Modeling the Performance of Hazardous Wastes Removal in Bioaugmented Activated Sludge Processes

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ABSTRACT: An enricher-reactor process in which acclimated biomass is grown in an offline reactor on high concentrations of enrichment substrates was used to bioaugment a conventional activated sludge process to a toxic compound, 1-amino naphthalene. Various levels of bioaugmentation, ranging from 1 to 16% mass ratio of augmented cells to indigenous cells, were evaluated in laboratory-scale reactors. The experimental results showed that bioaugmentation can enhance toxic compound removal, increase resistance to shock loading, and reduce the time required for acclimation to the toxic compound. A process model was developed and calibrated using the experimental data. This model was then used to compare the process to an in-situ bioaugmentation process using a reaeration reactor that receives a portion of the recycle activated sludge. The model predicted experimentally observed removals of toxic compound and decreasing relative benefits of bioaugmentation at higher levels. The model suggests that augmented biomass suffers higher decay, which likely is due to the effects of its removal from a substrate-rich to substrate-poor environment. The model shows that the enricher-reactor-process has advantages at lower mean cell retention time (MCRT), and the in-situ process is superior at higher MCRT. Both processes can remove the toxic compound when operating below the washout MCRT that would occur in an unaugmented activated sludge process. *Water Environ. Res.*, **81,** 2309 (2009).

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

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Introduction

Bioaugmenation is a method to improve removal of nutrients or hazardous wastes in activated sludge and fixed-film processes (Babcock et al., 1993; Parker and Wanner, 2007; Ro, et al., 1997). Bioaugmentation processes can be classified into three main methods: (1) adding enriched cultures from ''offsite'' sources; (2) adding cells produced from an ''onsite'' reactor, and (3) growing additional biomass in-situ with ordinary biomass using special reactor geometry. The first type of bioaugmentation, offsite or external, is challenging because enriched cultures can lose activity when introduced into the process or may require acclimation to new process conditions (i.e., different mean cell retention time (MCRT), dissolved oxygen, low substrate supply, or predation). Also, the required cell mass for effective bioaugmentation may be too high to acquire cost-effectively from an offsite facility. Babcock et al. (1993) reviewed the difficulties of offsite bioaugmentation for hazardous waste. Rittmann et al. (1996) and Lee (1997) simulated the high biomass requirements for bioaugmented nitrification, and Head and Oleszkiewicz (2004) demonstrated the requirements in laboratory-scale experiments. This research considers onsite bioaugmentation and in-situ methods because offsite bioaugmentation appears impracticable.

The benefit of the onsite strategy, such as the enricher-reactor, is that it can grow enriched cultures at the treatment plant, where they can be quickly produced and supplied intermittently or continuously, as needed. The enricher-reactor process has been described previously (Babcock et al., 1992; Babcock and Stenstrom, 1993; Stenstrom et al., 1989; Cardinal and Stenstrom, 1992;). Figure 1(a) shows the proposed process configuration whereby existing activated sludge systems can be upgraded to treat hazardous wastes that are introduced to the wastewater or to accept additional wastes that would have been unacceptable without bioaugmentation. The enricher-reactor process allows the existing activated sludge infrastructure to be used in the increased capacity of efficient treatment of hazardous wastewaters with reduced risk of impairing normal operation

Figure 1(b) shows an example of the online in-situ bioaugmenation method (also called Enricher Reactor with Return Activated Sludge or ER-RAS approach). This process originally was developed to improve nutrient removal and has been applied successfully in full-scale wastewater treatment plants (WWTPs) to improve nitrification (Bogusch, 1987; Krhutkova et al., 2005). In this method, a culture that is capable of degrading the target compounds is grown in a reactor that becomes an integral part of the activated sludge process through addition of recycled activated sludge. The recycled sludge reactor can be fed with high-strength wastewater (for example, digester supernatant high in ammonia concentration), and reintroduced into the main reactor. The loss in bacteria activity using this method is considered low, because the environment is similar to the main process (Parker and Wanner, 2007).

The enricher-reactor process culture can be maintained on either high concentrations of the hazardous target compound or using structurally similar or suspected degradation metabolite compounds (Babcock et al., 1992; Babcock and Stenstrom, 1993). Advantages of not using the target compound include greater ability to tolerate shock loads without breakthrough to the effluent

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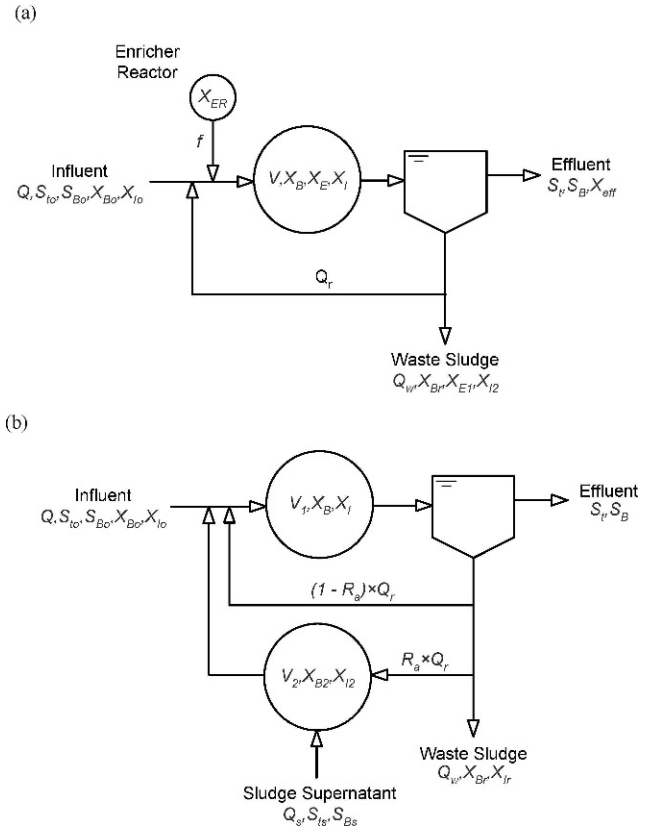

Figure 1—Concept schematic of two bioaugmenation processes: (a) off-line, onsite process using enricher reactor; and (b) online, in-situ process with reaeration.

and the ability to degrade structurally similar compounds without prior acclimation. Improvement in steady-state removal efficiency also occurs, but this benefit is less dramatic.

There were two goals for the modeling work to better understand the mechanisms involved and the relationships between observation and theory. The first goal was to examine whether kinetic simulations could predict accurately the experimentally observed dynamic response of complete-mix activated-sludge (CMAS) processes during shock loading and reacclimation periods. The CMAS processes are applications of continuous-flow stirred-tank reactors (CFSTR) with biosolids recycle. Degradation kinetics of bioaugmentation cultures following inoculation into CMAS are difficult to measure directly. A simulation model, however, can explain the underlying kinetic behavior. The second goal was to compare the benefits that might be obtained using the enricher-reactor and in-situ processes. Based upon the validated model, 1-NA degradation was simulated in an in-situ reaeration method.

