Development of an off-line enricher-reactor process for activated sludge degradation of hazardous wastes

Roger W. Babcock Jr., Kyoung S. Ro, Chu-Chin Hsieh, Michael K. Stenstrom

Development of an off-line enricher-reactor process for activated sludge degradation of hazardous wastes

Roger W. Babcock Jr., Kyoung S. Ro, Chu-Chin Hsieh, Michael K. Stenstrom

ABSTRACT: The improvement in degradation of a hazardous chemical using a novel bioaugmentation scheme was studied. Bench-scale off-line batch enricher-reactors (ERs) maintaining an enrichment culture were used to bioaugment bench-scale continuous-flow activated sludge reactors treating 1-naphthylamine (1NA). In batch experiments, one-time bioaugmentation inoculations of 1, 2, 5, 10, 20, and 50% by mass of a 1NA-degrading culture (mg mixed liquor volatile suspended solids [MLVSS] of 1NA-degrading culture/mg MLVSS of indigenous culture) increased degradation rates by approximately 0, 33, 100, 100, 100, and 300% respectively over an uninoculated control. In continuous-flow experiments, separate 13.7-L reactors received daily inoculations of 1.4, 2.5, 6.6, 11.4, and 18.3% by mass of 1NA-degrading culture. Cumulative target compound breakthrough reduction following a 50 mg 1NA/L spike was 13, 21, 11, 35, and 41% compared to an unacclimated control and 4, 13, 1, 27, and 35% compared to an acclimated control, respectively. Similarly, the reduction in breakthrough during reacclimation to 5 mg 1NA/L over six days was 66, 73, 85, 98, and 100%, respectively. A 6% bioaugmented continuous-flow reactor significantly reduced 1NA breakthrough following a step-loading increase from 1 to 5 mg 1NA/L compared to an uninoculated control. Effective bioaugmentation was achieved with additions of biomass equivalent to 14–25% of indigenous cell production rates. *Water Environ. Res.*, **64**, 782 (1992).

KEYWORDS: activated sludge, bioaugmentation, biodegradation, enricher-reactors, hazardous wastes, 1-naphthylamine.

Approximately 98% of the 747 million tons of hazardous wastes generated in the United States in 1986 occurred in the form of wastewaters (Baker *et al.*, 1992). Biological oxidation (mineralization) is an attractive treatment method because the hazardous materials are converted to innocuous endproducts (carbon dioxide and water) (Grady, 1985). Since nearly all wastewater treatment plants are required to have secondary treatment under the 1976 amendments to the Federal Clean Water Act, and since activated sludge (AS) is the most commonly employed process, it may be prudent to investigate the use of this enormous existing infrastructure to treat hazardous wastes (Grady, 1986, and Cardinal and Stenstrom, 1991).

Conventional AS systems (shown schematically in box in Figure 1) work well for the easily degraded components of the waste stream but not for the hazardous components which may be intermittently present, toxic to bacteria, or slow to degrade. An AS culture may, under certain circumstances, acclimate to some hazardous compounds (Kobayashi and Rittmann, 1982), but efficient degradation is often difficult to maintain if the compounds are discontinuous in the waste stream. The necessity to reacclimate following the absence of a hazardous compound results in a period of reduced treatment efficiency. In order to make the conventional system more amenable to degradation of hazardous wastes, we propose addition of one or more batch-type "enricher-reactors" (ERs) to ensure the presence of a continuously acclimated culture. In the proposed system, ERs produce an inoculum of acclimated organisms to augment degradation of hazardous compounds in the conventional system (Figure 1).

The idea of maintaining a separate, acclimated culture for addition to AS when necessary was reported previously by Stenstrom *et al.* (1989) and also by Arbuckle and Kennedy (1989). In order to maintain acclimation, the ER substrate should contain some combination of the hazardous compound(s), intermediates in the degradation pathway, inducer compounds, and/or nutrients. This differs from the common conception of "bioaugmentation."

Bioaugmentation via addition of biological "preparations" to AS has become popular (Beardsley and Coffey, 1985), and has been in use since the mid-1970s (Young, 1976). Many companies offer products designed to increase degradation of bulk organic material or a specific class of toxic compounds. Other products are designed to decrease filamentous bulking. Manufacturers usually recommend high initial doses to establish the product culture and sometimes maintenance doses, but relatively few researchers have performed controlled investigations on their efficacy. There are several favorable reports by authors associated with bioaugmentation product manufacturers on successful full-scale applications (Thibault and Tracy, 1979; Nyer and Bourgeois, 1980; and McDowell and Zitrides, 1979). However, the exact timing and quantities of doses are often omitted. Results of laboratory investigations with commercial products and adequate controls have generally been negative or inconclusive (Lewandowski *et al.*, 1986; Qasim and Stinehelfer, 1982; Hull and Kapuscinski, 1987; Martin and Zall, 1985; Lange *et al.*, 1987; Lynch *et al.*, 1987; and Senthilnathan and Ganczarczyk, 1988). Table 1 gives a summary of the published work involving some commercially available bioaugmentation products.

An effective bioaugmentation process should meet several requirements. First, viability of the ER bacteria should remain high after inoculation. Second, inocula should initiate degradation of the target compound(s) rapidly or degrade compounds not degraded by indigenous bacteria. Third, inocula should maintain a significant population in the main AS reactor (Lange *et al.*, 1987) for a time sufficient to degrade the target compound. Investigations of some commercially available bioaugmentation products have reported short-lived inocula, an acclimation period prior to onset of degradation, and no significant growth of inocula

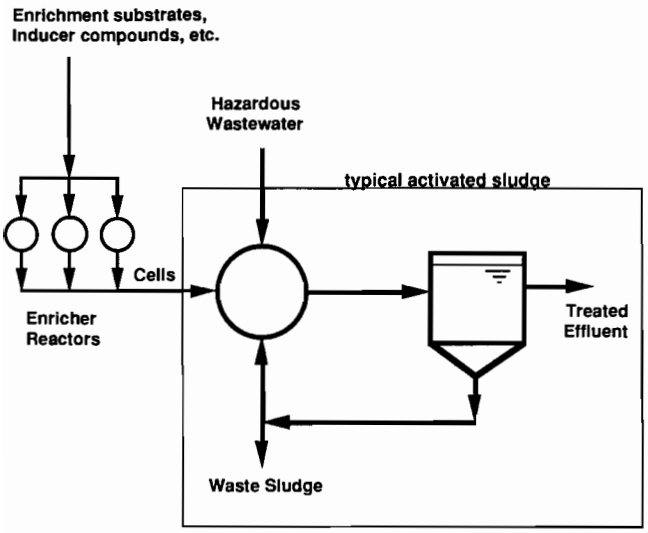

Figure 1—Enricher-reactor bioaugmentation system.

in the main reactor (Lange *et al.*, 1987; Lynch *et al.*, 1987; and Senthilnathan and Ganczarczyk, 1988).

Use of ERs potentially achieves all three of these requirements. First, if necessary the enrichment cultures can be maintained under substrate and environmental conditions similar to the main reactor so that viability remains high after inoculation and degradation is initiated quickly. Alternatively, isolation of ERs allows flexibility of substrate composition and environmental conditions for optimization of cell growth. Second, since ERs are permanent and inoculations continuous or semicontinuous, the requirement that inocula become established in the main AS reactor is reduced. Additionally, onsite ERs, operated by the same agency can likely produce high numbers of specific populations of cells at greatly reduced costs compared to commercial products.

