Kinetics of Uranium(VI) Reduction in an Indigenous Culture of Mine Soil Bacteria

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A batch study was performed to investigate the effect of different carbon sources on biological uranium-(VI) reduction. Two homogeneous carbon sources ethanol and acetate and a heterogeneous (natural) carbon source (sawdust) were used and their impact on the U(VI) reduction rate evaluated. These carbon sources acted as carbon sources and electron donors while generating metabolites such as acetate from ethanol that were further degraded to simpler compounds by the bacteria. The cultures showed rapid reduction during the first 3-6 hours of incubation. U(VI) reduction rate determined for the highest concentration, 400 mg/L at the 50 % of added point was as high as 286 mg/L/h. Acetate derived from the sodium acetate salt yielded the best results at high initial U(VI) concentration in batches (200 and 400 mg/L). U(VI) reduction rate kinetics obeyed the modified *Michaelis-Menten* enzyme kinetics with the values of k_u = 20.73 h^{-1}, K_u = 172.04 mg/L, and a cell deactivation rate coefficient T_c = 20.54 mg U(VI) reduced/mg cells deactivated. The model fitted well the experimental data with maximum χ^2 value of and less than 5 % deviation in the evaluated parameters when optimised at different initial U(VI) concentrations.

Keywords: uranium (VI) reduction, organic carbon sources, metabolites, facultative consortium.

Introduction

The utilisation of fossil fuels worldwide depletes the natural reserves and at the same time releases billions of tonnes of carbon dioxide and other green house gases into the atmosphere. Electricity generation from coal has contributed to the fast accumulation of greenhouse gases in the atmosphere. In order to reverse the negative effects of this accumulation, i.e., global warming and climatic changes, countries around the world are now considering nuclear energy as a substitute to the burning of fossil fuels. The deployment of the later technology has progressed slowly due to public opposition due to the discharge of radioactive waste from the nuclear power generation and processing cycle of the fuel elements. . The common fuel element, uranium, is mostly discharged from the nuclear generation processes as the highly toxic uranium-6, U(VI), which is mostly radioactive as well as highly toxic to aquatic life forms.

Natural attenuation processes such as bacterial reductive/precipitation and immobilization of soluble uranium are gaining much interest (Dodge and Francis, 2003). For example, dissimilatory metal-reducing microorganisms have been investigated for their capability to selectively remove uranium from aqueous solutions. These bacteria can use U(VI) as an electron acceptor thereby reducing the highly mobile and toxic U(VI) to U(IV) which is less toxic and easier to remove from solution by precipitation (Lovley and Phillips, 1992). Much research has been dedicated to exploring the mechanism of metal reduction in bacteria. However, the reaction kinetics that would help illucidate the underlying processes have not been researched sufficiently.

Biological treatment of metal pollutants is viewed as an environmentally friendly alternative to conventional physical/chemical treatment methods, especially in dilute solutions where physical/chemical methods may not be effective (Lovley and Phillips, 1992). Microbial processes may be applied both as *in situ* and/or *ex situ* processes. Microbial consortia, consisting of several species of microorganisms in the form of bioflocs for reducing/removing the pollutants have been used as they preserve the complex interrelationships that exist between species in the source.

Currently, four mechanisms have been identified by which bacteria immobilize uranium, namely; revoval by biosorption, removal by bioaccumulation into cells followed whereby the uranium is removed by separation processes such as sedimentation and filtration, precipitation by reaction with inorganic ligands such as phosphate, and microbial reduction of soluble metal ions to the insoluble elemental form (Nancharaiah et al., 2006). In the future, microbial U(VI) reduction may be engineered for the recovery of uranium and other heavy metals from spent nuclear fuel. Metal removal or recovery will help alleviate the toxic metal pollution problem in the environment (Kovacova and Sturdik, 2002).

A fundamental understanding of mechanisms of microbial transformation of uranium under a variety of environmental conditions will be valuable in developing appropriate remediation and waste management strategies as well as predicting the microbial impacts on the long-term stewardship of contaminated sites. The aim of this study is to utilize indigenous cultures of bacteria from the local environment to biologically reduce U(VI) to U(IV).

