The Characterization and Application of Biological Remediation Technology for Organic Contaminants

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ABSTRACT: The continuous addition of industrial, municipal, and agricultural effluents to the environment has led to a great increase of toxic pollutants. It is well known that these pollutants are hazardous to animals, plants, microorganisms, and other living organisms including humans. As a result, effective technologies should be developed to remove these contaminants. Biological remediation, as an economically feasible and environmentally friend approach, has been extensively studied and reported. In this review, the biological pathways and mechanism of most important contaminants, such as aromatic compounds, ionic liquids, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, etc, are described and discussed with some details according to a vast number literatures published in recent years.

Key words: Pollutants, Remediation technology, Applications, Environmental pollution, Review

INTRODUCTION

The continuous addition of industrial, municipal, and agricultural effluents to the environment has resulted in a considerable increase of toxic contaminants (Khler *et al.,* 2006; Roslev *et al.,* 1998). Clearly, significant amounts of such substances, which are hazardous to animals, plants, microorganisms, and other living organisms, are released annually into aquatic and terrestrial environments (Devic *et al.,* 2002). The inherent toxicity of these anthropogenic compounds indicates that they should not be permitted to remain in the environment; however, the widespread contamination of the soil and water worldwide denotes that inexpensive, harmless, and sustainable approaches to remove these toxic compounds need to be developed and employed (Olaniran *et al.,* 2008; Hale *et al.,* 2001). Current methods of remediating contaminated sites, such as incineration and composting, often are expensive and harmful. One effective solution to the problems, which is considered both economically feasible and environmentally friendly, is bioremediation. This is the use of organisms, such as microbes or plants, to degrade or detoxify hazardous compounds (Papadopoulos *et al.,* 2007; Stokes *et al.,* 2006; Symons & Bruce, 2006; Cherian and Jayachandran, 2009). Up to date, a substantial number of publications have been reported concerning the biodegradation of polymers, plastics and surfactants, as described by numerous studies (Cerniglia *et al.,* 1992; Spain *et al.,* 2000; Diaz *et al.,* 2001; Nishino *et al.,* 2006).

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A great variety of organic chemicals are now widespread in developing and developed countries, and each chemical may be unique in terms of its physicochemical properties and toxicological mode of action. Evidently, the risk assessment and remediation of such contaminated sites is a priority and continuing need early detection of pollutants, including various hydrocarbons, aromatic substances, nitroaromatic compounds, and heavy metals released from human activity. However, containment and remediation strategies are complicated in many cases by the range of contaminants present and the historical nature of the contaminations. Therefore, there is a urgent need to understand, where possible, and quantify the bioavailable fraction as well as the total concentration of contaminant present in environment. In this review, the biological pathways and mechanism of most typical contaminants, such as aromatic compounds, ionic liquids, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, etc, are described and discussed with some details according to a vast number literatures published in recent years.

Among the most abundant environmental pollutants, nitroaromatic compounds (NACs) represent a major class of soil and groundwater contaminants duo to their persistence, toxicity and their widespread use as pesticides, dyes, explosives, and industrial feedstocks (Kulkarnin & Chaudhari, 2007; Hofstetter *et al.,* 2008). Nowadays, a number of

nitroaromatic compounds such as nitrobenzenes, o- and p-nitrotoluene, 2, 4-dinitrotoluene, diphenylamine, and nitrobenzoates are massively produced and are widely employed as intermediates for the development of drugs, polyurethane foams, herbicides, dyes, pigments, pharmaceuticals, explosives, etc (Abraham *et al.,* 2001; Lessner *et al.,* 2003; Wu *et al.,* 2006; Shin & Spain 2009). The natural formation of NACs is rare, and most of these compounds are man-made from industrial productions and have been introduced into the environment for a relatively short period. Their occurrence in the environment has selected microorganisms that can utilize many NACs as carbon, nitrogen, and energy sources for growth. Examples include bacteria that can grow with nitrobenzene, mononitrotoluenes, and dinitrotoluenes (Nishino *et al.,* 2000; Lessner *et al.,* 2003; Wu *et al.,* 2004). However, the continuing persistence of some xenobiotics indicates that their biodegradative capacity is not expressed completely or effectively (Kulkarni *et al.,* 2007), and many NACs are harmful to animals, plants, microorganisms, and other living organisms (Abraham *et al.,* 2001; He *et al.,* 2000). In addition, use and disposal of NACs have led to extensive environmental contamination. As a consequence, it is necessary to remove them from the environment by developing molecular biology that can remediate previously un-biodegradable NACs or degrade them at a higher rate and/or to a greater extent than naturally occurring organisms. For this, the microbial degradation of NACs has been extensively investigated and the removal of the nitro group(s) is carried out via oxidative pathways with monooxygenases (Haigler *et al.,* 1994) or dioxygenases (Lessner *et al.,* 2003; Nadeau *et al.,* 2003) or a partial reductive pathway with nitroreductases (Hasegawa *et al.,* 2000; Meulenberg *et al.,* 1996; Haigler *et al.,* 1996). Obviously, most NACs are more recalcitrant than the raw material from which they are synthesized mainly due to the symmetric number and position of the nitro groups and other aromatic substituents, an arrangement that limits attack by classic dioxygenase enzymes involved in the microbial metabolism of aromatic compounds. Therefore, the chemical structure of these compounds affects its biodegradability. This makes it worth understanding the chemical structure of NACs and the mechanism of biodegradation of these molecules with some details. Next to glucosyl residues, the benzene ring is the most widely distributed unit of chemical structure in nature (Diaz *et al.,* 2001; Harwood & Parales 1996). The degradation of such chemicals is accomplished mainly by microorganisms (van der Meer *et al.,* 1992; Fournier *et al.,* 2004), and in recent years there has been increasing interest in exploring their ability to degrade and detoxify the amounts of aromatic compounds, as well as in developing efficient bioremediation technology that can transform NACs in the presence of oxygen to key metabolites that are amenable to further degradation. In such process, the nitro group can be released as nitrite following either monooxygenation to an epoxide, which leads to a phenolic compound, dioxygenation of the aromatic ring to a substituted catechol, or via partially reduce some NACs via nitroso- and hydroxylaminobenzene intermediates (Hofstetter *et al.,* 2008). The typical nitro group for general aromatic compounds can be released as nitrite following either monooxygenation to an epoxide, which leads to a phenolic compound (Fig 1, pathway A) or via dioxygenation of the aromatic ring to a substituted catechol (pathway B). An alternative nitro group removal has been observed after nucleophilic addition of hydride to form a hydride-Meisenheimer complex (pathway C). Finally, bacteria can partially reduce some NACs via nitroso- and hydroxylaminobenzene intermediates. The nitro group is later eliminated as ammonia during reactions of substituted *o*-aminophenols (pathway D) (Hofstetter *et al.,* 2008).

Some bacterial strains use specific hydrocarbon substrates as their primary sources of carbon and energy, and the number of such strains is continuously increasing (Fetzner & van der Meer 2000; Cafaro *et al.,* 2005). The wide ranges of substrates that can be transformed by these microorganisms make them a powerful tool for the bioremediation of environmentally harmful substances. Aerobic bacterial degradation of aromatic hydrocarbons is generally divided into two major routes (Arenghi *et al.,* 2001), the so-called upper pathway, which leads to the formation of partially oxidized aromatic intermediates, and a lower pathway, which uses dihydroxylated aromatic molecules. These activated aromatic compounds undergo ring cleavage reactions and are further processed to give molecules that can eventually enter the citric acid cycle (Cafaro *et al.,* 2004). Monooxygenases are key enzymes in the upper pathway and catalyze hydroxylation of the aromatic ring at different positions. Recently, it has been recognized that bacterial multicomponent monooxygenases (BMMs) constitute a family of enzymes which can be divided into six distinct groups, each with a characteristic subunit composition (Notomista *et al.,* 2003). BMMs are transcribed from single operons that code for four to six polypeptides. Analysis of the sequences from nucleotide and protein databases means that most bacterial strains possess only one BMM, but a few cases (3 out of 31) of bacterial genomes coding for more than one monooxygenase have been found (Notomista *et al.,* 2003). Two group 1 (phenol hydroxylases) and one group 2 (toluene-benzene monooxygenases) BMMs have been found
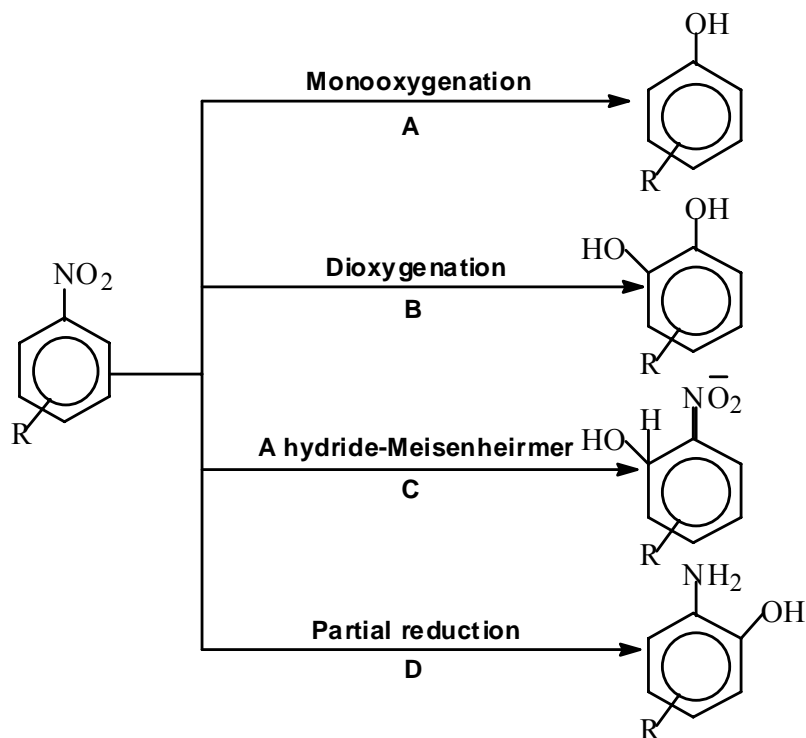
Fig. 1. General biodegradation pathways of typical NAC by bioremediation technology
(reproduced with permission from Hofstetter et al., 2008)

in the genome of Ralstonia metallidurans CH34 (Notomista *et al.,* 2003), and three BMMs belonging to groups 1 and 2 were detected in Burkholderia cepacia (formerly Pseudomonas cepacia) JS150 (Notomista *et al.,* 2003; Kahng *et al.,* 2001). *B. cepacia* JS150 instead is endowed with the ability to use different pathways for the metabolism of substituted aromatic compounds (Kahng *et al.,* 2001). This led to the suggestion that in *B. cepacia* JS150, toluene metabolism is initiated by one dioxygenase and two distinct BMMs with different specificities, whereas a third BMM acts downstream of the other two monooxygenases (Kahng *et al.,* 2001). *Pseudomonas stutzeri* OX1 is able to grow on a wide spectrum of aromatics, including phenol, cresols, and dimethylphenols, but also on nonhydroxylated molecules such as toluene, *o*-xylene (Bertoni *et al.,* 1996), and benzene (Cafaro *et al.,* 2004). These multicomponent monooxygenases catalyze the hydroxylation of aromatic ring and display a certain regioselectivity for hydroxylation (Bertoni *et al.,* 1998). For example, toluene-2-monooxygenase from *Burkholderia* (*Pseudomonas*) *cepacia* G4 (Shields *et al.,* 1995) and *Pseudomonas* sp. strain JS150 (Johnson & Olsen 1995), toluene-3-monooxygenase from *Burkholderia* (*Pseudomonas*) *pickettii* PKO1 (Olsen *et al.,* 1994), and toluene-4-monooxygenase from *Pseudomonas mendocina* KR1 (Whited & Gibson 1991) have been particularly studied. Other

multicomponent monooxygenases, active on phenols, were identified in phenol-degrading *Pseudomonas* and *Acinetobacter* strains (Bertoni *et al.,* 1998; Herrmann *et al.,* 1995).

