
ANAEROBIC BIOTRANSFORMATION OF AROMATIC AND POLYCYCLIC AROMATIC HYDROCARBONS IN SOIL MICROCOSMS: A REVIEW

¹R. Karthikeyan and ²A. Bhandari

¹Graduate Research Assistant, Biological and Agricultural Engineering, 147 Seaton Hall, Kansas State University, Manhattan, KS 66506; Phone: (785) 532-2923;

Fax: (785) 532-5585; email: karthi@ksu.edu. ²Assistant Professor, Civil Engineering, Kansas State University, Manhattan, KS 66506; Phone: (785) 532-1578; Fax: (785) 532-7717; email: bhandari@ksu.edu.

ABSTRACT

Industrial activities introduce a wide variety of xenobiotic aromatic compounds into the biosphere. These aromatic and polycyclic aromatic compounds find their way into anaerobic marine and freshwater sediments, saturated aquifers, and waterlogged soils where neither their impact on carbon flow nor their propensity to persist is clearly understood. Although some halogenated aromatic compounds are degraded via anaerobic pathways, anaerobic transformation of aromatics remains limited to a small range of chemicals. This paper reviews anaerobic transformation processes for aromatic compounds, particularly polycyclic aromatic hydrocarbons (PAHs) in soil microcosms. A simplified pathway of PAH biotransformation and a conceptual pathway of PAH degradation under different redox-conditions are presented.

Key words: *bioremediation, denitrification, PAHs, sulfidogenesis*

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) exhibit toxic properties at low concentrations and several have been listed as priority pollutants to be monitored in industrial effluents, natural waters, soils, and sediments (Watts, 1997). PAHs are fused-ring compounds that enter soil systems and natural waters via wastewater effluents from coke and petroleum refining industries, accidental spills and leakages, rainwater runoff from highways and roadways, or from intentional disposal in the past. Low aqueous solubilities of PAHs and high octanol-water partition coefficients (K_{ow}) often result in their accumulation in soils and sediments to levels several orders of magnitude above aqueous concentrations. PAHs can be potent carcinogens, and their presence in groundwater, streams, soil, and sediments may constitute a chronic human health hazard.

There has been tremendous interest in understanding the fate and transport of PAHs in subsurface environments that are largely microaerobic or anaerobic. Although vast information is available on aerobic biotransformation of PAHs (Atlas and Bartha, 1992; Bossert and Bartha, 1986; Bryniocck et al., 1990; Carmichael and Pfaender, 1993; Cerneglia, 1984; 1992; 1993; Mahro et al., 1994; Pradhan et al., 1997; Wilson and Jones, 1993), little is known about anaerobic biotransformation of these contaminants, particularly in the context of soil and groundwater contamination. Aerobic transformation of PAHs associated with soil and groundwater often leads to rapid depletion of dissolved oxygen and this eventually decreases the redox potential (Eh). Such decrease in the redox potential can result in favorable growth environments for denitrifying, sulfate-reducing, or even methanogenic [Eh < -0.3 V] microbial populations. Nearly 10 to 15% of the bacterial population in

soil, water, and sediments can be comprised of anaerobic organisms (Riser-Roberts, 1998). Anaerobic transformations may, therefore, play a significant role in oxygen-depleted natural habitats. This review paper summarizes anaerobic transformation processes affecting aromatic compounds and PAHs. Anaerobic metabolic processes are detailed, and laboratory studies on biotransformation of PAHs at different redox potentials are summarized. A conceptual pathway of PAH degradation under strict anaerobic conditions is also presented.

2. ANAEROBIC METABOLISM OF AROMATIC AND POLYAROMATIC HYDROCARBONS

Results from past studies on PAH biodegradation under strict anaerobic conditions have remained ambiguous (Al-Bashir et al., 1990; Barbaro et al., 1999; Bauer and Capone, 1985b; Bedessem et al., 1997; Cerniglia and Heitkamp, 1989; Coates et al., 1996a, 1996b, 1997; McNally et al., 1998; Meckenstock et al., 2000; Mihelcic and Luthy, 1988a, 1988b; Rockne and Strand, 1998; Rockne et al., 1999, 2000; Wilson et al., 1997; Zhang and Young, 1997). Indications that anaerobic metabolism of aromatic structures is possible in nature were first provided by Tarvin and Buswell (1934, cited by Taylor et al., 1970). However, until recently, anaerobic biodegradation of unsubstituted aromatics like benzene and PAHs had not been widely reported (Harwood and Gibson, 1997; Heider et al., 1998; Phelps et al., 1996, 1998; Reinhard et al., 1991). In fact, anaerobic biodegradation of these compounds was thought to be unlikely due to the lack of ring substituents such as the methyl group (Heider and Fuchs, 1997). Clark and Fina (1952, cited by Taylor et al., 1970) confirmed that the aromatic acid, benzoate, was anaerobically mineralized by mixed cultures. Using mixed cultures enriched from rumen fluid or anaerobic digestion tanks, Fina and Fiskin (1960, cited by Taylor et al., 1970) were able to demonstrate that $^{14}\text{CH}_4$ was produced from benzoate-1- ^{14}C under fermentative growth conditions.

Taylor et al. (1970) concluded that the anaerobic pathway for breakdown of the aromatic ring was different and quite distinct from the aerobic pathway. These researchers isolated a bacterium by selective culture with *p*-hydroxybenzoate (pHBz) as substrate and nitrate as the electron acceptor. The strain, *Pseudomonas PN-1*, grew either aerobically or by nitrate respiration on a range of aromatic compounds and released 4 to 5 μmoles of CO_2 and about 3 μmoles of N_2 per μmole of benzoate when grown on pHBz. Mineralization of 60 to 70% of the carbon associated with benzoate to CO_2 during nitrate respiration was indicative of benzene ring disruption and degradation under anaerobic conditions. This study may have been the first to conclusively demonstrate cleavage of benzene ring in the absence of molecular oxygen (Evans, 1977). Healy and Young (1979) also investigated anaerobic biodegradation of 11 simple aromatic lignin derivatives to methane, suggesting that more than half of the carbon associated with the 11 aromatic compounds could be potentially converted to methane gas.

Most biological transformations of aromatic ring structures are catalyzed by mono or dioxygenases and, therefore, proceed only when molecular oxygen is available for ring cleavage. It may thus appear that aromatic metabolism is restricted to aerobes possessing oxygenase enzymes. Microbial transformation of aromatic compounds under denitrifying, sulfate-reducing, and methanogenic conditions, however, is fundamentally different from degradation under aerobic conditions. Taylor et al. (1970) were unable to determine the mode of cleavage of the aromatic ring by *Pseudomonas PN-1* but suggested that aromaticity was destroyed upon addition of three molecules of water to benzene in preparation of ring cleavage. Disagreeing with Taylor's et al. (1970) hypothesis on theoretical grounds, Evans (1977) illustrated the operation of a reductive pathway in the anoxic transformation of aromatic substrates through nitrate respiration.

Evans' (1977) hypothesis stated that benzene undergoes an initial ring reduction followed by hydrolytic ring cleavage to yield aliphatic acids for cell growth. The aromatic ring is reduced to a substituted cyclohexane before hydrolytic ring cleavage. For aromatics with substituent groups, a modification or removal of substituents precedes ring reduction (Evans, 1977). Unlike phenols and benzoates, however, aromatic hydrocarbons have no activating substituent groups which facilitate hydration of the ring. Hydrogen saturates the aromatic ring under moderate conditions, whereas addition of water is thermodynamically impossible. Microorganisms, however, can utilize a remarkable biochemical pathway of initial ring reduction followed by ring disruption to initiate biotransformation of aromatic hydrocarbons (Evans, 1977).