Isolation of ERs from the main AS system has several other benefits, including: protection from periodic upsets and operating constraints of main-line reactors, and potential use for rapid establishment or restoration of efficient operation during start-up or after upsets. Additionally, ERs would likely only positively affect base-line treatment of bulk organic material in the main AS system and, if degradation rates can be increased, existing systems might be operated beyond their design capacities.

Previous work in this laboratory has established enhanced degradation of trace levels of naphthalene, phenanthrene, and high concentrations of most isomers of xylenol by AS using ERs (Cardinal and Stenstrom, 1991). Here we report results of more detailed bioaugmentation experiments with a model hazardous compound, 1-naphthylamine (1NA). 1-Naphthylamine is an amino substituted polyaromatic hydrocarbon, and an inter-

Table 1—Summary of published bioaugmentation studies using commercial products.

Bioaugmentation method	Comments	Results	Reference
Commercial product, mutant bacteria	Full-scale refinery AS, doses not given, reported by product manufacturers.	Favorable	McDowell and Zitrides (1979)
Commercial product, mutant bacteria	Full-scale refinery AS and full-scale municipal AS, doses not given, reported by product manufacturers.	Favorable	Nyer and Bourgeois (1980)
Commercial product, mutant bacteria	Full-scale refinery AS, doses given but not enough information to calculate concentrations, reported by product manufacturers.	Favorable	Thibault and Tracy (1979)
Enrichment culture and commercial products (2)	Full-scale extended aeration AS-reduced bulking, full-scale aerated lagoon-improved BOD5 removal, full-scale oxidation ditch-stopped bulking, doses not given.	Favorable	Chambers (1981)
Commercial product	Bench-scale CFSTRs, municipal wastewater, various daily doses of 0.3–10 mg/L.	Unfavorable	Qasim and Steinhelfer (1982)
Commercial products (2)	Bench-scale fill and draw AS, synthetic dairy wastewater, followed manufacturer recommendations.	Unfavorable	Martin and Zall (1985)
Commercial products (3)	Bench-scale batch AS, wastewater with phenol, 2-chlorophenol, and 2,4-dichlorophenol, unamended AS performed better than products.	Unfavorable	Lewandowski *et al.* (1986)
Commercial products (16)	Bench-scale batch AS, hazardous wastewater, found: degradation lag period and short lived inoculant viability.	Unfavorable	Lange *et al.* (1987)
Commercial product	Modeling approach, typical recommended doses of 0.005 mg cells/L are insufficient.	Unfavorable	Hull and Kapuscinski (1987)
Enrichment culture and speculation regarding commercial products	Bench-scale batch AS, wastewater with phenol, de-adaption of culture had negative effect.	Unfavorable	Senthilnathan and Ganczarczyk (1988)
Commercial product	Full-scale refinery AS, doses not given, increased resistance to shock loadings, and increased maximum substrate utilization rate.	Favorable	Wong and Goldsmith (1988)

mediate in the production of azo dyes. Azo compounds comprise about 60% of all organic coloring dyes in use today (Boeniger, 1980), and 1NA is a possible azo dye degradation product (Shaul *et al.*, 1985). It is also an intermediate in the production of herbicides, a rubber antioxidant (phenyl-alpha-naphthalene), rubber insulated cables, paints, plastics, pigments, and toning prints made with cerium salts (Boeniger, 1980; Dictionary, 1982; Proctor and Hughes, 1978; Scott, 1962; and Merck, 1989).

In addition, 1NA may be found in the waste from nearly all destructive distillation processes (oil refining and petrochemical products manufacture) (Staff, 1974) as well as direct coal-liquefaction wastes (Wilson *et al.*, 1985). 1-Naphthylamine is suspected to cause bladder cancer (Case *et al.*, 1954; Goldblatt, 1958; and Proctor and Hughes, 1978) and has been regulated as a carcinogen by OSHA since 1974 (Federal Register, 1974). Early investigations of 1NA found it to be generally resistant to biodegradation (Pitter, 1976, and Malaney, 1960), inhibitory to nitrification (Hockenbury and Grady, 1977), highly toxic to organisms (Giddings, 1979), and mutagenic (McCann *et al.*, 1975). Concurrent work with axenic cultures isolated from our enrichment showed that degradation does not occur by simple deamination followed by degradation of naphthalene by established pathways and actually proceeds via novel pathways (manuscripts in preparation).

This study investigates the potential utility of using ERs to bioaugment an AS system treating a wastewater containing low levels of a hazardous compound.

Materials and Methods

Chemicals and media. Aromatic compounds were obtained from Aldrich Chemical Co. (St. Louis, Mo.) and Sigma Chemical Co. (Milwaukee, Wis.). All solvents were high-performance liquid chromatography (HPLC) grade from Fisher Scientific Co. (Pittsburg, Pa.). Several media were used in this study, including a synthetic wastewater for all continuous-flow experiments and different enrichment substrates were used to select for cultures able to rapidly degrade 1NA. Table 2 shows the synthetic wastewater composition. Yeast extract, beef extract, and Bacto peptone were obtained from Difco Laboratories (Detroit, Mich.). Salicylic acid, all inorganic salts, and vitamins except biotin and pyridoxamine were obtained from Fisher Scientific Co. (Pittsburg, Pa.). Biotin and pyridoxamine were obtained from Sigma Chemical Co. (St. Louis, Mo.). The various enrichment media with salicylic acid as the primary carbon source are described elsewhere (Babcock, 1991). Table 2 also shows the ER maintenance substrate composition which includes succinic and pyruvic acids.

Analytical methods. Aromatic compounds were analyzed by capillary gas chromatography (GC) and/or HPLC (Babcock, 1991). Retention times and detection limits for 1NA using GC and HPLC were 16.8 minutes, 1.0 ng, and 4.4 minutes, 50 ng, respectively. Supernatant and mixed liquor samples were prepared for analysis using solid phase extraction with C18 bonded silica sorbent columns (Analytichem, Harbor City, Calif.) (Babcock, 1991). For some tests, cell pellets were extracted with methanol or toluene to quantify adsorption (Babcock, 1991). Mixed liquor total solids and biomass concentrations (MLVSS) were measured biweekly using methods 2540D and 2540E (Standard Methods, 1989), and bulk organic content of influents and effluents was monitored weekly as filtered COD using method 5220C (Standard Methods, 1989). Dissolved oxygen was monitored with Yellow Springs Inst. Co. model 51B meters and model 5720A probes (Yellow Springs, Ohio).

Table 2—CFSTR synthetic wastewater and ER substrate composition.