To achieve these goals with a manageable simulation model, we assumed the existence of three populations of organisms: (1) indigenous cells that can degrade the target compound; (2) exogenous cells from the enricher-reactor that have been acclimated to degrade the target compound; and (3) non-1-NA degrading cells that grow only on rapidly degradable chemical oxygen demand (COD) bulk substrate but not the target compound. The dynamic model incorporated mass balances for each group of microorganisms and each type of substrate. The response of bioaugmented CMAS to transient loadings of the hazardous target-compound (shock loads and changes in influent concentrations) were simulated using the dynamic model and compared to experimental results. The model was calibrated by minimizing the least square error between model and observed data from bench-scale experiments. The calibrated model was then used to investigate the dynamics of both the enricher-reactor and *in-situ* processes.

Experimental Methods

The hazardous waste used for the model consisted of a carcinogenic, amino-substituted polyaromatic hydrocarbon—1-naphthylamine (1-NA). This compound typically resists biodegradation and inhibits nitrifying organisms. It also is mutagenic in the Ames assay, associated with human bladder cancer, and regulated as a carcinogen by Occupational Safety and Health Administration (OSHA) (Federal Register, 1974; Hockenbury and Grady, 1977; McCann et al., 1975; Pitter, 1976; Scott, 1962). The 1-naphthylamine is an intermediate in the manufacture of approximately 150 organic azo-type coloring dyes and is probably a trace component of many petrochemical-product manufacturing waste streams (Colour Index, 2007; Staff, 1974). It was selected for study because of its industrial importance and the difficulty in eliminating it from use.

Previous sources have described in detail materials (chemicals and media), setup of activated sludge reactor (SBRs and CMASs), analytical methods, enrichment techniques, and bioaugmentation procedures (Babcock and Stenstrom, 1993; Babcock et al., 1992; Babcock et al., 1993). This paper describes conditions under which new experiments were conducted and results quantified to acquire the data collected for model calibration and validation.

Over nine months, an activated sludge enrichment culture was developed, which was then able to rapidly degrade 1-NA as a secondary substrate. Monod-type kinetic parameters of 1-NA degradation were determined, non-biological removal mechanisms were quantified, and mineralization (conversion of 1-NA to CO_2 when present as the sole carbon source) was demonstrated (Babcock et al., 1993). The enrichment culture was developed and maintained in sequencing-batch reactors (SBRs) where they were exposed to the target compound and selective pressures similar to the CMAS (i.e., ability to flocculate and settle well). The enrichment culture was used to bioaugment the main reactors by wasting cells from the SBRs to the main reactors.

The enricher reactors initially were seeded with an inoculum from a large municipal WWTP that also receives pretreated industrial wastewaters and an inoculum from a west-coast petroleum refinery activated sludge process. The reactors were operated for five months before they developed significant 1-NA removal. It was demonstrated that the enrichment culture could use 1-NA as a sole carbon source (Babcock et al., 1993). Figure 2 shows the laboratory setup of experiments. Five bioaugmented CMAS and two controls were operated continuously for 12 months (steady-state) prior to their use in these experiments. The unacclimated control was not exposed to 1-NA and did not receive bioaugmentation. The acclimated control was exposed continuously to 1-NA but received no bioaugmentation. The unacclimated CMAS represented the worst-case treatment system in which the least removal of 1-NA would be expected. The acclimated CMAS represented the best-case conventional system without bioaugmentation. The other CMAS received various daily

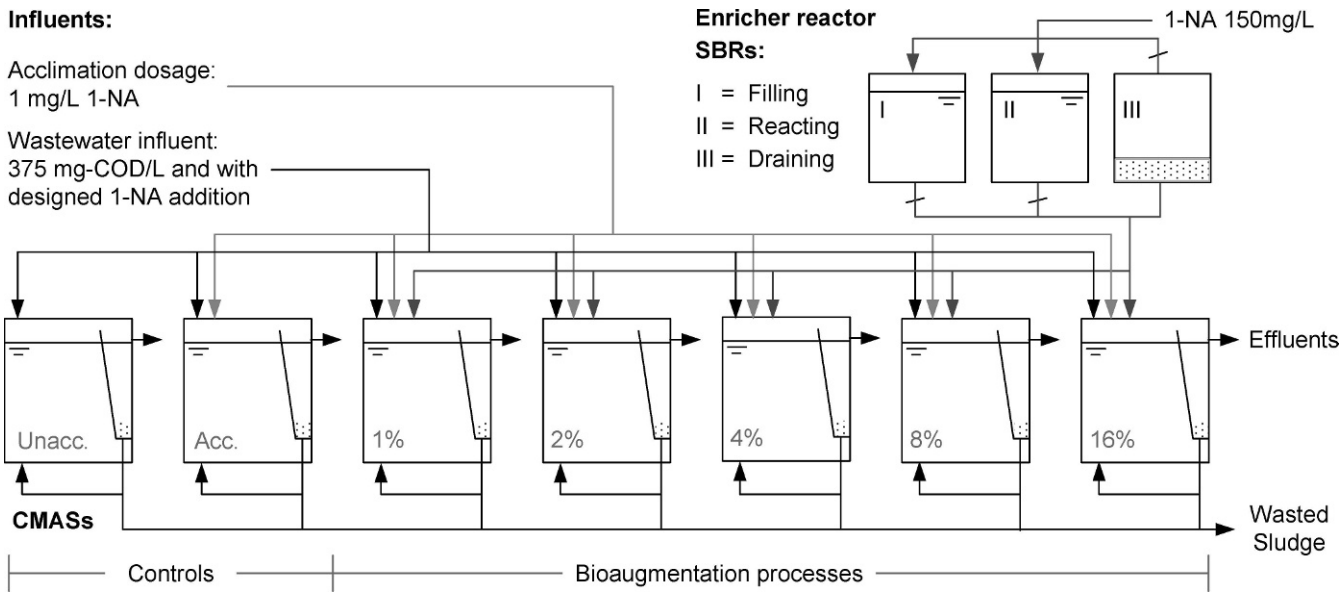

Figure 2—Laboratory setup of bioaugmentation experiments. Note: the bioaugmentation level was defined as percent mass exogenous biomass per unit mass of non-1-NA degrading biomass.

bioaugmentation doses. Bioaugmentation level was defined as the mass percent of exogenous biomass added per unit mass of non-1-NA degrading biomass (mg exogenous cells per mg mixed liquor volatile suspended solids [MLVSS]). Five different bioaugmentation levels were used: 1, 2, 4, 8, and 16%.

