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Modelling U(VI) Reduction by *Pseudomonas stutzeri*

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Pseudomonas stutzeri, a facultative anaerobe sourced from soil in a uranium mine in Limpopo, South Africa, reduced uranium-6 (U(VI)) to uranium-4 (U(IV)) in batches under a relatively high initial U(VI) concentration ranging from 30 to 400 mg/L (pH 5 to 6). U(VI) reduction was rapid during the first 4 to 6 h of incubation followed by slower reduction rates at incubation times longer than 6 hours. Equilibrium conditions were only obtained after incubation for 24 h. The reduced U(VI) was recovered in solution as a hydroxide which was determined to consist of U(IV).
A kinetic model based on enzyme kinetics produced the best fit of the optimised model to experimental data of U(VI) versus time in batch cultures of *Pseudomonas stutzeri*. The parameters: maximum specific uranium-6 reduction rate coefficient (k_u), half velocity concentration (K_u), and uranium-6 reduction capacity of cells (T_c) were estimated using the data from the 200 mg/L batch. The parameters obtained in the 200 mg/L batch were then used to simulate the concentration in the other batches at lower and higher initial U(VI) concentrations. The reduction capacity T_c remained stable but a loss of accuracy was observed with increasing initial U(VI) concentration.
Numerous batch experiments were conducted to establish kinetic parameters that will be used later for scale-up purposes. The knowledge gained from such processes will be of practical value in predicting effluent response to diverse loading conditions.

1. Introduction

1.1 Kinetic Model Calibration

Metal reduction in living bacterial cells can be linked to cellular metabolic processes as illustrated earlier by Wang et al. (1989) and others. In 1993, Shen and Wang (1993) demonstrated that hexavalent metal species such as Cr(VI) can be reduced under sterile conditions using NADH as a sole electron donor. NADH is a critical energy conservation molecule linked to metabolic processes in living cells. In these previous studies, it was assumed that the reaction was catalysed by the membrane bound NADH-dehydrogenase. Other researchers such as Lovley et al. (1993) observed that transmembrane electron carriers such as cytochrome c_3 may also be involved in electron transport coupled to U(VI) reduction in sulphate reducing bacteria.
The biochemical process of metal reduction was hypothesized to involve the transfer of electrons from cytosolic electron donors to the transmembrane electron carriers and protons pumps (Lovley and Phillips, 1994), which in turn transfer the electrons to target metal species (Bopp and Ehrich, 1988; Ishibashi et al., 1990). The metal could either be reduced directly by the membrane electron carriers or reduced indirectly via membrane associated reductases (Chirwa, 2011).
A model is developed in this study in which all U(VI) reduction activity in cells represented by reductase enzymes E_i is replaced by the activity of one representative enzyme (E) in a two-step enzyme reaction as shown in Eq. 1:

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$$U(VI) + E \xleftarrow[k_2]{k_1} E*U(VI) \xrightarrow{k_3} E + U(IV) \quad (1)$$

where E = *U(VI)* reductase, $E^*U(VI)$ = transitional enzyme-U(VI) complex, and k_1, k_2, k_3 are reaction rate constants in the directions indicated by the arrows.

If the U(VI) concentration is represented by U and the enzyme-U(VI) complex by E^* *U(VI)*, then the rate of U(VI) reduction should be equal to U(IV) formation. The overall rate of the reaction can thus be represented by:

$$r_u = -\frac{dU}{dt} = \frac{dU(IV)}{dt} k_3 \cdot E* \quad (2)$$

The formation of E^* can be described by:

$$-\frac{dE*}{dt} = k_1(E - E*)(U) - k_2(E*) - k_3(E*) \quad (3)$$

In the above equation, steady-state conditions prevail as long as E^* is formed and destroyed spontaneously such that $d(E^*)/d(t) \approx 0$. The above mass balance can thus be written as:

$$0 = k_1(E - E*)(U) - k_2(E*) - k_3(E*) \quad (4)$$

After rearranging Equation 4, E^* can be expressed as:

$$E* = \frac{U \cdot E}{U + \frac{k_2 + k_3}{k_1}} \quad (5)$$

Thus, the U(VI) reduction rate in Eq. 2 becomes:

$$r_u = -\frac{dU}{dt} = \frac{k_3 \cdot U \cdot E}{U + \frac{k_2 + k_3}{k_1}} \quad (6)$$

