UNIVERSITY OF CALIFORNIA

Los Angeles

Enricher-Reactor Bioaugmentation of Activated Sludge for Degradation of Hazardous Wastewaters

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Civil Engineering

by

Roger William Babcock Jr.

1991

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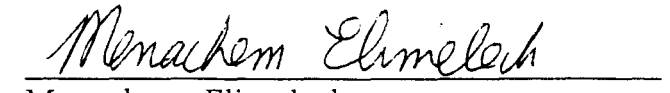

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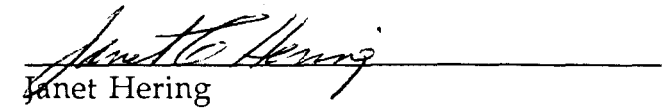

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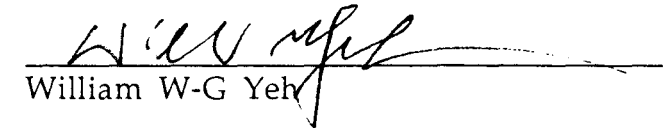

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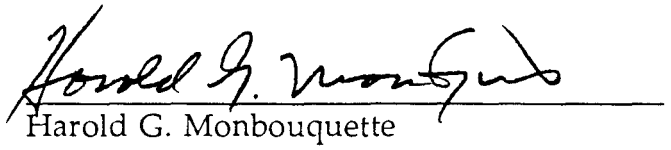

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1991

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ACKNOWLEDGMENTS

I am most grateful to have had the love and support of my wife, Jill, during my research.

I would like to thank my advisor Michael Stenstrom for his guidance and inspiration throughout the course of this research. I would like to thank Chu-Chin Hsieh who was always around working late when I was.

The whole group of students from the UCLA Water Quality Laboratory helped to make for an enjoyable experience, including, Wei-bo Yuan, Daylin Liu, Rolf Hesselman, Tom Fisher, Nagi Ozgur, Robert Cheng, David Ching, Chia-Ji Teng, Judy Libra, Lynne Cardinal, and especially Eddie Tzeng, Kenneth Wong, and Simlin Lau who did most of the daily maintenance of all activated sludge reactors.

Others who contributed directly to my research through discussions on relevant topics include Eric Duell, and especially Kyoung Ro and Wei Chen. I would also like to thank the members of my committee; Menachem Elimelech, John Dracup, Harold Monbouquette, and Robert Mah.

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PUBLICATIONS AND PRESENTATIONS

Babcock, R. W. Jr., W. Chen, K. S. Ro, R. A. Mah, and M. K. Stenstrom (1990). Enrichment and kinetics of degradation of 1-naphthylamine in activated sludge. Submitted to Applied and Environmental Microbiology, September 1990.

Babcock, R. W. Jr., K. S. Ro, and M. K. Stenstrom (October, 1990). Development of a novel off-line enricher reactor process scheme for the activated sludge degradation of hazardous wastes. Paper presented at the 63rd annual conference of the Water Pollution Control Federation, Washington D.C.

Babcock, R. W. Jr., K. S. Ro, C. C. Hsieh, and M. K. Stenstrom (1991). Development of an off-line enricher-reactor process for activated sludge degradation of hazardous wastes. Submitted to Research Journal of Water Pollution Control Federation, April 1991.

Babcock, R. W. Jr., C. C. Hsieh, C. J. Tzeng, K. S. Ro, and M. K. Stenstrom (July, 1991). Degradation of 1-naphthylamine by activated sludge using bioaugmentation. Proceedings of the 1991 American Society of Civil Engineers Specialty Conference on Environmental Engineering, Reno, Nevada, ASCE, New York, N.Y., 493.

Babcock, R. W. Jr., and M. K. Stenstrom (October, 1991). Off-line enricher-reactors for bioaugmentation of activated sludge treating hazardous organics. Paper presented at the 64th annual conference of the Water Pollution Control Federation, Toronto, Canada.

Babcock, R. W. Jr., and M. K. Stenstrom (1991). Use of inducer compounds in the enricher-reactor process for degradation of 1-naphthylamine. Submitted to Research Journal of Water Pollution Control Federation, November, 1991.

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ABSTRACT OF THE DISSERTATION

Enricher-Reactor Bioaugmentation of Activated Sludge for Degradation of Hazardous Wastewaters

by

Roger William Babcock Jr.
Doctor of Philosophy in Civil Engineering
University of California, Los Angeles, 1991
Professor Michael K. Stenstrom, Chair

A novel process scheme for biological treatment of hazardous wastewaters was developed and validated through bench-scale activated sludge (AS) experiments and model simulations. Hazardous wastes are difficult to degrade with conventional AS systems because of toxicity, unsteady composition, and discontinuities which make it difficult or impossible to maintain a continuously acclimated culture.

A continuously acclimated culture can be maintained using bioaugmentation, whereby enrichment cultures (ECs) are used to supplement the indigenous culture. An EC was developed to degrade 1-Naphthylamine (1NA) a regulated carcinogen and known biological inhibitor, degradation of which had not been extensively studied previously. The kinetics of 1NA degradation were quantified. Losses due to abiotic mechanisms (volatilization and adsorption) were quantified. The culture was shown to mineralize 1NA to CO_2 as a sole source of carbon and energy.

The 1NA-degrading EC, maintained on high concentrations of 1NA, was used to inoculate several continuous-flow reactors (CFSTRs) daily with different quantities of cells. Bioaugmentation was quantified as "bioaugmentation level" (mass of inocula added per day/mass of cells present in the CFSTR at steady-state). Bioaugmented and control CFSTRs were subjected to a step-increase loading, shock loading, and disacclimation followed by reacclimation. Reactors which received higher bioaugmentation levels outperformed those which received lower levels (decreased 1NA effluent breakthrough). All bioaugmented reactors outperformed acclimated and unacclimated controls.

Sub-cultures of the original EC which were maintained on inducer compounds (structurally similar but less hazardous than 1NA) and not exposed to 1NA, were shown to retain the ability to degrade 1NA. These cultures were then used to bioaugment several CFSTRs and their performance relative to the original EC was examined during several dynamic loading conditions. Several of the induced cultures were shown to be nearly as effective as the original EC.

The effects of bioaugmentation on the observed steady-state biomass concentration was examined in relation to that predicted by mass-balance considerations. A dynamic kinetic model was developed to simulate the proposed process and aid in scale-up design.

1. INTRODUCTION

1.1 Thesis Organization

This thesis is a compilation of papers written over the course of the dissertation research. An earlier version of the manuscript in Section 2 was submitted for publication and upon review found to be in need of further experimental. Considerable additional work was completed and the paper was recently resubmitted. Section 3 contains three manuscripts, the first of which has been favorably reviewed and is expected to be accepted for publication at any time. The other two papers in Section 3 have been submitted and are currently under review for publication. Section 5 outlines the experimental apparatus and analytical techniques used, and a discussion of accuracy and repeatability of the experimental data reported.

1.2 Overview of the Problem

Hazardous wastes threaten human life and health if not properly disposed. In addition, these materials have adverse effects on the biosphere. The United States generated 525 million metric tonnes of hazardous wastes in 1986 (Cohen, Y., and D. T. Allen *J. Hazardous Materials* , in press) as defined under the Resource Conservation and Recovery Act (RCRA). Of this total, 90% was identified as dilute aqueous streams (wastewaters). Thus, these wastes are mostly water (generally greater than 99%) and hence expensive to treat because of the large volume. Conventional wisdom dictates removal of the hazardous compounds from the water followed by proper treatment and disposal of residuals. Physical/chemical separation techniques have been developed which concentrate the hazardous compounds prior to final

treatment or disposal (see Wentz reference). Exactly what constitutes proper treatment and disposal is debatable.

Techniques which destroy hazardous wastes are favored over burial in landfills (disposal). Thermal destruction techniques are currently not favorable in most areas because residuals and secondary products formed during high-temperature combustion which could be released with stack gases are perceived as dangerous to public health. Separation techniques are expensive and often create hazardous solid-waste byproducts. Incineration of wastes which are mostly water is also very expensive. Biochemical processes, of the same type which cycle nearly all organic matter in the biosphere (Stanier et. al., 1986), are also capable of destroying many hazardous wastes. Under favorable conditions, bacteria can convert many organic hazardous wastes to a combination of new cell mass, CO_2 and water (Grady, 1985).

Because biochemical processes convert hazardous compounds to innocuous end-products, they are one of the most favorable potential treatment alternatives currently available. In addition, the these processes are well established and the required infrastructure is already in place.

Activated sludge (AS) is the most common form of biological secondary wastewater treatment in use today. Figure 1.1 shows a schematic representation of the typical two-step system which treats a continuous waste stream. In the first step (aeration), wastewater and bacteria are mixed and aerated and organics consumed (biodegradation). In the second step (clarification), bacteria are separated from the treated effluent via gravity sedimentation. Concentrated sludge is recycled from the clarifier to the aeration tank or wasted to control biomass concentration.

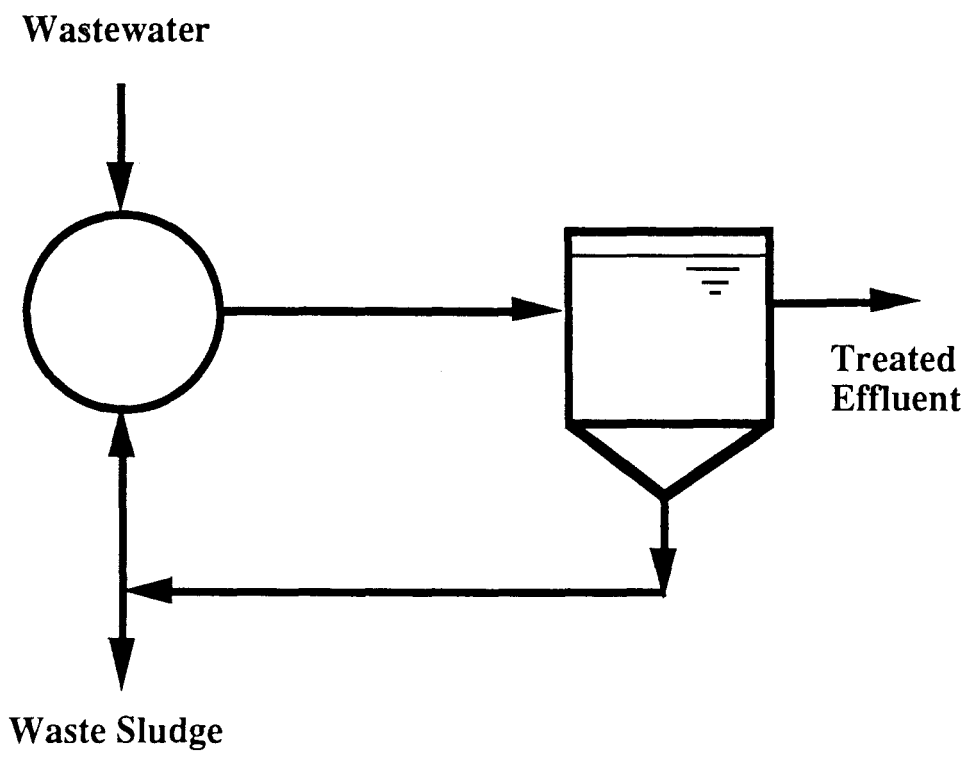

Figure 1.1 Typical activated sludge system.

Activated sludge is a biochemical conversion process which was developed to treat the relatively easily degraded organic material found in municipal wastewater primary effluent. The process has been in use for nearly 80 years in this capacity and has evolved into a predictable and efficient unit operation (Tchobanoglous, 1979). Under the 1976 amendments to the Clean Water Act, all municipalities are required to have biological secondary wastewater treatment. Thus, there has been a substantial national investment in AS infrastructure. Hazardous wastes are more problematic for AS systems than municipal wastes for several reasons.

First, hazardous compounds may be slow to degrade (because of size, complex structure, or the type or number of functional groups) and thus may

not remain in the system long enough to be efficiently degraded. Second, these materials may be toxic to some or all of the bacterial species present in the AS culture resulting in either a loss of treatment efficiency or worse (death of a significant portion of the culture). Third, the phenomenon of acclimation, whereby selective pressure is placed on the AS culture, results in a culture which is dominated by organisms which utilize the most abundant carbon sources the most rapidly. Since most hazardous wastes are present at low concentration, those organisms able to degrade it may be too low in number to efficiently degrade the waste before it flows out with the effluent. Fourth, many hazardous waste flows are discontinuous and there is usually a finite time required for induction of enzymatic activity prior to degradation. This can result in loss of acclimation and lag periods prior to efficient degradation during the reacclimation of systems.

Despite these problems, under certain more or less ideal conditions, some hazardous compounds can be treated efficiently by AS (Kobayashi and Rittmann, 1982). If the waste is always present in the waste stream at a concentration below that which is highly toxic to the majority of the culture, the sludge may acclimate to it with time. If, however, conditions are not ideal, then a new approach is required. The idea of using the existing infrastructure is appealing because it could be very cost effective (Grady, 1986). A method which will take advantage of the existing infrastructure and make the process more robust and hence amenable to hazardous waste treatment is desired.

The idea of bioaugmentation in which something is added to AS to improve degradation or decrease operational problems has been around for some time (Wojnowska-Baryla and Young, 1983, Young, 1976). Past emphasis

has been on the development of commercial preparations consisting of freeze-dried bacteria or enzymes or other "biocatalytic additives." Many such products are commercially available, but few have been subjected to rigorous testing by independent investigators using adequate controls. Those that have been, have not performed well at doses that are economically justifiable (Hull and Kapuscinski, 1987; Lange et. al., 1987; Lewandowski et. al., 1986; Lynch et. al., 1987; Martin and Zall, 1985; Qasim and Stinehelfer, 1982). Several reasons for this have been postulated (Young, 1976), but it is obvious that a new approach is required.

Addition of engineered off-line "enricher-reactors," (ERs) containing an on-site source of live cells to augment the existing AS system, offers a potential solution. Figure 1.2 shows a schematic representation of the proposed system. Enricher-reactors maintain and grow laboratory-developed cultures for regular inoculation into the main system. Enrichment cultures are developed which can rapidly degrade the chosen hazardous waste. This novel concept has been investigated in laboratory-scale experiments to prove its utility and feasibility. The relationship between required inoculum size and increase in degradation rate has been investigated, as well as the response of bench-scale AS systems to operational stress while being bioaugmented. Similar work which has been published is reviewed in Section 3.1.

1.3 Objectives

This thesis concerns the development of a novel biological hazardous wastewater treatment process. Several objectives were put forth:

1. To develop a suitable enrichment culture able to efficiently degrade the chosen model hazardous waste.

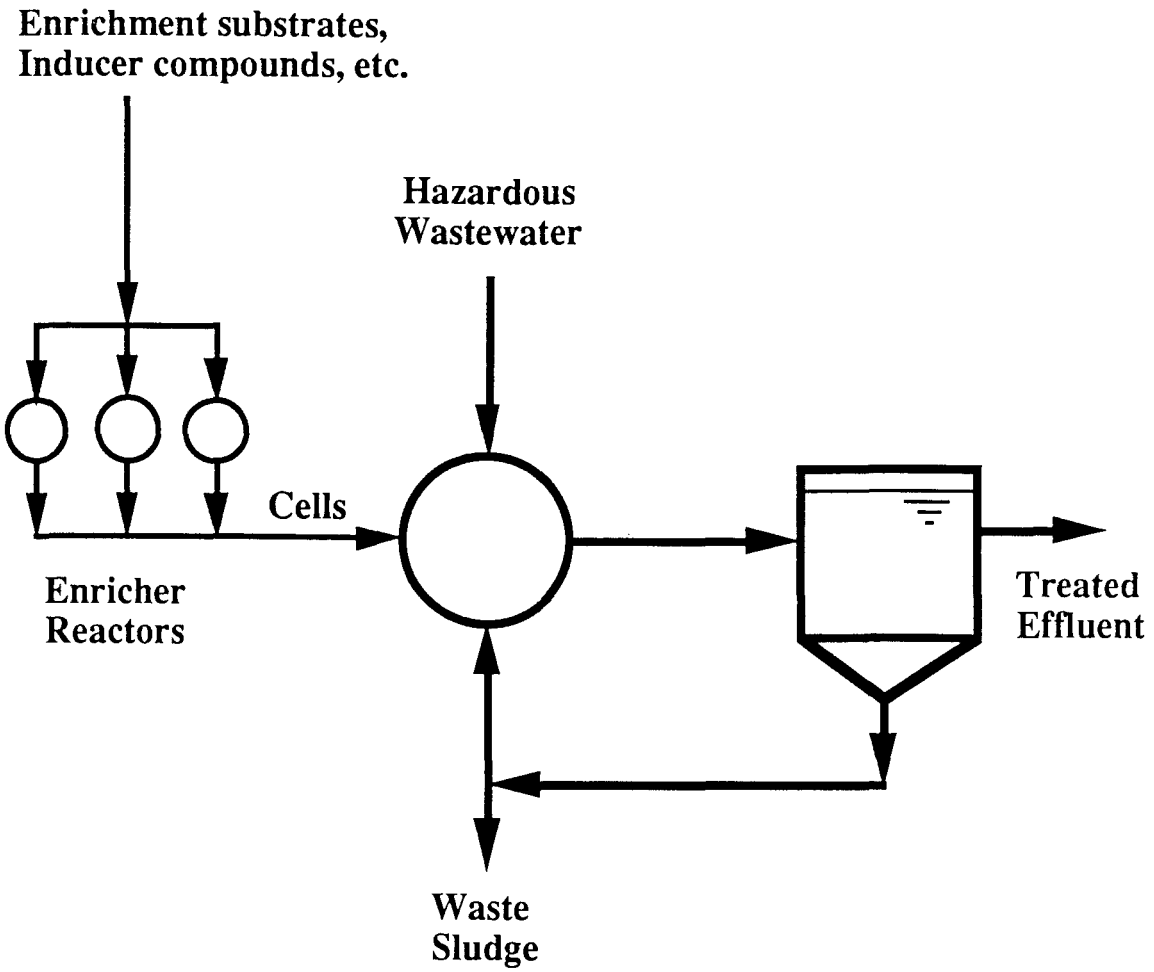

Figure 1.2 Enricher-reactor bioaugmentation process.

2. To demonstrate the efficacy of the proposed process using the developed enrichment culture maintained on high concentrations of the model hazardous waste.

3. To demonstrate the efficacy of the proposed process using cultures developed from the original enrichment culture which were maintained on compounds which were less toxic than the model hazardous waste.

4. To determine the relationship between dose and response in the proposed bioaugmentation process.

5. To demonstrate that the process could be understood mathematically, and to create a design tool for scale-up of the process.

The approach taken here was to utilize bench-scale equipment, operated under conditions analogous to full-scale conventional AS systems, to conduct experiments to determine the validity of the proposed bioaugmentation scheme. Synthetic wastewater and a model hazardous waste were used in order to provide for consistent and repeatable results.

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2. DEVELOPMENT OF AN ENRICHMENT CULTURE

Enrichment and Kinetics of Biodegradation of 1-Naphthylamine in Activated Sludge[1]

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ABSTRACT

An activated sludge enrichment culture capable of mineralizing 1-Naphthylamine (1NA) as a sole source of carbon was developed for use in bioaugmentation studies. After 9 mo acclimation using a sequencing-batch reactor mode, a deep-purple colored culture developed which degraded 1NA concentrations as high as 300 mg liter^{-1} with salicylic acid as the primary carbon source. The enrichment was able to mineralize 35 mg-1NA liter^{-1} as the sole source of carbon in 12 h. Kinetic determinations found that substrate removal followed Michaelis-Menten kinetics with values of K_m and V_m of 227 (+/- 15) μM and 1.82 (+/- 0.03) nmol hr^{-1} mg-cells^{-1}, respectively.

INTRODUCTION

Biological processes which convert hazardous compounds to non-hazardous byproducts are preferred over physico-chemical processes which are usually more expensive and often create hazardous byproducts. However, conventional biological wastewater treatment processes suffer from a general lack of reliability, especially for degradation of hazardous compounds. Because of intermittent exposure and changing complexity of hazardous wastewaters, the maintenance of a continuously acclimated culture is difficult.

A novel treatment scheme which maintains an enriched culture and protects it from the perturbations of the bulk waste stream is needed. The use of such enriched cultures from separate biological reactors to inoculate and hence bioaugment conventional activated sludge processes is relatively new and still largely undeveloped (2,11,18,21,L. J. Cardinal, and M. K. Stenstrom, J. Wat. Poll. Control Fed., in press). This bioaugmentation concept has several

advantages over conventional systems because of the use of separate off-line "enricher-reactors".

Enricher-reactors are not subject to periodic main-line upsets, constitute a ready supply of new biomass after upsets, can be designed to control air emissions, can be operated under flexible environmental conditions with known substrates including inducer compounds, and can be added on to existing systems.

We report here the use of sequencing batch reactors (SBRs) to develop an enrichment culture capable of degrading 1-Naphthylamine (1NA).

1-Naphthylamine (also known as 1, amino-naphthalene) is a chemical intermediate in the manufacture of azo dyes, herbicides, rubber covered cables, pigments, paints, plastics, and toning prints made with cerium salts (3, 4, 15, 16, 20). Other sources of 1NA include wastes from direct coal liquifaction processes (19), and probably all destructive distillation processes used in oil refining and in manufacturing petrochemical products (17). In 1974 the Occupational Health and Safety Administration (OSHA) regulated 1NA as a carcinogen (6). It is also controlled in the U.K. by the Carcinogenic Substances Regulations of 1967, QM400000 (4). Approximately 35,395 workers were exposed to 1NA in 1972-74 in the United States (3).

Unsubstituted naphthalene, is rapidly degraded aerobically by activated sludge, and the degradation pathway is well known (7). However, degradation of an amino-polyaromatic hydrocarbon may not follow a simple sequence of deamination to naphthalene followed by degradation by the corresponding pathway. We report here biodegradation of 1NA including conversion to CO_2, its percent removal by nonbiological mechanisms (volatilization and sorption), and its kinetics of removal by biodegradation.

Mineralization of 1NA has not been reported previously and there was conflicting data regarding its fate in activated sludge in the literature.

MATERIALS AND METHODS

Chemicals and Media. Aromatic compounds were obtained from Sigma Chemical Co. (St. Louis, MO) and Aldrich Chemical Co. (Milwaukee, WI). All solvents were high-pressure liquid chromatography grade and obtained from Fisher Scientific Co. (Pittsburg, PA). The inorganic mineral salts base used throughout this study consisted of (mg liter^{-1}); KH_2PO_4 (200), K_2HPO_4 (125), $(NH_4)_2SO_4$ (100), $CaCl_2 \cdot 2H_2O$ (10.52), $MgCl_2 \cdot 6H_2O$ (16.40), Na_3Citrate (1.765), $FeCl_3$ (0.195), $MnCl_2 \cdot 4H_2O$ (0.0475), $ZnCl_2$ (0.033), $CoCl_2 \cdot 6H_2O$ (0.029), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.021), $CuCl_2 \cdot 6H_2O$ (0.0205), $Na_2B_4O_7 \cdot 10H_2O$ (0.012) in tap water. After 5 mo enrichment, we also incorporated the following vitamin solution (mg liter^{-1}); pyridoxamine dihydrochloride (0.15), nicotinic acid (0.10), thiamine hydrochloride (0.10), Ca-D(+)-pantothenate (0.05), 4-aminobenzoic acid (0.04), D(+)-biotin (0.01). The concentrated salicylic acid medium consisted of mineral base and vitamins plus 1600 mg liter^{-1} salicylic acid and 160 mg liter^{-1} yeast extract. The dilute medium consisted of mineral base and vitamins plus 50 mg liter^{-1} salicylic acid, and 10 mg liter^{-1} yeast extract. The beef extract/Bacto peptone medium consisted of mineral base and vitamins plus 100 mg liter^{-1} Bacto peptone, and 75 mg liter^{-1} beef extract. Yeast extract, beef extract and Bacto peptone were obtained from Difco Laboratories (Detroit, MI); salicylic acid, all inorganic salts and all 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).

Analytical methods. Cell growth was monitored by dry weight determination (method 2540 E; total nonfilterable volatile residue at 550°C)(5); dissolved oxygen was monitored with a Yellow Springs Inst. Co. model 58 dissolved oxygen meter with model 5720A probe (Yellow Springs, OH). Aromatic compounds were analyzed by high-performance liquid chromatography (HPLC) and/or gas chromatography (GC).

The HPLC analysis was performed with a modified Dionex Corp. (Sunnyvale, CA) 4000i gradient ion-chromatography system equipped with either a Dynamax-Microsorb C18 or a Dynamax-Macro C8 column from Rainin Inst. Co. (Woburn, MA), a Linear Inst. Co. (Reno, NV) UV-200 Spectrophotometric detector, and a Hewlett Packard (Avondale, PA) 3396A integrator; the column temperature was 25°C, the mobile phase was 70% acetonitrile, 30% water at either 1.0 (C18 column) or 2.0 (C8 column) ml min^{-1} flow rate, the sample size was 100 µl, and the detector was at 244 nm. The ion chromatograph was modified to operate as an HPLC with the addition of a Valco C10U air-actuated sample loop injection valve (Alltech Inc. Deerfield, IL).

The GC analysis was performed on a Varian Inst. Group (Palo Alto, CA) Vista 6000 GC equipped with a 30-m X 0.25-mm RSL-200 Heliflex bonded Polydiphenyldimethylsiloxane 1.0-µm film capillary column (Alltech Associates, Inc., Deerfield, IL), a flame ionization detector, and a Shimadzu Corp. (Kyoto, Japan) C-R3A integrator. The injection technique was splitless with septum sweep (30 ml min^{-1}) flow injected automatically at 30 sec after injection of 2-µl samples. The following operating conditions were used: injector temperature, 280°C; column temperature, 55°C (hold 2 min) to 190°C at 20°C min^{-1} (no hold) then to 280°C at 8°C min^{-1} with a final hold of 3

min; detector temperature, 325°C; helium carrier gas flow velocity, 30 cm s^{-1} (0.88 ml/min flow rate). Under these GC conditions, 1NA had a retention time of 16.8 min and a detection limit of 1.0 ng or 0.007 nmol. For the same conditions, unsubstituted naphthalene eluted at 12.4 min. Under these HPLC conditions, the detection limit for naphthalene was higher at 50.0 ng or 0.350 nmol.

