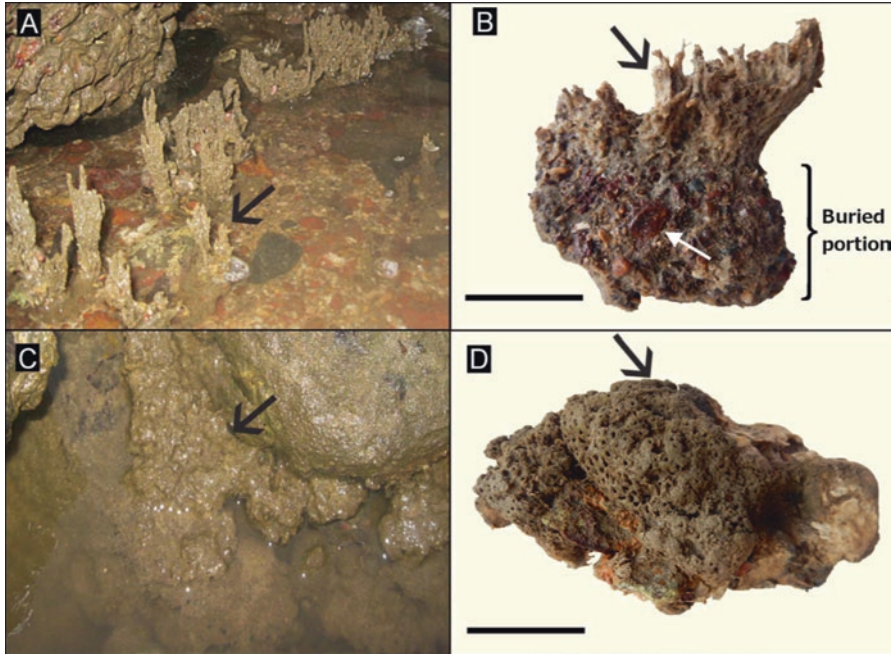


Fig. 9.2 *Biemna fortis* growth forms (Dahihande and Thakur 2017). (a) *Biemna fortis* growing (b) Partially buried growth form, with the underground, buried body mass (c) *Biemna fortis* growing (d) Massive growth form, most of the growth is above surface. Black arrows indicate the portion above ground surface, white arrow indicates the sediment inclusion in the buried body mass

The color of the sponge varies in live and in a preserved sample: yellowish-green to darkish-yellow color can be observed in the part buried in the ground; the tip of the projection is dull green to dim, and core is sandy gray. Differences in color are because of aggregated debris.

9.6 Sponge-Associated Microorganisms

Marine sponges inhabit diverse microbial communities with remarkable biological and biotechnological implications. Sponges are filter feeders and devour microorganisms from the surrounding seawater. Any microbes that endure the digestive and immune responses of sponges are symbiotically associated. In marine sponges, ten characterized bacterial phyla have been identified (Taylor et al. 2007; Hentschel et al. 2012; Webster and Taylor 2012). Majority of sponges are widely associated with α , β , γ , δ , and ϵ *Proteobacteria* and *Chloroflexi* bacteria (Hentschel et al. 2002; Taylor et al. 2007).

Sponges form harmonic relations with diverse bacteria and form the most vital portion of sponge's diet. In some cases, the bacterial cells make up to 38% of sponge

wet weight (Vacelet and Donadey 1977; Taylor et al. 2007; Hentschel et al. 2012). Albeit sponge-specific archaea, eubacteria subsist exclusively within sponge hosts but do not inhabit the surrounding marine environment (Hentschel et al. 2002). The microbiomes of marine sponge reliably constitute archaea and fungi exhibiting species-specific host-related functions (Taylor et al. 2007; Thomas et al. 2010).

Prabha et al. (2010) isolated and cultured several bacteria from the sponge *Halichondria* sp., collected from the Gujarat coast of the Indo-Pacific region, and the antibiotic activity was assayed against 16 strains of clinical pathogens. Further investigation was carried out on the most potent *Bacillus* sp. (SAB1) and the fungus *Aspergillus fumigatus* which was antagonistic to several clinically pathogenic Gram-positive and Gram-negative bacteria.

Four species of marine sponges, viz., *Echinodictyum* sp., *Spongia* sp., *Sigmatocia fibulatus*, and *Mycale mannarensis*, were found to harbor 75 bacterial strains from the Tuticorin coast, Gulf of Mannar region (Anand et al. 2006). Four bacteria, viz., *Bacillus subtilis*, *Escherichia coli*, *Vibrio parahaemolyticus*, and *Vibrio harveyi*, and one fungal pathogen, viz., *Candida albicans*, were used to screen for antibiotic production by these strains by the agar overlay method.