In this equation, k_1, k_2 and k_3 are constants, the groups of constants in Eq. 6 can be replaced by meaningful symbols from enzyme kinetics as follows: $(k_2 + k_3)/ k_1$ can be replaced by the half velocity concentration K_u (mg/L), and k_3 can be replaced by the maximum specific U(VI) reduction rate coefficient k_u (mg/mg/h) such that:

$$r_u = -\frac{dU}{dt} = \frac{k_u \cdot U \cdot E}{U + K_u} \quad (7)$$

For any amount of cells X, the amount of enzyme produced will be proportional to the cell concentration such that the enzyme E (Mulukutla et al., 2012) can be replaced by the total cell biomass term X, if cells are harvested during the log growth phase. This gives a Monod type equation:

$$r_u = -\frac{dU}{dt} = \frac{k_u \cdot U \cdot X}{U + K_u} \quad (8)$$

where U = U(VI) concentration (mg/L) as function of time, k_u = maximum specific U(VI) reduction rate coefficient (mg/mg/h), K_u = half velocity constant (mg/L), X = viable cell concentration (mg/L) at any particular time, and t = time (h).

A similar expression was derived previously by other researchers for Cr(VI) reduction in batch systems for pure cultures of bacteria by (Shen and Wang, 1994) and for mixed cultures (Boonchayaanant et al., 2008).

During non-inhibited state (at very low U(VI) concentration), the only unknown values could be K_u and k_u. Since the experiment was carried out under high biomass concentrations with induced space limitation, we can assume a stationary phase with respect to viable cells X. Under these conditions, the value of X is virtually constant, i.e., $X = X_o$. To determine kinetic parameters, the analytical solution of Eq. 8 could be used and expressed as a function of time as shown below:

$$t = -\frac{K_u}{X_o \cdot k_u} \ln\left(\frac{U_o}{U}\right) + \frac{1}{X_o + k_u}(U - U_o) \tag{9}$$

where X_o = initial biomass concentration (*mg/L*) and U_o = initial U(VI) concentration (mg/L). A simple algorithm can then be used to estimate the values of k_u and K_u using the least-squares minimisation procedure (Mathews, 1992).

In this study, a reaction scheme involving a selected rate equation and kinetic constants for the processes taking place in the batch reactor were chosen from published models on enzymatic toxic metal reduction. Wang and Shen (1997) demonstrated earlier that the rate of reduction of a hexavalent toxic metal (Cr(VI)) by enzymes can be expressed as the Monod form when enzyme activity is the predominant mechanism. However, the active cell concentration, X, decreases in proportion to the amount of toxic metal reduced due to the toxicity of toxic metal:

$$X = X_o - \frac{U_o - U}{T_c} \tag{10}$$

where U_0 (mg/L) is the initial concentration of U(VI); X_o (mg cells)/L is the initial cells density of U(VI)-reducing strains; and T_c (mg U(VI))/(mg cell) is the maximum U(VI) reduction capacity of cells. Substituting Eq. 10 into Eq. 8 yields the following equation:

$$-\frac{dU}{dt} = \frac{k_u \cdot U}{K_u + U}\left(X_o - \frac{U_o - U}{T_c}\right) \tag{11}$$

U(VI) reduction data obtained with the pure cultures and the mixed culture were analysed using Equation 11. The parameters k_u, K_u and T_c were searched within the range of typical values observed in previous studies (Zhuang et al., 2010; Zuo et al., 2012).

1.2 Model Calibration

The model was calibrated using data from the batch reactor which was operated for 48 hours at a time under micro-aerobic redox conditions. Samples were collected at 1 h intervals and analysed for U(VI) concentration using the Arsenazo-III method (Niazi et al., 2007) with samples analysed in a UV-Vis Spectrophotometer at a wavelength of 651 nm for U(VI) and 595 nm for U(IV) (Chabalala and Chirwa, 2010).