Gas samples were analyzed for CO_2 by GC. A Hewlett Packard (Avondale, PA) 5890A GC equipped with a 3 m X 3 mm stainless steel column packed with activated carbon, a thermal conductivity detector (TCD), and Hewlett Packard 3392A integrator. The following operating conditions were used: injector temperature, 60°C; detector temperature, 60°C; column temperature, 80°C; helium carrier gas flow rate, 60 ml min^{-1}; TCD helium reference gas flow rate, 75 ml min^{-1}; sample size, 1 ml. Under these GC conditions, CO_2 had a retention time of 1.09 min and a detection limit of less than 300 ppm (air).

Preparation of samples for analysis. Bulk liquid samples with cell pellets (5, 14, or 100 ml) were collected and centrifuged at 2575 X g for 5 min (5 and 14 ml sample sizes) or vacuum filtered through 1.5 µm Whatman 934AH glass microfiber filters (100 ml sample size). Four or 10 ml of the centrifuged samples or 100 ml of the filtered samples were extracted using solid-phase extraction.

Samples were extracted with a Vac-Elute (Analytichem Intl., Harbor City, CA) ten-place vacuum manifold at 20-22 in Hg vacuum onto 100 mg C18 bonded silica sorbent cartridges (1 ml). The adsorbed compounds were then eluted with 2 ml methanol into 1-dram amber serum vials and sealed with screw caps and teflon-lined neoprene septa until analysis. Most samples were

analyzed by both GC and HPLC within 8 h; others were stored at 4°C until analysis. The extraction columns were first conditioned with 1 ml methanol for 5 min, then washed with 2 ml deionized water. The sample (4, 10, or 100 ml) was then eluted through the column at 10-30 ml min^{-1}, the column was washed again with 2 ml deionized water and dried under vacuum for 10 min. Finally, the sample was eluted twice with 1.0 ml methanol, with a 2-min contact time for each ml of methanol before elution. At the highest sample volume, this gave a lower detection limit of 10.0 μg $liter^{-1}$.

Efficiency of recovery in the extraction procedure was a function of concentration, the lower concentrations gave lower recoveries. For this reason, standards were formulated in four separate concentration ranges for the extraction procedure, the GC, and the HPLC. Recovery efficiencies for the extraction procedure varied by less than 5% within each concentration range but were different for each range (between 80 and 100%). The use of four separate ranges contributed to correlation coefficients (r squared) in excess of 0.90 for all standard curves. For each of the GC and HPLC standard curves, a 95% central confidence interval equation for the regression line was determined and used to calculate error bars whenever replicate samples were not available.

Cell pellets were extracted with toluene or methanol. Ten ml of the chosen solvent was added to the previously centrifuged and decanted pellets in a centrifuge tube which was stoppered and shaken vigorously by hand for 1 min. Following mixing, the cells were centrifuged for 5 min and the supernatant analyzed by GC and HPLC.

Activated sludge enrichment cultures. Activated sludge was collected from Hyperion wastewater treatment plant in El Segundo, CA and a west

coast petroleum oil refinery. Hyperion is a large wastewater treatment plant which receives mostly municipal sewage and some industrial wastes. Naphthalene, but not 1NA, was reportedly detected in the Hyperion influent several times per year at concentrations of 2-20 μg liter^{-1}. The oil refinery is a large (300,000 bbl d^{-1}), diversified refinery with a wide variety of operations, including catalytic cracking, coking, and reforming. The possibility of prior environmental contamination from PAH's and from 1NA is probably high.

A 50/50 mixture of activated sludge from these two sites was used as inocula for one 5-liter and one 14-liter SBR (initial solids dry weight of 2000 mg liter^{-1}). In general, SBRs operate on a periodic cycle made up of 4 discrete time periods (10). These consist of (1) an aeration or reaction period, followed by a (2) settling period when the air or mixing is turned off and gravity separation of supernatant and solids occurs. The next step (3) is a drain period to withdraw supernatant and/or cells. Finally (4), fresh feed is added during the fill period, and the cycle repeats in the same sequence. The SBRs used here were operated on a 24-h cycle with a 22-h aeration time, 1-h settle time, and 0.5-h drain and fill times. The SBRs were rectangular high density polyethylene plastic reactors blackened with dark window tinting material. They were operated at 20-25°C, pH 6.5-8, and sparged with compressed air at the rate of 0.14 or 0.42 m^3 hr^{-1} using spherical fine-pore aeration stones. The dissolved oxygen concentration varied between 4.0 and 6.0 mg liter^{-1}. Solids retention time (SRT) was controlled by removal of enough bulk liquid from the SBRs just prior to the end of the aeration period to provide a nominal solids retention time of 14 d.

The 5-liter SBR was maintained on beef extract/Bacto peptone medium, and the 14-liter SBR received concentrated salicylic acid medium.

The assumptions used in the operation of these SBRs are the same as those used in actual practice; namely that the settle period is of sufficient duration to allow most of the floc-forming bacteria to settle by gravity into a thick sludge blanket on the bottom of the tank, and only settleable organisms are used since all suspended cells are wasted during the drain cycle. In the general case the drained volume would be a set value, however, we removed as much of the supernatant as possible (approximately 4 and 10 liters from the 5-liter and 14-liter SBRs respectively).

RESULTS

Acclimation of fed-batch and sequencing-batch cultures. A 14-liter SBR was fed concentrated salicylic acid medium and 3 mg-1NA liter^{-1} daily for 5 mo prior to the detection of significant depletion of 1NA by GC and HPLC analysis. Dry cell weight as well as 1NA concentrations were monitored weekly. Good growth was observed and cell mass remained between 3-6 g liter^{-1} (upper and lower limits for the entire period) during this phase while the SRT was maintained at 14 d.

After 5 mo the culture was able to deplete 3 mg liter^{-1} of 1NA in less than 22 h. The daily 1NA spike was then increased to 10 mg liter^{-1} for a period of 2 mo, after which the medium was changed from concentrated to dilute salicylic acid medium. Growth was not as vigorous, and dry cell weight decreased continuously during this period to approximately 1.1 g liter^{-1}. Concurrently, the concentration of 1NA was steadily increased and a deep-purple colored culture developed which was able to degrade 1NA concentrations as high as 50 mg liter^{-1}.

Separate 5-liter SBRs inoculated with the same enrichment culture and maintained on either concentrated salicylic acid medium or beef-extract/Bacto-peptone medium did not have the same degradative abilities as the dilute salicylic acid enrichment.

After 7 mo, a transfer was made to a second 5-liter reactor (2.5 liters from the 14-liter reactor plus 2.5-liter medium) and dilute salicylic acid medium was used in all subsequent experiments. The transferred culture was not operated in the SBR mode. Instead, it was inoculated with 1NA and monitored every 12 or 24 h for losses and not refed fresh media or 1NA until the supernatant 1NA concentration had decreased below the detection limit. Once 1NA was no longer detectable, aeration was stopped and cells allowed to settle for 1 h, followed by removal of supernatant, addition of fresh concentrated media, dilution with tap water, and reinoculation with 1NA. This is called the fed-batch mode operation. The culture was initially spiked with 50 mg liter^{-1} 1NA, followed by two cycles each of 100, 150, 200, and 300 mg liter^{-1}(see Figure 1 and Tables 1 and 2). This culture was incubated at 20-25^{o}C, pH 6.5-7.5, and with an air sparge rate of 0.14 m^{3} h^{-1}, and solids were not wasted daily as in the case of the SBRs.

An identical reactor with media, 1NA, the same air sparge rate, and no biomass was operated as a control to assess losses due to volatilization. Total losses from the control reactor during each feeding cycle varied between 8 and 15% irrespective of the initial concentration (data not shown). Supernatant and cell-adsorbed 1NA concentrations are shown in Figure 1 (error bars represent 95% confidence intervals of GC standard curves), and cell dry weights are given in Table 1 (single determinations, no replicates). The total time for depletion of 1NA to the detection limit (100 μg liter^{-1}) was always

shorter during the second feeding period (Table 2). During these experiments, dry cell weight increased from an initial value of 580 mg liter^{-1} to 2432 mg liter^{-1} after 43 days (no solids were wasted, see Table 1).

Figure 2 shows the results of refeeding the SBR culture at the highest cell dry weight with 40 mg liter^{-1} 1NA, and monitoring supernatant and cell concentrations for active and no-cell control reactors (error bars represent 95% confidence intervals of GC standard curves). In Figure 2, the zero time samples taken shortly after spiking with 1NA show significant adsorption. Adsorption of this hydrophilic compound is apparently very rapid (approaching instantaneous). The only removal mechanism possible from the no-cell control reactor was volatilization, which is seen to contribute a total removal of about 5 mg liter^{-1} or 12.5%. The total time for depletion of 40 mg liter^{-1} to the detection limit in the active SBR reactor was 10 h which is less than half the duration of the SBRs periodic aeration cycle.

Toxicity. The overall rate of 1NA depletion (initial 1NA mass added/time to reach detection limit) for the second incubation period at each concentration for the fed-batch experiments is given in Figure 3 (no replicates). The maximum rate is about 7.4 mg-1NA h^{-1} and occurs for 150 mg liter^{-1} 1NA initial concentration. At initial 1NA concentrations greater than 150 mg liter^{-1}, the overall depletion rate is less than the maximum value which may indicate some type of toxic effect.

Kinetics study. Degradation kinetics of 1NA for the SBR enrichment culture were estimated using 400 mg of washed cells resuspended in 200 ml of dilute salicylic acid medium (initial cell concentration, 2000 mg liter^{-1}) in 500 ml Erlenmeyer flasks covered with aluminum foil and placed on an enclosed orbital shaker table at 27^{o}C and 200 rpm. Triplicate active flasks and

uninoculated controls were incubated for each of the following initial 1NA concentrations: 1, 5, 10, 50, 100, 200, 300, and 500 mg liter^{-1}. Degradation of 1NA was monitored by taking 10 ml samples at appropriate intervals (0, 2, 4, 8, 12, 24, 48, 77, 97, 120, 145, and 168 hr) followed by extraction and GC or HPLC analysis. Losses from controls were negligible.

Kinetic parameters were estimated by transforming the data via Lineweaver-Burke, Hanes-Woolf, and Eadie-Hofstee plots. Good agreement was found among the three methods. Toxicity as indicated by a deviation from Michaelis-Menten kinetics occurred for the higher concentrations (200, 300, and 500 mg-1NA liter^{-1}) and thus only the lower concentrations were used for determination of kinetic constants. The initial degradation rates were measured and plotted by the three methods and linear curves were obtained indicating Michaelis-Menten kinetics. The values of K_m and V_m were estimated to be 32.5 +/- 2.2 mg liter^{-1} and 261 +/- 4.5 ng-1NA mg-cells^{-1} hr^{-1} (227 +/- 15 μM and 1.82 +/- 0.03 nmol hr^{-1} mg-cells^{-1}) respectively. The +/- values are the standard deviations of the values obtained from the three methods.

Sole carbon source experiment. To determine whether co-metabolism was occurring in the SBR enrichment culture, cells were exposed to 1NA in the absence of any other carbon sources. The media used was the inorganic salts base without citrate. Triplicate Erlenmeyer flasks (500 ml) were inoculated with 400 mg of washed cells in 200 ml of media (initial cell concentration, 2000 mg liter^{-1}), spiked with approximately 175 mg-1NA liter^{-1}, covered with aluminum foil and incubated at 27°C on a shaker table at 200 rpm (see Figure 4). Washed cells were prepared by centrifuging the pellets for 5 min and decanting the supernatant, then resuspending the pellets in

deionized water and repeating the procedure again, twice. A control with media, 1NA and no cells was also incubated. Cell adsorbed 1NA was quantified by extracting the cell pellets with methanol.

Figure 4 shows that the enrichment culture was able to transform 1NA without the presence of any other carbon sources and apparently at rates which are similar to or faster than those obtained in the presence of the other carbon sources which are normally present in the SBR. Error bars represent the standard deviation of triplicate flasks for the supernatant and cell adsorbed 1NA data. Error bars for the control flask are 95% confidence intervals for the HPLC 1NA standard curves.

CO_2 evolution experiment. To determine whether or not 1NA was degraded to CO_2 (mineralized), cells were exposed to 1NA as the sole source of carbon in sealed vials and the headspace gas was monitored for production of excess CO_2. Using 125-ml serum vials, 60 mg of washed cells were resuspended in 30 ml of the same media used for the sole carbon source experiment (initial cell concentration, 2000 mg liter^{-1}). Vials were inoculated with 1NA (except CO_2 control), sealed with Teflon-lined rubber septa and aluminum crimp tops, and placed on an enclosed orbital shaker-table at 27^{o}C and 200 rpm. There were four vials; duplicate active vials with approximately 35 mg-1NA liter^{-1}; a CO_2 control containing cells and media, but no 1NA; and a control without cells but containing 35 mg-1NA liter^{-1}.

Figure 5 shows the depletion of supernatant and cell-adsorbed 1NA in the active vials, and formation of CO_2 in excess of that formed in the CO_2 control. Losses from the uninoculated control were minimal. Cell pellets were extracted with methanol. Figure 5 shows that more CO_2 was evolved from sealed sample vials containing 1NA than from an identically prepared

vial without 1NA. Error bars represent standard deviations of duplicate vials for supernatant and cell-adsorbed 1NA as well as excess CO_2 data points. Error bars for the control vial are 95% confidence intervals for the HPLC 1NA standard curves. Although the error bars are quite large for the 12-h CO_2 data, the upward trend is conserved. The 12-h excess CO_2 concentration of 15,600 ppm corresponds to approximately 0.76 g of carbon and approximately 0.87 g of carbon in the form of 1NA was added to the serum vials. Thus, approximately 87% of the 1NA carbon added could be accounted for as CO_2 in 12 h.

DISCUSSION

Naphthalene was never detected as an intermediate degradation product. This suggested that either deamination occurred and the resulting naphthalene was degraded too rapidly to detect (detection limit = 100 mg liter^{-1}) or that degradation followed a different pathway than via naphthalene. When naphthalene was added as a substrate, it was degraded rapidly by the present cultures. However, the actual degradation pathway for 1NA cannot be determined from the experiments reported here. Since the enrichment culture is a consortium of organisms, degradation intermediates were presumably metabolized by the mixed population present. We have isolated organisms from the enrichment culture capable of 1NA degradation and are examining the degradation pathways in these isolates. Of these isolates one requires the presence of pyruvate to degrade 1NA and another is able to deplete and grow on 1NA without any other carbon sources.

Figures 4 and 5 show that 1NA can be degraded when present as the sole source of carbon. However, in our reactors, salicylic acid serves as the

primary carbon and energy source and 1NA is added at much lower concentrations. The rationale for using salicylic acid is three-fold. First, salicylate is a known intermediate in the degradation pathway for naphthalene and may be a coordinate inducer of enzymes capable of degrading 1NA (7). Second, salicylate is a relatively inexpensive carbon source with which to grow cells for use in bioaugmentation experiments. Third, 1NA cannot be added as the sole carbon and energy source because of its toxicity at substrate level concentrations.

Because, 1NA is apparently inhibitory to many microorganisms, its biodegradation by activated sludge was uncertain. 1-Naphthylamine significantly inhibited nitrification by a mixed population of *Nitrobacter* and *Nitrosomonas* species isolated from activated sludge; a concentration of 15 mg liter^{-1} gave a 50% inhibition (9). 1-Naphthylamine was also highly toxic to an axenic culture of the alga, *Selenastrum capricornutum*, even at low concentrations (1% of saturation)(8), and was mutagenic but non-carcinogenic in most studies using the *Salmonella*/microsome mutagenicity test (13).

1-naphthylamine as sole carbon source (200 mg liter^{-1})was not degraded after 20 d incubation in activated sludge regardless of a previous exposure to 1NA as a secondary substrate (14). 1-Naphthylamine was inhibitory to oxygen uptake of activated sludge as measured by Warburg respirometry at 20 mg liter^{-1}, but 82% of the 1NA added disappeared within 6 h (1). Apparently, in autoclaved control cultures, less than 3% loss was found, but no data on 1NA mineralization were included. An aniline-acclimated activated sludge fed 500 mg liter^{-1} of 1NA exhibited some oxidation as measured by incubation of Warburg vessels for 192 h (12); however, no quantification of any actual decrease in 1NA was reported so

comparison with the present results is difficult. Furthermore, our results do not agree with those of Baird et. al. (1) who reported significant removal of 1NA by unacclimated activated sludge.

Our findings showed that 1NA was initially resistant to degradation, and only after several months of enrichment was the culture able to degrade significant quantities of 1NA and to mineralize 1NA to CO_2. Based on our results, it should be possible to develop appropriate bioaugmentation systems utilizing enricher-reactors to maintain cultures for inoculation into wastewater systems which receive 1NA.

ACKNOWLEDGMENTS

We thank Kenneth Wong and Eddie Tzeng for their assistance with the daily maintenance of the cultures used in this study, and their help with GC and HPLC analysis.

This work was supported in part by the UCLA NSF Engineering Research Center for Hazardous Substances Control.

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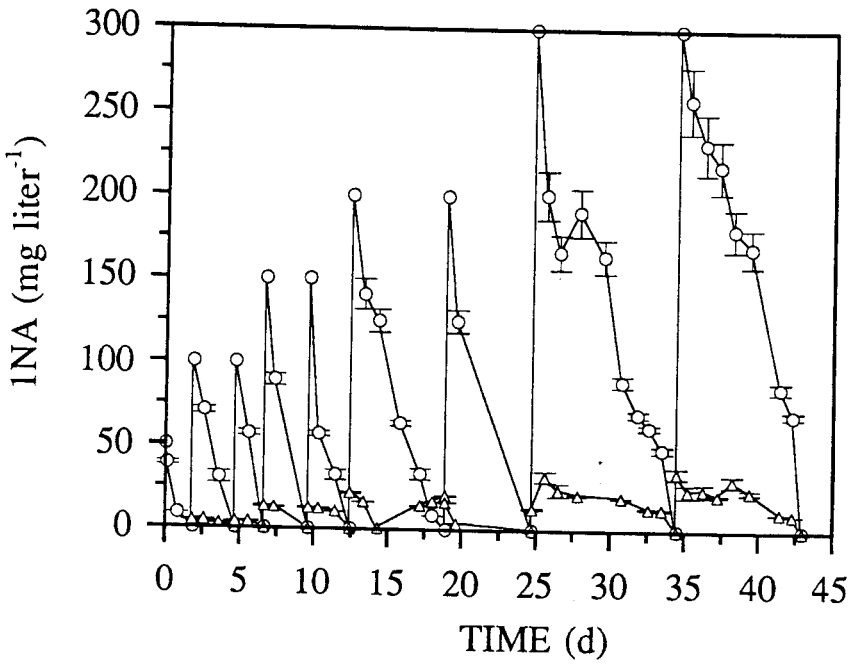

FIG. 1. Fed-batch removal of 1NA in dilute salicylic acid medium. Supernatant (O) and cell (Δ) 1NA concentrations.

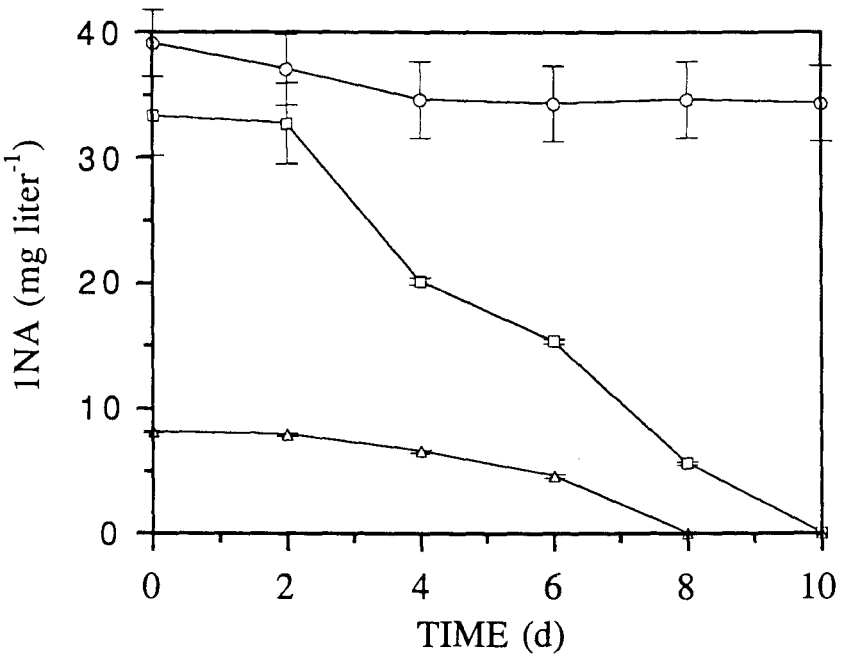

FIG. 2. Removal of 40 mg liter^{-1} 1NA in a 5-liter SBR. Supernatant (□) and cell-adsorbed (Δ) 1NA concentrations, active reactor. Supernatant 1NA concentrations, control reactor (O).

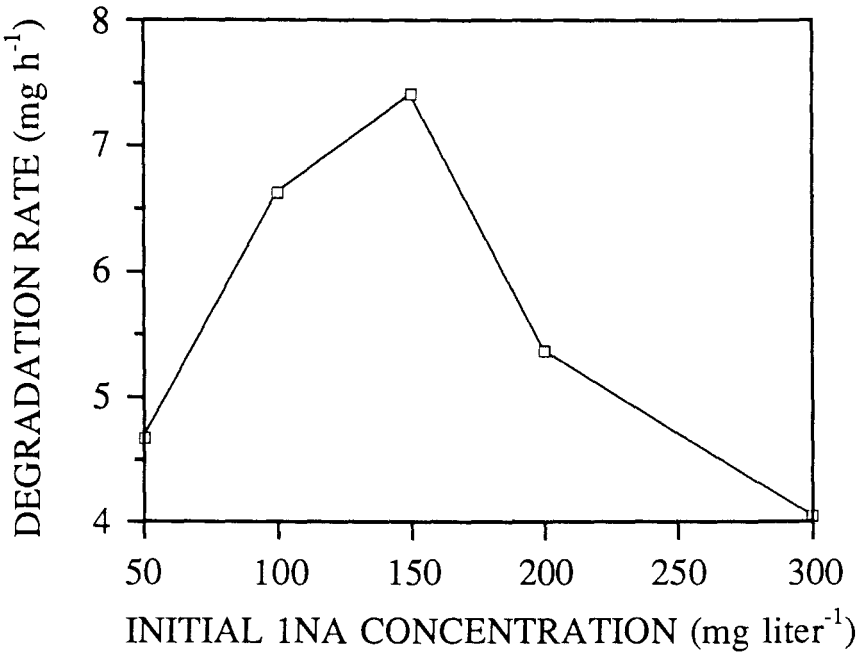

FIG. 3. Depletion rate of 1NA as a function of initial concentration. Rates shown are initial mass of 1NA / total degradation time during each subsequent growth period as shown in Fig 1.

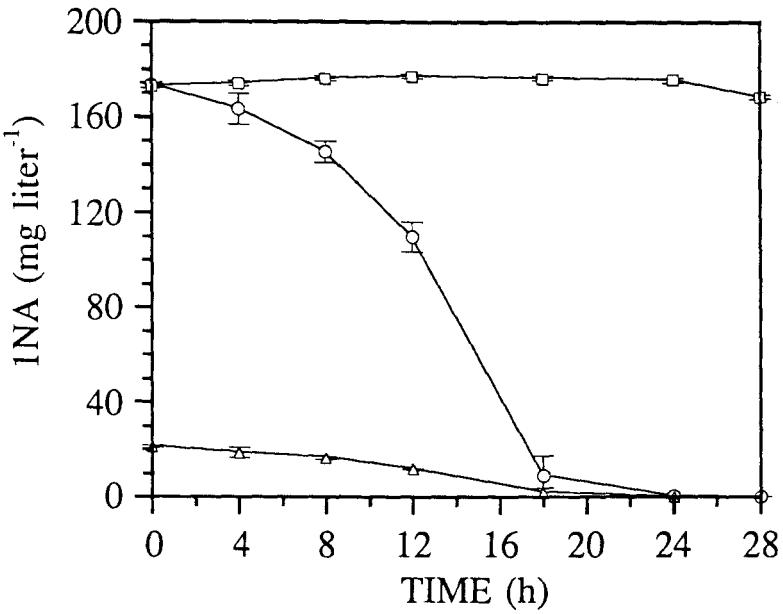

FIG. 4. Shake-flask removal of 175 mg-1NA liter-1 as the sole source of carbon at 27°C and 200 rpm. Supernatant (O) and cell-adsorbed (Δ) 1NA concentrations, active reactor. Supernatant 1NA concentrations, control reactor (□).

