BIOREMEDIATION OF SOLID TNT PARTICLES IN A SOIL SLURRY REACTOR: MASS TRANSFER CONSIDERATIONS

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ABSTRACT The bioreduction of solid TNT by a *Pseudomonas fluorescens* strain was investigated in a stirred tank reactor. Experiments in which TNT beads were the only solids present indicated that the biodegradation mechanism is dissolution followed by degradation in bulk solution. Dissolution may limit the overall rate, in which case degradation can be enhanced through increased agitation. Since soil slurries may contain high concentrations of solids other than TNT, Teflon chips were added to investigate two separate effects on TNT dissolution in slurries. First, Teflon solids increase the viscosity of the slurry, resulting in lower solid-liquid mass transfer coefficients. Second, the agitated Teflon slurry can grind or comminute TNT particles, creating additional surface area for mass transfer. Enhanced dissolution rates were observed for TNT beads in a Teflon slurry at higher agitator speeds. This suggests that the biodegradation of solid TNT nuggets in a soil slurry bioreactor may be enhanced under conditions that promote particle attrition.

KEYWORDS: TNT, bioremediation, solids, slurry, dissolution, attrition

INTRODUCTION

The bioremediation of 2,4,6-trinitrotoluene (TNT) contaminated soils is complicated by the fact that TNT can be present in the form of solid "nuggets" as large as 1 inch in diameter. Field trials of bioremediation processes have shown that these nuggets are slow to degrade [1]. Some studies suggest that solid contaminants must first dissolve in aqueous solution before biodegradation can occur [2-4]. Thus, the biodegradation of solid TNT particles may be limited by the dissolution rate. Slurry-phase reactors may offer an advantage in this case, since dissolution rates can be increased with agitation.

The focus of this research was the effect of solid-liquid mass transfer on the slurry phase biotreatment of solid TNT particles. In the first part of this study, we investigated the bioreduction of TNT solids in a stirred tank environment; no solids other than TNT were present in these initial experiments. Observed reactor profiles were compared with those predicted by a dissolution-degradation model to determine the role of dissolution in this process. Real soil slurry bioreactors contain up to 50 wt% solids, most of which are soil particles rather than TNT. Therefore, in the second part of this study, we investigated the effect of non-TNT solids at high concentrations on TNT dissolution. Teflon boiling chips were used as an ideal slurry of non-adsorbing, inert solids. Stirred-tank experiments with Teflon slurries were run to determine the effects of slurry concentration on the solid-liquid mass transfer coefficient (k_L) as well as on TNT particle attrition.

MATERIALS AND METHODS

Reactor setup

All experiments were performed in an Applikon 3 liter fermenter fitted with 3 baffles and a 45 or 60 mm marine impeller (vortexing). The working volume for all

experiments was 1.8 or 2.0 liters, and the temperature was controlled at 25 or 30ºC as noted. PTFE boiling chips (4-8 mesh) were used as an inert solid to study the effect of solids other than TNT.

Dissolution experiments

Solid TNT beads were agitated in either deionized water or a Teflon/water slurry, and the concentration of TNT in solution was monitored via HPLC. TNT beads were fabricated by melting TNT flakes in hot water (> 80ºC), agitating the molten TNT/hot water solution to create droplets, then quickly dumping the solution into ice water to freeze the TNT in spherical form. The TNT beads were then classified into size fractions using screen sieves.

Ion exchange experiments

k_L values in Teflon slurries were measured using cation exchange resin as described by Levins and Glastonbury [5]. Dowex 50W-X8 resin was classified by wet sieving (600-710 μm), regenerated to H^+ form with 1.0 N H_2SO_4, and added to the fermenter which contained 5 x 10^{-4} M NaOH and 0-30 vol% PTFE chips. The neutralization of NaOH by the cation resin was followed on-line with a conductivity probe. The volume of cation exchange resin (~8 ml) was chosen to ensure that the neutralization reaction would be film diffusion controlled. In these experiments the total reactor volume was 2.0 l, and the impeller diameter was 60 mm. The effective diffusivity for NaOH used to calculate k_L was 5.30 x 10^{-5} cm^2/s at 30ºC.

Attrition experiments

Approximately 0.5 g of TNT beads (600-700 μm) were agitated in a slurry containing 29 vol% PTFE chips and 71 vol% TNT-saturated water. After the specified agitation period, the Teflon particles were collected on a 1.18 mm screen sieve. TNT particles were washed from the Teflon with saturated solution and then collected on a 1 μm polycarbonate filter. Particle size analysis was performed by Particle Technology Labs, Ltd. (Downers Grove, IL). Screen sieves were used to classify the larger TNT fragments; subsieve size analysis was via the electrozone method (Coulter Counter technique).

