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Bioremediation of Wastewater Containing RDX

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ABSTRACT

A free suspension model of the biodegradation of RDX by a consortium has been developed as a part of an overall model that will describe the waste treatment for explosives in a continuous process. The free suspension model uses experimentally determined parameters to predict the concentrations of the bacteria cells, the substrate (yeast extract), RDX, and the mononitroso intermediate. The model correctly predicts the experimentally observed trends; however, the values predicted for the cell concentrations are consistently lower than those observed experimentally. This can be resolved by better characterizing the growth parameters of the consortium. The degradation of RDX was found to be first order in RDX concentration and assumed to be first order in cell concentration, X , i.e.

$$\frac{d(\text{RDX})}{dt} = -k_1(X)(\text{RDX})$$

The degradation rate constant, k_1 , was found to be 0.043 L/(g cells hr). Similarly, the degradation rate constant of the mononitroso intermediate was found to be 0.066 L/(g cells hr).

INTRODUCTION

As a brief reminder, the goal of this project is to develop a model that accurately describes the behavior of a packed-bed and/or fluidized bed waste treatment facility for explosives, i.e. RDX and TNT. This research project can be divided into three (3) main areas: batch bioremediation of explosive waste by a consortium in a free suspension, batch bioremediation of explosive waste by immobilized consortiums, and bioremediation of munition waste in a continuous process. Currently, our work focuses upon modeling the bioremediation of RDX by a consortium in a free suspension.

The physical system that the free suspension model is attempting to describe is the batch, anaerobic biodegradation of RDX by a consortium. These bacteria were grown with yeast extract as the substrate using the following three step process: an inoculum was initially grown for six (6) hours anaerobically (anoxically) then, switched to an aerobic atmosphere and grown for another five (5) hours then lastly switched back to anaerobic conditions until the end of the experiment. This last stage represents the stationary growth phase for the consortium. RDX degradation is basically only seen during this stationary growth phase. Experimental observation shows that degradation of RDX can be expressed by the following simple reaction scheme

THEORY

The free suspension model incorporates cell growth, substrate (yeast extract) consumption, the degradation of RDX, and the appearance and subsequent disappearance of the first degradation intermediate. Here, simple Monod type kinetics are used to describe the growth of the consortium. The degradation of RDX has been shown experimentally to behave as a first order decay; thus, the disappearance of the mono intermediate has been assumed to also follow a first order decay. The equations to describe these events are

$$\begin{array}{ll} \text{(cell growth)} & \frac{dX}{dt} = \mu X \quad \text{where} \quad \mu = \frac{\mu_{\max} S}{K_s + S} \end{array} \quad (2)$$

$$\begin{array}{ll} \text{(yeast extract consumption)} & \frac{dS}{dt} = \frac{-\mu X}{Y_{x/s}} \end{array} \quad (3)$$

$$\begin{array}{ll} \text{(RDX degradation)} & \frac{dR}{dt} = -k_1 X R \end{array} \quad (4)$$

$$\text{(Mono production)} \quad \frac{dM}{dt} = k_1XR - k_2XM \quad (5)$$

where

μ	rate of cell growth expression (hr^{-1})
$\mu_{\max}$	maximum rate of cell growth (hr^{-1})
K_s	half saturation constant (g/L)
X	cell concentration (g/L)
S	substrate concentration (g/L)
R	RDX concentration (g/L)
M	Mononitroso intermediate concentration (g/L)
k_1	RDX degradation constant (L/g hr)
k_2	Mononitroso degradation constant (L/g hr)

The rate of degradation of RDX and of the mononitroso intermediate are both assumed to have a linear dependence on the cell concentration. Since the degradation of RDX occur only in the stationary phase, the cell concentration is considered to be a constant in equations (4) and (5).

MATERIALS AND METHODS

The consortium, consisting of bacteria found in horse manure, was grown in an AM-1 medium with yeast extract as the carbon source. The RDX was obtained from Los Alamos National Laboratory with no further purification before usage. Anaerobic conditions were achieved by flushing samples with pure nitrogen for two minutes anytime the sample was exposed to air.

The parameters of this model were determined experimentally.

Growth parameters ($\mu_{\max}$ and K_s) were calculated for both anaerobic and aerobic conditions by measuring the initial growth rates for different substrate concentrations varying from 0.1 to 1.0 g/L. The growth parameters were then determined from a Lineweaver-Burk plot, i.e. plotting $1/\mu$ versus $1/S$.