1.3 Model Simulation and Parameter Optimization

Kinetic parameters were initialized by guessed values and values from batch studies found in literature for U(VI) reduction, followed by optimization. To ascertain that the values obtained using the mathematical model were reliable, upper and lower constraints were set for each parameter to allow the omission of nonsensical or invalid parameter values. Whenever optimization converged at/or very close to a constraint, the constraint was relaxed until the constraint did not force the model. The procedure was repeated until unique values lying away from the constraints but between set limits were found for each set of parameters (Nkhalambayausi-Chirwa and Wang, 2004).

2. Results and Discussion

Figure 1 shows the model simulations against experimental data of U(VI) versus time in batch cultures of *Pseudomonas stutzeri* at high initial U(VI) concentrations; 200 and 400 mg/L. The accuracy of the model in fitting data sets from different initial U(VI) concentrations is shown by the χ^2 values in Table 1. The data set from the 200 mg/L batch was used to estimate parameters k_u, K_u and T_c.

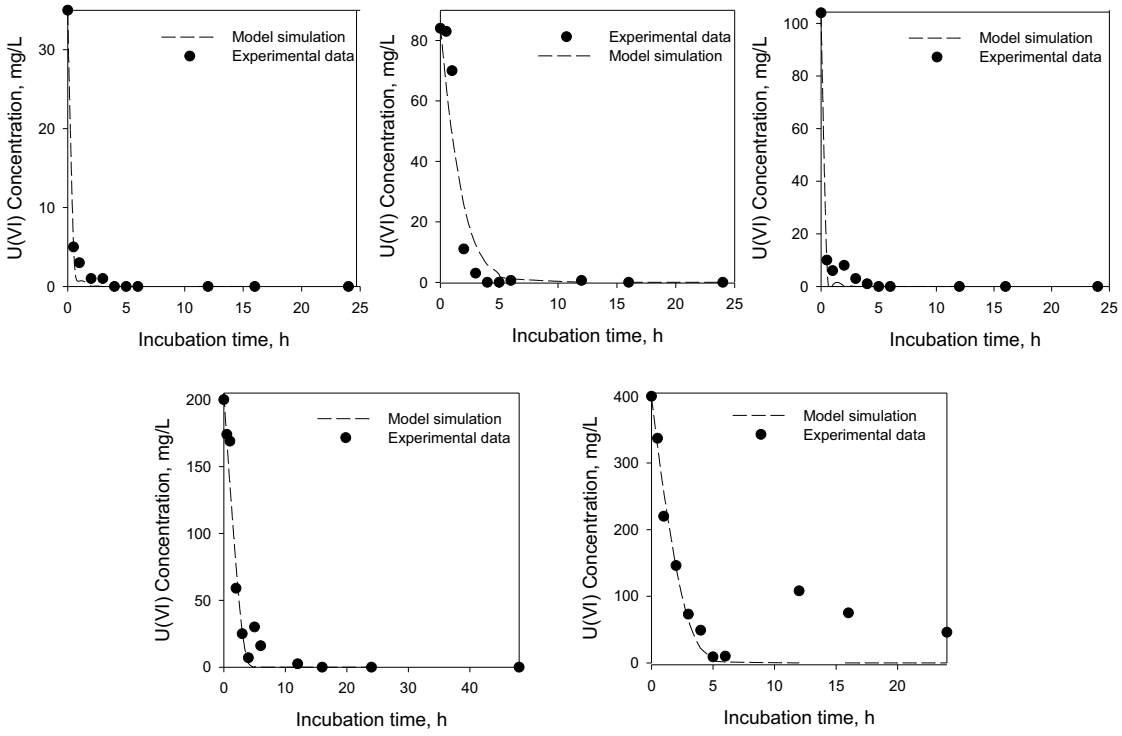

Figure 1: U(VI) reduction in batch cultures of Pseudomonas sp. for concentrations ranging from 30 to 400 mg/L

