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Cooxidation of naphthalene and other polycyclic aromatic hydrocarbons by the nitrifying bacterium, *Nitrosomonas europaea*

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Abstract

The soil nitrifying bacterium *Nitrosomonas europaea* has shown the ability to transform cometabolically naphthalene as well as other 2- and 3-ringed polycyclic aromatic hydrocarbons (PAHs) to more oxidized products. All of the observed enzymatic reactions were inhibited by acetylene, a selective inhibitor of ammonia monooxygenase (AMO). A strong inhibitory effect of naphthalene on ammonia oxidation by *N. europaea* was observed. Naphthalene was readily oxidized by *N. europaea* and 2-naphthol was detected as a major product (85%) of naphthalene oxidation. The maximum naphthol production rate was 1.65 nmole/mg protein-min in the presence of 240 μM naphthalene and 10 mM NH_4^+. Our results demonstrate that the oxidation between ammonia and naphthalene showed a partial competitive inhibition. The relative ratio of naphthalene and ammonia oxidation, depending on naphthalene concentrations, demonstrated that the naphthalene was oxidized 2200-fold slower than ammonia at lower concentration of naphthalene (15 μM) whereas naphthalene was oxidized only 100-fold slower than ammonia oxidation. NH_4^+- and N_2H_4-dependent O_2 uptake measurement demonstrated irreversible inhibitory effects of the naphthalene and subsequent oxidation products on AMO and HAO activity.

Introduction

Polyaromatic hydrocarbons (PAHs) are ubiquitous compounds that originate from processes involving the pyrolysis of organic matter. Representative processes include natural phenomena such as forest fires and anthropogenic processes such as the refining and combustion of hydrocarbon fuels (Bouchez 1995). Because many PAHs and their derivatives are recognized as either toxic, mutagenic or carcinogenic there is considerable interest in processes that can either remediate environmental contamination by these compounds or minimize release.

Biological processes have considerable potential for the degradation and detoxification of PAHs. Under aerobic conditions a wide variety of microorganisms are known to utilize simple PAHs as carbon and energy sources for growth. However, because PAHs often occur at low concentrations they may not support extensive growth and activity of organisms capable of fully mineralizing these compounds. The biodegradation of PAHs may also be further limited by the low bioavailability of these hydrophobic compounds resulting from their absorption to soils and sediments (Mihelcic 1988). In addition to organisms that utilize PAHs as growth substrates there are also several examples of microorganisms that can transform PAHs while utilizing other substrates to support growth. In the case of eukaryotic organisms PAHs are often subject to detoxification processes involving an enzymatic transformation by non-specific oxygenases followed by excretion of the transformed PAH as a derivatized water-soluble product (Walter 1991; Weissenfels 1991). In the case of prokayotic organisms PAHs are also known to be enzymatically transformed by non-specific oxygenases but in most instances these

appear to be fortuitous reactions that arise through a lack of enzyme specificity and consequently serve no apparent benefit to the organism. These reactions are commonly regarded as cometabolic transformations. The cometabolic transformation of PAHs are known to be catalyzed by bacteria grown on compounds either structurally related to PAHs such as other PAHs (Bouchez 1995) and phenol (Chang et al. 1997) and structurally unrelated compounds such as methane (Brusseau 1990).

In the present study we have examined the PAH-oxidizing activity of the soil nitrifying bacterium *Nitrosomonas europaea* as another example of a cometabolically active organism grown on a substrate structurally unrelated to PAHs. As an obligately lithoautotrophic soil nitrifying bacterium *N. europaea* obtains all of its energy for growth from the oxidation of ammonia (NH_3) to nitrite and satisfies all of its carbon requirements through the fixation of CO_2 (Wood et al. 1986). The overall oxidation of ammonia to nitrite is achieved by the activities of two enzymes. Ammonia is initially oxidized to hydroxylamine (NH_2OH) by the membrane-bound enzyme ammonia monooxygenase (AMO). The further oxidation of hydroxylamine to nitrite is catalyzed by the multi-heme-containing periplasmic enzyme, hydroxylamine oxidoreductase (HAO). During steady state ammonia oxidation the electrons required to sustain AMO activity are provided by electrons released during hydroxylamine oxidation (Wood et al. 1986). While the substrate range of HAO is restricted to small molecules such as hydroxylamine, hydrazine and N-methylhydroxylamine (Hyman & Wood 1983), AMO is known to oxidize a wide range of non-growth supporting hydrocarbons. For instance, it has been demonstrated that AMO in whole cells of *N. europaea* can oxidize, among other compounds, *n*-alkanes and *n*-alkenes (Hyman 1988), chlorinated hydrocarbons (Rasche 1990, 1991), alkyl ethers (Hyman 1994) and thioethers (Juliette 1993). There is also evidence that indicates AMO can catalyze reductive reactions in the absence of oxygen (Abeliovch & Vonshak 1992). Most recently attention on the substrate range of AMO has focused on the oxidation of monoaromatic compounds (Keener & Arp 1994). These studies have extended the substrate range of AMO from benzene and phenol to include a wide range of monosubstituted aromatics including iodobenzene, chlorobenzene and bromobenzene. One of these studies has also briefly indicated that *N. europaea* can oxidize naphthalene (Keener & Arp 1994). In the present study we have extended these preliminary observations and have examined the substrate range of AMO with respect to a range of simple PAH compounds. We have also examined the kinetics of naphthalene degradation including an investigation of the effects of substrate and co-substrate concentration on the cooxidation.

