Reprinted from RESEARCH JOURNAL WATER POLLUTION CONTROL FEDERATION
Alexandria, VA 22314-1994
Printed in U.S.A.

Enhanced biodegradation of polyaromatic hydrocarbons in the activated sludge process

Lynne J. Cardinal, Michael K. Stenstrom

ABSTRACT: Naphthalene and phenanthrene were degraded in a variant of the activated sludge process, called an enricher reactor system. In this system a second biological reactor is used to grow cells especially prepared to degrade the target compound. The cells are then added to the main reactor as a form of bioaugmentation. The new system showed enhanced removals and it is concluded that it is a promising way to improve activated sludge process efficiency. *Res. J. Water Pollut. Control Fed.*, **63,** 950 (1991).

KEYWORDS: activated sludge, biodegradation, enhancement, naphthalene, phenanthrene.

The passage of amendments to the Clean Water Act in 1972 has necessitated that municipalities construct some type of secondary wastewater treatment facility. Many use the activated sludge process to remove organic soluble and colloidal pollutants. Biodegradable material is oxidized to carbon dioxide and water or converted to biomass. Nonbiodegradable pollutants may pass through the process but are often removed from the liquid phase, either by adsorption onto biological flocs, which are subsequently removed in secondary clarifiers, or by volatilization to the atmosphere.

Secondary wastewater treatment facilities in publicly owned treatment works (POTWs) were designed to treat municipal wastewaters that are composed primarily of biodegradable materials from domestic sources such as human wastes, kitchen wastes, and washing byproducts. Consequently, the process has been optimized for treating these easily biodegraded materials. Industries often operate their own treatment or pretreatment facilities, but in large urban areas like Los Angeles, they frequently discharge their pretreated effluents directly into POTWs. For this reason, POTWs effectively become industrial treatment facilities and may be subjected to upsets and hazardous or toxic compounds because pretreatment facilities can never be 100% efficient.

The preferred method for treating liquid-borne hazardous substances is to remove them from wastewater, concentrate them to reduce volume, and recycle or destroy them in an approved facility. This approach is not possible in all cases because wastewaters that contain low concentrations of priority pollutants or regulated compounds are too numerous to economically treat. A good example is the controversy that surrounds the 301H ocean waivers for the Los Angeles City and County treatment plants. Many of the critics of ocean waivers seek full secondary treatment to control trace amounts of polynuclear aromatic hydrocarbons (PAHs) and other potentially hazardous compounds that may be contained in the combined domestic/industrial wastewater, as opposed to conventional pollutants such as oxygen-demanding materials and suspended solids.

Therefore, it is proposed that existing biological treatment facilities be reevaluated as a treatment technology for hazardous wastewater. There is currently no explicit legal definition of hazardous wastewater, but for the purposes of this manuscript, hazardous wastewater is defined as wastewater from domestic or industrial origin that contains low but potentially significant concentrations of priority pollutants or regulated compounds. Contaminants that can be biodegraded or removed by adsorption in the activated sludge process, cannot be conveniently isolated from municipal wastewater or urban runoff, and cannot be replaced in manufacturing processes by nonhazardous alternatives are the best candidates for treatment in POTWs.

In this study, a modification of the activated sludge process, called an enricher reactor system, was evaluated for its ability to enhance treatment of hazardous wastewater. Enricher reactors, which in this research were operated as sequencing batch reactors (Irvine, 1979), were used to produce biomass to biodegrade a specific compound or class of compounds. This biomass was then used to inoculate or seed continuous-flow reactors, which are typical of many existing activated sludge plants. The goal of inoculation or bioaugmentation was to produce enhanced biodegradation in the continuous-flow reactors (Figure 1). The term enricher is used, as opposed to enrichment, because the enricher reactor is used to modify or enrich the microbial culture in another reactor, the continuous-flow reactor (for example, activated sludge process).

This research addresses the removal of PAHs, specifically naphthalene and phenanthrene. These compounds and related compounds are commonly found in wastewater from the processing and combustion of fossil fuel, urban runoff, and many other processes. Our findings with respect to the removal of naphthalene and phenanthrene in the activated sludge treatment process, enricher reactor development, and the ability of the enricher reactor system to produce enhanced removal of these compounds are reported. The potential effect of enhanced biodegradation on naphthalene volatilization is also presented.

One of the objectives of this research was to test the feasibility of maintaining naphthalene- and phenanthrene-degrading cultures on other compounds. This is desirable to avoid potential undesirable environmental consequences of using naphthalene and phenanthrene and take advantage of the benefits of bioaugmentation provided in the enricher reactor, which can produce genetically prepared organisms for use in the continuous-flow reactor. In this way, a viable biological treatment process can

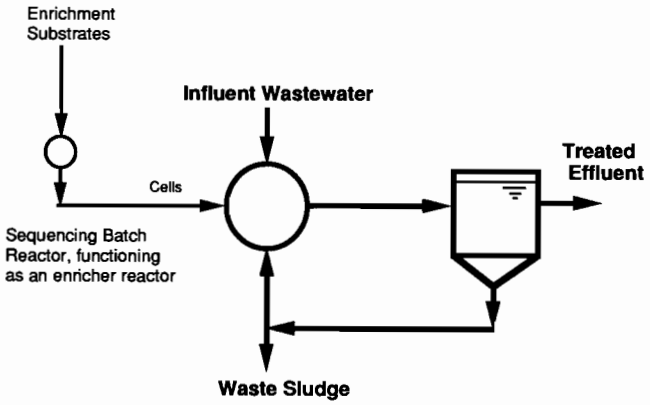

Figure 1—Enricher reactor system.

be maintained without requiring the target compounds to be routinely present in the wastewater.

Degradation of Polycyclic Aromatic Hydrocarbons

The initial steps for the oxidation of polycyclic aromatic hydrocarbons are similar to that of the monocyclic aromatic hydrocarbons xylenol and cresol (Cerniglia, 1984). Aromatic hydrocarbons in prokaryotes are initially oxidized to form dihydroxylated derivatives by incorporation of both atoms of molecular oxygen to the aromatic nucleus by a dioxygenase. Naphthalene [Figure 2 (Gibson and Subramanian, 1984)] in the presence of oxygen and coenzyme NADH is oxidized by incorporating both atoms of molecular oxygen into the aromatic nucleus to form the dihydrodiol 1,2-dihydroxynaphthalene.

