Use of inducer compounds in the enricher-reactor process for degradation of 1-naphthylamine wastes

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ABSTRACT: The feasibility of using less hazardous inducer compounds to maintain the ability of an enrichment culture to degrade a hazardous compound was investigated. Bench-scale (5 L) batch enricher-reactors (ERs) maintaining enrichment cultures were used to bioaugment bench-scale continuous-flow activated sludge reactors treating 1-naphthylamine (1NA). Potential inducer compounds tested were 1-acetate-naphthalene, 1-naphthoic acid, 1-naphthalene-sulfonic acid, and gentisic acid. In batch experiments, subcultures of the original enrichment culture that had been grown on potential inducer compounds for a period of 3 months without 1NA, maintained the ability to degrade 1NA but at reduced rates compared to a control maintained on 1NA. In continuous-flow experiments, separate 13.7-L reactors received daily inoculations of 2–4% by mass of 1NA-enrichment culture, or 3% by mass of the subcultures maintained on inducer compounds. Target compound breakthrough from augmented reactors was significantly reduced (relative to an acclimated control) following a step-increase from 1 to 5 mg 1NA/L, and reintroduction of 5 mg 1NA/L after its absence from the waste stream for 9 days (two separate experiments). A modest reduction of breakthrough was also observed following a 10 mg 1NA/L spike (a separate experiment). *Water Environ. Res.*, **65**, 26 (1993).

KEYWORDS: activated sludge, bioaugmentation, biodegradation, enricher-reactors, inducer compounds, 1-naphthylamine.

Wastewaters containing hazardous organic compounds are often difficult to treat effectively using conventional activated sludge (AS) processes. The difficulty is an inability to maintain a continuously acclimated culture, and is due to factors such as non-steady-state composition of the waste (both the easily degraded and the more resistant hazardous constituents), unsteady loading conditions (spikes and discontinuities), and compound recalcitrance (due to toxicity or slow degradation rates). If an enrichment culture can be developed to efficiently degrade the hazardous compounds in the waste stream, presence of the culture in sufficient quantity in the AS system treating the hazardous wastewater would ensure removal of the hazardous compounds prior to their release in the effluent. The enricher-reactor (ER) process, which has been described in detail elsewhere (Babcock *et al.*, 1992), utilizes off-line sequencing batch reactors (SBRs) maintaining AS enrichment cultures as a source of inocula for continuous bioaugmentation of conventional AS systems treating hazardous wastewaters. Figure 1 shows a diagram of the process.

In previous work, the ER process was tested in bench-scale experiments that examined the effects of inoculum size (bioaugmentation level) on improvement in treatment efficiency. Bench-scale continuous-flow stirred tank reactors (CFSTRs) received daily inoculations of an enrichment culture (Babcock *et al.*, 1992) that was maintained on a substrate containing possible degradation intermediates (salicylate, succinate, and pyruvate) as well as high concentrations of the target compound (1-naphthylamine [1NA]). Controls and bioaugmented reactors were subjected to 1NA spikes, step-increases, and discontinuities. The relationship between bioaugmentation level and reduction in 1NA breakthrough in effluent samples was quantified. Results were favorable, with higher bioaugmentation levels affecting greater treatment efficiency.

A potential drawback of scaling-up the process as previously tested is the requirement of large quantities of the hazardous compounds as ER substrate. This problem could be eliminated if less hazardous or nonhazardous compounds that would induce the same enzymes as the target compounds could be used as ER substrate. This may be possible due to the nature of bacterial enzyme systems.

Bacteria utilize two general types of enzyme systems; biosynthetic enzyme systems that provide amino acids and other intermediates that are essential for growth and maintenance of cellular activities, and catabolic enzyme systems that are associated with conversion of organic compounds into energy and simpler growth substrates. Biosynthetic enzymes are often constitutive (produced continuously), whereas degradative enzymes usually require induction by the compound degraded. These enzymes can be substrate specific but are often quite nonspecific and can be induced by compounds of similar structure, degradation products, or earlier precursors (Clarke, 1984, and Chapman, 1971).

An ideal inducer compound would maintain the degradation kinetics and growth characteristics of an enrichment culture without the presence of the enrichment substrate (target compound). Grady (1985 and 1986) previously identified the importance of this concept. Previous work has noted the possible benefits of inducer compounds, but always with at least a trace of the target compound present at all times.

Cardinal and Stenstrom (1991) found that an enrichment culture maintained on salicylate and just a trace of naphthalene maintained a high affinity for naphthalene for many months. Hickman and Novak (1984) found that AS acclimation to pentachlorophenol provided protection from shock loadings of related priority pollutants. Stenstrom *et al.* (1989) working with xylenols, cresols, and 2,4,6-trimethylphenol showed that bacteria acclimated to one compound were able to degrade a whole class of compounds.