Vogel and Grbic-Galic (1986) obtained evidence suggesting that benzene derivatives could be metabolized under anaerobic conditions through an oxidation pathway rather than a reduction pathway. The initial step of ring oxidation instead of ring reduction appeared to contradict Evans' (1977) reductive pathway theory. Vogel and Grbic-Galic (1986) argued that the anaerobic transformation of benzene and toluene to CO_2 and CH_4 was proceeded by a hydroxylation reaction. Phenol and cresol were identified as intermediates from benzene and toluene, respectively.

Berry et al. (1987) suggested that in the presence of sufficient organic carbon, the diversity of heterotrophic populations depended on the availability of external electron acceptors: NO_3^- , SO_4^{2-} , and CO_2 . By the time Berry et al. (1987) had published their review paper, only the reductive pathway for anaerobic metabolism of aromatic compounds (with one exception: Vogel and Grbic-Galic, 1986) had been elucidated. This pathway was believed to be common in all microorganisms involved in aromatic metabolism, including denitrifiers, sulfate reducers, and fermenters (Berry et al., 1987).

Erickson and Fan (1988) reviewed anaerobic degradation of toxic and hazardous wastes. According to them, several aspects of microbial degradation processes such as genetics, biochemical pathways, kinetics, bioenergetics, nutrition, stability, and reliability must be considered in the design and control of treatment systems. These authors have summarized information on anaerobic

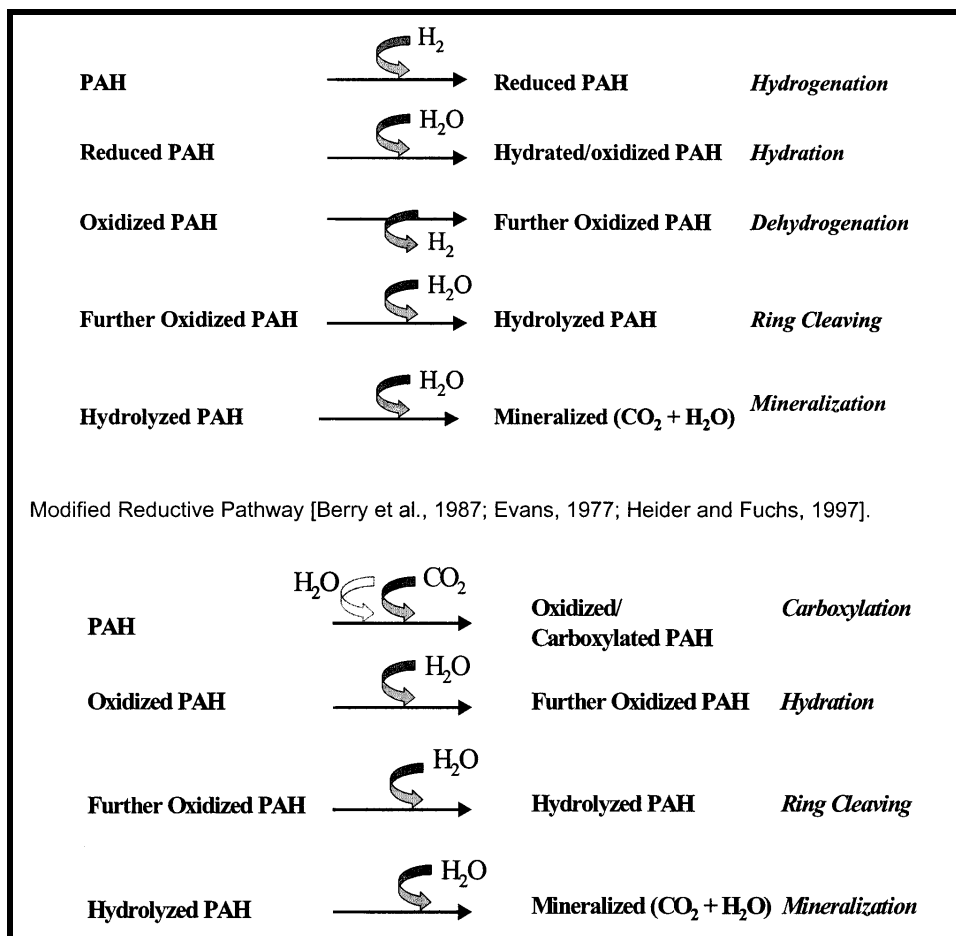


Figure 1. Simplified Pathways of PAH Biotransformation as Proposed by Various Researchers.

biodegradation of a number of aromatic compounds by means of photometabolism, anaerobic respiration using nitrate or sulfate, and methanogenic fermentation.

A recent review by Heider and Fuchs (1997) summarized pathways that allow bacteria to utilize aromatic compounds in the absence of oxygen. This article focused on previously unknown reactions and on enzyme characteristics of these novel metabolic pathways. CoA ligases, oxidoreductases, and decarboxylases were suggested enzymes involved in anaerobic transformation of aromatic compounds. Carboxylation, reductive dehydroxylation, reductive deamination, reductive dehalogenation, oxidation of carboxymethyl groups, methyl oxidation, *o*-demethylation, transhydroxylation, and decarboxylation were discussed as possible peripheral metabolic reactions that occur during the anaerobic transformation process. Carboxylation has been proposed as the initial step in PAH biotransformation under sulfate reducing conditions by other researchers (Meckenstock et al., 2000; Zhang and Young, 1997). Bedessem et al. (1997), however, have proposed hydroxylation as the initial step in naphthalene biotransformation under sulfate-reducing conditions. These proposed reductive and oxidative PAH transformation pathways are summarized in Figure 1.

3. BIOTRANSFORMATION OF PAHS UNDER DIFFERENT REDOX CONDITIONS

In the absence of molecular oxygen, redox potential becomes a critical factor in determining the metabolic diversity of microbial populations in soils, sediments, and aquifer systems. Rittman and McCarty (2001) present half reactions and Gibb's standard free energy for various biological oxidation processes (Table 1). Hambrick et al. (1980) investigated microbial mineralization rates of two petroleum hydrocarbons as affected by the redox potential. In their study, nearly 22.6% of [¹⁴C]naphthalene was mineralized at Eh = +130 mV; at Eh = -220 mV, the mineralization was about 0.62% over a 35-day period. Several other studies conducted on anoxic or anaerobic sediments, subsurface materials, and wastewater systems, have demonstrated PAH biotransformation in the absence of molecular oxygen (Cerniglia and Heitkamp, 1989; Heitkamp and Cerniglia, 1987; Heitkamp and Cerniglia, 1988; Herbes, 1981; Herbes and Schwall, 1978; Parker and Monteith, 1995). The following sub-sections summarize results from experimental studies on PAH biotransformation under various redox conditions.

3.1. Denitrification

Microbial reduction of NO₃⁻ to NO, N₂O, and N₂ is known as nitrate reduction or denitrification (Paul and Clark, 1996). Nitrate serves as the terminal electron acceptor in the oxidation of organic substrates, and nitrogen gas is usually the final product of denitrification. Denitrification is strongly dependent on the availability of carbon (Paul and Clark, 1996). Active nitrate-respiring microorganisms are found in a variety of anoxic habitats, including soils, lakes, rivers, and oceans (Berry et al., 1987). Denitrification, which may occur when dissolved oxygen concentrations drop below 10 μM, can mediate effective biotransformation of organic contaminants.