Table 1 lists common operating characteristics of the CMAS and SBRs. The nominal 8.9-day CMAS MCRT was controlled by daily removal of 1.37 L of mixed liquor. The required bioaugmentation inoculation was added to the aeration zone immediately following sludge wastage. Bioaugmentation inoculum 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 (1-NA) had been depleted below the detection limit. Cells used for inoculum were allowed to settle for 0.5 hours in graduated cylinders after which the supernatant was decanted and settled sludge added to the proper CMAS. Additional cells were removed as necessary to maintain the SBR at seven-day MCRT.

Table 1—Operational characteristics of laboratory reactors.

Parameter	Main reactor (CMAS)	Enricher-reactor (SBR)
Volume	12.2 L aeration zone 1.5 L clarifier	5.0 liter
HRT	0.458 d (Q = 26.64 L/d)	22 h aeration 1.0 h settle 0.5 h drain 0.5 h fill
MCRT	8.9 d	7 d
MLVSS	1588 to 3008 mg/L	2000–4000 mg/L (3415 average)
Loading Rate	810 mg COD/L·d	760 mg COD/L·d
1–NA Influent	1.0 mg/L	150 mg/L
Aeration Rate	7.1 L/min	2.4 L/min
DO	7.9 mg/L	1–4 mg/L
pH	6.5–7.5	6–7
Temperature	13–23°C	18–30°C

Two experimental regimes were developed to validate dynamic simulations: (1) shock load and (2) reacclimation to higher influent concentrations. In the shock-load experiments, sufficient mass of 1-NA was added to the aeration zone of each CMAS to raise the concentration of 1-NA to 15 mg/L. The reduction of 1-NA was recorded every two hours to calculate the rate of biodegradation. In the reacclimation experiments, CMAS were acclimated to 3 mg/L 1-NA until achieving steady state, and then the influent 1-NA concentration was decreased to 0 mg/L for 12 days or 1.3 MCRT. The influent 1-NA was then restored to 3 mg/L to observe the time to reclamation. Effluent concentration was monitored twice per day for the duration of the experiment.

Samples collected from aeration off-gas and treated effluent were analyzed by gas chromatography and high-performance liquid chromatography (HPLC). The detection limit for 1-NA in samples for data reported here was 0.01 mg/L for both gas chromatography and HPLC measurements (Babcock, et al. 1993). Bulk organic material (soluble substrate) was quantified as filtered chemical oxygen demand (COD) by Standard Method 5220B weekly (American Public Health Association [APHA], 1989). Biomass concentrations were measured as MLVSS by Standard Method 2540E twice per week (APHA, 1989). Triplicate measurements were obtained and mean values are presented.

Model Development

Model formulation typically used the unified approach of Lawrence and McCarty (1970) because the more complicated structure of advanced models, such as the structured biomass models or the ASMs, were not needed (Clifft and Andrews, 1981; Gujer et al., 1999; Henze et al., 1987). Two dynamic material balance equations were written for substrates: 1-NA (S_t) and the non-toxic bulk carbon source (bulk substrate, S). And three were written for cells: 1-NA degrading exogenous (X_E), 1-NA degrading indigenous (X_I), and non-1-NA degrading species (X). Four mechanisms facilitate removal of 1-NA: (1) volatilization/

stripping; (2) adsorption onto activated sludge; (3) biodegradation from the acclimated indigenous culture; and (4) biodegradation from exogenous culture supplied by the enricher-reactors. Adsorption was not considered an important mechanism because adsorbed substrate is metabolized quickly, and periodic solvent extraction of waste sludge samples never recovered adsorbed 1-NA. Previous work showed that biomass rapidly adsorbs 1-NA, but the sorbed 1-NA is degraded over time. It also showed that adsorbed 1-NA disappears before the bulk liquid 1-NA concentration decreases to the 1-NA detection limit. The five mass balances can be written as follows:

$$\frac{dS}{dt} = \frac{1}{\theta_H}\cdot(S_O - S) - r_{su} \quad (1)$$

$$\frac{dS_t}{dt} = \frac{1}{\theta_H}\cdot(S_{tO} - S_t) - K_L a\cdot S_t - r_{suE} - r_{suI} \quad (2)$$

$$\frac{dX}{dt} = \frac{1}{\theta_H}\cdot\left(X_O - \frac{X}{X+X_E}\cdot X_{eff}\right) - \frac{X}{\theta_X} + r_g \quad (3)$$

$$\frac{dX_E}{dt} = \frac{1}{\theta_H}\cdot\left(\frac{fX_{ER}}{Q} - \frac{X}{X+X_E}\cdot X_{eff}\right) - \frac{X_E}{\theta_X} + r_{gE} \quad (4)$$

$$\frac{dX_I}{dt} = \frac{1}{\theta_H}\cdot X_{IO} - \frac{X_I}{\theta_X} + r_{gI} \quad (5)$$

Where,

$K_L a$ = gas transfer coefficient, 1/h;
r_{suE} = substrate consumption rate of exogenous biomass, mg/L·h;
r_{suI} = substrate consumption rate of indigenous biomass, mg/L·h;
r_g = growth rate of bulk biomass, mg/L·h;
r_{gE} = growth rate of exogenous biomass, mg/L·h;
r_{gI} = growth rate of indigenous biomass, mg/L·h;
S = bulk substrate, mg/L;
S_t = target compound, mg/L;
S_{tO} = influent concentration of target substrate, mg/L;
X = concentration of non 1-NA degrading bio mass, mg/L;
X_{eff} = effluent suspended solids, mg/L;
X_E = 1-NA degrading exogenous biomass, mg/L;
X_O = influent concentration of bulk biomass, mg/L;
X_{IO} = influent concentration of 1-NA degrading indigenous biomass, mg/L;
θ_H = hydraulic retention time (HRT), d; and
θ_X = mean cell retention time (MCRT), d.

Figure 1(a) shows all of these variables and their relationship to the physical system. The effluent biomass, X_{eff}, which is sometimes neglected in ASP simulations, was needed in the mass balances because of significant loss of effluent suspended solids. In the CMAS, the suspended solids and process water were separated by a baffled, quiescent section inside the reactor, functioning as a clarifier (Figure 2). Bacteria flocs were lost in the effluent, and the loss was especially high when the reactors were highly augmented. The MLVSS in the reactor consisted primarily of two groups of biomass: the exogenous biomass, X_E, and the non-1-NA degrading biomass, X. The growth of indigenous species, X_I, from 1-NA consumption was considered negligible because the influent concentration of 1-NA was low (1 to 3 mg/L) compared to the bulk substrate, S (375 mg/L). The effluent volatile suspended solids (VSS) were divided into two terms by using Equation 3 and 4.