This paper addresses the use of inducer compounds to maintain activity over long periods without the presence of the target compound. Several inducer compounds are used to maintain subcultures of an enrichment culture. Inducer compounds are evaluated for their ability to induce enrichment culture degra-

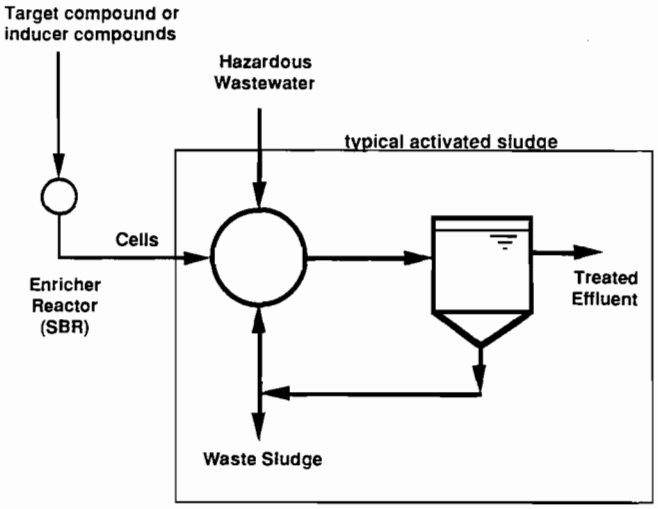

Figure 1—Enricher-reactor concept schematic diagram.

dation of 1NA. The performance of the induced cultures is compared to that of the original enrichment culture in CFSTR experiments.

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.). The synthetic wastewater media used for all continuous-flow experiments has been described previously (Babcock *et al.*, 1992). The ER media (Babcock *et al.*, 1992) which included beef extract, Bacto Peptone, yeast extract, salicylic acid, pyruvic acid, and succinic acid, was used as the base media for maintenance of all the subcultures described here. Experiments with 1NA as the sole source of carbon utilized a mineral salts media consisting of (numbers in parenthesis denote concentrations in mg/L); KH_2PO_4 (455), K_2HPO_4 (723), $(NH_4)_2SO_4$ (182), $CaCl_2 \cdot 2H_2O$ (10.5), $MgCl_2 \cdot 6H_2O$ (16.4), $FeCl_3$ (1.4), $MnCl_2 \cdot 4H_2O$ (0.34), $ZnCl_2$ (0.24), $CoCl_2 \cdot 6H_2O$ (0.21), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.15), $CuCl_2 \cdot 6H_2O$ (0.15), and $Na_2B_4O_7 \cdot 10H_2O$ (0.089) in tap water.

Analytical methods. Aromatic compounds were analyzed by capillary gas chromatography (GC) as previously described and/or high-performance liquid chromatography (HPLC) using a Dionex ion chromatograph operated as an HPLC (Babcock, 1991). The following HPLC operating conditions were used: sample volume, 10 μl; column, Dynamax 300A 12 μm C18 25-cm bed from Rainin Inst. Co. (Woburn, Mass.); mobile phase, isocratic 30% acetonitrile 70% water; flow rate, 1.5 mL/min; detector, UV-200 Spectrophotometer from Linear Inst. Co. (Reno, Nev.) set at 254 nm; integrator, HP3396A from Hewlett-Packard (Avondale, Pa.). The detection limit for 1NA using either GC or HPLC was 1.0 ng. Supernatant samples were prepared for analysis using centrifugation followed by solid phase extraction (Babcock, 1991). In shake-flask experiments, centrifuged cell pellets were extracted with methanol to quantify adsorption by resuspension in methanol and vigorous shaking for 1 minute, followed by centrifugation and HPLC analysis of decanted liquid. Biomass concentrations (mixed-liquor volatile suspended solids [MLVSS]) were measured biweekly using method 2540E (Standard Methods, 1989), and bulk organic content of CFSTR influent and effluents was monitored weekly as filtered COD using method 5220C (Standard Methods, 1989).

Activated sludge reactors and cultures. The eight rectangular 13.7-L laboratory-scale CFSTRs used in these experiments have been described previously (Babcock *et al.*, 1992). The physical setup as well as operation and maintenance procedures for the CFSTRs have been reported (Babcock *et al.*, 1992). Bulk system operating parameters for CFSTRs are given in Table 1. The various ERs (original enrichment culture and subcultures) were all operated as sequencing batch reactors (SBRs) with the same operating parameters as the original enrichment culture (see Table 1). Continuous cultures were easily maintained indefinitely without reseeding.

The AS seed culture sources and enrichment culture development procedures are described elsewhere (Babcock *et al.*, 1992). The enrichment culture has been shown to mineralize 1NA to CO_2, and abiotic removal mechanisms, volatilization, and adsorption have been quantified. Degradation was found to follow Michaelis-Menten kinetics and the values of V_m (6.3 μg 1NA/mg cells · d) and K_m (32.5 mg/L) have been reported (Babcock *et al.*, In press).

Enricher-reactor system. Two phases of experiments were conducted to show the utility of using inducer compounds in the ER process. The first phase involved development of subcultures maintained on potential inducer compounds (without 1NA) and determination of how well they retained the ability to rapidly degrade 1NA. The second phase of experiments involved use of the original enrichment culture and subcultures as inocula in bioaugmentation experiments. Continuous-flow experiments were conducted to determine improvements in 1NA treatment performance of CFSTRs due to bioaugmentation. Three transient treatment scenarios were investigated including a step increase, a shock loading, and loss of acclimation.

Results

Choice of potential inducer compounds. The catabolic enzymes responsible for degradation of 1NA have not been isolated at

Table 1—Laboratory reactor operating parameters.