In the process of biodegradation, monooxygenase adds a single oxygen atom and causes elimination of nitro groups from mono-nitrophenols (Kulkarni *et al.,* 2007). For instance, degradation of o-nitrophenol by Pseudomonas putida involves replacement of the nitro group with a hydroxyl group, and subsequent conversion of the catechol to Iketoadipate via cis,cis-muconate (Zeyer & Kearney 1984). Simpson and Evans (1953) suggested that the initial step in the bacterial degradation of p-nitrophenol (PNP) involved a similar oxidative removal of the nitro group. Munnecke and Hsieh (1974) investigated hydroquinone as an early metabolite in PNP degradation by a pseudomonad and proposed that it was hydroxylated to form 1,2,4-benzenetriol prior to ortho ring fission. Subsequently, Spain and Gibson (1979; 1991) used partially purified monooxygenase (flavoprotein) of Moraxella, which degraded 4-nitrophenol, with a concomitant release of nitrite and accumulation of hydroquinone at the expense of two moles of NADPH per mole of 4-nitrophenol. They proposed that though hydroquinone is the first detectable intermediate of the pathway, its formation takes place via 1,4-benzoquinone, an unstable intermediate in the monooxygenase-catalyzed

reaction. Hydroquinone formed in the above reaction served as a substrate for ring fission catalyzed by a ferrous ions dependent dioxygenase. It converted hydroquinone to g-hydroxy muconic semialdehyde, which was further oxidized to maleyl acetic acid. The latter is further reduced to b-ketoadipic acid (Kulkarni *et al.,* 2007). Similar pathways for 4-nitrophenol degradation have also been reported in Pseudomonas cepacia, (Prakash *et al.,* 1996) Sphingomonas (Leung *et al.,* 1997) and Pseudomonas putida (Kulkarni *et al.,* 2007). Molecular elucidation of these catabolic pathways has indicated that new pathways are continually generated by a variety of genetic mechanisms, such as gene transfer, mutational drift, genetic recombination, and transposition (Cafaro *et al.,* 2005; Antonie *et al.,* 1997). *Pseudomonas stutzeri* OX1 is one microorganism that is capable of growth on highly toxic aromatic compounds, such as benzene, toluene, *o*-xylene, and dimethylphenols, each of which can act as a sole source of carbon and energy (Cafaro *et al.,* 2005). This strain is an interesting model system for studying hydrocarbon catabolism because of its peculiar ability to transform various molecules than other similar microorganisms. In *P. stutzeri*, (methyl) benzenes are initially activated by sequential introduction of two adjacent hydroxyl groups to form (methyl)phenols and, eventually, (methyl)catechols. These metabolites are subsequently cleaved into 2-hydroxymuconic semialdehyde derivatives, which, upon further processing, are transformed into citric acid cycle intermediates (Arenghi et al., 2001). Recently, Donato et al. (2002, 2004)have demonstrated that the initial hydroxylation steps are carried out by two evolutionarily distinct bacterial multicomponent monooxygenases (BMMs), toluene *o*-xylene monooxygenase (ToMO), belonging to the family consisting of four-component aromatic/alkene monooxygenases (group 2 BMMs), and phenol hydroxylase (PH), belonging to the group consisting of toluene 2-monooxygenases (T2MO)/phenol hydroxylases (group 1 BMMs) (Cafaro *et al.,* 2002, 2004). The ring cleavage step that allows aromatic hydrocarbons access to the lower metabolic pathway is catalyzed by a catechol 2,3-dioxygenase (C2,3O) (Arenghi *et al.,* 2001). Genetic studies carried out with *P. stutzeri* OX1 have demonstrated that the genes coding for ToMO were recently acquired by horizontal gene transfer and incorporated into a preexisting catabolic route (Fournier *et al.,* 2004). Thus, the acquisition of ToMO by *P. stutzeri* OX1 is an example of expansion of a catabolic pathway. The result of this expansion is an apparent redundancy in enzymatic activity, since ToMO and PH hydroxylate both benzene and phenol (Arenghi *et al.,* 2001; Cafaro *et al.,* 2004). To elucidate the metabolic significance of the

coexpression of two multicomponent enzymes with very similar catalytic activities, Donato et al. (2004) investigated the kinetics of benzene oxidation catalyzed by ToMO and PH. The data obtained strongly supported the hypothesis that ToMO and PH form a metabolic chain, in which the depletion of phenol by PH enhances the use of benzene in *P. stutzeri*. However, this hypothesis needs to be extended to other substrates in order to establish, in general, whether the concerted use of the two different monooxygenases controls the metabolic flow of aromatic molecules that can be used by the microorganism as growth substrates which enter the lower pathway. In the further work, Donato et al (2005) investigated toluene and *o*-xylene oxidation using recombinant *Escherichia coli* cells expressing ToMO and PH complexes to collect data on how the biochemical properties of the two complexes could influence the efficiency of methylated aromatic utilization. Their results confirmed the general hypothesis that the two monooxygenases of *P. stutzeri* OX1 form a metabolic chain, thus expanding the catabolic potential of the microorganism by optimizing the degradation of benzene, toluene, and *o*-xylene.

Biodegradation of aromatic compounds by aerobic bacteria often begins with the initial oxidation of the substrate by dioxygenases which catalyze the incorporation of both atoms of molecular oxygen into the substrates to form arene *cis*-diols (Suen *et al.,* 1996; Gibson & Parales 2000; Boyd & Sheldrake 2005). They are currently members of the aromatic-ring-hydroxylating dioxygenase superfamily and are classified by the number and properties of the electron transport proteins that precede the catalytic oxygenase component (Gibson & Parales 2000). These dioxygenases are multicomponent enzyme systems. Two-component dioxygenases such as 4-sulfobenzoate 3, 4-dioxygenase, *o*-phthalate dioxygenase, and 2-halobenzoate 1,2- dioxygenase consist of an iron-sulfur flavoprotein reductase and an iron-sulfur oxygenase (Suen *et al.,* 1996; Mason & Cammack 1992). Three-component systems such as biphenyl dioxygenase, dibenzofuran dioxygenase and o-halobenzoate dioxygenase comprise a flavoprotein reductase, an iron-sulfur ferredoxin and a terminal iron-sulfur oxygenase where the reductase and ferredoxin components serve as a short electron transfer chain, shuttling electrons from NAD(P)H to the terminal oxygenase (Mason et al., 1992; Haddock *et al.,* 1993; Parales *et al.,* 1996).

Bacterial degradation of aromatic compounds under aerobic conditions involves the conversion of the initial substrates into intermediates with two or more hydroxyl groups on the aromatic ring. The

intermediates are subject to oxidative attack by the ring cleavage dioxygenases. For example, Bacteria degrade 2,4-dinitrotoluene (2,4-DNT), which are intermediates in the synthesis of explosives and expanded polyurethane foam, by a pathway involving two oxygenase reactions that lead to the removal of the nitro substituents. The pathway is initiated by a multicomponent hydroxylating dioxygenase that transforms 2, 4-dinitrotoluene to 4-methyl-5-nitrocatechol (Suen *et al.,* 1996). The methylnitrocatechol is then oxidized by a flavin-containing monooxygenase to yield 2- hydroxy-5-methylquinone (He & Spain 2000), which is enzymatically reduced to 2, 4, 5-trihydroxytoluene. Trihydroxytoluene is then oxidized in the ring cleavage reaction catalyzed by the extradiol dioxygenase trihydroxytoluene oxygenase. Burkholderia cepacia R34 mineralizes 2,4-dinitrotoluene via the degradative pathway used by strain DNT. Ring cleavage is a key step in the 2,4- dinitrotoluene degradative pathway. The trihydroxytoluene oxygenase from Burkholderia sp. strain DNT is phylogenetically distant from previously described ring cleavage enzymes and specifically attacks the trihydroxylated substrate (He *et al.,* 2000). The process was shown in Fig.2 (Nishino *et al.,* 2000; Johnson *et al.,* 2000, 2002). The ring

cleavage enzymes are classified into two groups based on the site of ring fission (Haigler *et al.,* 1999). Enzymes catalyzing ring fission between two hydroxyl groups are designated intradiol (*ortho-*) cleavage enzymes. Enzymes that cleave at a bond proximal to one of the two adjacent hydroxyl groups are designated extradiol (*meta-*) cleavage enzymes. The primary structures of many ring cleavage dioxygenases have been determined, and comparison of their amino acid sequences has revealed that they can be divided further into distinct gene families (Harayama *et al.,* 1992). The interest in bacterial metabolism of aromatic compounds has motivated a fast growing research on the biochemistry and molecular genetics (Lessner *et al.,* 2003).