Materials and methods

Materials

Naphthalene, 1-naphthol, 2-naphthol, 2-methylnaphthalene, 2-methylnaphthenol, 2-methylnaphthaldehyde, acenaphthalene, acenaphthene, fluorene, anthracene, phenanthrene, acenaphthenone and acetonitrile were obtained from Aldrich Co. (Milwaukee, WI). Allythiourea was obtained from Eastman Kodak Co. (Rochester, NY). All other chemicals were of reagent grade.

Growth of Nitrosomonas europaea

Cells of *N. europaea* (ATCC 19178) were grown in batch cultures (1.5 l) at 30 °C in mineral salts medium, as described previously (Hyman & Wood 1985). After growth for 3 days the cells were harvested by centrifugation and suspended in phosphate buffer (50 mM sodium buffer [pH 7.8], 2 mM $MgSO_4$), as described previously (Hyman et al. 1985). In all cases, cell suspensions (0.2 g wet wt/ml) were stored in the dark at 4 °C and used within 24 h of harvesting.

PAH degradation studies

Experiments examining the degradation of PAHs by *N. europaea* were conducted in glass serum vials (37 ml) sealed with Teflon-lined silicone septa (Sun Broker™, Wilmington, NC). In experiments with naphthalene the reaction vials contained sodium phosphate buffer (10 ml, as above) and the required concentration of naphthalene added from a stock solution (0.164 M) in dimethyl sulfoxide (DMSO). The vials were placed in shaking water bath (30 °C, 200 rpm) for 10 min to allow complete dissolution of naphthalene in the buffer. With all other PAHs examined saturated concentrations of each compounds were added. In all cases the reactions were initiated by addition of cells (100 μl; ca. 2 mg of protein) and the vials were then returned to the shaker. To establish the role of AMO in PAHs transformations control incubations were conducted as

above contained cells suspensions treated with acetylene (1% v/v), a specific and irreversible inactivator of AMO (Hyman & Wood 1985)

O_2 uptake measurement

The effects of PAHs and their oxidation products on the activities of the two enzymes involved in ammonia oxidizing activity were determined by examining rates of NH_4^+- and N_2H_4-dependent O_2 uptake, as described previously (Chang 1998). In summary, O_2 uptake measurements were made using a Clark-style O_2 electrode (Yellow Springs, OH) mounted in reaction chamber (1.8 ml) of a glass water-jacketed vessel. Cells (100 μl) exposed to PAHs, or their oxidation products (see individual experiments for details), were sedimented by centrifugation in a microfuge (10,000 rpm for 2 min) and resuspended in fresh buffer (1.5 ml). Samples of these washed cells (50 μl) were added to buffer in the O_2 electrode reaction chamber and the rate of O_2 uptake activity was determined after the addition of $(NH_4)_2SO_4$ (5 mM). This rate of O_2 uptake was taken as a measure of the AMO activity of the cells. Subsequently, allylthiourea (ATU) (100 μM) was added to fully inhibit further NH_4^+-dependent O_2 uptake activity and after 2 min N_2H_4 (600 μM) was added to the cells and the new rate of O_2 uptake was determined. This rate was taken as a measure of the HAO activity of the cells. All substrates and inhibitors were added from aqueous stock solutions using microsyringes. The solubility of O_2 in air-saturated buffer was taken as 230 μM (Truesdale & Dowing 1954).

Analytical methods

The consumption of PAHs and the accumulation of PAH oxidation products was determined using high-performance liquid chromatography (HPLC). The HPLC apparatus consisted of an Altex Model 110A pump, a UV detector, a reversed-phase ultramex C_{18} column (150 × 4.60 mm; Phenomex). 200 μl samples of the reaction mixtures were transferred into 1.5 ml Eppendorf tubes, and cells were removed by centrifugation. 100 μl samples of the supernant were injected into HPLC. The HPLC column eluted under isocratic conditions at a flow rate of 1.5 ml min^{-1} using an acetonitrile : deionized water mobile phase (1 : 1 vol : vol). The eluted compounds were detected by UV absorption at 254 nm and were identified by coelution with authentic compounds and by GC-MS analysis of eluted compounds.