Biological experiments

A pure culture of *Pseudomonas fluorescens* isolated from TNT-contaminated soils was used in all biological experiments. The starter culture medium was 1.0 liter of tryptic soy broth (30 g/l), which was inoculated with 1 ml of frozen culture and incubated for 10 hr at 30ºC. The culture was harvested by centrifuging at 3,000 rpm for 15 min. The recovered pellet was then used to inoculate the fermenter. A mineral salts medium [6] containing 2.0 g/l ethanol and no KNO_3 was used for the TNT bioreduction experiments. For intrinsic kinetic experiments, 100 mg/l TNT was dissolved in this medium prior to inoculation. A constant argon purge was used to maintain anoxic conditions during the experiments. O.D. (560 nm), pH, redox, cell concentration (BCA protein), aqueous TNT, and TNT metabolites were monitored during the experiment.

Sampling/analytical

HPLC samples were diluted 1:2 with acetonitrile; for biological experiments, the samples were centrifuged for 5 min at 15,000 rpm and then decanted to eliminate biological solids. When TNT solids were present, the samples were 1.0 μm filtered (glass fiber) prior to dilution with CH_3CN. HPLC and BCA protein assay methods were as described previously [6].

Chemicals

TNT was obtained from Chem Service, Inc. TNT, 2-amino-4,6-dinitrotoluene (2-ADNT), and 4-amino-2,6-dinitrotoluene (4-ADNT) standards were from Supelco. 2-hydroxlyamino-4,6-dinitrotoluene (2-HADNT), 4-hydroxylamino-2,6-dinitrotoluene (4-HADNT), 4,4',6,6'-tetranitro-2,2'-azoxytoluene (2,2'-AZ), 2,2',6,6'-tetranitro-4,4'-azoxytoluene (4,4'AZ), and 4,2',6,6'-tetranitro-2,4'-azoxytoluene (2,4'-AZ) standards were generously provided by Ron Spanggord. Chemware® PTFE boiling chips were from Norton.

RESULTS

Experiments without Teflon

Intrinsic kinetics

In order to determine whether dissolution or other mass transfer processes are rate limiting, the intrinsic biotransformation rate for TNT dissolved in aqueous solution must be known. The reductive pathway for this organism under anoxic conditions has been reported previously [6]. By measuring the disappearance of TNT from solution, the kinetics for the initial reduction of TNT to HADNT were determined. Cell concentration as measured by BCA protein assay remained constant during the course of the experiments. An average cell concentration was used to determine the kinetic parameters. TNT bioreduction was fitted reasonably well using a Michaelis-Menten expression:

$$dC/dt = -v'_{max} X C / (K_m + C) \quad (1)$$

where X = biomass concentration, v'_{max}, K_m = kinetic constants, and C = bulk aqueous TNT concentration.

At 25ºC, fitted parameters were v'_{max} = 0.142 (g TNT/g BCA protein·hr) and K_m = 85 (mg/l).

Standard curves for 2-HADNT, 4-HADNT, 2-ADNT, 4-ADNT, 2,2'-AZ, 2,4'-AZ, and 4,4'-AZ were used to quantify the amounts of TNT converted to these metabolites. At the point where less than 5% of the initial TNT remained, only 80% was accounted for as TNT or the above metabolites. Some of these metabolites, particularly the azoxy compounds, may have been adsorbed by the cell matter; biomass extractions will be performed in future experiments to see if this improves the TNT material balance.

Abiotic dissolution of solid TNT

Figure 1 shows the abiotic dissolution of TNT beads for two different size fractions at two different agitator speeds. The experimental data agree reasonably well with model dissolution curves predicted by the following equation:

$$dC/dt = k_L a (C^* - C) \quad (2)$$

where k_L = solid-liquid mass transfer coefficient, a = surface area/volume, and C^* = aqueous TNT conc. at interface (equilibrium).

k_L was obtained from the correlation of Levins and Glastonbury [5]:

$$Sh = k_L D_p/D_v = 2 + 0.47 Re_p^{0.62} Sc^{0.36} (D_S/D_T)^{0.17} \quad (3)$$

D_S, D_T = stirrer, tank diameter, Re_p = particle Reynold's number, Sc = Schmidt number, Sh = Sherwood number, D_p = TNT particle diameter, and D_v = diffusivity of TNT in water.