Component	CFSTRs, mg/L	ERs, mg/L
Bacto peptone	189	189
Beef extract	142	142
Salicyclic acid	0	71
Succinic acid	0	71
Pyruvic acid	0	71
Yeast extract	38	52
K_2HPO_4	95	455
KH_2PO_4	150	723
$(NH_4)_2SO_4$	38	182
$CaCl_2 \cdot 2H_2O$	12.5	10.5
$MgCl_2 \cdot 6H_2O$	19.4	16.4
$FeCl_3$	3.0×10^{-1}	1.4
$MnCl_2 \cdot 4H_2O$	7.2×10^{-2}	3.4×10^{-1}
$ZnCl_2$	5.0×10^{-2}	2.4×10^{-1}
$CuCl_2 \cdot 2H_2O$	3.1×10^{-2}	1.5×10^{-1}
$CoCl_2 \cdot 6H_2O$	4.4×10^{-2}	2.1×10^{-1}
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	3.2×10^{-2}	1.5×10^{-1}
Na_3 citrate	2.7	13
$Na_2B_4O_7 \cdot 10H_2O$	1.9×10^{-2}	8.9×10^{-2}
Pyridoxamine dihydrochloride	5.7×10^{-4}	2.7×10^{-3}
Nicotinic acid	3.8×10^{-4}	1.8×10^{-3}
Thiamine hydrochloride	3.8×10^{-4}	1.8×10^{-3}
D-Pantothenate	1.9×10^{-4}	9.1×10^{-4}
p-Aminobenzoic acid	1.5×10^{-4}	7.3×10^{-4}
d-Biotin	3.8×10^{-5}	1.8×10^{-4}
1-Naphthylamine	variable	150

Activated sludge reactors. Seven rectangular 13.7-L laboratory-scale continuous-flow completely-mixed reactors (CFSTRs) were used in these experiments. These plexiglass reactors had 12.2-L aeration zones, 1.5-L internal clarifiers and continuous sludge recycle and were covered with dark window tinting material to limit the growth of algae (Figure 2). Synthetic wastewater was prepared automatically in 15.3-L batches using a refrigerated (5°C) mix-tank with a system of float-valve controlled Masterflex pumps and a make-up water solenoid. The pumps delivered prescribed doses of refrigerated, concentrated substrates to the mix tank, and dilution water (tap water) was added via the solenoid. This system provided a continuous supply of wastewater. Feed was delivered to CFSTR aeration zones, and solids were continuously recycled from the internal clarifier. Sludge was manually wasted daily by removing sufficient mixed liquor from a sampling port located in the middle of the aeration zone. Daily maintenance included scraping to remove wall growth. Concentrated 1NA feed solution was also refrigerated at 5°C.

The pH was controlled automatically between 6.5 and 7.5 by the addition of 0.071 *M* Na_2CO_3 and 0.5 *M* HCl using Horizon model 5997 or Cole-Parmer Chemcadet pH controllers and 60 rpm Masterflex constant speed pumps. The temperature of all reactors was allowed to vary with ambient air. While the temperature of the ERs closely approximated ambient levels (18–30°C), because of feed refrigeration, CFSTR temperatures remained several degrees lower (13–23°C). Wastewater and concentrated 1NA were delivered to CFSTRs at flow rates of 18

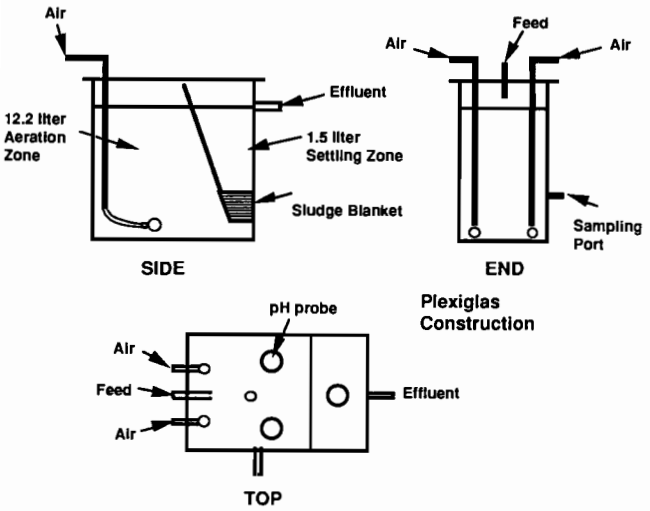

Figure 2—Polymethylmethacrylate laboratory reactors.

mL/minute and 0.5 mL/minute, respectively, using Masterflex 7524-00 microprocessor pump drives and 7519-00 multichannel cartridge pump heads, providing a hydraulic retention time (HRT) of 12.3 hours. The CFSTRs were operated with a nominal mean cell retention time (MCRT) of 10 days, a food to microorganism ratio (F/M) of 0.2–0.4, a volumetric loading rate of 0.5 kg COD m^{-3} d^{-1}, a biomass concentration (MLVSS) of 1 200–2 500 mg/L (average = 2 105). The CFSTRs were sparged with air through fine-bubble diffuser stones at 7.1 L/minute providing an average dissolved oxygen (DO) concentration of 7.9 mg/L and a well-mixed aeration zone. Average bulk influent and effluent COD concentrations (not including target compound) for all CFSTRs during these studies were 374.5 and 39.9 mg/L respectively, (base-line organic removal efficiency of 89%).

Smaller 5-L reactors of the same configuration as the CFSTRs, except without clarifiers, were used as batch ERs. The ERs were operated as sequencing batch reactors (SBRs) (Irvine and Busch, 1979) on a 24-hour cycle with a 22-hour aeration time, 1-hour settle time, and 0.5-hour drain and fill times. During the fill cycle, concentrated substrate was added along with tap water for dilution. At the end of the daily 22-hour aeration cycle, the volume of mixed liquor required for bioaugmentation was removed and allowed to settle for 30 minutes in graduated cylinders. Supernatant was then decanted and cell pellets resuspended in tap water followed by transfer of resuspended cells to the CFSTR. Clear supernatant (3.5–4 L) was removed from ERs during the drain cycle and discarded. The SBRs were sparged with air at 2.4 L/minute through fine-bubble diffuser stones which maintained DO concentrations at 1–3 mg/L. The pH was monitored and maintained between 6 and 7. Biomass concentration varied between 2 000 and 4 000 mg MLVSS/L (mean = 3 533). Mixed liquor was wasted daily to give a MCRT of 7 days.

Activated sludge cultures. City of Los Angeles' Hyperion treatment plant AS was used as seed culture for each CFSTR. The 1NA-degrading enrichment seed source was a mixture of AS from a large West Coast oil refinery and from Hyperion. The CFSTRs were acclimated to 1NA for periods of at least 6 months prior to bioaugmentation experiments to approximate the best case acclimation scenario for conventional (unaugmented) systems. Continuous cultures were easily maintained indefinitely without reseeding, using the synthetic wastewater formulation in Table 2.

Design of enricher-reactor system. Two phases of experiments were conducted to show the utility of the proposed ER bioaugmentation system. The first phase involved development of suitable enrichment culture. Seed cultures were acclimated over a 7.5-month period as described elsewhere (Babcock, 1991), which resulted in a deep-purple colored culture. This culture has been shown to degrade 1NA when present as the sole carbon source and to mineralize the 1NA to carbon dioxide. In batch ER experiments, volatilization and adsorption were quantified and kinetic coefficients (V_m and K_m) were determined. The enrichment culture degraded 1NA concentrations as high as 300 mg/L. The enrichment was able to mineralize 35 mg 1NA/L as the sole source of carbon in 12 hours. Kinetic determinations (using Lineweaver-Burk, Hanes-Woolf, and Eadie-Hofstee plots) found that substrate removal followed Michaelis-Menten kinetics with values of K_m and V_m of 32.5 (+/–2.2) mg/L and 261 (+/–4.5) ng 1NA/mg cells · hour, respectively. In numerous experiments, volatilization accounted for 8–15% of compound removal, and adsorption onto biomass accounted for 8–10% of initial 1NA mass added. However, as degradation proceeded, cell-adsorbed 1NA was depleted prior to that in the supernatant. Experiments with the enrichment culture failed to detect intermediate metabolites probably because of analytical techniques which were not designed to recover acidic metabolites and/or consortium effects which would tend to deplete such compounds.

