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DEMONSTRATION OF BIOAUGMENTATION IN A FLUIDIZED-BED PROCESS TREATING 1-NAPHTHYLAMINE

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Abstract—The feasibility of using a fluidized-bed reactor with bioaugmentation to treat low-strength wastewater or slightly contaminated groundwater was evaluated. Tap water spiked with 1.6 to 12.6 mg/L of 1-naphthylamine was treated in a fluidized-bed reactor with a hydraulic retention time of 6.2 to 13.3 h. With conventional operation, the reactor was unable to maintain consistent removal because of cell washout. Continuous removal efficiency was greater than 90% using bioaugmentation through periodic addition of acclimated cells from an off-line enricher-reactor. The concept demonstrated by this research presents an alternative for biological treatment of wastes (dilute wastewaters and contaminated groundwater) which are not normally considered good candidates for biological treatment. © 1997 Elsevier Science Ltd

Key words—bioaugmentation, enricher-reactor, wastewater, groundwater, naphthylamine, biological, fluidized bed

INTRODUCTION

Waters contaminated with low concentrations of hazardous organic compounds, such as contaminated groundwater, pose special treatment problems because a relatively large volume of water must be treated in order to remove a small mass of contaminants. Activated carbon adsorption has been successfully used on-site to remove adsorbable contaminants, but is expensive for pollutants that are poorly adsorbed. Additionally, if the contaminants are hazardous, the spent activated carbon is a hazardous by-product, which presents a disposal problem. Biological treatment processes which may destroy the hazardous compounds and produce the fewest byproducts are favored.

Conventional biological processes (activated sludge, trickling filters) can destroy a large fraction of biodegradable organic compounds found in wastewaters, and can remove additional materials by biosorption. Biological treatment costs can be as little as 5% of the cost of carbon adsorption (Nyer, 1985); however, many hazardous compounds are poorly removed in conventional biological processes due to toxicity, recalcitrance, or inhibition. Furthermore, conventional biological processes are not well-suited to treatment of waters with low concentrations of contaminants, because there is insufficient substrate and nutrients to support a viable biomass. For example, the activated sludge process requires high concentrations of carbon sources (generally greater than 50 mg/L BOD) to create a flocculent biomass that can be maintained in the reactor and recycled from the secondary clarifier. The wastewater must also contain sufficient nutrients, both macro and trace, to support biological growth.

Bioaugmentation is one method of maintaining sufficient biomass when adequate carbon substrates and nutrients are unavailable. Using purchased cells is one method of bioaugmentation; however, Babcock, *et al.* (1992) in their review could not find evidence of successful operation in experiments using purchased cells and well-defined controls. This research follows previous research (Cardinal and Stenstrom, 1991) using suspended cultures, where an off-line reactor, called an enricher-reactor, is used on-site to grow cells specifically prepared for use in the main reactor. The enricher-reactor operates separately from the main reactor, and conditions that are quite different from the main reactor can be maintained. The substrates being fed to the enricher-reactor can be the same as in the main reactor (i.e. target compounds) or other compounds designed to induce the desired operation in the

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enricher (Babcock and Stenstrom, 1993). The biomass is produced within the enricher-reactor where optimum growth conditions are maintained and growth-supporting substrates are added. Cells produced in the enricher-reactor are viable in the main reactor for several generations, which produces elevated biomass concentration. The acclimated cells can be either continuously or intermittently transferred to the main reactor, and an adequate or elevated biomass can be maintained. An elevated biomass can be maintained to increase treatment efficiency, or less frequent augmentation can be used to prevent washout or maintain the desired organism condition, such as acclimation to a specific substrate, wastewater or contaminated groundwater.

This manuscript describes the use of the enricher-reactor concept to bioaugment a fluidized-bed contact-reactor treating a synthetic groundwater containing a toxic compound. The fluidized-bed reactor was operated in such a way as to create conditions where bioaugmentation might improve operation. Our objective was not to conclusively demonstrate that a fluidized-bed reactor would not function in this application without bioaugmentation, but only to show that bioaugmentation could be used to promote biological treatment to applications where conventional operation might be difficult or unfavorable. We believe this research will help extend bioaugmentation concepts to fixed-film processes.