A 5-ml sample of the reaction mixtures was extracted by hexane (5 ml) and 2 μl of hexane solution was then injected into a Hewlett Packard model 5890 gas chromatograph fitted with an XTI-5 fused silica capillary column and a Hewlett Packard model 5988 mass spectrometer. The gas chromatograph was operated with a initial column temperature of 50 °C (1 min) which was subsequently increased to 300 °C, at a rate of 10 °C/min. The injector and detector temperatures were 290 and 315 °C respectively and helium (20 ml/min) was used as the carrier gas.

Nitrite was determined colorimetrically as described previously (Hageman & Hucklesby 1971). Protein concentrations were determined by the Biuret assay (Gornall 1949) after solubilization of cell protein in aqueous 3 N NaOH (30 min at 60 °C) and sedimentation of insoluble material by centrifugation (14, 000× g, 5 min).

Results

Inhibitory effect of naphthalene on the rate of ammonia oxidation

Several previous studies with *N. europaea* have demonstrated that alternative substrates for AMO can often be identified by determining whether the candidate compound exerts an inhibitory effect on the rate of ammonia oxidation (Juliette 1993; Keener & Arp 1993). This inhibitory effect occurs because of competitive interactions between ammonia and the alternative substrate for oxidation by AMO and the effects of this competition can be most easily detected by examining the effects of the alternative substrate on the rate of accumulation of nitrite as the ultimate product of ammonia oxidation (Juliette 1993). In the absence of naphthalene the cells oxidized all of the added ammonium (10 mM) to nitrite within 1 h (Figure 1). However, in the presence of 80 μM naphthalene only approximately 4 mM nitrite was generated after 1.5 h and cells incubated with 240 μM naphthalene generated less than 2 mM nitrite over the same time period. Cells incubated with acetylene and naphthalene (240 μM) did not produce nitrite (<25 μM) due to previously characterized inactivating effect of acetylene on AMO activity (Hyman & Arp 1992; Hyman & Wood 1985).

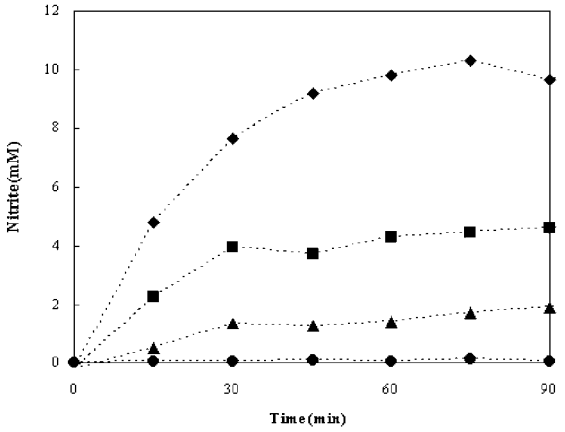

Figure 1. Time course of effect of naphthalene concentration on nitrite production by *N. europaea*. Cells were incubated in serum bottles with 10 mM NH_4^+ and various naphthalene concentrations of 0 μM (♦), 80 (■), 240 (▲), and 240 (●). In the case of symbol (●), acetylene (1% v/v) was also added. Aliquots (0.3 ml) of the reaction bottles were removed at the indicated time points, and the reaction was terminated with ATU (to 100 μM).

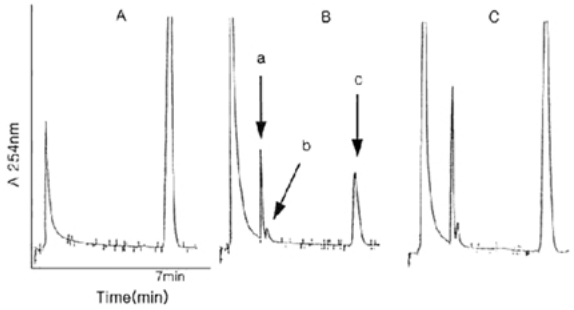

Figure 2. HPLC profile of naphthalene degradation. The samples were analyzed after 2 h incubation from previous experiment as described in the section "Materials and methods". Each chromatogram represents samples incubated in the presence of cells with (A) 240 μM naphthalene and $C2H_2$ (1% v/v) (B) 80 μM naphthalene (C) 240 μM naphthalene. (a) 2-naphthol, (b) 1-naphthol, (c) naphthalene.