Equations 4 and 5 indicate that both indigenous and exogenous cells consume 1-NA, and that daily additions of exogenous biomass are assumed to be continuous (fX_{ER}). Monod kinetics were assumed throughout this study for oxidation of both substrate types. The authors are aware that Monod kinetics was not developed for degradation of secondary substrates, but in the experiments and in reports from others, the Monod model fit secondary-substrate degradation data (Babcock et al., 1993). The correlation between the uptake rates of target substrate for exogenous enriched cultures can be expressed as:

$$r_{suE} = \frac{k_E X_E S_t}{K_t + S_t} \quad (6)$$

$$r_{gE} = Y_E\cdot r_{suE} - K_{dE}\cdot X_E \quad (7)$$

Where,

K_t = half-velocity coefficient of target compound, mg/L;
K_{dE} = decay coefficient of 1-NA degrading exogenous, 1/d; and
Y_E = yield coefficient of 1-NA degrading exogenous, g VSS per g 1-NA.

An important aspect in this model is that the indigenous 1-NA degrading species, X_I, in Equation 3 is an assumed variable that cannot be directly measured from experiments. Former studies (Babcock et al., 1992) showed that after acclimation (several months) and at long MCRT, 1-NA can be degraded by normal activated sludge processes without bioaugmentation, although at a slower rate and with consistent uninterrupted feeding of 1-NA. In the mainstream or an in-situ reactor, indigenous 1-NA degrading culture must grow with other heterotrophic organisms. It would be difficult to measure indigenous independently 1-NA degrading organisms. It is not required to measure these organisms independently, however, but only to simulate their effects in a way that is analogous to simulating nitrification in full-scale systems. The need for separate balances on the two groups of 1-NA degrading organisms is to quantify the difference in rates of metabolism that occurs because of their previous environmental conditions: within the enricher-reactor reactor at generally higher 1-NA concentrations, or in main reactor, at lower 1-NA concentration and in competition with other organisms.

Mathematically, it was assumed that the non-1-NA degrading biomass (X) degraded only the bulk substrate (S) and were unaffected by the presence of 1-NA or the 1-NA-degrading inoculum; and the growth of exogenous biomass was not affected by the concentration of bulk substrate. The exogenous species is a mixed culture and should be able to adapt to the bulk substrate, but for simplicity this growth was separated from X_E calculations. To verify competition between exogenous and indigenous species

Table 2.—Measured effluent biomass and soluble COD

		Bioaugmentation levels (%)					
Measurements	**Unit**	**0**	**1**	**2**	**4**	**8**	**16**
Average MLVSS	mg/L	1668	1604	2076	1586	2362	3008
Effluent VSS	mg/L	21.7	39.0	62.8	79.2	70.4	99.0
Effluent COD	mg/L	36.7	38.8	29.6	49.4	46.6	60.6
Rate X_E add	mg/day	0	431	861	1703	3400	6826
Rate X_E loss	mg/day	0	461	1095	1532	1297	2059
Loss/added	%	-	107	127	90	38	30

MLVSS=mixed liquor volatile suspended solids; VSS=volatile suspended solids; XE=enriched biomass

on bulk substrate, detailed experimental approaches would have been required but were not considered necessary, because this study is not focused on degradation of easily degradable substrate. Because the influent bulk substrate concentration is constant, the total production of the non-1-NA degrading biomass should be steady and independent of the source of the biomass. This assumption simplified the calculation so that Equation 3 is independent from Equations 4 and 5. The model neglects the effect of dissolved oxygen because it was always high in the reactors (greater than 4 mg/L).

The model was constructed based upon Matlab 7.0 (MathWorks, Natick, Massachusetts) using a fourth-order correct, variable-time-step Runge-Kutta technique (function code 45) to integrate Equations 1 through 5. Laboratory data from shock-load and reacclimation experiments were used for calibration based on sensitivity analysis. Then a pattern search algorithm was used to minimize the sum of squares errors between the model output and experimental results (Box, 1965; Tzeng, et al., 2003).

Results and Discussion

Steady-State Results. Table 2 shows the average MLVSS, effluent VSS, and effluent COD versus the bioaugmentation levels after several months of operation at steady state. The bioaugmented CMAS lost significantly more cells and discharged more COD than controls, and the loss increased with bioaugmentation levels. Daily losses of biomass in the reactor effluent were calculated and compared to the mass of cells added for bioaugmentation. The loss and addition rates of VSS are not linearly correlated. At low bioaugmentation level, the daily loss of biomass was more than 100% of the exogenous biomass; when the CMAS was highly bioaugmented (8 to 16%), however, loss was only 30% of the exogenous mass. Effluent VSS was composed of both indigenous cells as well as exogenous cells because the observed improvement in 1-NA removal would not have occurred if the effluent VSS were composed solely of exogenous cells. Degradation of 1-NA at low bioaugmentation level (1 to 4%) improved, even though the MLVSS concentration did not increase.

The steady state data were used to calibrate the model shown previously. At steady-state, Equation 3 can be rearranged to calculate the concentration of non-1-NA degrading biomass as follows:

$$X = \frac{\theta_X}{\theta_H} \cdot \left[Y(S_O - S) - \frac{X}{X + X_E} \cdot X_{eff} \right] \cdot \frac{1}{1 + K_d \cdot MCRT} \qquad (11)$$

Where,

K_d = decay coefficient, 1/d;
Y = yield coefficient, g VSS per g substrate; and
S_O = influent substrate concentration, mg/L.

For exogenous biomass, Equation 10 can be reduced to:

$$X_E = \frac{\theta_X}{\theta_H} \cdot \left[\frac{fX_{ER}}{Q} - \frac{X_E}{X + X_E} \cdot X_{eff} \right] \cdot \frac{1}{1 + K_{dE} \cdot MCRT} \qquad (12)$$

Where,

Q = volumetric flow rate, m^3/h; and
fX_{ER} = bioaugmentation flow, g/h.

First, production of the suspended solids because of consumption of bulk substrate was calculated. At zero bioaugmentation (acclimated and unacclimated controls), the MLVSS was assumed to be composed of non-1-NA degrading biomass and a much smaller mass of 1-NA-degrading cells. The steady-state results of the non-1-NA degrading species corresponded to Monod kinetics with the following values: MLVSS =1650 mg/L; X_{eff} = 22 mg/L; influent COD = 375 mg/L; and $MCRT$ = 8.9 days. Provided parameters were: k = 5/d; K_s = 30 mg/L; K_d = 0.06 mg/L; and Y = 0.4. These values were assumed for the bulk substrate kinetics for all CMASs regardless of bioaugmentation level. Equation 12 was used to calculate the exogenous biomass concentration; K_{dE} is the only parameter that needs to be identified.

Equation 11 and 12 were solved iteratively with given conditions. The calculated total biomass was plotted in Figure 3 with the measured data. The concentration of the non-1-NA degrading biomass (X) and exogenous biomass (X_E) are plotted against the bioaugmentation levels, and the summation of the two is shown as the total biomass. A stable production of non-1-NA degrading biomass is predicted regardless of the level of bioaugmentation: a steady feed of substrate supports a mixed culture with approximately 1000 to 1500 mg/L MLVSS. For the exogenous 1-NA degrading species, observed biomass was lower than predicted with the kinetic parameters described previously. A better fit between model predictions and observations is possible if the value of K_{dE} is increased to 0.12/d. This is reasonable because the exogenous cells have been removed from a 1-NA rich (1-NA = 150 mg/L) environment to an 1-NA poor (1-NA = 3mg/L) environment, and greater decay is to be expected.