The first part of the manuscript describes a series of single inoculation experiments and the subsequent cell washout. The second part describes continuous inoculation experiments, which maintained stable operation. A basic mathematical model was developed to help describe reactor operation.

MATERIALS AND METHODS

Synthetic wastewater

Tap water spiked with a model hazardous compound, 1-naphthylamine (1-NA CAS No. 134-32-7), was used to simulate contaminated groundwater. This synthetic groundwater was used as fluidized-bed reactor influent in all experiments. 1-NA 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 1-NA is a possible azo dye degradation product (Shaul *et al.*, 1985). 1-NA is a suspected bladder carcinogen (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 1-NA found it to be generally resistant to biodegradation (Pitter, 1976; Malaney, 1960), inhibitory to nitrification (Hockenbury and Grady, 1977), and mutagenic (McCann *et al.*, 1975). It has a log octanol/water partition coefficient of 2.22 and a maximum solubility of 1700 mg/L (Verschueren, 1983). Its Henry's Law coefficient has never been reported, which is probably because it is too low to be easily measured.

Reactors

A draft-tube fluidized-bed reactor (DTFB) was used as the main reactor (Tang and Fan, 1987). Figure 1 shows the experimental set-up. The DTFB reactor contained support particles (sand) for microorganism attachment. Air bubbles introduced from the bottom of the reactor rose through the draft tube providing mixing and oxygen transfer. Solids from the bottom were transported to the top by the air bubbles which created an internal circulation pattern.

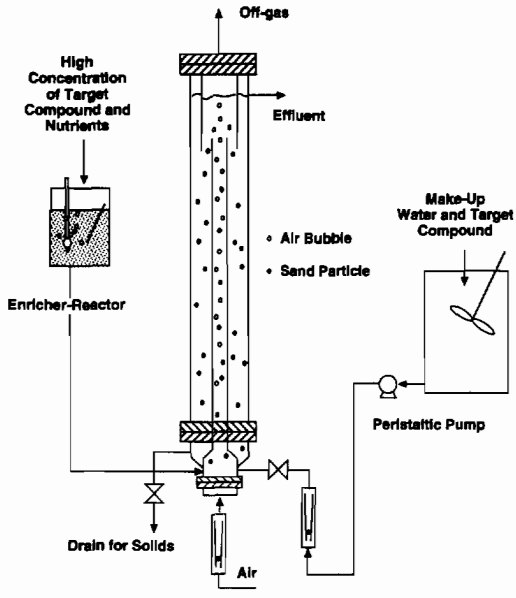

Fig. 1. Fluidized-bed draft-tube reactor with enricher reactor.

The 23-L DTFB reactor was fabricated from clear PVC pipe and consisted of a removable draft tube (5 × 91 cm) located concentrically inside a 15 × 122 cm tube. At the lower end of the draft tube, the inside diameter increased from 5 to 10 cm to trap air bubbles rising from an air diffuser into the draft tube. Clearance between the end of the draft tube and the bottom of the reactor was approximately 1.2 cm to allow unrestricted internal circulation of liquid and solids. At the top of the reactor, a 12.7 × 61 cm long Plexiglas tube was attached concentrically to a lid. This additional tube promoted quiescent conditions in the effluent (drain) region so that support particles would be retained in the reactor. Air at 4.7 L/min was introduced at the bottom of the reactor through a fine-pore plastic diffuser which provided a uniform distribution of air into the draft tube. The support medium was 2000 g of graded sand (US mesh 50/80, average diameter = 0.25 mm) with a density of 2.69 g/cm^3.

To determine the mixing characteristics of the DTFB reactor, a tracer study was performed using potassium chloride. Figure 2 shows the results. The smooth line is the theoretical washout for the actual reactor volume and flow rate. The slight shift in conductivity measurement represents

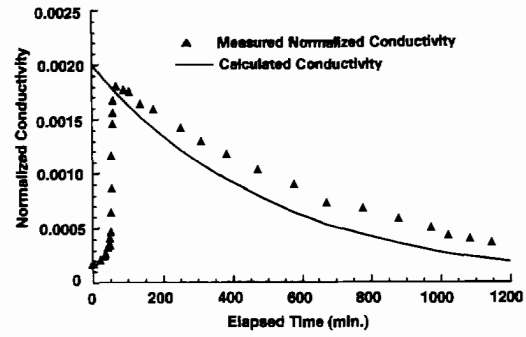

Fig. 2. Tracer experiment results.