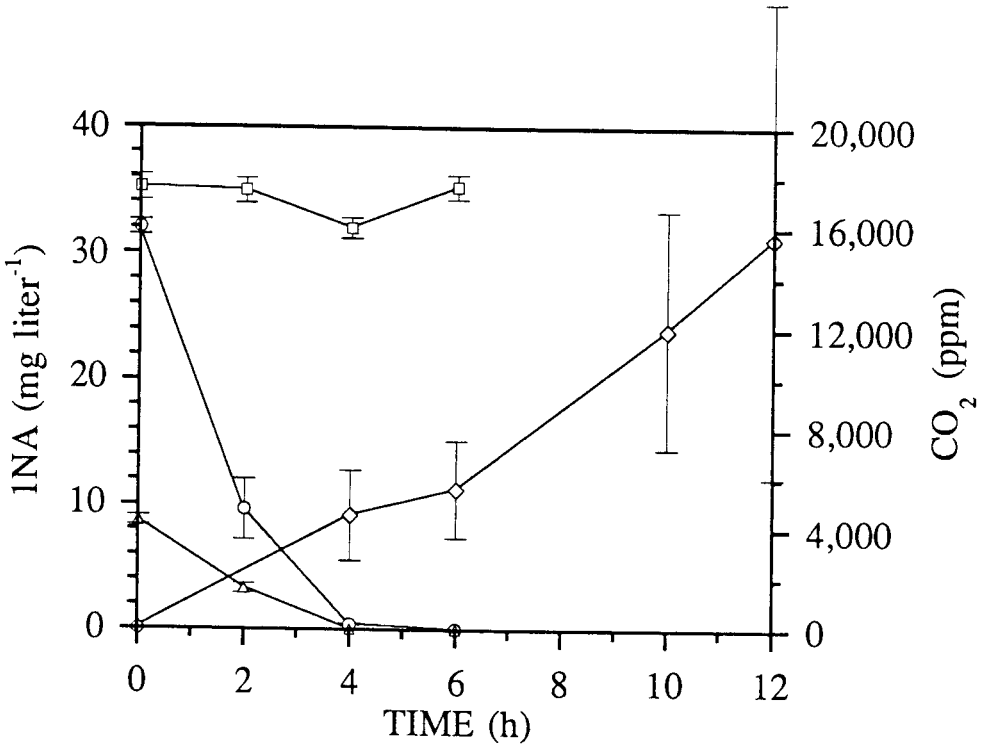

FIG. 5. Mineralization of 35 mg-1NA liter-1 as the sole source of carbon at 27°C and 200 rpm. Supernatant (O) and cell-adsorbed (Δ) 1NA concentrations, active vials. Supernatant 1NA concentrations, cell-free control vial (❑). Amount of excess CO_2 above CO2 control in headspace of active vials (◆).

TABLE 1. Cell dry weight during fed-batch experiments

Day	Dry weight (mg/liter)
0	580
5	796
10	1282
18	1630
25	1658
35	1960
43	2400

TABLE 2. Total time for depletion of supernatant 1NA to below detectable levels in fed-batch experiments

Initial Concentration (mg/liter)	Time (h)	
	First growth period	Second growth period
50	[a]	44
100	68	48
150	72	67
200	155	140
300	234	203

[a] Data not available

3. DEVELOPMENT OF A NOVEL HAZARDOUS WASTEWATER TREATMENT PROCESS

3.1 Development of an Off-Line Enricher-Reactor Process for Activated Sludge Degradation of Hazardous Wastes[1]

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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-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 to 25% of indigenous cell production rates.

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

INTRODUCTION

Approximately 90% of the 525 million metric tonnes of hazardous wastes generated in the United States in 1986 occurred in the form of

wastewaters (Cohen and Allen, in press). Biological oxidation (mineralization) is an attractive treatment method because the hazardous materials are converted to innocuous endproducts (CO_2 and H_2O) (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 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; Cardinal and Stenstrom, in press).

Conventional AS systems (shown schematically in box in Figure 1) work well for the easily degraded components of the waste stream but not for 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 insure 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, 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 (Thilbault and Tracy, 1979; Nyer and Bourgeois, 1980; McDowell and Zitrides, 1979). However, the exact timing and quantities of doses are often omitted. 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; 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 in the main reactor (Lange et. al., 1987; Lynch et. al., 1987; 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 semi-continuous, the requirement that inocula become established in the main AS reactor is reduced. Additionally, on-site 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 also 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, in press). 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 intermediate in the production of azo dyes. Azo compounds comprise about 60% of all organic coloring dyes in use today (Boeniger, 1980), and 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; Buckingham, 1982; Proctor and Hughes, 1978; Scott, 1962; 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; 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; 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, WI). 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 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, MI). 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 et.al. submitted). 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 et.al. submitted). Retention times and detection limits for 1NA using GC and HPLC were 16.8 min, 1.0 ng and 4.4 min, 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 CA)(Babcock et.al. submitted).

For some tests, cell pellets were extracted with methanol or toluene to quantify adsorption (Babcock et.al. submitted). Mixed liquor total solids and biomass concentrations (MLVSS) were measured bi-weekly 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, OH).

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 (see Figure 2). Synthetic wastewater was prepared automatically in 15.3-L batches using a refrigerated (5 ^{o}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 ^{o}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 ^{o}C), because of feed refrigeration, CFSTR temperatures remained several degrees lower (13-23 ^{o}C). Wastewater and concentrated 1NA were delivered to CFSTRs at flow rates of 18 and at 0.5 ml/min respectively using Masterflex 7524-00 microprocessor pump drives and 7519-00 multi channel cartridge pump heads, providing a hydraulic retention time (HRT) of 12.3 hr. 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 1200-2500 mg/L (average = 2105). The CFSTRs were sparged with air through fine-bubble diffuser-stones at 7.1 L/min 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-hr cycle with a 22-hr aeration time, 1-hr settle time, and 0.5-hr each 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-hr aeration cycle, the volume of mixed liquor required for bioaugmentation was removed and allowed to settle for 30 min 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/min 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 2000 and 4000 mg-MLVSS/L (mean = 3533). 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 re-seeding, 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 et.al. submitted), 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 CO_2. 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 hr. Kinetic determinations (using Lineweaver-Burk, Hanes-Woolf, and Eadie-Hofstee plots) found that

substrate removal followed Michaelis-Menten kinetics with values of Km and Vm of 32.5 (+/- 2.2) mg/L and 261 (+/- 4.5) ng-1NA/mg-cells•hr, 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 concentrations 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 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-hr HRT, highlighting the need for batch experiments. Significant losses observed from

the unacclimated controls (approximately 50%) seem to agree with the previous results (Babcock et.al., submitted), 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 to 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.

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 ^{o}C.

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 considerable 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 (see 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 hr. After 32 hours, the concentration of 1NA in all effluents was below the detection limit.

The eight 4-hr 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; i.e., 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 which 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 hr experiment. In a manner analogous to Figure 8, percent reductions in cumulative 1NA effluent concentrations compared 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.

Non-biological 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 CO_2, H_2O, and new cell mass), adsorption and subsequent removal with waste sludge, and volatilization to the atmosphere.

Losses due to all non-biological 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 non-biological 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 insure adequate mixing and non-limiting 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 asses 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 non-biological removal would be approximately 50%. However, because of the way the experiments were set-up (Figures 5, 8, 9, and 10), all losses due to non-biological 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 non-biological 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 CO_2 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 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 hr 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 and co-workers (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 non-hazardous 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 and co-workers (1990) found that very 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; Lynch et. al., 1987; Senthilnathan and Ganczarczyk, 1988; Hartman et. al., 1986; 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 semi-continuous 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 treatment of many hazardous wastes. The problem is an inability to maintain a continuously acclimated culture because of discontinuous, variable concentration 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:

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.

Acknowledgements

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 their help with GC and HPLC analysis. We are grateful 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.

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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 info to calculate concentrations, reported by product manufacturers	Favorable	Thilbault 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	Un-favorable	Qasim and Steinhelfer (1982)
Commercial products (2)	Bench-scale fill and draw AS, synthetic dairy wastewater, followed manufacturer recommendations	Un-favorable	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	Un-favorable	Lewandowski *et. al.* (1986)
Commercial products (16)	Bench-scale batch AS, hazardous wastewater, found: degradation lag period and short lived inoculant viability	Un-favorable	Lange *et. al.* (1987)
Commercial product	Modeling approach, typical recommended doses of 0.005 mg-cells/L are insufficient	Un-favorable	Hull and Kapuscinski (1987)
Enrichment culture and speculation regarding commercial products	Bench-scale batch AS, wastewater with phenol, deadaption of culture had negative effect	Un-favorable	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)

Table 2-CFSTR synthetic wastewater and ER substrate composition.

COMPONENT	CFSTRs (mg/L)	ERs (mg/L)
Bacto Peptone	189	189
Beef Extract	142	142
Salicylic 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}
$Na_3Citrate$	2.7	13
$Na_2B_4O_7 \cdot 10H_2O$	1.9×10^{-2}	8.9×10^{-2}
Pyrdoxamine Dihydrochloride	5.7×10^{-4}	2.7×10^{-3}
Nicotinic acid	3.8×10^{-4}	1.8×10^{-3}
Thaimine 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

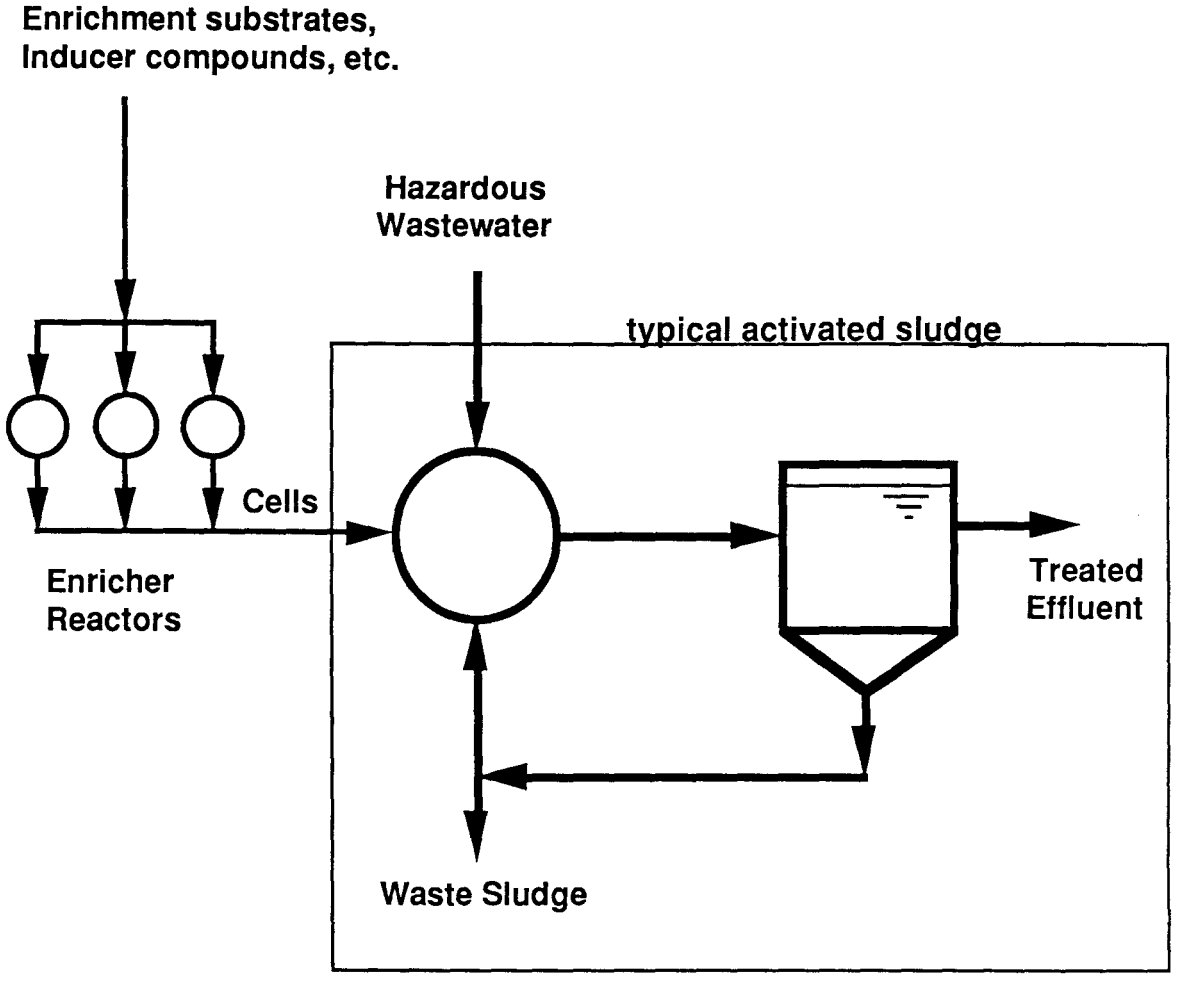

Figure 1-Enricher-reactor bioaugmentation system.

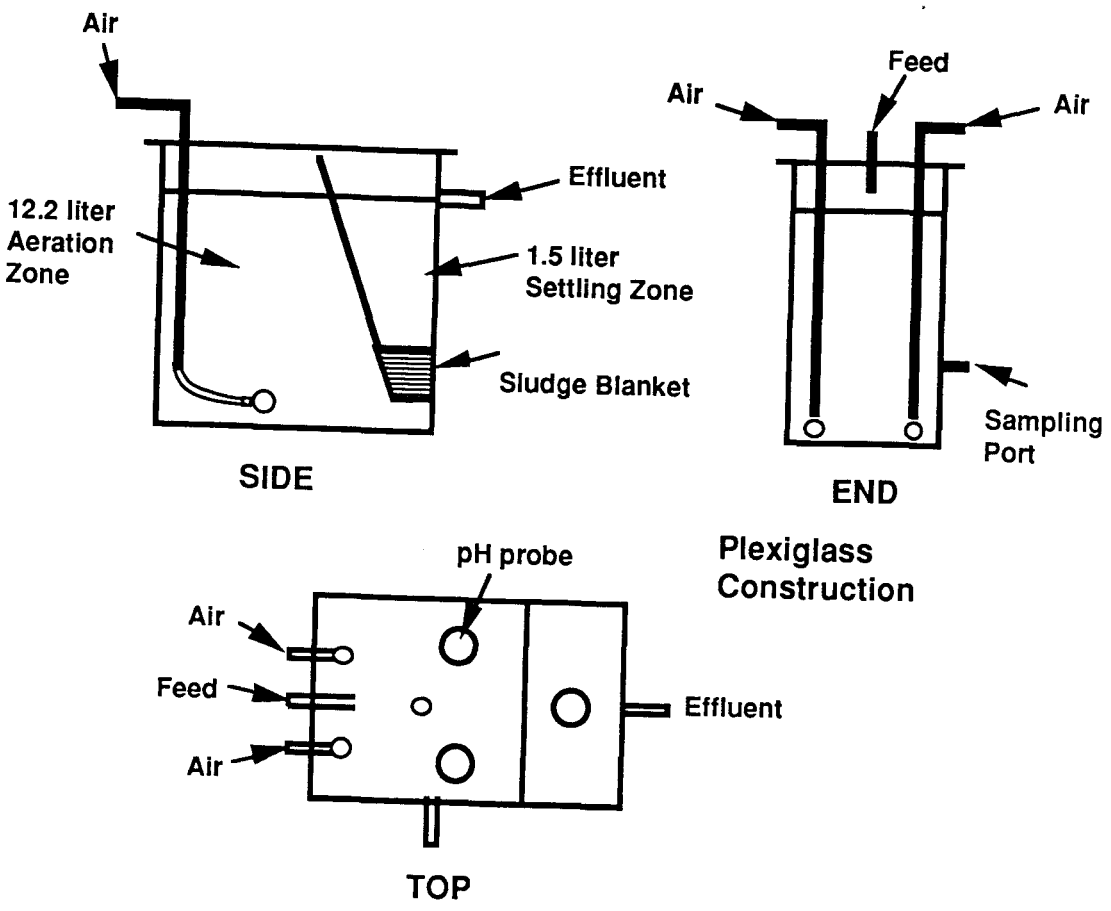

Figure 2-Plexiglass laboratory reactors.

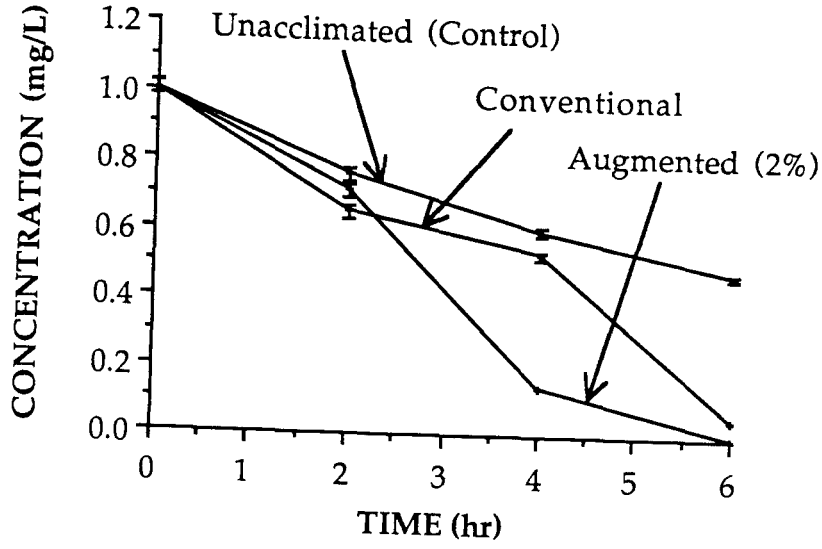

Figure 3-Batch kinetic response of unacclimated, acclimated, and 2% bioaugmented CFSTR cultures to a 1 mg-1NA/L spike.

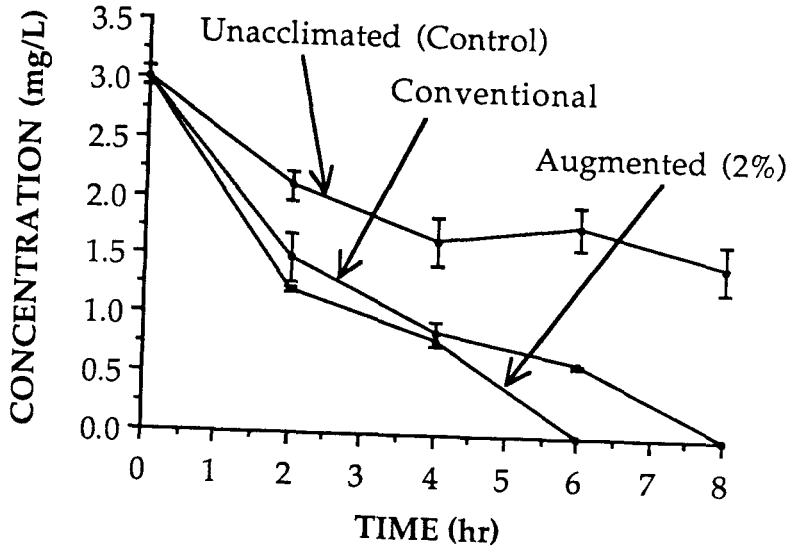

Figure 4-Batch kinetic response of unacclimated, acclimated, and 2% bioaugmented CFSTR cultures to a 3 mg-1NA/L spike.

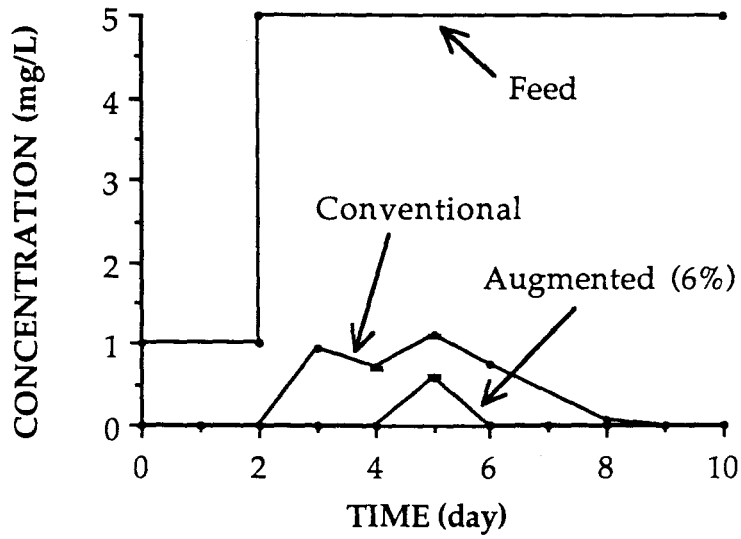

Figure 5-Bioaugmented and conventionally acclimated CFSTR responses to a step loading increase.

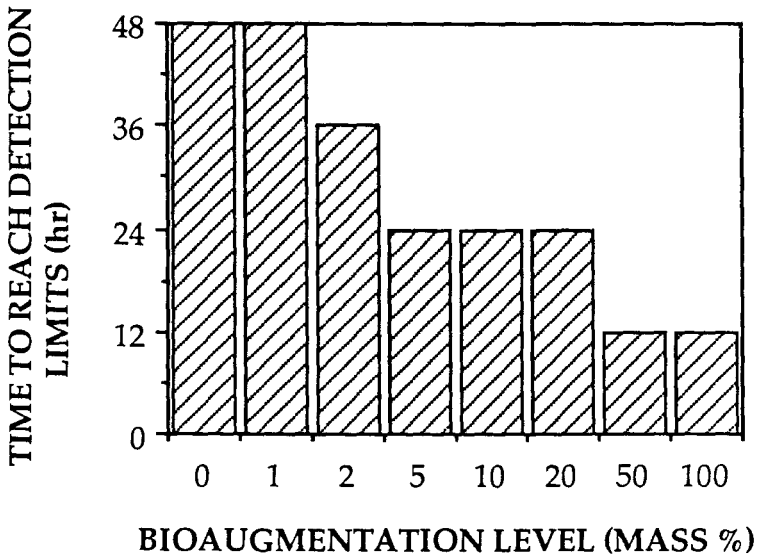

Figure 6-Effect of mass percent inoculum size on batch degradation of 10 mg-1NA/L.

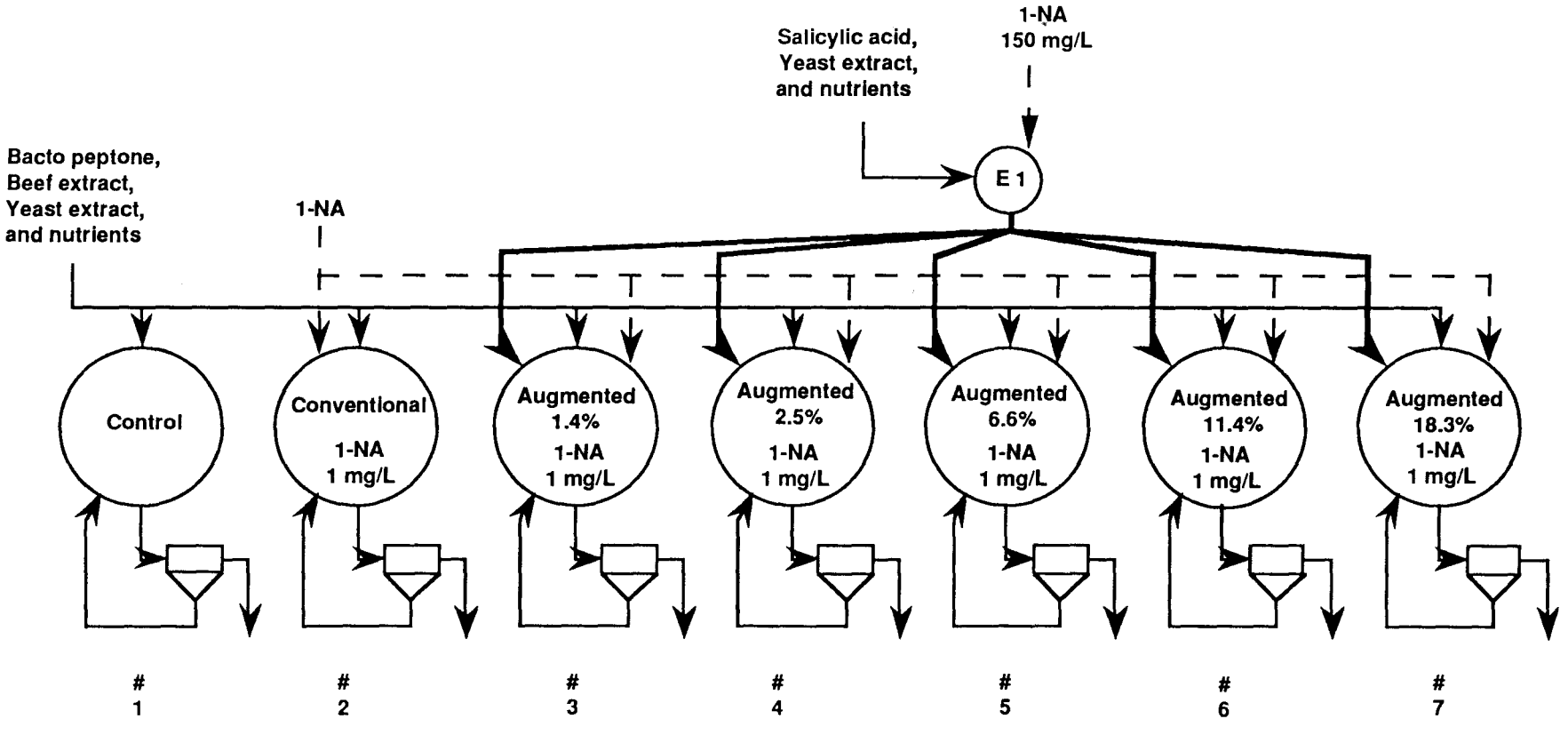
Salicylic acid,
Yeast extract,
and nutrients
1-NA
150 mg/L
E 1
Bacto peptone,
Beef extract,
Yeast extract,
and nutrients
1-NA
Control
Conventional
1-NA
1 mg/L
Augmented
1.4%
1-NA
1 mg/L
Augmented
2.5%
1-NA
1 mg/L
Augmented
6.6%
1-NA
1 mg/L
Augmented
11.4%
1-NA
1 mg/L
Augmented
18.3%
1-NA
1 mg/L
1
2
3
4
5
6
7

Figure 7

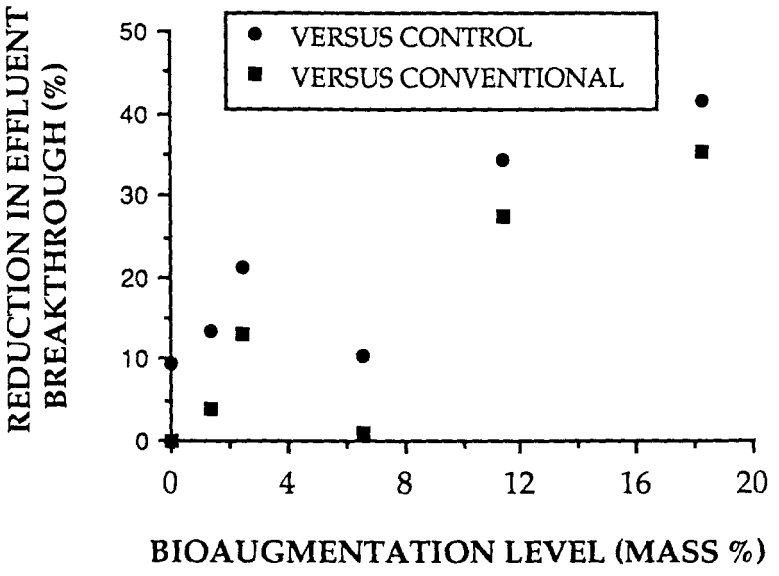

Figure 8-Effect of bioaugmentation level on CFSTR response to a 50 mg-1NA/L shock loading.