Equation 1 was solved numerically using a Runge-Kutta algorithm. The model also

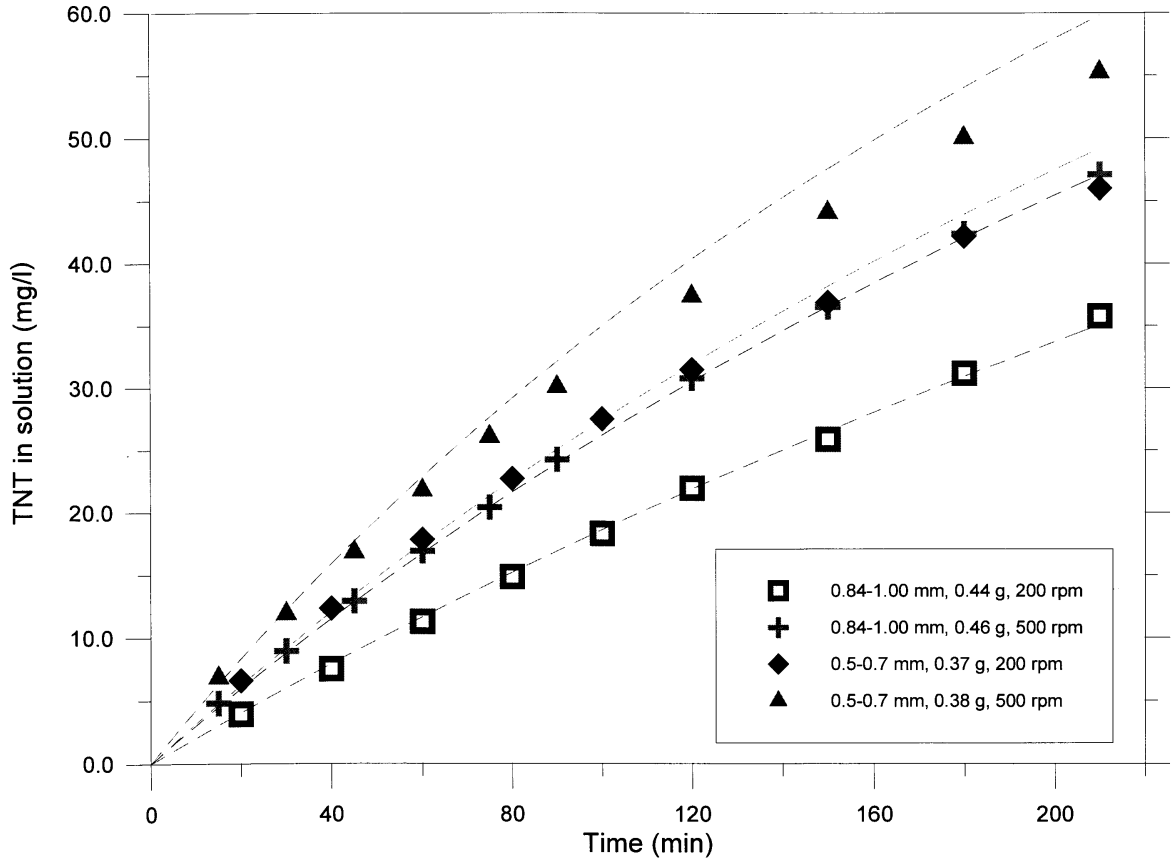

FIGURE 1. ABIOTIC DISSOLUTION OF SOLID TNT BEADS INTO WATER. VOLUME = 1.8 l, TEMP. = 25ºC, C^* = 121 mg TNT/l, D_S = 45 mm, $D_V = 0.74 \times 10^{-5}$ cm^2/s. SEE SYMBOL LEGEND FOR TNT PARTICLE SIZE FRACTIONS, MASS OF TNT, AND AGITATOR SPEED FOR EACH EXPERIMENT. DOTTED LINES SHOW THE PREDICTED DISSOLUTION RATE (EQUATIONS 2 & 3).

accounts for the fact that the surface area of the beads decreases as dissolution proceeds.

Bioreduction of solid TNT

Figures 2 and 3 show the bioreduction of solid TNT beads by *Pseudomonas fluorescens* under anoxic conditions. 0.54 g of TNT solids with an average diameter of 600 μm was used in each experiment. For the experiment shown in Figure 2, the biomass concentration (X) was 194 mg/l and the agitator speed (N_s) was 500 rpm; for Figure 3, X = 274 mg/l and N_s = 200 rpm. Biomass concentrations showed no appreciable change during the course of the experiments (reported values are averages). In each case, the concentration of TNT in solution was zero initially and increased until a plateau value was reached (60 mg/l for Figure 2, 30 mg/l for Figure 3). These plateau concentrations are well below the saturation concentration of 121 mg/l. The dotted lines are model predictions based on integration of the following expression:

$$dC/dt = k_La\,(C^* - C) - v'_{max}\,X\,C / (K_m + C) \qquad (4)$$

This model is based on the assumption that solid TNT must first dissolve before bioreduction can take place. Figures 2 and 3 show that the model predicts the