Shock Loading and Reacclimation Experiments. The effluent 1-NA concentrations from the shock loading experiments are shown in Figure 4(a) and are plotted for various levels of bioaugmentation (0, 1, 2, 4, 8, and 16%). In all shock load experiments, effluent 1-NA decreased exponentially to detection limits with increasing levels of bioaugmentation causing greater

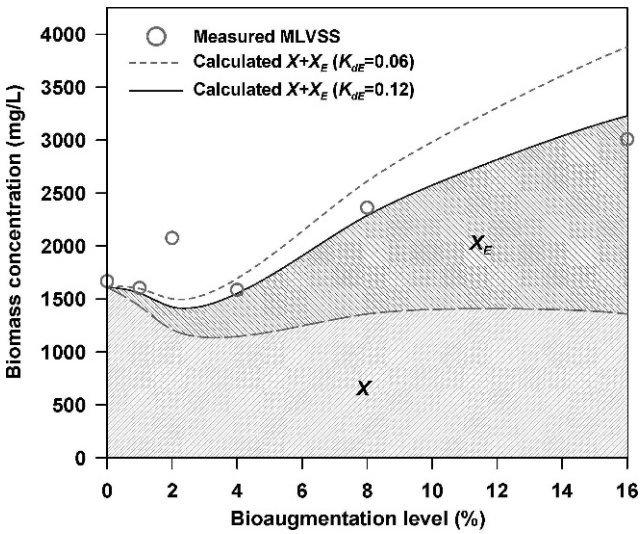

Figure 3—Biomass concentration versus bioaugmentation levels: concentrations of exogenous biomass, X_E, and non 1-NA degrading biomass, X, were calculated, and K_{dE} should be increased to fit the measured data.

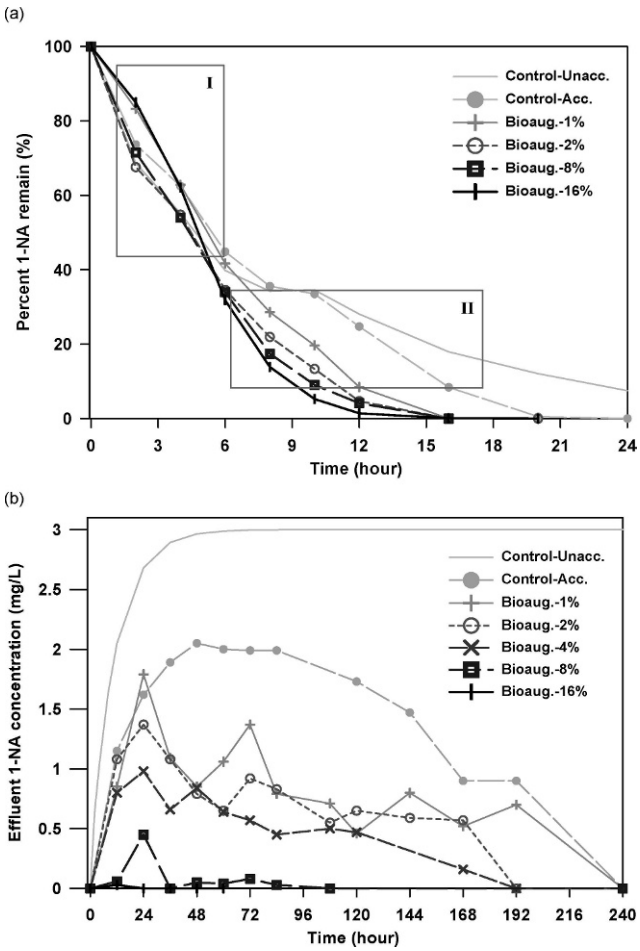

Figure 4—Effluent 1–NA breakthrough under different bioaugmentation levels in two experiments: (a) shock loading to 15 mg/L 1-NA; and (b) reacclimation—increase in influent from 0 to 3 mg/L 1-NA (unacc. = unacclimated control and acc. = acclimated control).

degradation rates. Degradation of 1-NA initially showed no correlation with bioaugmentation levels (box I). The difference in biodegradation rates became distinguishable only after the 1-NA concentration decreased to less than 35% of the initial value (box II). The 1-NA decreased to detection limits four to eight hours faster with increasing bioaugmentation levels. The CMAS operating at 4% bioaugmentation was upset during this experiment and results are not shown.

Figure 4(b) shows the reacclimation in response to a step increase of influent 1-NA to 3 mg/L after operating for 12 days (1.2 MCRT) with no 1-NA. For all CMAS (including the 4% acclimation level, which was operating properly), effluent 1-NA had decreased to detection limits within 240 hours (10 days). The patterns of breakthrough curves are similar: effluent 1-NA increased with influent concentration to reach a plateau and then decreased after a specific period until being totally degraded. The difference between each test was the peak effluent concentration and the time required for the effluent 1-NA to return to detection limits. As expected higher bioaugmentation reduced the peak 1-NA breakthrough concentration and decreased the time required to reach detection limits. In the case of no bioaugmentation, the effluent 1-NA concentration reached a plateau of approximately 2 mg/L and did not return to detection limits until after 10 days of operation. With low level bioaugmentation (1, 2, and 4%), the peak effluent concentrations were approximately 0.5 to 1 mg/L and the time to reach detection limits decreased to eight days. When highly bioaugmented (8 and 16%), there was almost no breakthrough, and no recovery period was needed.

Dynamic Simulations. Simulation results of shock loading experiments are presented in Figure 5(a) through (d) with the parameters shown in Table 3. Figure 5(a) shows the 1-NA breakthrough simulated (smooth curves) versus the measured data (open circles) for the unacclimated control experiment. Because no biological degradation was expected from the unacclimated cells, the removal mechanisms are due to washout and stripping. To illustrate the magnitude of stripping, a second simulation is presented with no stripping ($K_La = 0$). The figure shows that stripping of 1-NA is to be expected and the simulation fits better with the gas transfer term (solid line) than without (dash line) using a K_La of 0.4/d (Babcock, et al., 1992). Figure 5(b) shows the simulation results of 1-NA loss in the acclimated control. The dashed line shows the results of stripping only; the solid line simulates stripping and biodegradation provided by X_I. The acclimated control reduced the 1-NA to detection limits in approximately half the time required for the unacclimated control, from 48 to 24 hours. Figure 5(c) shows the results of the two low-level (1 and 2%) bioaugmentation experiments; Figure 5(d) shows the results from the two high levels (8 and 16%). The observations and simulation results fit well. The impact of 8 and 16% bioaugmentation are similar, and reduced the time to detection limits to 12 hours.