Parameter	CFSTRs	SBRs
Volume	12.2 L aeration zone, 1.5 L clarifier	5.0 L
HRT	0.458 day (Q = 18.5 mL/minute = 26.64 L/day)	22 hour aeration, 1.0 hour settle, 0.5 hour drain, 0.5 hour fill
MCRT	8.9 days	10 days
MLVSS	1200–1600 mg/L	2500–3500 mg/L
Loading rate	0.81 kg COD/m^3 · d	0.76 kg COD/m^3 · d
1NA influent	1–5 mg/L	0.0 mg/L of 1NA 100 mg/L of inducers
F:M	0.27–0.51	0.19–0.38
Aeration	7.1 L/minute average DO = 7.9 mg/L	2.4 L/minute DO = 1–4 mg/L
pH	6.5–7.5	6–7
Temperature	13–23°C	18–30°C

this time, but considerable work toward construction of the degradation pathway has been completed utilizing an axenic culture of organisms isolated from our enrichment culture. Experiments using the isolate and the considerable literature available with regard to degradation pathways for unsubstituted (Barnsley, 1976; Zuniga *et al.*, 1981; Ensley *et al.*, 1982; and Yen and Gunsalus, 1982) and some mono and disubstituted naphthalene compounds (Williams *et al.*, 1975; Brilon *et al.*, 1981a; Brilon *et al.*, 1981b; Nortemann *et al.*, 1986; and Wittich *et al.*, 1988) and other polyaromatic hydrocarbons (Gibson and Subramanian, 1984), have led to a proposed degradation pathway (to be published elsewhere).

The proposed pathway is similar to that of unsubstituted naphthalene, with the initial enzymatic attack occurring on the opposite end of the compound from the amino group. This results, after several steps, in 3-amino-salicylic acid (3AS). A compound isolated during degradation of 1NA by pure culture was identified as 3AS following purification and comparison to genuine 3AS. The isolate was unable to degrade naphthalene or salicylic acid. Based on the determination that high gentisate 1,2-dioxygenase enzyme activity and only low catechol 1,2- and 2,3-dioxygenases activities were found during growth on 1NA, we believe that 1NA catabolism probably is affected via the gentisate pathway. It should also be noted that breakage of the first benzene ring of 1NA probably results in the formation of pyruvate (a component of the enrichment culture substrate).

Based on the proposed pathway several potential inducer compounds were chosen, including: 1-acetate-naphthalene (1AN), 1-naphthalene-sulfonic acid (1SN), 1-naphthoic acid (1NO), 1-chloro-naphthalene (1CN), and gentisic acid (GA). Gentisic acid was chosen due to its presence in the proposed degradation pathway, and the others were chosen because of structural similarity. The proposed degradation pathway constituent 3AS was not used because of its high cost. If ingested, GA and 1AN are moderately toxic (Sax and Lewis, 1989). Additionally, since 2-naphthalene-sulfonic acid is poisonous if ingested and 2-naphthoic acid is mildly toxic by ingestion (Sax and Lewis, 1989), we can speculate that 1SN and 1NO are likely to be toxic by ingestion also. Both GA and 1AN are reported in the EPA Toxic Substances Control Act Inventory. Also, 1AN is used commercially as a plant growth regulator for propagation of roots, and 1NO is a skin irritant (Merck Index, 1989). The inducer compounds chosen are not exactly benign, but none of them are regulated carcinogens and could therefore be considered less hazardous than 1NA.

Subcultures grown on inducer compounds. Several subcultures of the original enrichment culture were initiated with a 20% inoculum (1 L diluted to 5 L). The subcultures were fed the same media as the enrichment culture and spiked with 100 mg/L of one of the following instead of 1NA daily; 1AN, 1NO, 1CN, 1SN, or GA. Another subculture was initiated which received neither 1NA nor any inducer compound. This subculture control was used to determine any background inducing effect of the other ER substrate components which included pyruvate (thought to be included in the degradation pathway), succinate, and salicylic acid (a known naphthalene degradation inducer). The subcultures were grown under conditions identical to those of the enrichment culture (5-L SBRs with 22-hour aeration cycle, and MCRT of 10 days). Good growth was observed for all subcultures and all had good settling characteristics except for 1CN which was eliminated from further evaluation for this reason.

After 3 months acclimation to the inducer compounds (equivalent to nine MCRTs), the subcultures were tested for their ability to degrade 1NA. Batch aliquots containing 400 mg of cells were removed from each subculture and centrifuged for 5 minutes. The supernatant was then decanted and the cells resuspended in tap water and shaken. The cells were centrifuged again (5 minutes), decanted, and finally resuspended in 200 mL of fresh ER media in 500-mL Erlenmeyer flasks (initial MLVSS = 2 000 mg/L). Flasks (the original enrichment culture, one each of the inducer compound subcultures, and a no-cell control) were spiked with 5 mg/L of 1NA, covered tightly with aluminum foil, and placed on a shaker-table at 200 rpm and 28°C. Samples were taken every 12 hours, centrifuged, and the supernatant extracted for HPLC analysis (detection limit 0.020 mg/L).

