Microbial diversity: Application of micro-organisms for the biodegradation of xenobiotics

Rakesh Kumar Jain[1,*], Manisha Kapur[1], Sumeet Labana[1], Banwari Lal[2], Priyangshu Manab Sarma[3], Dhruva Bhattacharya[3] and Indu Shekhar Thakur[4,†]

[1]Institute of Microbial Technology, Sector 39-A, Chandigarh 160 036, India
[2]Microbial Biotechnology, The Energy and Resources Institute, Habitat Place, Lodhi Road, New Delhi 110 003, India
[3]Center of Bioresources and Biotechnology, TERI School of Advanced Studies, Habitat Place, Lodhi Road, New Delhi 110 003, India
[4]Department of Environmental Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar 263 145, India
[†]Present address: School of Environmental Sciences, Jawaharlal Nehru University, New Delhi 110 067, India

Environmental pollution caused by the release of a wide range of compounds as a consequence of industrial progress has now assumed serious proportions. Thousands of hazardous waste sites have been generated worldwide resulting from the accumulation of xenobiotics in soil and water over the years. Nitroaromatic compounds (NACs), polycyclic aromatics and other hydrocarbons (PAHs) that are constituents of crude oil, and halogenated organic compounds together constitute a large and diverse group of chemicals that are responsible for causing widespread environmental pollution. The physico-chemical remedial strategies to clean up sites contaminated by these compounds are not cost effective or adequate enough. Therefore, research is increasingly being focused on biological methods for the degradation and elimination of these pollutants. Sites contaminated by these compounds need urgent remedial solutions, the search for which has revealed a diverse range of bacteria that can utilize these xenobiotics as substrates, often mineralizing them or converting them into harmless products, and in the process helping to clean up the environment. New genes, enzymes and metabolic routes involved in bacterial degradation of PAHs, NACs and halogenated organic compounds (HOCs) have been discovered, and new methods have been developed which allow the discovery and broad flexibility of microorganisms in environmental clean up. Studies to understand the interaction between xenobiotics and microorganisms in the environment have to intersect with biochemical and genetic engineering areas. Such a strategy will provide the ground for successful interventions into environmental processes and ultimately lead to optimized strategies for tapping of microbial diversity for efficient and effective bioremediation of xenobiotics.

MICROBIAL diversity constitutes the most extraordinary reservoir of life in the biosphere that we have only just begun to explore and understand. Diversity is composed of two elements: richness and evenness, so that the highest diversity occurs in communities with many different species present (richness) in relatively equal abundance (evenness)[1]. Microorganisms represent the richest repertoire of molecular and chemical diversity in nature, as they comprise the most diverse forms of life. Over millennia, they have adapted to extremely diverse environments and have developed an extensive range of metabolic pathways. This metabolic wealth has traditionally been exploited by man in processes such as fermentation, production of antibiotics, vitamins, etc. More recently, this largely unexplored reservoir of resources has begun to be harnessed for innovative applications useful to mankind. These include the use of microorganisms for bioproduction of novel as well as difficult-to-synthesize compounds, monitoring pollutant levels and biodegradation of xenobiotic pollutants. The bane of industrial progress has been the generation and release into the environment of huge amounts of toxic compounds which have caused widespread contamination of land and water. Large amounts of chemicals such as herbicides, insecticides and fertilizers are used in agricultural activities and synthetic chemicals such as plasticizers, dyes, pigments, agrochemicals, solvents, pharmaceuticals, hydraulics, fire retardants, halogenated compounds, etc. are produced by industrial activities[2]. Over the last few decades, significant quantities of these chemicals have been released into the environment creating countless number of contaminated sites. The major contaminants are halogenated and nitrated alicylic, aliphatic, aromatic and polyaromatic chemical compounds. Removal of these xenobiotics involves physical and chemical processes like landfilling, excavation, incineration, etc., which are expensive and sometimes difficult to execute. Microorganisms are nature's original recyclers, converting toxic organic compounds to harmless products, often carbon dioxide and water. Ever since it was discovered that microbes have the ability to transform and/or degrade xenobiotics, scientists have been exploring the microbial diversity, particularly of contaminated areas in search for organisms that can degrade a wide range of pollutants. In this context, the present review deals with microbial diversity of degradative organisms with specific emphasis on microorganisms de-

*For correspondence. (e-mail: rkj@imtech.res.in)

grading hydrocarbons such as aliphatic, aromatic, polycylic aromatics, nitroaromatics and halogenated organic compounds.

Biodegradation of xenobiotics: Role of microbial diversity

Bioremediation, a biodegradation process in which sites contaminated with xenobiotics are cleaned up by means of bacterial bio-geochemical processes, preferably *in situ*, exploits the ability of microorganisms to reduce the concentration and/or toxicity of a large number of pollutants. It is an economical, versatile, environment-friendly and efficient treatment strategy, and a rapidly developing field of environmental restoration. Bioremediation utilizes the microbial ability to degrade and/or detoxify chemical substances such as petroleum products, aliphatic and aromatic hydrocarbons (including polycyclic aromatic hydrocarbons and polychlorinated biphenyls), industrial solvents, pesticides and their metabolites, and metals. The presence of a large number of diverse bacterial species in nature expands the variety of chemical pollutants that can be degraded and the extent to which pollutant sites can be decontaminated. A well-known example of bioremediation which highlighted the usefulness of this treatment strategy and accelerated its development is the biological clean up (in addition to physico-chemical methods) of a large accidental oil spill by the tanker *Exxon Valdez* which ran aground on Bligh reef in the Gulf of Alaska in March 1989, spilling approximately 41,000 m^3 of crude oil and contaminating about 2000 km of coastline. The *Exxon Valdez* and other similar incidents demonstrated the usefulness of bioremediation as a fairly complete solution to oil contamination[3]. Thus, the use of microorganisms for degradation of pollutants is now being increasingly applied as the technology of choice for clean up or restoration of polluted sites as it can be self-sustaining and inexpensive.

Microbial diversity offers an immense field of environment-friendly options for mineralization of contaminants or their transformation into less harmful non-hazardous compounds. There is a general interest in studying the diversity of indigenous microorganisms capable of degrading different pollutants because of their varied effects on the environment. Efforts have been made to characterize bacterial communities and their responses to pollutants, to isolate potential degraders and to identify the genes involved in degradation processes[4,5]. The detailed analysis of microbial diversity within an environment can be divided into two broad categories: culture-dependent studies and culture independent studies[6]. It has been established that contaminated environments harbour a wide range of unidentified pollutant-degrading microorganisms that have crucial role in bioremediation[7] that can be assessed only by the culture independent techniques. Conventional characterization of microbial strains has been subjected to debate, as it is dependent on the ability of the strains to grow under specific environmental conditions[8]. In the past two decades, molecular tools exemplified by 16S rRNA analyses have facilitated the study of natural microbial populations without cultivation, which has made quantitative assessment of microbial diversity now conceivable[9]. However, no single tool allows definitive assessment of the soil microbial community. Therefore, the use of a polyphasic approach (Figure 1) involving a combination of molecular biology techniques, microbiological methods and geochemical techniques or microsensors[10,11] is necessary to obtain a better understanding of the interaction between the microorganisms and their natural environment.

Molecular biology methods are now being employed to study bioremediation since a comprehensive understanding of microbial ecology is required to gain maximum benefits from this process. A number of studies have reported the occurrence of conjugative gene transfer between bacteria in soil through appropriate plasmid-borne catabolic genes into competitive indigenous bacterial populations during bioremediation of contaminants at the industrial sites. Therefore, we need to understand the role of catabolic genes by molecular cloning and characterization for degradation of a particular organic compound so that it can be applied for bioremediation of contaminated ground water and soil.

The relatively new field of molecular microbial ecology which makes use of molecular biology techniques to study microbial ecology is increasing our understanding of microbial diversity as it has revealed the presence of viable but non-culturable bacteria. Molecular microbial ecology describes the microbial diversity based on DNA sequences without cultivation. DNA is extracted directly from environmental samples so that the cultivation bias is eliminated. The ability to monitor expression of specific catabolic genes may be of great importance in determining the feasibility of bioremediation *in situ*.