Several oxygenated hydrocarbons that are typical biodegradation intermediates of fuel hydrocarbons have been shown to degrade under denitrifying conditions. However, the biotransformation of parent aromatic hydrocarbons under denitrifying conditions is less established and often the reports are conflicting (Al-Bashir et al., 1990; Bauer and Capone, 1985b; Cerniglia and Heitkamp, 1989; Hutchins et al., 1991; McNally et al., 1998; Mihelcic and Luthy, 1988a, 1988b; Rockne and Strand, 1998; Rockne et al., 2000). Much of the available literature on

Table 1. Half Reactions and Gibb's Standard Free Energy for Various Biological Oxidation Processes.

Oxidation Process	Half Reaction	? G ⁰ kJ/e ⁻ equivalent
Aerobic Respiration	0.25O ₂ + H ⁺ + e ⁻ = 0.5 H ₂ O	-78.72
Iron Reduction	Fe ³⁺ + e ⁻ = Fe ²⁺	-74.27
Denitrification	0.2NO ₃ ⁻ + 1.2H ⁺ + e ⁻ = 0.1N ₂ + 0.6H ₂ O	-72.20
Sulfate Reduction	0.125SO ₄ ²⁻ + 1.1875H ⁺ + e ⁻ = 0.0625H ₂ S + 0.0625HS ⁻ + 0.5H ₂ O	20.85
Methanogenesis	0.125CO ₂ + H ⁺ + e ⁻ = 0.125CH ₄ + 0.25H ₂ O	23.53

[Adapted from Rittman and McCarty (2001)]

microbial transformation of aromatic compounds under nitrate-reducing conditions has been obtained with phenolic or benzoate derivatives (Evans, 1977; Sleat and Robinson, 1984; Taylor et al., 1970).

Molecular oxygen is necessary for aromatic catabolism as it is directly incorporated into the aromatic ring structure. It has been observed that oxygen-containing benzoates are degraded anaerobically by initial ring reduction followed by hydrolytic cleavage (Evans, 1977). However, similar mechanisms have not been reported for PAH breakdown. A few investigations on microbial denitrification have addressed degradation of non-oxygenated aromatic compounds (Al-Bashir et al., 1990; Bauer and Capone, 1985a; McNally et al., 1998; Mihelcic and Luthy, 1988a, 1988b; Rockne and Strand, 1998; Rockne et al., 1999, 2000). Bauer and Capone (1985a) performed a laboratory study on the degradation of anthracene and naphthalene by the microbiota of intertidal marine sediments. Rates and extent of PAH mineralization were strongly correlated to oxygen concentration and the incubation temperature. However, no mineralization of either PAH was observed in the absence of oxygen.

Bauer and Capone (1985b) evaluated effects of four aromatic compounds including two PAHs, anthracene and naphthalene, on two microbial activities common in oxic and anoxic coastal marine sediments. The activities studied were uniformly radio-labeled glucose (d-[U-¹⁴C]glucose) metabolism and tridiated thymidine ([methyl-³H]thymidine([³H]TdR)) incorporation. Cell-specific rates of [¹⁴C] glucose metabolism averaged 1.7×10^{-21} and 0.5×10^{-21} mol/min per cell for aerobic and anoxic sediment slurries, respectively. [³H]thymidine incorporation rates averaged 43×10^{-24} and 9×10^{-24} mol/min per cell for aerobic and anoxic slurries, respectively. These researchers concluded that anoxic sediments were more sensitive than aerobic sediments to contaminant additions based on d-[U-¹⁴C]glucose metabolic activity and [methyl-³H]thymidine incorporation activity.

Mihelcic and Luthy (1988a) were the first to demonstrate naphthalene biodegradation under denitrifying conditions. These researchers studied microbial degradation of acenaphthene and naphthalene under denitrifying conditions at soil-water ratio of 1:25 and 1:50. Under excess nitrate conditions, both acenaphthene and naphthalene were degraded to nondetectable levels (<0.01 mg/L) in less than nine weeks. An acclimation period of 12 to 36 days was required in soils with no previous PAH contamination history, while no acclimation period was observed in soils contaminated with PAHs in the past. These researchers reported that naphthalene and acenaphthene were degraded under denitrifying conditions but were stable under other anaerobic test conditions (Mihelcic and Luthy, 1988b). In a separate study, Mihelcic and Luthy (1988a) observed nitrate depletion during naphthalene degradation from about 40 to 24 mg/L, while aqueous naphthalene concentration reduced from about 4.5 mg/L to nondetectable levels. Results from this study also pointed out that PAH degradation under denitrifying conditions depended on the ratio of the PAH compound to other mineralizable carbon sources (Mihelcic and Luthy, 1988a).

Naphthalene biodegradation rates under denitrifying conditions were in the same range as those under aerobic conditions.

Al-Bashir et al. (1990) studied the biodegradation of naphthalene in a soil-slurry system under denitrifying conditions. At an initial naphthalene concentration of 50 mg/L (near saturation), about 90% of the total naphthalene was mineralized within 50 days, at a maximum mineralization rate of 1.3 mg/L-day achieved after a lag period of approximately 18 days. When added at concentrations greater than the aqueous-phase saturation level (200 and 500 mg/L), similar naphthalene mineralization rates (1.8 mg/L-day) were observed until about 50 mg/L of naphthalene were mineralized. Thereafter, mineralization rates decreased exponentially to a minimum of 0.24 mg/L-day for the remaining 160 days of the experiment.

Rockne and Strand (1998) used a fluidized-bed reactor (FBR) to enrich naphthalene, biphenyl, dibenzofuran, and phenanthrene-degrading anaerobes in sediment-free cultures with nitrate and sulfate as terminal electron acceptors. Enrichment using an FBR approach allowed for long cell residence times at high mass loading of dissolved substrates – a desirable technique for enriching slow growing anaerobes on sparingly soluble substrates like PAHs (Rockne and Strand, 1998). Removal of naphthalene and phenanthrene was observed within 100-200 days of operation. Batch incubations of FBR cells under strict anaerobic conditions confirmed the transformation of naphthalene and phenanthrene with stoichiometric removal of nitrate by the nitrate-reducing enrichment, and production of sulfide by sulfate-reducing enrichment. Although results confirmed the ability of PAHs to undergo biotransformation under nitrate- and sulfate-reducing conditions, degradation rates under these conditions were observed to be 10 to 100 times lower than those reported for aerobes (Rockne and Strand, 1998).

McNally et al. (1998, 1999) utilized three microorganisms in pure culture in a biodegradation study of three- and four-ring PAHs under denitrifying conditions. These researchers also compared aerobic and anaerobic degradation rates and adaptation periods by isolated organisms (McNally et al., 1998). Two microorganisms, strains SAG-R and W-2, were isolated from hydrocarbon-contaminated sites, and the third microorganism, KBM-1, was isolated from a site with no previous direct contamination to PAHs. All three strains were pseudomonads capable of growing on PAHs aerobically and under denitrifying conditions (McNally et al., 1998). Degradation proceeded to nondetectable levels (<0.001 mg/L) in 12-80 hr for anthracene, 12-44 hr for phenanthrene, and 24-72 hr for pyrene. The rates of anaerobic degradation were typically slower than under aerobic conditions in almost all cases, except for strain SAG-R which had similar removal rates for all PAHs (McNally et al., 1998). Anaerobic degradation rates under denitrifying conditions varied from being similar to being approximately 0.5 times the aerobic rates (McNally et al., 1998). Results from this study support growing evidence that anaerobic degradation rates under denitrifying conditions may approach those of aerobic degradation when cell densities are similar (McNally et al., 1998).