Ocky Karna Radjasa (2007) isolated 90 bacterial isolates found associated with sponges collected from the marine regions of Indonesia.

Saravanakumar et al. (2011) reported antibiotic-producing bacteria, isolated from 14 species of sponges from Indian waters. One hundred and nine bacterial strains were screened for antibiotic production against five fish pathogens, namely, *Vibrio fischeri*, *Vibrio vulnificus*, *Vibrio harveyi*, *Aeromonas hydrophila*, and *Aeromonas sobria*.

Santos et al. (2010) isolated and characterized bacteria with antimicrobial activities against pathogenic bacteria from Brazilian sponges. The sponge-associated bacterial strains were subdivided into three different clusters based on the comparative sequence analysis of 16S rRNA genes, among which, three with alpha-Proteobacteria (*Pseudovibrio* sp.), four with gamma-Proteobacteria (genera *Pseudomonas* and *Stenotrophomonas*), and five strains were affiliated with Firmicutes (genera *Bacillus* and *Virgibacillus*).

9.7 Antimicrobial Activity of Sponge-Associated Bacteria

The microorganisms associated with numerous sedentary marine sponges serve as food particles and chemical defenses against potential predators (Albrecht et al. 2007), inhibiting cancerous growths (Belinda et al. 2005). Sponge-associated bacterial strains represent a rich source of bioactive metabolites (Kalirajan et al. 2013) and are regarded as gold quarry due to their vast applications in pharmaceuticals, nutritional supplements, cosmetics, agrochemicals, molecular probes, enzymes, and fine chemicals (Isaac et al. 2012). Only a few of the bioactive compounds discovered in sponges have been commercialized (Archana et al. 2005, Kalirajan et al. 2013).

The quantitative and qualitative condition of the surrounding water varies according to the bacterial colonies (Albrecht et al. 2007). The bacteria developing on the surface of sponges reside in a highly competitive environment and have limited access to space and nutrients (Burgess et al. 1999). Sponge-associated bacteria produce secondary metabolites which exceed planktonic bacterial metabolite production (Lemos et al. 1986; Jensen and Fenical 1994). A number of impending therapeutic substances of the sponges have remarkable similarities to metabolites derived from their associated microorganisms and are a rich source for the manufacture of antibiotics (Proksch et al. 2002).

The microbes associated with sponges have the novel genes such as polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS) for synthesizing a broad range of structurally diverse natural compounds (Isaac et al. 2012). These bioactive substances have significant medical and industrial applications and are important for the epibiotic defense of the marine invertebrates (Archana et al. 2005). *Pseudomonas* sp. 1531-E7 was isolated from the marine sponge *Homophymia* sp. leading to the discovery of antiviral compound 2-undecyl-4-quinolone (1) (Bultel-Poncé et al. 1999).

Fourteen isoprenylated cyclohexanols and truncateols A-N isolated from the sponge-associated fungus *Truncatella angustata* were tested in vitro against the influenza A (H1N1) virus reported by Zhao et al. (2015). Reimer et al. (2015) isolated *Streptomyces* sp. that was associated with the marine sponge *Dysidea tupa*.

Hundred heterotrophic, halophilic bacterial bionts isolated from one bivalve, five, and nine corals sponges were investigated for the antagonistic activities (Sheryanne and Irene 2012). Among these 46 bionts were active against human pathogenic bacteria, namely, *E. coli*, *A. aerogenes*, *S. marcescens*, *S. citreus*, *P. vulgaris*, and *S. typhi*. Due to the immense activity, biochemical accessibility, and stability than the terrestrial counterparts, marine sponge-related microorganisms have drawn tremendous consideration as a reserve for new secondary metabolites (Skariyachan et al. 2014; Kiran et al. 2014).

9.8 Biodegradation of PAHs Using Sponge *Biemna fortis*-Associated Bacteria

Virtually, all aquatic sponges contain a numerous microorganisms in their tissues. From the marine sponge *Callyspongia diffusa*, a total of 101 microbial isolates were obtained, and the biosurfactant producers were *B. subtilis* MB-7, *B. amyloliquefaciens* MB-101, *Halomonas* sp. MB-30, and *Alcaligenes* sp. (Asha et al. 2015). The sponge-associated microorganisms in aquatic environments can be effectively used in the bioremediation of PAH.