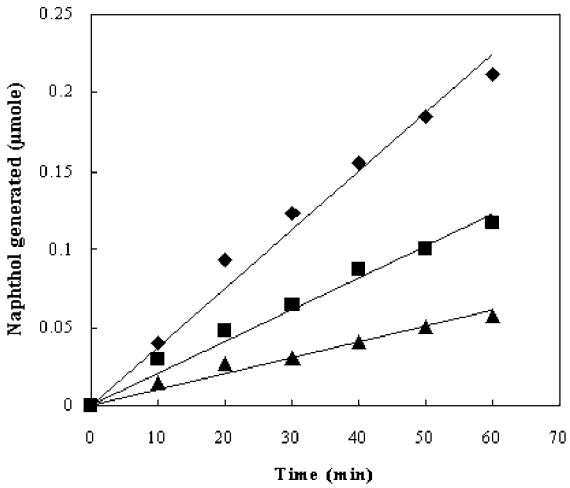

Figure 3. Time course of naphthalene oxidation to naphthols by *N. europaea*. Assays were conducted as described in Materials and Methods in the presence of 80 (▲), 120 (■), and 240 (♦) μM of naphthalene with 10 mM NH_4^+ and cells.

The oxidation of naphthalene by N. europaea

A subsequent analysis of the reaction media generated during the experiment described in Figure 1 confirmed that naphthalene was oxidized by *N. europaea* and that the degree of inhibition of nitrite production was related to the quantity of naphthalene oxidized (Figure 2). For example, For cells incubated with 80 μM naphthalene we observed the production of two compounds (Figure 2B) that were identified as 1- and 2-naphthol by coelution with the authentic compounds and by HPLC and GC-MS analyses. Our HPLC analysis indicates 2-naphthol represented 85% of the detected two naphthols. The 5.5 : 1 ratio of 2- and 1-naphthol production was also observed for the incubation conducted with 240 μM naphthalene, although 2-fold higher concentrations of both compounds were generated relative to the incubation conducted with 80 μM naphthalene (Figure 2C). No oxidations products were detected for cells incubated with both acetylene and 240 μM naphthalene (Figure 2A).

The effect of ammonia and naphthalene concentration on the production rate of nitrite and naphthols

As our preliminary experiments indicated naphthalene was readily degraded by *N. europaea* we were interested to determine the conditions that would support the maximal rate of naphthalene oxidation. To establish true rates of naphthalene degradation it was necessary to determine the period of time over which the rate of naphthol production was constant. The time course of naphthol production was initially investigated for cells incubated with NH_4^+ (10 mM) and a range of naphthalene concentrations, up to the limit of the aqueous solubility of this compound at 30 °C (240 μM). Our results (Figure 3) demonstrate that the rate of naphthol production increased with increasing concentrations of naphthalene. However, the rate of naphthol production was only constant during the initial portion (0–20 min) of the reaction and tended to decrease as the reaction progressed, especially in the incubation with the highest naphthalene concentration.

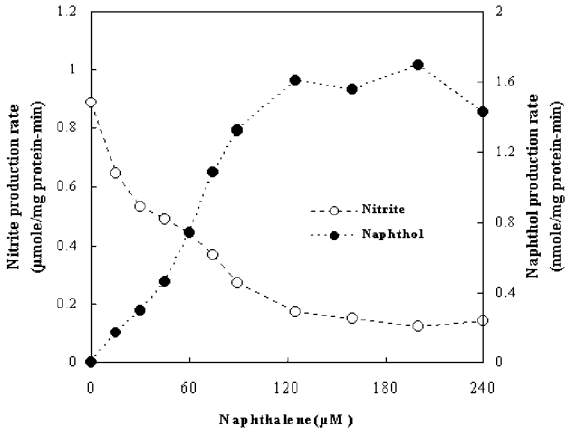

Figure 4. Effect of naphthalene concentration on naphthol and nitrite production rate after exposure of cells to different naphthalene amount for 2 h. Cells were exposed to a range of naphthalene, and naphthol (●) and nitrite(○) productions generated in the same incubations were measured.

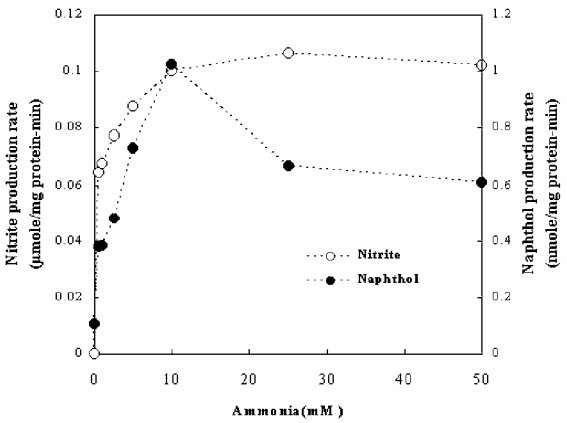

Figure 5. Effect of NH_4^+ concentration on naphthalene oxidation rate after exposure of cells to 150 μM naphthalene for 2 h. Cells were exposed to naphthalene, and naphthol (●) and nitrite (○) productions generated in the same incubation conditions were measured as described in the section "Materials and methods".