Figure 2 shows 1NA disappearance. Error bars represent 95% confidence intervals based on the HPLC standard curve regression lines and the recovery efficiency of the extraction procedure as described previously (Babcock, 1991). The 1NA enrichment culture degraded the 5 mg 1NA/L spike in approximately 24 hours. All of the subcultures retained the ability to degrade 1NA, although slower than the original enrichment culture. The control flask indicated that volatilization losses were minimal. Figure 2 shows that the inducer compounds did not induce 1NA-degrading enzymes to the same degree as 1NA.

Subculture respiking experiment. Figures 3 through 6 show the effect of reexposure to 1NA on the subcultures following depletion of supernatant 1NA concentrations to below the detection limit (0.050 mg/L). For this experiment, 400 mg of the appropriate cells were washed as described above and resuspended in 200 mL mineral media in 500-mL Erlenmeyer flasks (initial MLVSS = 2 000 mg/L). Flasks were then spiked with 15–20 mg/L of 1NA from a 1NA-in-water stock solution, covered with aluminum foil, and placed on a shaker-table at 27°C and 200 rpm (1NA was the sole carbon source). Samples were taken at the appropriate intervals and centrifuged. Decanted supernatant samples were extracted and analyzed by HPLC and/or GC. Cells were extracted with methanol and analyzed by HPLC. There were eight flasks; the original 1NA enrichment culture (data not shown), the 1AN enrichment culture (Figure 3), the 1SN enrichment culture (data not shown), the 1NO enrichment culture (data not shown), the GA enrichment culture (Figure 4), cells from the acclimated CFSTR (Figure 5), the subculture control (data not shown), and a no-cell control (Fig-

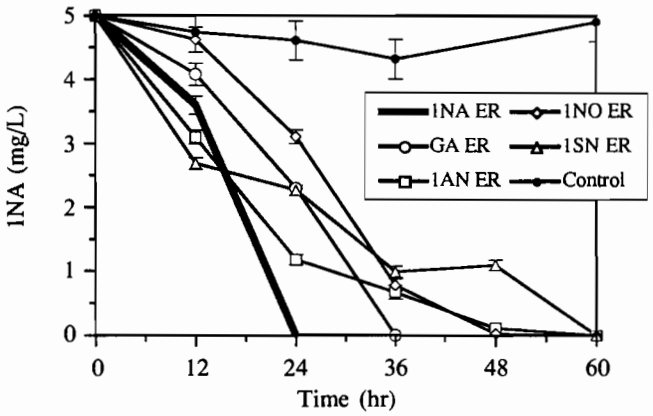

Figure 2—Initial rate comparison.

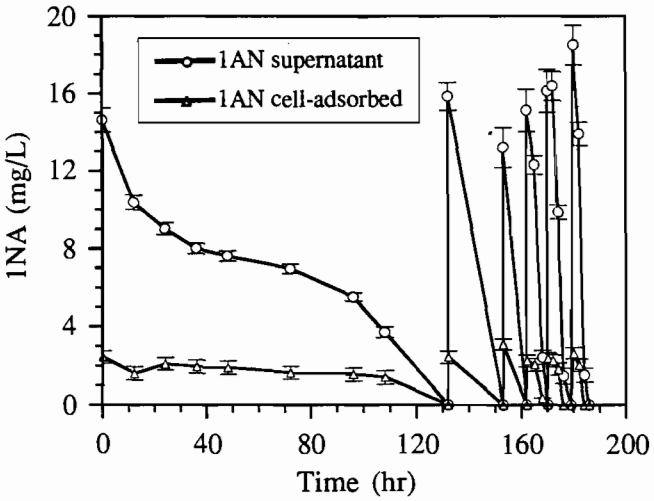

Figure 3—Effect of respiking on the 1-acetate-naphthalene subculture.

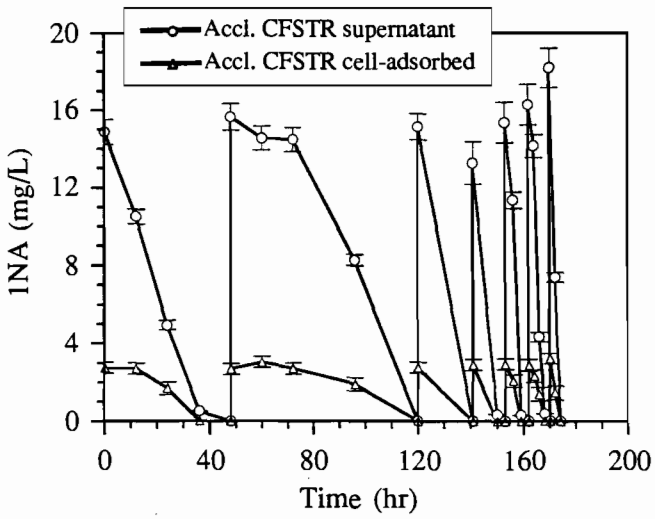

Figure 5—Effect of respiking acclimated CFSTR cells.

ure 6). For all figures, error bars represent 95% confidence intervals.