Expanding the catabolic repertoire of bacteria: genetic and metabolic engineering

In recent years, a number of compounds previously considered non-degradable are also being degraded by microorganisms, suggesting that under selective pressure of environmental pollution, microbes develop the ability to degrade recalcitrant xenobiotics. However, the fact that many pollutants are still persistent in the environment reflects the inadequacy of the current microbial catabolic capacity to deal with such pollutants. This is where molecular biologists step in to engineer bacteria for more efficient remediation of contaminated areas, and to degrade hitherto recalcitrant organic pollutants. Advances in genetic engineering have enabled a far more rational approach for the development of microbes for the degradation of xenobio-

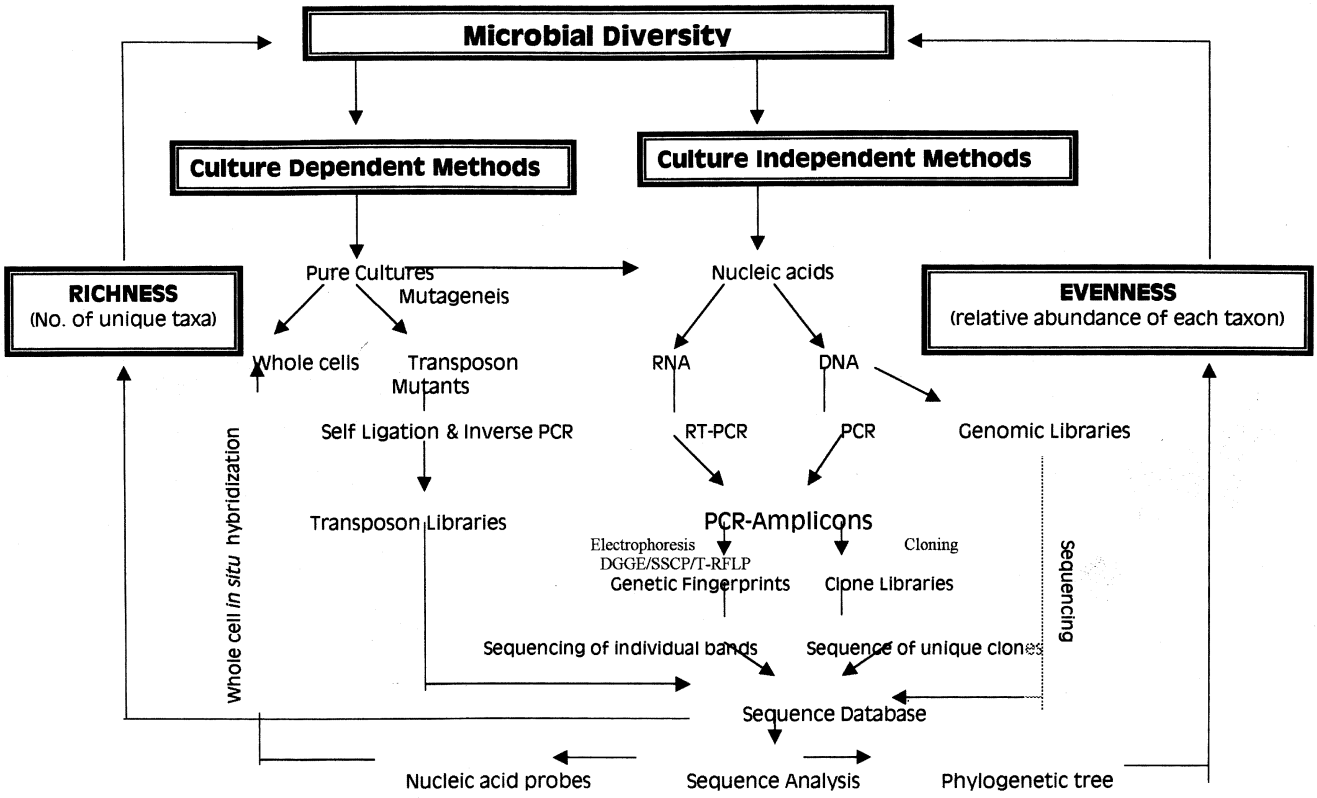

Figure 1. Polyphasic approaches for the analysis of microbial diversity.

tics, than the classical approach via mutagenesis and screening. This has led to the development of a new field of metabolic engineering that involves the improvement of cellular activities by manipulation of enzymatic, transport and regulatory functions of the cell using recombinant DNA technology. Metabolic engineering has emerged in the past decade as an interdisciplinary field aiming to improve cellular properties by using modern genetic tools to modify pathways[12]. With rapid developments in new analytical techniques and cloning procedures, it is now possible to introduce directed genetic changes in microbes and subsequently analyse the consequences of the introduced changes at the cellular level. It may therefore involve continuous improvements of the cellular properties through several rounds of genetic engineering. Advances in the field of genetic engineering, sequencing of whole genomes of several organisms and developments in bioinformatics have speeded up the process of gene cloning and transformation. Furthermore, a number of very powerful analytical techniques have been developed for metabolic pathway analysis and analyses of cellular function, e.g. gas chromatography, gas chromatography–mass spectrometry (GC–MS), nuclear magnetic resonance (NMR), two-dimensional gel electrophoresis, matrix-assisted laser desorption ionization-time of flight (MALDI-TOF), liquid chromatography-mass spectrometry (LC-MS) and DNA chips. Metabolic engineering is therefore concerned with modifying pathways and assessing the physiological outcome of such genetic modifications in an effort to improve the degradative abilities of microorganisms.

Biodegradation of organic compounds

Nitroaromatic compounds

The versatile chemistry of the nitro group makes nitroaromatic compounds (NACs) important industrial feedstocks. They are extensively used in many industrial processes as solvents, as precursors for amino aromatic derivatives and in the synthesis of dyes, plasticizers, pharmaceuticals, pesticides (parathion, methyl parathion, dinoseb, dinitrocresole, nitrofen) and explosives {2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazine (HMX)[13]. However, the very properties that make NACs valuable to industry, viz. stability, persistence and toxicity, render them hazardous when they are released into the environment. On entering mammalian systems, NACs are readily reduced to more reactive and potentially more carcinogenic or mutagenic derivatives due to conversion of nitro groups to more harmful nitroso and hydroxylamino groups[14]. Owing to

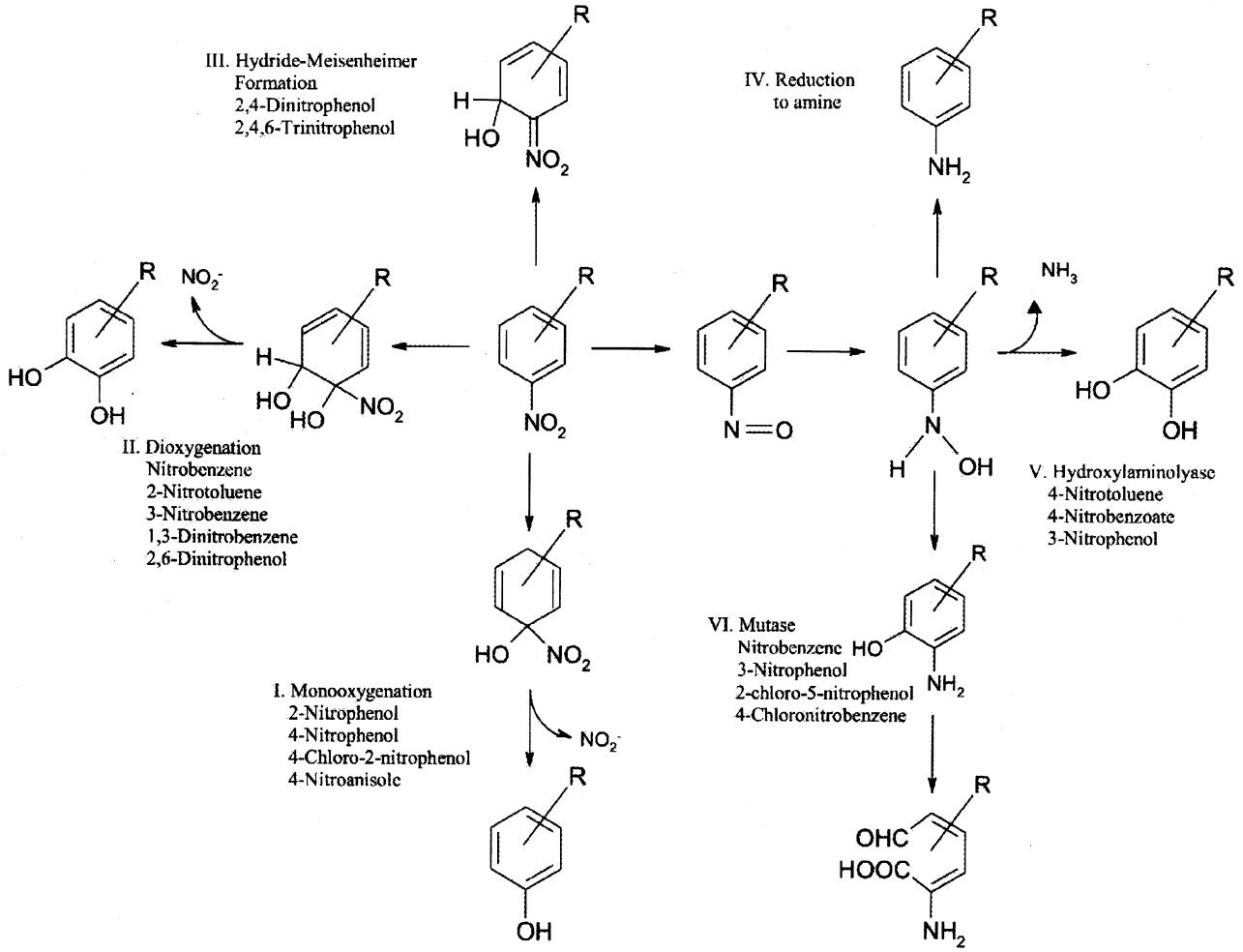

Figure 2. General mechanisms for the degradation of nitroaromatic compounds. Convincing evidence for pathway IV is only known from anaerobic systems (taken from Nishino *et al.*[16]).

their high toxicity, a number of NACs including nitrobenzenes, dinitrotoluenes and mono- and dinitrophenols have been classified as priority pollutants[15]. Presence of NACs in the environment creates intense selective pressure which has led to the evolution of microorganisms capable of degrading a growing number of NACs.