Using initial rates of naphthol production (0–20 min) we subsequently examined the effect of naphthalene concentration on both the rate of naphthol production and the concurrent rate of nitrite production. The rate of naphthol production generally increased with increasing concentrations of naphthalene but tended to saturate at higher naphthalene concentrations (Figure 4). As expected from our previous experiment (Figure 1) the rate of nitrite production also progressively decreased with increasing naphthalene concentration. The maximal rate of naphthalene oxidation observed in this experiment was approximately 1.6 nmole/min/mg protein. An analysis of the relative rates of ammonia and naphthalene oxidation indicates that naphthalene was oxidized greater than 3000-fold slower than ammonia at the lowest concentration of naphthalene tested (17.6 μM) whereas at the highest concentration (240 μM) naphthalene was only oxidized 100-fold slower than ammonia.

Because of the apparent competitive interactions between ammonia and naphthalene we were also interested to determine the effect of ammonia concentration on the rate of naphthol production. To prevent the complete oxidation of low concentrations of ammonia we examined naphthol production using a fixed concentration of naphthalene (150 μM) in short term incubations (10 min) using ammonia concentrations between 0 and 50 mM (Figure 5). Our results demonstrate that the rate of ammonia oxidation progressively increased with increases in ammonia concentration up to 10 mM NH_4^+. Above this concentration the rate of nitrite production remained almost constant despite a 5-fold increase in ammonia concentration. In contrast, the naphthol production rate progressively increased with increases in ammonia concentration up to 10 mM and subsequently decreased to a lower but constant rate with higher ammonia concentrations.

Toxic effects associated with naphthalene oxidation on AMO and HAO

Our previous studies with several alternative hydrocarbon substrates for AMO have indicated that toxic effects of these compounds often limit the ability of *N. europaea* to sustain cometaobolic transformation reactions (Keener & Arp 1993). Potential toxic effects associated with naphthalene oxidation were examined by incubating cells with a range of concentrations of naphthalene and an equivalent range of concentrations of the two identified naphthalene oxidation products, 1- and 2-naphthol. After exposure to these compounds the cells were washed and the residual levels of AMO and HAO-dependent O_2 uptake activities were determined. Our results (Figure 6) demonstrate that all of the compounds led to a small and roughly equivalent irreversible inhibitory effect on AMO activity, as measured by NH_4^+-dependent O_2 uptake (Figure 6A). The maximal inhibitory effect (ca. 35%) was observed with the highest concentrations of naphthalene or naphthols added (200 μM). In contrast, a larger effect of naphthols relative to naphthalene as observed when we examined HAO activity, as measured by N_2H_4-dependent O_2 uptake activity (Figure 6B).

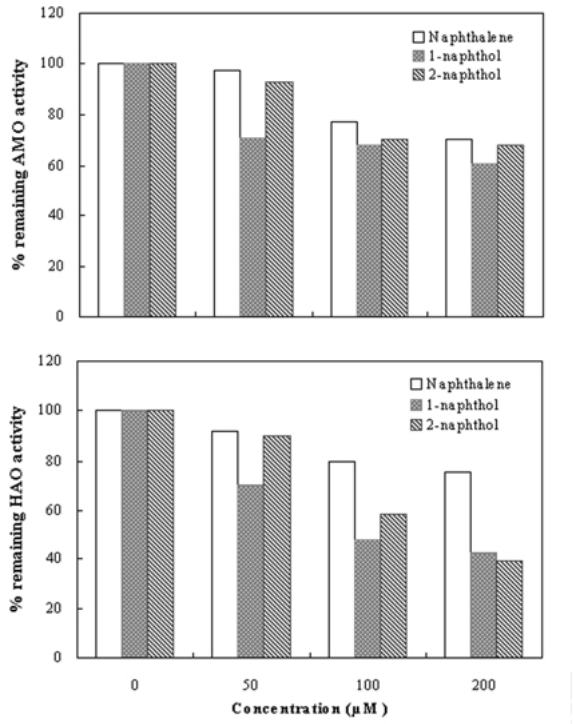

Figure 6. Inhibition of ammonium-dependent O_2 uptake in whole cells of *N. europaea* by naphthalene. O_2 uptake was monitored as described in the section "Materials and methods". The reaction was initiated by the addition of 5 mM $NH_4(SO_4)_2$ with whole cells to a final concentration of 1.1 mg of protein per ml. After a steady rate of O_2 uptake was established, 100 μM ATU was added to stop AMO reaction. And then, 600 μM hydrazine was added to catalyze HAO reaction. Finally, The % activities of AMO and HAO for different naphthalene amounts were calculated.