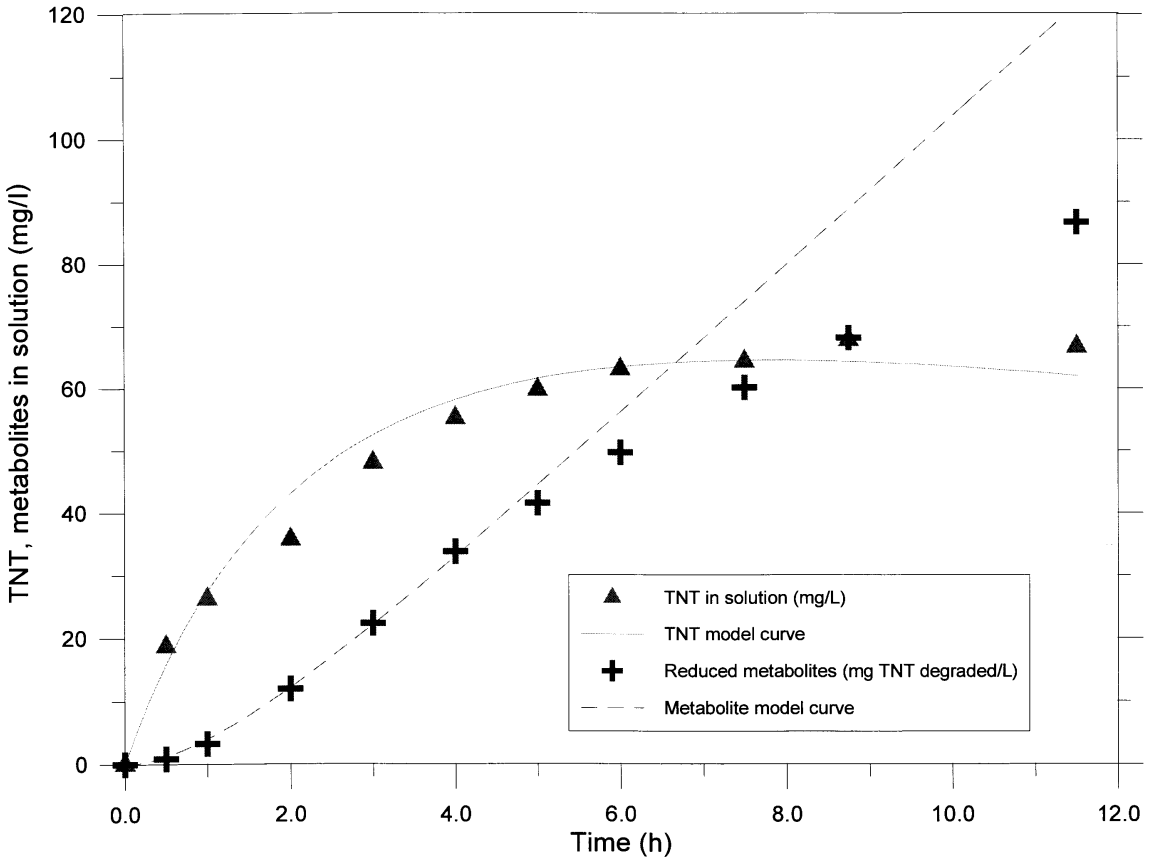

FIGURE 2. BIOREDUCTION OF SOLID TNT PARTICLES AT 500 RPM. X_{AVG} = 194 mg/l. 0.54 g SOLID TNT BEADS, 500-700 μM IN DIAMETER. VOLUME = 2.0 l, TEMP. = 25ºC, D_S = 45 mm. MODEL CURVES OBTAINED FROM THE INTEGRATION OF EQUATION 4. FOR MODEL PARAMETERS, C^* = 121 mg TNT/l, v'_{MAX} = 0.142 g TNT/g BCA PROTEIN·HR, AND K_M = 85 mg/l.

concentration of TNT in solution quite well. The model also assumes that 100% of the TNT degraded can be accounted for as reduced metabolites. The observed concentration of reduced metabolites matches model predictions initially, but later falls below the model curve. The model TNT curve drops slowly after 8 hours due to decreasing solid surface area, but the experimental values were constant during this period.

Experiments with Teflon

k_L values for ion exchange beads

Teflon was chosen as an inert solid material to study the effects of solids other than TNT on TNT dissolution. The effect of volume % Teflon in the reactor on the solid-liquid mass transfer coefficient is shown in Figure 4. k_L values were measured by monitoring the neutralization of NaOH in solution by cation exchange beads (H^+ form). This experimental system was chosen to eliminate the effects of TNT particle attrition in a Teflon slurry (see below). Figure 4 shows that at 800 rpm, as the volume % Teflon is increased from 0 to 30%, the measured k_L