Successful bioremediation requires not only the knowledge of which microorganisms can degrade a particular compound, but also an understanding of the pathways involved in degradation both at physiological and molecular levels. The degradative pathways which have evolved toward the catabolism of nitro-substituted aromatic rings are catalysed by common enzyme types (mono- and dioxygenases, dehydrogenases) produced by common microbial groups belonging to *Pseudomonas, Nocardia* and *Arthrobacter*[16] (Figure 2). However, there is a great diversity observed in funneling mono- and di-nitro aromatic molecules through oxygenase-based pathways[17].

The majority of NACs being anthropogenic have been present in the environment for a relatively short time period giving bacteria little time to evolve pathways for their degradation. Nevertheless, during the past decade or so, bacteria capable of degrading a number of NACs by aerobic pathways, once thought unlikely have been discovered. Our research group has been exploring the microbial diversity of different geographical regions of India in search of bacteria that are capable of mineralizing/degrading NACs. Soil samples from agricultural fields sprayed with parathion/methyl parathion (nitro-pesticides) were used for isolation of a bacterial strain identified as *Arthrobacter protophormiae* which was capable of utilizing nitroaromatic compounds *o*-nitrobenzoate (ONB), *p*-nitrophenol (PNP) and 4-nitrocatechol (NC) as the sole source of carbon, nitrogen and energy. The pathway for ONB degradation in this organism was elucidated and found to be a novel one and was determined to be encoded on a large 65 kb plasmid[18]. The pathways for degradation of PNP and NC were also elucidated in this strain and the genes involved in degradation of these compounds were found to reside on the same 65 kb plasmid[19]. Thus, this organism could

be useful for bioremediation of sites contaminated with multiple NACs. Another PNP- and NC-degrading organism was isolated from these soil samples and identified as *Burkholderia cepacia*[20,21]. The PNP-degradation pathway in this organism was elucidated[21] and it was seen that degradation of PNP in both *B. cepacia* and *A. protophormiae* occurs via formation of benzoquinone and hydroquinone (Figure 3). The oxidative degradation of 4-nitrocatechol was found to occur by a novel pathway in this strain involving a reductive dehydroxylation step which is a well-known reaction in anaerobic metabolism of aromatic compounds, but was reported to be operative aerobically in *B. cepacia* for the first time. Involvement of plasmid in PNP and NC metabolism in this strain was also established[21]. The kinetics of biodegradation of PNP was studied in *A. protophormiae* and *B. cepacia.* These kinetic studies provided useful information which could be used in the designing of biological treatment plants[22] .

Bioremediation studies have been carried out to study the efficacy of *A. protophormiae* in degrading PNP in soil microcosms and also in small-scale field studies. This organism has shown promising potential for bioremediating PNP under natural environmental conditions (unpublished data). One aspect of bioremediation of a contaminated area by the addition of exogenous microorganisms is the possible effect of added organisms on the native microbial diversity of the area being treated. Therefore, efforts are being made to study the total microbial diversity (culturable and non-culturable) of PNP-contaminated soil treated with this organism.

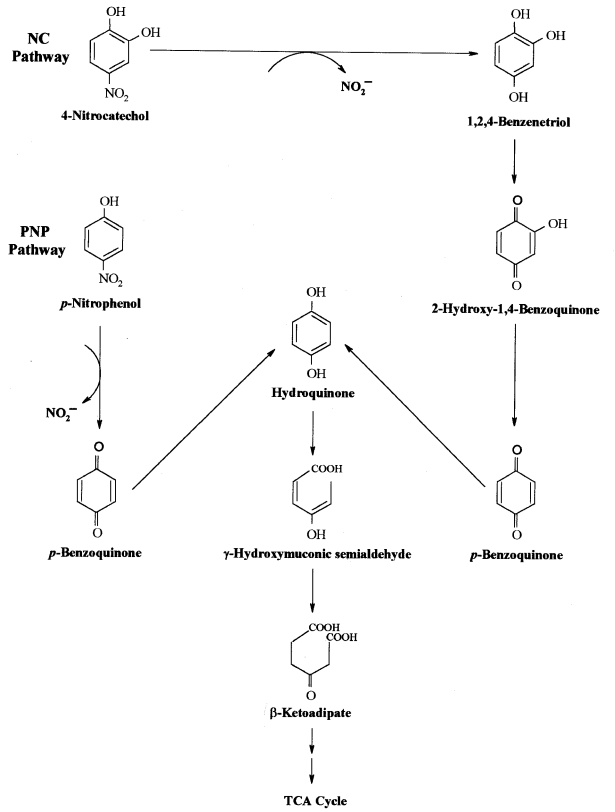

Figure 3. Pathway for the degradation of PNP and NC by *A. protophormiae* and *B. cepacia*.

Role of chemotaxis in biodegradation

The *in situ* biodegradation of a contaminant is a function of the catabolic activity of bacteria and bioavailability of the contaminant to bacteria. Chemotaxis, i.e. the movement of bacteria under the influence of a chemical gradient has been postulated to play an important role in enhancing biodegradation as it increases bioavailability of pollutants to bacteria. Some toxic organic compounds are chemoattractant for different bacterial species, which can lead to improved biodegradation of these compounds. A *Ralstonia* sp. was isolated by a chemotactic enrichment technique in our laboratory from a pesticide-contaminated agricultural field of Assam. It was chemotactic towards different NACs, i.e. PNP, 3-methyl 4-nitrophenol, 4-nitrocatechol, ONB and *p*-nitrobenzoate (PNB) which were subsequently degraded completely by this organism[23,24]. It was also chemotactic towards *o*-dinitrobenzoate, *m*-dinitrobenzoate, 2,4-dinitrophenol, 2,5-dinitrophenol, 2,6-dinitrophenol and 3,5-dinitrobenzoate which do not serve as sole source of carbon and energy but are co-metabolized[25,26]. These NACs are partially transformed in the presence of an alternate carbon source such as succinate. On the other hand, this *Ralstonia* strain did not degrade *o*-nitrophenol, *p*-nitroaniline, 2,3-dinitrotoluene, naphthalene, phenanthrene or salicylic acid, nor was it chemotactic towards these compounds[24], clearly indicating a relation between chemotaxis and biodegradation.

Crude oil constituents

Crude oil is a complex mixture of hydrocarbons, basically composed of aliphatic, aromatic and asphaltene fractions along with nitrogen, sulfur and oxygen-containing compounds. The constituent hydrocarbon compounds are present in varied proportion resulting in great variability in crude oils from different sources[27]. There are several reports indicating the recalcitrance and potential health hazards of the different constituents of crude oil[28]. These compounds have been reported to be carcinogenic, mutagenic and have immunomodulatory effects on humans, animals and plant life[29,30]. The sites contaminated with hydrocarbons are ecologically important locations as one may encounter microbial flora of diverse nature, which may be potential candidates for important industrial processes.

There is a plethora of cultivable microbes with the ability to utilize hydrocarbons as sole source of carbon or to transform them to a less toxic form[28,31,32]. Earlier reports based on cultivable bacteria suggested that hydrocarbon-contaminated soil is predominated by Gram-negative bacte-

ria[33]. The culture independent studies from contaminated soil samples have revealed existence of new bacterial lineages[8,9,34]. Studies with molecular tools indicate that specific groups of bacteria commonly occur in oil-contaminated environment[35]. Many *g* and *b* proteobacterial groups (*Holophaga–Geothrix–Acidobacterium*) and green non-sulfur bacteria with a strong potential to degrade hydrocarbons were present in benthic cyanobacterial mats as determined by DGGE (Denaturant Gradient Gel Electrophoresis) analysis of 16S DNA from soil[7]. Similar molecular approach-based study on spilled oil bioremediation conducted at a sandy beach showed that the phylotypes were affiliated with the *a*-proteobacterial group, suggesting their importance in bioremediation of oil spills[33]. Studies on Norwegian arctic oil sands showed that 16S DNA types affiliated with the *g*-proteobacteria, specially belonging to the group *Pseudomonas* and *Cycloclasticus*, were most abundant bacterial strains with high biodegradation throughput[35]. Stoffels *et al.*[36] also reported the significance of *g*-proteobacterial population in hydrocarbon degradation.