Table 1. Enricher–reactor feed mixture

Component	Concentration (mg/L)
Bacto peptone	189
Beef extract	142
Salicylic acid	71
Succinic acid	71
Pyruvic acid	71
Yeast extract	52
K_2HPO_4	455
KH_2PO_4	723
$(NH_4)_2SO_4$	182
$CaCl_2 \cdot 2H_2O$	10.5
$Mgl_2 \cdot 6H_2O$	16.4
$FeCl_3$	1.4
$McCl_2 \cdot 4H_2O$	3.4×10^{-1}
$ZnCl_2$	2.4×10^{-1}
$CuCl_2 \cdot 2H_2O$	1.5×10^{-1}
$CoCl_2 \cdot 6H_2O$	2.1×10^{-1}
$(NH_4)Mo_7O_{24} \cdot 4H_2O$	1.5×10^{-1}
Na_3 Citrate	13
$Na_2B_4O_7 \cdot 10H_2O$	8.9×10^{-2}
Pyrdoxamine dihydrochloride	2.7×10^{-3}
Nicotinic acid	1.8×10^{-3}
Thiamine hydrochloride	1.8×10^{-3}
D-pantothenate	9.1×10^{-4}
p-Aminobenzoic acid	7.3×10^{-4}
d-Biotin	1.8×10^{-4}
1-Naphthylamine	150

the time required for the tracer to travel through the reactor entrance and exit structure and tubing. The small delay is insignificant for this application since all reaction rates are very slow compared to this delay. Otherwise there is very good agreement between the tracer and the theoretical washout curve.

A 5 L rectangular Plexiglass enricher-reactor was operated as a sequencing-batch reactor (SBR) on a 24 h cycle with a 22 h aeration, 1 h settle, and 0.5 h drain and fill times (Babcock *et al.*, 1992). During the fill cycle, concentrated substrate was diluted with tap water to create the mixture shown in Table 1. At the end of the daily 22 h aeration cycle, cells were removed and washed with deionized water and transferred into the DTFB reactor. An air flow rate of 2.4 L/min through a ceramic fine-pore diffuser maintained the DO concentration in the enricher-reactor between 1–3 mg/L. The pH was monitored and maintained between 6 and 7. Mixed-liquor solids varied between 1500 and 4812 mg/L-MLVSS over the course of all experiments. Mixed-liquor solids were wasted daily to provide a mean cell retention time (MCRT) of 7 days.

The original seed culture was 50% municipal activated sludge and 50% petroleum refinery wastewater activated sludge. Nine months were required before the culture began to degrade significant quantities of 1-NA. After acclimation the culture was able to degrade 150 mg/L of 1-NA to non-detectable concentrations in 22 h. Depletion was shown to be due to mineralization conversion to CO_2 (Babcock *et al.*, 1993).

Analytical protocols

Two ml influent samples were filtered in series through a 0.5 μm and then a 0.2 μm HPLC syringe filter (Corning) into an amber 4 mL serum vial. Effluent samples (10 mL) were passed through a C-18 bonded silica sorbent column (Analytichem) and then 1-NA was eluted with 1.0 mL methanol. Fifty μL samples, were injected into a high performance liquid chromatograph (Varian 5020) equipped with a reverse phase C-18 column (10 μ Adsorbosphere, Alltech) and a variable UV–VIS detector operated at 254 nm wavelength. The mobile phase was 40% water, 30% methanol, and 30% acetonitrile and the flowrate was 1.0 mL/min which yielded a 1-NA retention time of 4.5 min. The detection limit using this protocol was approximately 0.05 mg/L.