In oil spilled environment, microorganisms are the most active primary oil degraders (Rahman et al. 2003; Brooijmans et al. 2009) and feed exclusively on hydrocarbons (Yakimov et al. 2007). Twenty five genera of hydrocarbon-degrading bacteria and fungi were enlisted by Floodgate, 1984, which were isolated from

marine environment. Kiran et al. (2010) reported that cultivable microorganisms from marine sponge may act as potent sources of glycolipid and lipopeptide biosurfactant.

The *Biemna fortis*-associated bacterial strains, namely, *E. coli*, *P. aeruginosa*, *S. aureus*, *S. typhi*, *S. flexneri*, *K. pneumoniae*, *V. cholera*, *A. baumannii*, methicillin-resistant *S. aureus*, *P. macquariensis*, *K. varians*, *M. luteus*, *C. xerosis*, and *M. varians* were successfully isolated using the enrichment process. The bacteria, viz., *C. xerosis*, *K. varians*, *P. macquariensis*, *M. luteus*, and *M. varians*, were the most influential and significantly biodegraded-specific PAHs like phenanthrene, fluoranthene, naphthalene, pyrene, and anthracene (Farhan and Mahesh 2015). These four bacterial isolates were utilizing fluoranthene, pyrene, and naphthalene as a sole source of carbon and energy for growth. Naphthalene being the simplest PAH has a fused pair benzene rings and has increased water solubility at 25 °C resulting in greater accessibility of the substrate to the microorganisms.

The majority of the isolates from marine sponge *Biemna* were *P. macquariensis*, *K. varians*, *M. luteus*, *C. xerosis*, and *M. varians* grows on or mineralizes pyrene as reported by Farhan and Mahesh (2015). Since pyrene is structurally similar to several carcinogenic PAHs, it has been used as a model compound for biodegradation of high molecular weight PAH.

Paenibacillus macquariensis was an effective degrader to metabolize three PAHs with more complex structure, i.e., naphthalene, fluoranthene, and pyrene, and consequently may be employed in metabolizing different recalcitrant PAHs having lesser solubility. Hence, it can play an efficient role in cleaning up of numerous PAHs in the contaminated sites (Farhan and Mahesh 2015). Xuezhong Zhu et al. (2016) recommended that phenanthrene, fluoranthene, and naphthalene contributed as co-substrates, and the degradation of these compounds proceeded at a moderately faster rate when compared to the biodegradation of substrates alone. Thavamani et al. (2012) reported that *Paenibacillus* sp. PHE-3 could biodegrade PAHs through co-metabolism and degrade benzo[a]pyrene utilizing phenanthrene as a co-substrate. Daane et al. (2002) investigated the degradation of naphthalene from petroleum hydrocarbon-contaminated sediment and salt marsh rhizosphere from the isolated *Paenibacillus* sp. which were able to use aromatic substrates.

Bacillus gordonae sp. (*P. validus* by Heyndrickx et al. 1995) described by Pichinoty et al. (1986) utilized phthalate, protocatechuate, p-hydroxybenzoate, isophthalate, phenol, trimellitate, p-cresol, quinate, and naphthalene as a sole source of carbon. *Paenibacillus* sp. was isolated as a PAH-degrading microorganism from tar oil-contaminated soil (Meyer et al. 1999). Daane et al. (2001) revealed that *Paenibacillus* sp. (strain PR-P1) facilitated pyrene degradation in sediment slurry microbes utilizing naphthalene or phenanthrene as a sole source of carbon.

Kocuria varians was found to degrade naphthalene (Farhan and Mahesh 2015). Tumaikina et al. (2008) recognized the ability of other *Kocuria* species to grow on oil and other hydrocarbons as a sole carbon and energy sources. For example, naphthalene, phenanthrene, fluoranthene, and crude oil were degraded by *K. flava* and *K. rosea*.

Micrococcus sp. was reported to have high interaction on naphthalene and was apparent that the antagonistic incident may result in blocking appropriate degradation pathways for other PAHs (Farhan and Mahesh 2015). The rates of degradation for these compounds delayed considerably when more than one compound was present in the same sample showing an antagonistic effect on the degrading abilities of isolated strains which could co-metabolize other PAHs. *M. luteus* degraded the compounds at a faster rate than *K. rosea*, and the utmost degradation was observed for naphthalene followed by phenanthrene, fluoranthene, and pyrene (Haritash and Kaushik 2016). However, the mechanism of biodegradation of benz- α anthracene, benz- α pyrene, and pyrene is still unclear. Othman et al. (2010) revealed that under optimum conditions, only *M. diversus* had a high tendency for degradation of two ring naphthalenes. Additionally, similar results were obtained by Narasimhulu and Setty (2011) who isolated and characterized the naphthalene-degrading bacteria in soil.