Further oxidation of the dihydrodiols leads to the formation of catechols that are substrates for other dioxygenases that bring about enzymatic cleavage of the aromatic ring. Cleavage of the catechol can proceed by way of the ortho pathway, which involves cleavage of the bond between carbon atoms of the two hydroxyl groups to yield *cis-cis*-muconic acid, or by way of the meta pathway, which involves cleavage of the bond between a carbon atom with a hydroxyl group and the adjacent carbon atom without a hydroxyl group.

Bacteria commonly associated with this type of naphthalene degradation are fluorescent *Pseudomonas putida,* other *Pseudomonas* sp. (Stainer *et al.,* 1986, and Jeffrey *et al.,* 1975), and *Aeromonas* sp. (Cerniglia, 1984). Prokaryotic and eukaryotic photosynthetic algae can also hydroxylate aromatic compounds such as naphthalene (Cerniglia, 1984).

The degradative plasmids "NAH" and "SAL," commonly found in *P. putida* and related species, are responsible for the degradation of naphthalene and salicylate, respectively. The NAH plasmid is involved in the degradation of naphthalene by a series of reactions through salicylate, which is then metabolized further through catechol by way of the ortho or meta pathways (Figure 2).

Enzymes that are produced by these plasmids and whether they are actually the same plasmid are matters of debate. Dunn and Gunsalus (1973), and Williams *et al.* (1974), found that the loss of this plasmid caused total disappearance of all meta enzymes. Other studies have found that the presence of a plasmid is directly related to the ability of *P. putida* to use naphthalene and salicylic acid as the sole carbon sources. Salicylic acid degradation by meta cleavage was found to be entirely plasma encoded, whereas naphthalene degradation, which proceeds through salicylate, requires some chromosomal genes and genes on the plasmid (Zuniga *et al.,* 1981).

The characterization of these plasmids is relevant because of the role they play in the evolution of metabolic diversity among pseudomonades. Plasmids can increase the rate and degree of removal by degradation of these compounds using of transduction or conjugation. By transferring plasmids between pseudomonades of the same and different species, the number of organisms capable of using naphthalene increases at a faster rate than in the case of cell multiplication. As a result, the removal of these compounds, which is first-order with respect to the microbial population, occurs at a much faster rate. This is similar to the nutritional versatility by plasmid transfer found in the genera of bacteria that develop drug resistance.

Enricher Reactor Substrate Development

Production of enzymes of broad specificity. Previous studies have shown that early reactions involved in biodegradation of xylenols are mediated by enzymes of broad specificity, which are inducible by structurally related compounds (structural analogues). Studies by Chapman (1971) show that a strain of *P. putida* capable of degrading 2,4-xylenol readily oxidizes 3,4-xylenol and *p*-cresol and slowly oxidizes meta methyl substituents of phenols such as *m*-cresol and 3,5-xylenol. Stenstrom *et al.* (1989) showed that activated sludge acclimated to 2,4-xylenol was capable of degrading many isomers of xylenol, the three isomers of cresol, and 2,4,6-trimethylphenol. In these cases, exposure of bacteria to a single compound induced the production of enzymes that can partially or completely metabolize a whole class of compounds.

Numerous studies have been performed to determine the ability of naphthalene-degrading pseudomonades to metabolize other polyaromatic hydrocarbons. *P. putida* grown on naphthalene oxidizes phenanthrene as rapidly as naphthalene (Jeffrey *et al.,* 1975). Cell extracts of *P. putida* showed 30–80% relative activity with benzene, substituted benzenes and naphthalenes, and anthracene (Ribbons *et al.,* 1984). In Jeffrey's study, only naphthalene supported significant growth. The principle product of phenanthrene oxidation by naphthalene-degrading pseudomonades was *cis* 3,4-dihydrophenanthrene.

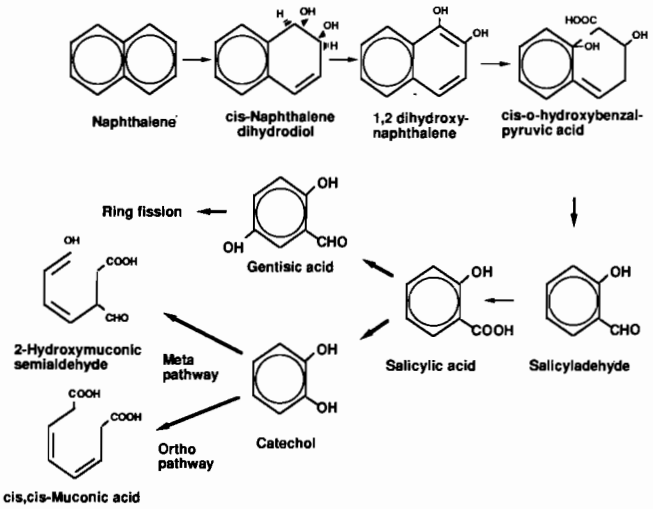

Figure 2—Naphthalene pathway (Chapman, 1971, and Gibson and Subramanian, 1984).

Phenanthrene undergoes complete catabolism with protocatechuate as an intermediate when naphthalene and phenanthrene are both used as primary energy sources by *Aeromonas* sp. and fluorescent pseudomonades (Kiyohara and Nagao, 1978). They believed that although the pathways included the same intermediate, they were separate. Results by Barnsley (1983) provide further evidence by showing that an organism that can grow on both naphthalene and phenanthrene induces a separate enzyme for the initial oxidation of each hydrocarbon.

Enzyme induction. The microbial population present in activated sludge is diverse, which results in the presence of numerous and distinct genomes that can be induced by more than one compound. These compounds induce specific genes, which in turn produce the enzymes needed for many degradation pathways. This genetic coding for the enzymes that are used in these pathways can be located on the main bacterial chromosome or on plasmids.