Reactor operation

Tap water spiked with 1-NA was used to simulate contaminated groundwater. Over the course of the experimental program this influent, ranging in 1-NA concentration from 1.6 to 12.6 mg/L, was pumped into the DTFB reactor, at rates ranging from 28.9 to 61.8 mL/min. Different quantities of acclimated culture (1.2 to 13.2 g VSS), removed from the enricher–reactor and washed with deionized water, were transferred into the DTFB reactor at the beginning of each experiment (single inoculation) or every three days (multiple inoculations). Influent and effluent 1-NA concentrations were monitored daily. Between single inoculation experiments, the DTFB reactor was cleaned with chlorine bleach to remove residual biomass from previous experiments. Frequent analysis of 1-NA and solids concentrations in the enricher-reactor were performed to ensure optimal operating conditions.

Abiotic loss of 1-NA

Experiments were performed to estimate the 1-NA removed by air stripping and physical adsorption to the sand particles. A 15 cm diameter × 91 cm tall PVC column without a draft tube was used to determine the rate of 1-NA stripping. Air was introduced through a spherical fine-bubble diffuser placed at the bottom of the column containing 19 L of tap water and various initial concentrations of 1-NA. The volumetric air flow rate was 3.9 L/min, which yielded the same air flow rate per unit volume used in the DTFB reactor. Samples were taken after 24 h to determine the rate of stripping. Three samples were withdrawn from three locations across the diameter of the column at 30 cm submergence. The percentage of 1-NA stripped from the column was calculated from a mass balance of 1-NA in the liquid phase. Figure 3 (top) shows the loss of 1-NA due to stripping at various initial 1-NA concentrations. Stripping losses of 1-NA are quite small and are within the range of experimental error, as shown by the high standard deviation of the results.

In the adsorption experiment, triplicate samples of 60 mL tap water, spiked with the same influent 1-NA concentrations used in the DTFB reactor, were placed into 60 mL glass serum vials. The vials contained 5.21 g of sand and

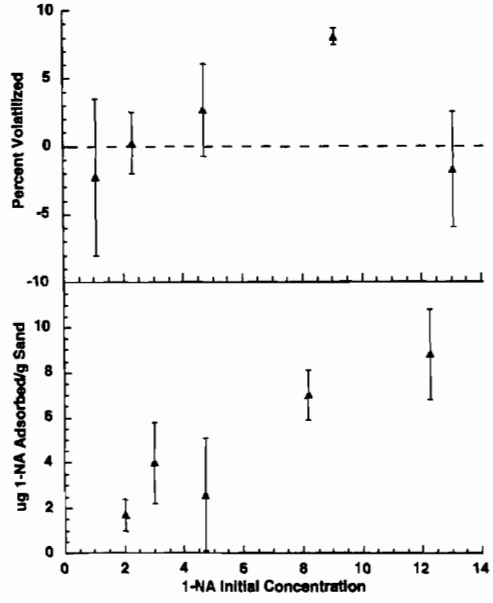

Fig. 3. Abiotic losses of 1-NA: Stripping (top) and adsorption to sand (bottom). Error bars represent the range of the data.

Table 2. Experimental conditions for single inoculumn experiments

Experiment	HRT (h)	Influent concentration (mg/L)	Biomass added (g VSS)	Initial F/M (1/d)	Time to breakthrough (h)*	m (1/d)
A	6.2	3.5	1.4	0.22	<1	0.26
B	6.4	4.4	1.2	0.32	<1	0.19
C	6.2	1.6	2.4	0.06	1.6	0.32
D	11.6	12.6	6.0	0.10	4	0.18
E	8.4	2.2	3.7	0.04	2.8	0.38
F	13.3	3.5	13.2	0.01	6	0.29

*Estimated from Fig. 4.

were sealed without head space. This ratio of 1-NA to sand was the same as used in the DTFB reactor. The vials were sealed with teflon-lined rubber septa and aluminum crimp tops. The vials were shaken vigorously for 1.0 min and then placed on an orbital shaker table at 200 rpm for 36 h. The vials were again shaken vigorously for 1.0 min and opened to measure equilibrium concentrations. The amount of 1-NA adsorbed per unit mass of sand was calculated from a mass balance of 1-NA in the liquid phase.

Figure 3 (bottom) shows the isotherm, and suggests that the sand-adsorbed 1-NA, at the effluent concentration observed in the experiments, would be approximately 1 to 2 mg. This small mass is negligible compared to the influent 1-NA mass; which ranged from approximately 900 to 3600 mg for a 6 day experiment.