Corynebacterium sp., a GC-rich Gram-positive bacterium, employed in the development of bio-production of diverse compounds such as amino acids, alcohols, and organic acids can utilize naphthalene as the main source of carbon and energy (Farhan and Mahesh 2015). *Pseudomonas*, *Sphingomonas*, *Nocardia*, *Beijerinckia*, *Rhodococcus*, and *Mycobacterium* can completely mineralize anthracene forming the dihydriol as an initial oxygenated intermediate (Sudip et al. 2002).

Mycobacterium sp. has been reported to degrade >95% fluoranthene efficiently in a mineral medium supplemented with organic nutrients. *Mycobacterium* sp., *Rhodococcus* sp., and *Gardona* sp. isolated from numerous actinomycetes bacteria utilize fluoranthene from varying hydrocarbon polluted soils (Sudip et al. 2002).

9.9 Microbial Biodegradation Mechanism of PAH

Bacteria have developed several approaches for imbibing energy from nearly all compounds and have been considered as nature's vital scavengers due to their rapid flexibility to degrade or remediate ecological hazards.

Numerous bacteria can biodegrade PAHs, and few can consume low-MW PAHs as their carbon source. To investigate PAH degradation, culture-based approaches have been extensively employed, and several bacterial species have been capable of doing it.

The biochemical pathways for the bacterial biodegradation of PAHs have been thoroughly studied for anthracene and acenaphthene (Dean-Ross et al. 2001; Pinyakong et al. 2004), naphthalene (Resnick et al. 1996; Annweiler et al. 2000), and phenanthrene (Menn et al. 1993; Kiyohara et al. 1994; Pinyakong et al. 2003a, b). The most representative genera responsible for PAH degradation are *Acidovorax*, *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Comamonas*, *Corynebacterium*, *Flavobacterium*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Pseudomonas*, *Rhodococcus*, *Sphingomonas*, *Stenotrophomonas*, *Streptomyces*, and *Xanthomonas* (Doyle et al. 2008). These monoculture studies

have been extremely valuable since the microorganisms metabolize different compounds in pure cultures.

Based on the presence or absence of oxygen, two main approaches (aerobic and anaerobic degradation) are being followed to degrade PAHs. Oxygen is not only the final electron acceptor in the aerobic catabolism of aromatics but also acts as a co-substrate for the hydroxylation and oxygenolytic aromatic ring cleavage. On the contrary, based on reductive reactions, the anaerobic catabolism of aromatic substances employs a completely different strategy to cleave the aromatic ring (Carmona et al. 2009). The anaerobic catabolism of aromatic compounds is unclear with respect to microbial potentiality.

Under aerobic conditions, the degradation of the majority of organic pollutants by microorganisms is more rapid. The foremost step in aromatic hydrocarbon degradation is the accumulation of one or two oxygen atoms which are then converted into phenol (aliphatic) or alkanol (aromatic). The first intermediate is an epoxide in some species activating the hydrocarbon to make it more soluble in water and tags and commence a reactive site for the next reaction. The energy required for the reaction is generated by the oxidation of a reduced bio-intermediary, for instance, NADH is re-oxidized by an electron acceptor. HMW PAHs (two and three rings) are readily degradable substrates which are less specifically metabolized by the catabolic enzymes due to their low solubility when compared to LMW PAHs (Cerniglia and Heitkamp 1989; Molina et al. 1999). These aromatic hydrocarbons get transformed into products like alkanes, alkenes, and cycloalkanes. The degradation pathway for alkanes and cycloalkanes includes subsequent formation of alcohol, aldehyde, and fatty acids. Different enzyme systems accomplish the primary attack on alkanes for the degradation.