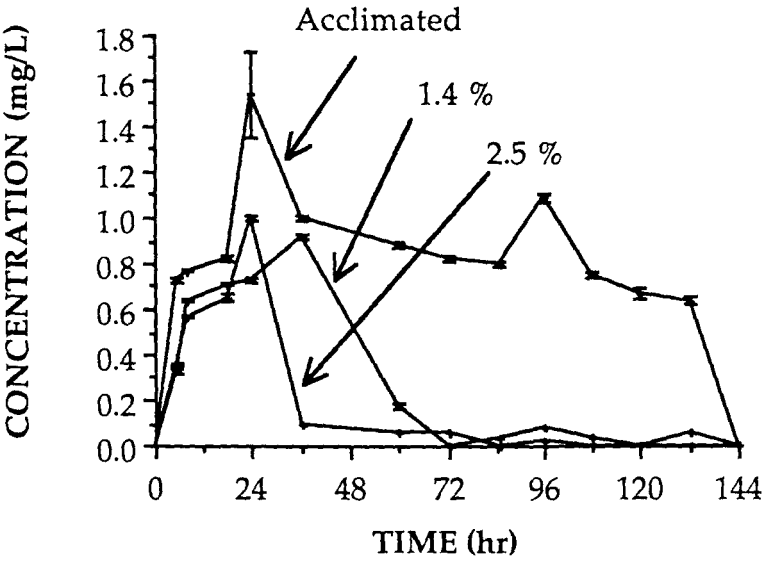

Figure 9-Effect of low bioaugmentation level on CFSTR reacclimation to 5 mg-1NA/L after its absence from the waste stream for 15 days.

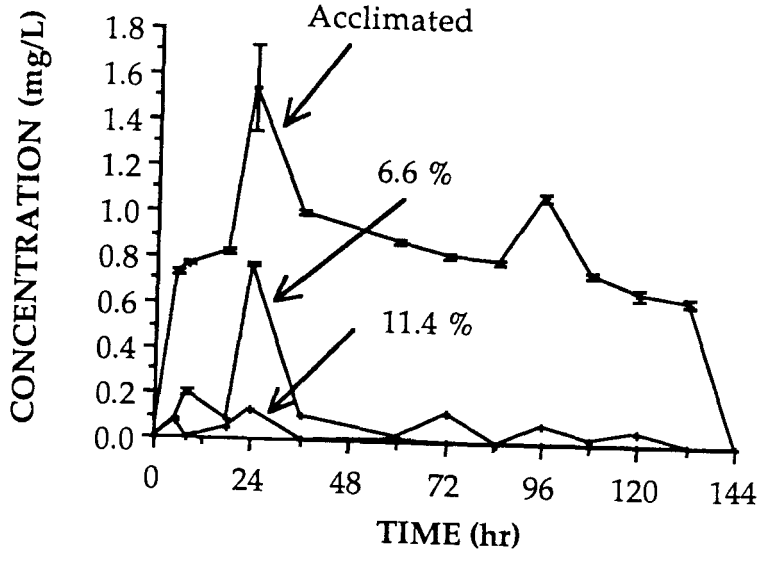

Figure 10-Effect of high bioaugmentation level on CFSTR reacclimation to 5 mg-1NA/L after its absence from the waste stream for 15 days.

3.2 Use of Inducer Compounds in the Enricher-Reactor Process for Degradation of 1-Naphthylamine[1]

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[1] Submitted for publication in Research Journal of Water Pollution Control Federation, (November, 1991)

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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, sub-cultures of the original enrichment culture which had been grown on potential inducer compounds for a period of three months without 1NA showed decreased 1NA-degradation rates which improved significantly after several re-exposures. In continuous-flow experiments, separate 13.7-L reactors received daily inoculations of 2 to 4% by mass of 1NA-enrichment culture, or 3% by mass of the sub-cultures maintained on inducer compounds. Cumulative target compound breakthrough reduction was significant following a 10 mg-1NA/L spike, 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.

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

INTRODUCTION

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 such factors as nonsteady-state composition of the waste (both the easily degraded and the more resistant hazardous portions),

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 insure 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., submitted-a), 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 which 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 (described elsewhere, Babcock et. al., submitted-b) which 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 non-

hazardous compounds which 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 which provide amino acids and other intermediates which are essential for growth and maintenance of cellular activities, and catabolic enzyme systems which 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 very substrate specific but are often quite nonspecific and can be induced by compounds of similar structure, degradation products, or earlier precursors (Clarke, 1984; 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 (in press), 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 sub-cultures of an enrichment culture. Inducer compounds are evaluated for their ability to induce enrichment culture degradation 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, WI). 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. submitted-a). The ER media (also described previously) 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 sub-cultures described here. Experiments with 1NA as the sole source of carbon utilized a mineral salts media consisting of (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 (Babcock et.al. submitted-b) and/or HPLC using a Dionex ion chromatograph operated as an HPLC. The following HPLC operating conditions were used: sample volume, 10 µl; column, Dynamax 300A 12µm C18 25cm bed from Rainin Inst. Co. (Woburn, MA); mobile phase, isocratic 30% acetonitrile-70% water; flow rate, 1.5 ml/min; detector, UV-200 Spectrophotometer from Linear Inst. Co. (Reno, NV) 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 et.al. submitted-b). In shake-flask experiments, centrifuged cell pellets were extracted with methanol to quantify adsorption by resuspension in methanol and vigorous shaking for 1 min, followed by centrifugation and HPLC analysis of decanted liquid. Biomass concentrations (MLVSS) were measured bi-weekly using methods 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. The physical set-up as well as operation and maintenance procedures and bulk system operating parameters for the CFSTRs have been reported. The various ERs (original enrichment culture and sub-cultures) were all operated as SBRs with the same operating parameters as the original enrichment culture (values reported previously). Continuous cultures were

easily maintained indefinitely without re-seeding using the media formulations given (Babcock et.al. submitted-a).

The AS seed culture sources and enrichment culture development procedures are described elsewhere. 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 and K_m have been reported (Babcock et.al. submitted-b).

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 sub-cultures 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 sub-cultures 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 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; Yen and Gunsalus, 1982) and some mono- and di-substituted naphthalene compounds (Williams et. al., 1975; Brilon et. al., 1981a and 1981b; Nortemann et. al., 1986; Wittich et. al., 1988) and other poly-aromatic 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 this information 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 known degradation pathway constituent 3AS was not used because of high costs. 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 necessarily non-hazardous (we would not want them in our drinking water), but none of them are regulated carcinogens and could therefore be called less hazardous than 1NA.

Sub-cultures grown on inducer compounds. Several sub-cultures of the original enrichment culture were initiated with a 20% inoculum (1 L diluted to 5 L). The sub-cultures 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 sub-culture was initiated which received neither 1NA nor any inducer compound. This sub-culture 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 sub-cultures were grown under conditions identical to those of the enrichment culture (5-L SBRs with 22-hr aeration cycle, and MCRT of 10 days). Good growth was observed for all sub-cultures and all had good settling characteristics except for 1CN which was eliminated from further evaluation for this reason.

After three months acclimation to the inducer compounds (equivalent to nine MCRTs), the sub-cultures were tested for their ability to degrade 1NA. Batch aliquots containing 400 mg of cells were removed from each sub-

culture and centrifuged for 5 min. The supernatant was then decanted and the cells resuspended in tap water and shaken. The cells were centrifuged again (5 min), decanted, and finally resuspended in 200 ml of fresh ER media in 500 ml erlenmeyer flasks (initial MLVSS = 2000 mg/L). Flasks (original enrichment culture, one each of the inducer compound sub-cultures, 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 hr, 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 et. al., submitted-a). The 1NA enrichment culture degraded the 5 mg-1NA/L spike in approximately 24 hr. All of the sub-cultures 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.

Sub-culture respiking experiment. Figures 3 through 6 show the effect of re-exposure to 1NA on the sub-cultures following depletion of supernatant 1NA concentrations 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 = 2000 mg/L). Flasks were then spiked with 15 to 20 mg/L of 1NA from a 1NA-in-water stock solution, covered with aluminum foil, and placed on a shake 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 sub-culture control (data not shown), and a no cell control (Figure 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 14.5 and 15.1 mg/L each within 3 hr (data not shown). Samples were taken every 3 hr, and in each of the three spikings, the 1NA had been depleted to below 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 detection limit at time 3 hr for each spike. Respiking had no measurable effect on 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 detection limit was 132 hr, and subsequent spikes were degraded in progressively shorter time periods (21, 9, 9, 9, and 7 hr). After five exposures to 1NA, the 1AN sub-culture 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 sub-cultures (data not shown). The 1SN culture responded faster

requiring only 102 hr for the first spike and 28, 12, 9, 9, and 6 hr for subsequent spikes. The 1NO culture behaved very similarly to the 1AN culture requiring 132, 21, 9, 6, 8, and 8 hr 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 hr to degrade the 1NA spikes. Figure 5 shows slightly different results for cells from an acclimated CFSTR (exposed to 1 to 5 mg-1NA/L continuously for 12 months). The acclimated cells which did not receive any of the inducer compounds but which had continuously received 1NA required 48, 72, 21, 12, 9, 8, and 4 hr to degrade the 1NA spikes. The flask with sub-culture control cells (maintained without inducer compounds or 1NA) only degraded approximately 35% of the first 1NA spike during the time period of the 186-hr respiking experiment (data not shown). The no-cell blank flask (Figure 6) indicates that volatilization was not a significant removal mechanism.

Several points can be made regarding the respiking experimental results. While it appears that the GA-induced culture may have performed better than the others, after 3 exposures to 1NA, each sub-culture was able to degrade the 1NA spike in about 9 hr and subsequent spikes were degraded in 6 to 8 hr 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 is seen to account for about 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 sub-culture control, there is some maintenance of affinity

of the inducer compound-maintained sub-cultures 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) which 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. 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 sub-cultures was due to continuous exposure to the inducer compound.

While the inducer compound-maintained cultures took longer to degrade the first spike of 1NA, all the induced cultures degraded 1NA as well or better than the acclimated cells after the second exposure. 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, however at diminished rates.

Bioaugmentation experiments. Figure 7 shows the laboratory set-up used to examine the performance of the inducer compound sub-cultures under bioaugmentation conditions. There were eight 13.7-L CFSTRs. The control reactor had been operated continuously for about 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 to 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 1200-1600 mg-MLVSS/L. All other operating parameters were as previously reported. Sub-culture SBRs had also reached steady state (3 MCRTs) with cell concentrations of 2500-3500 mg-MLVSS/L.

At this point, bioaugmentation was begun by adding 200 ml of SBR-cells per 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 sub-cultures 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 was 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 (i.e. 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 (see 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 hr. After 48 hr, 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 which can be removed by non-biological 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 is what would be expected from the best-case conventional unaugmented system). The two 1NA CFSTRs (2% and 4%) reduced effluent breakthrough by 15 and 21% over the acclimated reactor, respectively.

The performance of the various CFSTRs which received inducer compound-maintained sub-culture cells was varied. The 1AN CFSTR was able to reduce effluent 1NA breakthrough by about 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 (i.e. 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 respectively (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 successfully used in the ER bioaugmentation process 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 loading increase from 1 to 5 mg-1NA/L was examined (see 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 hr. Within 2 days, the effluent 1NA breakthrough from all reactors was below the detection limit (error bars are 95% confidence intervals).

Figure 11 shows effluent 1NA for each CFSTR during the loading increase. Breakthrough from the acclimated CFSTR is due to re-acclimation to the increased 1NA loading rate and represents the best case treatment for conventional unacclimated 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% for the 1AN CFSTR, 99% for the GA CFSTR, and 100% for the 1NO CFSTR. This indicates that two of the CFSTRs which received bioaugmentation from the sub-cultures reduced breakthrough during acclimation to a higher degree than the

original enrichment culture. This also provides further 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 (see 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 hr 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 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. 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 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 20% for the 1NO CFSTR. Thus, GA, 1AN, and 1SN appear to be the best candidates for use as inducer compounds.

CONCLUSIONS

Conventional AS systems are not well suited to treatment of many hazardous wastes because of inability to continuously maintain an acclimated culture due to discontinuous, variable concentration and shock loadings. 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 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 sub-cultures maintained on inducer compounds were compared under various treatment conditions to CFSTRs supplemented with the original enrichment culture. The inducer compound sub-culture augmented reactors treatment performance was comparable to the enrichment culture augmented reactors.

We anticipate that these results will make the proposed ER process more attractive by eliminating the need to store hazardous compounds on-site for ER substrate. A potential design scenario would be a stepwise process where an enrichment culture is first developed through enrichment with concentrated hazardous compounds, followed by maintenance with non-hazardous, 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.

ACKNOWLEDGEMENTS

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 Simlin Lau for her assistance with the daily maintenance of the AS units used in these experiments, and her help with GC and HPLC analysis. Portions of this paper were presented at the 64th Annual Conference, Water Pollution Control Federation, Toronto, Canada, October, 1991.

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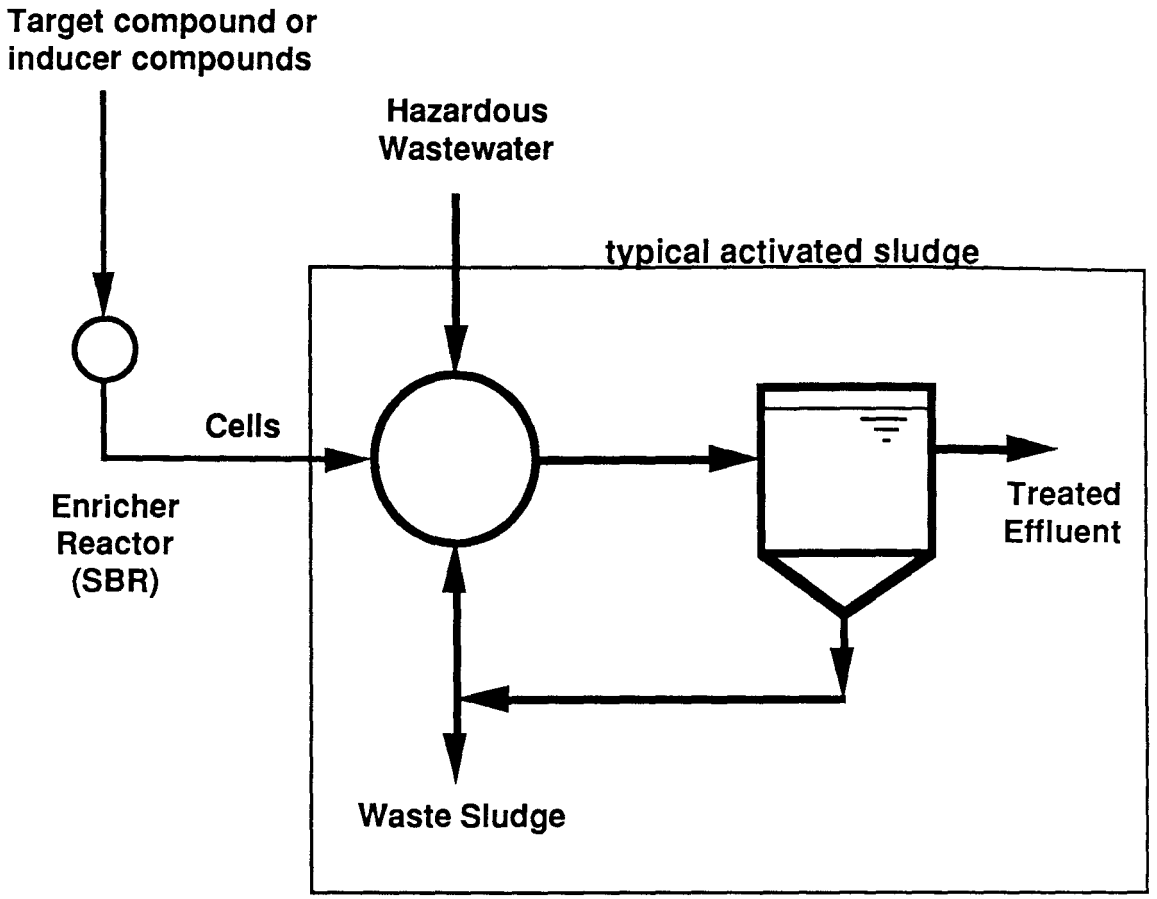

Figure 1-Enricher-reactor concept schematic diagram

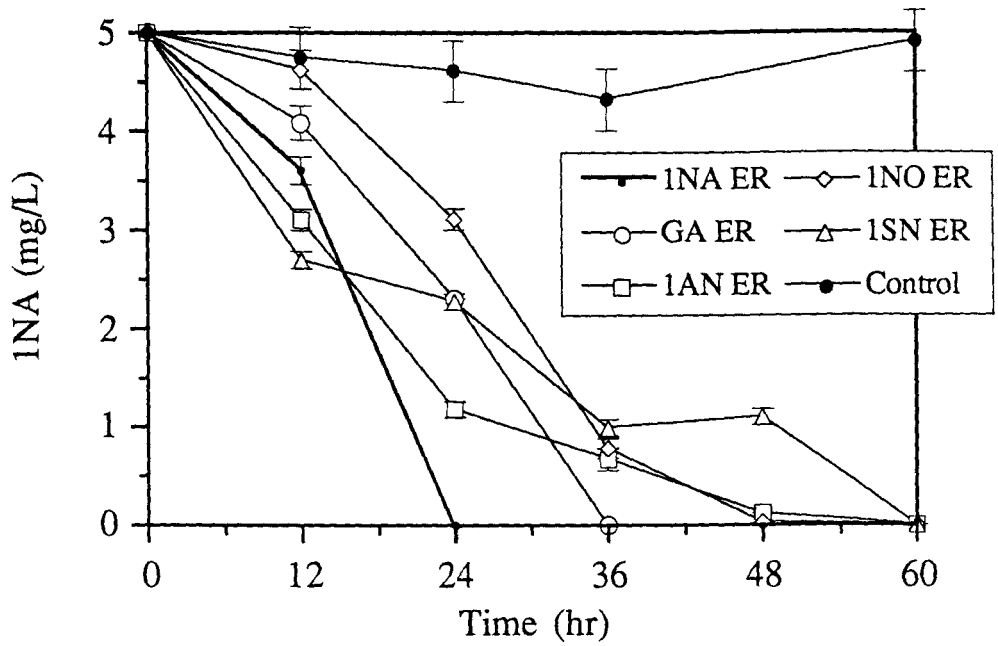

Figure 2-Initial Rate Comparison

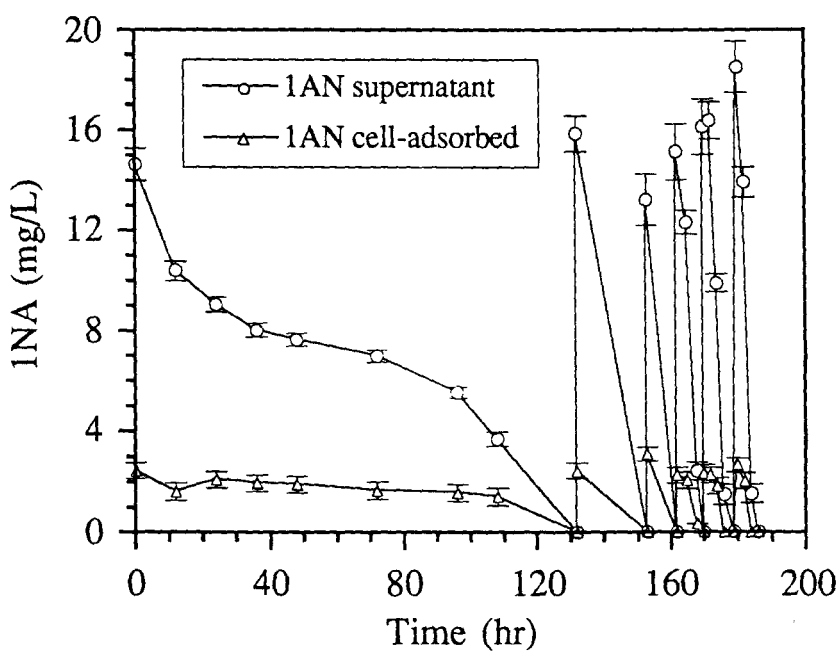

Figure 3-Effect of respiking on the 1-acetate-naphthalene sub-culture

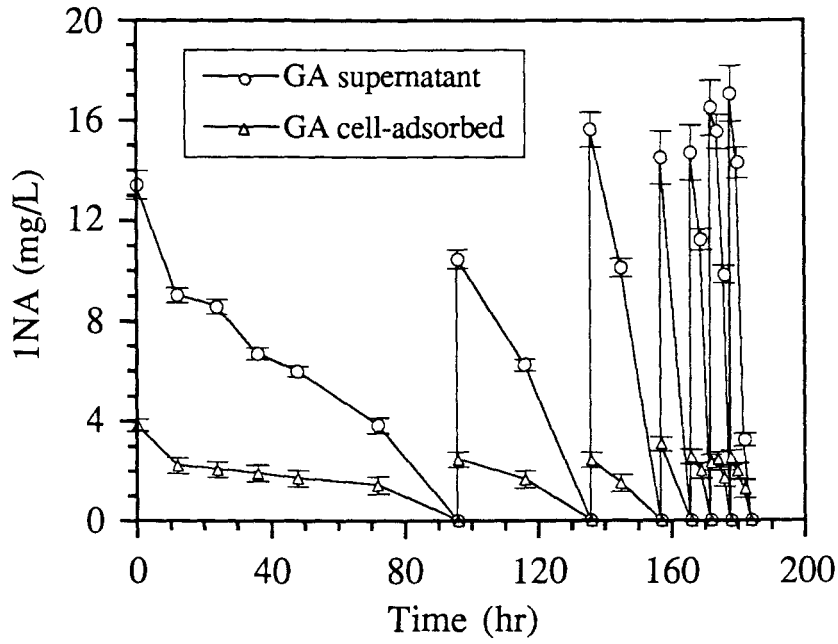

Figure 4-Effect of respiking on the gentisic acid sub-culture

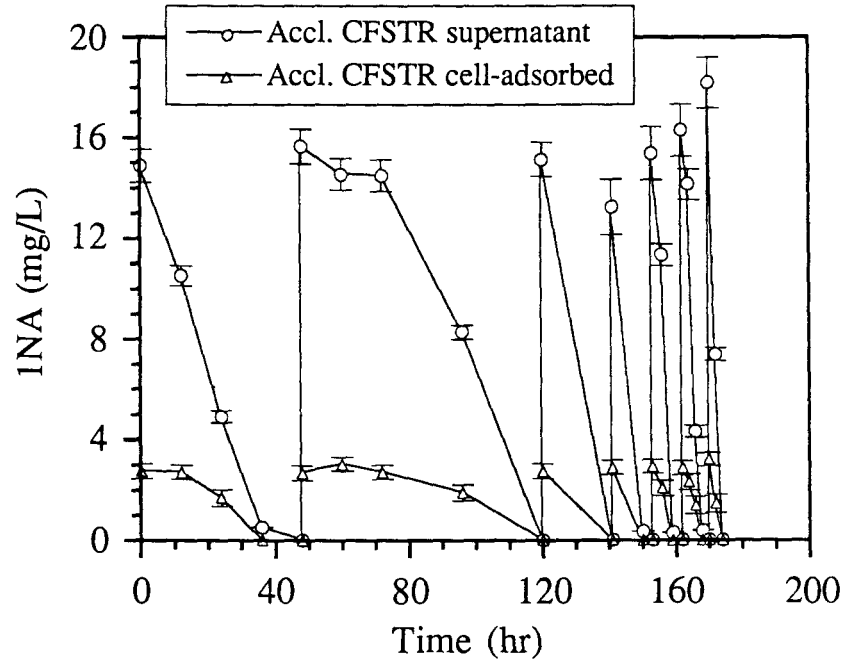

Figure 5-Effect of respiking acclimated CFSTR cells

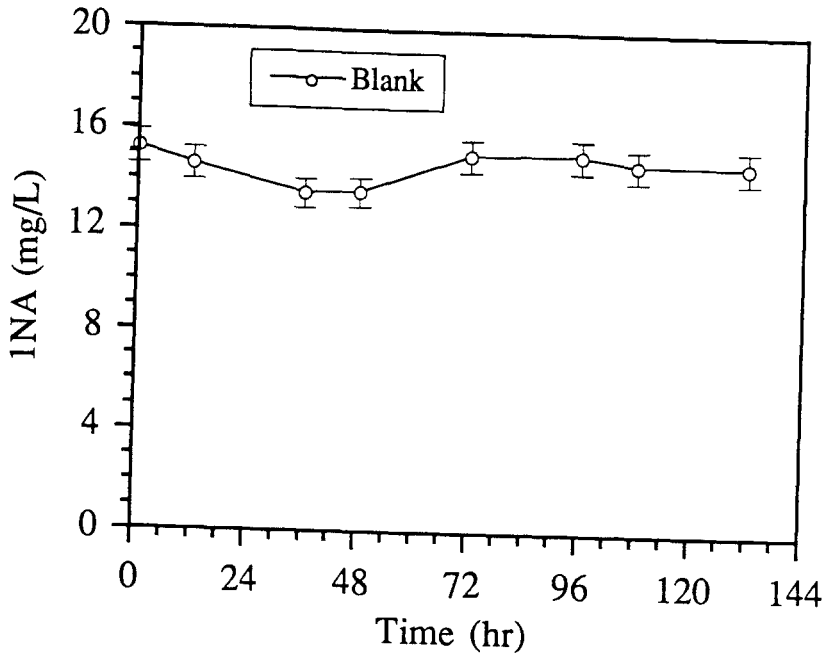

Figure 6-Respiking experiment control flask

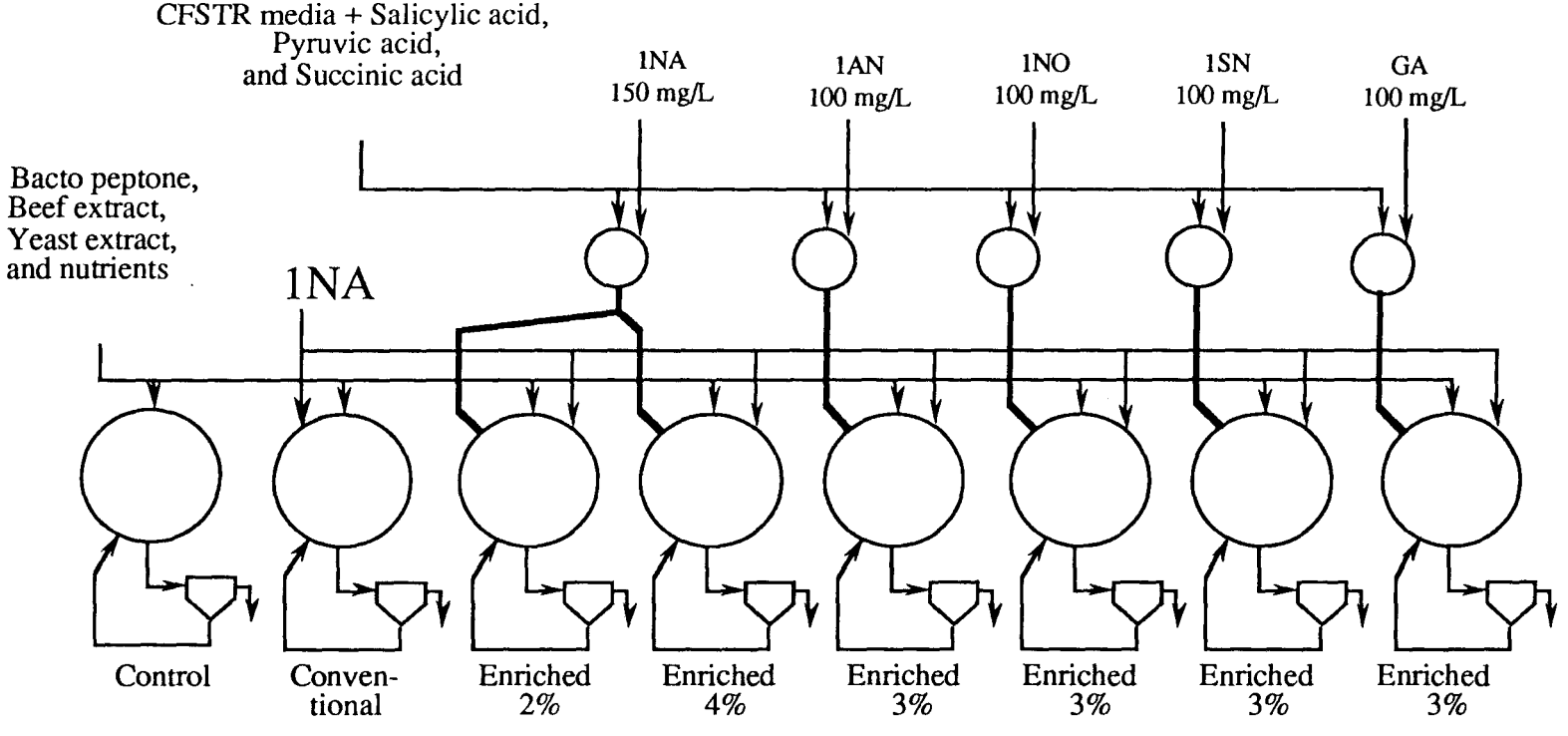

Figure 7-Laboratory set-up for examination of transient effects of bioaugmentation using induced cultures