Materials and Methods

Isolation of Indigenous Bacteria

Bacterial cultures were isolated from cultures grown aerobically and anaerobically at 25-30°C under shaking at 120 rpm in a Rotary Environmental Shaker (Labotec, Gauteng, South Africa). Aerobic cultures were grown in cotton plugged Erlenmeyer flasks whereas anaerobic cultures were grown in 100 mL serum bottles bubbled with pure N_2 gas (99% pure grade) and sealed with silicon rubber stoppers and aluminium seals. After 24 hrs, the cultures were streaked onto nutrient agar plates after making up serial dilutions. Single colonies were then picked and transferred to new nutrient agar plates. All chemicals were analytical reagent grade and deionized water was used in all experiments.

Sample collection and Preparation

For uranium analysis, 0.5 mL of the homogenous solution was collected using a syringe and then centrifuged using a Minispin® Microcentrifuge (Eppendorf, Hamburg, Germany). The 0.5 mL sample was then diluted with 4.5 mL of BMM (1:10 dilution) prepared according to Chabalala 2010, mixed with 2 mL of complexing reagent and analyzed for U^{6+} immediately. For microbial analysis, manual counting was performed through the use of a Petroff-Hausser counting chamber employing dark-field microscopy. A 1:100 dilution of bacterial cultures were prepared using distilled water. Diluted culture samples were then loaded individually into the counting chamber and enumerated under the dark-field microscope at a magnification of 400x. Each manual count was performed with a freshly cleaned and loaded chamber.

U(VI) Reduction experiments

U(VI) reduction experiments were conducted under anaerobic conditions as described earlier in Chabalala and Chirwa (2010). These were inoculated from single colonies of *Pseudomonas* sp., *Pantoea* sp. and *Enterobacter* sp. All batch studies were carried out in 100 mL Serum Bottles using Mineral Salt Medium (MSM). Batches were purged with N_2 for 5 minutes before closing the bottles. Uranium solutions of different concentrations (30 – 400 mg/L) were prepared in Basal Mineral Medium. These bottles were incubated at 30°C for a predetermined time interval at 120 rpm on the orbital shaker (Labotec, Gauteng, South Africa).

Preparation of Sample for Measurement

Arsenazo III (Sigma-Aldrich, St. Louis, MO) (1,8-dihydroxynaphthalene-3,6-disulphonic acid-2,7-bis[(azo-2)-phenylarsonic acid]), a non-specific chromogenic reagent, was selected as the complexing agent for facilitating uranium(VI) detection. The oxidized fraction of uranium was measured from a sample (0.5 mL) of the homogenous solution collected using a syringe and then centrifuged using a Minispin® Microcentrifuge (Eppendorf, Hamburg, Germany).

The 0.5 mL sample was then diluted with 4.5 mL of BMM (1:10 dilution), mixed with 2 mL of complexing reagent and analyzed for U(VI) immediately at a wavelength of 651 nm against a reagent blank. Total uranium level in each sample (U(IV) and U(VI)) was determined by oxidizing an unfiltered sample with nitric acid prior to uranium measurement. This treatment converted U(IV) in the sample to U(VI) which was then measured colorimetrically as described above.

The accuracy and precision of the method was determined by measuring the

concentration of standard uranium solutions in the range of 0.02 mg L^{-1} to 1 mg L^{-1} after appropriate dilution. The results showed that recovery of uranium was quantitative with good precision (92-100%). The percentage deviation was found to be at a maximum (0.4%) at dilution 0.5 mg L^{-1} whereas, the deviation decreased to zero when the concentration was decreased to 0.02 mg L^{-1}.

U(VI) Determination

A colorimetric method was used to measure the U(VI) and U(IV) concentrations using a UV/Vis spectrophotometer (WPA Lightwave II, Biochrom, Cambridge, England). The concentration of hexavalent uranium U(VI) in the sample complexed to a chromatogen was measured by absorbance of light with a wavelength of 651 nm.

Total Organic Carbon

Total organic carbon (TOC) was measured using Total Organic Carbon Analyzer (Model TOC-VWP, Shimadzu Corporation, Kyoto, Japan) after acidifying a 40 mL of sample with one drop of concentrated orthophosphoric acid (Merck, SA). 0.5 mL of the sawdust solution was dissolved in basal mineral medium and filled up to 1 L with ultrapure water to make a 0.5 % solution. Standards were prepared from different dilutions of a potassium hydrogen phthalate stock solution. The potassium hydrogen phthalate stock solution was prepared by dissolving 2.1254 g (anhydrous) (Merck, SA) in 1 L of ultrapure water which is equivalent to 1000 mg/L. TOC was determined with a precision of ±0.5 mg carbon/L in all samples.