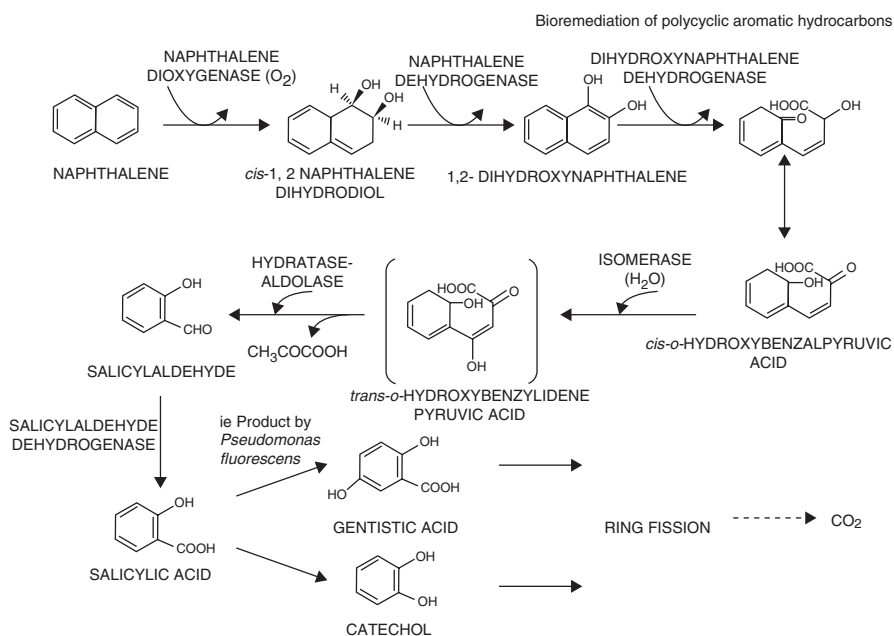
Depending on the chain length, specific enzyme system attacks PAH rings and relies on molecular oxygen for the biodegradation (Table 9.2). The chief mechanism for aerobic bacterial metabolism of PAHs relies on the preliminary oxidation of the benzene ring by the action of multicomponent enzyme systems (oxygenases, peroxidases, and dioxygenase) to form *cis*-dihydrodiols as a preliminary by-product. *Mycobacterium* sp. is competent of oxidizing PAHs by the enzyme cytochrome P-450 monooxygenase to form *trans*-dihydrodiols (Kelley et al. 1990). Dioxygenases cleave these intermediates resulting in the formation of intradiol or extradiol ring (Cerniglia 1992; Eaton and Chapman 1992; Gibson and Paraes 2000), which is then metabolized to carbon dioxide and water via catechols (Kelly et al. 1990). The enzymatic reactions and metabolic pathways implicated in the microbial degradation of naphthalene have been illustrated in Fig. 9.3.

The microbial degradation of oil, chlorinated hydrocarbons, fuel additives, and many other compounds are efficiently degraded by cytochrome P450 alkane hydroxylases belonging to the superfamily of heme-thiolate monooxygenases (Van Beilen and Funhoff 2007).

Pseudomonas along with diversified bacteria is capable of oxidizing naphthalene utilizing dioxygenase enzymes. Ensign (2001) isolated and characterized three proteins of omega-hydroxylase system (the rubredoxin reductase, a rubredoxin, and an omega-hydroxylase) from *Pseudomonas*. Fatty acids are the intermittent prod-

Table 9.2 Enzymes implicated in biodegradation of aromatic hydrocarbons

Enzymes	Substrates	Microorganisms
Soluble methane Monooxygenases	C ₁ to C ₈ alkanes, alkenes, and cycloalkanes	<i>M. cella</i> <i>M. coccus</i> <i>M. cystitis</i> <i>M. monas</i> <i>M. sinus</i>
Particulate Methane Monooxygenases	C ₁ to C ₅ alkanes and cycloalkanes	<i>M. bacter</i> <i>M. coccus</i> <i>M. cystitis</i>
AlkB-related Alkane Hydroxylases	C ₅ to C ₁₆ alkanes, fatty acids, alkyl benzenes, cycloalkanes, etc.	<i>Burkholderia</i> <i>Mycobacterium</i> <i>Pseudomonas</i> <i>Rhodococcus</i>
Eukaryotic P450	C ₁₅ to C ₁₆ alkanes, fatty acids	<i>C. maltosa</i> <i>C. tropicalis</i> <i>Y. lipolytica</i>
Bacterial P450 Oxygenase system	C ₅ -C ₁₆ alkanes, cycloalkanes	<i>Acinetobacter</i> <i>Caulobacter</i> <i>Mycobacterium</i>
Dioxygenases	C ₁₀ -C ₃₀ alkanes	<i>Acinetobacter</i> sp.

**Fig. 9.3** Mechanism of polycyclic aromatic hydrocarbon degradation by microbes. (Bamforth and Singleton 2005)

ucts of the alkane degradation produced from the alkanols via aldehydes and further decomposed by carboxylic acid degradation pathway. These acids are excreted by the cells and accumulate in the environment and thus, serve as a carbon source for microbial community.