Some of the most recent work by Rockne et al. (1999, 2000) illustrates further progress in understanding PAH transformation under denitrifying conditions. This was achieved by the isolation and identification of pure cultures of anaerobic, PAH-degrading bacteria. Pure bacterial cultures isolated from a denitrifying enrichment were shown to anaerobically degrade phenanthrene, naphthalene, and biphenyl with stoichiometric nitrate reduction. Two pure cultures, NAP-3-1 and NAP-4, demonstrated unambiguous naphthalene biodegradation ability. Naphthalene transformation was nitrate dependent and no significant removal of naphthalene occurred in nitrate-limited incubations or in controls. Isolate NAP-3-1 was a denitrifier, which produced a significant amount of nitrogen gas. In contrast, NAP-4 did not produce nitrogen gas, but did produce significant amounts of nitrite. DNA sequencing showed that NAP-4 was phylogenetically related to *Vibrio spp.* and NAP-3-1 was phylogenetically related to *Pseudomonas spp.* These results suggest that aromatic degradation abilities under denitrifying conditions may be more widespread among the proteobacteria. Rockne et al. (1999, 2000) were the first to report nitrate-dependent anaerobic degradation and mineralization of naphthalene by pure cultures.

3.2. Iron Reduction

Iron reduction involves the oxidized form of iron, Fe(III), acting as a terminal electron acceptor, when a suitable carbon and energy source is available. At the onset of anaerobic conditions in most freshwater sediments, Fe(III) is the most abundant potential electron acceptor for organic matter oxidation (Lovley et al., 1989). The observed accumulation of Fe(II) during anaerobic transformation of organic materials in pristine and contaminated aquifers suggests that Fe(III) can be a potential electron acceptor in a wide variety of sub-surface environments. Few investigations have studied biodegradation of aromatic compounds under iron-reducing conditions (Beller et al., 1992; Lovley et al., 1989, 1994; Coates et al., 1996a). However, no study to date has demonstrated biotransformation of PAHs coupled to iron reduction.

3.3. Sulfidogenesis

Dissimilatory sulfate reduction is a process mediated by anaerobic, organotrophic organisms that use low molecular weight organic acids, alcohols, and often H₂ as electron donors (Nedwell, 1982; Odom and Singleton, 1993; Paul and Clark, 1996). Sulfate reducers, also known as sulfidogens, are described as strict anaerobes that utilize organic substrates as sources of carbon and energy. Sulfate reducers are most commonly associated with freshwater and marine sediments but can also be found in soils (Riser-Roberts, 1998). These bacteria are capable of degrading aromatics such as benzoate, catechols, indol derivatives, and chlorophenols. The ability of sulfidogens to transform aromatic compounds all the way to CO₂ is indicative of the diverse microbial enzymatic capabilities associated with these organisms (Nedwell, 1982; Odom and Singleton, 1993; Paul and Clark, 1996). Due to the rapid depletion of oxygen, nitrate, and ferric

iron in marine sediments, and an abundance of sulfate in seawater, sulfate reduction often serves as the predominant terminal electron-accepting process (TEAP) in the marine environment.

Lovley et al. (1995) evaluated benzene oxidation under sulfate-reducing conditions. Highly reduced sediments from San Diego Bay, California, that were incubated under strict anaerobic conditions metabolized benzene within 55 days when 1 μM benzene was introduced. Benzene transformation was enhanced when more benzene was added to the benzene-adapted sediments. Addition of [^{14}C]benzene to benzene-adapted sediments resulted in the transformation of 92% of the added radioactivity to $^{14}\text{CO}_2$. Molybdate, an inhibitor of sulfate reduction (Newport and Nedwell, 1988), inhibited benzene uptake and production of $^{14}\text{CO}_2$. Benzene metabolism stopped when the sediments were depleted of sulfate, and benzene uptake resumed when sulfate was added to the sediments. The stoichiometry of benzene uptake and sulfate reduction was consistent with the hypothesis that sulfate was the principal electron acceptor for benzene oxidation. Isotope trapping experiments performed with [^{14}C]benzene revealed no production of intermediates such as phenol, benzoate, catechol, and acetate. Results from this study illustrated the ability of benzene to be completely oxidized to CO_2 in the absence of O_2 by sulfate-oxidizing bacteria without production of extracellular intermediates. This was the first study providing evidence of complete mineralization of simple aromatic hydrocarbons with sulfate as an electron acceptor (Lovley et al., 1995).

Past studies demonstrating PAHs transformation under denitrifying conditions have contended that PAHs tend to persist under sulfate-reducing or methanogenic conditions (Al-Bashir et al., 1990; Mihelcic and Luthy, 1988a, b). Although anaerobic oxidation of benzene coupled to the reduction of chelated Fe(III) was demonstrated by Lovley et al. (1994), transformation of PAH compounds under iron-reducing and sulfate-reducing conditions was not reported until the mid 1990's (Coates et al., 1996a,b). Coates et al. (1996b) were the first to demonstrate the oxidation of PAHs under sulfate-reducing conditions. These researchers documented the oxidation of [^{14}C]naphthalene and phenanthrene to $^{14}\text{CO}_2$ under strict anaerobic conditions in sediments from San Diego Bay, California. Sulfate reduction was necessary for PAH oxidation in these marine sediments. Results from this study suggest that the self-purification capacity of PAH-contaminated, sulfate-reducing environments may be greater than previously recognized (Coates et al., 1996b).

Further investigations with the San Diego Bay sediments revealed that methylnaphthalene, fluorene, and fluoranthene were also anaerobically transformed to carbon dioxide, while pyrene and benzo[a]pyrene were not (Coates et al., 1997). Studies with naphthalene indicated that PAH oxidation was sulfate dependent. Incubating the sediments with additional naphthalene for one month resulted in a significant increase in the transformation of [^{14}C]naphthalene. In sediments from a less heavily contaminated site in San Diego Bay where PAHs were not readily degraded, naphthalene degradation could be stimulated through inoculation with active PAH-degrading sediments from the more heavily contaminated site. This illustrated that benzene- and naphthalene-

degrading microbial populations were quite different (Coates et al., 1997). Results from this study point to the possibility of utilizing sulfate reduction as a treatment strategy for dredged sediments contaminated with PAHs. Although PAHs entering pristine anaerobic marine sediments may not be degraded immediately, microbial populations capable of utilizing the abundant sulfate as an electron acceptor can develop over time to metabolize these compounds.

In another study, Bedessem et al. (1997) used sediments from two sulfate-rich, coal tar-contaminated aquifers enriched over a three-year period to illustrate that naphthalene was microbially transformed in sulfate-reducing laboratory microcosms established under strict anaerobic conditions. A series of nine, sediment-free enrichment cultures were maintained with naphthalene as the sole carbon source for over 21 months. Sulfate reduction was the predominant electron-accepting process in the microcosms. Initial rates of naphthalene transformation varied greatly, ranging from less than one week to about five months for complete degradation, and abiotic controls exhibited no loss of naphthalene over the same period. Naphthalene added to actively degrading enrichments was rapidly biodegraded. Even microcosms with the slowest initial degradation rates manifested naphthalene disappearance at rates comparable to the fastest degrading microcosms upon subsequent naphthalene addition. The addition of molybdate, a competitive inhibitor of sulfate reduction, resulted in a 45% reduction in naphthalene degradation in enrichment cultures. Radio-labeled experiments confirmed naphthalene mineralization to CO_2 with simultaneous reduction of sulfate to sulfide. Inhibition of sulfidogenesis by molybdate and a concomitant decrease in naphthalene oxidation provided convincing evidence that naphthalene mineralization was coupled to sulfate reduction (Bedessem et al., 1997).