Bacterial multicomponent dioxygenase enzyme systems carry out the first reaction in the aerobic degradation of a variety of aromatic compounds. These enzymes have been of interest to researchers for several reasons. First, aromatic hydrocarbons, such as benzene, toluene, naphthalene, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and nitroaromatics, are common contaminants of soils and groundwaters (Parales *et al.,* 1999). The removal of these compounds from polluted environments by
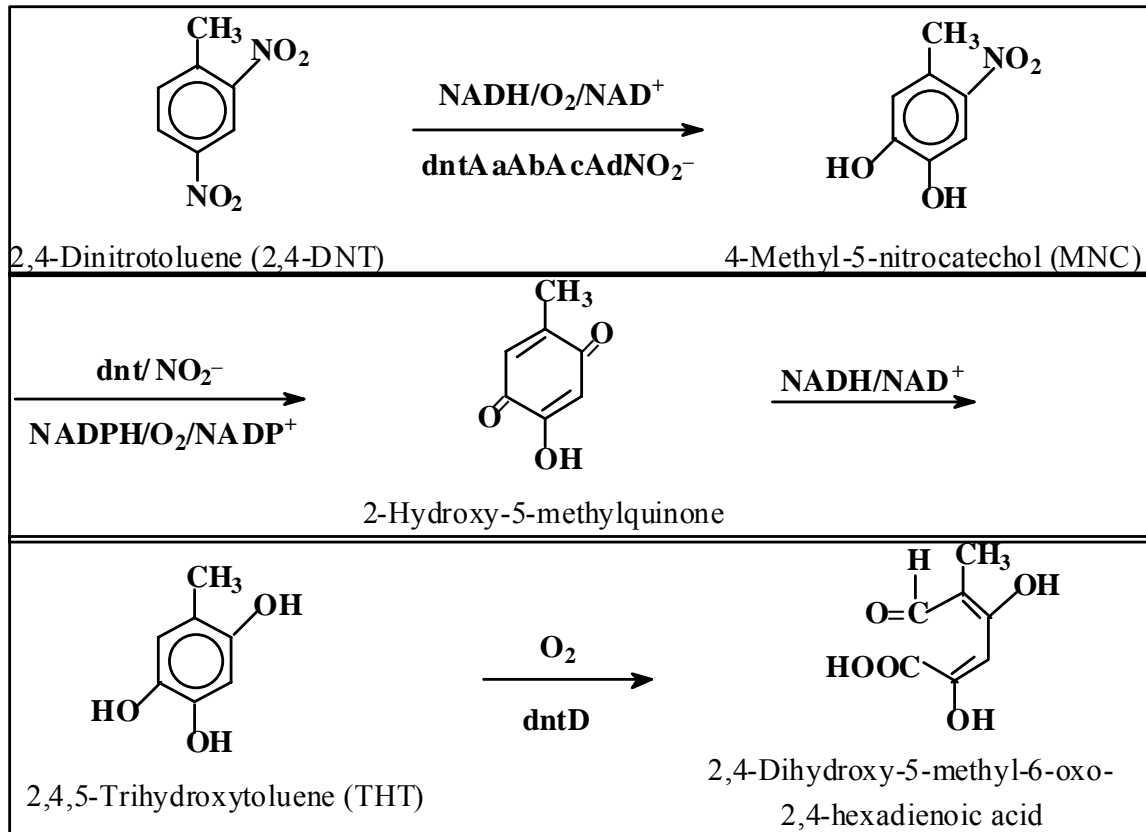

Fig. 2. Pathway for degradation of 2,4-DNT in Burkholderia cepacia R34

microorganisms represents a potential solution to the environmental problems posed by these pollutants. Dihydroxylation of the aromatic ring is a prerequisite for oxidation of the aromatic nucleus by bacterial ring-fission dioxygenases (Timmis & Pieper 1999). Aromatic hydrocarbons contain only the elements of carbon and hydrogen. Studies on the multicomponent dioxygenases that oxidize aromatic hydrocarbons to vicinal arene *cis*-diols are of paramount importance in providing the scientific foundations necessary for the development of bioremediation technology (Timmis *et al.,* 1999; Lau & De Lorenzo 1999). The second, the ability to produce enantiomerically pure products from a wide range of substrates has brought dioxygenases to the attention of organic chemists for the production of chiral synthons used in the preparation of biologically active chemicals and pharmaceuticals (Parales *et al.,* 1999). The third reason for interest in aromatic hydrocarbon dioxygenases is related to industry's search for environmentally benign procedures for the development of useful chemicals. The dioxygenases fulfill this 'green chemistry' requirement because they are a source of new enantiopure arene *cis*-diols that are not attainable by conventional chemical synthesis. The diols are used as synthons in the development of new compounds of industrial and medical importance (Gibson *et al.,* 2000). Two examples are polyphenylene and prostaglandin E2a. The extent of interest in arene *cis*diols, including those formed from aromatic acids, in asymmetric synthesis can be seen in the publication of several extensive literature in this field (Gibson *et al.,* 2000; Boyd *et al.,* 1998; Hudlicky *et al.,* 1999).

Besides two major bioremediation technologies discussed above, other pathways, such as meisenheimer complex formation and partial reduction, are also effective methods for removing the contaminants of aromatic compounds in soil and groundwater, in which, the nitro group in aromatic compounds is partially reduced to corresponding hydroxylamine and upon hydrolysis yields ammonia or to formation of a hydride-Meisenheimer complex through addition of hydride ions (Kulkarni *et al.,* 2007; He *et al.,* 2000; Spain 1995). Subsequently, the hydroxylamino intermediates can be transformed to catechols which enter the ring cleavage pathways of aerobic degradation of aromatic compounds, as observed during degradation of 4-nitrobenzoate, 3-nitrophenol, 4-nitrotoluene, etc (He *et al.,* 2000; Meulenberg *et al.,* 1996; Spain *et al.,* 1995). Alternatively, the hydroxylamino intermediates can be rearranged, by an intramolecular transfer of the hydroxyl group (He *et al.,* 2000), to ortho-aminophenols, as introduced during biodegradation of nitrobenzene, 3-nitrophenol, and 2-chloro-5-nitrophenol, by various

microorganisms (Park et al., 1999; Schenzle *et al.,* 1999). The initial steps of each of the degradation pathways have been elucidated; however, the lower pathway has been determined only for the metabolism of 2-aminophenol during the degradation of nitrobenzene by pseudomonas pseudoalcaligenes JS45. The pathway is parallel to the catechol extradiol ring cleavage pathway, except that 2-aminophenol is the ring cleavage substrate. Mycobacterium strain HL 4-NT-1 is able to grow on 4-nitrotoluene as the sole source of nitrogen, carbon, and energy. In the process of reduction, 4-NT is partially reduced to 4-hydroxylaminotoluene, which is then converted to 6-AC by an enzymecatalyzed Bamberger-type rearrangement, after that, ring cleavage of 6-AC yields 2-amino-5-methylmuconic semialdehyde, which is further degraded via an NAD-dependent step (Spiess *et al.,* 1998). In further work, Spain et al (2000)rigorously determined the steps in the lower pathway including the mechanism on release of ammonia in Mycobacterium strain HL 4-NT-1 grown on 4-nitrotoluene, and compared the substrate specificities of the relevant enzymes in strains JS45 and HL 4-NT-1 to provide a preliminary evaluation of the relationship between the two pathways.

The remarkable number of substrates oxidized to single enantiomers by monooxygenases and dioxygenases has led to their extensive use in asymmetric synthesis of many biologically active products. Only a few of these have been targeted for commercial use. The aromatic hydrocarbon dioxygenases, together with many other Rieske non-heme iron oxygenases, initiate the biodegradation of environmental pollutants. The pros and cons of strategies used to develop hybrid dioxygenases that have potential use in the development of bioremediation technology for improved aromatic compounds degradation. Additionally, although a substantial number of bacteria that are able to degrade synthetic nitroaromatic compounds have been isolated, and the degradation mechanisms have been established (Nishino & Spain 2004), little information is available on the biodegradation of natural nitroaromatic compounds such as 3-nitrotyrosine (3NTyr). Therefore, the pathways and mechanism of biodegradation of natural nitroaromatic compounds will also be a promising interest research field for academic application, as described by Nishino and Spain (2006).

IONIC LIQUIDS (ILs)
Ionic liquids (ILs) are deemed greener solvent alternatives in chemical synthesis (Harjani et al., 2008), catalysis (Fellay 2007), biocatalysis (Zhao 2005), and electrochemistry (Silvester & Compton, 2006). This wide applicability of ionic liquids is mainly responsible

for the beneficial physico-chemical properties (e.g. high thermal and electrochemical stability, high conductivity, extraction behaviour, etc.) of certain compounds out of this diverse substance class (Stolte *et al.,* 2008). Furthermore, the negligible vapor pressure can effectively reduce air emission and nonflammability. In this respect, the operational safety of ionic liquids is improved as compared to conventional solvents (Harjani *et al.,* 2008; Stolte *et al.,* 2008). Thus, these materials are often branded as "environmentally friendly," and have being driven industrial and academic research teams to redesign chemical processes in order to reduce, or eliminate, losses of solvents, particularly volatile organic compounds (Wells & Coombe 2006; Swatloski *et al.,* 2003).

In general, the high structural variability of the head group (positively charged core structure, e.g. ammonium, pyridinium, and imidazolium cations) (Gathergood & Scammells 2002), the substituent (s) and the corresponding anion leads to an enormous number of accessible ionic liquids (general structure Fig.3), such core-shell structure can exhibit many excellent advantages, the reader interested in obtaining further information is referred to a our recent review on the fabrication and progress of core-shell composite materials (Cao *et al.,* 2009). The combination of these different structural elements allows at best for an optimization of physico-chemical properties of ionic liquids necessary for a defined technical application (Stolte *et al.,* 2008). Not surprisingly, with their high structural variability these neoteric solvents have attracted the great attention of chemists from a multitude of disciplines and the domain of their applications has substantially broadened. Although the nonvolatility of ILs under operational conditions minimizes their impact on air quality during their life cycle, their impact on soil and water is certainly of considerable concern at the time of their disposal. As a result, it is necessary to evaluate the effects on man and the environment for numerous ILs, as introduced by Stolte et al. (2007), Ranke et al. (2007) and Zhao et al. (2007). These studies indicate that ionic liquids can cause adverse effects on organisms. Especially for cations substituted with long (C>8) alkyl side chains (Stolte *et al.,* 2007; Matzke *et al.,* 2007) or for anions showing lipophilicity or a susceptibility to hydrolysis (Stolte *et al.,* 2006; Matzke *et al.,* 2007) partially drastic effects have been observed. Nevertheless, by an appropriate choice of (eco)toxicologically favorable structural elements as short and functionalised side chains, avoiding the quinolinium and the 4-(dimethylamino) -pyridinium head group and by using, for example, chloride, tetrafluoroborate or octylsulfate as the anion, (Stolte *et al.,* 2007; Matzke *et al.,* 2007)

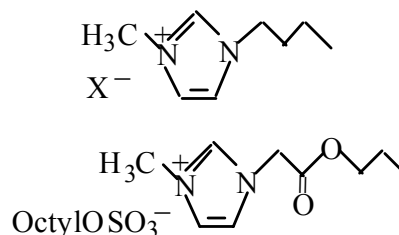the (eco)toxicity of an ionic liquid can be reduced remarkably in the test systems investigated so far.