Figure 6(a) through (d) shows the simulation results of reacclimation experiments. Figure 6(a) shows the 1-NA breakthrough with degradation from indigenous species versus the observations. The simulation output agrees well with the experimental data. The growth kinetics of indigenous biomass collected from this experiment was used throughout all simulations. Figure 6(b) shows the simulation of 1% endogenous bioaugmenation. To fit the biomass concentrations, it was necessary to increase K_{dE} as discussed previously. The base value

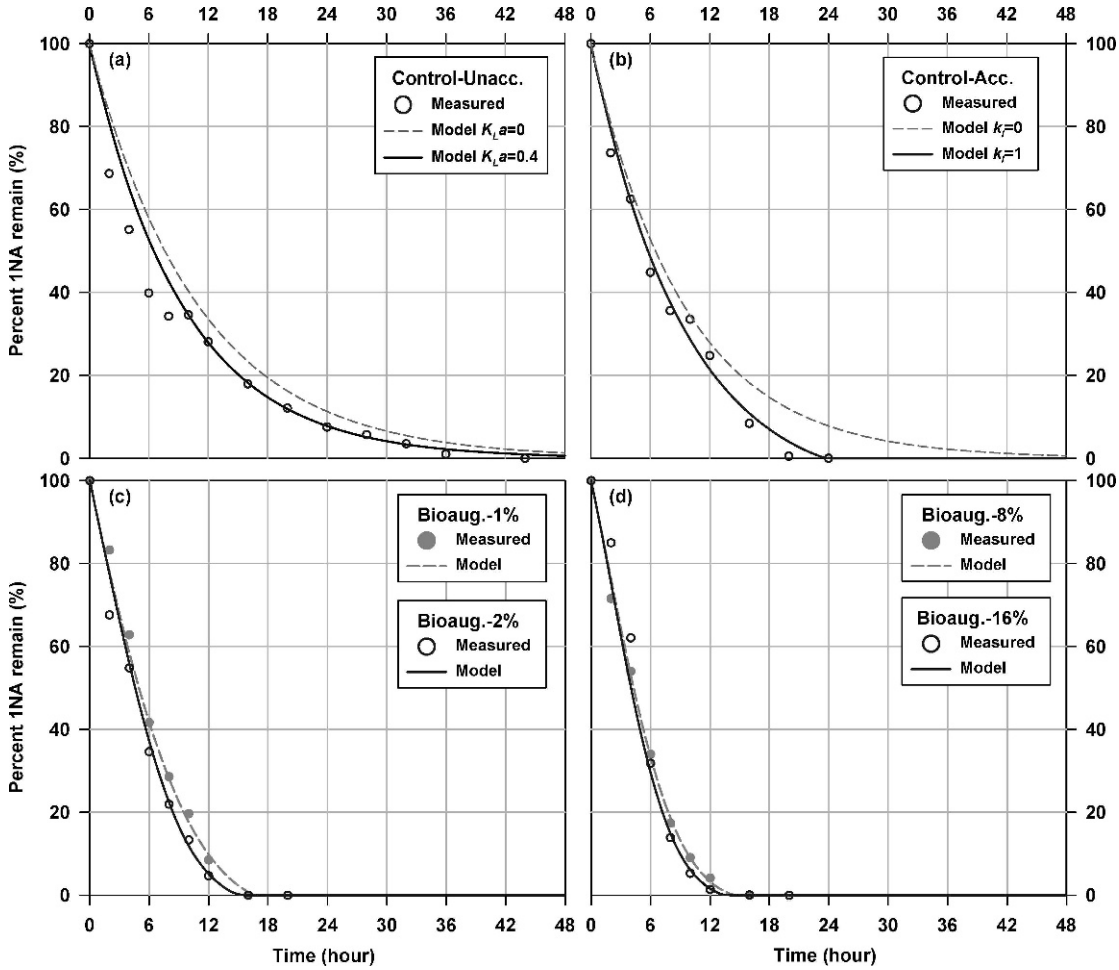

Figure 5—Simulation results of 1-NA breakthrough in the shock loading experiments: (a) unacclimated control with gas transfer; (b) acclimated control; (c) 1% and 2% bioaugmentation; and (d) 8% and 16% bioaugmentation.

of K_{dE} of 0.06/d was used for the acclimated control and 1% bioaugmentation; K_{dE} was increased to 0.20 and 0.32/d for the 2% and 4 to 16%, respectively. The simulations at 8 and 16% bioaugmentation are relatively less accurate because the effluent 1-NA concentrations were small and approaching detection limits. Higher values of K_{dE} were needed for the higher bioaugmentation levels to prevent the model from predicting too great 1-NA removal efficiency. The fit between observed and predicted solids concentrations is not shown but is roughly the same as in Figure 3.

Model Calibration. The parameters used in the models were validated separately; Table 3 shows the average values used in the simulations. Validated parameters were performed in two groups: (1) defined from references (Y, k, K_s) or based on the authors' previous studies (Y_I, Y_E) or general assumptions (K_t, K_{dI}); and (2) calibrated from results of experiments designed to identify specific parameters, including gas transfer (K_La) in unacclimated control experiments; growth kinetics of indigenous species (k_I) in acclimated control experiments; average growth kinetics of exogenous species (k_E) from shock loading experiments; and decay coefficient of exogenous species (K_{dE}) from reacclimation experiments.

Yield coefficients of acclimated indigenous species, X_I, and enriched cultures, X_E, were calculated based on previous observations, which showed that enricher cultures converted approximately 87% of the carbon in 1-NA to CO_2 (Cardinal and Stenstrom, 1991; Babcock et al., 1992). This yields a stoichiometry of the microbial growth of:

$$C_{10}H_7NH_2 + 10.2O_2 \rightarrow 8.7CO_2 + 2.48H_2O + 0.74NH_3 + 0.26C_5H_7NO_2 \quad (8)$$

The yield coefficient of the enriched species, X_I and X_E, is 0.26 molar biomass per molar 1-NA, or 0.33 mass biomass per mass of 1-NA. This value of the yield coefficient confirms the assumption of the authors that the increase in steady-state total biomass is predominantly because of the continuous additions of exogenous biomass and not growth on 1-NA. The influent 1-NA concentration of 1.0 mg/L would only support a steady-state growth of 8.8 mg/d or, at most, 6 mg/L MLVSS, whereas the minimum dosage of exogenous biomass (1% bioaugmentation) was approximately 50 times higher (431 mg/d). For the other parameters, Monod kinetics of the non-1-NA degrading biomass was defined previously; decay coefficient of acclimated 1-NA degrading culture was assumed to be the same as the non-1-NA degrading biomass because both cultures were reproduced in the main reactor but not from the enricher-reactor.

Monod kinetics of indigenous and exogenous cultures was calibrated by the breakthrough curve of 1-NA. The Monod function represents the growth of microorganisms on the substrate,

Table 3—Monod kinetics used in the simulations.