Using the obtained parameter values, the model simulated U(VI) reduction well at high concentrations only as shown in Figure 1. The reduction capacity T_c remained stable and was much lower than that of the other two species examined under the same conditions. Also, a loss of accuracy with increasing initial U(VI) concentration as illustrated by an increase in the value of chi-square (χ^2).
Pseudomonas sp. viable cells decreased very slowly from an initial concentration of 12.9 to 0.05 (mg cells)/L during U(VI) reduction. Despite such significant cell death, the rate of U(VI) reduction in this culture increased accordingly even after 3 h incubation and eventually approaching zero. In addition, the model only requires the input of the initial biomass and describes the experimental data very well without the need for further consideration of biomass growth or death.

The reduction capacity varied significantly with the initial U(VI) concentration. The removal capacity increased with increasing initial concentration. All the values observed from experimental data (ranging from 35-320 mg/L) fell within the limits of the model and it was found that the higher the X_o, the higher the reduction capacity of the microorganism.

Table 1: Kinetic parameters for U(VI) reduction in Pseudomonas stutzeri

Concentration	K_u	k_u	T_c	X_o	χ^2
mg U(VI)/L	mg U(VI)/L	mg U(VI)/(mg cells)/h	mg U(VI)/(mg cells)	(mg cells)/L	mg^2/L^2
30	99.4	0.01	0.080	42,837	7.6
75	99.8	0.01	0.079	9,288	1,106
100	99.8	0.01	0.081	65,243	105
200	99.6	0.01	0.089	12,213	2,776
400	99.8	0.01	0.083	19,674	21,835

3. Discussion

Currently, the model does not fit certain ranges of data. The parameters are not constant and the model is not fitting certain data ranges properly, therefore further investigation is required. The concentration values in most of the batches show an escalation in the U(VI) concentration after 6 hours followed by a decrease after 15 h suggesting a transitional oxidation process during a certain period of incubation. This is currently not captured by the model. Moreover, the parameters have to be optimized further and constraints be studied properly. The modelling work is on-going so as to address these concerns.

Despite the limitations, we expect that the kinetic expressions and parameters obtained from this study will prove useful for engineering applications. They can be incorporated into reactive transport models used for the design and operation of remediation systems. Because these cultures reduce uranium at a rate comparable to or better than rates found in literature for other microorganisms, reduction rates reported in this paper can be used to assess the applicability of bioreduction for uranium removal processes. Lovely and Phillips (1992) showed that *Desulfovibrio desulfuricans* could reduce an initial 1 mM U(VI) down to 0.1 mM in 3 to 4 h.

It is believed that biological activities are the main mechanisms for U(VI) reduction in these cultures. Such enzymatic reduction of U(VI) was described very well by the developed model. The model's ability to analyze U(VI) reduction can be extended to other strains that are known to reduce U(VI). The model demonstrates a vital characteristics of microbial U(VI) reduction, that is, the rate and extent of U(VI) in batch cultures are dependent upon the reduction capacity, which is regulated by the initial cell density regardless of subsequent growth or death. During U(VI) reduction, a significant decrease in cell density were observed in the pure cultures as well as the mixed culture. For the observed cell density decrease during U(VI) reduction, the reduction capacity in batch cultures may have been caused by termination of metabolic activity due to U(VI) toxicity.

4. Summary

The kinetics of U(VI) in batch cultures of several bacterial species may be adequately described by an enzyme-based mathematical model. Model analysis of U(VI) reduction data indicates that the investigated U(VI)-reducing species (*Pseudomonas stutzeri*) possessed a very high U(VI) reduction capacity. U(VI) reduction in this species may be part of the defence mechanism against U(VI) toxicity or may be independent to the resistance to the toxic metal – this relationship needs to be investigated further. Results from this experimental work will be instrumental in the development of a more predictive model applicable to continuous flow systems. With further improvements, it will be possible to optimise the operation of reactor systems and predict effluent conditions during failure and recovery times. Improvements to the proposed model in this study will include a detailed investigation of the impact of active metabolic processes such as cell growth kinetics.

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