Fig. 3. the common 1-butyl-3- methylimidazolium ionic liquids

Although ionic liquids have created a great deal of interest as green reaction media, the first reports regarding their biodegradability have only appeared relatively recently (Gathergood *et al.,* 2002; 2006). the principles of green chemistry and engineering teach us that we should consider the whole process (life cycle analysis) rather than individual components of a reaction ("single issue sustainability"), and thus avoid replacement of materials with those that could turn out to be more damaging to the environment (Wells *et al.,* 2006). According to this principle, chemicals should also be designed to break down to innocuous substances after their use so that they do not accumulate in the environment (Stolte *et al.,* 2008). Therefore, designing biodegradable chemicals are significant because it would decrease the bioaccumulation of the parent compound and its metabolic products, reducing its toxic effects. The design of potentially biodegradable ionic liquids focused on individual components that comprise most ionic liquids: the cation core, cation side chain(s), and the anion (Gathergood *et al.,* 2002). As a result, the logical design of ILs may result in such compounds that would not only be ideal solvents for chemical processes, but would also be safe for disposal. Research in this area is currently vital as ILs are likely to make a transition from academic laboratories to large scale operations where disposal of any chemical is a major concern (Adam 2000; Seddon 2003). Enormous structural variations are possible in the ILs by changing either the cation and/or the anion. This leads us to believe that it should be possible to manipulate their chemical architecture to achieve high biodegradability (Gathergood *et al.,* 2004, 2006). Unfortunately, the tendency of certain ionic liquid cations to be thermally and chemically very stable is mirrored in their stability to biological degradation processes. Up to date, only a few fundamental studies have introduced the biodegradability of ionic liquids (Jastorff et al., 2005; Stepnowski & Storoniak 2005). However, no compound

could be classified as readily biodegradable. In a later study, Gathergood et al. (2006) reported their efforts toward the design, synthesis, and evaluation of biodegradable ionic liquids that were constructed to contain moieties that are metabolizable, namely an ester side chain attached to the cationic core and an alkylsulfate anion as the counter ion, and identified two new ionic liquids that proved to be 'readily biodegradable' in the CO_2 headspace test (ISO 14593) (Gathergood et al., 2006; Bouquillon *et al.,* 2007). Then, they sought to investigate the utility of these ionic liquids as solvents in reactions of general interest and found that the side chain ester moiety proved to be a key feature in improving the biodegradability of the dialkylimidazolium class of ionic liquids. Recently, Wells and Coombe (2006) chose to look at a range of ionic liquids (ammonium, imidazolium, phosphonium and pyridinium compounds) to explore molecular weight and size effects on their potential fate and ecotoxicological properties by measuring the biological oxygen demand (BOD). No biodegradability of cations with short chains (C<4) was observable within this test series. For phosphonium and imidazolium cations with longer chains (C12, C16 and C18) a strong inhibitory potential to the inoculum used was found, indicating the toxicity of these ionic liquids towards the microorganisms used. Recently, Kulpa and co-workers (2000) examined the biodegradability of N-methylimidazolium and 3-methylpyridinium compounds substituted with butyl, hexyl and octyl side chains and bromide as the anion. The test revealed that only the long octyl side chain in ionic liquids resulted in an improved biodegradability, which in turn would create a conflict of aims between minimizing the toxicity and maximizing the biodegradability as afore-described. Consequently, the biodegradability seems to be a bottle-neck in the development of inherently safer ionic liquids. To fabricate such ionic liquids, considerable efforts have been devoted to overcoming this inherent problem and to enlarging the restricted knowledge in the field of biodegradation of ionic liquids. For example, Stolte et al (2008) analyzed systematically the influence of the structural elements 'head group' and 'side chain' on the biodegradability of 27 compounds. The experiments strongly suggested that a certain lipophilicity of the test compounds was an essential criterion for biodegradable ionic liquid cations. However, a high lipophilicity corresponds to an increased (eco) toxicity in different test systems. Thus, a conflict of goals between (eco) toxicologically favorable compounds (short and functionalized side chains) on the one hand and inherently biodegradable substances on the other hand needs to be solved for the development of more sustainable ionic liquids. Although Ionic liquids have created a great deal of interest as green reaction media, for regarding the hazard assessment of ionic liquids, this structural variability represents an almost insurmountable problem since it is impossible to generate a profound knowledge of the effects on man and the environment for every single compound. Although many studies were conducted to evaluate the (eco) toxicity of certain ionic liquids in *in vitro* assays and in some selected organism studies comprising, certain data, especially ecotoxicity data, could not be readily located. The lack of readily accessible ecotoxicity data is a big gap in our knowledge when considering the employment of ionic liquids on a pilot or manufacturing scale. Therefore, the design and synthesis of inherently biodegradable ionic liquids is still challenge for the development and application of ILs in chemical synthesis.

POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) constitute a class of hazardous organic chemicals comprising three or more fused benzene rings in linear, angular and cluster arrangements (Fig. 4). PAHs mostly are produced mainly by incomplete combustion of petroleum based on chemicals or organic matter through natural and anthropogenic activities (Alexander, 2000; Semple *et al.,* 2003; Kim *et al.,* 2008). PAHs enter the environment from a multiplicity of sources which include: the burning of fossil fuels, the use of wood preservatives such as creosote, direct aerial fallout, chronic leakage of industrial or sewage effluents, accidental discharges during the transport, use and disposal of petroleum products, or from natural sources such as oil seeps and surface water run-off from forest and prairie fire sites (Cerniglia 1992; NýChadhain *et al.,* 2006).

PAHs are hydrophobic chemicals which can readily associate with organic matter and/or clay fractions after entering the soil through natural processes, disposal and/or spillage (Papadopoulos *et al.,* 2007; Hilyard *et al.,* 2008; Semple *et al.,* 2001). More specifically, PAHs have the capacity to sorb onto and/or diffuse into the 3-dimensional structure of organic matter and mineral fractions; these processes are collectively known as sequestration and these interactions increase with increasing soil-PAH contact time (Papadopoulos *et al.,* 2007; Hilyard *et al.,* 2008; Semple *et al.,* 2001). In other words, these compounds persist in the environment and, due to their hydrophobicity, become associated with particulate matters that are deposited in soils and sediment. Evidently, these persistent PAHs, especially the higher molecular weight compounds, are toxic to living

organisms, and this toxicity is enhanced by their intrinsic chemical stability and resistance to many forms of degradation (N Chadhain *et al.,* 2006; Li *et al.,* 2001). Therefore, there is great interest in developing strategies to remove PAHs from contaminated sites. Many of these remediation strategies employ microorganisms which can degrade PAHs. Bioremediation of contaminated sites relies on either the activity of microorganisms already present at the site or the addition of selected microorganisms with desired catabolic traits in bioaugmentation techniques (N Chadhain *et al.,* 2006).

PAHs are widespread in nature due to both their natural production in the environment and input from anthropogenic activities (NýChadhain *et al.,* 2006; Johnsen *et al.,* 2002). The use of conventional remediation approaches, such as dredging and incineration, can be costly and may cause further damage to the environment by dispersing PAHs and making them more bioavailable (Hilyard *et al.,* 2008). Biodegradation of PAHs is a possible way to clean up polluted soils and water systems, for example, several types of bacteria with the ability to degrade PAHs, the nocardioform actinomycetes, including species of *Mycobacterium*, *Nocardioides*, and *Rhodococcus*, are of particular interest because of their marked ability to aerobically degrade high-molecular-weight (HMW) PAHs to ring isomeric dihydroxylated metabolites and in some cases to complete oxidation to carbon dioxide and water (Kim *et al.,* 2006; Sho *et al.,* 2004; Saito *et al.,* 2000; Dean-Ross *et al.,* 2001). Compared with incineration, storage, or soil washing, Biological treatments are cheaper (Eriksson *et al.,* 2003). However, degradation of PAHs *in situ* is often slow, and research over the last decades has shown that these compounds very often are persistent (Ný Chadhain *et al.,* 2006; Eriksson *et al.,* 2003; Kanaly & Harayama 2000). This persistence may be due to several factors such as nutrients, bioavailability of PAHs (sorption to particles), temperature, oxygen, and presence of PAH-degrading microorganisms. The water solubilities of most PAHs are in the lower parts-per-million range, and this is a major problem when studying and implementing aerobic degradation of PAHs. Therefore,

enhancement of biodegradation rate is the basis of bioremediation technologies. The use of surfactants may increase PAH solubility (Abalos *et al.,* 2004) but may also be toxic to microorganisms (Eriksson et al., 2003; Gonthier *et al.,* 2010). Another important factor in bioremediation of contaminated soils is the availability of nitrogen and phosphorus, which allows the necessary increase in the size of the hydrocarbon-degrading microbial populations. Taking into the fact that each contaminated site can respond in a different way to distinct parameters that affect microbial biodegradation, laboratory-scale bioremediation protocols have been developed in order to determine the effects of different conditions (Sabate *et al.,* 2004). However, most studies on PAH biodegradation have focused on microorganisms that can grow on common laboratory media at neutral pH (Uyttebroek *et al.,* 2007), data on the existence of bacteria able to degrade PAHs under acidic conditions are scarce, as introduced by Stapleton et al (1998), Dore et al (2003), and Springael et al (2007). On the other hand, the rate-limiting step in the microbial degradation of petroleum hydrocarbon pollutants in open systems, such as lakes, oceans, and wastelands, is generally an utilizable source of nitrogen (Koren *et al.,* 2003). Since petroleum contains only traces of nitrogen, the required nitrogen must come from the surrounding environment. Although the nitrogen requirement for optimum growth of hydrocarbon oxidizers can be readily satisfied with urea or salts that contain ammonium or nitrate ions in the laboratory, these nitrogen sources have a high water solubility, which reduces their effectiveness in open systems because of rapid dilution. Accordingly, there is practical challenge for microbial solution of the reoccurring problem of petroleum pollution in the sea. To overcome the N limitation for petroleum degradation in open systems, one approach has been the use of a water-insoluble polymer, based on a urea-formaldehyde formulation, which adheres to oil (Rosenberg *et al.,* 1996). Other approach is to use uric acid as a source of nitrogen to improve the bioremediation of petroleum pollutants in open systems, as described by Rosenberg et al (2003). Besides, in some Arctic and temperate regions, soil temperature remains below 10°C year-
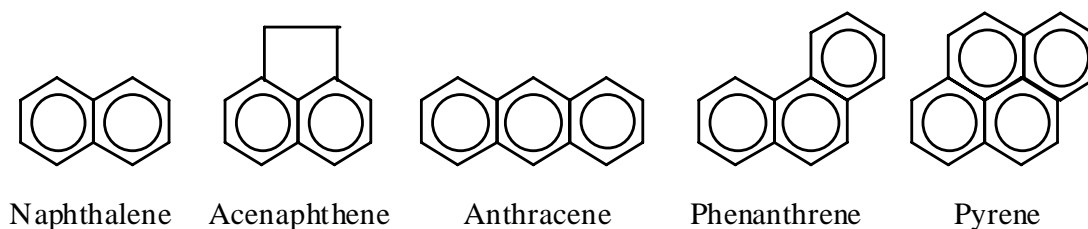

Naphthalene   Acenaphthene   Anthracene   Phenanthrene   Pyrene

Fig. 4. Chemical structures of polycyclic aromatic hydrocarbons(PAHs)

round, and wet conditions limit oxygen availability. The cost of increasing the temperature may be prohibitive, so it is desirable to optimize a treatment system for low temperature. In a word, it is necessary to evaluate the possibility of obtaining enrichment cultures capable of efficient PAH degradation at low temperature under aerobic or anaerobic conditions (Eriksson *et al.,* 2003; Aislabie *et al.,* 2000).