The original 1NA enrichment culture flask was able to degrade a 1NA spike of 13.7 mg/L, followed by spikes of 14.5 and 15.1 mg/L, each within 3 hours (data not shown). Samples were taken every 3 hours, and in each of the three spikings the 1NA had been depleted to below the detection limit within that time. Cell-adsorbed 1NA was 2.6, 2.8, and 2.3 mg/L at time zero for spikes one through three, respectively, and below the detection limit at time 3 hours for each spike. Respiking had no measurable effect on the 1NA degradation rate for the original enrichment culture.

Figure 3 shows the effect of respiking the 1AN-induced enrichment culture. The time to deplete the first spike to below the detection limit was 132 hours, and subsequent spikes were degraded in progressively shorter time periods (21, 9, 9, 9, and 7 hours). After five exposures to 1NA, the 1AN subculture still required more than twice as long as the parent culture to degrade the 1NA spike. Further exposures were not tested, but it is believed that the rate would increase further and eventually approach the degradation rate of the original enrichment culture. Nearly identical data were taken for the 1SN and 1NO subcultures (data not shown). The 1SN culture responded faster, requiring only 102 hours for the first spike and 28, 12, 9, 9, and 6 hours for subsequent spikes. The 1NO culture behaved very similarly to the 1AN culture requiring 132, 21, 9, 6, 8, and 8 hours to degrade the 1NA spikes. It is not clear why the 1NO culture was able to degrade the 4th spike more rapidly than the 5th and 6th.

Figure 4 shows that the GA-induced culture may have performed better than the others, requiring 96, 40, 21, 9, 6, 6, and 6 hours to degrade the 1NA spikes. Figure 5 shows slightly different results for cells from an acclimated CFSTR (exposed to 1–5 mg 1NA/L continuously for 12 months). The acclimated cells that did not receive any of the inducer compounds but which had continuously received 1NA required 48, 72, 21, 12, 9, 8, and 4 hours to degrade the 1NA spikes. The flask with subculture control cells (maintained without inducer compounds

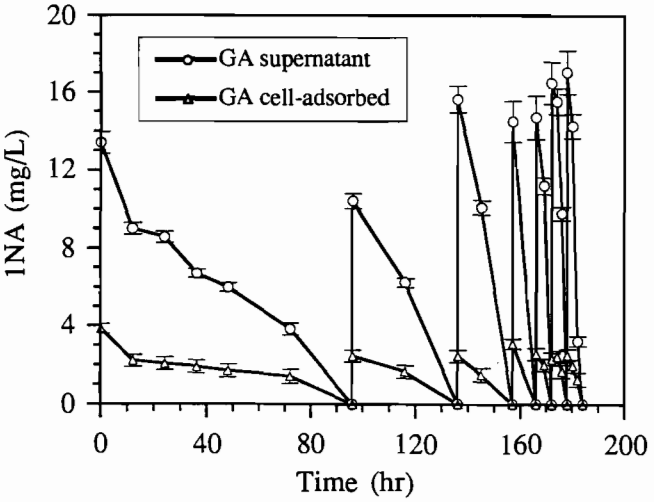

Figure 4—Effect of respiking on the gentisic acid subculture.

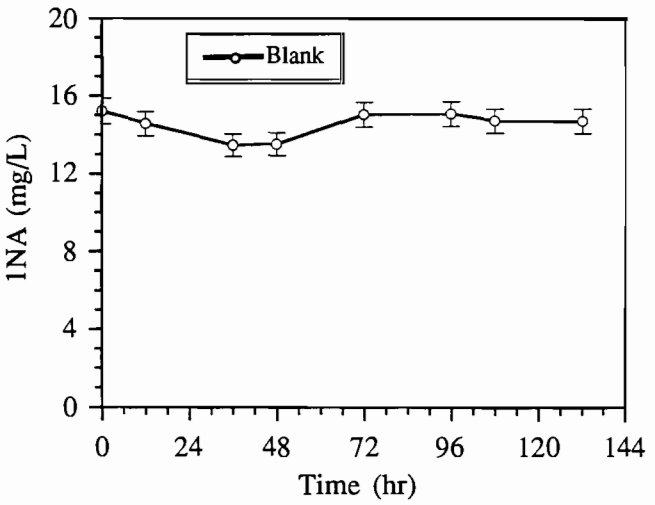

Figure 6—Respiking experiment control flask.

or 1NA) only degraded approximately 35% of the first 1NA spike during the time period of the 186-hour respiking experiment (data not shown). The no-cell control flask (Figure 6) indicates that volatilization was not a significant removal mechanism.

Several points can be made regarding the respiking experimental results. None of the induced subcultures were able to achieve the same batch degradation rates as the original enrichment culture even after repeated exposures. While it appears that the GA-induced culture may have performed better than the others, after 3 exposures to 1NA, each subculture was able to degrade the 1NA spike in approximately 9 hours and subsequent spikes were degraded in 6–8 hours in all cases. Thus, any of these inducer compounds may be of about equal utility. Volatilization can be ruled out as a significant removal mechanism (Figure 6), as can adsorption which accounts for approximately 20% of initial uptake, followed by disappearance of cell-adsorbed 1NA coincident with supernatant-1NA depletion in all cases. Based on the performance of the subculture control, there is some maintenance of affinity of the inducer compound-maintained subcultures for 1NA which cannot be attributed to the base media components.