If necessary genetic coding is located on plasmids, special consideration must be exercised when choosing a substrate for maintenance and growth of the desired microbial population. In the case of pseudomonades that use naphthalene, the genetic coding necessary for the degradation is located in two places: the main bacterial chromosome and the plasmid. All the genetic material must be present for naphthalene degradation to occur. The microorganism may cease to produce the plasmid during replication if naphthalene is not present in the substrate. Kiyohara and Nagao (1978) noted that pseudomonades with the phenotype to assimilate naphthalene and/or phenanthrene lost the ability to assimilate both compounds during prolonged storage on nutrient agar slants. This may be the mechanism that causes activated sludge to lose its ability to degrade the target compound after being maintained for an extended period on other substrates. The possibility then exists that the plasmid, hence the genetic coding necessary to produce enzymes for the degradation of naphthalene, may be lost by the microbial population if the maintenance substrate does not induce the necessary genes.

Substrate for maintenance and growth. Maintaining activated sludge that has been enriched in microorganisms that can mineralize a toxic compound by using the toxic compound itself will ensure the retention of the genetic information, that is, plasmids. Unfortunately, the constant use of the toxic compound is undesirable because of economic or safety reasons. It is more desirable to find a nontoxic compound that induces necessary enzymes, resulting in the retention of necessary genetic information.

Enzymes required to degrade naphthalene may be induced by intermediates formed during the catabolic process and compounds not directly in the metabolic pathway. Shamsuzzman and Barnsley (1974) showed that early enzymes of naphthalene metabolism—naphthalene oxygenase, 1,2 dihydroxynaphthalene oxygenase, and salicyladehyde dehydrogenase—are induced when the organism is grown on naphthalene or salicylate, but not catechol. Later studies using *P. putida* by Williams *et al.* (1975) and Barnsley (1983) showed induction of early enzymes of naphthalene oxidation when degrading salicylate or 2-aminobenzoate. Therefore, we speculated that it should be possible to maintain a naphthalene-degrading activated sludge culture on substrates containing naphthalene itself, salicylate, or 2-aminobenzoate and succinate serving as an energy sources. These compounds induce early enzymes of naphthalene oxidation, some of which are encoded on plasmids, resulting in the retention of necessary genetic information to degrade naphthalene. They were chosen for evaluation in conjunction with varying amounts of the target compounds, naphthalene and phenanthrene. Salicylate and 2-aminobenzoate were selected because they gratuitously induce early enzymes for naphthalene degradation, and salicylate and succinate serve as energy sources.

Experimental Methods

Reactor description. Six laboratory-scale reactors were used in the experimental program. All were rectangular in shape and contained removable internal clarifiers. The reactors have been previously described in detail by Ng *et al.* (1987). Aeration was provided by bubbling compressed air through ceramic diffuser stones. The pH was automatically controlled to within 7.5 ± 0.5. Three reactors were 15 L in volume and were operated as continuous-flow reactors, simulating the activated sludge part of the enricher reactor system (Figure 1). Three 5-L reactors with clarifiers removed were operated as batch-sequencing reactors (Herzbrun *et al.*, 1985) and functioned as enrichers, providing bioaugmentation to continuous-flow reactors. Figure 3 shows the experimental arrangement. Additional details are provided by Cardinal (1989).

Various substrates were used to maintain continuous-flow and enricher reactors. Table 1 shows the concentrated feed mixtures and is divided into components common to all three mixtures and components unique to each mixture. Naphthalene and phenanthrene were fed to the reactors from a separate system of pumps, feed lines, and reservoirs. This was necessary to vary their concentration with respect to other substrates. Because of the low solubility of naphthalene in water, a concentrated mixture of naphthalene and methanol (40.4 mg naphthalene/mL methanol) was pumped into the reactors at timed intervals. In this way, a wide range of influent naphthalene concentrations were obtained (0.005 7 to 26.7 mg/L). Naphthalene concentrations associated with each experimental condition are discussed later with the experimental results.

Reactors were initially seeded with waste activated sludge from the City of Los Angeles' Hyperion facility. This is a large plant

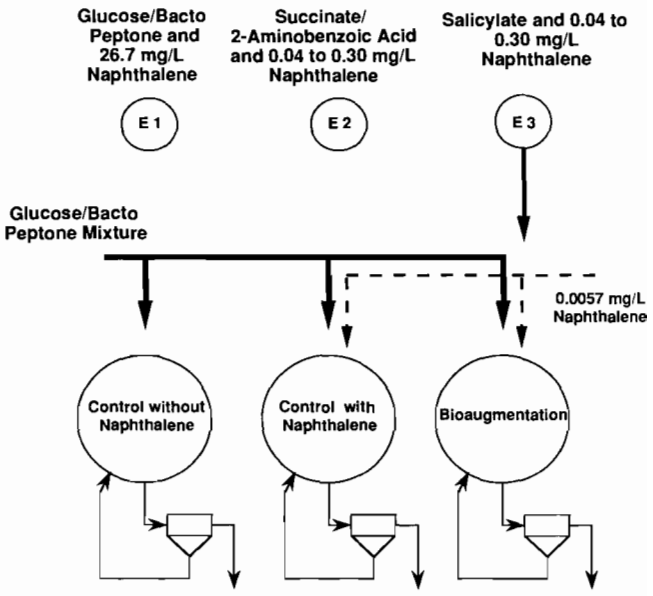

Figure 3—Reactor experimental schematic.

Table 1—Substrate mixture description.

Constituents common to all three mixtures	Amount
H_2O	1000 mL
Trace mineral solution	2 mL
K_2HPO_4	25.00 g
$(NH_4)_2SO_4$	20.00 g
Constituents in specific mixtures	
Glucose/nutrient broth	
Glucose	53.50 g
Beef extract	18.75 g
Bacto peptone	31.25 g
Yeast extract	5.00 g
Succinate/2-aminobenzoic acid	
2-Aminobenzoic acid	35.00 g
Succinate	70.00 g
Yeast extract	10.00 g
Salicylate	
Salicylate	100.00 g
Yeast extract	10.00 g

(31 000-m^3/h secondary treatment flow rate) and receives municipal and a variety of pretreated industrial wastewater. Enricher reactor 1, which was acclimated to a high concentration of naphthalene, was also seeded with waste activated sludge from a west coast petroleum oil refinery activated sludge plant. After acclimation, all reactors were operated for 3 months before testing was performed.