The yield coefficients ($Y_{R/S}$) were also determined for both anaerobic and aerobic conditions with and without RDX. Samples were grown in the desired media until they reached stationary growth phase as determined by measuring the optical

density on a spectrophotometer. The samples were then filtered with a $0.2\ \mu\text{m}$ filter and weighed to determine the amount of growth per amount of substrate consumed.

Since degradation was observed only for the anaerobic stationary phase, the rate constants were analyzed only in that regime. Concentrations of RDX and the mono intermediate were measured by HPLC using a C18 column. The mobile phase of the HPLC was a methanol/water mixture (30/70) flowed at $0.7\ \text{mL/min}$.

The experimentally determined parameters are summarized in Table 1.

These parameters were implemented into the model from which the amounts of bacteria, substrate, RDX, and mononitroso intermediate were calculated over time. This model was then compared to data from kinetic experiments to determine its accuracy. A kinetic experiment consisted of growing a consortium for six hours anaerobically then growing five hours aerobically and finally returned to anaerobic conditions for the extent of the experiment which lasted anywhere from 60 to 140 hours with samples being taken every four to eight hours. The model was designed to account for each of these three periods.

RESULTS

After inputting the experimentally determined parameters into the model, a computer simulation was then made and compared to the results of a kinetic experiment. The particular experiment that was modeled ran for 71 hours which included the time for all three phases.

The comparison for the growth of bacteria, calculated and experimentally observed, is shown in Figure 1. The theoretically predicted values are consistently lower than those that are seen experimentally; however, the model correctly predicts the observed trends. As can be seen in Figure 1, most of the growth occurs in the aerobic growth phase. Thus, most likely, the difference between experimental and predicted values results from inaccuracies in the aerobic growth parameters, μ_{max} and K_s .

In the calculation of RDX and its degradation products, two different sets of rate constants were employed. In the first case the rate constants, $k_1 X$ and $k_2 X$, were based on cell concentrations predicted from the model. In the second case the rate constants, $k_1 X'$ and $k_2 X'$, were based on cell concentrations observed from the experiment. The second set of rate constants were employed to show how the model would behave if the correct cell concentration could be predicted. The predicted value of the cell concentration during the

stationary phase, X , is 0.450 g/L, and the average experimentally observed value of the cell concentration, X' , is 0.706 g/L.

The predicted values for the degradation of RDX using both sets of rate constants agree very well with the trend observed experimentally as shown in Figure 2. As expected, the model predictions for RDX concentrations using predicted cell concentrations, X , are higher than the observed RDX concentrations. This results from the fact that the model predicts a lower cell concentration which intuitively leads to a lower degradation rate. When the average stationary phase cell concentration, X' , was used in the model, the predicted values for RDX concentrations matched the experimentally observed values very well.

The predicted values for the amount of the mononitroso intermediate during the experiment again agree well the experimentally observed trends as shown in Figure 3. The results and explanations for the mononitroso intermediate values are similar to those found for the RDX values.

CONCLUSIONS

Overall the model predicts the free suspension behavior of the biodegradation of RDX by a consortium fairly well. The predicted values for the cell concentration are lower than those values observed experimentally most likely due to inaccuracies in the aerobic growth parameters. The rate of degradation of RDX has been found to follow first order kinetics where the concentration of bacteria is linearly related to the rate and has been included into the rate constant. The model does a very good job of predicting the concentrations of RDX and the mononitroso intermediate when the correct cell concentration is used. Once, the cell concentrations can be predicted well, this part of the overall model will be complete.

TABLE 1: Experimentally Determined Model Parameters

Yield Coefficients

Anaerobic (no RDX)	$Y_{an_{x/s}}$	0.03256 g cells/g yeast extract
Aerobic (with RDX)	$Y_{a_{x/s}}$	0.2042 g cells/g yeast extract

Growth Parameters

Aerobic	μ_{max}	0.6246 hr ⁻¹
Aerobic	K_s	1.320 g/L
Anaerobic	μ_{max}	0.08401 hr ⁻¹
Anaerobic	K_s	0.8085 g/L

Rate Constants

RDX degradation constant	k_1	0.04230 L/(g cells hr)
Mono degradation constant	k_2	0.065692 L/(g cells hr)

Figure 1: Cell Concentration

Figure 2: RDX Degradation

Figure 3: Mono Intermediate

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