The response of the acclimated CFSTR cells (Figure 5) to the respiking experiment can be interpreted as an enrichment for cells (increase in number) that could degrade 1NA due to the fact that the other media components that were normally present had been removed (1NA was the sole source of carbon). Those organisms able to degrade 1NA were able to grow at the expense of those that could not and hence their numbers increased rapidly and degradation proceeded more rapidly with subsequent exposures (at least after the second exposure). Presumably, the reason the culture was able to degrade the 1NA initially was that it was acclimated to 1NA because of continuous exposure to 1NA. It follows that the similar performance noted for the inducer compound-maintained subcultures was due to continuous exposure to the inducer compound.

While the inducer compound-maintained cultures took longer than the acclimated cells (132, 102, 132, and 96 hours versus 48 hours) to degrade the first spike of 1NA, all the induced cultures degraded 1NA as well as, or better than the acclimated cells after the second exposure. However, all the induced cultures required significantly more time to degrade all spikes than the original enrichment culture (which only required 3 hours each time). It should be noted that unacclimated AS initially took 6 months to acquire the ability to degrade 1NA. This experiment shows that inducer compounds may be used to maintain the enrichment cultures ability to degrade 1NA, although at diminished rates.

Bioaugmentation experiments. Figure 7 shows the laboratory setup used to examine the performance of the inducer compound subcultures under bioaugmentation conditions. There were eight 13.7-L CFSTRs. The control reactor had been operated continuously for approximately 12 months without exposure to 1NA. The acclimated reactor and each of the augmented reactors had been seeded with 2 L of acclimated sludge (exposed to 1–5 mg/L of 1NA continuously for 12 months) and allowed to grow for 3 MCRTs, at which time they had reached steady-state conditions with biomass concentrations of 1 200–1 600 mg MLVSS/L. Subculture SBRs had also reached steady state (3 MCRTs) with cell concentrations of 2 500–3 500 mg MLVSS/L.

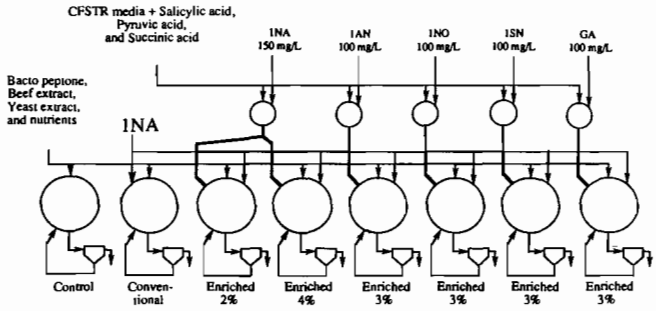

Figure 7—Laboratory setup for examination of transient effects of bioaugmentation using induced cultures.

At this point, bioaugmentation was begun by adding 200 mL of SBR-cells/day from each SBR to the appropriate CFSTR. After a period of several weeks (3 MCRTs), steady-state bioaugmentation levels of 3% using each of the inducer compound subcultures were achieved, as well as two different levels (2% and 4%) using the original enrichment culture (6 bioaugmented CFSTRs). The reported bioaugmentation percentages are mass of inocula added per day per steady-state total mass of CFSTR cells (mg MLVSS of inocula/mg MLVSS of receiving CFSTR). During the period approaching steady state, all CFSTRs except the control received 1 mg 1NA/L continuously. Prior to the start of CFSTR experiments all CFSTRs had steady-state effluent 1NA concentrations below the detection limit (0.020 mg/L).

Once steady state was achieved, a series of three experiments were initiated. Experiments were conducted using a spike loading of 10 mg/L of 1NA, a step-increase from 1 to 5 mg/L of influent 1NA, and reacclimation to 5 mg 1NA/L following its absence from the waste stream for 1.0 MCRT. In the following discussion each CFSTR will be identified by the origin of inoculum cells it received (for example, the CFSTR which received 1AN-induced cells will be referred to as the 1AN CFSTR).

Shock loading. The response of the CFSTRs (which were accustomed to 1 mg 1NA/L) to a 10 mg 1NA/L spike was investigated (Figure 8). Prior to the spike, the effluent concentration of 1NA was below the detection limit (0.020 mg/L) for all of the reactors. During the experiment, the influent 1NA concentration remained at 1 mg/L to all CFSTRs (except the control reactor which only received the spike). Effluent 1NA (breakthrough) concentrations for control (unacclimated), acclimated, 2 and 4% original enrichment culture, 3% 1AN, 3% 1NO, 3% 1SN, and 3% GA bioaugmented CFSTRs were monitored every 4 hours. After 48 hours, the concentration of 1NA in all effluents was below the detection limit.