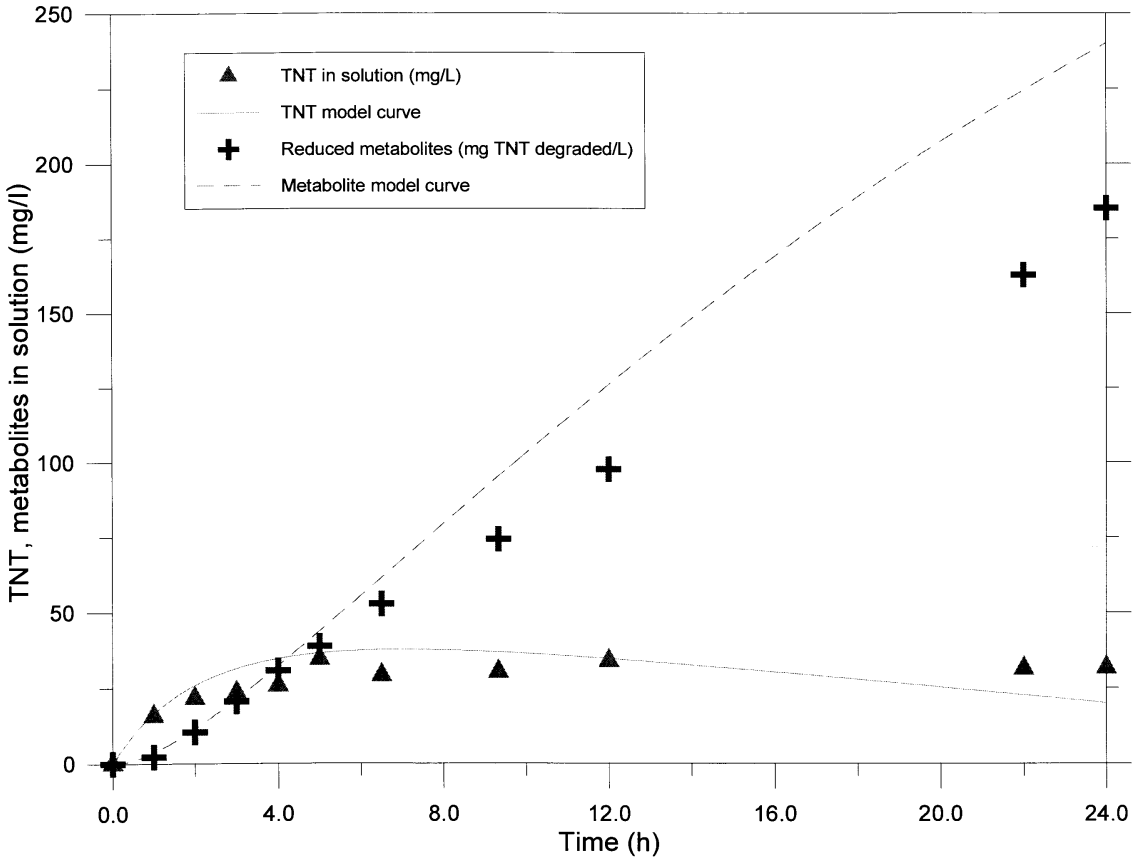

FIGURE 3. BIOREDUCTION OF SOLID TNT PARTICLES AT 200 RPM. X_{AVG} = 274 mg/l. ALL OTHER VALUES SAME AS IN FIGURE 2. MODEL CURVES OBTAINED FROM THE INTEGRATION OF EQUATION 4.

value drops from 2.29 x 10^{-2} to 1.72 x 10^{-2} (cm/s), a 25% decrease. At 500 rpm, k_L drops from 1.76 x 10^{-2} at 0 volume % Teflon to 1.25 x 10^{-2} cm/s at 20 volume % Teflon. The method of Kikuchi, *et al.* [7], was used to predict k_L in a Teflon slurry. This method uses the same correlation for k_L as described above (Equation 3), but the particle Reynold's number (Re_p) is defined as follows to account for the increased kinematic viscosity of the slurry:

$$Re_p = \varepsilon^{1/3} D_p^{4/3}/\upsilon_m \quad (5)$$

ε = power per unit mass of water, not total mass, $\upsilon_m = \mu_m/\rho_m$ = kinematic viscosity of slurry, and D_p = diameter of ion exchange particle.

$$\mu_m = \mu_w\{1 - (\alpha/\alpha_{max})\}^{-2.5\alpha max} \quad (6)$$

α = vol. frac. PTFE, α_{max} = max. vol. frac. PTFE = 0.554, and μ_w = viscosity of water.

$$\rho_m = (1 - \alpha)\rho_w + \alpha\rho_p \quad (7)$$

ρ_w = density of water and ρ_p = density of PTFE = 2.09.

The Schmidt number in the Levins and Glastonbury correlation (Equation 3) was defined using the kinematic viscosity of water. At 500 rpm and 20 vol% PTFE (Figure 4), the experimental k_L values are lower than predicted; at this point the Teflon chips were no longer in suspension. The Teflon slurry was suspended under the