The second phase of experiments involved use of the enrichment culture as an inoculum source in bioaugmentation studies. Both batch and continuous-flow experiments were performed. Batch experiments were necessary to determine kinetics of degradation. Continuous-flow experiments were conducted to determine improvements in performance of CFSTRs due to bioaugmentation. Various stressful treatment scenarios were investigated including step loading increases, shock loading conditions, and loss of acclimation. Steady-state comparisons of bioaugmented systems to unacclimated and conventionally acclimated systems, treating the same influent substrates, were made.

Results and Discussion

Steady-state effects of bioaugmentation. Performance of conventional AS can be assessed by both batch-aliquot kinetic experiments and CFSTR effluent monitoring. Preliminary experiments were performed using two different daily inoculum sizes or bioaugmentation levels (2% and 6% as mg MLVSS/L). This is analogous to an initial mass concentration ratio of 1NA-degrading cells to acclimated indigenous cells of 1:50 and 1:16.7, respectively. Four identical CFSTRs were operated continuously, and one (unacclimated control) received no 1NA. The other three received 1 mg 1NA/L. One of these did not receive ER inoculations (conventional control). The others received daily inoculations of cells (2 or 6%) from an ER.

After 6 months (>24 MCRTs) the system was presumed to be at steady state and all CFSTRs effluents contained concen-

trations of 1NA below the detection limit of 0.01 mg/L. This represented greater than 99% 1NA removal efficiency. At this steady state, the CFSTR clarifiers were temporarily removed and influent wastewater flow was interrupted to facilitate batch kinetic analyses. Figures 3 and 4 show results of experiments in which the 2% bioaugmented cultures were spiked with 1 and 3 mg 1NA/L, respectively.

Figure 3 shows that the reactor receiving regular inoculations (2% by mass daily) of a 1NA-degrading enrichment culture did not significantly outperform the conventionally acclimated reactor when the 1NA spike was equivalent to that which it had been acclimated (1 mg/L). This may be due to the small ratio of inoculated to indigenous organisms (1:50, a ratio which is still well above that achieved with recommended doses of commercial bioaugmentation products). Figure 4 shows the augmented reactor degraded the larger 1NA spike (3 mg/L; 300% of acclimation concentration) to below the detection limit 25% faster than the conventional reactor. When spiked with 10 mg/L, the augmented reactor performed similarly to the conventional control (about 60% removal in one HRT, data not shown).

In Figures 3 and 4, the conventional and augmented reactors degraded the 1NA to below detectable concentrations well before the 12-hour HRT, highlighting the need for batch experiments. Significant losses observed from the unacclimated controls (approximately 50%) seem to agree with the previous results (Babcock, 1991), which showed that adsorption and volatilization could account for 8–10% and 8–15%, respectively, when using a much lower air sparge rate (1/3).

Transient effects of bioaugmentation. Figure 5 shows the hazardous compound breakthrough response to a step loading increase (1–5 mg/L) for conventionally acclimated and 6% bioaugmented CFSTRs. Prior to day 2, effluent 1NA concentrations were below the detection limit for both reactors. While the acclimated reactor responded fairly well with transient effluent 1NA concentrations of 1 mg/L or less, the augmented reactor response was much better allowing detectable 1NA residuals in the effluent for only one day. The conventional reactor required 7 days to acclimate to the increased hazardous compound loading: a period when it could have been out of compliance.

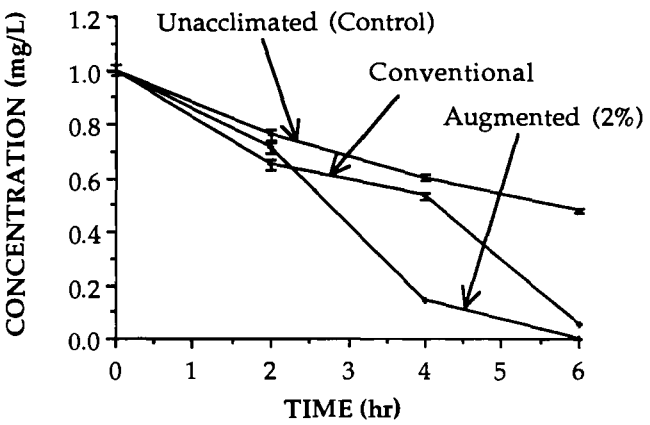

Figure 3—Batch kinetic response of unacclimated, acclimated, and 2% bioaugmented CFSTR cultures to a 1 mg 1NA/L spike.

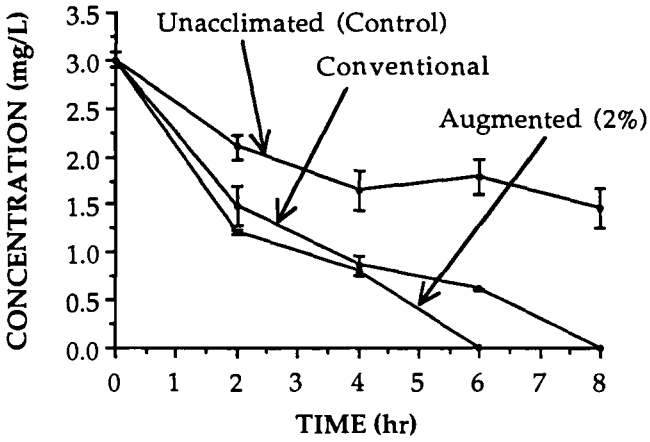

Figure 4—Batch kinetic response of unacclimated, acclimated, and 2% bioaugmented CFSTR cultures to a 3 mg 1NA/L spike.

Effect of bioaugmentation level. The effect of inoculum size on a one-time augmentation of AS (from a conventionally acclimated CFSTR receiving 5 mg 1NA/L for three MCRTs) which had not previously received ER inoculations was determined. Seven aliquots were prepared by centrifugation and separation of supernatant followed by resuspension of cells in 100 mL fresh media of the same composition as the CFSTR influent in 250-ml Erlenmeyer flasks. The prescribed amount of 1NA degrading enricher-reactor cells were used to replace an equivalent amount of indigenous biomass (0, 1, 2, 5, 10, 20, 50, and 100%). These represented mass concentration ratios of 1NA-degrading cells to indigenous cells of 0:1, 1:100, 1:50, 1:20, 1:10, 1:5, 1:2, and 1:0, respectively. The two controls (0% and 100%) represent the performance of a conventionally acclimated CFSTR, and the performance of the enrichment culture, respectively. Flasks were all spiked with 10 mg 1NA/L, covered tightly with aluminum foil, and placed on a shaker table at 200 rpm and 27°C.