Aliphatic hydrocarbon degradation

The aliphatic fraction is the major constituent of crude oil. The aliphatic fraction of hydrocarbons consists of straight chain, branched chain and cyclic chain carbon moieties. This fraction contains the most readily degraded hydrocarbon compounds[37] and most of the reported hydrocarbon-degrading bacteria have shown relatively good growth on these compounds[38,35,39]. There are reports on degradation of short carbon chain (C_8–C_{16})[40] to very long carbon chain (C_{44}) hydrocarbons[41]. The bacterial species of *Acinetobacter*, *Pseudomonas*, *Alcaligenes*, *Burkholderia*, *Arthrobacter*, *Flavobacterium*, *Bacillus*, etc. are a few of the well-known degraders of aliphatic fractions of petroleum hydrocarbons[38–40]. There are several reports on the isolation of different hydrocarbon-degrading bacteria from petroleum-contaminated sites[41]. Herman *et al.*[42] mentioned the role of various surface-active compounds released by hydrocarbon degrading bacteria, which further enhanced the bioremediation potential by overcoming mass transfer limitation and dissolution rates of these hydrocarbon compounds. Studies on cycloalkanes suggest that these cyclic hydrocarbons are most resistant to microbial degradation due to their toxic effects on cellular structures[43,44]. There are reports on utilization of cycloalkanes by *Pseudomonas citronellolis*, *Brevibacterium erythrogenes* and *Saccharomyces cerevisiae*[43]. Yakimov *et al.*[45] reported a novel hydrocarbon degrading and surfactant-producing bacterium, *Alcanivorax borkumensis*. The sequence analysis of 16S rDNA indicated the presence of different species of *Ralstonia*, *Commamonas* and *Flavobacterium* along with the common soil bacteria from contaminated groundwater sources[46].

Aromatic hydrocarbon degradation

The aromatic hydrocarbon compounds basically contain benzene ring in their structure and are ubiquitous in the environment[47]. Aromatic hydrocarbons being derivatives of benzene are very stable and this leads to the relative inertness of these molecules to different fate processes[48]. However, many bacterial species have evolved to use these compounds as source of energy[49]. Kerr and Capone[50] have reviewed the microbial degradation of aromatic fraction of petroleum hydrocarbons. It was observed that the light aromatics were most susceptible to microbial attack. Owing to hydrophobicity, higher aromatic hydrocarbons get rapidly associated with organic material rendering them less available to biological uptake[51]. Watanabe *et al.*[52] reported the presence of phylotypes associated with *a* subclass of proteobacteria in sites contaminated with crude oil. The bacteria affiliated to *e*-proteobacterial subgroup were reported to be associated with petroleum-contaminated groundwater[52]. As petroleum hydrocarbons are persistent under anaerobic conditions, contamination of groundwater is a serious environment problem. Stapleton *et al.*[53] demonstrated significant bioremediation potential of degradative bacteria from hydrocarbon-contaminated aquifers which had phylogenetic resemblance with common soil bacteria. This included members from the group *Pseudomonas, Ralstonia, Burkholderia, Sphingomonas, Flavobacterium* and *Bacillus*. Kniemeyer *et al.*[54] also demonstrated anaerobic degradation of aromatic hydrocarbons by marine sulfate reducing bacteria that use sulfate as the electron acceptor. While studying hydrocarbon degradation by archaeal population, Ficker *et al.*[55] came across a genus *Methanospirillum* that was unrelated to any previously described genus. Another study revealed that the culturable methanogenic archaeal population was much less than the total anaerobic organisms in the biomass[56].

Polycyclic aromatic hydrocarbons

Among the aromatic fraction, polycyclic aromatic hydrocarbons (PAHs) are compounds of intense public concern owing to their persistence in the environment and potential deleterious effects on human health. They represent a unique class of petroleum hydrocarbons because of their pyrogenic nature and the complexity of the assemblages in which they occur. There has been intense research pertaining to bacterial biodegradation of PAHs and degradation of PAHs composed of three rings has been well documented[28]. Many bacterial, fungal and algal strains have been shown to degrade a wide variety of PAHs. The most commonly reported bacterial species include *Acinetobacter calcoaceticus, Alcaligens denitrificans, Mycobacterium* sp., *Pseudomonas putida, Pseudomonas fluorescens, Pseudomonas vesicularis, Pseudomonas cepacia, Rhodococcus* sp., *Corynebacterium renale, Moraxella* sp., *Bacillus*

cereus, *Beijerinckia* sp., *Micrococcus* sp., *Pseudomonas paucimobilis* and *Sphingomonas* sp.[28]. Though most of these bacterial species are reported to degrade low-molecular weight PAHs, there are limited reports on degradation of high-molecular weight PAHs with more than four benzene rings. In general, high-molecular weight PAHs are degraded slowly by indigenous or augmented microorganisms as persistence of PAHs increases with their molecular size.

Early reports are available on the ability of the microorganisms to degrade PAHs dating back to 1975 where Gibson *et al.* showed the involvement of *Beijerinkia* sp. in oxidation of benzo(*a*)pyrene and benzo(*a*)anthracene to dihydrodiols[57]. However, it was not until late 1980s when Mahaffey *et al.*[58], Heitkamp and Cerniglia[59] and Mueller *et al.*[60] reported biodegradation of high-molecular weight (HMW) PAHs. Mueller *et al.*[60] demonstrated for the first time the utilization of PAH containing four or more aromatic rings as a sole source of carbon and energy by bacterial isolates. They showed that a seven-member bacterial consortium isolated from creosole-contaminated soil was capable of utilizing fluoranthene and was also capable of co-metabolically biotransforming other HMW PAHs. Boldrin *et al.*[61] reported fluoranthene mineralization by a consortium of *Mycobacterium*, *Pseudomonas* and *Alcaligens* sp. They also postulated that the growth rates were not dependent on the solubility of the substrates, contradicting the general observations that degradation rates depend upon the dissolution rate of hydrocarbon.

Although many of the PAH-degrading bacteria described are actinomycetes, a variety of non-actinomycete bacteria have also been reported to metabolize fluoranthene, pyrene, chrysene, and benz[*a*]anthracene. *P. putida*, *P. aeruginosa*, *Flavobacterium* sp. and an unidentified strain were isolated from a soil-derived mixed culture which was capable of metabolizing fluoranthene and pyrene when supplemented with other forms of organic carbon[62]. The aromatic hydrocarbon-degrading ability of *Sphingomonas yanoikuyae* was recently reviewed and it has been shown to oxidize chrysene[63]. The analysis of 16S DNA sequence types represented organisms closely related to known HMW PAH-degrading bacteria (*Burkholderia*, *Sphingomonas* and *Mycobacterium*)[28]. Lindstrom *et al.*[64], in their 20-year experiment on long-term effects on microbial communities observed that communities exposed to petroleum hydrocarbons exhibited diminished genetic diversity relative to pristine reference communities, but displayed increased physiological tolerance and substrate utilization capabilities. It was also observed by Vila *et al.*[65] that a *Mycobacterium* strain, which could use pyrene as a sole source of carbon, could also grow on hexadecane, phenanthrene and fluoranthene and could transform several other PAHs. Hedlund *et al.*[66] isolated a novel PAH-degrading species of *Neptunomonas naphthovorans*.

A *Pseudomonas putida* strain isolated from oil fields of Gujarat could use naphthalene as sole source of carbon and energy and mineralized naphthalene via salicylate. This strain was also chemotactic towards these compounds. An 83 kb mega plasmid was found to contain all the genes responsible for degradation of naphthalene and salicylate in this strain[67]. The plasmid was transferred into a plasmid-free strain of *P. putida* KT2442 which resulted in the acquisition of chemotaxis and degradation properties by this strain, thus establishing the role of this plasmid in chemotaxis associated with complete mineralization of these two compounds[68]. Another organism, *Arthrobacter sulfureus*, which was also isolated from Gujarat oil fields, could utilize phenanthrene as the sole source of carbon and energy. The pathway for degradation of this compound was determined in this strain along with that in three other strains, *Acidovorax delafieldi* P4-1, *Brevibacterium* sp. HL4 and *Pseudomonas* sp. DLC-P11. Novel pathways for phenanthrene degradation in *Brevibacterium* HL4 and *Pseudomonas* DLC-P11 were proposed. These organisms could utilize several other PAHs such as acenaphthene, anthracene, fluorine, fluoranthene, naphthalene and pyrene, making them potentially useful in cleaning up petroleum-contaminated soils containing a mixture of different PAHs[69].

Pseudomonas putida is a good candidate for metabolic engineering and genetic manipulation applications for expression of genes encoding several degradative enzymes. Therefore, a *P. putida* strain was engineered to increase the efficiency of degradation of naphthalene and salicylate[70]. In this strain, utilization of glucose (a simple carbon source) was blocked through metabolic engineering making it primarily dependent on the more complex carbon source, i.e. the organic compound in question (naphthalene/salicylate) thus degrading it more efficiently.

Halogenated organic compounds

Halogenated organic compounds constitute one of the largest and diverse groups of environmental chemicals. Although some of these chemicals are generated by naturally occurring biotic and abiotic processes in the oceans and atmosphere[71], the widespread use of halogen-based chemistry in industrial-scale chemical processing over the past 100 years has introduced many additional man-made halocarbons into the environment[72]. The occurrence of incomplete pathways and the accumulation of toxic intermediates are important factors in the apparent recalcitrance of environmental pollutants such as chloroaromatics, haloalkanes and chloroethenes. The recalcitrant nature of these compounds is due to low electron density at the aromatic ring, and the enzyme oxygenase that usually initiates the degradation of other aromatic compounds, cannot attack these halogenated compounds. This reaction does not proceed through an electrophilic attack but via a nucleophilic attack. A variety of microbial enzyme systems have been found to effect cleavage of carbon–

halogen bonds, providing the means for these compounds to be utilized as carbon sources or as alternative electron acceptors[73].