Carboxylation has been shown as an initial reaction in the anaerobic transformation of naphthalene and phenanthrene by a sulfidogenic consortia (Zhang and Young, 1997). Anaerobic biotransformation of naphthalene and phenanthrene was investigated in sediment collected from the Arthur Kill in the New York/New Jersey harbor. The utilization of both compounds was inhibited in the presence of 20 mM molybdate. Isotope tracer studies showed that naphthalene and phenanthrene were mineralized to CO_2 . Carboxylation of PAHs leads to the formation of benzoate-like analogs, which are ideal substrates for further activation by coenzyme A ligation followed by ring reduction. PAH carboxylation supports the notion that reductive hydrogenation is possible only after the aromatic ring is destabilized or activated by a reaction such as carboxylation (Zhang and Young, 1997). Whether carboxylated PAHs undergo similar sequential ring reduction and ring fission as that seen with benzoate under anaerobic conditions remains unknown. Thus, it appears that a sufficiently long adaptation period and the availability of appropriate electron acceptors may be a necessity for the biotransformation of PAHs under microaerobic or anaerobic conditions.

In another recent study, anaerobic transformation of naphthalene by a sulfate-reducing enrichment culture was confirmed by substrate utilization tests and identification of metabolites by

gas chromatography-mass spectroscopy (GC/MS)(Meckenstock et al., 2000). Meckenstock et al. (2000) enriched a sulfate-reducing culture from a contaminated aquifer material by adding naphthalene as the sole carbon and energy source, and sulfate as the electron acceptor. These researchers observed 2-naphthoic acid as a prominent metabolite in all naphthalene-degrading cultures investigated. In an earlier study, Zhang and Young (1997) also reported 2-naphthoic acid to be an intermediate of anaerobic naphthalene degradation in a marine sulfate-reducing enrichment culture. Those researchers stated that 2-naphthoic acid was a product of naphthalene carboxylation, as shown by incorporation of [¹³C]bicarbonate (Zhang and Young, 1997). Meckenstock et al. (2000) also observed incorporation of [¹³C]bicarbonate into the carboxylic group of 2-naphthoic acid, indicating that naphthalene was activated through addition of a C1 compound. Two other 2-naphthoic acid derivatives were identified by Meckenstock et al. (2000). The metabolites identified suggested a stepwise reduction of 2-naphthoic acid to tetralin-2-carboxylic acid and subsequently to decahydro-2-naphthoic acid by the enriched culture (Meckenstock et al., 2000). These researchers concluded that carboxylation of naphthalene was the initial step in anaerobic naphthalene biotransformation due to the GC/MS evidence of the incorporation of [¹³C]bicarbonate into the carboxyl group of 2-naphthoic acid. This conclusion supported the carboxylation theory proposed by Zhang and Young (1997).

In another study, naphthalenol was detected consistently by GC/MS analysis of microcosms that exhibited naphthalene transformation (Bedessem et al., 1997). Bedessem et al. (1997) suggested that naphthalenol was an observed intermediate in a sulfidogenic sediment supporting the argument that hydroxylation was the initial step in naphthalene transformation. However, hydroxylated intermediates were not identified by GC/MS in the study by Meckenstock et al. (2000), and the enriched culture could not grow on 1- or 2-naphthalenol.

3.4. Methanogenesis

Methanogenesis is a strictly anaerobic process and methanogens are obligate anaerobes that ferment simple, low molecular weight organic acids to methane. The availability of electron donors can affect the competition between sulfate-reducing bacteria and methanogens. When electron donor availability is limited, sulfate-reducing bacteria generally out compete methanogens for available substrate at low sulfate concentrations (< 1mg/L). In the presence of an abundant electron donor, methanogens are able to sequester part of the electron flow even if there is sufficient sulfate to support sulfate reduction (Riser-Roberts, 1998). Although substituted monoaromatic compounds have been shown to completely transform to CO₂ and CH₄ under fermentative and methanogenic conditions (Healy and Young, 1979; Kaiser and Hanselmaan, 1982), no published reports pertaining to methanogenic transformation of PAHs by enrichment cultures are available to date (2001).

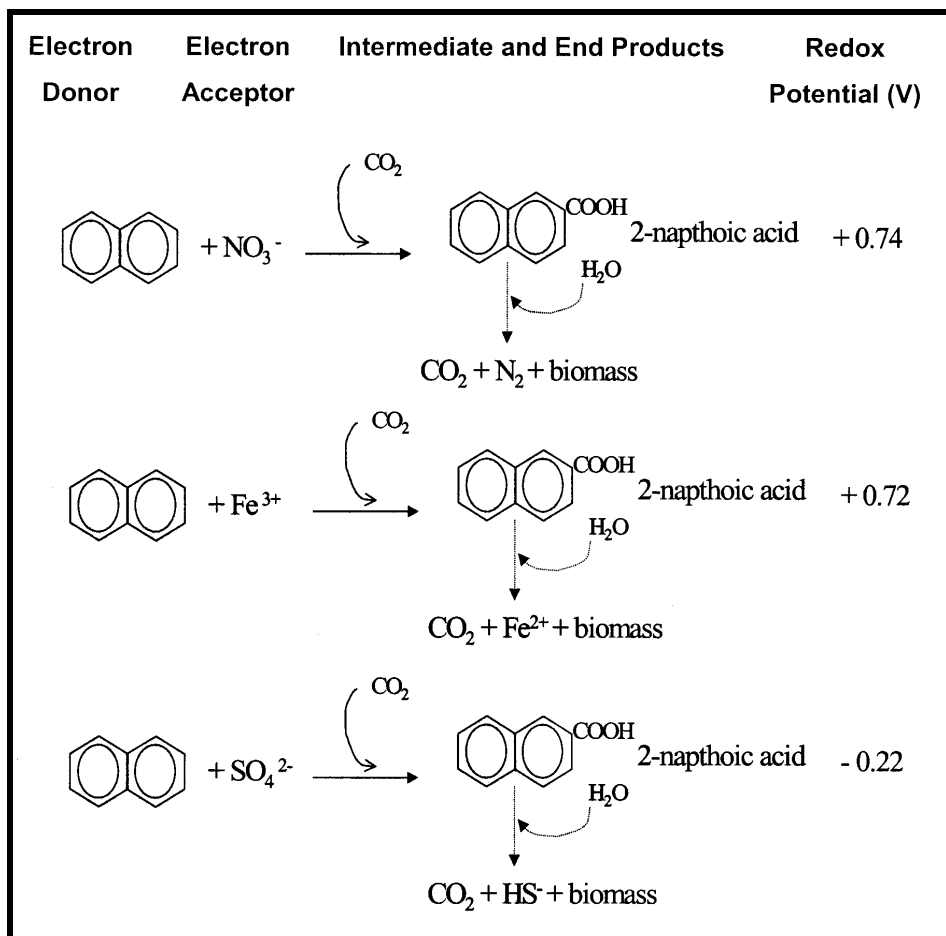


Figure 2. Conceptual Pathway of PAH Degradation Under Different Redox Conditions Using Naphthalene as a Model PAH.

4. CONCEPTUAL PATHWAY OF ANAEROBIC PAH MINERALIZATION

Although some pathways for anaerobic mineralization of PAH compounds have been discussed in the literature, an established pathway still alludes the research community. Figure 2 illustrates pathways for anaerobic transformation of naphthalene as suggested by the authors of this paper and based on a thorough review of the literature. Since there is strong evidence exhibited by both marine and aquifer enrichments (Meckenstock et al., 2000; Zhang and Young, 1997), carboxylation is considered as the initial step in naphthalene biotransformation under anaerobic conditions.