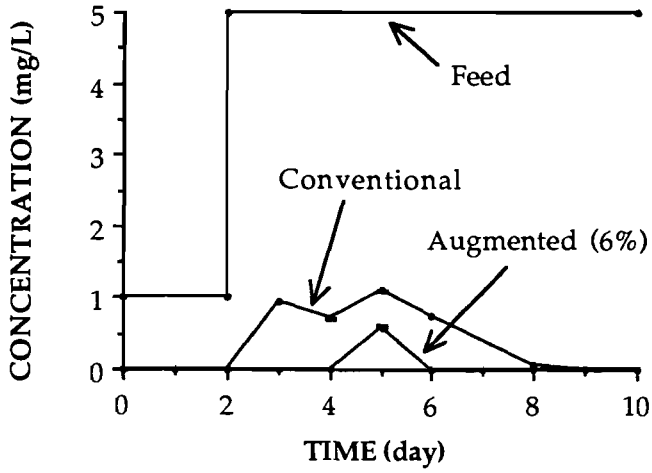

Figure 5—Bioaugmented and conventionally acclimated CFSTR responses to a step loading increase.

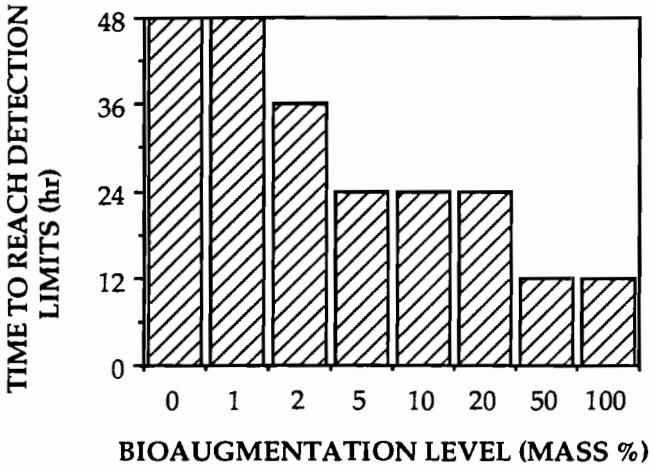

Figure 6—Effect of mass percent inoculum size on batch degradation of 10 mg 1NA/L.

Figure 6 shows total time versus inoculum size for supernatant 1NA concentrations to decrease below the detection limit. Total degradation time is shown to be a function of the inoculum size, with larger inocula providing faster degradation. Figure 6 shows that a 1% inoculum size was not enough to improve the average degradation rate, but a 2% inoculum resulted in a 33% increase in average degradation rate. A 5% inoculum increased the average degradation rate 2-fold, but there was no further increase until the inoculum size was increased to 50% (which behaved the same as the enrichment culture itself: 4-fold increase in average rate). By comparison, recommended doses of commercial products typically result in inoculum sizes of considerably less than 1%.

Figure 7 shows a schematic representation of the set-up used to examine transient effects of bioaugmentation. The response of CFSTRs acclimated to 5 mg 1NA/L for one month (3 MCRTs) to a shock loading of 50 mg 1NA/L was investigated (Figure 8). During the experiment, the influent concentration of 1NA remained at 5 mg/L in all CFSTRs (except for the unacclimated control which only received the 50 mg/L spike). Effluent 1NA concentrations for unacclimated control, conventional (0%), 1.4, 2.5, 6.6, 11.4, and 18.3% bioaugmented CFSTRs were monitored every 4 hours. After 32 hours, the concentration of 1NA in all effluents was below the detection limit.

The eight 4-hour effluent 1NA concentrations for each CFSTR were summed to determine the cumulative breakthrough for each reactor. Next, the percent reduction in breakthrough for each of the bioaugmented reactors was computed relative to both the control and the conventional CFSTRs. Figure 8 shows the reductions plotted versus inoculum size. With the exception of the 6.6% level, there was a larger decrease in cumulative 1NA breakthrough with increasing inoculum size. Also, there seems to be diminishing return with the larger inoculum sizes, that is, a 2.5% inoculum reduces effluent concentrations by about 13% (a 5-fold increase), but an 18.3% inoculum only reduces breakthrough by about 35% (a 1.9-fold increase). The poor response of the 6.6% bioaugmented reactor cannot be easily explained.

Loss of acclimation experiments. When a waste component is absent for a period of time, there is often some loss of acclimation to the compound and a lag period prior to reinstatement of adequate degradation of the compound after its reappearance. Factors that could be important include: length of the absence, type of compound, MCRT, and initial degree of acclimation. Figures 9 and 10 show the reacclimation response of the CFSTRs to 5 mg 1NA/L after 1NA was absent from the influent for 1.5 MCRT (15 days). Prior to the experiment, all CFSTRs were highly acclimated and effluent 1NA concentrations were below the detection limit. At the highest bioaugmentation level used (18.3%), no 1NA was detected in effluent samples during the 144 hour experiment. In a manner analogous to Figure 8, percent reductions in cumulative 1NA effluent concentrations compared

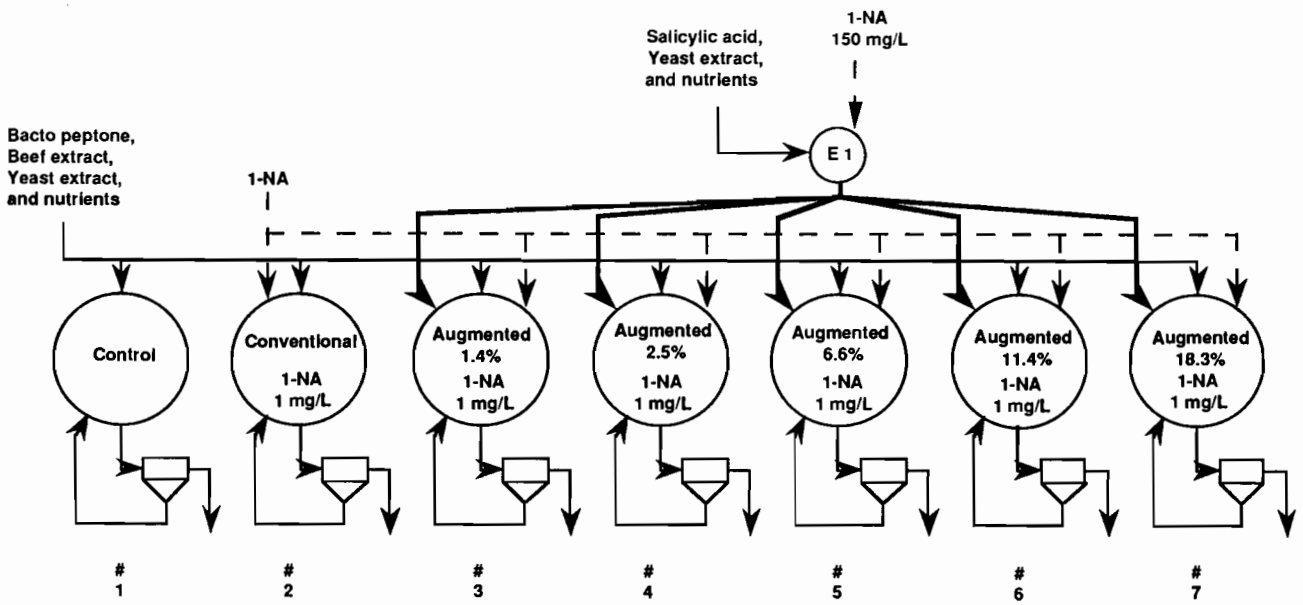

Figure 7—Laboratory setup for determining transient effects of bioaugmentation.