Table 2 shows the operating conditions for the reactors. The enricher reactors were manually fed on a daily basis. During the bioaugmentation phase of the experiments, cells were manually removed from the enrichers and transferred to the continuous-flow reactors.

Batch assays. To assess the degradation of naphthalene and phenanthrene, batch assays were performed using 60-mL serum bottles. For each analysis, 30-mL aliquots were collected from the experimental and control reactors and placed in 60-mL serum bottles. Each bottle was centrifuged and the supernatant was discarded. Thirty mL of an aqueous solution containing only naphthalene and a phosphate buffer solution (to maintain the pH at 7.0–7.3) were added to each bottle. Approximately 30 mL of air space remained in each bottle, which ensured that the culture would remain aerobic. We calculated the maximum potential naphthalene volatilization to be 1.74% of the original naphthalene mass, assuming a Henry's law constant of 0.017 6 [dimensionless (Mackay and Shiu, 1981)]. The bottles were immediately sealed and placed on a shaker table for the duration of the time test. At the end of the incubation period, the bottles were centrifuged and 5 mL of supernatant was removed and extracted. Cell extractions were performed at various intervals during the time test to determine naphthalene adsorbed to cells.

Analytical methods. Naphthalene and phenanthrene were extracted using a liquid/solid extraction using Bond Elute (C8) (Chladek and Marano, 1984). The pH of the sample was adjusted to 6.5–7.5 as required for a neutral extraction. The elution solvent for naphthalene was acetylnitrile. The elution solvent for phenanthrene was methanol. This reverse-phase procedure provided more reproducible results and better recovery (<90%) than liquid–liquid extractions and was faster and less expensive.

Naphthalene and other polyaromatic hydrocarbons adsorbed onto the surface of suspended solids in the activated sludge were extracted using a modified lipid extraction method adopted from Bligh and Dyer (1959). In this procedure, volumes of chloroform, methanol, and water, before and after dilution, were kept in the proportions 1:2:0.8 and 2:2:1.8, respectively.

Gas chromatography for naphthalene was performed on a Varian Vista 6000 with a fused silica Altech RSL 200 capillary column. The temperature program for naphthalene was run both as a gradient and isothermally. The gradient began at 56°C, was held at this temperature for 6 minutes, and was then increased by 20°C/min until 130°C. For isothermal analysis, the oven temperature was 120°C. In both cases, the injector temperature was 250°C and the detector temperature was 300°C. Naphthalene was also quantified in acetylnitrile using ultraviolet spectrophotometry. A linear response for absorbence of naphthalene was found at 276 nm for concentrations ranging from 1.5 to 35 mg/L. Acetonitrile extracts of naphthalene in reactor effluent were linear from 8 to 35 mg/L. GC/MS analyses were occasionally performed using a Finnigan 4000 instrument.

Phenanthrene was quantified using a Dionex ion chromatograph converted to work as a high-performance liquid chromatograph with a 250 mm × 4 mm reverse-phase column (BIO-

Table 2—Reactor operating parameters.

Parameter	Continuous-flow reactors	Enricher 1	Enricher 2	Enricher 3
Mean cell retention time, days	13.9	13.9	28	13.9
Hydraulic retention time, hours	13.9	34	34	34
Mixed liquor volatile suspended solids concentration (MLVSS), mg/L	1500–2000	1500–2000	1500–2000	1500–2000
F:M ratio, day^{-1}, COD basis	0.48–0.64	0.22–0.30	0.50–0.67	0.22–0.30
pH	7–8	6–8	6–8	6–8
DO, mg/L	>3.0	>3.0	>3.0	>3.0
Flow rate, L/day	24	3.5	3.5	3.5
Influent COD, mg/L	253 + 290	253 + 290 + 85	1175	1450
Primary substrate(s)	Glucose + bacto peptone	Glucose + bacto peptone + naphthalene	Succinate + 2-aminobenzoic acid	Salicylate

SIL ODS-55). Analyses were performed under isocratic conditions at a flow rate of 1.0 mL/min. The mobile phase consisted of a 1:10 water:methanol solution. The HPLC detector was an ultraviolet spectrophotometer operating at 254 nm. Under these conditions a linear response was observed for the range of phenanthrene concentrations from 0.002 to 2.0 mg/L.

Results and Discussion

The results are presented in two parts. The first part describes the success of using alternative substrates to maintain a population of organisms that were capable of degrading the target compounds. These experiments were performed using enricher reactors and the batch assay procedure described previously. Degradation rates were compared among the enrichers and continuous-flow reactors, which had not yet been subjected to bioaugmentation. The second part describes the results of bioaugmentation of continuous-flow reactor 3 with cells from enricher reactor 3 (salicylate). These two coupled reactors represented a functioning enricher reactor system.

Biodegradation of target compounds. Based on the acclimation methods, one would expect enricher number 1, which had been receiving high concentrations (26.7 mg/L) of naphthalene to have high degradation rates. If the enrichment substrates were indeed providing the necessary stimulation to induce the appropriate enzymes, then high degradation rates should be expected from enrichers 2 and 3 also. Otherwise one would expect little or no ability to degrade naphthalene because they were only being fed 5.7 μg/L.

Figure 4 shows the results of a typical assay. Cells from enricher 1 were exposed to 14 mg/L naphthalene. Error bars on Figure 4 represent absolute concentration differences in replicate samples performed in the development of analytical procedures. The culture from enricher 1, as expected, rapidly removed the naphthalene to nondetectable concentrations (<6 μg/L) in fewer than 1.4 hours. Adsorption of naphthalene onto cells from the control reactor, as determined from cell extractions, was approximately 5.5% of the original naphthalene mass, suggesting a partitioning