Previous work (Babcock, and Stenstrom, 1993) showed that 1-NA will adsorb to acclimated cells at about 1 to 2 μg/mg at liquid phase 1-NA concentrations ranging from 15 to 20 mg/L, and that it will biodegrade after the liquid-phase 1-NA concentration is exhausted. Therefore, cell-adsorbed 1-NA is also small and is not considered a significant abiotic loss.

RESULTS AND DISCUSSION

Single inoculation experiment

A series of seven single inoculation experiments were performed to determine the ability of the enricher–reactor culture to degrade 1-NA within the DTFB reactor. Experimental conditions and removal efficiencies are shown in Table 2 and Figure 4, respectively. Cells introduced into the DTFB reactor removed more than 90% of the influent 1-NA for a period of 3 to 7 days, after which the removal efficiency rapidly declined, suggesting that the cells decayed or washed out of the reactor. The value of 90% removal efficiency was selected arbitrarily, but is a reasonable goal for biological treatment and the purpose of this research. The term 'breakthrough' will be used to describe the time when removal decreases to less than 90%. Longer hydraulic retention times and larger inoculums increased the time to breakthrough. The results of experiments C, D, E, and F show that operation with longer hydraulic retention times extended the time to breakthrough, regardless of the dosage of acclimated culture or the influent concentration. This suggests that cell washout is an important mechanism in the loss of treatment. In the DTFB reactor cell washout is related to hydraulic retention and upward liquid velocity. None of the combinations of influent 1-NA concentration, hydraulic retention time, or inoculum mass produced a sustainable biomass in the DTFB reactor.

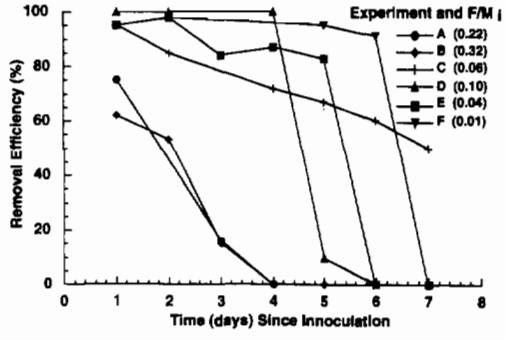

Fig. 4. Single inoculation experimental results.

A modified food-to-microorganism ratio (F/M), initial F/M, is proposed to quantify the combined effect of the different operating conditions. Initial F/M is similar to conventional F/M, except that steady-state microorganism mass is replaced by the initial inoculum mass of added to the DTFB reactor, as follows:

$$\text{Initial F/M} = (\text{F/M})_i = \frac{C_o Q}{M_{ac}} \qquad (1)$$

where

$(F/M)_i$ = applied food to microorganism ratio, d^{-1}
M_{ac} = inoculum mass (enricher-reactor cells), g
C_o = influent 1-NA concentration, g/m^3
Q = influent flow rate, m^3/d

Figure 4 shows that the time-to-breakthrough is greater at lower $(F/M)_i$. In the experiments with the highest $(F/M)_i$, (A and B, 0.22 and 0.32 $days^{-1}$, respectively) breakthrough occurred after less than 1 day. In experiments C, D, and E, with $(F/M)_i$ ranging from 0.04 to 0.10, breakthrough occurred after 1 to 4 days. In the last experiment, F, with an $(F/M)_i$ of 0.01 day^{-1}, breakthrough did not occur for 6 days. The F/M concept will be used later to model and interpret experimental results.

Biomass washout

The single inoculation experiments showed that the biomass washed out of the reactor and did not establish itself. This was expected due to the inhibitory nature of 1-NA as well as the difficulty in growing biomass on sand particles. It was not possible to model the washout and decay separately,

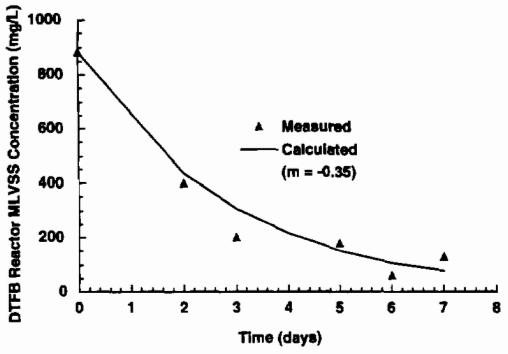

Fig. 5. DTFB reactor MLVSS concentration versus time.

because of the complex interactions of the biomass with the sand, as well as organism growth.