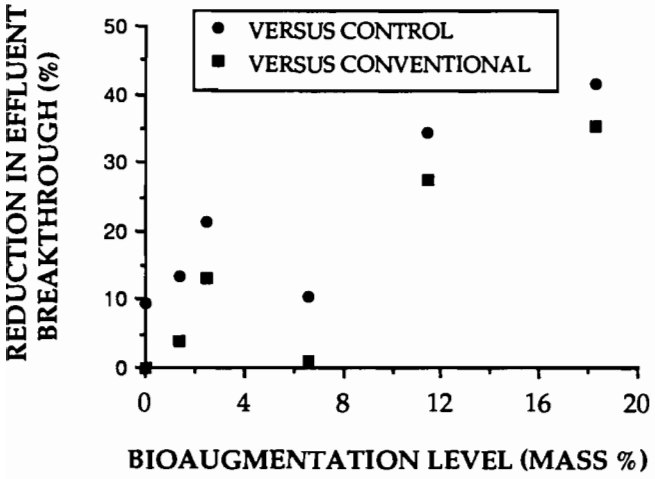

Figure 8—Effect of bioaugmentation level on CFSTR response to a 50 mg 1NA/L shock loading.

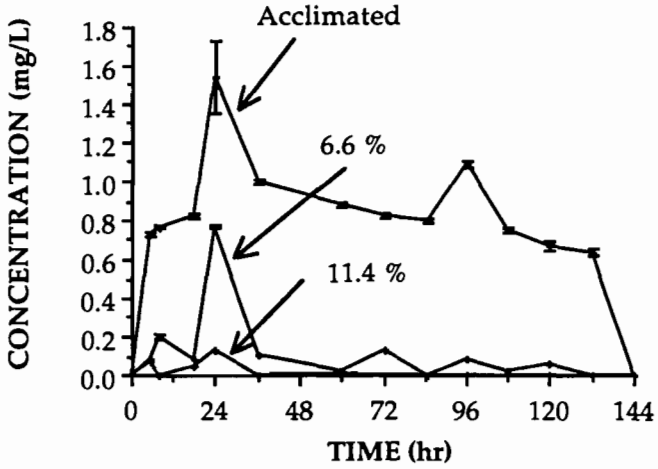

Figure 10—Effect of high bioaugmentation level on CFSTR reacclimation to 5 mg 1NA/L after its absence from the waste stream for 15 days.

to the conventional control were calculated. Results were 66, 73, 85, 98, and 100% reductions for the 1.4, 2.5, 6.6, 11.4, and 18.3% bioaugmented reactors, respectively. These results indicate that larger inocula impart greater stability, but the greatest increase of effect was provided by smaller inoculum sizes.

Nonbiological removal mechanisms: adsorption and volatilization. In the AS treatment process, there are three dominant removal mechanisms which compete for the 1NA. These include biodegradation (conversion to carbon dioxide, water, and new cell mass), adsorption and subsequent removal with waste sludge, and volatilization to the atmosphere.

Losses due to all nonbiological removal mechanisms in the CFSTRs when operated in the batch mode can be assessed from Figures 3 and 4. Any depletion from the unacclimated reactor during these tests is assumed to be due to nonbiological removal mechanisms (predominantly volatilization because no cell-adsorbed 1NA was detected in sludge samples). The large losses observed (approximately 50% during the course of the experiment) can be attributed to the high air sparge rates used to ensure adequate mixing and nonlimiting DO concentrations.

Volatilization of 1NA from the CFSTR vessels when operated in the continuous-flow mode was not directly measured, but periodic sampling of waste sludges to assess losses due to adsorption revealed no detectable 1NA (supernatant or cell-adsorbed). Removal by volatilization during continuous-flow operation can be assumed to be no greater than that observed during batch-mode operation. This means that the maximum possible nonbiological removal would be approximately 50%. However, because of the way the experiments were setup (Figures 5, 8, 9, and 10), all losses due to nonbiological mechanisms have been factored out of the results. In each case 1NA breakthrough or residuals from augmented CFSTRs are compared to residuals from unaugmented (but acclimated) CFSTRs. Thus, assuming that the nonbiological losses from all of the CFSTRs are equivalent, the reductions in effluent residuals shown are due to biodegradation.

It should be noted again that for the enrichment culture used here, biological removal has been determined to be due to mineralization of 1NA to carbon dioxide rather than conversion to possibly more toxic degradation products.

Previous similar work. Several investigators have performed bioaugmentation experiments similar to those reported here and have found a variety of results. We studied a mixed enrichment culture and a range of small to large inoculum sizes. We also tested the effect of bioaugmentation on both acclimated and unacclimated AS, with three different types of stressful loading conditions.

Unlike the present investigation, Edgehill and Finn (1983) used a pure culture of pentachlorophenol-metabolizing *Arthrobacter* to augment bench-scale continuous-flow AS degradation of a synthetic PCP-containing wastewater. They found that an initial inoculation with the pure culture amounting to 10% of indigenous biomass drastically reduced the period of acclimation to the PCP-waste. They also reported that the response to a step loading increase from 40 to 120 mg/L of PCP caused an upset condition in which effluent levels rose to 60 mg/L rapidly and

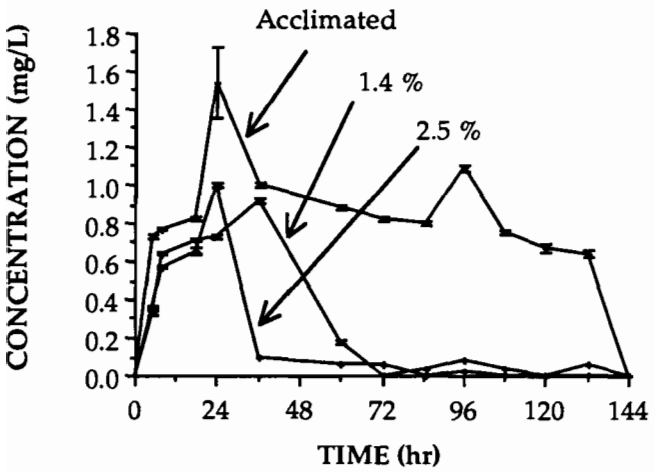

Figure 9—Effect of low bioaugmentation level on CFSTR reacclimation to 5 mg 1NA/L after its absence from the waste stream for 15 days.

only recovered after 48 hours with acclimated AS. However, when continuously augmented at a rate equal to 5 or 7% of the indigenous culture sludge production rate, the response to the same shock loading was much better with recovery occurring within 18 hours and effluent PCP concentrations only reaching 15 mg/L. The reported bioaugmentation levels can be converted to 0.8 and 1.1% as mass added divided by indigenous mass. These results are very similar to ours and indicate that smaller inocula may be possible when using a pure culture which has a greater affinity for the target compound. However, the maintenance of a pure culture large enough to augment a full-scale AS system may not be practical. In addition, they used much higher concentrations of the target compound which may have some beneficial effect on biodegradation.

Hartman *et al.* (1986) conducted bench-scale bioaugmentation experiments using SBRs and a hazardous wastewater. They used several unspecified specialized pure cultures and mixtures of pure cultures with established abilities to degrade the target compounds. Various unspecified bioaugmentation schemes were used. However, the bioaugmented reactors failed to perform better than unaugmented controls. The reasons for this are unknown but may include small inocula or inhibition of inocula. Once again, even if favorable results had been achieved, pure cultures would probably not be practical to maintain in a full-scale operation.