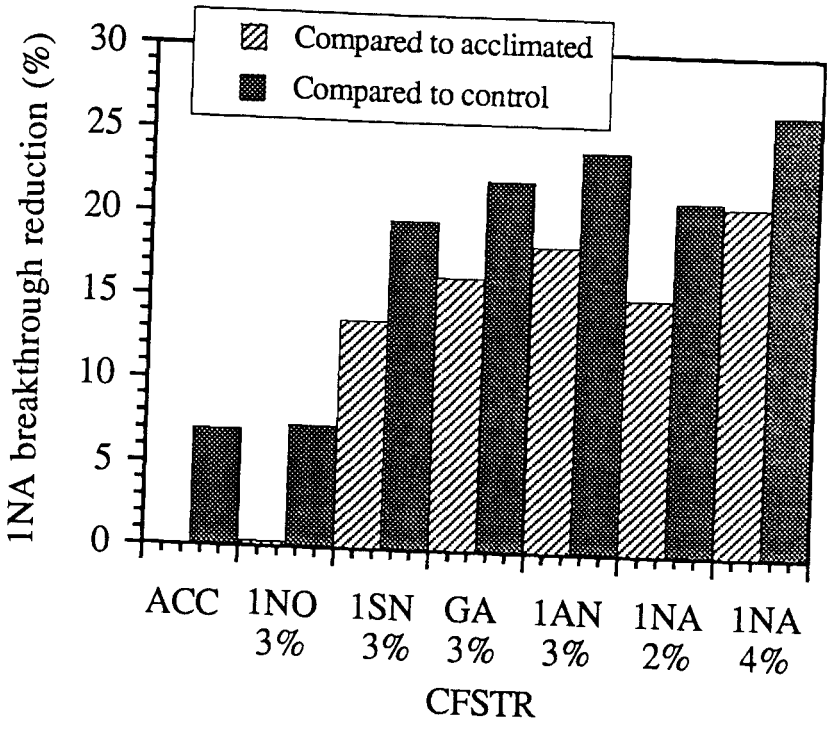

Figure 8-Effluent 1NA breakthrough reduction compared to the acclimated CFSTR following a 10 mg/L spike

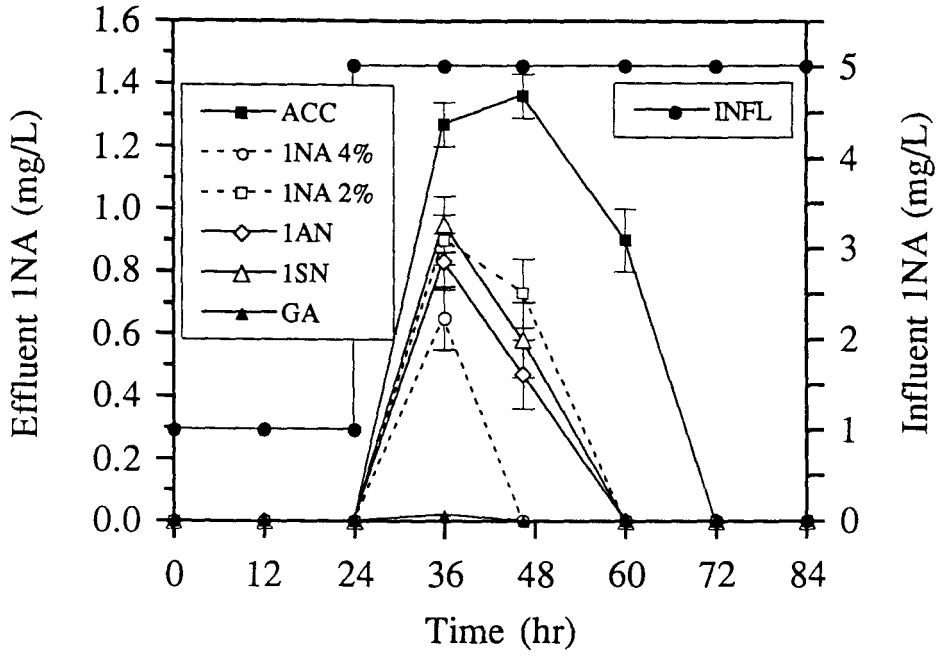

Figure 9-Transient effects of 1 to 5mg-1NA/L step loading increase

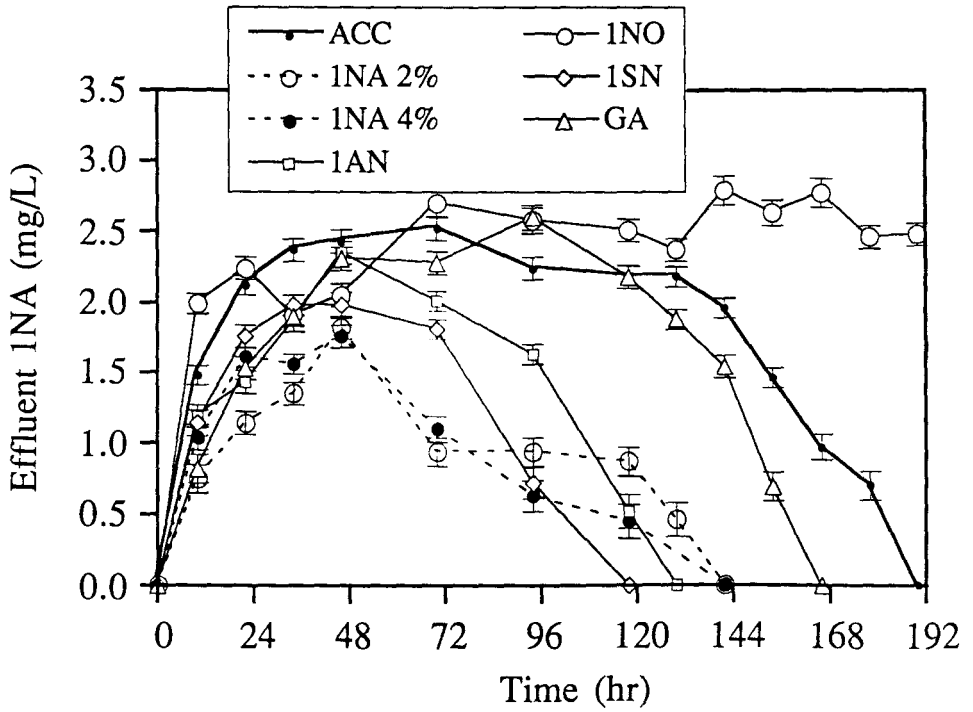

Figure 10-Breakthrough during reacclimation to 5 mg-1NA/L

3.3 Use of Models to Predict the Efficacy and Operational Characteristics of the Enricher-Reactor Bioaugmentation Process[1]

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[1] Submitted for publication in Research Journal of Water Pollution Control Federation, (November, 1991)

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ABSTRACT: Steady-state mass balances using typical Monod-kinetic coefficients were unable to accurately predict steady-state biomass concentrations measured in laboratory activated sludge systems bioaugmented using the enricher-reactor process. Explanations for observed biomass concentrations in augmented reactors which were not as large as predicted via steady-state assumptions are explored. Modification of the Monod decay coefficient to 6 times the typical value permitted good correlation with experimental results. A kinetic model was developed to simulate the dynamic response of a conventional activated sludge system bioaugmented using the enricher-reactor process. A relatively simple model was able to accurately predict the dynamic response of bench-scale bioaugmented activated sludge reactors to transient loading conditions. Again, however, the decay coefficient had to be modified in order for simulations to fit observations at high bioaugmentation levels.
KEYWORDS: Wastewater, activated sludge, biodegradation, enricher-reactors, bioaugmentation, 1-naphthylamine, modeling.

INTRODUCTION

The enricher-reactor (ER) process, in which enrichment cultures are used to inoculate (bioaugment) conventional activated sludge (AS) systems treating hazardous wastewaters, has been described previously (Stenstrom et. al., 1989; Cardinal and Stenstrom, in press; Babcock et. al., submitted [a]). Figure 1 shows a diagram of the proposed process scheme whereby existing AS systems could be upgraded to treat previously undegraded hazardous compounds or to accept additional wastes that would have been unacceptable

prior to the upgrade. Use of off-line ERs operated as sequencing batch reactors (SBRs) would also allow the enormous existing AS infrastructure to be utilized in an increased capacity (efficient treatment of low strength hazardous wastewaters) presumably without infringement of normal operation (degradation of conventional municipal wastes).

Previous work has demonstrated concept utility in bench-scale AS systems using enrichment cultures maintained on either high concentrations of the hazardous target compound (Babcock et. al., submitted [a]) or structurally similar or suspected degradation metabolite compounds in absence of the target compound (Babcock et. al., submitted [b]). To examine the mechanisms involved and relationships between observation and theory, some modeling work was initiated. There were two goals.

The first goal was to examine whether conventional steady-state mass-balances and typical AS kinetic parameters could be used to predict steady-state biomass concentrations observed in bench-scale experiments. The second goal was to examine whether kinetic simulations could accurately predict the experimental dynamic response of continuous-flow reactors (CFSTRs) during shock loading and reacclimation conditions. Degradation kinetics of bioaugmentation cultures following inoculation into CFSTRs cannot be directly measured experimentally, whereas a simulation model could be useful to estimate the underlying kinetic behavior.

Other investigators have examined bioaugmentation effects using steady-state (Hull and Kapuscinski, 1987) and dynamic (Edgehill and Finn, 1983) models. Edgehill and Finn were unable to accurately simulate the response of bioaugmented AS units treating a synthetic wastewater containing pentachlorophenol to a step increase from 40 to 120 mg/L. Hull

and Kapuscinski used a detailed steady-state model of bioaugmented AS to show that steady-state removal of 10 mg/L of a hypothetical hazardous component of a wastewater would not be significantly enhanced using typically prescribed dosages of commercial bioaugmentation products.

EXPERIMENTAL METHODS

Materials (chemicals and media), AS reactor operating conditions (SBRs and CFSTRs), analytical methods, enrichment techniques, and bioaugmentation procedures have been described in detail previously (Babcock et. al, submitted [a], [b], and [c]). However, since these descriptions have not been published, a minimum of repetition will be necessary to describe conditions under which experiments were conducted and results quantified to acquire data presented here for model correlation.

The model hazardous waste consisted of a carcinogenic, amino-substituted polyaromatic hydrocarbon; 1-naphthylamine (1NA). Prior to our work, this compound was noted to be generally resistant to biodegradation (Pitter, 1976; Malaney, 1960) and in fact inhibitory to nitrifying organisms (Hockenbury and Grady, 1977), mutagenic in the Ames assay (McCann et. al., 1975), associated with human bladder cancer (Case et. al., 1954; Scott, 1962), and regulated as a carcinogen by OSHA (Federal Register, 1974). 1NA is an intermediate in the manufacture of approximately 150 organic coloring dyes (azo-type) in use today (Colour Index, 1975) and is probably a trace component of many petrochemical-product manufacturing waste streams (Staff, 1974).

An AS enrichment culture was developed over a period of approximately 9 months which was then able to rapidly degrade 1NA. Monod-type kinetic parameters of 1NA degradation were determined, non-

biological removal mechanisms were quantified, and mineralization (conversion of 1NA to CO_2) was demonstrated (Babcock et. al., submitted [c]). The enrichment culture was developed and maintained in sequencing-batch reactors (SBRs), which offer several benefits over other operating modes (Irvine and Busch, 1979). The enrichment culture was used to continuously bioaugment several CFSTRs.

Seven 13.7-L CFSTRs were operated continuously for 12 months. There were two controls; an unacclimated control which was not normally exposed to 1NA and did not receive bioaugmentation, and an acclimated control which was continuously exposed to 1NA and also unaugmented. The unacclimated CFSTR represented worst-case existing treatment systems where the least removal of 1NA would be expected. The acclimated CFSTR represented best-case conventional systems without addition of ER technology. Five other CFSTRs received various daily bioaugmentation doses. Table 1 lists the bioaugmentation status of each CFSTR, and Table 2 lists common operating characteristics of CFSTRs and SBRs.

Sampling procedures, solid-phase extraction methodology, and gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) protocals are detailed elsewhere (Babcock et. al., submitted [a], [b], and [c]). The detection limit for 1NA in samples for data reported here was 0.010 mg/L for both GC and HPLC measurements. Error bars for all experimental data reported here are 95% confidence intervals based upon GC or HPLC external standard regression lines. Bulk organic material was quantified as filtered chemical oxygen demand (COD) by method 5220B (Standard Methods, 1989) weekly. Biomass concentrations were measured as mixed-liquor volatile

suspended solids (MLVSS) by method 2540E (Standard Methods, 1989) twice per week.

MODEL DEVELOPMENT

The ER process was developed with ERs (source of inocula) operated as SBRs. Reactors which received bioaugmentation were CFSTRs with clarifiers and sludge recycle. The nominal 8.91-d CFSTR solids retention time (MCRT) of was controlled by daily removal of 1.37 L of aerated mixed liquor. Immediately following sludge wastage, the required bioaugmentation inoculation was added to the aeration zone. Bioaugmentation inocula consisted of aerated mixed liquor from the SBRs which was removed just prior to the end of the aeration cycle when the concentration of the target compound (1NA) or inducer compounds had been depleted below the detection limit. Additional cells were also removed to maintain the established SBR 7-day MCRT. Cells used for inocula were allowed to settle for 0.5 hr in graduated cylinders after which the supernatant was decanted and settled sludge added to the proper CFSTR.

The benefits of using ERs operated in the SBR mode are two-fold. First, SBRs have several operating parameters that can be varied for optimization of operation and maintenance of enrichment cultures (they are not constrained by the operating requirements of the augmented CFSTR). Second, because it is a batch process, there are times when the mixed liquor contains cells and little if any residual carbon sources. Thus, cells can be harvested at the end of the aeration cycle so that inoculations (bioaugmentation) would not contribute to CFSTR organic loading rates. If

the ERs were operated as CFSTRs, cells might have to be separated somehow from residual carbon and residual target compounds prior to inoculation.

Steady-state effects of bioaugmentation. Bioaugmentation may be considered as an equivalent continuous influent biomass concentration, and the conventional mass-balance approach can be used to model the process. Using the mass balance approach of Lawrence and McCarty (1970), the dynamic variation of substrate and microorganism concentrations can be expressed as follows:

$$V\frac{dC}{dt} = QC_o - QC + Vr_{su} \quad (1)$$

$$V\frac{dX_1}{dt} = QX_{1o} - Q_wX_1 + Vr_g \quad (2)$$

where C and C_o are 1NA concentrations in clarifier effluent and feed, respectively; Q is wastewater flow rate; V is volume of aeration zone; r_{su} is rate of substrate utilization; X_1 is aeration-zone concentration of cells originating from the ER; X_{1o} is concentration of ER cells in the influent; Q_w is waste flow rate; and r_g is biomass growth rate.

Using Monod kinetics and assuming steady-state, Equation 1 can be solved for a quadratic equation in C, the solution of which is as follows:

$$C = \frac{(C_o - k_1X_1\theta - K_{s1}) + \sqrt{(k_1X_1\theta + K_{s1} - C_o)^2 + 4K_{s1}C_o}}{2} \quad (3)$$

where k_1 is maximum rate of substrate utilization per unit mass of organisms, K_{s1} is the Monod half-velocity constant, and θ is hydraulic retention time (V/Q).

Similarly, Equation 2 can be solved for X_1 using Monod kinetics to get:

$$X_1 = \frac{X_{1o} + Y_1(C_o - C)}{\theta(\frac{1}{\theta_c} + k_{d1})} \quad (4)$$

where Y_1 is the maximum yield coefficient, and k_{d1} is the endogenous decay coefficient. Equations 3 and 4 combined with values for X_{1o}, θ_c, θ, C_o, Y_1, k_1, k_{d1}, and K_{s1}, can be solved iteratively for C and X_1.

To examine the effects of bioaugmentation on total biomass concentration, we assume that there is also a group of indigenous organisms (X_2) which are exposed to a bulk substrate (C_B). As a first approximation, we assume that indigenous cells cannot degrade 1NA and are unaffected by its presence or the presence of the 1NA-degrading inocula. Equation 4 can also be used to calculate X_2, concentration of indigenous organisms (with $X_{2o} = 0$). Reasonable values can be assumed for indigenous organism maximum-yield (0.4) and decay (0.06) coefficients and laboratory-reactor experimental parameters can be used for C_{Bo} (375 mg-COD/L), C_B (30 mg-COD/L), θ (0.458 d), and θ_c (8.91 d). These values result in a steady-state indigenous biomass concentration (X_2) of 1749 mg/L.

Equations 3 and 4 were solved iteratively with various values of X_{1o}; C_o = 1 mg/L; k = 0.10, and K_s = 30.0 (derived from batch ER culture experiments); Y=0.4 (assumed); and various values of k_d. Figure 2 shows theoretical steady-state total biomass concentration ($X_1 + X_2$) and actual measured values versus bioaugmentation level. Bioaugmentation level has been defined previously as mass percent of ER-cells added per day per unit mass of organisms in the CFSTR (mg MLVSS added per day/mg MLVSS in receiving CFSTR at steady-state). The assumptions required to derive Figure 2 are that ER-inocula grow only on 1NA, are completely viable and utilize the same degradation kinetics as in batch mode, flocculate well and remain in the system. Additionally, it is assumed that steady-state is achieved. The experimental data are average

MLVSS values for each CFSTR from biweekly measurements made over 12 months.

Figure 2 indicates that the steady-state model does not work well (upper curve) because the steady-state measured biomass concentrations are much lower than predicted using the commonly accepted decay coefficient value of 0.06 day^{-1}. Exaggerated values of the decay coefficient can be used to pull the curve down to agree with the measured values offering a predictive tool. However, it is extremely unlikely that large decay rates are the actual mechanism responsible for the observed data. There are several possible explanations regarding why cells do not accumulate to concentrations predicted.

First, ER cells may not flocculate well and leave the system in the effluent. This explanation fits with observations made during experimental work where higher solids were noted in effluents of CFSTRs with high bioaugmentation levels. On two occasions, effluent was collected from all CFSTRs for 24 hr and analyzed for solids content. Average values from the two determinations for each CFSTR were as follows (reported as X_e, effluent MLVSS): unacclimated and acclimated combined, 21.7 mg/L; 2.2% augmented, 39 mg/L; 3.4% augmented, 62.8 mg/L; 8.8% augmented, 79.2 mg/L; 11.8% augmented, 70.4 mg/L; and 18.6% augmented, 99.0 mg/L. From this data, it is apparent that bioaugmented CFSTRs lost significantly more cells than controls.

Table 3 shows average biomass in each bioaugmented CFSTR and the combined average for controls. Also shown are calculated excess biomass lost per day from each augmented CFSTR, biomass added per day as ER inocula, and excess cells lost calculated as a percentage of cells which were added for

bioaugmentation. The percentages indicate that excess solids in bioaugmented CFSTR effluents can account for all of the discrepancy between theoretical and observed biomass concentrations for the lower bioaugmentation levels but not for the higher levels.

Excess organic content was also observed in weekly effluent COD determinations made throughout the study period. Table 4 shows average effluent CODs from all CFSTRs during the 12 month test period. The trend of increased effluent COD for increased bioaugmentation level is not strictly met, but with the exception of the 3.4% bioaugmented CFSTR, there is greater effluent COD from augmented reactors than from controls. This may indicate that some portion of bioaugmentation inocula were unable to grow and were subsequently reduced to soluble decay products. Some of these products would have been taken up as a carbon source, but some could also end-up in the effluent. Based on effluent MLVSS data, we would not expect excess COD in the effluent from the 2.2 and 3.4% bioaugmented CFSTRs. However, we would perhaps expect greater effluent CODs from the other CFSTRs as shown.

Apparently, there were mechanisms occurring in the bioaugmentation process which are not accounted for in the simple additive steady-state model developed thus far. However, the biomass concentration that will result from a given amount of bioaugmentation can be predicted if higher than normal values of the decay coefficient are employed. A dynamic model that accounts for interactions between inocula and indigenous cells would be more likely to accurately predict operating characteristics.

Kinetic model. There are several aspects of the ER process which cannot be easily examined experimentally. For example, it is not possible to determine directly whether ER bioaugmentation inocula remain in the

CFSTR system for longer or shorter periods than indigenous cells or if they flocculate well. The question of how ER inocula behave kinetically following inoculation is even more important. There could be a lag followed by attainment of the same degradation kinetics found in the ER, or a change in degradation rate. Because of organism interactions, it is probably not possible to determine experimentally the degradation kinetics of the different types of organisms on the different types of substrates in an operating CFSTR. However, if a simulation model can accurately predict experimental results, then the underlying mechanisms or relationships can be postulated.

A kinetic model was developed in an attempt to better understand the dynamic response of the ER process to shock loadings, and reacclimation. Formulation of our kinetic model also follows the mass-balance approach of Lawrence and McCarty (1970). We assumed two types of substrate (bulk COD and 1NA) and two types of organisms (ER cells and indigenous cells). Thus, we used a set of two substrate mass balances and two cell mass balances with Monod growth and degradation kinetics. The system of four coupled non-linear ordinary differential equations was as follows:

$$V\frac{dC_t}{dt} = QC_{to} - QC_t + Vr_{su1t} + Vr_{su2t} \quad (5)$$

$$V\frac{dC_B}{dt} = QC_{Bo} - QC_B + Vr_{su2B} \quad (6)$$

$$V\frac{dX_1}{dt} = f\,X_{ER} - Q_wX_1 + Vr_{g1t} \quad (7)$$

$$V\frac{dX_2}{dt} = -\,Q_wX_2 + VYr_{su2t} + Vr_{g2B} \quad (8)$$

where the "t" and "B" subscripts denote 1NA and bulk COD, respectively; and the "1" and "2" subscripts denote inoculum and indigenous cells, respectively. The terms "f" and "X_{ER}" are volume of ER inocula added per day and biomass concentration in ER, respectively. Equations 5 through 8

indicate that both indigenous cells and inocula consume 1NA, and only indigenous cells consume bulk substrate. In Equation 8, VYr_{su2t} appears instead of Vr_{g2t} to avoid using two decay coefficients. A forth-order correct, variable-time-step Runge-Kutta integration solution technique was used. Data from a spike experiment and a reacclimation experiment will be reported in conjunction with simulation model output. Prior to the simulations, the model was calibrated with steady-state biomass concentrations and bioaugmentation levels.