Halogenated aliphatic hydrocarbons

These are prevalent groundwater contaminants and are significant components of hazardous wastes and landfill leachates. Many hazardous halogenated aliphatic compounds released from industrial, commercial and agricultural sources are chlorinated or brominated alkanes and alkenes that contain one to three carbon atoms such as halogenated alkanoic acids (HAA), haloalkanes, trichloroethane and ethylene dibromide (EDB). A number of soil microorganisms which synthesize dehalogenases are capable of utilizing HAA[74–76]. Hardman *et al.*[74] examined four *Pseudomonas* and two *Alcaligenes* species capable of growth on 2-monochloropropionic acid and monochloroacetic acid. They found that all isolates contained a single plasmid of molecular size of 53 kb (pUU204) or more.

Chlorinated ethenes such as vinyl chloride, trichloroethylene (TCE) and tetrachloroethylene have been frequently detected in drinking water aquifers[77]. TCE is one of the major industrial solvents used for degreasing and cleaning metals and electronic components. Currently, there is much concern regarding the microbial metabolism of TCE. Bouwer and McCarty[78] demonstrated that under anerobic conditions in the laboratory, TCE and tetrachloroethylene could be degraded during vigorous methanogenesis supported by growth on acetate. Kleopfer *et al.*[79] demonstrated that reductive dechlorination of TCE to 1,2-dichloroethylene occurred in soil. Recently, an aerobic methane-oxidizing bacterium that degrades TCE in pure cultures was isolated. TCE biodegradation in this bacterium appeared to be a co-metabolic process[80]. Another aerobic microorganism degraded TCE in the presence of phenol[81]. Freitas dos Santos *et al.*[82] isolated a mixed culture that exhibited slow growth on 1,2-dibromoethane under aerobic conditions. After repeated subcultivation, during which the growth rate increased slowly, a pure culture was isolated that utilized 1,2-dibromoethane as a sole source of carbon[83]. The organism was classified as *Mycobacterium* sp. strain GP1, making it the first *Mycobacterium* sp. capable of growth on a short chain haloalkane. The dehalogenase genes in this strain encode enzymes that are very similar to dehalogenases found in other microorganisms.

EDB is a brominated hydrocarbon that has been used as a soil fumigant and in anti-knock gasoline. Pignatello[84] has reported the microbial degradation of EDB in aquatic environments. However, no information is available on microorganisms and their genetic traits involved in EDB transformation. A general trend with regard to the degradation of chlorinated aliphatic hydrocarbons is that greater the chlorinated the aliphatic hydrocarbon, the higher the relative rate of reduction with lesser the chlorination, higher the rate of oxidation[85].

Chlorinated aromatic compounds

Chlorinated aromatic compounds are major environmental pollutants because they are often released in substantial quantities, are toxic, resistant to degradation and accumulate in sediment and biota.

Chlorinated phenols and their derivatives are widely used in the environment. The toxicity of these compounds tends to increase with the degree of chlorination. The chlorinated phenolics are contaminated with chlorinated dibenzofuran and dioxins which are highly toxic compounds produced as contaminants during the manufacture of pesticides and during incineration. The pulp and paper mill is a major industrial sector utilizing huge amounts of lignocellulosic materials and water during the manufacturing process, and releases chlorinated lignosulphonic acids, chlorinated resin acids, chlorinated phenols and chlorinated hydrocarbons in the effluent[86]. Tetrachlorodibenzo-*p*-dioxin and dibenzofuran are major contaminants of coloured wastewater of pulp and paper mill effluent[87]. Mineralization of chlorinated dioxins or dibenzofurans by single bacterial strains has not been reported so far. Strain *Sphingomonas* sp. RW1 is able not only to degrade the unchlorinated structures but also to transform lower chlorinated congeners. The transformation of lower chlorinated congeners by strain RW1, however, leads to the accumulation of intermediates, which prevent further transformation of the substrates[88]. A strategy for reducing environmental pollution by chlorinated dioxins and dibenzofurans was developed in the chemostat in which microbial populations in a sediment core contaminated by chlorinated aromatics were enriched and a stable microbial consortium was obtained in the presence of 4-chlorosalicylic acid as sole carbon source[89]. The stable consortium comprising *Klebsiella pneumoniae, Pseudomonas fluorescens, P. mendocina* and *P. cichhori* had the potency to degrade chlorinated benzoic acid, salicylic acid, phenoxyacetic acid and dibenzofuran. These strains degraded chlorinated aromatic compounds by *ortho* and *meta* ring cleavage[90].

Pentachlorophenol (PCP) is a general biocide used primarily as preservative of wood, leather, textile and related commercial products. The intermediary metabolites formed during aerobic and anaerobic degradation of PCP are toxic and recalcitrant[91]. The PCP-degrading organisms have been isolated such as *Pseudomonas* sp. strain IST 103 (*Acinetobacter* sp. by 16S rDNA sequence analysis) which was capable of utilizing PCP as a carbon source. The first step in PCP degradation by bacteria is oxidative dehalogenation to tetrachlorohydroquinone, which then dechlorinates to chlorohydroquinone[92]. The enzyme PCP-4 monooxygenase[92] was responsible for the dechlorination of PCP[93].

PCP-degrading microbial strains have been isolated but they proved ineffective for *in situ* bioremediation, therefore, two bacterial consortia were developed by continuous enrichment of sediment core of pulp and paper mill and tannery, contaminated by PCP in the chemostat[90]. The microbial consortium from paper mill pulp contained *E. coli*, *Pseudomonas aeruginosa* and *Acinetobacter* sp. and that from tannery contained *Serratia* sp. (three isolates) and *Pseudomonas fluorescens*, identified on the basis of 16S rDNA sequence analysis[94]. Another microbial consortium was developed by continuous enrichment of microbial population of sediment core of pulp and paper mill effluent under alkaline conditions in the presence of 4-chlorosalicylic acid. The consortium contained three isolates identified as *Micrococcus diversus*, *Deinococcus radiophilus* and *Alloiococcus otitis*. One of the strains, *Micrococcus diversus*, which produces xylanase and lignin peroxidase (no cellulase) is currently under investigation for biopulping and biobleaching.

The genes involved in PCP degradation have been reported from genomic DNA[95]. There is limited information regarding involvement of plasmid in degradation of PCP. Two different-sized plasmids were found to be responsible for carrying genes for degradation of PCP[92] in the strain IST 103. The plasmid isolated from the bacterium was subjected to shotgun cloning and transformed into *E. coli* XLBlue1*a*. The recombinant clones having higher potentiality to degrade PCP were selected by utilization of PCP as the sole source of carbon and it released various intermediary metabolites during degradation. The recombinant clones contained an insert of 3 kb that was found to have a homology with *thdf* gene of monooxygenase of thiophene and furan. Southern blot analysis performed by developing gene probes indicated presence of PCP-monooxygenase gene in plasmid of the bacterium[93].

Recently, bacteria such as *Alcaligenes* sp. and *Pseudomonas* sp., that utilize chlorobenzene[96,97], 1,2-dichlorobenzene[98], 1,3-dichlorobenzene[99] and 1,4-dichlorobenzene[100] as the sole source of carbon have been isolated or constructed. However, the genetic basis of degradation of chlorobenzenes in these microorganisms has not been described.

Pierce *et al.*[101] reported that *P. cepacia* and several other *Pseudomonas* strains were capable of utilizing mono- and di-chlorinated toluenes as the sole source of carbon and energy. These strains contained plasmids of about 72 MDa which coded for chlorotoluene degradation.

Chlorinated polycyclic hydrocarbons

Polycyclic chlorinated hydrocarbons occur as natural constituents and combustion products of fossil fuels and are widespread environmental contaminants[102]. Out of the chlorinated polycyclic hydrocarbons, DDT, polychlorinated biphenyls (PCBs) and *p*-chlorobiphenyls (*p*-CBs) are of general interest because of their widespread occurrence in the environment. It has been reported that *p*-CBs and PCBs are subject to biodegradation. Furukawa and Chakrabarty[103] isolated an *Acinetobacter* sp. capable of utilizing *p*-CB. They showed that the degradation of this compound is encoded on an 82-kb plasmid (pKF1). Several other *p*-CB-degrading plasmids such as SS50 (53 kb) from *Alcaligenes* sp., have also been isolated[104]. Another *p*-CB degrading plasmid was isolated from *Klebsiella pneumoniae*[105]. Barton and Crawford[106] have isolated a *Pseudomonas* sp. that is capable of utilizing *p*-CB as the sole carbon and energy source. No genetic information about this species is available.

PCBs are water insoluble, non-polar, lipid soluble, inert and highly toxic compounds[107]. Both aerobic and facultative anaerobic bacteria capable of utilizing PCBs have been isolated from the environment[108–110]. Takase *et al.*[109] isolated a *Pseudomonas cruciviae* strain that could grow on more than 10 biphenyl related compounds including *p*-CB. Physiological and genetic studies have shown that the genes for the degradation of PCBs may be plasmid encoded[103,104] or present on the chromosome[108,111].