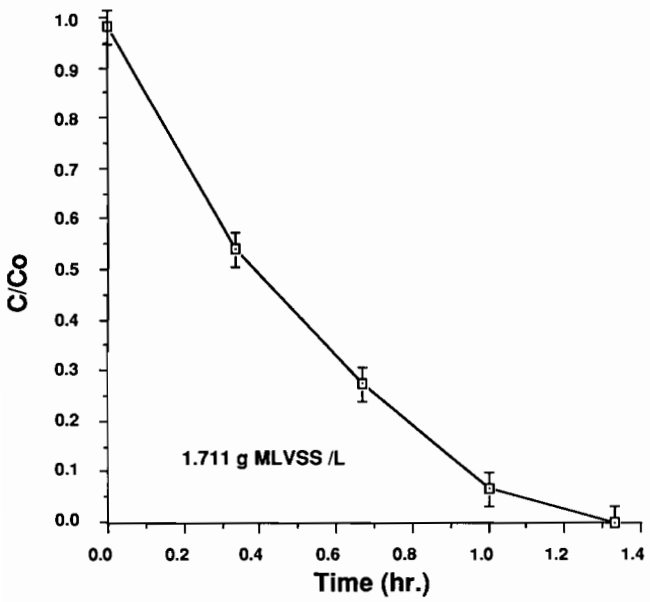

Figure 4—Degradation of naphthalene in acclimated activated sludge.

coefficient of 106, using the correlation provided by Karickhoff *et al.* (1979). This compares to reported values of octanol/water partitioning coefficients of 3890 (Mackay *et al.*, 1982), 2290 (Casserly *et al.*, 1983), and 1023 (Vershueren, 1983). Little or no naphthalene was found on acclimated cells.

Gas-phase analyses in serum bottles used for the batch assays to verify volatilization were never performed. Controls with unacclimated cells were used to verify degradation and volatilization. Naphthalene disappearance in control samples containing only distilled water showed less than 10% disappearance, which represents volatilization into the head space of serum bottles and recovery error. This agreed well with Henry's law prediction (maximum 2% mass loss). The additional disappearance of naphthalene in batch assays from unacclimated cells (from continuous-flow reactor number 1) was slightly greater (~2%) as compared to assays with no cells.

GC/MS analysis of the reactor effluent showed no stable intermediates within the limits of detection, which was 6 μg/L. Radio tracer assays were not performed to confirm degradation to CO_2; previous research has confirmed the complete mineralization of naphthalene under similar conditions, and the procedures to extract cells and quantify naphthalene volatilization confirm its disappearance. If naphthalene was not mineralized, it could only have been converted to a nonchromatographical byproduct, which is unlikely in view of its well-known degradation pathways.

The reaction rate for naphthalene was found to be first-order with respect to MLVSS concentration (Figure 5). The initial concentration of naphthalene in the feed solution was 21 mg/L. Using activated sludge acclimated to naphthalene at 2.24 g/L MLVSS, approximately 75% of the naphthalene was destroyed after 1.167 hours; at 0.56 g/L MLVSS, approximately 13% of the naphthalene was destroyed after the same time period.

Figure 6 shows the results of three separate batch tests for the removal of naphthalene by the enricher reactor maintained on salicylate, the enricher reactor maintained on 2-aminobenzoic acid/succinate, and the enricher reactor acclimated to naphthalene. The results of the three tests are plotted together for ease of comparison. Activated sludge from the enricher reactor maintained on salicylate plus 0.3 mg/L naphthalene for more than 3 months showed approximately 99%+ removal in approximately 4 hours. The activated sludge from the enricher reactor maintained on 2-aminobenzoic acid and succinic plus 0.3 mg/L naphthalene influent for 3 months showed fair removal of naphthalene in approximately 6.5 hours.

Later assays showed poorer and poorer removal rates in the enricher maintained on 2-aminobenzoic acid and succinate. Eventually the removal rate was not significantly different than in the continuous-flow reactor fed only a trace (5.7 μg/L) of naphthalene. By contrast, the enricher maintained on salicylate continued to degrade naphthalene at an efficient rate even after the naphthalene concentration was reduced to 40 μg/L, for up to 9 months, at which time experiments were discontinued.

A possible reason for this difference is that succinate, which is an intermediate compound in the Krebs cycle can serve as an energy source for many organisms, whereas salicylate supports a more restricted range of organisms. Therefore, in the enricher maintained on 2-aminobenzoic acid and succinate, other succinate-degrading organisms may have proliferated, diluting out naphthalene-degrading organisms.

Activated sludge acclimated to naphthalene and maintained

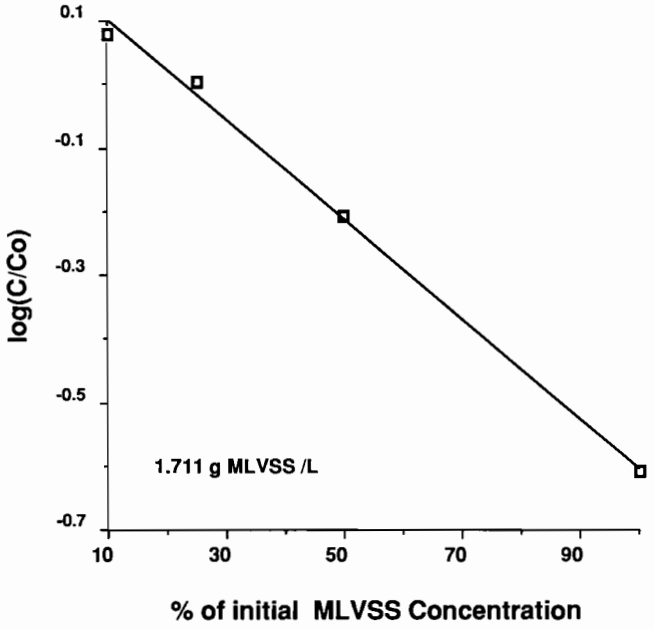

Figure 5—Naphthalene degradation over 1.17 hours with varying MLVSS concentrations.

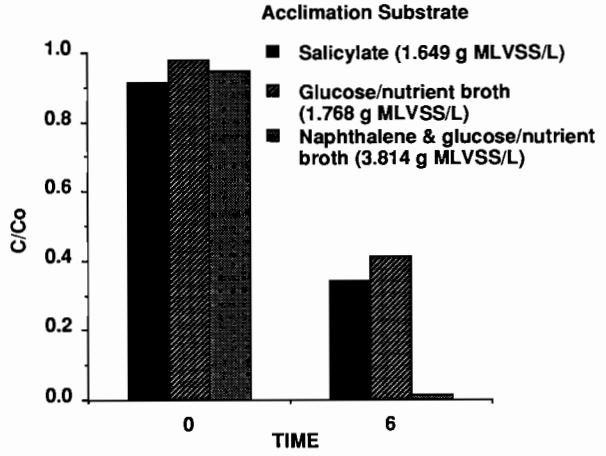

Figure 7—Degradation of phenanthrene by activated sludge acclimated to naphthalene and maintained on different substrates.

on naphthalene or salicylate was tested for its ability to degrade phenanthrene (Figure 7). The enricher fed naphthalene at 26.7 mg/L degraded phenanthrene. The enricher maintained on salicylate and 40 μg/L naphthalene did not reduce the concentration of phenanthrene any better than the continuous-flow control reactor. The difference in concentration between the control reactor and the salicylate reactor over the 6-hour interval represents the adsorption of phenanthrene onto the cells.