5. SUMMARY

Anaerobic degradation of aromatic hydrocarbons is often presumed to be slow and of minor ecological significance. However, anaerobic biotransformation may play a key role in the transformation of aromatic and PAH compounds when oxygen demand exceeds supply in natural environments. Under such conditions, anoxic or anaerobic degradation mediated by denitrifying or sulfate-reducing bacteria can become a key pathway for the cleanup of contaminated sites.

Our understanding of anaerobic biodegradation of aromatic compounds has increased tremendously over the past decade. Isolation of denitrifying and sulfate-reducing organisms capable of degrading toluene has led to the elucidation of several biodegradation pathways and phylogenetic relationships between bacterial strains (Beller and Spormann, 1997a, 1997b; Biegert et al., 1996; Dolfing et al., 1990; Edwards et al., 1991; Evans et al., 1991; McNally et al., 1998; Meckenstock, 1999; Rockne et al., 1999, 2000; Rueter et al., 1994; Zeyer et al., 1986). These studies have demonstrated that the metabolic ability of microorganisms to degrade aromatic compounds anaerobically is widespread. In the past three years, however, anaerobic transformation of PAHs such as naphthalene, phenanthrene, fluorene, and fluoranthene has also been demonstrated in marine sulfate-reducing and denitrifying enrichments derived from Puget Sound, Washington (Rockne and Strand, 1998; Rockne et al., 1999; 2000); Arthur Kill, New Jersey (Zhang and Young, 1997); and San Diego Harbor, California (Coates et al., 1996b; Coates et al., 1997). Some recent studies have also demonstrated the mineralization of PAHs in groundwater aquifers (Al-Bashir et al., 1990; Bedessem et al., 1997; McNally et al., 1998, 1999; Meckenstock et al., 2000) and sediments (Johnson and Ghosh, 1998; Langenfoff-Alette et al., 1996) under strict anaerobic conditions. A summary of the published research on anaerobic biotransformation of PAHs in soil microcosms at different redox conditions is presented in Table 2. However, despite the recent progress in elucidating microbe-mediated transformation of PAHs during denitrification, iron reduction, and sulfidogenesis, more work is needed to establish PAH degradation pathways under these conditions. Understanding the factors affecting anaerobic transformation of aromatic and PAH compounds holds the key to extending the possibility of bioremediation processes to natural environments that have a limited supply of molecular oxygen.

6. BIBLIOGRAPHY

- Al-Bashir, B., T. Cseh, R. Leduc, and R. Samson. 1990. Effect of Soil/Contaminant Interactions on the Biodegradation of Naphthalene in Flooded Soil Under Denitrifying Conditions. *Applied Microbiology and Biotechnology*, 34(3): 414-419.
- Atlas, R.M., and R. Bartha. 1992. Hydrocarbon Biodegradation and Oil Spill. Bioremediation, *Advances in Microbial Ecology*, 12: 287-338.
- Barbaro, J.R., B.J. Butler, and J.F. Barker. 1999. Biotransformation of Gasoline-Contaminated Groundwater Under Mixed Electron-Acceptor Conditions. *Project Summary, US EPA/600/SR-99/012*, National Risk Management Research Laboratory, Ada, OK 74820. 4 p.
- Bauer, J.E., and D.G. Capone. 1985a. Effects of Four Aromatic Pollutants on Microbial Glucose Metabolism and Thymidine Incorporation in Marine Sediments. *Applied and Environmental Microbiology*, 49: 828-835.
- Bauer, J.E., and D.G. Capone. 1985b. Degradation and Mineralization of the Polycyclic Aromatic Hydrocarbons Anthracene and Naphthalene in Intertidal Marine Sediments. *Applied and Environmental Microbiology*, 50: 81-90.

- Bedessem, M.E., N.G. Swoboda-Colberg, and P. J. S. Colberg. 1997. Naphthalene Mineralization Coupled to Sulfate Reduction in Aquifer-Derived Enrichments. *FEMS Microbiology Letters*, 152(2): 213-218.
- Beller, H.R., and A.M. Spormann. 1997a. Benzylsuccinate Formation as a Means of Anaerobic Toluene Activation by Sulfate-Reducing Strain PRTOL1. *Applied and Environmental Microbiology*, 63: 3729-3731.
- Beller, H.R., and A.M. Spormann. 1997b. Anaerobic Activation of Toluene and O-xylene by Addition of Fumerate in Denitrifying Strain T. *Journal of Bacteriology*, 179: 670-676.
- Beller, H.R., D. Grbic-Galic, and M. Reinhard. 1992. Microbial Degradation of Toluene Under Sulfate-Reducing Conditions and the Influence of Iron on the Process. *Applied and Environmental Microbiology*, 58: 786-793.
- Berry, D.F., A.J. Francis, and J.M. Bollag. 1987. Microbial Metabolism of Homocyclic and Heterocyclic Aromatic Compounds Under Anaerobic Conditions. *Microbiology Reviews*, 51: 43-59.
- Biegert, T., G. Fuchs, and J. Heider. 1996. Evidence that Anaerobic Oxidation of Toluene in the Denitrifying Bacterium *Thauera aromatica* Is Initiated by Formation of Benzylsuccinate from Toluene and Fumerate. *European Journal of Biochemistry*, 238: 661-668.

Table 2. Research Papers on Anaerobic Biotransformation of PAHs in Soil Microcosms at Different Redox Conditions.

Research Papers	PAH	Redox Status	Description
Al-Bashir et al., 1990	[¹⁴ C]Naphthalene	Denitrifying	Complete mineralization; Pristine and oil-contaminated soils
Bedessem et al., 1997	[¹⁴ C]naphthalene	Sulfate reducing	Complete mineralization; Sulfate-rich, coal tar-contaminated aquifers
Coates et al., 1996b	[¹⁴ C]naphthalene and [¹⁴ C]phenanthrene	Sulfate reducing	Complete mineralization; Marine sediments contaminated with PAHs
Coates et al., 1997	[¹⁴ C]naphthalene, phenanthrene, methylnaphthalene, fluorene, and fluoranthene	Sulfate reducing	Complete mineralization; Marine sediments contaminated with PAHs
McNally et al., 1998; 1999	Anthracene, phenanthrene, and pyrene	Denitrifying	Biotransformation; Three pseudomonad strains (denitrifiers)
Meckenstock et al., 2000	Naphthalene	Sulfate reducing	Biotransformation to by-products; Sulfate-reducing enrichment culture
Mihelcic and Luthy, 1988a	Naphthalene and acenaphthalene	Denitrifying	Biotransformation of PAHs
Mihelcic and Luthy, 1988b	Naphthalene and acenaphthalene	Denitrifying	Biotransformation of PAHs; Nitrate-reducing enrichment culture
Rockne and Strand, 1998	Naphthalene and phenanthrene	Denitrifying and Sulfate reducing	Biotransformation of PAHs; Coal tar creosote-contaminated sediments
Rockne et al., 1999	Naphthalene	Denitrifying	Biotransformation of naphthalene; Four denitrifying pure cultures
Rockne et al., 2000	Naphthalene and [¹⁴ C]naphthalene	Denitrifying	Biotransformation and complete mineralization; Three denitrifying pure cultures
Zhang and Young, 1997	[¹⁴ C]naphthalene and [¹⁴ C]phenanthrene	Sulfate reducing	Complete mineralization; Marine sediments