The examples cited above once again illustrate the diversity of solutions that microorganisms have found to deal with hostile conditions and turn a threat, a toxic compound into a benefit, a growth substrate.

Conclusion

Microbial diversity, the richness of species in environmental sites, provides a huge reservoir of resources which we can utilize for our benefit. However, little is known about the true diversity of bacterial life. Despite the acknowledged value of microorganisms, our understanding of their diversity and many of their key roles in sustaining global life support systems is still very scarce. This is because the vast majority of bacteria are non-culturable by standard methods and we have only recently acquired the skills to explore this aspect of microbial biodiversity. Exploring the range of microbial biodiversity is the key to developing effective and environment friendly 'green' technologies. Bioremediation is one such process that exploits the catabolic abilities of microorganisms to degrade harmful and toxic xenobiotics. We have been able to restore what once were irreversibly polluted sites in some cases, attesting to the usefulness of this clean-up process. However, to maximize the potential benefits of microbial community in combating pollution problems, it is vital that we have fundamental understanding of a microbe's degradative potential under various conditions, its biochemical systems and its molecular biology.

1. Huston, M. A., *Biological Diversity: The Coexistence of Species on Changing Landscapes*, Cambridge University Press, Cambridge, UK, 1994.
2. Reineke, W. and Knackmuss, H. J., Microbial degradation of haloaromatics. *Annu. Rev. Microbiol.*, 1988, **42**, 263–287.

3. Margesin, R. and Schinner, F., Biological decontamination of oil spills in cold environments. *J. Chem. Technol. Biotechnol.*, 1999, **74**, 1–9.
4. Watanabe, K., Futamata H. and Harayama, S., Understanding the diversity in catabolic potential of microorganisms for the development of bioremediation strategies. *Anton. Van Leeuwen*., 2002, **81**, 655–663.
5. Greene, E. A., Kay, J. G., Jaber, K., Stehmeier, L. G. and Voordouw, G., Composition of soil microbial communities enriched on a mixture of aromatic hydrocarbons. *Appl. Environ. Microbiol*., 2000, **66**, 5282–5289.
6. Juck, D., Charles, T., Whyte, L. G. and Greer, C. W., Polyphasic community analysis of petroleum hydrocarbon contaminated soils from two northern Canadian communities. *FEMS Microbiol. Ecol*., 2000, **33**, 241–249.
7. Margesin, R., Labbe, D., Schinner, F., Greer, C. W. and Whyte L. G., Characterization of hydrocarbon degrading microbial population in contaminated and pristine alpine soils. *Appl. Environ. Microbiol*., 2003, **69**, 3985–3092.
8. Bakonyi, T., Derakhshifar, I., Grabensteiner, L. and Nowotny N., Development and evaluation of PCR assays for the detection of *Paenibacillus larvae* in honey samples: Comparison with isolation and biochemical characterization. *Appl. Environ. Microbiol*., 2003, **69**, 1504–1510.
9. Kubicek, C. P., Bissett, J., Druzhinina, I., Kullnig-Gradinger, C. and Szakacs, G., Genetic and metabolic diversity of Trichoderma: a case study on South-East Asian isolates. *Fungal Genet. Biol.*, 2003, **38**, 310–319.
10. Ramsing, N. B., Fossing, H., Ferdelman, T. G., Anderson, F. and Thamdrup, B., Distribution of bacterial populations in a stratified fjord (Mariager Fjord, Denmark) quantified by *in situ* hybridization and related to chemical gradients in the water column. *Appl. Environ. Microbiol*., 1996, **62**, 1391–1404.
11. Teske, A., Wawer, C., Muyzer, G. and Namsing, N. B., Distribution of sulfate-reducing bacteria in a stratified fjord (Mariager Fjord, Denmark) as evaluated by most-probable-number counts and denaturing gradient gel electrophoresis of PCR-amplified ribosomal DNA fragments. *Appl. Environ. Microbiol.*, 1996, **62**, 1405–1415.
12. Nielsen, J., Metabolic engineering. *Appl. Microbiol. Biotechnol*., 2001, **55**, 263–283.
13. Spain, J. C., Biodegradation of nitroaromatic compounds. *Annu. Rev. Microbiol*., 1995, **49**, 523–555.
14. Venulet, J. and Van Etten, R. L., *The Chemistry of the Nitro and Nitroso Groups* (ed. Fever, H.), Interscience, New York, 1970, pp. 201–289.
15. Keith, L. H. and Teillard, W. A., Priority pollutants. I. A perspective view. *Environ. Sci. Technol.*, 1979, **13**, 416–423.
16. Nishino, S. F., Spain, J. C. and He, Z., Strategies for aerobic degradation of nitroaromatic compounds by bacteria: process discovery to field application. *Biodegradation of Nitroaromatic Compounds and Explosives* (eds Spain, J. C., Hughes, J. B. and Knackmuss, H. J.), Lewis Publishers, Boca Raton, 2000, pp. 7–61.
17. Crawford, R. L., The microbiology and treatment of nitroaromatic compounds. *Curr. Opin. Biotechnol*., 1995, **6**, 329–336.
18. Chauhan, A. and Jain, R. K., Degradation of *o*-nitrobenzoate via anthranilic acid (*o*-aminobenzoate) by *Arthrobacter protophormiae*: A plasmid-encoded new pathway. *Biochem. Biophys. Res. Commun.*, 2000, **267**, 236–244.
19. Chauhan, A., Chakraborti, A. K. and Jain, R. K., Plasmid-encoded degradation of *p*-nitrophenol and 4-nitrocatechol by *Arthrobacter protophormiae*. *Biochem. Biophys. Res. Commun*., 2000, **270**, 733–740.
20. Prakash, D., Chauhan, A. and Jain, R. K., Plasmid-encoded degradation of *p*-nitrophenol by *Pseudomonas cepacia*. *Biochem. Biophys. Res. Commun.*, 1996, **224**, 375–381.
21. Chauhan, A., Samanta, S. K. and Jain, R. K., Degradation of 4-nitrocatechol by *Burkholderia cepacia:* a plasmid-encoded novel pathway. *J. Appl. Microbiol*., 2000, **88**, 764–772.
22. Bhushan, B., Chauhan, A., Samanta, S. K. and Jain, R. K., Kinetics of biodegradation of *p*-nitrophenol by different bacteria. *Biochem. Biophys. Res. Commun.*, 2000, **274**, 626–630.
23. Bhushan, B., Samanta, S. K., Chauhan, A., Chakraborti, A. K. and Jain, R. K., Chemotaxis and biodegradation of 3-methyl-4-nitrophenol by *Ralstonia* sp. SJ98. *Biochem. Biophys. Res. Commun*., 2000, **275**, 129–133.
24. Samanta, S. K., Bhushan, B., Chauhan, A. and Jain, R. K., Chemotaxis of a *Ralstonia* sp. SJ98 toward different nitroaromatic compounds and their degradation. *Biochem. Biophys. Res. Commun*., 2000, **269**, 117–123.
25. Pandey, G., Chauhan, A., Samanta, S. K. and Jain, R. K., Chemotaxis of a *Ralstonia* sp. SJ98 toward co-metabolizable nitroaromatic compounds. *Biochem. Biophys. Res. Commun*., 2002, **299**, 404–409.
26. Pandey, G. and Jain, R. K., Bacterial chemotaxis toward environmental pollutants: role in bioremediation. *Appl. Environ. Microbiol*., 2002, **68**, 5789–5795.
27. Speight, J. G., *Chemistry and Technology of Petroleum*, Marcel Dekker Inc, USA, 1999, vol. 4, pp. 145–146.
28. Kanaly, R. A. and Harayama, S., Biodegradation of high molecular weight polycyclic aromatic hydrocarbons by bacteria. *J. Bacteriol.*, 2000, **182**, 2059–2067.
29. Van Gestel, C. A. M. *et al.*, The use of acute and chronic bioassays to determine the ecological risk and bioremediation efficiency of oil-polluted soils. *Environ. Toxicol. Chem.*, 2001, **20**, 1438–1449.
30. Miller, E. C. and Miller, J. A., Search for the ultimate chemicals carcinogens and their reaction with cellular macromolecules. *Cancer*, 1981, **47**, 2327–2345.
31. Leahy, J. G. and Colwell, R. R., Microbial degradation of hydrocarbons in the environment. *Microbiol. Rev.*, 1990, **54**, 305–315.
32. Cerniglia, C. E., Biodegradation of polycyclic aromatic hydrocarbons. *Curr. Opin. Biotechnol*., 1993, **4**, 331–338.
33. Macnaughton, S. J., Stephen, J. R., Venosa, A. D., David, G. A., Chang, Y. J. and White, D. C., Microbial population changes during bioremediation of an experimental oil spill. *Appl. Environ. Microbiol*., 1999, **65**, 3566–3574.
34. Delong, E. F., Marine microbial diversity: the tip of the iceberg. *Trends Biotechnol*., 1997, **15**, 2–9.
35. Watanabe, K., Microorganisms relevant to bioremediation. *Curr. Opin. Biotechnol*., 2001, **12**, 237–241.
36. Stoffels, M., Amman, R., Ludwig, W., Hekmat, D. and Schleifer, K. H., Bacterial community dynamics during start up of a trickle bed bioreactor degrading aromatic hydrocarbons. *Appl. Environ. Microbiol*., 1998, **64**, 930–939.
37. Atlas, R. J., Microbial hydrocarbon degradation–bioremediation of oil spills. *Chem. Tech. Biotechnol*., 1991, **52**, 149–156.
38. Mishra, S., Jyot, J., Kuhad, R. C. and Lal, B., Evaluation of inoculum addition to stimulate *in situ* bioremediation of oily sludge contaminated soil. *Appl. Environ. Microbiol.*, 2001, **67**, 1675–1681.
39. Lal, B. and Khanna, S., Mineralization of [^{14}C] octacosane by *Acinetobacter calcoaceticus* S30. *Can. J. Microbiol.*, 1996, **42**, 1225–1231.
40. Bhattacharya, D., Sarma, P. M., Krishnan, S., Mishra, S. and Lal, B., Evaluation of the genetic diversity among some strains of *Pseudomonas citronellolis* isolated from oily sludge contaminated sites. *Appl. Environ. Microbiol*., 2003, **69**, 1435–1441.
41. Tsao, C. W., Song, H. G. and Bartha, R., Metabolism of benzene, toluene, and xylene hydrocarbons in soil. *Appl. Environ. Microbiol*., 1998. **64**, 4924–4929.
42. Herman, D. C., Zhang, Y. and Miller, R. M., Rhamnolipid (biosurfactant) effect on cell aggregation and biodegradation of residue hexadecane under saturated flow conditions. *Appl. Environ. Microbiol*., 1997, **63**, 3622–3627.