For Figure 8, effluent 1NA concentrations for each CFSTR were summed and the percent reductions in the sum from the bioaugmented reactors compared to the control and compared to the acclimated reactor were plotted. Breakthrough from the control is presumably due to a combination of dilution and the maximum that can be removed by nonbiological mechanisms (since the competitive biodegradation mechanism is not present). Any reduction in breakthrough from the other CFSTRs is due to biodegradation. For this case, the acclimated CFSTR was only able to decrease the cumulative breakthrough by approximately 7% over the unacclimated control (this represents the best-case conventional unaugmented system). The two 1NA

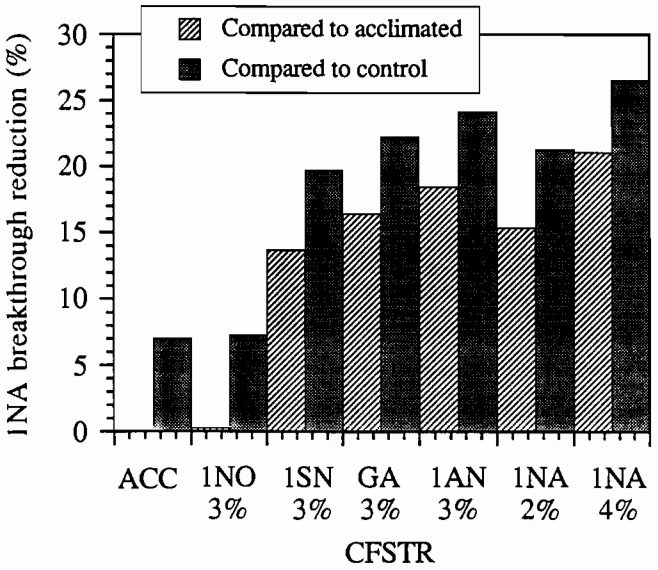

Figure 8—Effluent 1NA breakthrough reduction compared to the acclimated CFSTR following a 10 mg/L spike.

CFSTRs (2% and 4%) reduced effluent breakthrough by 15 and 21% over the acclimated reactor, respectively.

The performance of the various CFSTRs that received inducer compound-maintained subculture cells was varied. The 1AN CFSTR was able to reduce effluent 1NA breakthrough by approximately 18.5% over the acclimated CFSTR. This value is roughly half-way between the values observed for the 2 and 4% 1NA CFSTRs and is what would be expected from a 3% 1NA CFSTR (it performed as well as the 1NA-augmented CFSTRs). The GA CFSTR performed nearly as well as the 1AN CFSTR (16.5% reduction over acclimated reactor). The 1SN CFSTR performed respectably (14% reduction over acclimated CFSTR) and the 1NO CFSTR only performed about as well as the unaugmented (acclimated) CFSTR (7.3% reduction over unacclimated reactor). This experiment implies that inducer compounds can be used in the ER bioaugmentation process (with modest success) to reduce effluent releases of toxics during shock loading conditions.

Step-increase loading. The response of the CFSTRs (which were accustomed to 1 mg 1NA/L) to a step loading increase from 1 to 5 mg 1NA/L was examined (Figure 9). Prior to the spike, the effluent concentration of 1NA had been below the detection limit (0.020 mg/L) for all of the reactors for 1 MCRT (9 days). Effluent 1NA concentrations for acclimated, 2 and 4% original enrichment culture, 3% 1AN, 3% 1NO, 3% 1SN, and 3% GA bioaugmented CFSTRs were monitored every 12 hours. Within 2 days, the effluent 1NA breakthrough from all reactors was below the detection limit (error bars are 95% confidence intervals).

Figure 9 shows effluent 1NA for each CFSTR during the loading increase. Breakthrough from the acclimated CFSTR is due to reacclimation to the increased 1NA loading rate and represents the best case treatment for conventional unaugmented systems under this transient loading condition. Percent reductions in breakthrough for each CFSTR compared to the acclimated reactor were calculated. The percent reductions were 54 and 82% for the 2 and 4% 1NA CFSTRs, 57% for the 1SN CFSTR, 63%

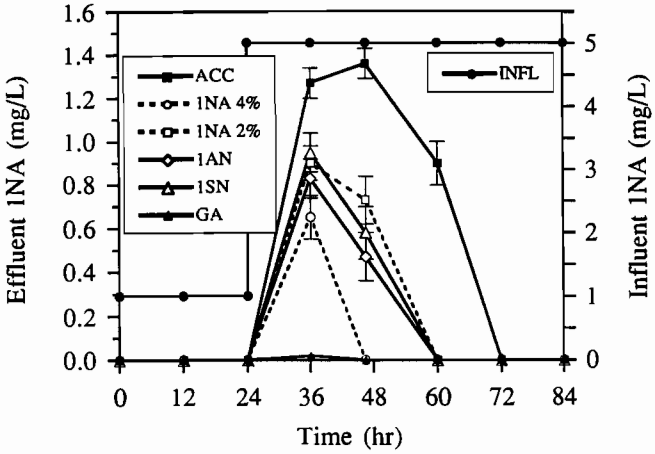

Figure 9—Transient effects of 1–5 mg 1NA/L step loading increase.

for the 1AN CFSTR, 99% for the GA CFSTR, and 100% for the 1NO CFSTR. This indicates that two of the CFSTRs that received bioaugmentation from the subcultures reduced breakthrough during acclimation to a higher degree than the original enrichment culture. This provides evidence that inducer compounds can be successfully used in the ER process to greatly lessen toxic releases during loading transients.