Enricher reactor system results. Figure 8 shows the result of batch tests for the removal of naphthalene in an enricher reactor system and two continuous-flow reactors. The enricher system had been operated for a period of 2 months, with the addition of approximately 300 mL of cells per day from enricher number 3 (salicylate and a 5.7 μg/L feed). Continuous-flow reactor 2 and the continuous-flow reactor in the enricher reactor system received 5.7 μg/L naphthalene feed. Continuous-flow reactor 1 served as the control and received only glucose. The reactor that received the enricher activated sludge showed a significantly enhanced ability to degrade naphthalene compared to the reactor fed only low levels of naphthalene. The control reactor fed only glucose showed no ability to degrade naphthalene.

If the genes for naphthalene removal are in fact plasmids, encoded naphthalene removal by the continuous-flow reactor in the enricher reactor system may be due to either to an increased cell population capable of using naphthalene and/or the transmission of plasmids (Zuniga *et al.*, 1981, and Heitkamp *et*

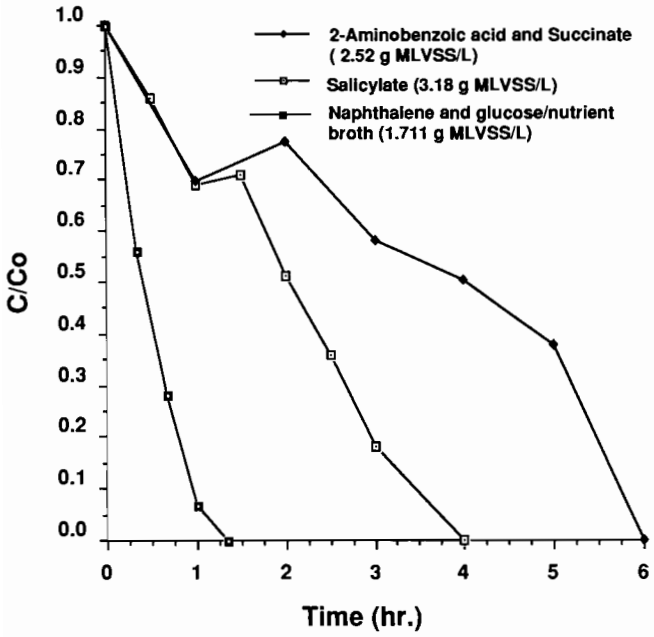

Figure 6—Degradation of naphthalene by activated sludge acclimated in different ways.

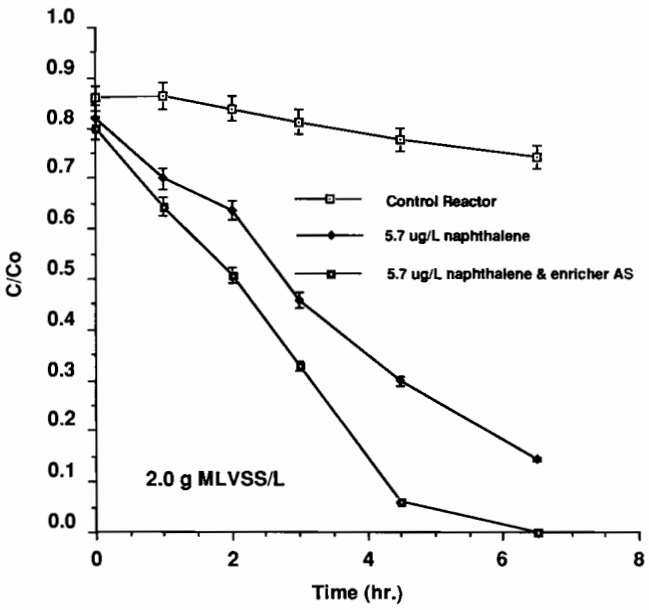

Figure 8—Degradation of naphthalene by an enricher reactor system.

al., 1987). In the latter case, exposure of naphthalene to cells capable of oxidizing it may not necessarily increase the actual number of heterotrophic microorganisms but may selectively increase, by plasmid transmission, the hydrocarbon-degrading ability of the microbial population already present.

To further evaluate the enricher reactor concept, naphthalene was removed from the feed and the experiment continued. After approximately 3 months, the continuous-flow reactors, including the one operating as an enricher reactor system, lost the ability to degrade naphthalene. This suggests that even with the addition of acclimated cells, a small trace of naphthalene is required to maintain the microbial population required for its degradation.

Volatilization. The effects of biodegradation rate on the rate of naphthalene volatilization was modeled using the materials balance equations for a single volatile species (Roberts *et al.*, 1984, and Stenstrom *et al.*, 1989). Steady-state conditions and a first-order biodegradation rate were assumed. The minimum degradation rate, which was estimated from the batch assay tests using zero-order kinetics with respect to naphthalene, was 0.4 h^{-1}. Mackay and Shiu's (1981) estimate for Henry's law coefficient for naphthalene (0.017 6, dimensionless) was used.

Operating conditions for bench-scale reactors used in this study and for a typical domestic wastewater plant were simulated. The typical plant was assumed to have an influent flow rate of 1000 m^3/h (6.5 mgd) and a 6-hour hydraulic retention time. Air flow rate in the domestic wastewater plant, which might range from 500 m^3/h for the highest efficiency subsurface aeration system to 12 000 m^3/h for the lowest efficiency spiral roll system (Stenstrom *et al.*, 1989), was set at 12 000 m^3/h. In these simulations, the remaining mass of compound, which is not volatilized or biodegraded, leaves the reactors untreated.