To show the interaction of the sand particles, a single experiment was performed without sand (not reported in Table 2). The experiment was performed at an $(F/M)_i$ of 0.047 day^{-1} (5.4 g inoculum, 7.5 h HRT, 3.5 mg/L influent 1-NA concentration). The removal efficiency was almost negligible after one day and the inoculum was completely washed out after 30 h. For similar conditions with sand particles, the time-to-breakthrough was at least four days, as shown in Fig. 4. From this information it was concluded that the sand particles assist in retaining biomass in the reactor, presumably by microbial attachment, although other interactions cannot be ruled out.

The biomass washout and decay, in the presence of sand particles, was modeled as first-order with respect to biomass concentration, as follows:

$$X = X_o e^{(-mt)} \tag{2}$$

where

X = inoculum concentration at time t, g VSS/m^3
X_o = initial inoculum concentration, g VSS/m^3 M_{ac} divided by the reactor volume
m = inoculum washout and decay coefficient, d^{-1}
t = time, d

The value of m was determined by linearizing equation 2 and determining the slope of the resulting line. Figure 5 shows cell washout and is typical of all results. Table 2 also shows the value of m for all experiments.

Model development

To characterize the reactor performance the washout rate described above can be combined with the reaction rate for 1-NA biodegradation in a mass balance. Babcock *et al.* (1993), have shown that the removal rate of 1-NA at high concentrations (>50 mg/L) is zero-order with respect to 1-NA. The reaction rate decreases at lower concentrations and can be considered first-order with respect to 1-NA. For all conditions, the reaction is first-order with respect to biomass, which makes the overall reaction rate second-order at low 1-NA concentration. The rate expression is shown in equation 3, as follows:

$$-r_{(1-NA)} = kCX \tag{3}$$

where

$-r_{(1-NA)}$ = degradation rate, g/m^3 d
k = second-order rate coefficient, m^3/g VSS d
C = 1-NA concentration, g/m^3
X = inoculum concentration, g VSS/m^3

A mass balance on 1-NA for the DTFB reactor can be written using equations 2 and 3. After substituting equation 1 to remove the X_o term, the following equation is obtained

$$\frac{dC}{dt} = \frac{C_o - C}{\theta} + \frac{KC_o e^{-mt} C}{\theta (F/M)_i} = 0 \tag{4}$$

where

θ = hydraulic retention time, d

Equation 4 was solved with a FORTRAN code using a fourth-order Runge-Kutta integration method. Figure 6 shows the model results and the experimental data (also shown in Fig. 4). The results were calculated using a second-order rate coefficient, k, of 0.1 m^3/g VSS d, which is the best fit to minimize the sum of squares error for all six experiments. An internal search was used to find the best value of k. This rate coefficient is in the range observed earlier for other 1-NA experiments (Babcock *et al.*, 1992, 1993). The model fits the data relatively well for the early parts of the experiments, but cannot explain the rapid washout observed in experiments D, E and F. The rapid loss of biomass may be due to nutrient exhaustion or increase in 1-NA concentration to an inhibitory level. The poor fit in experiments A and B may be due to the very small inoculum. The added biomass may not have been able to reduce the 1-NA to non-inhibitory concentration.

Multiple inoculation experiments

For the multiple inoculation experiment a hy-

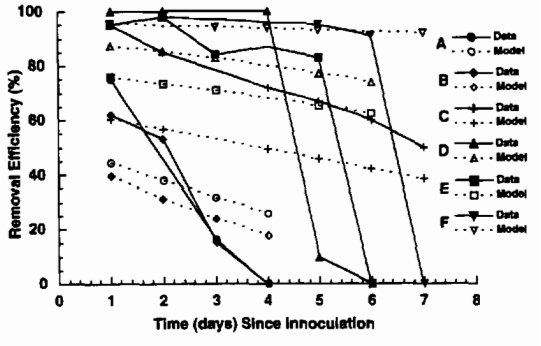

Fig. 6. Removal efficiency of single inoculation experiments (model, open symbols, dotted lines; data, closed symbols, straight lines).