Degradation of other PAH compounds by N. europaea

As a final aspect of this study we were interested to determine how extensive the substrate range of AMO is with respect to PAH compounds. To investigate this individual PAHs were incubated with *N. europaea* for 24 h in incubations containing approximately 25 μM of the more water soluble PAHs (2-methylnaphthalene, acenaphthalene and acenaphthene) or saturating concentrations of the larger, more insoluble compounds (fluorene, anthracene, phenanthrene). After 24 h the cells were removed from the reaction mixture by centrifugation and the supernatant was extracted and analyzed by GC-MS, as descibed in the Methods section. We observed the production of two metabolites for cells incubated with 2-methylnaphthalene. One product (35% of the total ion chromatogram) had an Rt of 19.5 min, an ion peak (M^+ at m/z 158), and fragmentation ions (130 [M^+–CO]). These properties were identical to those of authentic 2-methyl naphthalenol. The other metabolite was identified 2-methylnaphtaldehyde on the basis of molecular weight (M^+ at m/z 156), mass spectrum (128 [M^+–CO], 101 [M^+–COOH]) and Rt of 17.4 min. Cells incubated with acenaphthalene and acenaphthene both yielded one major metabolite when the reaction medium was extracted under neutral conditions. In both cases the metabolite eluted with an Rt of 19.9 min and exhibited a mass spectrum (M^+ at m/z 168), and fragmentation ions (at m/z 140 [M^+–CO], 113, and 98). These properties were identical to those exhibited by authentic acenaphthenone. The generation of all of these metabolites was subsequently shown to be inhibited by the presence of acetylene (data not shown). In contrast, no oxidation products were observed for cells incubated with fluorene, anthracene or phenanthrene either in the presence of absence of acetylene.

Discussion

Previous studies of the substrate range of AMO in *N. europaea* have established that this enzyme is capable of oxidizing a wide range of non-growth supporting substrates, including many aromatic compounds. The results of this study now extend the known susbstrate range of AMO to include 4 PAH compounds. Our evidence that these oxidation reactions are catalyzed by AMO and the broader implications of our observations are discussed below.

Inhibitory effects of PAHs

Alternative substrates for AMO have been demonstrated to have several effects on the ammonia-oxidizing activity of *N. europaea*. An inhibition of nitrite production by alternative substrates for AMO is one of these effects. The simplest model to account for this inhibition of nitrite production is that the alternative AMO substrate excludes the binding and oxidation of ammonia at the active site of AMO. This in turn leads to a decreased rate of nitrite production from hydroxylamine, the immediate product of ammonia oxidation by AMO. While a simple competitive model has been shown to account for the interactions between ammonia and small substrates such as methane and ethylene, the effects of larger substrates on nitrite production have been shown to exhibit more complex behaviors (Hyman & Wood 1983, 1984). Part of the complexity of these interactions is due to the fact

Table 1. Oxidation products of other PAHs by *N. europaea*

Substrate	Amount (μM) added	Product detected	Trans-formation	% of O_2 uptake activity remaining after incubation with individual PAH and ammonia for 2 h	
				NH_3-dependent	N_2H_4-dependent
Naphthalene	27.3	1-Naphthol (14%) 2-Naphthol (86%)	Yes	81.1	88.9
2-Methyl naphthalene	24.3	2-Methyl naphthalenol (84%) 2-Methyl aphthaldehyde (16%)	Yes	85.8	79.0
Acenaphthalene	23[1]	Acenaphthenone	Yes	82.8	79.3
Acenaphthene	23[1]	Acenaphthenone	Yes	73.6	74.7
Fluorene	9	ND	No	95.0	87.0
Anthracene	0.45[1]	ND	No	86.3	86.9
Phenanthrene	8.4[1]	ND	No	89.4	98.6

[1] Saturated amount were added.
ND: Not detected.

that AMO is a reductant-requiring enzyme and that the ultimate source of reductant for AMO is generated by hydroxylamine oxidation.

Oxidation of naphthalene and other PAHs by N. europaea

An important contribution of this study has been to further characterize the substrate range of AMO towards aromatic compounds. In most instances the products we have identified in this study are compatible with a single monooxygenase-catalyzed reaction. For example, the oxidation of naphthalene to naphthols is substantially similar to the previously characterized oxidation of benzene to phenol and the hydroxylation reactions that occur with other substituted aromatics (Keener & Arp 1994). The production of 2-methyl naphthalenol from 2-methyl naphthalene is also compatible with AMO activity whereas the production of 2-methyl naphthaldehyde is likely due to a dehydrogenase activity similar to the activity previously suggested to be responsible for the transformation of toluene to benzaldehyde (Keener & Arp 1994).