Lynch *et al.* (1987) investigated the bioaugmentation of organically stressed anaerobic filters treating cheese whey with an acetogen-methanogen enrichment culture. They found that arbitrarily small augmentation inocula did not improve recovery from an organic overloading. They hypothesized that this may have been due to small inoculum sizes or that the suspended culture supplement could not compete well with the indigenous fixed-film organisms. Although these investigators studied an anaerobic system and a nonhazardous wastewater, they did use an enrichment culture and small inocula. The authors felt that with larger inoculations and a CFSTR hydraulic regime, bioaugmentation would have provided better results: an assessment that agrees with our results.

Kennedy *et al.* (1990) found that high bioaugmentation levels (25, 33, 71, and 83%) improved the degradation of parachlorophenol shock loadings in laboratory chemostats. Similar to the results reported herein, an enrichment culture rather than a pure culture was used for inocula and performance was significantly enhanced. Unlike our work, they augmented only unacclimated AS and with 4.5–18 times as many cells as we used; this would not be practical on a larger scale. In addition, they only investigated the improvement in performance under shock loading conditions.

The literature review showed that bioaugmentation may not always work (Lewandowski *et al.*, 1986; Qasim and Stinehelfer, 1982; Hull and Kapuscinski, 1987; Martin and Zall, 1985; Lange *et al.*, 1987; Senthilnathan and Ganczarczyk, 1988; Hartman *et al.*, 1986; and Lynch *et al.*, 1987) and that if it does, it may require impractical inoculation volumes (Kennedy *et al.*, 1990). The bioaugmentation scheme we are proposing is different than commercial bioaugmentation preparations of cells which have generally shown poor results (except possibly at doses that would be prohibitively expensive; Lewandowski *et al.*, 1986). Enricher-reactors should produce cells at a reduced cost. Also, enrichment cultures should be much easier to maintain than pure cultures and hence are probably more practical.

We found that relatively small bioaugmentation levels can be used effectively and that bioaugmented reactors respond more favorably than acclimated and unacclimated AS systems under stressful operating conditions. There was no attempt to determine growth kinetics during this study because we eliminated this variable from the bioaugmentation process by advocating continuous or semicontinuous inoculation with an enrichment culture to be maintained on site. Thus, the inocula need not increase its numbers in the mainline reactor.

Indigenous cell production rates for each of the CFSTRs can be roughly approximated based on the nominal MCRT utilized. Since 10% of the cells were wasted daily to provide for the operating MCRT of 10 days and the MLVSS was constant throughout these experiments, cell growth was at least 10% (mass of new cells/mass of cells present at the start of the day) per day. Thus, because bioaugmentation level has been defined as mass of ER cells added per day divided by the mass of indigenous cells (expressed as a percentage), the bioaugmentation levels tested here (1.4, 2.5, 6.6, 11.4, and 18.3%) correspond to approximately 14, 25, 66, 114, and 183% of indigenous cell production rates, respectively.

This report detailed experiments designed to verify the utility of the ER bioaugmentation scheme proposed. It was necessary to choose a single model hazardous compound and develop a specific enrichment culture. However, a general approach can be suggested for further research and development. In general, the first step is to develop an enrichment culture with high target compound degradation efficiency using appropriate seed sources, substrates, and enrichment techniques. Second, determine an appropriate ER maintenance substrate which fosters rapid growth of the enrichment culture (to minimize required ER volume) and still maintains its affinity for the target compound. Third, conduct bench-scale experiments to determine the inoculum size which gives the main AS system the required increase in resistance to shock loadings, expected step-loading increases, and/or loss of acclimation. The final step would be scale-up.

Conclusions

Conventional AS systems are not well suited to the treatment of many hazardous wastes. The problem is an inability to maintain a continuously acclimated culture because of discontinuous, variable concentrations and shock loadings. The idea of maintaining a separate culture on site which is continuously acclimated to the problem wastes and can be added to the main system continuously or when required is one possible solution. Bench-scale CFSTRs supplemented daily with various quantities of an enrichment culture were compared with control cultures under different treatment conditions. The augmented reactors performed much better than controls. The economic viability of the proposed process remains to be determined, but this approach is probably less expensive than other approaches using cultures from off-site. Specific conclusions include the following. (1) Use of an off-line ER to maintain a 1NA-degrading culture for use as an inoculum source to conventional AS is a feasible method for improvement of biodegradation of 1NA. (2) The

relationship between the inoculum size of 1NA-degrading culture and increased average degradation rates is that larger inocula provide greater increases in rate. (3) Use of the proposed bioaugmentation scheme can make conventional AS more resistant to 1NA shock loadings and step loading increases. (4) ER bioaugmentation can reduce the effects of loss of acclimation due to absence of 1NA from the waste stream.

We anticipate that these results may be generalizable to varied hazardous wastewaters. In which case, the proposed ER system would allow existing conventional AS systems, after upgrading, to biologically treat many hazardous wastes. Treatment objectives include reduction or elimination of target compound breakthrough during shock loading events and step loading increases. By minimizing or eliminating the reacclimation period required in conventional systems, the ER system would also limit hazardous waste breakthrough during treatment of discontinuous hazardous waste flows.

Acknowledgments

Credits. This research was supported in part by the Engineering Research Center for Hazardous Substances Control, a National Science Foundation industry-university cooperative center for hazardous waste research at the University of California, Los Angeles. We thank Kenneth Wong and Eddie Tzeng for their assistance with the daily maintenance of the AS units used in these experiments, and with GC and HPLC analysis, and to Robert A. Mah for helpful suggestions on this study. Portions of this paper were presented at the 63rd Annual Conference, Water Pollution Control Federation, Washington, D. C., October 1990.

Authors. Roger W. Babcock Jr. and Chu-Chin Hsieh are research assistants, Kyoung S. Ro is a postdoctoral researcher, and Michael K. Stenstrom is a professor in the Civil Engineering Department at the University of California, Los Angeles. Correspondence should be addressed to M. K. Stenstrom, University of California, Los Angeles, Dep. of Civil Engineering, 4173 Engineering I, Los Angeles, CA 90024-1600.

Submitted for publication December 30, 1990; revised manuscript submitted September 16, 1991; accepted for publication April 27, 1992. The deadline for discussions of this paper is January 15, 1993. 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

Arbuckle, W. B., and Kennedy, M. S. (1989) Activated sludge response to a parachlorophenol transient. *J. Water Pollut. Control Fed.*, **61**, 476.

Babcock, R. W. Jr. (1991) Enricher-Reactor Bioaugmentation of Activated Sludge for Degradation of Hazardous Wastewaters. Ph.D. thesis, Univ. Calif., Los Angeles.

Baker, R. D., *et al.* (1992) Management of Hazardous Waste in the United States. *Haz. Waste Haz. Mater.*, **9**, 37.

Beardsley, M. L., and Coffey, J. M. (1985) Bioaugmentation: Optimizing Biological Wastewater Treatment. *Pollut. Eng.*, **17**, 30.

Boeniger, M. (1980) The Carcinogenicity and Metabolism of Azo Dyes, Especially Those Derived from Benzidine. NIOSH Technical Rep., U.S. Dep. Health and Human Serv., Washington, D.C., 3.