A wide variety of bacteria, such as naphthalene dioxygenase, fungi, algae, etc, have the ability to metabolize PAHs. Nevertheless, in almost all of these studies monitoring of the process is based on chemical analysis of contaminants. A better understanding of the diversity of the microbial communities inhabiting PAH-contaminated soils and their response to different biostimulation or bioaugmentation strategies could provide clues about the type of bacteria that are able to adapt to and exploit such habitats. It is well known that the majority of microbes in environmental samples cannot be cultured at present in laboratory media, which are biased for the growth of specific microorganisms (Abalos *et al.,* 2004; Orsvik *et al.,* 2002). In light of this, molecular biological techniques offer new opportunities. These techniques, combined with traditional laboratory enrichments, are often utilized to identify bacterial populations that are functionally important in the biodegradation of organic pollutants. For example, denaturing gradient gel electrophoresis (DGGE) allows us to directly determine the presence and relative levels of different 16S rRNA gene amplicons both qualitatively and semiquantitatively in order to perform a community analysis (Fromin *et al.,* 2002). Molecularbased techniques have also been used to follow the establishment of a soil-derived consortium capable of mineralizing benzo[a]pyrene during the biodegradation of a complex hydrocarbon mixture (Kanaly *et al.,* 2000). While these studies suggest that diverse microorganisms are present in polluted environments, a greater understanding of the diversity, ecology, and biochemistry of these PAH-degrading microorganisms is necessary in order to effectively remediate contaminated environments.

OTHER CONTAMINATES

Besides some important contaminates, other contaminates were also be investigated such as Fluoroacrylate Polymer (Russell *et al.,* 2008), Bisphenols (Inoue *et al.,* 2008), Benzothiazoles (Bunescu *et al.,* 2008), etc. Among them, polychlorinated biphenyls (PCBs), as a class of the most important environmental contaminants, are organic chemicals belonging to a class of hydrocarbons, are allowed to persist in the environment and bioaccumulate due to their physicochemical properties (Quensen *et al.,* 1988; Parnell *et al.,* 2006). PCBs accumulate in lipid-rich tissues of all organisms, including humans, where they alter immune functions, developmental, respiratory, and reproductive problems as well as cancer due to estrogenic activity (Demers *et al.,* 2002; Smithwick *et al.,* 2003; Parnell *et al.,* 2006). These health concerns prioritize PCBs as major targets for environmental cleanup. The bioremediation strategy with the highest potential for destruction of PCB mixtures is a sequential anaerobic-aerobic process (Seto *et al.,* 1996; Rodrigues *et al.,* 2006; Parnell *et al.,* 2006). Recently a sequential anaerobic-aerobic process was shown at the laboratory scale to remove over 50% of Aroclor 1242 from river sediments (Rodrigues *et al.,* 2006). Two of the most effective aerobic PCB-degrading bacteria, Burkholderia xenovorans strain LB400 and Rhodococcus sp. strain RHA1, vary greatly in their levels of PCB toxicity resistance (Seto *et al.,* 1996), yet the sources of this difference are unknown. Indeed recent studies of PCB-tolerant LB400 (Denef *et al.,* 2005; Denef *et al.,* 2006) suggest that cell systems other than the biphenyl pathway contribute to the efficient degradation of biphenyl and PCBs. As PCBs have been insinuated to impair microbial growth, viability, and degradation (Kim *et al.,* 2001; Vaillancourt *et al.,* 2002; Camara *et al.,* 2004), successful remediation should be developed to limit or overcome these toxic effects (Hrywna *et al.,* 1999; Cjhebrou *et al.,* 1999; Parnell *et al.,* 2008). Another important microbial degradation is organophosphate pesticides due to the high mammalian toxicity of such compounds and their widespread usability. For some organophosphates such as parathion, it has been relatively easy to isolate degrading bacteria (Singh *et al.,* 2004). In addition, Phthalate esters are synthetic compounds used predominantly as additives in plastic to improve the mechanical properties of plastic resin, particularly its flexibility. In order to provide the required flexibility, phthalate ester plasticizers are not bound covalently to plastic resins and are able to migrate into the environment (Ganning *et al.,* 1984; Hara *et al.,* 2007). Decades of global industrial use as plasticizers, phthalate esters are now recognized as ubiquitous environmental pollutants detected in every environment, in which they have been sought, with the highest concentrations detected adjacent to phthalate ester production or processing facilities (Staples *et al.,* 2000). There are now serious concerns about the impacts of these compounds on human health and the environment due to wide-ranging adverse effects on animal cells (Nakai *et al.,* 1999; Hara *et al.,* 2007). Phthalate esters are generally considered biodegradable. However, some studies indicated that these biodegradation processes was extremely limited and largely based on observations and experiments

with undefined biological systems (Gu *et al.,* 2005; Chang *et al.,* 2005). Therefore, further works should be undertaken to investigate mechanisms for biodegradation of the PTH.

CONCLUSION

Although the ultimate goal of biological remediation is conversion of toxic organic contaminants to simple, less-toxic constituents, this approach does not consider that end products or intermediates produced during remediation may be toxic. Furthermore, the potential exists that remediation may result in products for which the toxic response is greater over the parent compound or which the target of toxicity is different, and these possibilities would not be detected. It is, therefore, important that further processes of detection and monitoring of the contaminants be developed to determine whether the toxic pollutants have been totally mineralized or at least degraded to their stable intermediates that pose no threat to the environment. Similarly, chemical remediation may lead to products with increased biological activity. As a result, from the standpoint of assessing risk, it is necessary to understand the biological activity or toxicity of the end products and stable intermediates. That is to say, the products or intermediates of bioremediation should be less toxic than the starting materials. However, there is a dearth of evidence to support this assumption, some evidence even suggests that products formed during remediation or breakdown of environmental chemicals have greater biological activity than the starting materials. These emphasize the need for investigators to consider the biological activity or toxicity of the end products and stable intermediates during biological remediation.

REFERENCES

Abalos, A., Vinas, M., Sabate, J., Manresa, M. A. and Solanas, A. M. (2004) Enhanced Biodegradation of Casablanca Crude Oil by A Microbial Consortium in Presence of a Rhamnolipid Produced by Pseudomonas Aeruginosa AT10. Biodegradation **15**, 249-260.

Abraham, E., Caballero, A. and Ramos, J. L. (2001) Biological Degradation of 2,4,6-Trinitrotoluene. Microbiology and Molocular Biology Reviews, **65**, 335-352.

Adam, D. (2000). Clean and green. .but are they mean? , Nature, **407**, 938-940.

Aislabie, J., Foght, J. and Saul, D. (2000) Aromatic hydrocarbon-degrading bacteria from soil near Scott Base, Antarctica. Polar Biology, **23**, 183-188.

Alexander, M. (2000) Aging, Bioavailability, and Overestimation of Risk from Environmental Pollutants. Environmental Science & Technology, **34**, 4259-4265.

Arenghi, F. L., Berlanda, D., Galli, E., Sello, G. and Barbieri, P. (2001) Organization and Regulation of meta Cleavage Pathway Genes for Toluene and o-Xylene Derivative Degradation in Pseudomonas stutzeri OX1. Applied and Environmental Microbiology, **67**, 3304-3308.

Bertoni, G., Bolognesi, F., Galli, E. and Barbieri, P. (1996) Cloning of the genes for and characterization of the early stages of toluene and o-xylene catabolism in Pseudomonas stutzeri OX1. Applied and Environmental Microbiology, **62**, 3704-3711.

Bertoni, G., Martino, M., Galli, E. and Barbieri, P. (1998) Analysis of the Gene Cluster Encoding Toluene/o-Xylene Monooxygenase from Pseudomonas stutzeri OX. Applied and Environmental Microbiology, **64**, 3626-3632.

Bouquillon, S., Courant, T., Dean, D., Gathergood, N., Morrissey, S., Pegot, B., Scammells, P. J. and Singer, R. D. (2007) Biodegradable Ionic Liquids: Selected Synthetic Applications. Australian journal of chemistry, **60**, 843-847.

Boyd, D. R. and Sheldrake, G. N. (1998) The dioxygenase-catalysed formation of vicinal cis-diols. Natural Product Reports, **15**, 309-324.

Boyd, D. R., Sharma, N. D., Odowd, C. R., Carroll, J. G., Loke, P. L. and Allen, C. C. R. (2005) cis-Dihydrodiol, arene oxide and phenol metabolites of dictamnine: key intermediates in the biodegradation and biosynthesis of furoquinoline alkaloids. Chemical Communications, 3989-3991.

Bunescu, A., Besse-Hoggan, P., Sancelme, M., Mailhot, G. and Delort, A. M. (2008) Comparison of Microbial and Photochemical Processes and Their Combination for Degradation of 2-Aminobenzothiazole. Applied and Environmental Microbiology. **74**, 2976-2984.

Cao, S. S, Chen, J. R, Hu, J. (2009). The fabrication and progress of core-shell composite materials. Australian journal of chemistry, **62**, 1561-1576

Cafaro, V., Notomista, E., Capasso, P. and Donato, A. D. (2005) Regiospecificity of Two Multicomponent Monooxygenases from Pseudomonas stutzeri OX1: Molecular Basis for Catabolic Adaptation of This Microorganism to Methylated Aromatic Compounds. Applied and Environmental Microbiology, **71**, 4736-4743.

Cafaro, V., Izzo, V., Scognamiglio, R., Notomista, E., Capasso, P., Casbarra, A., Pucci, P. and Donato, A. D. (2004) Phenol Hydroxylase and Toluene/o-Xylene Monooxygenase from Pseudomonas stutzeri OX1: Interplay between Two Enzymes. Applied and Environmental Microbiology, **70**, 2211-2219.

Cafaro, V., Scognamiglio, R., Viggiani, A., Izzo, V., Passaro, I., Notomista, E., Dal Piaz, F., Amoresano, A., Casbarra, A., Pucci, P. and Donato, A. D. (2002) Expression and purification of the recombinant subunits of toluene/o-xylene monooxygenase and reconstitution of the active complex. European Journal of Biochemistry, **269**, 5689-5699.