Figures 9 and 10 show a 5 and 20% loss due to volatilization in a typical wastewater treatment plant and the bench-scale reactors, respectively, for the 0.4 h^{-1} biodegradation rate. It is apparent from this analysis that maximizing rates of biodegradation minimize volatilization. These simulations are not intended to predict absolute volatilization rates. They are included to show benefits of enhanced biodegradation.

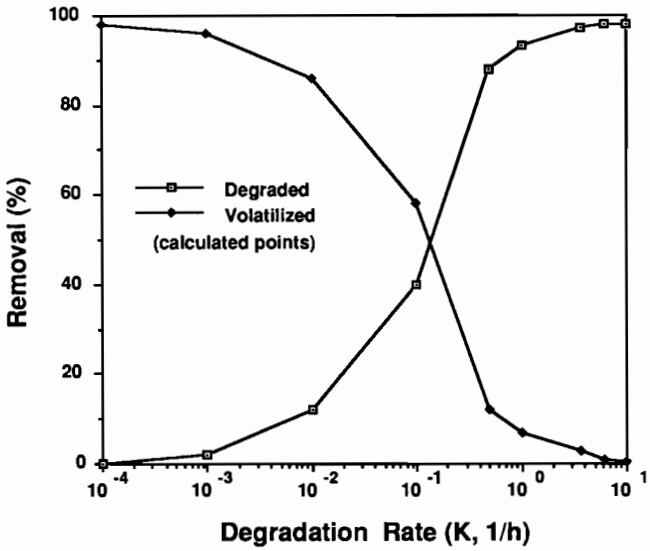

Figure 9—Effect of degradation rate on the volatilization rate of naphthalene in laboratory-scale reactors.

Conclusions

This paper has demonstrated the concept of using a separate enricher reactor to improve the performance of a larger, continuous-flow reactor. The research was divided into two parts: maintenance of the cells on substrates other than the target compound and the impact of bioaugmentation from the enricher reactor on the main activated sludge reactor. In this investigation, sequencing batch reactors were used as enricher reactors, but other types could also be useful for this purpose. The following specific conclusions are made.

An enricher reactor maintained for more than 9 months on small amounts of naphthalene and salicylate was able to efficiently degrade naphthalene. In contrast an enricher reactor maintained on 2-aminobenzoic acid, succinate, and 0.16 mg/L naphthalene lost its ability to efficiently degrade naphthalene, providing no better degradation than a reactor maintained on glucose and the same trace of naphthalene.

It is suggested that the enzymes required for naphthalene degradation may be effectively induced by the degradation of salicylate and traces of naphthalene. Conclusive microbiological evidence to substantiate this cause-and-effect relationship where not provided and were beyond the scope of this research.

Continuous-flow reactors maintained on high concentrations of naphthalene (26.7 mg/L) readily degraded phenanthrene. Cells maintained in an enricher reactor on a trace of naphthalene and salicylate were not able to degrade phenanthrene. This is consistent with the findings that the pathways for naphthalene and phenanthrene degradation are separate. Only if the necessary genetic information exists on separate genes could the microbial population maintained on salicylate retains its ability to degrade naphthalene but lose its ability to degrade phenanthrene.

An enricher reactor system, composed of a batch-sequencing reactor providing cells to a continuous-flow activated sludge reactor, showed enhanced removal rates when compared to an activated sludge reactor without cell addition. The enricher reactor system lost its ability to degrade the naphthalene when the trace concentration (5.7 μg/L) was removed from the continuous-flow reactor influent. This suggests that even when acclimated cells are continuously added to the activated sludge process, they will not significantly enhance the removal of naphthalene if it is totally absent from the influent for lengthy periods of time.

The enricher reactor's separation from the main process allows it to be operated under different conditions. Design and operating parameters, such as the mean cell retention time, hydraulic retention time, mixed liquor volatile suspended solids concentration, and influent feed concentration, can be adjusted to best accommodate the desired microbial population. Sequencing batch reactors are ideal for this application because of their versatility, low maintenance requirements, and ability to handle shock loading. The enricher could be covered to control volatilization. In this fashion it could produce a microbial population acclimated to volatile compounds, which could provide enhanced volatile compound removal in the main process.

The results of this study have important implications for the future treatment of hazardous waste in POTWs and other plants treating complex wastewater mixtures. The development of the enricher reactor concept could result in the construction of new activated sludge processes or the modification of existing activated sludge processes to enhance their ability to degrade haz-

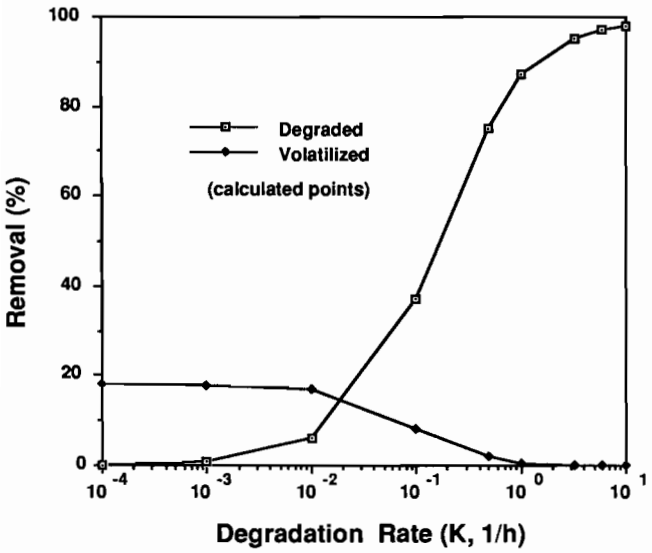

Figure 10—Effect of degradation rate on the volatilization rate of naphthalene in a hypothetical, full-scale activated sludge process.

ardous wastewater. Implementing this process could result in large savings to industry resulting from reduced cost of pretreating dilute wastes, protection of POTWs from upsets caused by uncontrolled discharges to the sewer system, and protection of receiving waters from effluent containing low concentrations of hazardous compounds.

Acknowledgments

Credits. This work was supported in part by the NSF funded Engineering Research Center for the Control of Hazardous Substances, the Center for the Engineering and Systems Analysis for the Control of Toxics, and an environmental fellowship from BP Research. The authors are grateful for the assistance of Roger Babcock, Chu-Chin Hsieh, and Kyoung Ro.