Loss of acclimation experiment. The response of the CFSTRs during reacclimation to 5 mg 1NA/L following its absence from the waste stream for 9 days (1 MCRT) was examined (Figure 10). Prior to the disacclimation/reacclimation experiment, the CFSTRs had been acclimated to 5 mg 1NA/L such that effluent 1NA was below the detection limit (0.020 mg/L) for 1 MCRT. The influent 1NA was then shut off for a period of 9 days to allow for disacclimation to occur. The influent was then returned to 5 mg 1NA/L and the effluent 1NA breakthrough monitored at 12 or 24 hour intervals. After approximately 9 days, the effluent breakthrough had decreased to below the detection limit for all CFSTRs except the 1NO CFSTR.

Figure 10 shows 1NA breakthrough during reacclimation to

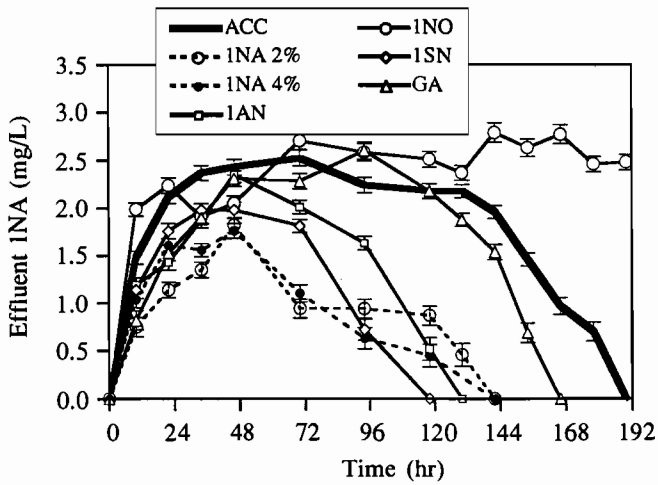

Figure 10—Breakthrough during reacclimation to 5 mg 1NA/L.

1NA. The two 1NA 2% and 1NA 4% CFSTRs performed the best. With the exception of the 1NO CFSTR, all of the CFSTRs augmented with inducer-compound maintained cultures had reduced 1NA breakthrough compared to the acclimated CFSTR. The calculated reductions were 63% and 64% for the 1NA 2% and 4% CFSTRs, 51% for the 1AN CFSTR, 58% for the 1SN CFSTR, and 22% for the GA CFSTR. The poor performance of the 1NO CFSTR cannot be easily explained (presumably there was an upset condition). These results indicate that none of the inducer-compound maintained cultures used for bioaugmentation were able to reduce the 1NA breakthrough during reacclimation as well as the original enrichment culture. However, the 1SN and 1AN cultures did perform reasonably well.

When all three transient loading conditions are considered equally, the overall average breakthrough reduction (compared to the acclimated control) for each CFSTR was; 44% for the 1NA 2% CFSTR, 56% for the 1NA 4% CFSTR, 46% for the GA CFSTR, 44% for the 1AN CFSTR, 43% for the 1SN CFSTR, and 33% for the 1NO CFSTR. Thus, GA, 1AN, and 1SN appear to be the best candidates for use as inducer compounds. These percentages were calculated by multiplying the percent reductions for each experiment by ⅓ and then adding them together (for the 1NO culture, a reduction of 0% was assumed for the reacclimation experiment). This is admittedly a coarse way to determine which inducer compounds are best, which is not a goal of this paper. The idea is that any of the compounds may be a potential choice, and another factor such as cost, availability, or ease of handling, might dictate the best choice of inducer substrate.

Conclusions

Conventional AS systems are not well suited to treatment of many hazardous wastes because of discontinuities, variable concentrations, and shock loadings that inhibit maintenance of a continuously acclimated culture. The ER process, whereby a continuously acclimated culture is maintained by virtue of inoculations from an enrichment culture, is a potential solution to this problem. The idea of maintaining enrichment cultures on inducer compounds in order to keep them continuously acclimated to the problem wastes is a feasible alternative to continuous exposure to high concentrations of the target compound. Bench-scale CFSTRs supplemented daily with subcultures maintained on inducer compounds were compared under various treatment conditions to CFSTRs supplemented with the original enrichment culture. The inducer compound subculture augmented reactors treatment performance was comparable to the enrichment culture augmented reactors. All of the experiments reported here have been repeated and similar results have been observed.

We anticipate that these results will make the proposed ER process more attractive by eliminating the need to store hazardous compounds onsite for ER substrate. A potential generalized design scenario would be a stepwise process where an enrichment culture is first developed through enrichment with concentrated hazardous compounds, followed by maintenance with nonhazardous, readily available, and inexpensive inducer substrates and none of the hazardous target compounds. This would preclude the need to handle the hazardous materials during ER substrate formulation.

This paper is meant to show the potential of the ER process relative to the do-nothing alternative (natural acclimation) and not relative to other approaches. As such, a detailed economic assessment of this approach relative to other possibilities is beyond the scope of this paper. However, biological processes that mineralize hazardous organic compounds to carbon dioxide and water are usually preferred over physicochemical processes that merely concentrate the chemicals which must then be disposed of via suitable methods (which are becoming increasingly difficult to define). The enrichment culture used here has been shown to mineralize 1NA to carbon dioxide.

The applicability of the ER process to any given situation would need to be determined experimentally. The limitations of the process (that is, maximum expected improvements in treatment efficiencies, which will vary with the type of loading transient) would presumably depend heavily on system-specific parameters (that is, hazardous wastewater composition/variability, and treatment system configuration).

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