Camara, B., Herrera, C., Gonzalez, M., Couve, E., Hofer, B. and Seeger, M. (2004) From PCBs to highly toxic metabolites by the biphenyl pathway. Environmental Microbiology, **6**, 842-850.

Cerniglia, C. E. (1992) Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation, **3**, 351-368.

Chang, B. V., Liao, C. S. and Yuan, S. Y. (2005) Anaerobic degradation of diethyl phthalate, di-n-butyl phthalate, and di-(2-ethylhexyl) phthalate from river sediment in Taiwan. Chemosphere, **58**, 1601-1607.

Cherian, E. and Jayachandran, K. (2009) Microbial Degradation of Natural Rubber Latex by a novel Species of Bacillus sp. SBS[25] isolated from Soil. International journal of environmental research. **3**, 599-604.

Cjhebrou, H., Hurtubise, Y., Barriault, D. and Sylvestre, M. (1999) Heterologous Expression and Characterization of the Purified Oxygenase Component of Rhodococcus globerulus P6 Biphenyl Dioxygenase and of Chimeras Derived from It. Journal of Bacteriology, **181**. 4805-4811.

Dean-Ross, D., Moody, J. D., Freeman, J. P., Doerge, D. R. and Cerniglia, C. E. (2001) Metabolism of anthracene by a Rhodococcus species. FEMS. Microbiology Letters, **204**, 205-211.

Demers, A., Ayotte, P., Brisson, J., Dodin, S., Robert, J. and Dewailly,E.(2002) Plasma Concentrations of Polychlorinated Biphenyls and the Risk of Breast Cancer: A Congener-specific Analysis. American Journal of Epidemiology, **155**, 629-635.

Denef, V. J., Patrauchan, M. A., Florizone, C., Park, J., Tsoi, T. V., Verstraete, W., Tiedje, J. M. and Eltis, L. D. (2005) Growth Substrate- and Phase-Specific Expression of Biphenyl, Benzoate, and C_1 Metabolic Pathways in Burkholderia xenovorans LB400. Journal of Bacteriology, **187**, 7996-8005.

Denef, V. J., Klappenbach, J. A., Patrauchan, M. A., Florizone, C., Rodrigues, J. L. M., Tsoi, T. V., Verstraete, W., Eltis, L. D. and Tiedje, J. M. (2006) Genetic and Genomic Insights into the Role of Benzoate-Catabolic Pathway Redundancy in Burkholderia xenovorans LB400. Applied and Environmental Microbiology, **72**, 585-595.

Devic, E., Li, D., Dauta, A., Henriksen, P., Codd, G. A., Marty, J. and Fournier, D. (2002). Detection of Anatoxin-a(s) in Environmental Samples of Cyanobacteria by Using a Biosensor with Engineered Acetylcholinesterases. Applied and Environmental Microbiology, **68**, 4102-4106.

Diaz, E., Ferrandez, A., Prieto, M. A. and Garcia, J. (2001) Biodegradation of Aromatic Compounds by Escherichia coli. Microbiology and Molocular Biology Reviews, **65**, 523-569.

Dore, S. Y., Clancy, Q. E., Rylee, S. M. and Kulpa, C. F. (2003). Naphthalene-utilizing and mercury-resistant bacteria isolated from an acidic environment. Applied Microbiology and Biotechnology, **63**, 194-199.

Eriksson, M., Sodersten, E., Yu, Z., Dalhammar, G. and Mohn, W. W. (2003) Degradation of Polycyclic Aromatic Hydrocarbons at Low Temperature under Aerobic and Nitrate-Reducing Conditions in Enrichment Cultures from Northern Soils. Applied and Environmental Microbiology, **69**, 275-284.

Fellay, C. (2007). Biphasic Catalysis in Aqueous and Ionic Liquid Media, CHIMIA International Journal for Chemistry, **61**, 172-174.

Fetzner, S. and Meer, J. R. (2000) Enzymes Involved in the Aerobic Bacterial Degradation of N -Heteroaromatic Compounds: Molybdenum Hydroxylases and Ring-Opening 2,4-Dioxygenases. Naturwissenschaften, **87**, 59-69.

Fournier, D., Halasz, A., Spain, J., Spanggord, R. J., Bottaro, J. C. and Hawari, J. (2004). Biodegradation of the Hexahydro-1,3,5-Trinitro-1,3,5-Triazine Ring Cleavage Product 4-Nitro-2,4-Diazabutanal by Phanerochaete chrysosporium. Applied and Environmental Microbiology, **70**, 1123-1128.

Fromin, N., Hamelin, J., Tarnawski, S., Roesti, D., Jourdain-Miserez, K., Forestier, N., Teyssier-Cuvelle, S., Gillet, F., Aragno, M. and Rossi, P. (2002) Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns. Environmental Microbiology, **4**, 634-643.

Ganning, A. E., Brunk, U. and Dallner, G. (1984) Phthalate Esters and Their Effect on the Liver. Hepatology **4**, 541-547.

Gathergood, N. and Scammells, P. J. (2002) Design and preparation of room-temperature ionic liuids containing biodegradable side chains, Australian Journal of Chemistry, **55**, 557-560.

Gathergood, N., Garcia, M. T. and Scammells, P. J. (2004) Biodegradable ionic liquids:Part I. Concept, preliminary targets and evaluation, Green Chemistry, **6**, 166-175.

Gathergood, N., Scammells, P. J. and Garcia, M. T. (2006) Biodegradable ionic liquids. Part III. The first readily biodegradable ionic liquids. Green Chemistry, **8**, 156-160.

Gibson, D. T, and Parales, R. E. (2000) Aromatic hydrocarbon dioxygenases in environmental biotechnology. Curent Opinion in Biotechnology, **11**, 236-243.

Gonthier, P., Nicolotti, G Rettori, A., Paoletti, E. and Gullino, M. L. (2010) Testing Nerium Oleander as a Biomonitor for Surfactant Polluted Marine Aerosol. International journal of environmental research, **4**, 1-10.

Gu, J. D., Li, J. and Wang, Y. (2005) Biochemical pathway and degradation of phthalate ester isomers by bacteria. Water Science Technology. **52**, 241-248.

Haddock, J.D., Nadim, L.M. and Gibson, D.T. (1993) Oxidation of biphenyl by a multicomponent enzyme system from Pseudomonas sp. strain LB400. Journal of Bacteriology. **175**, 395-400.

Haigler, B. E., Nishino, S. F. and Spain, J. C. (1994) Biodegradation of 4-methyl-5-nitrocatechol by Pseudomonas sp. strain DNT. Journal of Bacteriology. **176**, 3433-3437.

Haigler, B., Suen, W. N, and Spain, J. C. (1996) Purification and sequence analysis of 4-methyl-5-nitrocatechol oxygenase from Burkholderia sp. strain DNT. Journal of Bacteriology, **178**, 6019-6024.

Haigler, B. E., Johnson, G. R., Suen, W. and Spain, J. C. (1999) Biochemical and Genetic Evidence for meta-Ring

Cleavage of 2,4,5-Trihydroxytoluene in Burkholderia sp. Strain DNT. Journal of Bacteriology, **181**, 965-972.

Harayama, S., Kok, M. and Neidle, E. L. (1992) Functional and Evolutionary Relationships Among Diverse Oxygenases. Annual Reviews of Microbilogy, **46**, 565-601.

Hale, R. C., Guardia, M. J. L., Harvey, E. P., Gaylor, M. O., Mainor, T. M. and Duff, W. H. (2001) Flame retardants: Persistent pollutants in land-applied sludges. Nature, **412**, 140-141.

Hara, H., Eltis, L. D., Davies, J. E. and Mohn, W. W. (2007) Transcriptomic Analysis Reveals a Bifurcated Terephthalate Degradation Pathway in Rhodococcus sp. Strain RHA1. Journal of Bacteriology, **189**, 1641-1647.

Harjani, J. R., Singer, R. D., Garcia, M. T. and Scammells, P. J. (2008) The design and synthesis of biodegradable pyridinium ionic liquids. Green Chemistry, **10**, 436-438.

Harwood, C. S. and Parales, R. E. (1996) The β-ketoadipate pathway and the biology of self-identity. Annual Reviews of Microbilogy, 50, 553-590.

Hasegawa, Y., Muraki, T., Tokuyama, T., Iwaki, H., Tatsuno, M. and Lau, P. C. K. (2000) A novel degradative pathway of 2-nitrobenzoate via 3-hydroxyanthranilate in Pseudomonas fluorescens strain KU-7. FEMS. Microbiology Letters, **190**, 185-190.

He, Z. and Spain, J. C. (2000) Reactions Involved in the Lower Pathway for Degradation of 4-Nitrotoluene by Mycobacterium Strain HL 4-NT-1. Applied and Environmental Microbiology, **66**, 3010-3015.

He, Z., Nadeau, L. J. and Spain, J. C. (2000) Characterization of hydroxylaminobenzene mutase from pNBZ139 cloned from Pseudomonas pseudoalcaligenes JS45. European Journal of Biochemistry, **267**, 1110-1116.

Herrmann, H., Muller, C., Schmidt, I., Mahnke, J., Petruschka, L. and Hahnke, K. (1995) Localization and organization of phenol degradation genes ofPseudomonas putida strain H. Molcular and General Genettics MGG., **247**, 240-246.

Hilyard, E. J., Jones-Meehan, J. M., Spargo, B. J. and Hill, R. T. (2008) Enrichment, Isolation, and Phylogenetic Identification of Polycyclic Aromatic Hydrocarbon-Degrading Bacteria from Elizabeth River Sediments. Applied and Environmental Microbiology, **74**, 1176-1182.

Hofstetter, T. B., Spain, J. C., Nishino, S. F., Bolotin, J. and Schwarzenbach, R. P. (2008) Identifying Competing Aerobic Nitrobenzene Biodegradation Pathways by Compound-Specific Isotope Analysis. Environmental Science & Technology. **42**, 4764-4770.

Hrywna, Y., Tsoi, T. V., Maltseva, O. V., Quensen, J. F. and Tiedje, J. M. (1999) Construction and Characterization of Two Recombinant Bacteria That Grow on ortho- and para-Substituted Chlorobiphenyls. Applied and Environmental Microbiology, **65**. 2163-2169.

Hudlicky, T., Gonzalez, D. and Gibson, D. T. (1999). Enzymatic dihydroxylation of aromatics in enantioselective synthesis: expanding asymmetric methodology. Aldrichimica

Acta, **32 (2)**, 35-62.

Inoue, D., Hara, S., Kashihara, M., Murai, Y., Danzl, E., Sei, K., Tsunoi, S., Fujita, M. and Ike, M. (2008). Degradation of Bis(4-Hydroxyphenyl)Methane (Bisphenol F) by Sphingobium yanoikuyae Strain FM-2 Isolated from River Water. Applied and Environmental Microbiology, **74**. 352-358.