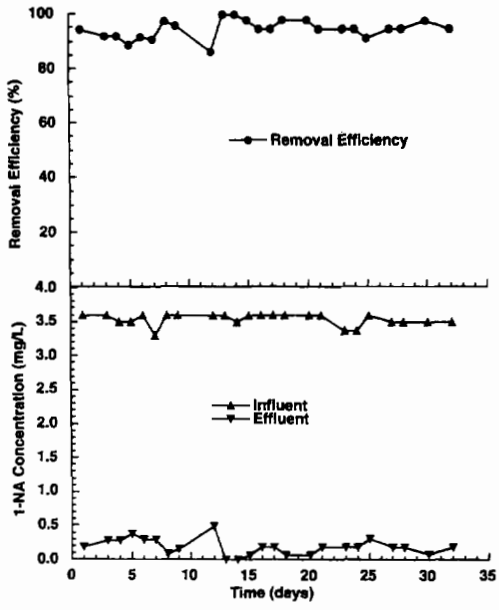

Fig. 7. Multiple innoculation experimental results.

draulic retention time of 7.5 h, average influent 1-NA concentration of 3.5 mg/L, and inoculum mass of 2.3 to 3.0 g, were selected. This produced an average $(F/M)_i$ of 0.11 d^{-1}. The time-to-breakthrough for a single inoculation experiment for similar conditions would range from 2.5 to 9 days, depending on the rate of cell washout. The experiment was continued for 32 days. A conservative frequency of every 3 days was selected for inoculations.

Treatment efficiency for most of the 32 days of continuous operation was in excess of 90%, as shown in Fig. 7 (top). Figure 7 (bottom) shows the influent and effluent 1-NA concentrations. Microscopic examination of sand particles removed from the DTFB reactor during this experiment showed only a slight biofilm accumulation. This was expected since the single inoculation experiments failed to establish a stable biomass in the DTFB reactor.

CONCLUSIONS

Treatability of tap water contaminated with low concentrations (1.6 to 12.6 mg/L) of a model hazardous compound (1-naphthylamine, 1-NA) using bioaugmentation in a fluidized-bed, draft tube biological reactor was demonstrated. Initial experiments showed that the reactor was unable to maintain treatment efficiency without bioaugmentation, due to an inability to retain and grow biomass. The removal efficiency after inoculation from a sequencing-batch reactor was related to the influent concentration, mass of inoculum, and hydraulic retention time, which were all characterized by calculating the initial, food-to-microorganism ratio, $(F/M)_i$. Continuous operation with 90% or greater removal efficiency at an $(F/M)_i$ of 0.11 d^{-1} was achieved by continually inoculating the fluidized-bed reactor every 3 days with 2.3 to 3.0 g of cells. The three day period was arbitrarily selected; lesser frequency might also have been satisfactory.

This study has demonstrated one method of biologically treating low-strength wastewaters and contaminated groundwaters which have heretofore been considered poor candidates for biological treatment. The use of an off-line enricher–reactor to continually inoculate the continuous flow reactor overcomes the difficulty of growing adequate biomass on inhibitory or toxic substrates, or waste with too little organic carbon and nutrients to sustain a viable biomass, or other conditions that may not be conclusive to treatment. The experiments have demonstrated that bioaugmentation is possible for improving treatment. Other benefits of bioaugmentation, such as improved removal and reactor stability may be obtained, but have not been demonstrated in this research.

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Authors. Kyoung Sin Ro was a postdoctoral fellow in the Civil Engineering Department at the University of California, Los Angeles at the time of the study. He is presently an assistant professor in the Civil Engineering Department at Louisiana State University. Roger W. Babcock, Jr. was a postdoctoral fellow at UCLA and is now an assistant professor in the Civil Engineering Department at the University of Hawaii, Honolulu, HI. Michael K. Stenstrom is a professor in the Civil and Environmental Engineering Department at the University of California, Los Angeles. Correspondence should be sent to Kyoung S. Ro, Department of Civil Engineering, LSU, Baton Rouge, LA 70803-6405.

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