43. Uribe, S., Rangel, P., Espinola, G. and Aguirre, G., Effects of cyclohexane, an industrial solvent, on the yeast *Saccharomyces cerevisiae* and an isolated yeast mitochondria. *Appl. Environ. Microbiol*., 1990, **56**, 2114–2119.
44. Hernandez, L. A. R., Gieg, L. M. and Suflita, J. M., Biodegradation of an alicyclic hydrocarbon by sulphate-reducing enrichment from a gas condensate-contaminated aquifer. *Appl. Environ. Microbiol*., 2003, **69**, 434–443.
45. Yakimov, M. M., Golyshin, P. N., Lang, S., Moore, E. R., Abraham, W. R., Lunsdorf, H. and Timmis K. N., *Alcanivorax borkumensis* gen. sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. *Int. J. Syst. Bacteriol*., 1998, **48**, 339–348.
46. Kodama, Y. and Watanabe, K., Isolation and characterization of a sulfur-oxidizing chemolithotroph growing on crude oil under anaerobic conditions**.** *Appl. Envir. Microbiol*., 2003, **69**, 107–112.
47. Dagley, S., Microbial metabolism of aromatic compounds. In *Chemical and Biochemical Fundamentals*, University of Minnesota, USA, 1990, pp. 483–503.
48. Kastner, M., Degradation of aromatic and polyaromatic compounds. In *Biotechnology: The Science and the Business* (eds Moses, V. and Cape, R. E.), Harwood Academic Press, Switzerland, 1991, pp. 274–304.
49. Gibson, D. T. and Subranian, V., Microbial degradation of aromatic hydrocarbons. In *Microbial Degradation of Organic Compounds* (ed. Gibson, D. T.), Marcel Dekker, Inc., New York, 1984, pp. 181–252.
50. Kerr, R. P. and Capone, D. G., The effect of salinity on the microbial mineralization of two polycyclic aromatic hydrocarbons in estuarine sediments. *Mar. Environ. Res*., 1988, **26**, 181–198.
51. Manilal, V. B. and Alexander, M., Factors affecting the microbial degradation of phenanthrene in soil. *Appl. Microbiol. Biotechnol*., 1991, **35**, 401–405.
52. Watanabe, K., Watanabe, K., Komama, Y., Syutsubu, K. and Harayama S., Molecular characterization of bacterial population in petroleum contaminated groundwater discharged from underground crude oil storage cavities. *Appl. Environ. Microbiol*., 2000, **66**, 4803–4809.
53. Stapleton, R. D., Bright, N. G. and Sayler, G. S., Catabolic and genetic diversity of degradative bacteria from hydrocarbon contaminated aquifers. *Microbial Ecol*., 2000, **39**, 211–221.
54. Kniemeyer, O., Fischer, T., Wilkes, H., Glöckner, F. O. and Widdel, F., Anaerobic degradation of ethylbenzene by a new type of marine sulfate-reducing bacterium. *Appl. Environ. Microbiol*., 2003, **69**, 760–768.
55. Ficker, M., Krastel K., Orlicky, S. and Edwards, E., Molecular characterization of a toluene degrading methonogenic consortium. *Appl. Environ. Microbiol*., 1999, **65**, 5576–5585.
56. de Nardi, I. R., Varesce, M. B., Zaiat, M. M. and Foresti, E., Anaerobic degradation of BTEX in a packed bed reactor. *Water Sci. Technol*., 2002, **45**, 175–180.
57. Gibson, D. T., Venkatanarayana, D., Jerina, M., Yagi, H. and Yeh, H., Oxidation of carcinogens benzo(a)pyrene and benzo(a) anthracene to dihydrodiols by a bacterium. *Science*, 1975, **189**, 295–297.
58. Mahaffey, W. R., Gibson, D. T. and Cerniglia, C. E., Bacterial oxidation of chemical carcinogens: formation of polycyclic aromatic acids from benz[a]anthracene. *Appl. Environ. Microbiol*., 1988, **54**, 2415–2423.
59. Heitkamp, M. A. and Cerniglia, C. E., Mineralization of polycyclic aromatic hydrocarbons by a bacterium isolated from sediment below an oil field. *Appl. Environ. Microbiol*., 1988, **54**, 1612–1614.
60. Mueller, J. G., Chapman, P. J. and Pritchard, P. H., Action of a fluoranthene-utilizing bacterial community on polycyclic aromatic hydrocarbon components of creosote. *Appl. Environ. Microbiol*., 1989, **55**, 3085–3090.
61. Boldrin, B., Andreas, T. and Fritzche, C., Degradation of phenanthrene, fluorene, fluoranthene and pyrene by a *Mycobacterium* spp. *Appl. Environ. Microbiol*., 1993, **59**, 1927–1930.
62. Trzesicka-Mlynarz, D. and Ward, O. P., Degradation of polycyclic aromatic hydrocarbons (PAHs) by a mixed culture and its component pure cultures obtained from PAH-contaminated soil. *Can. J. Microbiol*., 1995, **41**, 470–476.
63. Ye, D., Siddiqi, Maccubbin, M. A., Kumar, A. E. and Sikka, H. C., Degradation of polynuclear aromatic hydrocarbons by *Sphingomonas paucimobilis*. *Environ. Sci. Technol*., 1996, **30**, 136–142.
64. Lindstrom, J. E., Barry, R. P. and Braddock, J. F., Long-term effects on microbial communities after a subartic oil spill. *Soil Biol. Biochem*., 1999, **31**, 1677–1689.
65. Vila, J., Lopez, Z., Sabate, J., Minguillon, C., Solanas, A. M. and Grifoll, M., Identification of a novel metabolite in the degradation of pyrene by *Mycobacterium* sp. strain AP1: actions of the isolate on two and three ring PAH. *Appl. Environ. Microbiol*., 2001, **67**, 5497–5505.
66. Hedlund, B. P., Geiselbrecht, A. D., Blair, T. J. and Staley, J. T., Polyaromatic hydrocarbon degradation by a new marine bacterium, *Neptunomonas naphthovorans* gen sp. nov. *Appl. Environ. Microbiol*., 1999, **65**, 251–259.
67. Samanta, S. K., Rani, M. and Jain, R. K., Segregational and structural instability of recombinant plasmid carrying genes for naphthalene degrading pathway. *Lett. Appl. Microbiol*., 1998, **26**, 265–269.
68. Samanta, S. K. and Jain, R. K., Evidence for plasmid-mediated chemotaxis of *Pseudomonas putida* towards naphthalene and salicylate. *Can. J. Microbiol*., 2000, **46**, 1–6.
69. Samanta, S. K., Chakraborti, A. K. and Jain, R. K., Degradation of phenanthrene by different bacteria: evidence for novel transformation sequences involving the formation of 1-naphthol. *Appl. Microbiol. Biotechnol*., 1999, **53**, 98–107.
70. Samanta, S. K., Bhushan, B. and Jain, R. K., Efficiency of naphthalene and salicylate degradation by a recombinant *Pseudomonas putida* mutant strain defective in glucose metabolism. *Appl. Microbiol. Biotechnol*., 2001, **55**, 627–631.
71. Gschwend, P. M., MacFarlane, J. K. and Newmann, K. A., Volatile halogenated organic-compounds released to seawater from temperate marine macroalgae. *Science*, 1985, **227**, 1033–1035.
72. Weissermel, K. and Arpe, H. J., *Industrial Organic Chemistry*, VCH Publishers Inc., New York, 1997.
73. Slater, J. H., Bull, A. T. and Hardman, D. J., Microbial dehalogenation of halogenated alcanoic acids, alcohols and alkanes. *Adv. Microbiol. Physiol*., 1997, **38**, 133–176.
74. Hardman, D. J., Gowland, P. C. and Slater, J. H., Large plasmids from soil bacteria enriched on halogenated alkanoic acids. *Appl. Environ. Microbiol*., 1986, **51**, 44–51.
75. Hardman, D. J. and Slater, J. H., Dehalogenase in soil bacteria. *J. Gen. Microbiol*., 1981, **123**, 117–128.
76. Hardman, D. J. and Slater, J. H., The dehalogenase complement of a soil pseudomonad grown in closed and open cultures on haloalkanoic acids. *J. Gen. Microbiol*., 1981, **127**, 399–405.
77. Love, O. T. Jr. and Eilers, R G., Treatment of drinking water containing trichloroethylene and related industrial solvents. *J. Am. Water Works Assoc*., 1982, **74**, 413–425.
78. Bouwer, E. J. and Mc Carty, P. L., Transformations of 1- and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions. *Appl. Environ. Microbiol*., 1983, **45**, 1286–1294.
79. Kleopfer, R. D., Easley, D. M., Haas, B. B. Jr., Deihl, T. G., Jackson, D. E. and Wurrey, C. J., Anaerobic degradation of trichloroethylene in soil. *Environ. Sci. Technol.*, 1985, **19**, 277–280.
80. Little, C. D., Palumbo, A. V., Herbes, S. E., Lidstrom, M. E., Tyndall, R. L. and Gilmer, P. J., Trichloroethylene biodegrada-