Jastorff, B., Molter, K., Behrend, P., Bottin-Weber, U., Filser, J., Heimers, A., Ondruschka, B., Ranke, J., Schaefer, M., Schro der, H., Stark, A., Stepnowski,P., Stock, F., Stormann, R., Stolte, S., Welz-Biermann, U., Ziegert, S. and Thoming, J. (2005). Progress in evaluation of risk potential of ionic liquids—basis for an eco-design of sustainable products, Green Chemistry, **7**, 362-372.

Johnson, G. R. and Olsen, R. H. (1995) Nucleotide sequence analysis of genes encoding a toluene/benzene-2-monooxygenase from Pseudomonas sp. strain JS150. Applied and Environmental Microbiology, **61**, 3336-3346.

Johnson, G. R., Jain, R. K. and Spain, J. C. (2000) Properties of the trihydroxytoluene oxygenase from Burkholderia cepacia R34: an extradiol dioxygenase from the 2,4-dinitrotoluene pathway. Archives of Microbiology, **173**, 86-90.

Johnsen, A. R., Bendixen, K. and Karlson, U. (2002) Detection of Microbial Growth on Polycyclic Aromatic Hydrocarbons in Microtiter Plates by Using the Respiration Indicator WST-1. Applied and Environmental Microbiology, **68**, 2683-2689.

Johnson, G. R., Jain, R. K. and Spain, J. C. (2002) Origins of the 2,4-Dinitrotoluene Pathway. Journal of Bacteriology, **184**, 4219-4232.

Kahng, H. Y., Malinverni, J. C., Majko, M. M. and Kukor, J. J. (2001) Genetic and Functional Analysis of the tbc Operons for Catabolism of Alkyl- and Chloroaromatic Compounds in Burkholderia sp. Strain JS150. Applied and Environmental Microbiology, **67**, 4805-4816.

Kanaly, R. A. and Harayama, S. (2000) Biodegradation of High-Molecular-Weight Polycyclic Aromatic Hydrocarbons by Bacteria. Journal of Bacteriology, **182**, 2059-2067.

Kanaly, R. A., Bartha, R., Watanabe, K. and Harayama, S. (2000) Rapid Mineralization of Benzo[a]pyrene by a Microbial Consortium Growing on Diesel Fuel. Applied and Environmental Microbiology, **66**, 4205-4211.

Khler, A., Hellweg, S., Escher, B. I. and Hungerbhler, K. (2006) Organic Pollutant Removal versus Toxicity Reduction in Industrial Wastewater Treatment: The Example of Wastewater from Fluorescent Whitening Agent Production. Environmental Science & Technology, **40**, 3395-3401.

Kim, I. S., Lee, H. and Trevors, J. T. (2001) Effects of 2,22 ,5,52 -tetrachlorobiphenyl and biphenyl on cell membranes of Ralstonia eutropha H850. FEMS. Microbiology Letters, **200**, 17-24.

Kim, S., Kweon, O., Freeman, J. P., Jones, R. C., Adjei, M. D., Jhoo, J., Edmondson, R. D. and Cerniglia, C. E. (2006) Molecular Cloning and Expression of Genes Encoding a Novel Dioxygenase Involved in Low- and High-Molecular-Weight

Polycyclic Aromatic Hydrocarbon Degradation in Mycobacterium vanbaalenii PYR-1. Applied and Environmental Microbiology, **72**, 1045-1054.

Kim, J. M., Le, N. T., Chung, B. S., Park, J. H., Bae, J. W., Madsen, E. L. and Jeon, C. O. (2008) Influence of Soil Components on the Biodegradation of Benzene, Toluene, Ethylbenzene, and o-, m-, and p-Xylenes by the Newly Isolated Bacterium Pseudoxanthomonas spadix BD-a59. Applied and Environmental Microbiology, **74**, 7313-7320.

Koren, O., Knezevic, V., Ron, E. Z. and Rosenberg, E. (2003). Petroleum Pollution Bioremediation Using Water-Insoluble Uric Acid as the Nitrogen Source. Applied and Environmental Microbiology, **69**, 6337-6339.

Kulkarni, M. and Chaudhari, A. (2007). Microbial remediation of nitro-aromatic compounds: An overview. Journal of Environmental Management, **85**, 496-512.

Lau, P. C. K. and De Lorenzo, V. (1999) Genetic engineering: the frontier of bioremediation. Environmental Science & Technology, **4**, 124A-128A.

Lessner, D. J., Parales, R. E., Narayan, S. and Gibson, D. T. (2003). Expression of the Nitroarene Dioxygenase Genes in Comamonas sp. Strain JS765 and Acidovorax sp. Strain JS42 Is Induced by Multiple Aromatic Compounds. Journal of Bacteriology, **185**, 3895-3904.

Leung, K. T., Tresse, O., Errampalli, D., Lee, H. and Trevors, J. T. (1997). Mineralization of p-nitrophenol by pentachlorophenol-degrading Sphingomonas spp.. FEMS. Microbiology Letters. **155**, 107-114.

Li, Q, Ogawa, J, Schmid, R, and Shimizu. D. S. (2001). Engineering Cytochrome P450 BM-3 for Oxidation of Polycyclic Aromatic Hydrocarbons. Applied and Environmental Microbiology, **67**, 5735-5739.

Mason, J. R. and Cammack, R. (1992). The Electron-Transport Proteins of Hydroxylating Bacterial Dioxygenases. Annual Reviews of Microbilogy, **46**, 277-305.

Matzke, M., Stolte, S., Thiele, K., Juffernholz, T., Ranke, J., Welz-Biermann, U. and Jastorff, B. (2007). The influence of anion species on the toxicity of 1-alkyl-3-methylimidazolium ionic liquids observed in an (eco)toxicological test battery. Green Chemistry, **9**,1198-1207.

Meulenberg, R., Pepi, M. and de Bont, J. A. (1996) Degradation of 3-nitrophenol by Pseudomonas putida B2 occurs via 1,2,4-benzenetriol. Biodegradation, **7**, 303-311.

Munnecke, D. M. and Hsieh, D. P. H. (1974). Microbial Decontamination of Parathion and p-Nitrophenol in Aqueous Media. Applied and Environmental Microbiology, **28**, 212-217.

Nadeau, L. J., He, Z. and Spain, J. C. (2003). Bacterial Conversion of Hydroxylamino Aromatic Compounds by both Lyase and Mutase Enzymes Involves Intramolecular Transfer of Hydroxyl Groups. Applied and Environmental Microbiology, **69**, 2786-2793.

Nakai, M., Tabira, Y., Asai, D., Yakabe, Y., Shimyozu, T., Noguchi, M., Takatsuki, M. and Shimohigashi, Y. (1999). Binding Characteristics of Dialkyl Phthalates for the Estrogen Receptor. Biochemical and Biophysical Research Communications, **254**, 311-314.

Nýchadhain, S. M., Norman, R. S., Pesce, K. V., Kukor, J. J. and Zylstra, G. J. (2006). Microbial Dioxygenase Gene Population Shifts during Polycyclic Aromatic Hydrocarbon Biodegradation. Applied and Environmental Microbiology, **72**, 4078-4087.

Nishino, S., Paoli, G. C. and Spain, J. C. (2000). Aerobic Degradation of Dinitrotoluenes and Pathway for Bacterial Degradation of 2,6-Dinitrotoluene. Applied and Environmental Microbiology, **66**, 2139-2147.

Nishino, S. F. and Spain, J. C. (2004). Catabolism of nitroaromatic compounds, In J.-L. Ramos (ed.), Pseudomonas, vol. 3. Kluwer Academic/Plenum Publishers, New York, N.Y. p. 575-608.

Nishino, S. F. and Spain, J. C. (2006). Biodegradation of 3-Nitrotyrosine by Burkholderia sp. Strain JS165 and Variovorax paradoxus JS171. Applied and Environmental Microbiology, **72**, 1040-1044.

Notomista, E., Lahm, A., Donato, A. D. and Tramontano, A. (2003). Evolution of Bacterial and Archaeal Multicomponent Monooxygenases. Journal of Molecular Evolution, **56**, 435-445.

Olaniran, A. O., Motebejane, R. M. and Pillay, B. (2008). Bacterial biosensors for rapid and effective monitoring of biodegradation of organic pollutants in wastewater effluents. Journal of Environmental Monitoring, **10**, 889-893.

Olsen, R. H., Kukor, J. J. and Kaphammer, B. (1994). A novel toluene-3-monooxygenase pathway cloned from Pseudomonas pickettii PKO1. Journal of Bacteriology, **176**, 3749-3756.

Orsvik, V., Ovreas, L. and Thingstad, T. F. (2002). Prokaryotic Diversity—Magnitude, Dynamics, and Controlling Factors. Science, **296**, 1064-1066.

Quensen, J. F., Tiedje, J. M. and Boyd, S. A. (1988) Reductive Dechlorination of Polychlorinated Biphenyls by Anaerobic Microorganisms from Sediments. Science, **242**, 752-754.

Papadopoulos, A., Paton, G. I., Reidc, B. J. and Semple, K. T. (2007). Prediction of PAH biodegradation in field contaminated soils using a cyclodextrin extraction technique. Journal of Environmental Monitoring, **9,** 516-522.

Parales, J. V., Kumar, A., Parales, R. E. and Gibson, D. T. (1996). Cloning and sequencing of the genes encoding 2-nitrotoluene dioxygenase from Pseudomonas sp. JS42. Gene., **181**, 57-61.

Parales, R. E., Parales, J. V. and Gibson, D. T. (1999). Aspartate 205 in the Catalytic Domain of Naphthalene Dioxygenase Is Essential for Activity. Journal of Bacteriology, **181**. 1831-1837.

Park, H., Lim, S., Chang, Y. K., Livingston, A. G. and Kim, H. (1999). Degradation of Chloronitrobenzenes by a

Coculture of Pseudomonas putida and a Rhodococcus sp. Applied and Environmental Microbiology, **65**, 1083-1091.

Parnell, J. J., Park, J., Denef, V., Tsoi, T., Hashsham, Quensen III, S., J. and Tiedje, J. M. (2006).Coping with Polychlorinated Biphenyl (PCB) Toxicity: Physiological and Genome-Wide Responses of Burkholderia xenovorans LB400 to PCB-Mediated Stress. Applied and Environmental Microbiology, **72**, 6607-6614.

Prakash, D., Chauhan, A. and Jain, R. K. (1996). Plasmid-Encoded Degradation ofp-Nitrophenol byPseudomonas cepacia, Biochemical and Biophysical Research Communications, **224**, 375-381.

Ranke, J., Stolte, S., Sto¨rmann, R., Arning, J. and Jastorff, B. (2007). Design of Sustainable Chemical Products-The Example of Ionic Liquids, Chemical Review, **107**, 2183-2206.