Simulation and experimental results for spike experiment. Six CFSTRs (unacclimated, acclimated, 2.2%, 3.4%, 11.8%, and 18.6% bioaugmentation levels) were acclimated to 1 mg-1NA/L for a period of at least 3 MCRT and effluent 1NA concentrations for all reactors were below detection prior to the experiment. Enough 1NA was added to each CFSTR to instantaneously make the 1NA effluent concentration 12 to 15 mg/L (shock loading). Effluent 1NA breakthrough curves (experimental) are shown in Figure 3. Effluent 1NA had decreased below detectable concentrations within 48 hr for all CFSTRs. Figure 3 shows that CFSTRs which had greater bioaugmentation levels also had greater reduction of 1NA breakthrough.

Figures 4 through 8 show simulation model output along with experimental data for each CFSTR individually. Figure 4 shows the correlation attained for the unacclimated CFSTR in which no degradation was assumed to take place. The smooth curve is model output. The presumed reason that all the data fall below the curve is due to volatilization (stripping). The kinetic model only accounts for dilution and ignores stripping. The high aeration rates and headspace ventilation used in all CFSTR experiments would result in lower 1NA concentrations in the water

phase than would be predicted by dilution alone or a combination of dilution and biodegradation. Adsorption can be essentially ruled out as a removal mechanism because periodic solvent extraction of waste sludge samples did not recover and adsorbed 1NA. The experimental data follows the general shape of model output very well.

Figure 5 shows the acclimated CFSTR simulation. Here we have assumed that indigenous microorganisms degrade 1NA at 25% the maximum rate of the enrichment culture. There was no reason for this assumption other than that is seemed reasonable. Again, volatilization could account for any points which fall below the curve. However, volatilization from the acclimated CFSTR should be less than from the unacclimated CFSTR because of the actively competitive biodegradation removal mechanism. For this curve, the following 1NA-degradation kinetic parameters were used; k, 0.025; K_s, 30; k_d, 0.06. These values were chosen because they are the same as the parameters used for the enrichment culture except for k. Large deviations occur toward the end of the experiment. Experimental data shows that effluent 1NA decreases to below detectable concentrations in approximately 24 hr. However, any kinetic model will result in residual concentrations that persist. The discrepancy can be attributed again to volatilization which is not accounted for in the model. In practice, we do not measure detectable residuals at steady-state because of the highly-aerated, well-mixed system.

Figures 6 and 7 show simulations for the 2.2 and 3.4% bioaugmented CFSTRs. During kinetic simulations bioaugmentation inocula did not affect normal operation (effluent bulk COD concentrations did not change significantly). Again correlations are quite good between experimental and

simulated data. Also, the residuals problem is still prevalent. Here, the kinetic parameter values used for indigenous microorganisms were the same as for the acclimated CFSTR and the values used for ER cells were as follows; k, 0.1; K_s, 30; k_d, 0.06. The close fit implies that the assumption that inocula and indigenous cells do not affect each other is correct. This is not the case for the 11.8% and 18.6% bioaugmented CFSTRs.

Figure 8 and 9 show simulation output along with experimental data for the 11.8% and 18.6% bioaugmented CFSTRs, respectively. Values used for indigenous microorganism degradation kinetics were the same as for the acclimated CFSTR. However, much higher decay rates were required for ER inocula in order to get reasonable correlations. In Figure 8, the upper curve (better fit) has a k_d of 0.36 which is 6 times the value normally used. In Figure 9, the upper curve has an even larger k_d of 0.54. These figures indicate that at these higher bioaugmentation levels, there are simply too many cells added per day to be supported by the influent substrate. Additionally, we can determine that the problem is not with degradation kinetics. The large values of k_d are required to limit the steady-state cell concentration to a value which is representative of observed steady-state values. If instead, we assume that ER inocula were simply operating at diminished rates, then unrealistic excessive cell concentrations occur along with any shift of the simulation curve upward toward the experimental data.

Spike experiment simulations show that biodegradation using the ER bioaugmentation process occurs in a predictable manner. Reasonable correlations indicate the effect of ER bioaugmentation is to add to the degradative ability of the CFSTR which is manifested as a reduction in effluent breakthrough during a transient loading condition. Simulations also

indicate that dynamic behavior can be reasonably predicted using the assumption that ER inocula utilize the same degradation kinetics observed in ERs.

Simulation and experimental results for reacclimation experiment. Six CFSTRs (acclimated, 2.2%, 3.4%, 8.8%, 11.8%, and 18.6% bioaugmentation levels) were acclimated to 3 mg-1NA/L for a period of at least 3 MCRT and effluent 1NA concentrations for all reactors were below detection prior to the experiment. The influent 1NA concentration was decreased to 0.0 mg/L for 12 days (1.3 MCRT) then restored to 3 mg/L. Effluent 1NA breakthrough curves (experimental) during reacclimation are shown in Figure 10. Effluent 1NA had returned to below detectable concentrations within 240 hr (10 day) for all CFSTRs. Figure 10 shows that CFSTRs which had greater bioaugmentation levels also had greater reduction of 1NA breakthrough.

Figures 11 through 14 show simulations of reacclimation along with experimental data for acclimated, 2.2%, 3.4%, and 8.8% bioaugmented CFSTRs. The approach used was to first achieve a reasonable fit for the acclimated CFSTR (which only had indigenous organisms). Once satisfied with the correlation, the determined kinetic parameters were carried-over to all of the augmented CFSTRs for behavior of indigenous organisms. Figure 11 shows the acclimated reactor simulation, which has a nice fit except at the end of the experiment where there is a residual predicted by the model which was not observed in the experimental reactor. Again, this is presumably due to a combination of the effects of volatilization and the inherent limitations of a simple kinetic model.

In order to achieve the fit shown in Figure 11, the maximum substrate utilization rate (k) had to be varied during the course of the simulation. An

initial value of 0.01875 was held constant for 110 hr then increased linearly to 0.15 between hr 110 and hr 240. The variation of k is in accordance with the presumed effects of disacclimation followed by reacclimation. The first 110 hr (4.5 days) can be viewed as a lag period during which degradation proceeded via highly diminished rates. The nearly 10-fold increase of k during the last 5.5 days can be perceived as reacclimation.

Figures 12, 13, and 14 show simulations of the 2.2%, 3.4%, and 8.8% bioaugmented CFSTRs, respectively. During kinetic simulations, bioaugmentation inocula did not affect normal operation (effluent bulk COD concentrations did not change significantly). For each augmented CFSTR, the variation of k for indigenous organisms was the same as that used for the acclimated CFSTR, and k for ER inocula was varied linearly from an initial value of 0.1 to 0.4 between hr 0 and hr 96. The value of k was maintained at 0.4 between 96 and 240 hours. As in the case of the spike experiment, the ER-inocula decay rate of 0.06 day-1 was sufficient to achieve good correlations for the two low-level bioaugmented CFSTRs. However, a higher decay rate (0.18 day-1) was required for the 8.8% bioaugmented CFSTR. Again, there was a problem with residuals which were predicted by the kinetic model and not observed in the experimental data.

The very small breakthroughs shown in Figure 10 for the 11.8% and 18.6% bioaugmented CFSTRs proved difficult to simulate because of the problem of persistent residuals. The general shape of the simulation curves did mimic the shape of the experimental breakthrough curves, but residuals made the fit look poor (data not shown). However, the simulation model fit of reacclimation experimental data indicate that the presumption of disacclimation can be modeled. Also, the reduced breakthrough observed in

bioaugmented CFSTRs can be modeled effectively using the assumption that less disacclimation occurs in bioaugmented CFSTRs.

CONCLUSIONS

Kinetic simulations have added to knowledge regarding the forces at work in the ER bioaugmentation process. It seems that there is a limit to the beneficial effect which can be attained with bioaugmentation. This limit is controlled directly by two factors. First, the degradation rate of the inocula on the target compound directly affects how much of the compound can be degraded within the fixed hydraulic retention time of the augmented CFSTR. Second, is the limitation of the influent wastewaters ability to support the growth of the additional organisms added during the bioaugmentation process. Thus, for a given influent wastewater, there is a limitation on the amount of cells (added as inocula) which can be supported and excesses apparently are expelled or die. The only way then to achieve better results from bioaugmentation for a given system would be to develop a more robust enrichment culture (i.e. faster degradation rates) because CFSTR system parameters control the amount of inocula which can be active.

These conclusions are supported by both the steady-state model and the dynamic model. The steady-state model indicated that most of the cells added during bioaugmentation must be expelled in some way (effluent solids or COD). The dynamic model indicted that the behavior of the ER system under transient loading conditions can best be explained by the loss of a considerable amount of inocula rather than a decrease in kinetic degradation rates.

ACKNOWLEDGEMENTS

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 Simlin Lau and Kenneth Wong for their assistance with the daily maintenance of the AS units used in these experimental work, and their help with GC and HPLC sample analysis.

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Table 1-Laboratory CFSTR bioaugmentation status

CFSTR	Bioaugmentation Status	Constant influent 1NA (mg/L)
1	Unacclimated Control	0.0
2	Acclimated Control	1.0
3	2.2% Bioaugmented	1.0
4	3.4% Bioaugmented	1.0
5	8.8% Bioaugmented	1.0
6	11.8% Bioaugmented	1.0
7	18.6% Bioaugmented	1.0

Table 2-Operational characteristics of laboratory reactors

Parameter	CFSTRs	SBRs
Volume	12.2 Liter aeration zone 1.5 Liter clarifier	5.0 Liter
HRT	0.458 day (Q = 18.5 ml/min = 26.64 L/day)	22 hr aeration 1.0 hr settle 0.5 hr drain 0.5 hr fill
MCRT	8.9 day	7 day
MLVSS	1588-3008 mg/L	2000-4000 mg/L (3415 average)
Loading Rate	0.81 kg COD/m^3 day	0.76 kg COD/m^3 day
1NA Influent	1.0 mg/L	150 mg/L
F/M	0.27-0.51	0.19-0.38
Aeration	7.1 L/min average DO = 7.9 mg/L	2.4 L/min DO = 1-4 mg/L
pH	6.5-7.5	6-7
Temp	13-23 C	18-30 C

Table 3-Measured CFSTR effluent biomass

CFSTR	MLVSS (mg/L)	Excess MLVSS lost (mg/day)	MLVSS added (mg/day)	Lost/added (%)
Accl+Unaccl	1668	0	0	
2.2% Bioaug	1604	461	431	107
3.4% Bioaug	2076	1095	861	127
8.8% Bioaug	1586	1532	1703	90
11.8% Bioaug	2362	1297	3400	38
18.6% Bioaug	3008	2059	6826	30

Table 4-Measured CFSTR effluent COD

CFSTR	Effluent COD (mg/L)	Increase over controls (%)
Controls	36.7	
2.2% augmented	38.8	6
3.4% augmented	29.6	-19
8.8% augmented	49.4	35
11.8% augmented	46.6	27
18.6% augmented	60.6	128

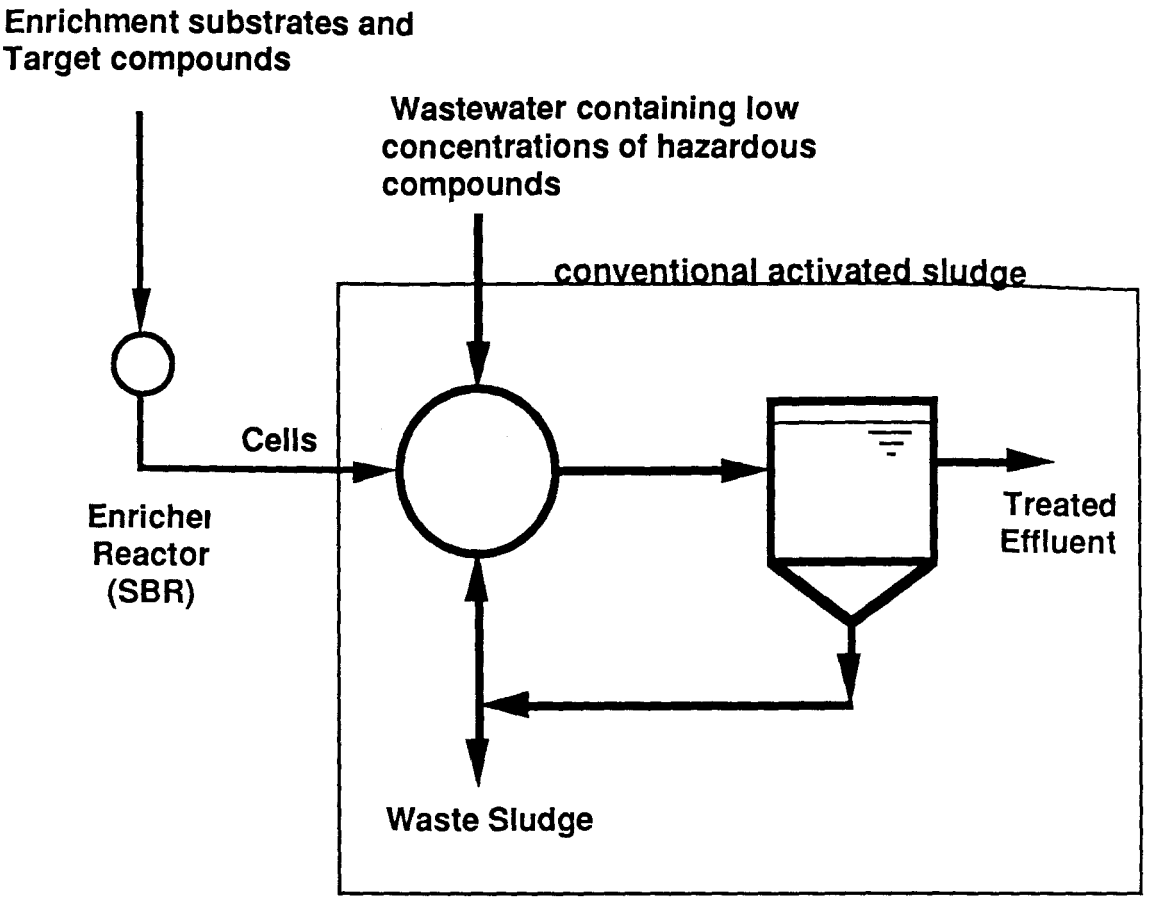

Figure 1-Enricher-reactor concept schematic diagram

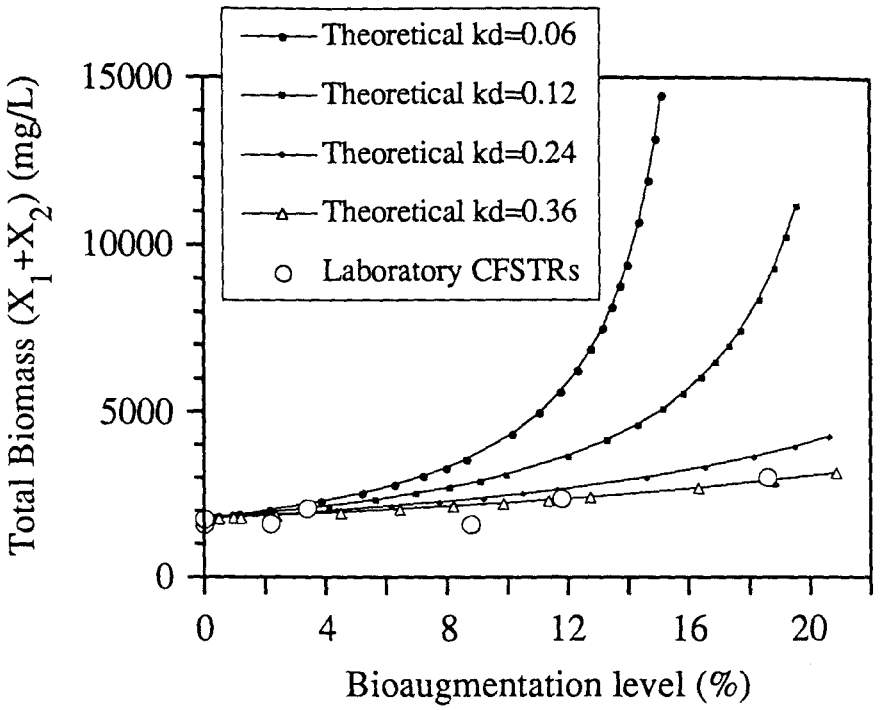

Figure 2-Theoretical and experimental biomass concentrations

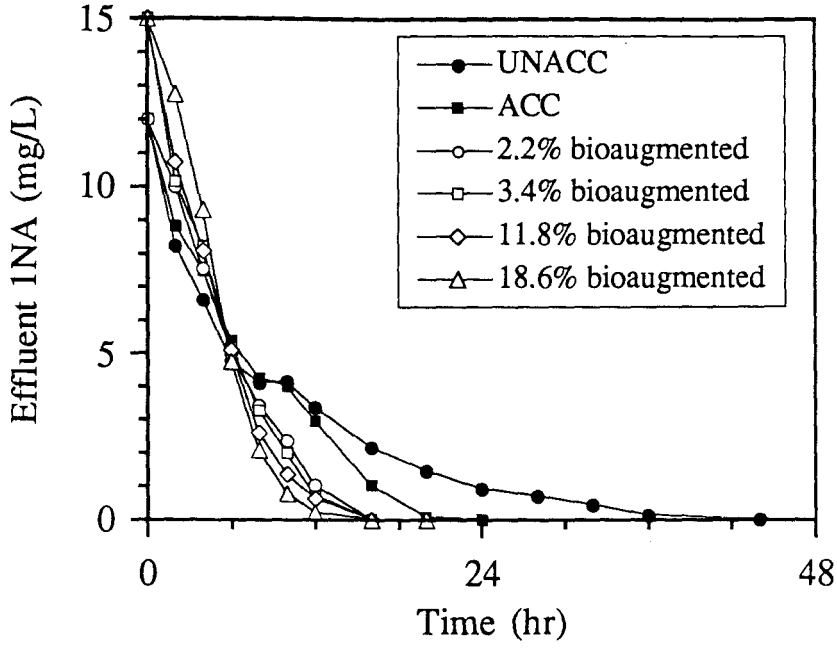

Figure 3-Effluent 1NA breakthrough data from spike experiment

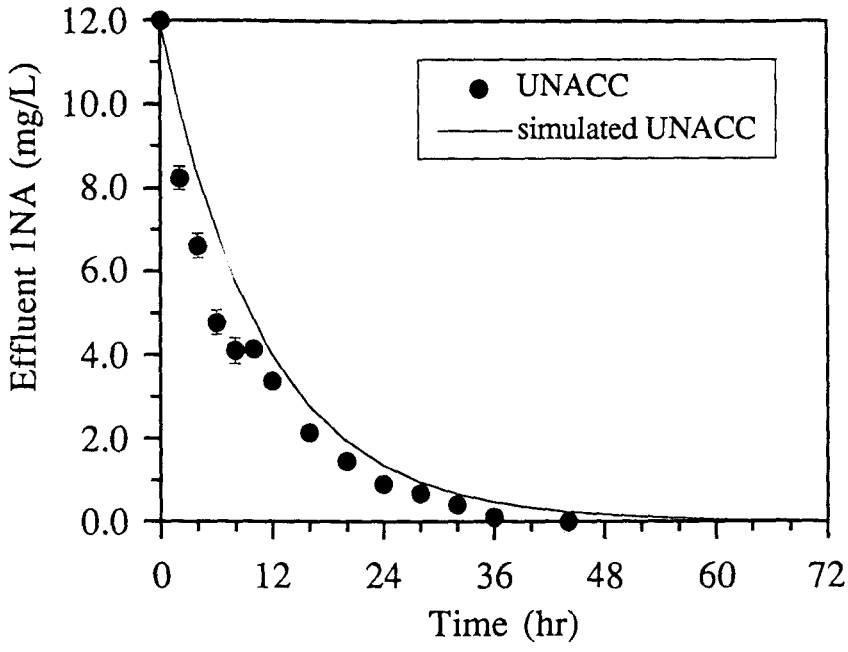

Figure 4-Correlation of simulation model output with breakthrough data for the unacclimated CFSTR

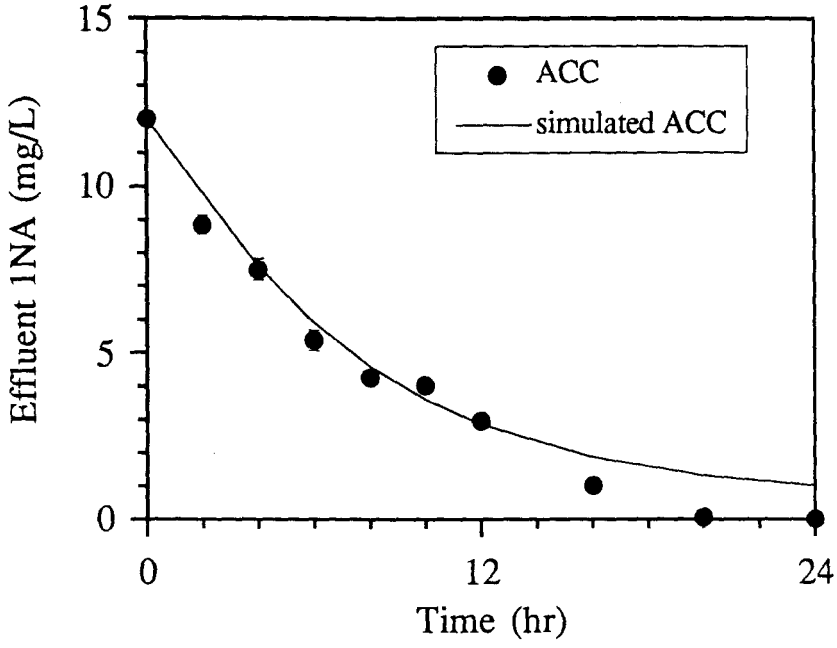

Figure 5-Correlation of simulation model output with breakthrough data for the acclimated CFSTR

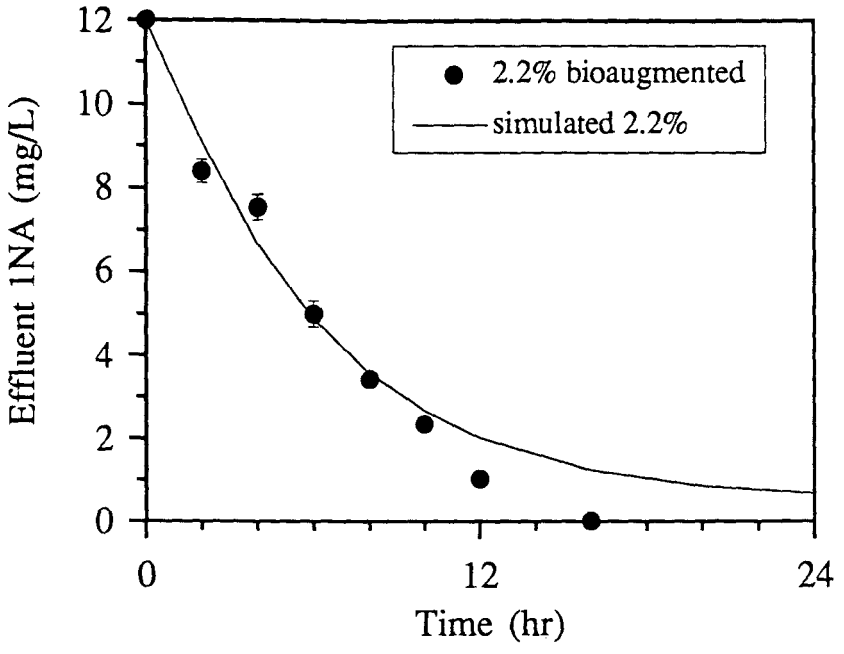

Figure 6-Correlation of simulation model output with breakthrough data for the 2.2% bioaugmented CFSTR

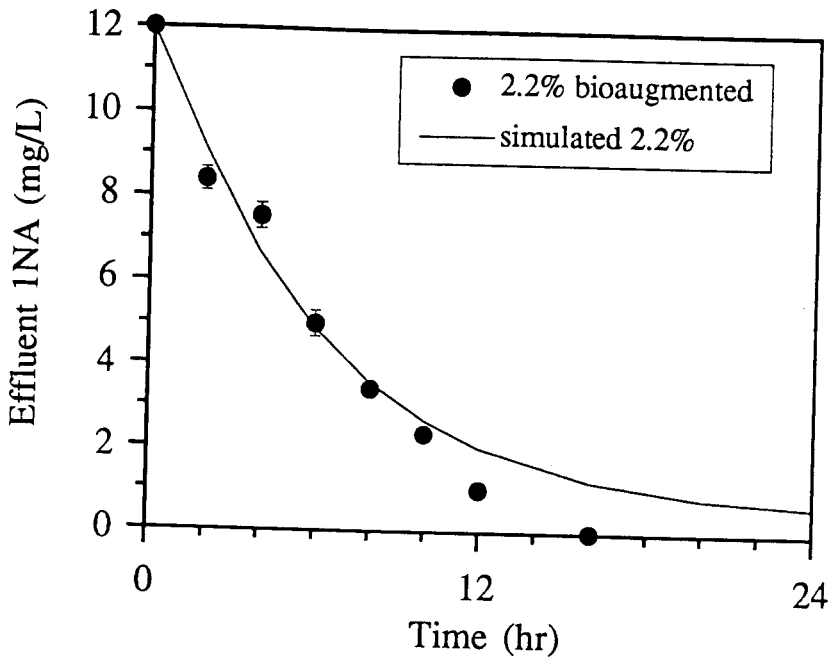

Figure 6-Correlation of simulation model output with breakthrough data for the 2.2% bioaugmented CFSTR