Parameters		Units
1. Defined parameters		
Yield of bulk biomass on bulk substrate, Y	0.40	–
Yield of acclimated culture on target compound, Y_I, Y_E	0.33	–
Maximum substrate uptake rate of bulk biomass, k	5	1/day
Half velocity coefficient of bulk biomass, K_s	30	mg/L
Half velocity coefficient on target compound, K_t	0.02	mg/L
Decay coefficient of bulk and indigenous biomass, K_d, K_{dI}	0.06	1/day
2. Calibrated parameters		
(1) Stripping and growth		
Gas transfer coefficient of 1–NA, K_La	0.374	1/day
Maximum uptake rate of target compound indigenous biomass, k_I	0.950	1/day
Maximum uptake rate of target compound by enriched culture, k_E	0.147	1/day
(2) Decay of enriched culture, K_{dE}		
Bioaugmentation level 1%	0.06	1/day
Bioaugmentation level 2%	0.20	1/day
Bioaugmentation level 4%	0.32	1/day
Bioaugmentation level 8%	0.32	1/day
Bioaugmentation level 16%	0.32	1/day

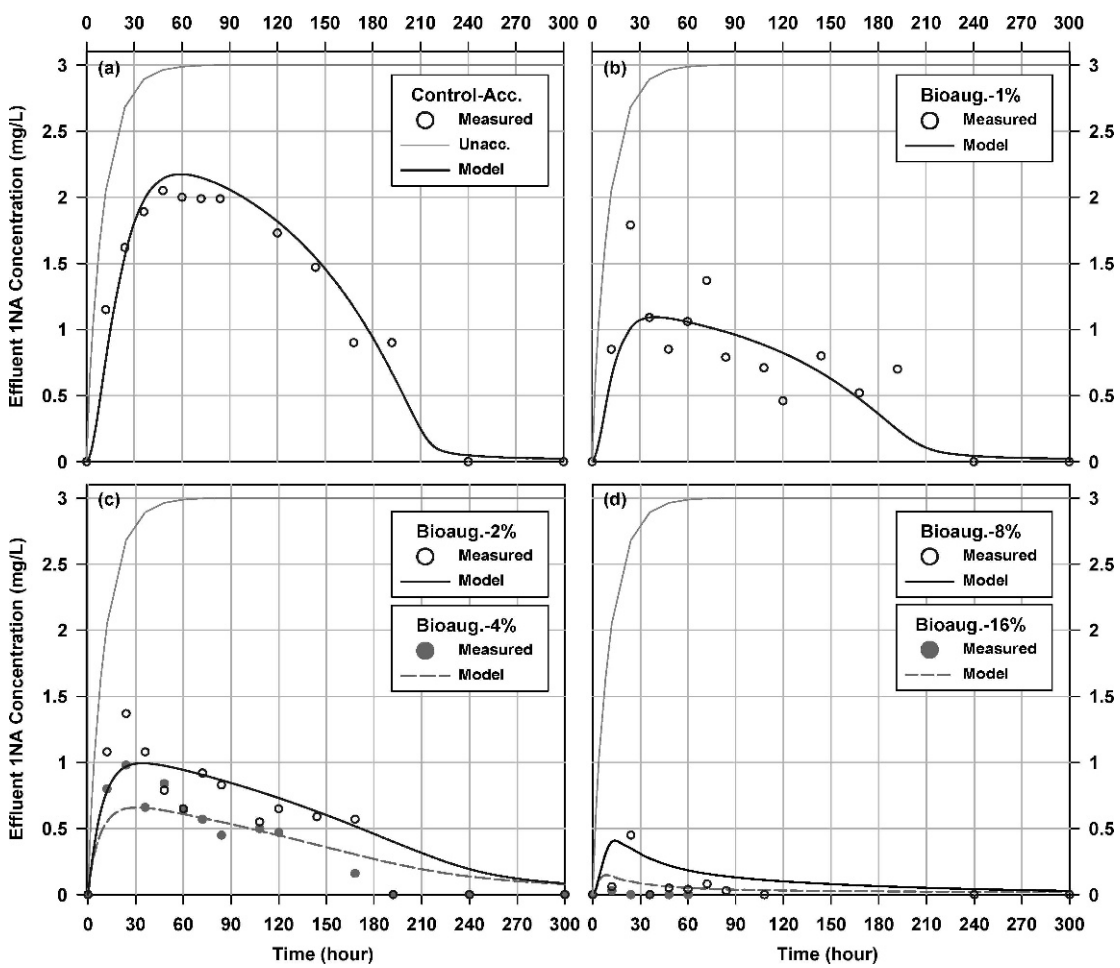

Figure 6—Simulation results of 1-NA breakthrough in the reacclimation experiments: (a) acclimated control; (b) 1% bioaugmentation; (c) 2 % and 4% bioaugmentation; and (d) 8% and 16% bioaugmentation.

and each term in the function performs a specific behavior. For example, in the reacclimation experiments, the maximum substrate uptake rate, k_E, describes the peak concentration of 1-NA and the time to achieve this value; the half-velocity coefficient, K_t, represents the residuals of 1-NA; and decay coefficient, K_{dE}, is correlated to the degradation curves after peak discharge. The calibration approach used in this paper is similar to the method used to calibrate structural ASM models. Yuan et al. (1993) applied oxygen uptake rates (OUR) to calibrate the kinetics of substrate consumption in a high purity oxygen (HPO) treatment plant; and Koch et al., (2000) applied the same index to examine the kinetics of the storage function in ASM 3.

We defined a low K_t, which is close to the detection limit, because 1-NA is degraded to the detection limit at the end of all acclimated experiments. Mathematically, this parameter controls the simulation when 1-NA concentration is low. The maximum uptake rate, k, is defined with other parameters based upon sensitivity analysis: the optimal simulations should lie on a linear function of k, K_t, and K_d. Because the ranges of K_t and K_{dE} were already defined and k_E should not vary significantly throughout the study, the value of 0.147/d was used and fit all experiments equally well.

The decay coefficient of exogenous biomass, K_{dE}, is one of the more sensitive parameters affecting simulation. The approach to define K_{dE} separately in each experiment is convenient because it allows model to fit the data and provides a plausible physical explanation. The endogenous decay parameter is actually a lumped parameter that encompasses substrate use for maintenance of cellular activity (energy) and cell losses because of death (cell lysis) and predation (Metcalf and Eddy, 2003). Increasing K_{dE} should occur with increasing bioaugmentation levels or decrease of the F/M ratio. This represents a change of conditions for the cells from ''growth-dominate'' conditions to ''respiration-dominate'' conditions. The K_{dE} parameter increased from 0.06 to 0.32/d as the bioaugmentation increased from 0 to 16%.