While the products obtained from PAH oxidation by *N. europaea* are compatible with a monooxygenase type reaction, the position of the hydroxylation of naphthalene is unusual in that 2-naphthol represents the major hydroxylated product. In contrast, the cytochrome P-450-dependent oxidation of naphthalene by various fungi is known to generate 1-naphthol in excess over 2-naphthol. Similarly, naphthalene cometabolism by both phenol-oxidizing (Lee 1998) and methantrophic (Brusseau 1990) bacteria is known to generate predominantly 1-naphthol. In the case of methanotrophs it is also important to note that naphthalene-oxidizing activity is restricted to organisms expressing the soluble methane monooxygenase system, not the particulate form of this enzyme which is structurally very similar to AMO in *N. europaea*. It is also important to note that the unusual production of 2-naphthol by AMO is another example of the unusual catalytic activity of this enzyme. For example, the relative proportions of 1- and 2-ols generated by *N. europaea* during the oxidation of *n*-alkanes is the opposite to that encountered with sMMO-catalyzed reactions (Brusseau 1990).

Potential applications

Much of the interest in the microbial transformation of PAH compounds aims to address the potential application of microorganisms for the remediation of PAH contamination. Our observation that a soil nitrifying bacterium can readily transform several PAH compounds suggests that these organisms in general may be able to promote the degradation. In general unmodified PAH compounds are thought to be largely resistant to degradation under anaerobic conditions (Galushko 1999). The biological stability of these compounds is a reflection of the chemical stability that is conferred by the delocalized electron

distribution within the underivatized aromatic nuclei. A clear example of this is seen with benzene and phenol. Whereas benzene is very poorly degradable under anaerobic conditions (Wilson 1986), there are numerous reports of anaerobic phenol-utilizing anaerobes (Lovley & Lonergan 1990; Bossert 1989). While ammonia-oxidizing bacteria are clearly incapable of mineralizing PAHs under aerobic conditions they may be able to contribute to the overall degradation of PAHs by catalyzing hydroxylation reactions that effectively destabilize the polyaromatic nuclei and thereby make these compounds more susceptible to degradation under anaerobic conditions. In the case of ammonia-oxidizing bacteria this "priming" activity could also be potentially coupled with denitrifying systems that could utilize the hydroxylated PAHs as electron donors and could utilize the nitrite or nitrate generated by the initial nitrification reaction as electron acceptors. Again, there is a clear president set for this in that phenol utilizing denitrifiers are well known (Tschech & Fuches 1987). Given the limited PAH substrate range demonstrated in this study this sort of coupled process could not be applied to efforts to remediate contamination by larger and often more toxic PAHs. However, this approach could potentially be applied to environments where the predominant PAHs are simple compounds such as naphthalene and other two carbon PAHs such as those found in creosote.

References

Abeliovich A & Vonshak A. (1992) Anaerobic metabolism of *Nitrosomonas europaea*. Archives of Microbiol. 158: 267–270.

Bossert ID, Whited G, Gibson DT & Young LY (1989) Anaerobic oxidation of p-cresol mediated by a partially purified methylhydroxylase by a denitrifying bacterium. J. Bacteriol. 171: 2956–2962.

Bouchez M, Blanchet D & Vandecasteele JP (1995) Degradation of polycyclic aromatic hydrocarbons by pure strains and by defined strain associations: Inhibition phenomena and cometabolism. Appl. Microbiol. Biotechnol. 43: 156–164.

Brusseau GA, Tsien Hsien-Chyang, Hanson RS & Wackett LP (1990) Optimization of trichloroethylene oxidation by methanotrophs and the use of a colorimetric assay to detect soluble methane monooxygenase activity. Biodegradation 1: 19–29.

Chang SW (1997) Cometabolic biodegradation of polycyclic aromatic hydrocarbons (PAHs) and aromatic ether by phenol- and ammonia-oxidizing bacteria. Ph.D. Dissertation, Oregon State University, Corvallis, Oregon.

Chang SW, Hyman MR & Williamson KJ (1998) Nitrifying bacteria as "priming catalysts" for the "biodegradation of xenobiotics" In: Wickramanayake GB & Hinchee RE (Eds) Natural Attenuation: Remediation of Chlorinated and Recalcitrant Compounds (pp 51–55). Battelle Press, Columbus, OH.

Galushko A, Minz D, Schink B & Widdel F (1999) Anaerobic degradation of naphthalene by a pure culture of novel type of marine sulfate-reducing bacterium. Environ. Microbiol 1(5): 415–420.

Gornall AG, Bardawill CJ & David MM (1949) Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177: 751–766.