tion by a methane-oxidizing bacterium. *Appl. Environ. Microbiol*., 1988, **54**, 951–956.
81. Nelson, M. J. K., Montgomery, S. O. and Pritchard, P. H., Trichloroethylene metabolism by microorganisms that degrade aromatic compounds. *Appl. Environ. Microbiol.*, 1988, **54**, 604–606.
82. Freitas dos Santos, L. M., Leak, D. J. and Livingston, A. G., Enrichment of mixed cultures capable of aerobic degradation of 1,2-dibromoethane. *Appl. Environ. Microbiol*., 1996, **62**, 4675–4677.
83. Poelarends, G. J., van Hylckama Vlieg, J. E. T., Marchesi, J. R., Freitas dos Santos, L. M. and Janssen, D. B., Degradation of 1,2-dibromoethane by *Mycobacterium* sp. strain GP1. *J. Bacteriol*., 1999, **181**, 2050–2058.
84. Pignatello, J. J., Microbial degradation of 1,2-dibromoethane in shallow aquifer materials. *J. Environ. Qual*., 1987, **16**, 307–312.
85. Vogel, T. M., Criddle, C. S. and Mc Carty, P. L., Transformations of halogenated aliphatic compounds. *Environ. Sci. Technol*., 1987, **21**, 722–736.
86. Leuenberger, C., Giger, W., Coney, R., Grayder, J. M. and Molnar-Kubica, E., Persistent chemicals in pulp mill effluents: Occurrence and behaviour in an activated sludge treatment plant. *Water Res*., 1985, **19**, 885–894.
87. Wittich, R. M., Wilkes, H., Sinwell, V., Franke, W. and Fortnagel, P., Metabolism of dibenzo-*p*-dioxin by *Sphingomonas* sp. strain RW1. *Appl. Environ. Microbiol*., 1992, **58**, 1005–1010.
88. Armengaud, J., Happe, B. and Timmis, K. N., Genetic analysis of dioxin dioxygenase of *Sphingomonas* sp. strain RW1: Catabolic genes dispersed on the genome. *J. Bacteriol*., 1998, **180**, 3954–3966.
89. Thakur, I. S., Structural and functional characterization of a stable, 4-chlorosalicylic acid degrading, bacterial community in a chemostat. *World J. Microbiol. Biotechnol*., 1995, **11,** 643–645.
90. Thakur, I. S., Biodegradation of chlorinated benzoic acid by a *Pseudomonas fluorescens* from a chemostat. *Indian J. Microbiol*., 2001, **41**, 27–32.
91. Apajalahti, J. H., Karpanoja, P. and Salkinoja-Salonen, M. S., *Rhodococcus chlorophenoliticus* sp. nov., a chlorophenol-mineralizing Actinomycete. *Int. J. Syst. Bacteriol*., 1986, **36**, 246–251.
92. Thakur, I. S., Verma, P. K. and Upadhayaya, K. C., Involvement of plasmid in degradation of pentachlorophenol by *Pseudomonas* sp. from a chemostat. *Biochem. Biophys. Res. Commun*., 2001, **286,** 109–113.
93. Thakur, I. S., Verma, P. K. and Upadhayaya, K. C., Molecular cloning and characterization of pentachlorophenol-degrading monooxygenase genes of *Pseudomonas* sp. from the chemostat. *Biochem. Biophys. Res. Commun*., 2002, **290**, 770–774.
94. Shah, S. and Thakur, I. S., Enrichment and characterization of a microbial community from tannery effluent for degradation of pentachlorophenol. *World J. Microbiol. Biotechnol*., 2002, **18**, 693–698.
95. Xun, L. and Orser, C. S., Purification of a *Flavobacterium* pentachlorophenol-induced periplasmic protein (PcpA) and nucleotide sequence of the corresponding gene (pcpA). *J. Bacteriol*., 1991, **173**, 2920–2926.
96. Reineke, W., Microbial degradation of halogenated aromatic compounds. *Microbial Degradation of Aromatic Compounds* (ed. Gibson, D. T.), Marcel Dekker Inc., New York, 1984, pp. 319–360.
97. Reineke, W. and Knackmuss, H. J., Microbial metabolism of haloaromatics: isolation and properties of a chlorobenzene-degrading bacterium. *Appl. Environ. Microbiol*., 1984, **47**, 395–402.
98. Haigler, B. E., Nishino, S. F. and Spain, J. C., Degradation of 1,2-dichlorobenzene by a *Pseudomonas* sp. *Appl. Environ. Microbiol*., 1988, **54**, 294–301.
99. de Bont, J. A. M., Vorage, M. J. A. W., Hartsman, S. and Van den Tweel, W. J. J., Microbial degradation of 1,3-dichlorobenzene. *Appl. Environ. Microbiol*., 1986, **52**, 677–680.
100. Spain, J. and Nishino, S. F., Degradation of 1,4-dichlorobenzene by a *Pseudomonas* sp. *Appl. Environ. Microbiol*., 1987, **53**, 1010–1019.
101. Pierce, G. E., Robinson, J. B. and Colaruotolo, substrate diversity of *Pseudomonas* spp. containing chlorotoluene degradative plasmids. *Dev. Ind. Microbiol*., 1983, **24**, 499–507.
102. Heitkamp, M. A., Franklin, W. and Creniglia, C. E., Microbial metabolism of polycyclic aromatic hydrocarbons: isolation and characterization of a pyrene-degrading bacterium. *Appl. Environ. Microbiol*., 1988, **54**, 2549–2555.
103. Furukawa, K. and Chakrabarty, A. M., Involvement of plasmids in total degradation of chlorinated biphenyls. *Appl. Environ. Microbiol.*, 1982, **44**, 619–626.
104. Shields, M. S., Hooper, S. W. and Sayler, G. S., Plasmid-mediated mineralization of 4-chlorobiphenyl. *J. Bacteriol*., 1985, **163**, 882–889.
105. Kamp, P. F. and Chakrabarty, A. M., Plasmids specifying *p*-chlorobiphenyl degradation in enteric bacteria. In *Plasmids of Medical, Environmental, and Commercial Importance* (eds Timmis, E. N. and Puhler, A.), Elsevier/North Holland Biomedical Press, New York, 1979, pp. 275–285.
106. Barton, M. R. and Crawford, R. L., Novel biotransformations of 4-chlorobiphenyl by a *Pseudomonas* sp. *Appl. Environ. Microbiol*., 1988, **54**, 594–595.
107. Chaudhary, G. R. and Chapalamadugu, S., Biodegradation of halogenated organic compounds. *Microbiol. Rev.*, 1991, **55**, 59–79.
108. Khan, A., Tewari, R. and Walia, A., Molecular cloning of 3-phenylcatechol dioxygenase involved in the catabolic pathway of chlorinated biphenyl from *Pseudomonas putida* and its expression in *Escherichia coli*. *Appl. Environ. Microbiol*., 1988, **54**, 2664–2671.
109. Takase, I., Omori, T. and Minoda, Y., Microbial degradation products from biphenyl-related compounds. *Agric. Biol. Chem*., 1986, 681–686.
110. Yagi, O. and Sudo, R., Degradation of polychlorinated biphenyls by microorganisms. *J. Water Pollut. Control Fed*., 1980, **52**, 1035–1043.
111. Pritchard, P. H., Fate of pollutants. *J. Water Pollut. Control Fed*., 1986, **58**, 635–645.