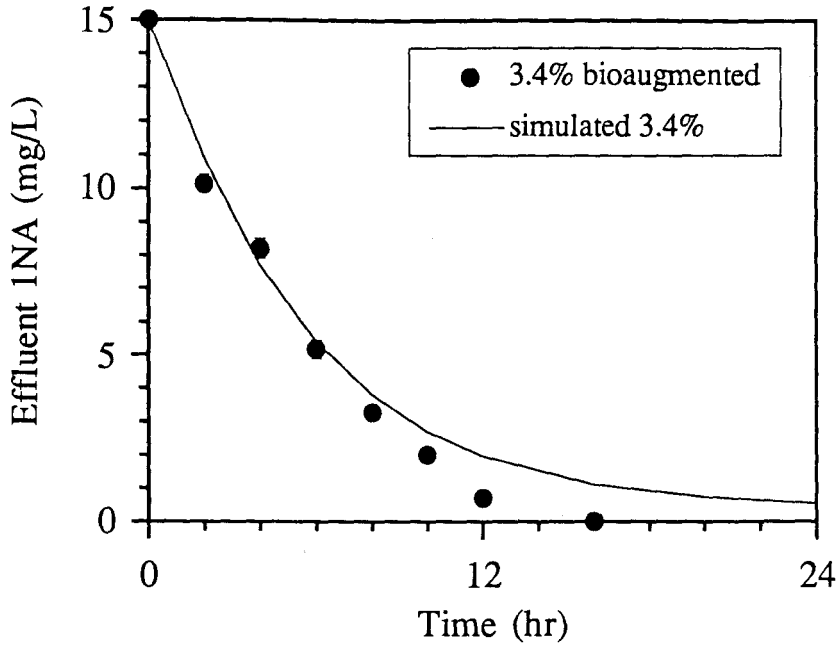

Figure 7-Correlation of simulation model output with breakthrough data for the 3.4% bioaugmented CFSTR

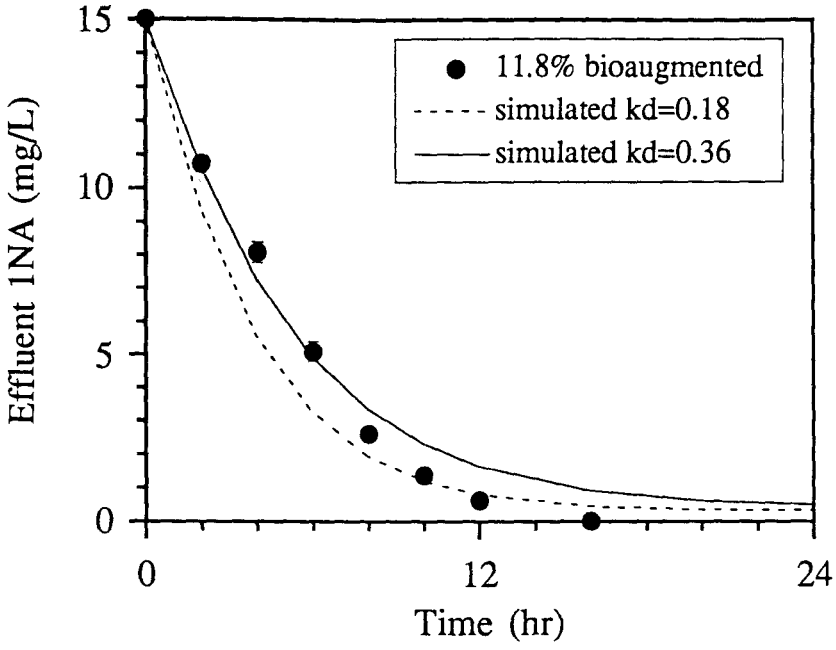

Figure 8-Correlation of simulation model output with breakthrough data for the 11.8% bioaugmented CFSTR

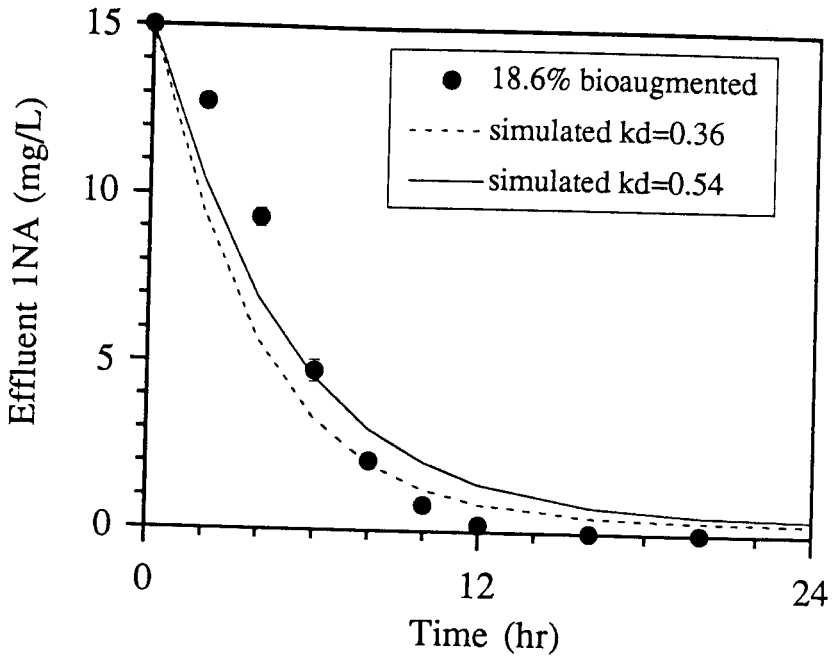

Figure 9-Correlation of simulation model output with breakthrough data for the 18.6% bioaugmented CFSTR

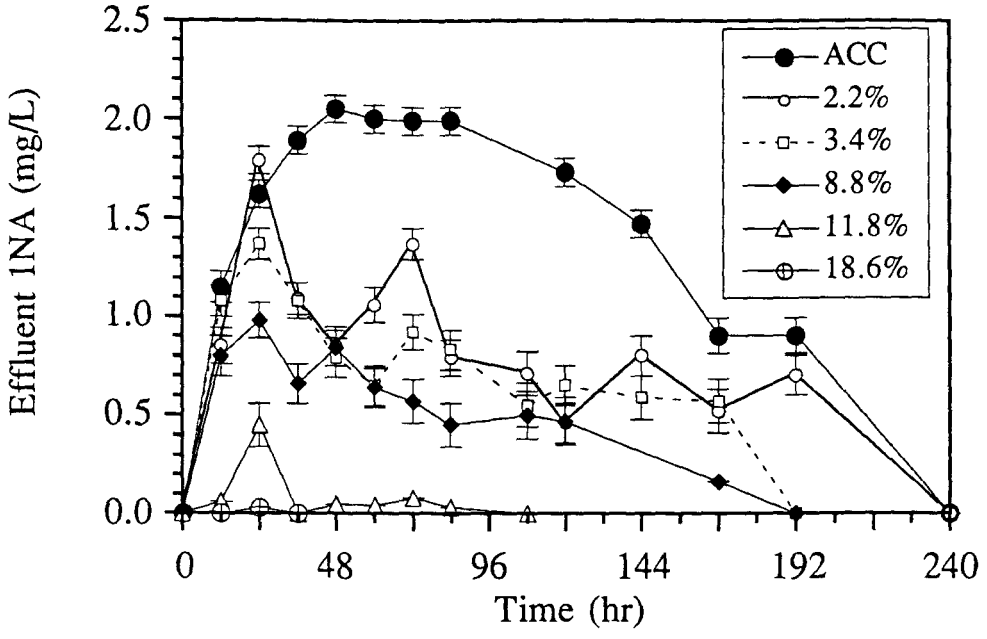

Figure 10-Effluent 1NA breakthrough data from reacclimation experiment

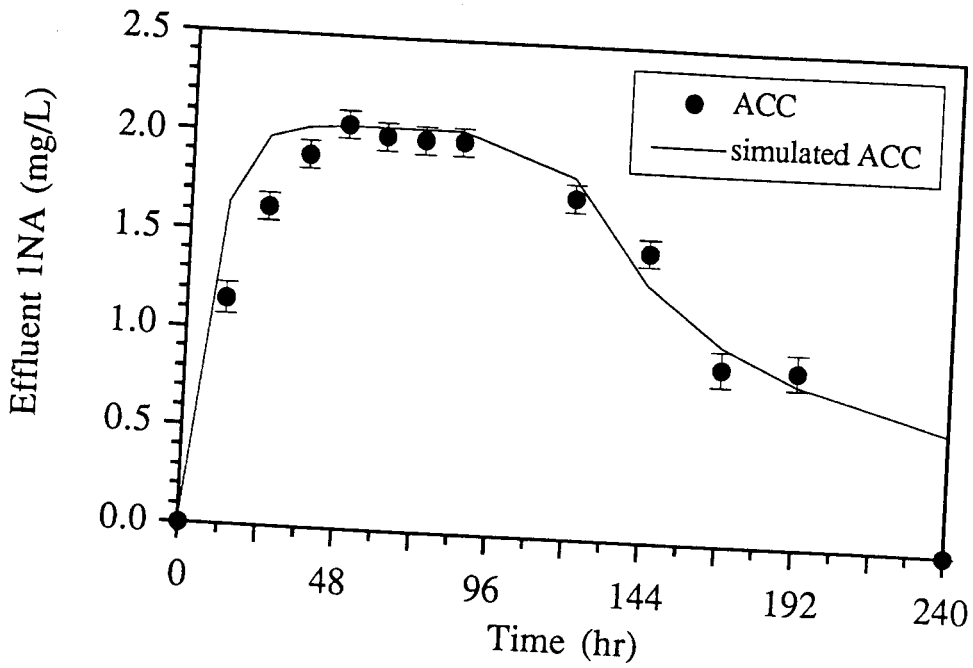

Figure 11- Simulation of acclimated CFSTR during reacclimation experiment

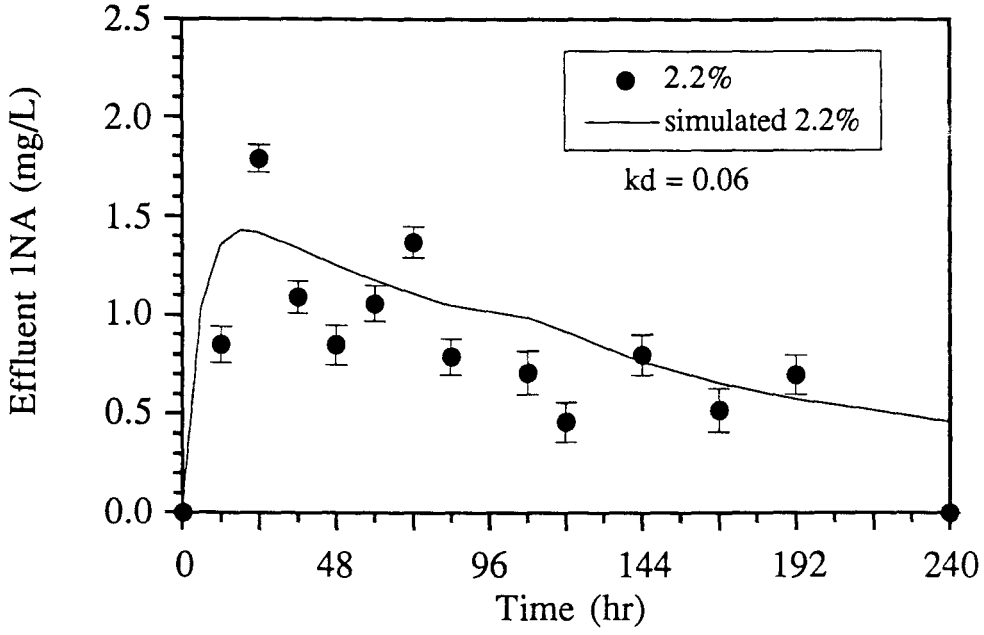

Figure 12- Simulation of the 2.2% bioaugmented CFSTR during reacclimation experiment

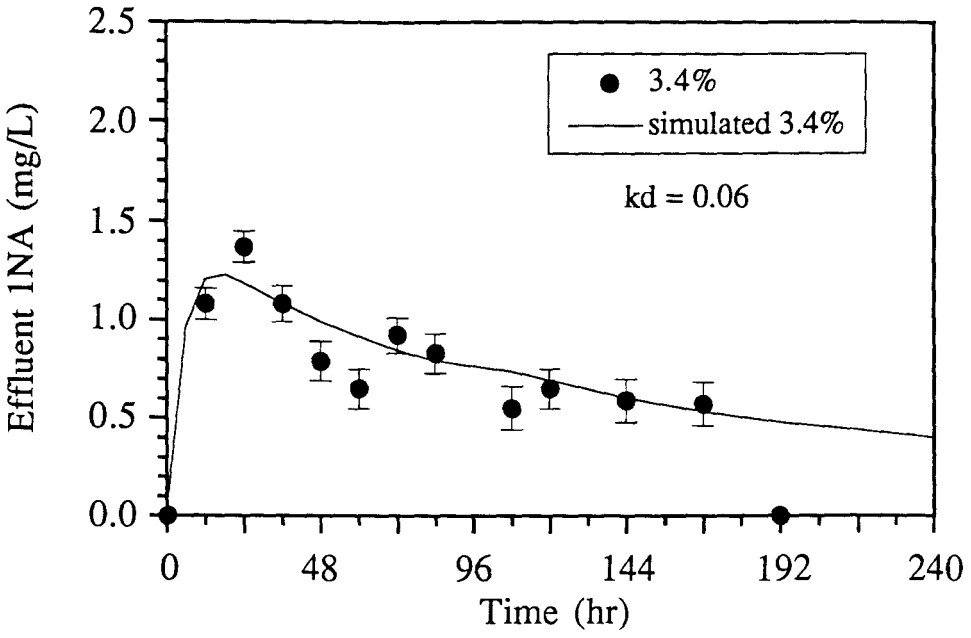

Figure 13- Simulation of the 3.4% bioaugmented CFSTR during reacclimation experiment

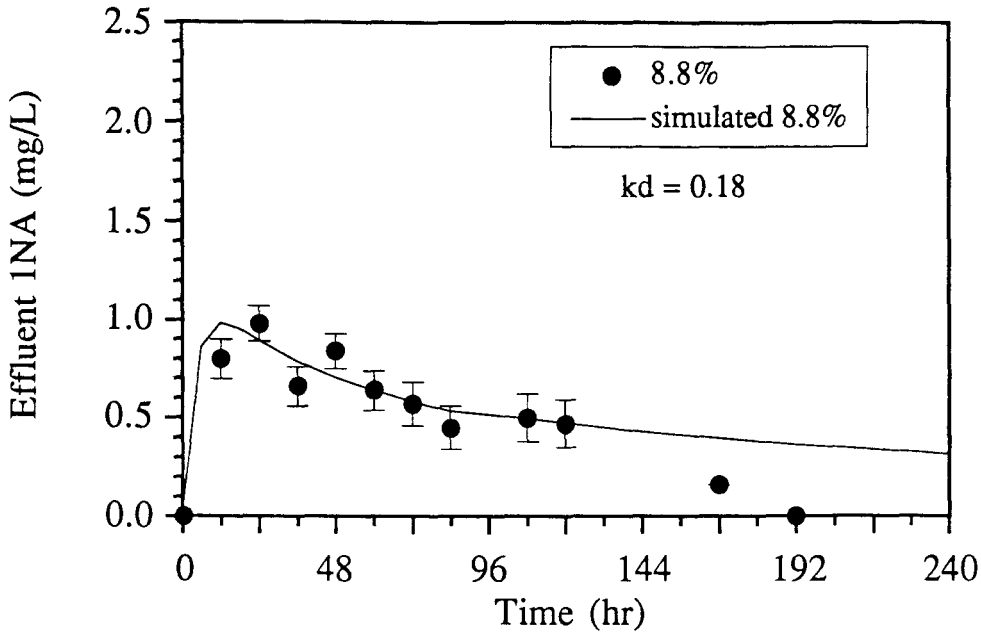

Figure 14- Simulation of the 8.8% bioaugmented CFSTR during reacclimation experiment

4. CONCLUSIONS

This section reiterates some of the most important findings of this thesis. Environmental awareness and the associated issue of hazardous waste treatment and disposal have clearly been at the forefront of the political and economic climate of the United States and the world for some time. Much of the attention has focused on remediation of existing contaminated soil and water which directly threatens human health and the enormous costs associated with clean-up. The size of the current disposal problem is just being pieced together. The regulatory climate has become such that classified hazardous wastes are usually shipped long distances for expensive isolation-type disposal or high-temperature thermal destruction. While the current trend of waste minimization is an excellent approach, there will always remain the problem of low concentration hazardous-waste residuals. These dilute waste streams are expensive to treat by physico-chemical technologies which merely concentrate them, but many may be amenable to treatment via biological oxidation. A technology was needed which would address this problem.

The proposal to upgrade the enormous existing AS infrastructure in place under mandate of the Clean Water Act, such that it could be utilized to effectively degrade low strength organic hazardous wastewaters has been put forth. With this in mind, a model hazardous waste was chosen in the compound 1NA which would be challenging to degrade using conventional treatment systems. Laboratory-scale experiments were then conducted to verify and validate the proposed process.

An enrichment culture was developed using AS inocula from a municipal wastewater treatment plant and a petrochemical oil refinery treatment plant in SBRs. After a period of several months, a culture able to rapidly degrade 1NA had evolved. Many experiments were conducted to verify that biodegradation was occurring and to determine the kinetics of biodegradation. The target compound was found to be most rapidly degraded at concentrations at or below 150 mg/L. Above this concentration, degradation proceeded at reduced rates indicating inhibition presumably due to toxicity. At concentrations below the inhibitory level, degradation was shown to follow Michaelis-Menten (also referred to as Monod) kinetics. The culture was shown to have the ability to degrade 1NA as its sole source of carbon and energy. Experiments verified that 1NA was indeed mineralized to CO_2 rather than merely transformed to perhaps equally toxic unknown intermediate compounds.

The developed enrichment culture was first used to show that a one-time inoculation of 1NA-acclimated AS with different doses of the enrichment culture would result in increased 1NA degradation rates. The culture was then used to continuously inoculate several CFSTRs. Each CFSTR was operated at different bioaugmentation levels from just over 1% to greater than 18% for approximately 12 months. During that period, several stressful treatment scenarios were set-up for the AS system and the benefits of bioaugmentation determined. Bioaugmented CFSTRs allowed less breakthrough of 1NA than controls following a step-increase loading, a shock loading, and during reacclimation to 1NA following its absence from the waste stream for an extended period. Both unacclimated (worst-case treatment) and continuously acclimated (best-case conventional treatment)

control reactors were operated for comparison. This set of experiments verified the utility of the ER process concept and showed that increased bioaugmentation gave an increased benefit.

During the first set of experiments, the enrichment culture had been maintained on high concentrations of 1NA which would be an unattractive requirement for a full-scale system. Thus, the potential to use less hazardous compounds to induce 1NA-degradation activity in cultures maintained in a media free of 1NA was investigated. Subcultures of the original enrichment culture were grown on structurally similar compounds or a proposed degradation metabolite for several months and then shown to retain the ability to degrade 1NA in batch experiments. The induced sub-cultures were then used to continuously bioaugment several CFSTRs which were again subjected to the same stressful treatment scenarios as before. The CFSTRs augmented with inducer-compound maintained cells performed nearly as well and in some cases as well as the original enrichment culture. This development was anticipated to make the process appear more attractive.

It was found that steady-state mass balances using typically accepted kinetic parameters were ineffective at predicting steady-state biomass concentrations observed during CFSTR experiments. Better correlations were achieved by assuming excessively high biomass decay rates. Bioaugmented CFSTRs apparently lost proportionally more cells and organic material in effluent than unaugmented reactors. A kinetic model was formulated to examine the underlying kinetics of the ER process which are unapproachable experimentally. The model was used to simulate the dynamic response of the CFSTRs to a spike loading. When a provision was made to allow inocula to loose the ability to degrade 1NA, good correlations with experimental data

were achieved. The model could be used to determine the operating characteristics and dynamic response of a scaled-up system.

5. METHODOLOGY

5.1 Reactors and equipment

Two sizes of plexiglass-constructed AS reactors were used. Continuous-flow experiments were conducted in eight 13.7-L reactors constructed of 0.5-in plexiglass (see Figure 2, Section 3.1). These reactors had 12.2-L aeration zones separated from 1.5-L clarification zones by sliding baffles. Substrates, pH probes and adjustment solutions, hazardous compounds, and air were added via access holes in the reactor covers. Batch cultures were maintained in seven 5-L reactors constructed of 0.25-in plexiglass (Figure 5.1). Three of the larger reactors were constructed of black plexiglass and all other reactors were covered with dark window tinting material to minimize growth of algae. All reactors had low-flow vapor extraction via 1-in I.D. reinforced-plastic hose and a fume-hood blower. A hole in the side of each reactor in the aeration zone was used to withdraw samples.

The 13.7-L reactors were operated as individual single-unit two-stage continuous-flow stirred-tank activated sludge systems (CFSTRs). The CFSTR operating mode is described in detail by Tchobanoglous and Schroeder (1985). The 5-L reactors were operated in sequencing batch mode (SBRs) described by Irvine and Busch (1979). SBRs are operated on five sequential periods including: fill (addition of fresh waste), react (aeration of system and degradation of waste), settle (separation of bacteria from treated effluent), drain (removal of treated effluent), and idle (time prior to addition of fresh feed). The duration of each period can be varied to optimize treatment efficiency and the idle period is optional. One cycle consists of completion of

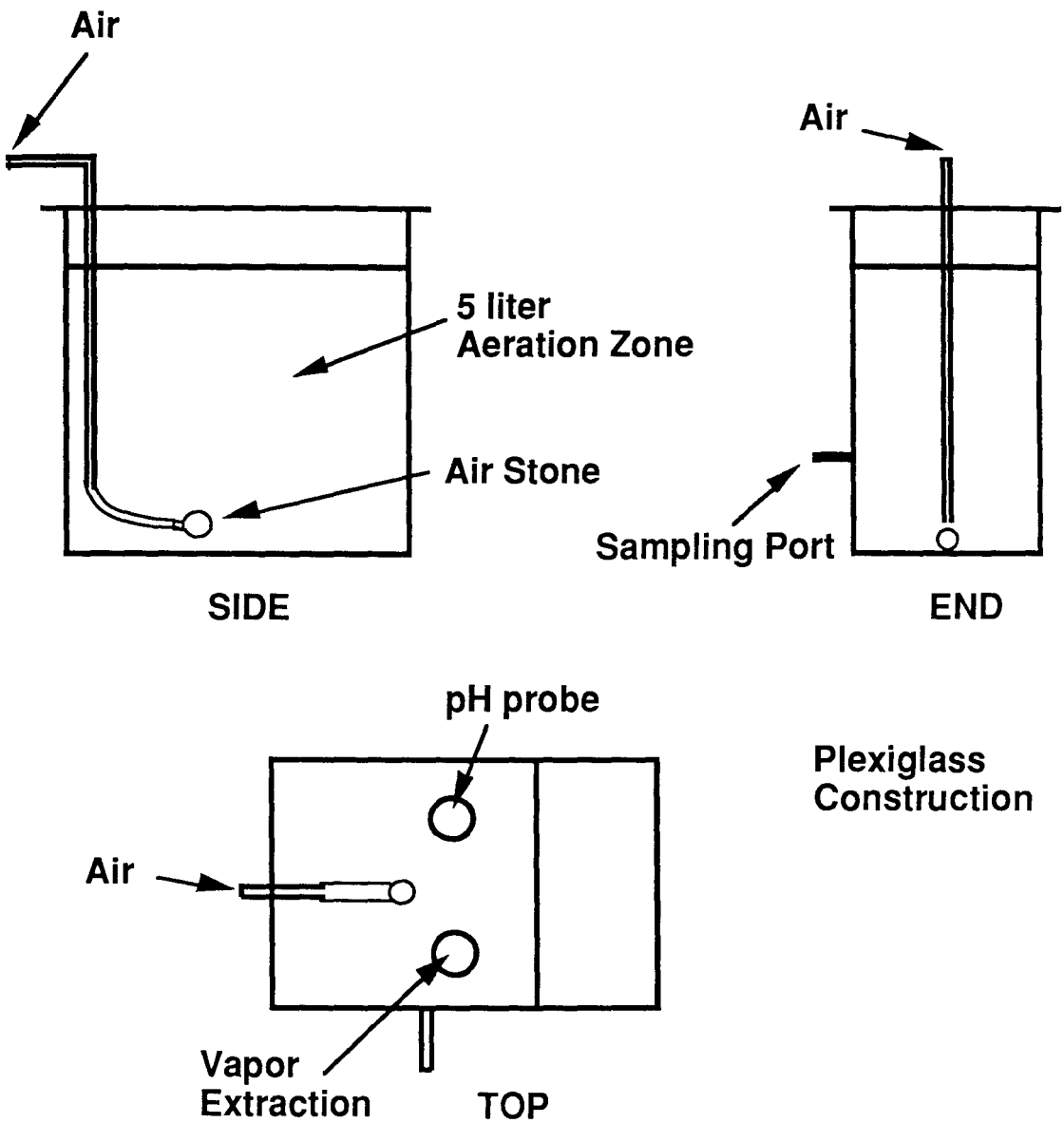

Figure 5.1 Laboratory-scale enricher reactors.

all chosen periods in sequence. Section 2 gives the duration of each period for all experiments conducted.

The pH adjustment solutions and associated hardware, which regulated the pH between 6.5 and 7.5 in each reactor independently, are described in Section 3.1. Compressed air was passed through separate rotameters for each reactor to control flow rate to the ceramic fine-bubble-diffuser aeration stones. Flow rates and resultant dissolved oxygen levels are given in Section 3.1.