Rodrigues, J. L. M., Kachel, C. A., Aiello, M. R., Quensen, J. F., Maltseva, O. V., Tsoi, T. V. and Tiedje, J. M. (2006). Degradation of Aroclor 1242 Dechlorination Products in Sediments by Burkholderia xenovorans LB400(ohb) and Rhodococcus sp. Strain RHA1(fcb). Applied and Environmental Microbiology, **72**, 2476-2482.

Roslev, P., Madsen, P. L., Thyme, J. B. and Henriksen, K. (1998).Degradation of Phthalate and Di-(2-Ethylhexyl)phthalate by Indigenous and Inoculated Microorganisms in Sludge-Amended Soil. Applied and Environmental Microbiology, **64**, 4711-4719.

Rosenberg, E., Legmann, R., Kushmaro, A., Adler, E., Abir, H. and Ron, E. Z. (1996). Oil bioremediation using insoluble nitrogen source. Journal of Biotechnology, **51**, 273-278.

Russell, M. H., Berti, W. R., Szostek, B. and Buck, R. C. (2008). Investigation of the Biodegradation Potential of a Fluoroacrylate Polymer Product in Aerobic Soils. Environmental Science & Technology, **42**, 800-807.

Sabate, J., Vinas, M. and Solanas, A. M. (2004). Laboratory-scale bioremediation experiments on hydrocarbon-contaminated soils. International Biodeterioration & Biodegradation, **54**, 19-25.

Saito, A., Iwabuchi, T. and Harayama, S. (2000). A Novel Phenanthrene Dioxygenase from Nocardioides sp. Strain KP7: Expression in Escherichia coli. Journal of Bacteriology, **182**, 2134-2141.

Schenzle, A., Lenke, H., Spain, J. C. and Knackmuss, H. J. (1999). Chemoselective Nitro Group Reduction and Reductive Dechlorination Initiate Degradation of 2-Chloro-5-Nitrophenol by Ralstonia eutropha JMP134. Applied and Environmental Microbiology, **65**, 2317-2323.

Seddon, K. R. (2003) Ionic liquids: A taste of the future. Nature Materials, **2**, 363-365.

Semple, K. T., Reid, B. J. and Fermor, T. R. (2001). Impact of composting strategies on the treatment of soils contaminated with organic pollutants, Environmental Pollution, **112**, 269-283.

Semple, K. T., Morriss, A. W. J. and Paton, G. I. (2003). Bioavailability of hydrophobic organic contaminants in soils:

fundamental concepts and techniques for analysis, European Journal of Soil Science, **54**, 809-818.

Seto, M., Okita, N., Sugiyama, K., Masai, E. and Fukuda, M. (1996). Growth inhibition of Rhodococcus sp. strain RHA1 in the course of PCB transformation. Biotechnology Letters, **18**, 1193-1198.

Shields, M. S., Montgomery, S. O., Cuskey, S. M., Chapman, P. J. and Pritchard, P. H. (1995). TOM, a new aromatic degradative plasmid from Burkholderia (Pseudomonas) cepacia G4. Applied and Environmental Microbiology, **61**, 1352-1356.

Sho, M., Hamel, C. and Greer, C. W. (2004). Two distinct gene clusters encode pyrene degradation in Mycobacterium sp. strain S65. FEMS Microbiology Econogy, **48**, 209-220.

Singh, B. K., Walker, A., Morgan, J. A. W. and Wright, D. J. (2004). Biodegradation of Chlorpyrifos by Enterobacter Strain B-14 and Its Use in Bioremediation of Contaminated Soils. Applied and Environmental Microbiology, **70**, 4855-4863.

Spiess, T., Desiere, F., Fischer, P., Spain, J. C., Knackmuss, H. J. and Lenke, H. (1998). A New 4-Nitrotoluene Degradation Pathway in a Mycobacterium Strain. Applied and Environmental Microbiology, **64**, 446-452.

Shin, K. A. and Spain, J. C. (2009) Pathway and Evolutionary Implications of Diphenylamine Biodegradation by Burkholderia sp. Strain JS667. Applied and Environmental Microbiology. **75**, 2694-2704.

Simpson, J. R. and Evans, W. C. (1953). Biochemical. Journal, **55**, XXIV.

Silvester, D. S. and Compton, R. G. Z. (2006). Electrochemistry in Room Temperature Ionic Liquids: A Review and Some Possible Applications. Phys. Chem., **220**, 1247-1274.

Smithwick, L. A., Smith, A., Quensen, J. F., Stack, A., London, L. and Morris, P. J. (2003). Inhibition of LPS-induced splenocyte proliferation by ortho-substituted polychlorinated biphenyl congeners. Toxicology, **188**, 319-333.

Spain, J. C., Wyss, 0. and Gibson, D. T. (1979). Enzymatic oxidation of p-nitrophenol. Biochemical and Biophysical Research Communications, **88**, 634-641.

Spain, J. C. and Gibson, D. T. (1991). Pathway for Biodegradation of p-Nitrophenol in a Moraxella sp. Applied and Environmental Microbiology, **57**, 812-819.

Spain, J. C. (1995). Biodegradation of Nitroaromatic Compounds. Annual Reviews of Microbilogy, **49**, 523-555.

Stapleton, R. D., Savage, D. C., Sayler, G. S. and Stacey, G. (1998). Biodegradation of Aromatic Hydrocarbons in an Extremely Acidic Environment. Applied and Environmental Microbiology, **64**, 4180-4184.

Staples, C. A., Parkerton, T. F. and Peterson, D. R. (2000). A risk assessment of selected phthalate esters in North American and Western European surface waters. Chemosphere, **40**, 885-891.

Stepnowski, P. and Storoniak, P. (2005). Lipophilicity and

Metabolic Route Prediction of Imidazolium Ionic Liquids. Environmental Science and Pollution Research, **12**, 199-204.

Stokes, J. D., Paton, G. I. and Semple, K. T. (2006). Behaviour and assessment of bioavailability of organic contaminants in soil: relevance for risk assessment and remediation. Soil. Use. and Managent. **21**, 475-486.

Stolte, S., Arning, J., Bottin-Weber, U., Matzke, M., Stock, F., Thiele, K., Uerdingen, M., Welz-Biermann, U., Jastorff, B. and Ranke, J. (2006). Anion effects on the cytotoxicity of ionic liquids. Green Chemistry, **8**, 621-629.

Stolte, S., Arning, J., Bottin-Weber, U., Muller, A., Pitner, W. R., Welz-Biermann, U., Jastorff, B. and Ranke, J. (2007). Effects of different head groups and functionalised side chains on the cytotoxicity of ionic liquids. Green Chemistry, **9,** 760-767.

Stolte, S., Matzke, M., Arning, J., Boschen, A., Pitner, W. R., Welz-Biermann, U., Jastorff, B. and Ranke, J. (2007). Effects of different head groups and functionalised side chains on the aquatic toxicity of ionic liquids. Green Chemistry, **9**, 1170-1179.

Stolte, S., Abdulkarim, S., Arning, J., Nienstedt, A. B., Weber, U. B., Matzke, M., Ranke, J., Jastorff, B. and Thoming, J. (2008). Primary biodegradation of ionic liquid cations, identification of degradation products of 1-methyl-3-octylimidazolium chloride and electrochemical wastewater treatment of poorly biodegradable compounds. Green Chemistry, **10**, 214-224.

Suen, W., Haigler, B. and Spain, J. C. (1996). 2,4-Dinitrotoluene dioxygenase from Burkholderia sp. strain DNT: similarity to naphthalene dioxygenase. Journal of Bacteriology, **178**, 4926-4934.

Swatloski, R. P., Holbrey, J. D. and Rogers, R. D. (2003). Ionic liquids are not always green: hydrolysis of 1-butyl-3-methylimidazolium hexafluorophosphate. Green Chemistry, **5**, 361-363.

Symons, Z. C. and Bruce, N. C. (2006). Bacterial pathways for degradation of nitroaromatics. Natural. Product. Reports, **23**, 845-850.

Timmis, K. N. and Pieper, D. H. (1999). Bacteria designed for bioremediation. Trends Biotechnology, **17**, 201.

Uyttebroek, M., Vermeir, S., Wattiau, P., Ryngaert, A. and Springael, D. (2007). Characterization of Cultures Enriched from Acidic Polycyclic Aromatic Hydrocarbon-Contaminated Soil for Growth on Pyrene at Low pH. Applied and Environmental Microbiology. **73**, 3159-3164.

Vaillancourt, F. H., Labbe, G., Drouin, N. M, Fortin, P. D. and Eltis, L. D. (2002). The Mechanism-based Inactivation of 2,3-Dihydroxybiphenyl 1,2-Dioxygenase by Catecholic Substrates. The Journal of Biological Chemistry, **277**, 2019-2027.

van der Meer, J. R., de Vos, W. M., Harayama, S. and Zehnder, A. J. B. (1992). Molecular mechanisms of genetic adaptation to xenobiotic compounds. Microbiology and Molecular Biology Reviews, **56**, 677-694.

van der Meer, J. R. (1997). Evolution of novel metabolic pathways for the degradation of chloroaromatic compounds. Antonie. Van Leeuwenhoek., **71**,159-178.

Wells, A. S. and Coombe, V. T. (2006). On the Freshwater Ecotoxicity and Biodegradation Properties of Some Common Ionic Liquids. Organic Process Research & Development, **10**, 794-798.

Whited, G. M. and Gibson, D. T. (1991). Toluene-4-monooxygenase, a three-component enzyme system that catalyzes the oxidation of toluene to p-cresol in Pseudomonas mendocina KR1. Journal of Bacteriology, **173**, 3010-3016.

Wu, J. f., Jiang, C., Wang, B., Ma, Y., Liu, Z. and Liu, S. (2006). Novel Partial Reductive Pathway for 4-Chloronitrobenzene and Nitrobenzene Degradation in Comamonas sp. Strain CNB-1. Applied and Environmental Microbiology, **72**. 1759-1765.

Wu, J.F., Shen, X.H., Zhou, Y.G. and Liu, S.J. (2004). Characterization of p-chloronitrobenzene-degrading Comamonas sp. CNB1 and its degradation of p-chloronitrobenzene. Acta.Microbiol. Sin. 2004. **44**, 8.

Zeyer, J. and Kearney, P. C. (1984). Degradation of o-nitrophenol and m-nitrophenol by a Pseudomonas putida. J. Journal of Agricultural and Food Chemistry, **32**, 238-242.

Zhao, H. (2005). Effect of ions and other compatible solutes on enzyme activity, and its implication for biocatalysis using ionic liquids, Journal of Molecular Catalysis B: Enzymatic, **37**, 16-25.

Zhao, D. B., Liao, Y. C. and Zhang, Z. D. (2007). Toxicity of Ionic Liquids. Clean Soil Air Water, **35**, 42-48.