Authors. Lynne J. Cardinal is currently a project engineer at Engineering-Science in Pasadena. At the time of this work she was a graduate student research assistant in the civil engineering department at the University of California, Los Angeles (UCLA). Michael K. Stenstrom is a professor of civil engineering at UCLA. Correspondence should be addressed to Michael K. Stenstrom, Civil Eng. Dept., UCLA, 4173 Eng. I, Los Angeles, CA 90024-1600.

Submitted for publication November 30, 1989; revised manuscript submitted June 3, 1991; accepted for publication June 30, 1991. The deadline for discussions of this paper is March 15, 1992. Discussions should be submitted to the Executive Editor. The authors will be invited to prepare a single Closure for all discussions received before that date.

References

Barnsley, E. A. (1983) Bacterial Oxidation of Naphthalene and Phenanthrene. *J. Bacteriol.*, **153,** 2, 1069.

Bligh, E. G., and Dyer, W. J. (1959) A Rapid Method of Total Lipid Extraction and Purification. *J. Biochem. Physiol.*, **37,** 911.

Cardinal, L. J. (1989) Degradation and Biotransformation of Isophorone, Xylenols, Cresols, and Polyaromatic Hydrocarbons in Acclimated Activated Sludge: Use of Enrichment Reactors to Enhance this Process. Ph.D. thesis, Univ. Calif., Los Angeles (1989).

Casserly, D. M., *et al.* (1983) Sorption of Organics by *Selenastrum capricornutum. Water Res.*, **17,** 11, 1591.

Cerniglia, C. E. (1984) Microbial Metabolism of Polycyclic Aromatic Hydrocarbons. In Applied Microbiology. Allen I. Laskin (Ed.), Acad. Press, Inc., Orlando, Fla.

Chapman, P. J. (1971) An Outline of Reaction Sequences Used for the Bacterial Degradation of Phenolic Compounds. In Degradation of Synthetic Molecules in the Biosphere. Printing and Publishing Office, Natl. Acad. Sci., Washington, D. C.

Chladek, E., and Marano, R. S. (1984) Use of Bonded Phase Silica Sorbents for the Sampling of Priority Pollutants in Wastewaters. *J. Chrom. Sci.*, **22,** 313.

Dunn, N. W., and Gunsalus, I. C. (1973) Transmissible Plasmid Coding Early Enzymes of Naphthalene Oxidation in *Pseudomonas putida. J. Bacteriol.*, **114,** 3, 974.

Gibson, D. T., and Subramanian, V. (1984) Microbial Degradation of Aromatic Hydrocarbons. In Microbial Degradation of Organic Compounds. D. T. Gibson (Ed.), Marcel Dekker, Inc., New York, 181.

Heitkamp, M. A., *et al.* (1987) Naphthalene Biodegradation in Environmental Microcosms: Estimates of Degradation Rates and Characterization of Metabolites. *Appl. Environ. Microbiol.*, **53,** 1, 129.

Herzbrun, P. A., *et al.* (1985) Biological treatment of hazardous waste in sequencing batch reactors. *J. Water Pollut. Control Fed.*, **57,** 1163.

Irving, R. L. (1979) Sequencing batch biological reactors—an overview. *J. Water Pollut. Control Fed.*, **51,** 235.

Jeffrey, A. M., *et al.* (1975) Initial Reactors in the Oxidation of Naphthalene by *Pseudomonas putida. Biochemistry,* **14,** 3, 575.

Karickhoff, S. W., *et al.* (1979) Sorption of Hydrophobic Pollutants on Natural Sediments. *Water Res.*, **13,** 3, 241.

Kiyohara, H., and Nagao, K. (1978) The Catabolism of Phenanthrene and Naphthalene by Bacteria. *J. Gen. Microbiol.*, **105,** 69.

Mackay, D. (1982) Correlation of Bioconcentration Factors. *Environ. Sci. Technol.*, **116,** 5, 274.

Mackay, D., and Shiu, W. Y. (1981) A Critical Review of Henry's Law Constants for Chemicals of Environmental Interest. *J. Phys. Chem. Ref Data,* **10,** 4, 1175.

Mackay, D., *et al.* (1979) Determination of Air–Water Henry's Law Constants for Hydrophobic Pollutants. *Environ. Sci. Technol.*, **13,** 3, 333.

Metcalf and Eddy (1979) Wastewater Engineering Treatment Disposal and Reuse. McGraw-Hill, Boston, Mass., 339.

Ng, A., *et al.* (1987) Nitrification enhancement in the powdered activated carbon-activated sludge process for the treatment of petroleum refinery wastewaters. *J. Water Pollut. Control Fed.*, **59,** 199.

Ribbons, D. W., *et al.* (1984) Microbial Degradation of Phthalates. In Microbial Degradation of Organic Compounds. E. T. Gibson (Ed.), Marcel Dekker, Inc., New York, 371.

Roberts, P. V., *et al.* (1984) Modeling volatile organic solute removal by surface and bubble aeration. *J. Water Pollut. Control Fed.*, **56,** 157.

Shamsuzzman, K. M., and Barnsley, E. A. (1974) The Regulation of Naphthalene Metabolism in Pseudomonads. *Biochem. Biophys. Res. Commun.*, **60,** 2, 582.

Stainer, R. Y., *et al.* (1986) Microbial World. Prentice Hall, Englewood Cliffs, N. J., 292.

Stenstrom, M. K., *et al.* (1989) Treatment of Hazardous Substances in Conventional Biological Treatment Plants. *Environ. Prog.*, **8,** 107.

Verschueren, K. (1983) Handbook of Environmental Data on Organic Chemicals. Van Nostrand Reinhold Co., New York.

Williams, P. A., *et al.* (1975) Metabolism of Naphthalene, 2-Methylnaphthalene, Salicylate, and Benzoate by *Pseudomonas* PG: Regulation of Tangential Pathways. *J. Bacteriol.*, **124,** 2, 679.

Zuniga, M. C., *et al.* (1981) Plasmid- and Chromosome-Mediated Dissimilation of Naphthalene and Salicylate in *Pseudomonas putida* PMD-1. *J. Bacteriol.*, **147,** 3, 836.