Cardinal, L. C., and Stenstrom, M. K. (1991) Enhanced biodegradation of polyaromatic hydrocarbons in the activated sludge process. *J. Water Pollut. Control Fed.*, **63**, 950.

Case, R. A., *et al.* (1954) Tumors of the Urinary Bladder in Workmen Engaged in the Manufacture and Use of Certain Dyestuff Intermediates in the British Chemical Industry. Part I. The Role of Aniline, Benzidine, Alpha-Naphthylamine, and Beta-Naphthylamine. *Br. J. Industr. Med.*, **11**, 75.

Chambers, J. V. (1981) Improving Waste Removal Performance Reliability of a Wastewater Treatment System Through Bioaugmentation. *Proc. 36th Ind. Waste Conf., Purdue Univ.*, West Lafayette, Ind., 631.

Dictionary of Organic Chemicals (1982). J. Buckingham (Ed.), Chapman and Hall, New York, N.Y., 4167.

Edgehill, R. U., and Finn, R. K. (1983) Activated Sludge Treatment of Synthetic Wastewater Containing Pentachlorophenol. *Biotechnol. Bioeng.*, **25**, 2165.

Federal Register (1974) OSHA Rules and Regulations, Sec. 1910.93d alpha-Naphthylamine. **39**, 23548.

Giddings, J. M. (1979) Acute Toxicity to *Selenastrum capriconutum* of Aromatic Compounds from Coal Conversion. *Bull. Environ. Contam. Toxicol.*, **23**, 360.

Goldblatt, M. W. (1958) Occupational Carcinogenesis. *Br. Med. Bull.*, **14**, 137.

Grady, C. P. L., Jr. (1985) Biodegradation: Its Measurement and Microbial Basis. *Biotechnol. Bioeng.*, **27**, 660.

Grady, C. P. L., Jr. (1986) Biodegradation of Hazardous Wastes by Conventional Biological Treatment. *Haz. Wastes Haz. Mater.*, **3**, 333.

Hartman, J. R., *et al.* (1986) Effects of Bioaugmentation on Hazardous Waste Treatment. *Proc. 18th Mid-Atlantic Ind. Waste Conf.*, Virginia Tech. Univ., Blacksburg, Va., 94.

Hockenbury, M. R., and Grady, C. P., Jr. (1977) Inhibition of nitrification-effects of selected organic compounds. *J. Water Pollut. Control Fed.*, **49**, 768.

Hull, S. J., and Kapuscinski, R. B. (1987) Efficacy of Bioaugmentation Products as Predicted by a Model of Steady-State Flocculent Cultures. *Proc. 42rd Ind. Waste Conf., Purdue Univ.*, West Lafayette, Ind., 297.

Irvine, R. L., and Busch, A. W. (1979) Sequencing batch biological reactors—an overview. *J. Water Pollut. Control Fed.*, **51**, 235.

Kennedy, M. S., *et al.* (1990) Parachlorophenol degradation using bioaugmentation. *J. Water Pollut. Control Fed.*, **62**, 227.

Kobayashi, H., and Rittmann, B. E. (1982) Microbial Removal of Hazardous Organic Compounds. *Environ. Sci. Technol.*, **16**, 170A.

Lange, C. R., *et al.* (1987) Constraints of Bioaugmentation in Enhancing Biological Treatment Process Performance. *Proc. 42th Ind. Waste Conf., Purdue Univ.*, West Lafayette, Ind., 275.

Lewandowski, G., *et al.* (1986) Biodegradation of Toxic Chemicals Using Commercial Preparations. *Environ. Prog.*, **5**, 212.

Lynch, N., *et al.* (1987) Bioaugmentation of Stressed Anaerobic Filters with Methanogenic Enrichment Cultures. *Proc. 42th Ind. Waste Conf., Purdue Univ.*, West Lafayette, Ind., 285.

Malaney, G. W. (1960) Oxidative abilities of aniline-acclimated activated sludge. *J. Water Pollut. Control. Fed.*, **32**, 1300.

Martin, J. H., and Zall, R. R. (1985) Dairy Processing Wastewater Bioaugmentation—An Evaluation of Effectiveness. *Proc. 40th Ind. Waste Conf., Purdue Univ.*, West Lafayette, Ind., 351.

McCann, J., *et al.* (1975) Detection of Carcinogens as Mutagens in the Salmonella/Microsome Test: Assay of 300 Chemicals. *Proc. Natl. Acad. Sci. USA, Med. Sci.*, **72**, 5135.

McDowell, C. S., and Zitrides, T. G. (1979) Accelerating the Dynamic Response of Bacterial Populations in Activated Sludge Systems. *Proc. 34th Ind. Waste Conf., Purdue Univ.*, West Lafayette, Ind., 664.

Merck Index (1989). 9th Ed., Merck and Co., Inc., Rahway, N.J., 6315.

Nyer, E. K., and Bourgeois, H. J., Jr. (1980) Operational Troubleshooting

in Industrial Biological Treatment Systems. *Proc. 35th Ind. Waste Conf., Purdue Univ.*, West Lafayette, Ind., 849.

Pitter, P. (1976) Determination of Biological Degradability of Organic Substances. *Water Res.* (G.B.), **10,** 231.

Proctor, N. H., and Hughes, J. P. (1978) Chemical Hazards of the Workplace. J. B. Lippincott Co., Philadelphia, Pa., 368.

Qasim, S. R., and Stinehelfer, M. L. (1982) Effect of a bacterial culture product on biological kinetics. *J. Water Pollut. Control Fed.*, **54,** 255.

Scott, T. S. (1962) Carcinogenic and Chronic Toxic Hazards of Aromatic Amines. Elsevier Publishing Co., New York, N.Y., 16.

Senthilnathan, P. R., and Ganczarczyk, J. J. (1988) Adaption and Deadaption Kinetics of Activated Sludge. *Proc. 43rd Ind. Waste Conf., Purdue Univ.*, West Lafayette, Ind., 301.

Shaul, G. M., *et al.* (1985) Removal of Azo Dyes by the Activated Sludge Process. *Proc. 40th Ind. Waste Conf., Purdue Univ.*, West Lafayette, Ind., 847.

Staff. (1974) Final Rules Set for Exposure to Carcinogens. *Chem. Eng. News,* **52,** 12.

Standard Methods for the Examination of Water and Wastewater (1989). 17th Ed., Am. Public Health Assoc., Washington, D.C., 2.75.

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

Thibault, G. T., and Tracy, K. D. (1979) Demonstration of a Mutant Bacterial Additive for Enhancement of Operational Stability in Oxygen Activated Sludge. *Proc. 34th Ind. Waste Conf., Purdue Univ.*, West Lafayette, Ind., 235.

Wilson, B. W., *et al.* (1985) Chemical Analysis and Mutational Assay of Distilled Oils from the H-Coal Direct Liquefaction Process: A Status Report. U.S. Dep. Energy, Washington, D.C.

Wong, A. D., and Goldsmith, C. D. (1988) The Impact of a Chemostat Discharge Containing Oil Degrading Bacteria on the Biological Kinetics of a Refinery Activated Sludge Process. *Water Sci. Tech.*, **20,** 131.

Young, C. Y. (1976) The Use of Enzymes and Biocatalytic Additives for Wastewater Treatment Processes. *Water Pollut. Control Fed. Highlights,* **13,** 5.