Hageman RH & Hucklesby DP (1971) Nitrate reductase from higher plants. Methods Enzymol. 23: 491-503.

Hyman MR & Arp DJ (1992) $^{14}C_2H_2$- and $^{14}CO_2$-labeling studies of the *de novo* synthesis of polypeptides by *Nitrosomonas europaea* during recovery from acetylene and light inactivation of ammonia monooxygenase. J. Bacteriol. 267(3): 1534–1545.

Hyman MR & Wood PM (1983) Methane oxidation by *Nirosomonas europaea*. Biochem. J. 212: 31-37.

Hyman MR & Wood PM (1984) Ethylene oxidation by *Nirosomonas europaea*. Arch. Microbiol. 137: 155–158.

Hyman MR & Wood PM (1985) Suicidal inactivation and labelling of ammonia mono-oxygenase by acetylene. Biochem. J. 227: 729–725.

Hyman MR, Murton IB & Arp DJ (1988) Interaction of ammonia monooxygenase from *Nitrosomas europaea* with alkanes, alkenes, and alkynes. Appl. Environ. Microbiol. 54(12): 3187–3190.

Hyman MR, Page CL & ARP DJ (1994) Oxidation of methyl fluoride and dimethyl ether by ammonia monooxygenase in *Nitrosomonas europaea*. Appl. Environ. Microbiol. 60(8): 3033–3035.

Juliette LY, Hyman MR & Arp DJ (1993) Inhibition of ammonia oxidation in *Nitrosomonas europaea* by sulfure compounds: Thioethers are oxidized to sulfoxides by ammonia oxygenase. Appl. Environ. Microbiol. 59(11): 3718–3727.

Keener WK & Arp DJ (1993) Kinetic studies of ammonia monooxygenase inhibition in *Nitrosomonas europaea* by hydrocarbons and halogenated hydrocarbons in an optimized whole-cell assay. Appl. Environ. Microbiol. 59(8): 2501–2510.

Keener WK & Arp DJ (1994) Transformations of aromatic compounds by *Nitrosomonas europaea*. Appl. Environ. Microbiol. 60(6): 1914–1920

Lee TJ, Chang SW, Hyman MR & Williamson KJ (1998) Cometabolic degradation of polycyclic aromatic hydrocarbons (PAHs) by a phenol-oxidizing bacteria. In: Proceeding of the 2nd International Conference of Advanced Wastewater Treatment, Recycling and Reuse, Vol. 2 (pp 607–615).

Lovley DR & Lonergan DJ (1990) Anaerobic oxidation of toluene, phenol, *p*-cresol by the dissimilatory iron-reducing organism, GS-15. Appl. Environ. Microbiol. 56(6): 1858–1864.

Mihelcic JR & Luthy RG (1988) Degradation of polycyclic aromatic hydrocarbon compounds under various redox conditions in soil-water systems. Appl. Environ. Microbiol. 54: 1182–1187.

Rasche ME, Hicks RE, Hyman MR & Arp DJ (1990) Oxidation of monohalogenated ethanes and *n*-chlorinated alkanes by whole cells of *Nirosomonas europaea*. J. Bacteriol. 172(9): 5368–5373.

Rasche ME, Hyman MR & Arp DJ (1990) Biodegradation of halogenated hydrocarbon fumigants by nitrifying bacteria. Appl. Environ. Microbiol. 56(8): 2568–2571.

Rasche ME, Hyman MR & Arp DJ (1991) Factors limiting aliphatic chlorocarbon degradation by *Nitrosomonas europaea*: Cometabolic inactivation of ammonia monooxygenase and substrate specificity. Appl. Environ. Microbiol 57(10): 2986–2994.

Truesdale GA & Downing AL (1954) Solubility of oxygen in water. Nature (London) 173: 1236.

Tschech A & Fuches G (1987) Anaerobic degradation of phenol by pure cultures of newly isolated denitrifying pseudomonas. Arch Microbiol. 148: 213–217.

Walter U, Beyer M, Klein J & Rehm HJ (1991) Degradation of pyrene by *Rhodococcus* sp. UW1. Appl. Microbiol. Biotechnol. 34: 671–676.

Weissenfels WD, Klein M, Beyer J & Rehm HJ (1991) Microbial metabolism of fluoranthene: Isolation and identification of ring fission products. Appl. Microbiol. Biotechnol. 34: 528–535.

Wilson BH, Smith GB & Rees JF (1986) Biotransformation of selected alkylbenzenes and halogenated aliphatic hydrocarbons in methanogenic and aquifer materials: A microcosm study. Environ. Sci. Technol. 20: 997–1002.

Wood PM (1986) Nitrification as a bacterial energy source. In: Prosser JI (Ed) Nitrification (pp 39–62). IRL Press, Oxford.