9.10 Application of Bioremediation Agents

The bioremediation agents defined by the United States Environmental Protection Agency (USEPA 2002) include microbial cultures, enzyme/nutrient additives enlisted in Table 9.3. considerably enhance the rate of biodegradation to alleviate the effects of the discharge (Nichols 2001).

The bioremediation product may be efficient in the laboratory but less efficient in the field (Venosa et al. 1996; Lee et al. 1997; Mearns 1997). Since laboratory studies always cannot imitate change in the macro environmental conditions, field studies are the most convincing expression of the efficacy of these products.

Inipol EAP22 is a nutrient additive consisting of urea as a nitrogen source (microemulsion), phosphorus source (sodium laureth phosphate), 2-butoxy-1-ethanol (surfactant), oleic acid (hydrophobic agent), and oil spill cleanup agent famous for bioremediation (Table 9.3). The merits of Inipol EAP22 include (1) prevention of water-in-oil emulsification by minimizing the interfacial tension and oil viscosity, (2) controlling the release of phosphorus and nitrogen for oil biodegradation, and (3) nontoxicity to living organisms and superior biodegradability (Ladousse and Tramier 1991).

Table 9.3 Commercially available bioremediation agents

Sl. No.	Bioremediation agents/products
1	BET BIOPETRO
2	BIOCATALYSTIOS-500
3	BIO-D NUTRIENTS
4	BIOREN 1 AND 2
5	ENVIROZYME BR
6	HYDROCARBON D-GRADER
7	INIPOL EAP22
8	IOS-500
9	LAND AND SEA
10	MEDINA MICROBIAL ACTIVATOR
11	MICRO-BLAZE
12	OIL SPILL EATER –II
13	PETRO-CLEAN
14	WAPED
15	WMI-2000

Oil Spill Eater II (OSEII) is an enzyme/nutrient stabilizer consisting of “nitrogen, phosphorus (ready carbon availability), and vitamins (quick bacterial colonization)” (Table 9.3). A field investigation was carried in a fuel-contaminated area of Marine Corps Air Ground Combat Center (MCAGCC) in California to test the efficacy of OSEII for enhancing hydrocarbon biodegradation (Zwick et al. 1997).

BIOREN 1 and 2 are the derivatives of fish meal with urea and superphosphate (in a granular form as nitrogen source) and phosphorus sources and proteinaceous material (carbon source). BIOREN 1 contains a biosurfactant leading to enhanced oil degradation, while BIOREN 2 without biosurfactant attributes to greater bio-availability of hydrocarbons to microbial attack (Le Floch et al. 1997, 1999).

9.11 Conclusion and Future Prospective

The main threat to the aquatic environment is through oil leakage and by lethal polycyclic aromatic hydrocarbons into the food chain which is due to the toxic, mutagenic, and carcinogenic properties (Sei and Fathepure 2009).

The quick elimination and cleanup of PAHs by the physicochemical methods such as volatilization, photochemical oxidation, and bioaccumulation are seldom successful when compared to microbial bioremediation (Prince 1997; Zhao et al. 2008).

Bacteria are omnipresent and predominantly found in the marine environment and are considered as a potent hydrocarbon-degrading agents (Dasgupta et al. 2013). The degradation of oil-rich and potentially toxic environments solely depends on the novel microorganisms associated with the sponge *Biemna fortis*. The native and exogenous microbes used as inoculants can be applied to hydrocarbon-polluted environments depending on their biodegrading capabilities (Venosa and Zhu 2003; Díaz-Ramírez et al. 2008).

The major requirement for the bioremediation of oil spill depends on the microorganisms with suitable metabolic competence (Venosa et al. 2001). An array of microorganisms has been isolated from the marine sponge *Biemna fortis* for PAH degradation yielding beneficiary result (Farhan and Mahesh 2015). The degradation of aromatic hydrocarbons by PAH-degrading bacteria may detoxify or even contribute to the nutrition of the sponge remains uncertain.

Therefore, several PAH-contaminated sites require the cleansing, impending remediation methods that have to be explored and applied. Further investigations required to evaluate the functions of other invertebrates for the associations of PAH-degrading bacteria in oil-seep environs should yield remarkable outcome. The associations of a multifaceted bacterial community in sponge *Biemna fortis* capable of scavenging PAHs have been translated. Thus, we could suggest that these microbial populations may pave to the success of the sponge *Biemna fortis* living in such unique ecosystems by degrading PAHs.

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