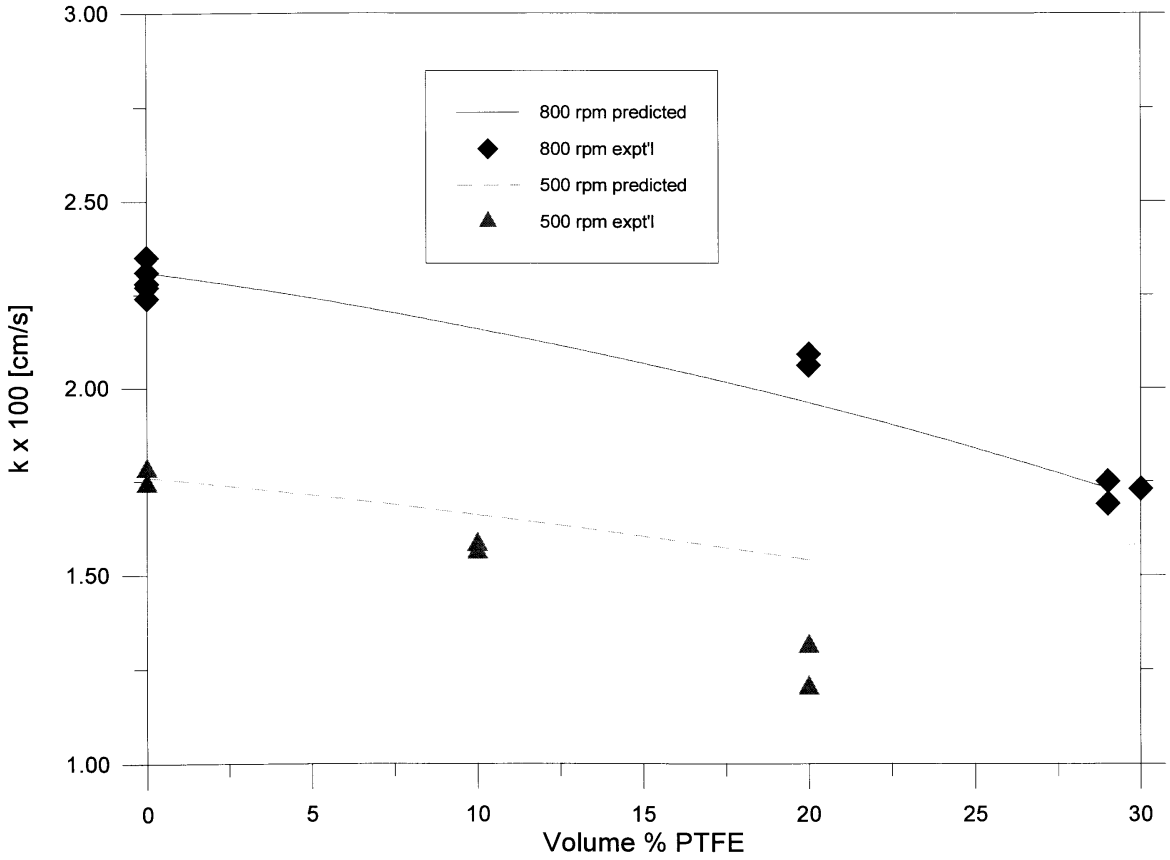

FIGURE 4. SOLID-LIQUID MASS TRANSFER COEFFICIENT (k_L) AS A FUNCTION OF VOLUME % TEFLON CHIPS IN SLURRY. EXPERIMENTAL VALUES OBTAINED FROM THE NEUTRALIZATION OF NaOH IN SOLUTION WITH DOWEX 50W-X8, H^+. PREDICTED VALUES FROM EQUATION 3 USING EQUATIONS 5-7 TO ACCOUNT FOR VISCOSITY OF SLURRY.

remainder of the conditions investigated. Predicted and experimental values were in good agreement for these experiments.

Dissolution of TNT beads

Figure 5 shows the dissolution of TNT beads in 29 volume % PTFE slurries at 200, 500, and 800 rpm. PTFE chips were not suspended at 200 or 500 rpm, but were near the suspension point at 800 rpm. TNT particles were collected at the end of each experiment; TNT attrition was visibly apparent at 500 and 800 rpm, but not at 200 rpm. Dissolution curves as predicted by the Levins & Glastonbury/Kikuchi method are also shown. At 200 rpm, dissolution was slightly lower than predicted, and at 500 and 800 rpm, the observed dissolution rates were well above the predicted values.

Attrition of TNT beads

Figure 6 shows TNT particle size distributions for 800 rpm and 29 volume % Teflon after 7 (Figure 6a) and 15 minutes (Figure 6b). These experiments were run in saturated solution to eliminate dissolution effects; therefore, the observed TNT size reduction is due to attrition effects only. At time zero all particles were between 600 and 700 μm in size. After 7 minutes, 64% of the