Simulation of the In-situ Process. After calibration, the model was used to simulate the in-situ bioaugmentation process [Figure 1(b)]. Because the growth environment in the reaeration zone is close to the main reactor, the same growth kinetics and decay coefficient for indigenous biomass was used in both reactors: k_I = 0.95/d; K_t = 0.02 mg/L; and K_{dI} = 0.06/d. Influent conditions were similar to the reacclimation experiments. An increase in influent 1-NA from 0 to a constant 3 mg/L was simulated. Four scenarios were evaluated: (1) acclimated control with no bioaugmentation; (2) with 1% exogenous bioaugmentation; (3) with 4% exogenous bioaugmentation; and (4) with the in-situ process. The enricher-reactor process and in-situ process were compared as closely as possible using the same conditions. For example, to create sufficient biomass in the enricher-reactor process to provide 1% bioaugmentation, approximately 26.5 mg/d of 1-NA is required for cell production. Therefore, in the simulation for the in-situ process, the same dosage of 1-NA was used to feed the reaeration tank.

Simulation results of the different scenarios are shown in Figure 7, which shows the percent removal of 1-NA as a function of MCRT. The effluent 1-NA mass decreased with increasing MCRT in all scenarios. For the acclimated control simulation (no enricher-reactor cells and no 1-NA added to the reaeration tank), the reactors performed equivalently and were able to degrade 1-NA for MCRTs larger than six days. Above the washout MCRT, the degradation of 1-NA improved with MCRT, removing approximately 60% of the feed 1-NA mass at MCRT = 10 days.

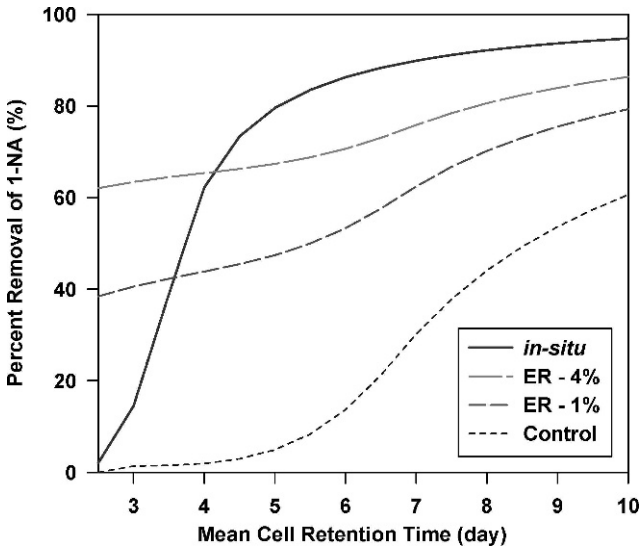

Figure 7—Simulation of 1-NA treatment performance of different bioaugmentation methods.

With bioaugmentation, enricher-reactor process and the in-situ processes both improved 1-NA degradation but performed differently. For the enricher-reactor process with 1% bioaugmentation, the removal of 1-NA was approximately 40% at MCRT = 2.5 days, and was 50% at six days MCRT. The benefit of this bioaugmentation method over the in-situ process is that 1-NA removal occurs at lower MCRTs. Because of the high decay rate of exogenous biomass, however, this improvement of 1-NA degradation is not a linear function of the bioaugmentation level; at 4% bioaugmentation removal increases from approximately 40 to 60% at MCRT = 2.5 days, and from approximately 50 to 65% at MCRT = six days. This is only a minor improvement for the increased (300%) biomass.

The in-situ process did not degrade 1-NA well at low MCRT, but excelled at higher MCRTs. The in-situ process begins to achieve significant 1-NA removal at MCRT = four days and outperforms the enricher-reactor process at both 1 and 4% bioaugmentation for MCRT greater than 4 days. One disadvantage of the in-situ process is the several-times larger volume required for the reaeration reactor compared to the enricher-reactor reactor. A second disadvantage is that a significant fraction of biomass in the in-situ process is exposed to higher 1-NA concentration as it is recycled through the reaeration reactor. The 1-NA is inhibitory or toxic to nitrifiers and other organism which may impact nitrification efficiency or other removal efficiencies (Hockenbury and Grady, 1977). In the enricher-reactor process, only the bioaugmentation mass is exposed to the high 1-NA concentrations. Furthermore, multiple enricher reactors can be provided; for example, the first enricher-reactor reactor could provide cells acclimated to 1-NA and the second could provide nitrifier biomass or biomass acclimated to a different organic compound.

Conclusions

Several conclusions emerged from these experiments as outlined below.

(1) The benefits of bioaugmentation using the enricher-reactor process was demonstrated, showing that enhanced target

compound removal, resistance to shock loads, and more rapid reacclimation to a toxic compound are possible. The benefits occur with low amounts of bioaugmentation (1 to 2% by reactor biomass). At higher bioaugmentation levels, a large fraction of the added mass decays, reducing the benefits of bioaugmentation.

(2) A simulation model is a useful tool to describe microbial behavior that is too difficult or expensive to measure experimentally. In this case, the simulation model was useful to quantify the biomass decay rate, which obviously must occur, but is not easy to measure. The increased decay rate explains the reduced benefits of bioaugmentation at higher bioaugmentation levels. The beneficial effects of bioaugmentation using the enricher-reactor process are limited, and the model can become a useful design tool.

(3) An alternative strategy to improve the degradation of hazardous waste is to enrich the target culture in the main reactor system using a reaeration reactor, called ''in-situ'' bioaugmentation. The calibrated model was used to compare the enricher-reactor process and the in-situ process to show that the latter is superior at longer MCRTs. The enricher-reactor process has the advantage of not exposing the entire biomass to high concentrations of potentially toxic enrichment substrates and can accommodate more than one target compound.

(4) Both bioaugmentation methods provided target compound removals at MCRTs below the normal washout MCRT associated with CAS. This may be an important feature, especially for more typically encountered treatment goals such as nitrification.

Notation

K_d	decay coefficient, T^{-1}
K_La	gas transfer coefficient, T^{-1}
K_s	half velocity coefficient, ML^{-3}
K_t	half velocity coefficient of target compound, ML^{-3}
Q	volumetric flow rate, L^3T^{-1}
S	substrate, ML^{-3}
V	volume of aeration zone, L^3
X	cell concentrations, ML^{-3}
X_{eff}	effluent suspended solids, ML^{-3}
X_{ER}	biomass concentration from enricher–reactor, ML^{-3}
Y	yield coefficient, mass VSS per mass substrate, MM^{-1}
f	volume of exogenous biomass added per d, L^3T^{-1}
k	maximum substrate uptake rate, T^{-1}
r_{su}	substrate uptake rate, $ML^{-3}T^{-1}$
r_g	growth rate of microbial strains, $ML^{-3}T^{-1}$
θ_H	hydraulic retention time (HRT), T
θ_X	mean cell retention time (MCRT), T

Subscripts

$_E$	exogenous microorganisms
$_I$	indigenous microorganisms
$_o$	influent concentrations
$_t$	target compound (1–NA)

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