- Bossert, I.D., and R. Bartha. 1986. Structure-Biodegradability Relationships of Polycyclic Aromatic Hydrocarbons in Soil. *Bulletin of Environmental Contamination and Toxicology*, 37: 490-495.
- Bryniocck, D., B. Eichler, A. Kohler, W. Clemens, K. MacKenbrock, D. Freier-Schroder, and H. J. Knackmuss. 1990. Development of a Biological Process for the Decontamination of Coking Plant Sites: Microbial Degradation of Polycyclic Aromatic Hydrocarbons, *In Contaminated Soil*, K. Wolf, J. Van de Brink, and F. J. Colon (Editors), Kluwer Academic Publishers, 1037-1038.
- Carmichael, L.M., and F.K. Pfaender, 1993. Polynuclear Aromatic Hydrocarbon Metabolism in Soils: Relationship to Soil Characteristics and Pre-Exposure. *Environmental Toxicology and Chemistry*, 16: 666-675.
- Cerniglia, C.E., 1984. Microbial Metabolism of Polycyclic Aromatic Hydrocarbons. *Advances in Applied Microbiology*, 30: 31-76.
- Cerniglia, C.E., 1992. Biodegradation of Polycyclic Aromatic Hydrocarbons. *Biodegradation*, 3: 351-368.
- Cerniglia, C.E., 1993. Biodegradation of Polycyclic Aromatic Hydrocarbons. *Current Opinions in Biotechnology*, 4: 331-338.
- Cerniglia, C.E., and M.A. Heitkamp. 1989. Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAH) in the Aquatic Environment. *In Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment*, U. Varanasi (Editor). CRC Press, Boca Raton, FL. 41-68 pp.
- Coates, J.D., R.T. Anderson, J.C. Woodward, E.J.P. Phillips, and D.R. Lovley. 1996a. Anaerobic Hydrocarbon Degradation in Petroleum-Contaminated Harbor Sediments Under Sulfate-Reducing and Artificially Imposed Iron-Reducing Conditions. *Environmental Science and Technology*, 30: 2784-2789.
- Coates, J.D., R.T. Anderson, and D.R. Lovley. 1996b. Oxidation of Polycyclic Aromatic Hydrocarbons Under Sulfate-Reducing Conditions. *Applied and Environmental Microbiology*, 62: 1099-1101.
- Coates, J.D., J. Woodward, J. Allen, P. Philip, and D.R. Lovley. 1997. Anaerobic Degradation of Polycyclic Aromatic Hydrocarbons and Alkanes in Petroleum-Contaminated Marine Harbor Sediments. *Applied and Environmental Microbiology*, 63(9): 3589-3593.
- Dolfing, J., J. Zeyer, P. Binder-Eicher, and R.P. Schwarzenbach. 1990. Isolation and Characterization of a Bacterium that Mineralizes Toluene in the Absence of Molecular Oxygen. *Archives in Microbiology*, 154: 336-341.
- Edwards, E.A., L.E. Wills, and D. Grbic-Galic. 1991. Anaerobic Degradation of Toluene and Xylene—Evidence for Sulfate as the Terminal Electron Acceptor. *In In situ Bioreclamation: Applications and Investigations for Hydrocarbon and Contaminated Site Remediation*, R.E. Hinchee and R. Olfenbuttel (Editors). Butterworth-Heinemann, Stoneham, MS. 463-471 pp.

- Erickson, L.E., and L.T. Fan. 1988. Anaerobic Degradation of Toxic and Hazardous Wastes. *In Handbook on Anaerobic Fermentations* L. E. Erickson and D. Y-C. Fung (Editors). Marcel Dekker, NY. 695-732 pp.
- Evans, W.C. 1977. Biochemistry of the Bacterial Catabolism of Aromatic Compounds in Anaerobic Environments. *Nature (London)*, 270: 17-22.
- Evans, P.J., D.T. Mang, K.S. Kim, and L.Y. Young. 1991. Anaerobic Degradation of Toluene by a Denitrifying Bacterium. *Applied and Environmental Microbiology*, 57: 1139-1145.
- Hambrick, G.A., R.D. DeLaune, and W.H. Patrick. 1980. Effect of Estuarine Sediment pH and Oxidation Reduction Potential on Microbial Hydrocarbon Degradation. *Applied Environmental Microbiology*, 40: 365-369.
- Harwood, C.S., and J. Gibson. 1997. Shedding Light on Anaerobic Benzene Ring Degradation: A Process Unique to Prokaryotes? *Journal of Bacteriology*, 179(2): 301-309.
- Healy, J.B., and L. Y. Young. 1979. Anaerobic Degradation of Eleven Aromatic Compounds to Methane. *Applied and Environmental Microbiology*, 38: 84-89.
- Heider, J., and G. Fuchs. 1997. Anaerobic Metabolism of Aromatic Compounds. *European Journal of Biochemistry*, 243: 577-596.
- Heider, J., A.M. Spormann, H.R. Beller, and F. Widdel. 1998. Anaerobic Bacterial Metabolism of Hydrocarbons. *FEMS Microbiology Reviews*, 22: 459-473.
- Heitkamp, M.A., and C.E. Cerniglia. 1987. Effects of Chemical Structure and Exposure on the Microbial Degradation of Polycyclic Aromatic Hydrocarbons in Freshwater and Estuarine Ecosystems. *Environmental Toxicology and Chemistry*, 6: 535-546.
- Heitkamp, M.A., and C.E. Cerniglia, 1988. Mineralization of Polycyclic Aromatic Hydrocarbons by a Bacterium Isolated from Sediment Below an Oil Field. *Applied and Environmental Microbiology*, 54: 1612-1614.
- Herbes, S.E. 1981. Rates of Microbial Transformation of Polycyclic Aromatic Hydrocarbons in Water and Sediments in the Vicinity of a Coal-Coking Wastewater Discharge. *Applied and Environmental Microbiology*, 41: 20-28.
- Herbes, S.E., and L.R. Schwall. 1978. Microbial Transformation of Polycyclic Aromatic Hydrocarbons in Pristine and Petroleum-Contaminated Sediments. *Applied and Environmental Microbiology*, 35(2): 306-316.
- Hutchins, S.R., G.W. Sewell, D.A. Kovacs, and G.A. Smith. 1991. Biodegradation of Aromatic Hydrocarbons by Aquifer Microorganisms under Denitrifying Conditions. *Environmental Science and Technology*, 25: 68-76.
- Johnson, K., and S. Ghosh. 1998. Feasibility of Anaerobic Biodegradation of PAHs in Dredged River Sediments. *Water Science and Technology*, 38(7): 41-48.
- Kaiser, J.P., and K.W. Hanselmaan. 1982. Fermentative Metabolisms of Substituted Monoaromatic Compounds by a Bacterial Community from Anaerobic Sediments. *Archives of Microbiology*, 133: 185-194.