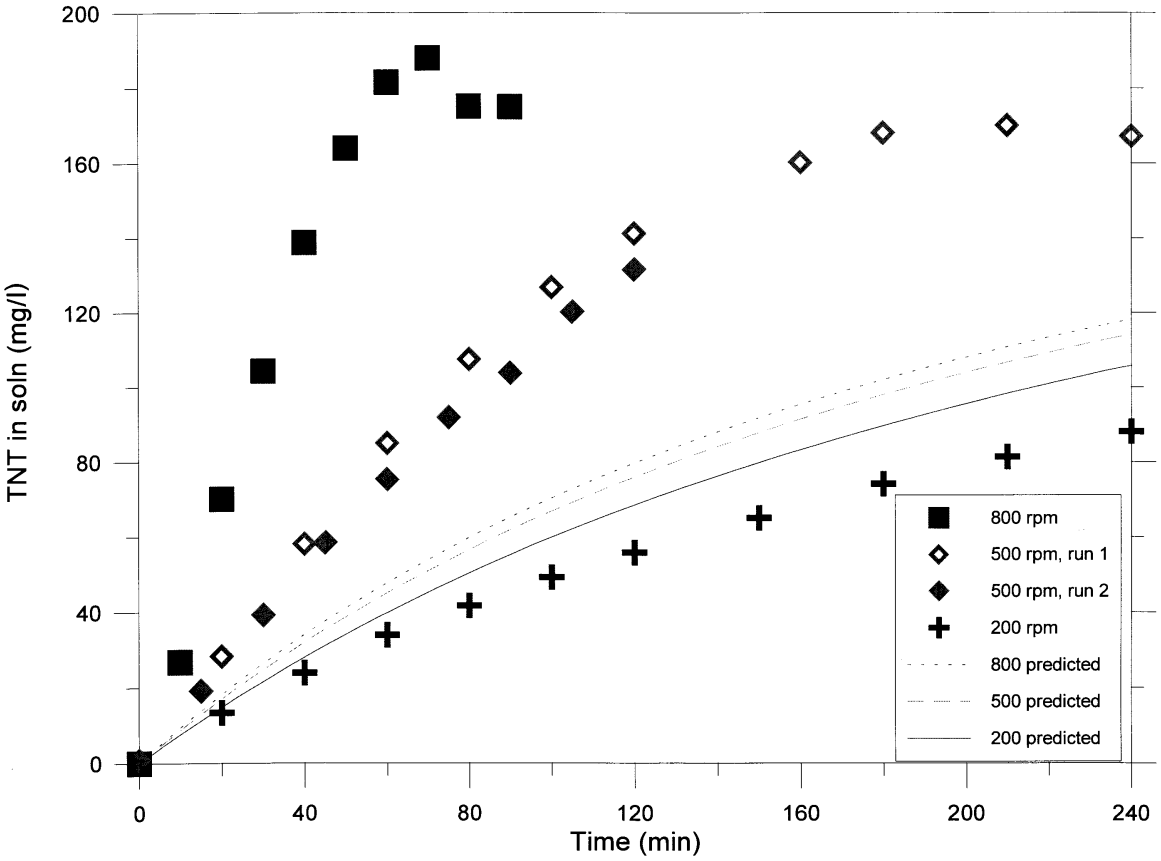

FIGURE 5. ABIOTIC DISSOLUTION OF TNT BEADS IN A 29 VOLUME % TEFLON SLURRY. 0.4 g TNT BEADS, 600-700 μM IN DIAMETER. 30ºC, 2.0 l WORKING VOLUME, 60 mm IMPELLER. PREDICTED CURVES FROM EQUATION 2 USING EQUATIONS 3 AND 5-7.

TNT remained in the original size range, and the estimated surface area was 3.7 times the initial area. After 15 minutes, only 52% of the TNT consisted of particles greater than 600 μm in size, and the surface area was 6.0 times greater than at time zero. The size distribution appears to be bimodal, with one set of fragments in the 300-600 μm size range and a second group in the 47-174 μm size range.

DISCUSSION

Experiments without Teflon

These experiments indicate that the initial bioreduction of solid TNT beads can be modeled using a dissolution-degradation model. This model assumes that solid TNT must first dissolve in aqueous solution before it can be reduced by the microorganisms. The agreement between model and experimental data does not eliminate the possibility that microbes may also act on solid TNT directly; microscopic examination of TNT beads showed what appeared to be a surface biofilm. Goswami, *et al.*, report that an *Arthrobacter* species exhibited growth on dissolved cholesterol, as well as on solid cholesterol via direct uptake from attached cells [8]. However, no decrease in aqueous cell concentration (O.D. and BCA protein measurements) was observed in our

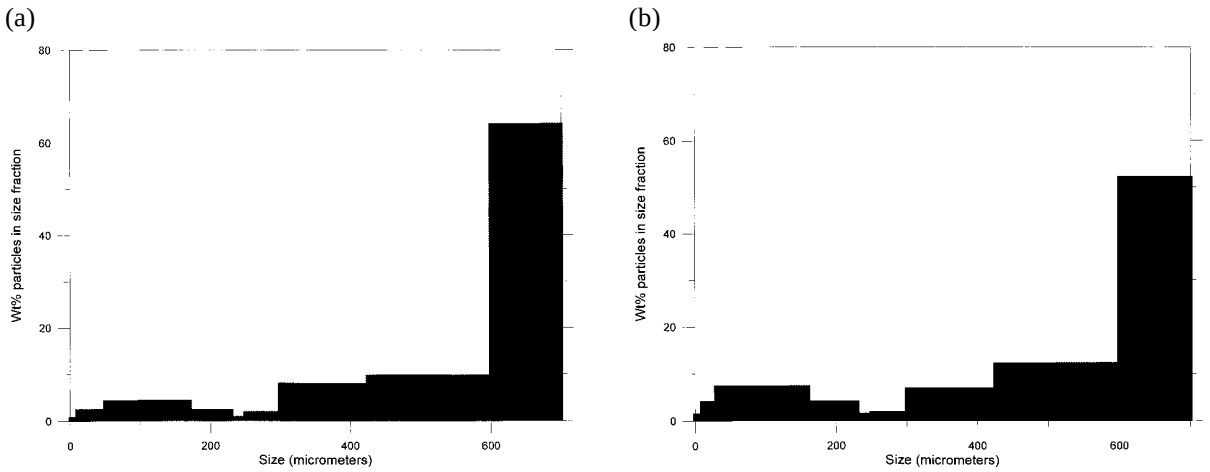

FIGURE 6. TNT BEAD ATTRITION IN A 29 VOLUME % TEFLON SLURRY AT 800 RPM. 0.51 g TNT BEADS, 600-700 µM IN DIAMETER INITIALLY. 2.0 l WORKING VOLUME, 60 mm IMPELLER. AGITATION TIME = 7 MIN (a), 15 MIN (b).