In these studies, an automated feed dilution system was used to continuously prepare substrate for all of the continuous-flow reactors (see Figure 5.2). Concentrated feed, calcium-magnesium (Ca/Mg), and target

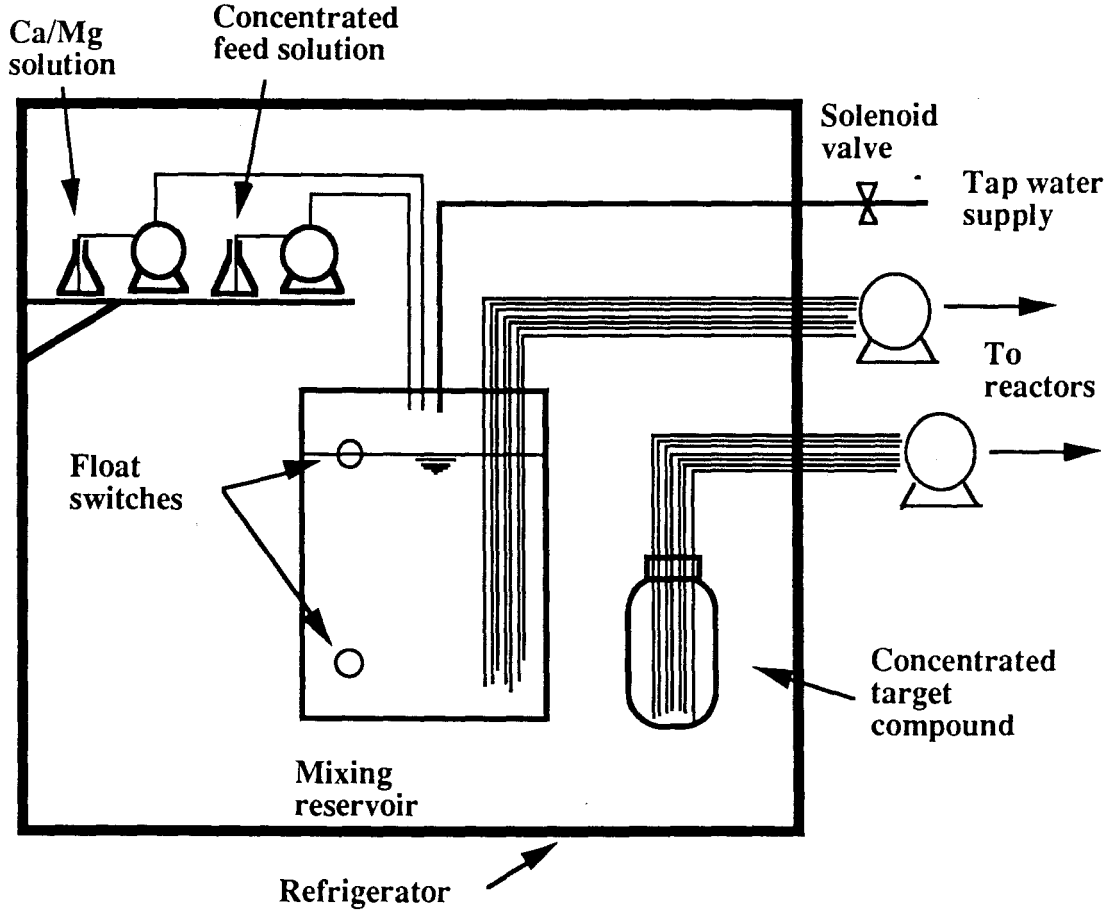

Figure 5.2 Automated feed dilution system.

compound solutions as well as the mixing reservoir and pumps were contained in a refrigerator at 5 °C. High and low liquid levels were sensed by two float switches. The low-level switch activated a controller which operated the concentrated feed solution pumps for 14.5 seconds and opened the external solenoid for flow of dilution water. The high-level switch closed the dilution-water solenoid. In each cycle, 58-ml of concentrated feed and 7.25-ml of concentrated Ca/Mg solution were added to 15.3-L of dilution water; this gave dilutions of 1/264 and 1/2110 respectively. Diluted substrate was pumped from the mixing reservoir to the CFSTRs through silicone and Tygon tubbing by an external pump. Concentrated target compound was pumped separately through chemical-resistant Viton tubbing by an external pump from a storage reservoir which was replenished every 24 hours.

5.2 Chemicals and substrates

Sources of all chemicals and aromatic compounds are reported in Sections 2 and 3.1. Several substrates were used in the studies. During the first several months of acclimation of both CFSTRs and SBRs, the primary carbon source used was dextrose (concentrated feed formulation given in Table 5.1). Simple experiments were conducted to determine that the dextrose was the cause of persistent bulking problems (a condition which causes loss of bacteria and prevention of stable long-term operation). Dextrose was thus eliminated from the substrate formulation. Table 5.2 shows the reformulated concentrated feed solution. Tables 5.3 and 5.4 show the concentrated calcium-magnesium and the trace mineral solutions used, respectively. Table 5.5 gives the concentrated vitamin solution formulation. Calcium and magnesium salts were fed separate of concentrated feed to avoid

Table 5.1 Concentrated dextrose feed

H_2O	2000	ml
Dextrose	107	g
Bacto peptone	62.5	g
Beef extract	37.5	g
Yeast extract	10	g
K_2HPO_4	50	g
KH_2PO_4	79	g
$(NH_4)_2SO_4$	20	g
$FeSO_4 \cdot 7H_2O$	22	g
Trace mineral solution	4	ml

Table 5.2 Concentrated feed solution

H_2O	2000	ml
Bacto peptone	100	g
Beef extract	75	g
Yeast extract	20	g
K_2HPO_4	50	g
KH_2PO_4	79.4	g
$(NH_4)_2SO_4$	20	g
Trace mineral solution	4	ml
Vitamin solution	10	ml

Table 5.3 Calcium-magnesium solution

H_2O	400	ml
$CaCl_2 \cdot 2H_2O$	10.52	g
$MgCl_2 \cdot 6H_2O$	16.40	g

Table 5.4 Trace mineral solution

H_2O	500	ml
$FeCl_3$	19.5	g
$MnCl_2 \cdot 4H_2O$	4.75	g
$ZnCl_2$	3.30	g
$CuCl_2 \cdot 2H_2O$	2.05	g
$CoCl_2 \cdot 6H_2O$	2.90	g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	2.10	g
Na_3 Citrate	176.5	g
$Na_2B_4O_7 \cdot 10H_2O$	1.20	g

Table 5.5 Vitamin solution

H_2O	1000	ml
Pyridoxamine dihydrochloride	30	mg
Nicotinic acid	20	mg
Thiamine hydrochloride	20	mg
d-pantothenate	10	mg
p-aminobenzoic acid	8	mg
d-biotin	2	mg

precipitation of the phosphate buffer present in the feed solution. Table 1 in Section 3.1 gives the final CFSTR and ER influent substrate concentrations of all components after mixing and dilution. Other ER substrate formulations used during the acclimation process are given in Section 2.

5.3 Activated sludge cultures

Seed sources for all cultures used as well as a chronological description of the enrichment program are given in Section 2. The operational characteristics of all AS reactors used (including; hydraulic retention times, cell retention times, operating temperatures, food to microorganism ratios, volumetric loading rates, biomass concentrations, and influent and effluent chemical oxygen demands) are given in Section 3.1.

Bioaugmentation was performed by making transfers of cells daily as described in Section 3.1.

5.4 Maintenance and measurements

Daily maintenance of CFSTRs involved removal of 1370 ml of mixed liquor (sludge wasting), followed by stoppage of feed pumps for 1 hr. During this idle period, the inner walls of the reactors were brushed to remove wall growth, the pH probe was washed, effluent lines cleared of any blockage, and ER cells added if appropriate. Daily maintenance of the SBRs included re-feeding during the appropriate period and removal of accumulated wall growth. Air flow rates were monitored and adjusted as necessary.

Weekly maintenance included a more thorough cleaning of all reactors and cleaning of the feed dilution system and feed delivery lines. Lines were cleaned with 2-L of a dilute bleach solution followed by 8-L of tap water to clear any microbial growth. The mixing tank was thoroughly scrubbed to remove apparent growth using bleach and rinsed with tap water. Pump cartridge tubbing was inspected and replaced if required. The pH probes were cleaned with bleach and recalibrated.

Dissolved oxygen was measured in all reactors weekly. Mixed-liquor suspended solids (MLSS) and mixed-liquor volatile suspended solids (MLVSS) were measured weekly for the first 6 months and bi-weekly thereafter. Section 3.1 gives the methods used for measurement of MLSS and MLVSS (a measure of biomass concentration). The pH was monitored continuously in the CFSTRs as described above and weekly in the SBRs. Influent and effluent filtered chemical oxygen demand (COD) of all the CFSTRs were monitored weekly by methods given in Section 3.1. Quantification of target compound in CFSTR and SBR effluent samples was conducted weekly during the first 11 months of this study and daily thereafter.

5.5 Analytical methods

The solid-phase extraction procedure used to concentrate and remove the target compound (1NA) from the sample matrix is described in Section 2. The approximately 2-ml methanol samples from the extraction procedure were stored until analysis by gas chromatography (GC) and/or high-performance liquid chromatography (HPLC) in one dram amber vials with screw caps and teflon-coated silicone septa. Every effort was made to analyze samples the same day as collected, but occasionally samples had to be stored at 4 C prior to analysis. Cell adsorbed target compounds were extracted with toluene or methanol as described in Section 2. Section 2 also gives protocals for GC and HPLC analysis.

5.6 Accuracy and repeatability

The external standard method (linear regression of 1NA concentration versus peak area) was used to quantify unknown samples. GC standard-curves were constructed using a wide range of 1NA concentrations (from the detection limit of 0.5 mg/L to 250 mg/L). The best linear fit (regression) was obtained by dividing this large range into 4 smaller ranges; 0.5 to 5 mg/L, 5 to 25 mg/L, 25 to 50 mg/L, and 50 to 250 mg/L. There were 5 or 6 different concentrations in each range, and three GC-sample injections for each concentration (e.g. Figure 5.3, where each plotted point represents the average area from three injections). Correlation coefficients (r squared) were very high (0.970 to 0.998). The same procedure was used for construction of HPLC standard curves. For the HPLC, the range of standards was larger (0.1 to 500

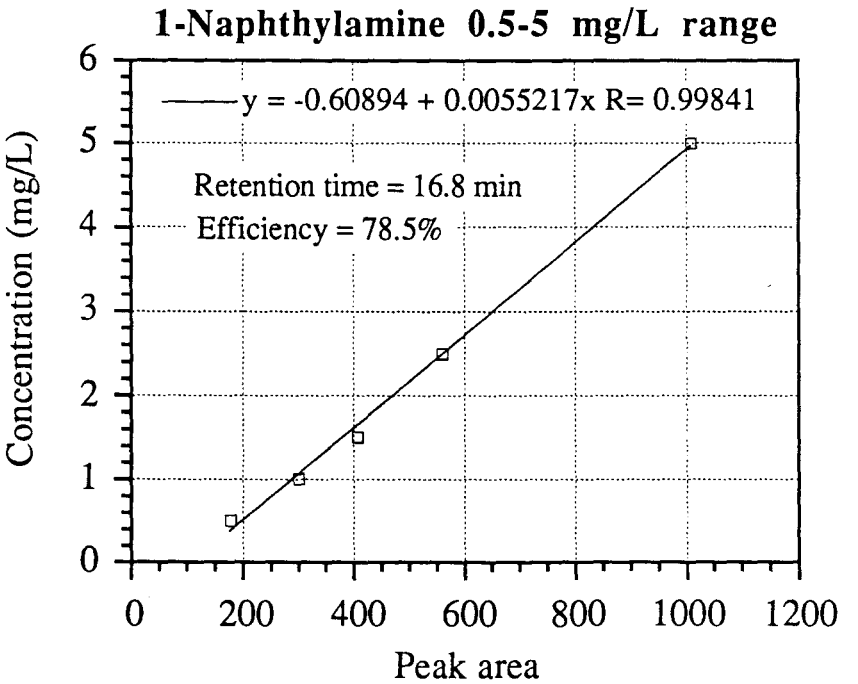

Figure 5.3 Standard curve for 1NA (0.5-5 mg/L) on the GC.

mg/L) but only three ranges were necessary to get good correlations (0.1-1.0 mg/L, 0.995; 1.0-20 mg/L, 0.985; and 20-500 mg/L, 1.00).

The efficiency of the solid phase extraction procedure was also considered. The extraction columns are very similar in design to HPLC columns except that they are smaller and discarded after one use. Each contains 1 mg of silica gel particles coated with the chosen solid phase (which can be polar or non-polar) which is held in place on each end by a 0.22 micron glass-fiber frit that also acts as a filter. The packing (coated silica and frits) forms a plug at the bottom of a 1-ml plastic column through which samples were pulled by vacuum. Following selection of the best solid-phase coating (C18) from the many that were available, it was necessary to determine the reproducibility of the extraction procedure. It was found that the recovery efficiency (found by processing carefully-prepared known-concentration samples through the extraction procedure) was dependent on the concentration of the sample, with lower concentrations giving lower efficiencies. This was to be expected, because at the lower concentrations there was less of the chemical to work with and thus errors have a larger relative effect.

The extraction efficiency was determined in triplicate for each GC-standard concentration (each point on each of the regression lines), and the values were averaged to get a final extraction efficiency for each of the four GC ranges. Recovery efficiencies for individual concentration values within each range varied by less than 5%. The efficiencies for the various GC ranges were: 0.5-5 and 5-25 mg/L, 78.5%; 25-50 mg/L, 90.5%; 50-250 mg/L, 100%. Sample volumes from 4 to 200 ml were extracted using the solid-phase extraction procedure during the course of this research with very few

problems. Occasionally, biomass from samples which were not centrifuged well enough would clog the columns.

In order to report the statistical quality of the data from the analytical procedures employed herein, 95% confidence intervals (CIs) were determined for each of the GC and HPLC regression curves. The CI estimate for a regression-predicted concentration value corresponding to a future area value, X_f is (McCuen, 1985);

$$CI = +/- \; t_{\alpha/2} \, S_e \sqrt{1 + \frac{1}{n} + \frac{(X_f - X_m)^2}{\Sigma(X_i - X_m)^2}}$$

where $t_{\alpha/2}$ is the value of a random variable having a t distribution with (n-2) degrees of freedom with a level of significance α. Here, "n" is the degrees of freedom, which is the number of points used to make the regression line. The term "X_f" is the future value of the predictor variable (area). The term "X_i" represents each of the area values used to make the regression line. The term "X_m" is the mean value of all the areas used to make the standard curve. The term "S_e" is the standard error of estimate (standard deviation of regression line errors):

$$S_e = \sqrt{\frac{1}{(n-2)} \; \Sigma(Y_{i_{est}} - Y_i)}$$

where $Y_{i_{est}}$ represents the 1NA concentration values predicted (estimated) by the regression curve for each area used to make the regression line. The term Y_i represents the actual 1NA-standard concentrations for each area.

Each regression line has a unique value of Se, and the value of the CI is unique for each future value of area (predictor variable). Thus, a small spreadsheet was set-up (see Figure 5.4) to calculate 1NA concentrations and CIs for any area value determined from either GC or HPLC. To use the spreadsheet shown in Figure 5.4, one must enter the GC or HPLC area in the correct range (row) along with the initial and final volumes from the extraction procedure. The appropriate values of 1NA concentration (in mg/L) and 95% CI will then be calculated. The values of recovery efficiency are fixed.

GC

Range of areas	Rec. Eff.	Area	Extraction Vi	Extraction Vf	Conc. (mg/L)	C.I. (+/-)
5,375 to 34,200	100	21314	10	1.90	32.88	0.89
1,664 to 5,375	90.5	4000	4	2.10	24.01	0.27
1,009 to 1,664	78.5	1550	5	2.15	11.82	0.21
177 to 1,009	78.5	600	10	1.95	0.67	0.01

HPLC

Range of areas	Rec. Eff.	Area	Extraction Vi	Extraction Vf	Conc. (mg/L)	C.I. (+/-)
1,877 to 60,523	78.5	21314	100	2.00	0.01	0.00
60,523 to 6,513,821	78.5	187721	4	2.05	1.26	0.28
6,513,821 to 16,277,184	90.5	6675181	10	1.90	3.79	0.30
16,277,184 to 156,562,160	100	126297824	4	1.95	195.20	0.82

Figure 5.4 Concentration/confidence interval spreadsheet.

The accuracy of the methods used to measure biomass concentration (MLVSS) and organic content (COD) were are also examined. The procedures used were 2540E and 5220C from Standard Methods (1989) for MLVSS and COD, respectively (as reported in Section 3.1). To determine the accuracy of

the MLVSS test, several samples were taken and either concentrated or diluted in order to encompass the whole range of values encountered in this research. Then the MLVSS of triplicate samples for each concentration were determined. In Figure 5.5 the different MLVSS values are plotted versus the average value (which is denoted the actual value) to give an idea of the type

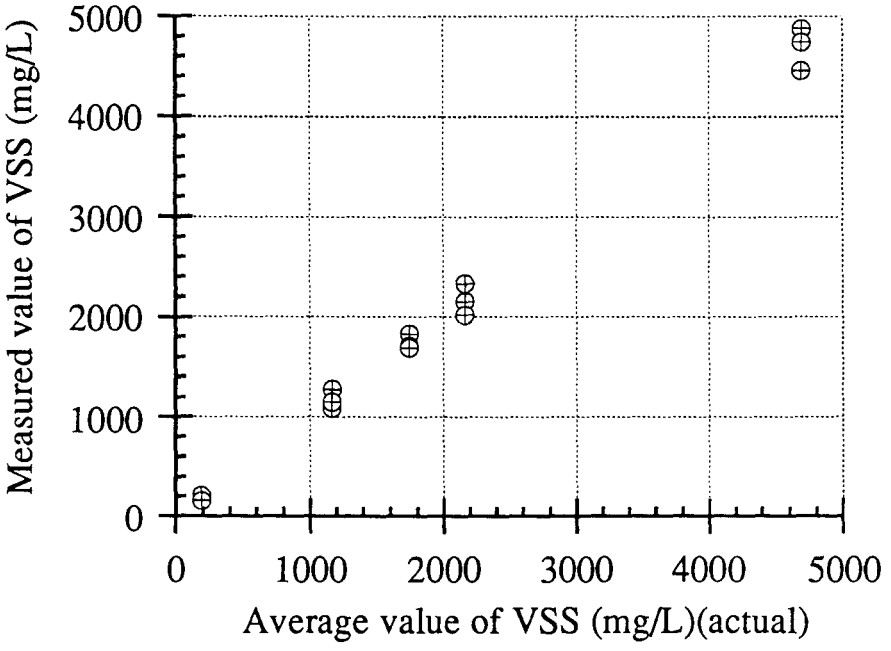

Figure 5.5 Accuracy of MLVSS test, average of replicates versus concentration of replicates.

of accuracy that was achieved. The standard deviations (approximately 68% confidence intervals) of the replicates were calculated and plotted versus the average value in Figure 5.6. In Figure 5.6, the solid lines indicate percentages of the mean value. For example, at the highest average concentration (approximately 4700 mg/L), the standard deviation is slightly less than 220 mg/L, which is less than 5% of the mean value. At the lower concentrations,

the standard deviations are smaller, yet they are larger compared to the mean value (nearly 20%). This indicates that it was the lower concentrations which were the least accurate.

The same procedure was used to determine the accuracy of the COD test. The COD of triplicates of three samples with different organic carbon contents were determined. A plot of standard deviation of replicates versus average COD value is shown in Figure 5.7. Figure 5.7 shows the same trend as for the MLVSS test, with the lower concentrations giving higher errors. In both tests, the errors are reasonable and show that the methods are quite repeatable.

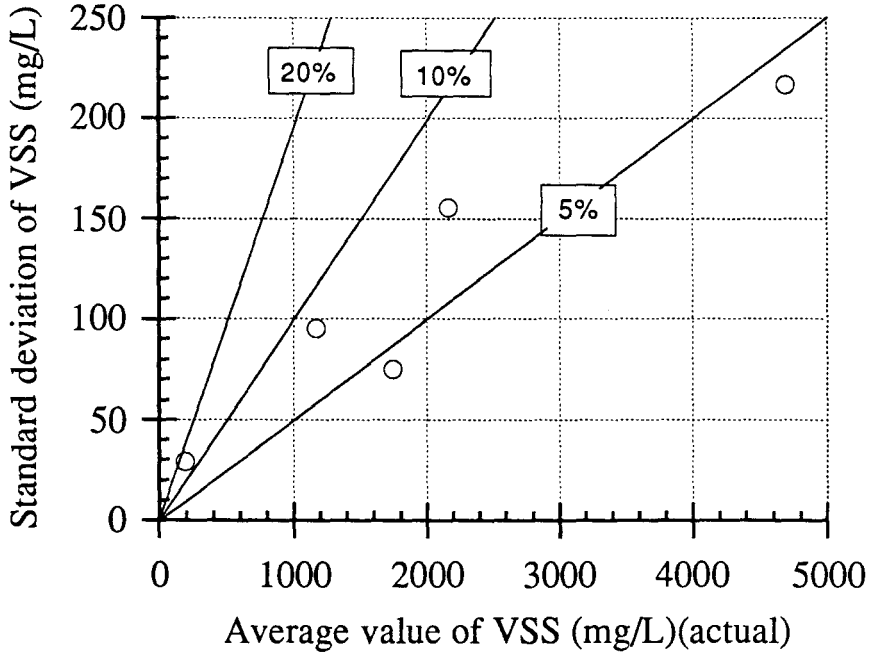

Figure 5.6 Accuracy of MLVSS test, standard deviation of replicates versus average of replicates.

There is much concern over the repeatability of any scientific experiment, and in particular for biological experiments where there are living organisms and a large number of variables concerned. In general, the problem can be addressed by employment of proper controls which isolate certain variables (removal mechanisms) and allow their separation and quantification. In most cases however, it is impossible to set up enough controls to look at all of the variables separately. Often, there are accepted

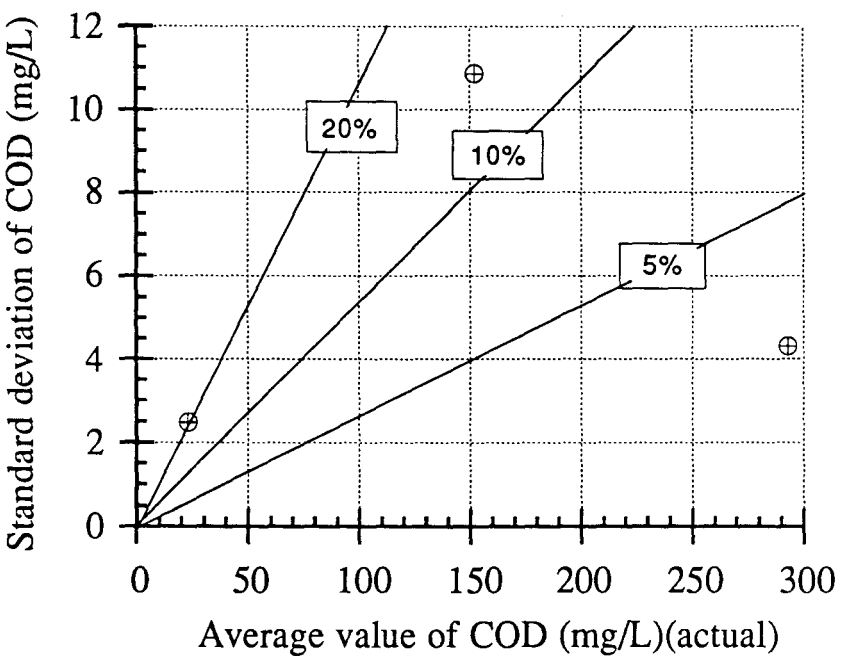

Figure 5.7 Accuracy of the COD test, standard deviation of replicates versus average value of replicates.

minimum controls which must the used to make the results defendable. For the work described herein, the minimum controls needed are those required to determine losses due to non-biological mechanisms (volatilization and

adsorption). In all of the work described here, these losses have been addressed using controls.

In a biological system like AS there is a fair amount of variability, which can be a problem. While most of the experiments reported herein have been repeated, it should be noted that exactly the same results are never found between any two experiments, or samples, or measurements. In general, batch-aliquot experiments were very reproducible. In these experiments, duplicates or triplicates were usually used and the plotted data represents the average value and the standard deviation of replicates was used for error bars. Continuous-flow experiments, by the nature of the process and the experimental set-up, are not as easily duplicated (each CFSTR is essentially a different culture with slightly different characteristics). However, the same relationships or trends were preserved in replicate CFSTR experiments (i.e. repetition of a spike experiment). It is felt that the results presented herein are representative of the performance of the experimental system tested, and additionally that they are repeatable.

A good example of the type of variability which is to be expected in the operation of biological reactors over a long period of time is the variation of effluent COD values. Figure 5.8 shows bi-weekly measured values of effluent COD from the CFSTRs for the first five months of 1991. Figure 5.8 shows that there is considerable variability in treatment efficiency from day to day and week to week even during so-called "steady-state" operation. Just like in full-scale AS systems, this data is indicative of normal operation.

5.7 References

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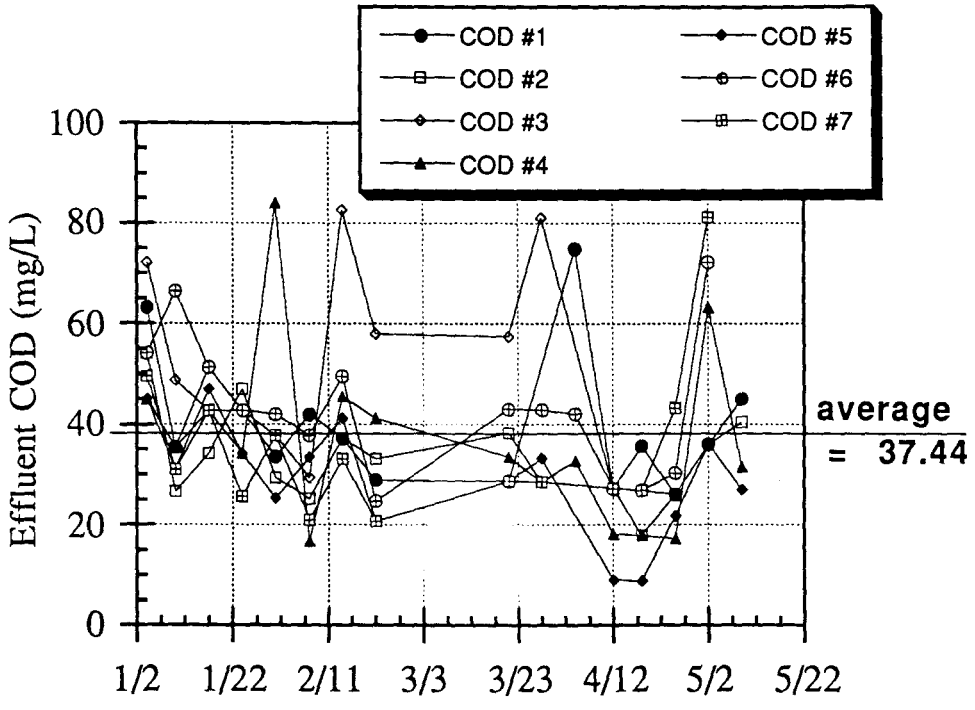

Figure 5.8 Effluent COD concentrations for the first part of 1991.