- Langenhoff-Alette, A.M., A.J.B. Zehnder, and G. Schraa. 1996. Behavior of Toluene, Benzene, and Naphthalene Under Anaerobic Conditions in Sediment Columns. *Biodegradation*, 7(3): 267-274.
- Lovley, D.R., M.J. Baedeker, D.J. Lonergan, I.M. Cozzarelli, E.J.P. Phillips, and D.I. Siegel. 1989. Oxidation of Aromatic Compounds Coupled to Microbial Iron Reduction. *Nature (London)*, 339: 297-299.
- Lovley, D.R., J.C. Woodward, and F.H. Chapelle. 1994. Stimulated Anoxic Biodegradation of Aromatic Hydrocarbons Using Fe(III) Ligands. *Nature (London)*, 370: 128-131.
- Lovley, D.R., J.D. Coates, J.C. Woodward, and E.J.P. Phillips. 1995. Benzene Oxidation Coupled to Sulfate Reduction. *Applied and Environmental Microbiology*, 61: 953-958.
- Mahro, B., G. Schaefer, and M. Kastner. 1994. Pathways of Microbial Degradation of Polycyclic Aromatic Hydrocarbons in Soils, *In Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbons Compounds*, Hinchee, R. E., A. Leeson, L. Semprini, and S. K. Ong (Editors), Lewis Publishers, Boca Raton, FL.
- McNally, D.L., J.R. Mihelcic, and D.R. Lueking. 1998. Biodegradation of Three- and Four-Ring Polycyclic Aromatic Hydrocarbons Under Aerobic and Denitrifying Conditions. *Environmental Science and Technology*, 32: 2633-2639.
- McNally, D.L., J.R. Mihelcic, and D.R. Lueking. 1999. Biodegradation of Mixtures of Polycyclic Aromatic Hydrocarbons Under Aerobic and Nitrate-Reducing Conditions. *Chemosphere*, 38(6): 1313-1321.
- Meckenstock, R.U. 1999. Fermentative Toluene Degradation in Anaerobic-Defined Syntrophic Cocultures. *FEMS Microbiology Letters*, 177: 67-73.
- Meckenstock, R.U., E. Annweiler, W. Michaelis, H.H. Richnow, and B. Schink. 2000. Anaerobic Naphthalene Degradation by a Sulfate-Reducing Enrichment Culture. *Applied and Environmental Microbiology*, 66(7): 2743-2747.
- Mihelcic, J.R., and R.G. Luthy. 1988a. Degradation of Polycyclic Aromatic Compounds Under Various Redox Conditions in Soil-Water Systems. *Applied and Environmental Microbiology*, 54(5): 1182-1187.
- Mihelcic, J.R., and R.G. Luthy. 1988b. Microbial Degradation of Acenaphthene and Naphthalene Under Denitrification Conditions in Soil-Water Systems. *Applied and Environmental Microbiology*, 54(5): 1188-1198.
- Nedwell, D.B. 1982. The Cycling of Sulfur in Marine and Freshwater Sediments. *In Sediment Microbiology*, Nedwell, D. B. and C. M. Brown (Editors). Academic Press, New York, NY. 73-106 pp.
- Newport, P.H., and D.B. Nedwell. 1988. The Mechanisms of Inhibition of *Desulfovibrio* and *Desulfotomaculum* Species by Selenate and Molybdate. *Journal of Applied Bacteriology*, 65: 419-423.

- Odom, J.M., and R. Singleton, Jr. (Editors). 1993. *The Sulfate-Reducing Bacteria: Contemporary Perspectives*. Springer-Verlag, New York, NY. 289 p.
- Parker, W.J., and H.D. Monteith. 1995. Fate of Polynuclear Aromatic Hydrocarbons During Anaerobic Digestion of Municipal Wastewater Sludges. *Water Environment Research*, 67: 1052.
- Paul, E.A., and F.E. Clark. 1996. *Soil Microbiology and Biochemistry*. Academic Press, New York, NY. 340 p.
- Phelps, C.D., J. Kazumi, and L.Y. Young. 1996. Anaerobic Degradation of Benzene in BTEX Mixtures Dependent on Sulfate Reduction. *FEMS Microbiology Letters*, 145: 433-437.
- Phelps, C.D., L.J. Kerkhof, L.Y. Young. 1998. Molecular Characterization of a Sulfate-Reducing Consortium Which Mineralizes Benzene. *FEMS Microbiology Ecology*, 27: 269-279.
- Pradhan, S.P., J.R. Paterek, B.Y. Liu, J.R. Conrad, and V.J. Srivatsava. 1997. Pilot-Scale Bioremediation of PAH-Contaminated Soils. *Applied Biochemistry and Biotechnology*, 63-65(0): 759-773.
- Reinhard, M., L.W. Wills, H.A. Ball, and T. Harmon. 1991. A Field Experiment for the Anaerobic Biotransformation of Aromatic Hydrocarbons at Seal Beach, California. *In In situ Bioreclamation: Applications and Investigations for Hydrocarbon and Contaminated Site Remediation*, R. E. Hinchee and R. Olfenbuttel (Editors). Butterworth-Heinemann, Stoneham, MS. 487-496 pp.
- Rittman, B.E., and McCarty P.L. 2001. *Environmental Biotechnology: Principles and Applications*, McGraw Hill Publishers, New York. 754 p.
- Riser-Roberts, E. 1998. *Remediation of Petroleum-Contaminated Soils: Biological, Physical, and Chemical Processes*. Lewis Publishers, Boca Raton, FL. 542 p.
- Rockne, K.J., and S.E. Strand. 1998. Biodegradation of Bicyclic and Polycyclic Aromatic Hydrocarbons in Anaerobic Enrichments. *Environmental Science and Technology*, 32: 3962-3967.
- Rockne, K.J., J.C. Chee-Sanford, R.A. Sanford, B. Hedlund, J.T. Staley, and S.E. Strand. 1999. Naphthalene Degradation and Mineralization by Nitrate-Reducing and Denitrifying Pure Cultures. *In Bioremediation Technologies for Polycyclic Aromatic Hydrocarbon Compounds*, A. Leeson and B.C. Alleman (Editors). Battelle Press, Columbus, OH. 191-196 pp.
- Rockne, K.J., J.C. Chee-Sanford, R.A. Sanford, B.P. Helund, J.T. Staley, and S.E. Strand. 2000. Anaerobic Naphthalene Degradation by Microbial Pure Cultures Under Nitrate-Reducing Conditions. *Applied and Environmental Microbiology*, 66(4): 1595-1601.
- Rueter, P., R. Rabus, H. Wilkes, F. Aeckersberg, F.A. Rainey, H.W. Jannasch, and F. Widdel. 1994. Anaerobic Oxidation of Hydrocarbons in Crude Oil by New Types of Sulphate-Reducing Bacteria. *Nature (London)*, 372: 455-458.

- Sleat, R., and J.P. Robinson. 1984. The Bacteriology of Anaerobic Degradation of Aromatic Compounds. *Journal of Applied Bacteriology*, 57: 381-394.
- Taylor, B.F., W.L. Campbell, and I. Chinoy. 1970. Anaerobic Degradation of the Benzene Nucleus by a Facultatively Anaerobic Microorganism. *Journal of Bacteriology*, 102: 430-437.
- Vogel, T.M., and D. Grbic-Galic. 1986. Incorporation of Oxygen from Water into Toluene and Benzene During Anaerobic Fermentative Transformation. *Applied and Environmental Microbiology*, 52: 200-202.
- Watts, R. J. 1997. Hazardous Wastes: Sources, Pathways, and Receptors. John Wiley & sons, Inc, New York, 201-203 pp.
- Wilson, S., and K. Jones. 1993. Bioremediation of Soil Contaminated with Polynuclear Aromatic Hydrocarbons (PAHs): A Review. *Environmental Pollution*, 81: 229-249.
- Wilson, L.P., P.C. D'Adamo, and E.J. Bouwer. 1997. Bioremediation of BTEX, Naphthalene, and Phenanthrene in Aquifer Material Using Mixed Oxygen/Nitrate Electron Acceptor Conditions. *Project Summary, US EPA/600/SR-97/103*, National Risk Management Research Laboratory, Ada, OK 74820. 5 p.
- Zeyer, J., E.P. Kuhn, and R. P. Schwarzenbach. 1986. Rapid Microbial Mineralization of Toluene and 1,3-dimethylbenzene in the Absence of Molecular Oxygen. *Applied and Environmental Microbiology*, 52: 944-947.
- Zhang, X., and L.Y. Young. 1997. Carboxylation as an Initial Reaction in the Anaerobic Metabolism of Naphthalene and Phenanthrene by Sulfidogenic Consortia. *Applied and Environmental Microbiology*, 63(12): 4759-4764.

Original manuscript received: September 18, 2000

Revised manuscript received: October 3, 2001