experiments. This indicates that almost all the biomass remained in bulk aqueous phase and that the fraction of attached cells was insignificant. Thus, the physical separation of TNT solids and aqueous microbes dictates that for this system, dissolution followed by bioreduction is the only significant pathway.

At both 200 and 500 rpm (Figures 3, 2), the aqueous TNT concentration increased from zero initially; thus, at low bulk concentrations the dissolution rate was higher than the degradation rate. As the aqueous TNT concentration rose, the dissolution rate slowed, and the bioreduction rate increased until both rates were equal. At this point the TNT concentration curve plateaued. The TNT model curve eventually dropped due to decreasing surface area, but the experimental data remained flat. This result may be related to the observation that some TNT particles were broken during these experiments; such particles would provide more surface area for dissolution. The metabolite concentration curve was lower than predicted, again because some of the TNT metabolites may have been associated with the biomass fraction.

The higher plateau concentration in Figure 2 is due to the higher agitator speed as well as the lower biomass concentration. Curves in Figures 2 and 3 cannot be compared directly, since the biomass concentrations are not the same. However, the model clearly indicates that for the same biomass and TNT solids concentrations, the aqueous TNT plateau is higher for increased agitator speeds. This result is due to the positive effect of agitation on the mass transfer coefficient. Since a higher concentration of TNT in solution also leads to a higher rate of bioreduction (Equation 1), an increase in the overall consumption of TNT accompanies increased agitation.

Experiments with Teflon

These experiments indicate that the presence of solids other than TNT can affect the dissolution rate of solid TNT. Ion exchange experiments indicate that the solid-liquid mass transfer coefficient decreases as the concentration of Teflon is increased. This decrease can be attributed to the fact that as the Teflon concentration increases, the effective viscosity of the slurry also

increases. For a given agitator speed, the particle Reynold's number, and thus the level of turbulence in the reactor, decreases as the percent Teflon increases. The k_L value in a Teflon slurry can be predicted using the same correlation used for no-Teflon systems (Equation 3), provided that the kinematic viscosity of the slurry is accounted for (Equations 6 & 7) and that the slurry is in suspension. The correlation overpredicts k_L values when the Teflon slurry is not suspended.

The abiotic dissolution of TNT beads in a 29 volume % Teflon slurry at 200 rpm was slightly less than predicted (Figure 5). Since the Teflon was not in suspension at this speed, we expect the predicted dissolution rate to be somewhat higher than the actual rate. However, at 500 and 800 rpm, the dissolution of TNT was much faster than predicted. The latter result is most likely due to the TNT particle attrition that was observed at the end of these experiments. Attrition experiments conducted under the same conditions (600-700 μm TNT beads, 800 rpm, 29 volume % Teflon) indicated that significant particle size reduction occurred after only 7 minutes (Figure 6a). Since the attrition of TNT particles creates more surface area, enhanced dissolution rates are to be expected.

While this study indicates that the biodegradation of solid TNT can be enhanced through increased agitation, there is most likely an upper limit to benefits from agitation. In a stirred tank, substantial increases in k_L can be obtained by increasing the agitation level to the point where all solids are "just suspended." However, further increases in agitator speed beyond this point give only slightly larger k_L values [9]. In preliminary biological experiments with 29 volume % Teflon at 500 and 800 rpm, a significant drop in O.D. was observed that did not occur in the same experiments without Teflon. This suggests that shear stresses associated with the Teflon slurry may cause damage or even rupture of the microbial cells. Thus, particle attrition benefits from increased agitation may have to be weighed against detrimental effects on culture viability.

CONCLUSIONS

Experiments without Teflon solids demonstrate that dissolution followed by degradation is the predominant mechanism for bioreduction of solid TNT in a stirred tank. The dissolution rate can limit the overall biotransformation rate, in which case higher rates can be obtained through increased agitation.

Experiments with Teflon solids show that high concentrations of solids other than TNT (up to 30 volume %) can affect TNT dissolution in a slurry reactor by two mechanisms. First, solid-liquid mass transfer coefficients decrease with higher solids concentrations due to the increased kinematic viscosity of the slurry. This is offset by the second effect; namely, that high slurry concentrations promote TNT particle attrition, creating more surface area for dissolution. Attrition in slurry reactors may prove to be an important factor in the bioremediation of soils containing TNT nuggets.

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