Electron Donor Variation Study

Three different carbon sources were chosen for this study; Ethanol (Merck, SA), Sodium Acetate (Merck, SA) and Organic Carbon source in the form of Sawdust from a wood workshop. 0.5 % solutions of these electron donors were made in basal mineral medium and used for reduction experiments.

Results and Discussion

Impact of Electron Donor on Uranium U(VI) Reduction

Reduction and immobilization of microbially-reduced U(VI) is of great concern for *in situ* uranium bioremediation. This study investigated the effect of carbon sources; Sodium Acetate (NaAc), Ethanol (EtoH) and Sawdust (Organic carbon source) on uranium reduction all at 5 g/L for each experiment.

Influence of Different Carbon Sources on Rate of U(VI) Reduction

At Low U(VI) Concentrations

At 50 % of added U(VI), the rate of reduction for Sawdust was at 30 mg/L/h which was the highest compared to that of EtOH and NaAc, also Sawdust reached zero after 3 hours of incubation. At 75 % of added U(VI), the mixed culture was reducing the fastest in Sawdust carbon source, at 42 mg/L/h, closely followed by NaAc and EtOH. Total U(VI) values were varying from 40 - 78 %, after an hour, the experiments carried out in NaAc had a uranium recovery of 78 %, after 3 hours, a recovery of 67 % and lastly after 6 hours, a recovery of 52 %, there was a definite decrease in total uranium recovery, whereas the experiments carried out in Sawdust there was an increase in total uranium recovery. EtOH on the other hand, in the first hour total uranium concentration had been half the amount initially began with, then wnet up to 73 % and finally after the experiment, it went back to 50 %.

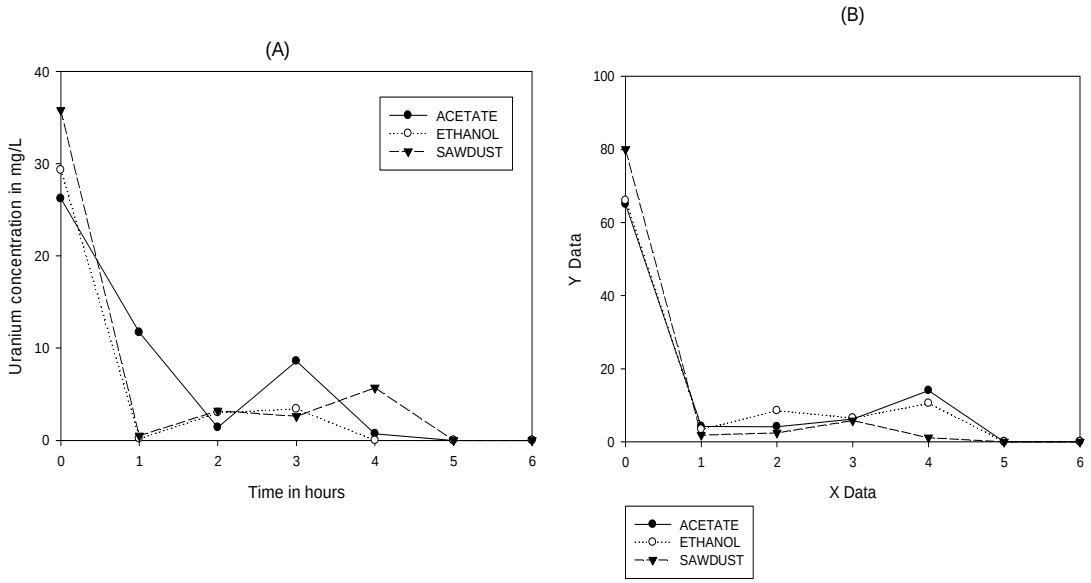

Figure 1.1. Mixed culture under varied carbon sources, A: 30 mg/L, B: 75 mg/L.

The culture grown in medium with Sawdust as an electron donor reduced all the U(VI) within 4 hours of incubation, the other two carbon sources only reached zero after 5 hours of incubation. Recovery of uranium at the end of the experiment was very low in NaAc and EtOH, and moderate in Sawdust. By the end of the experiment all the carbon sources total uranium concentrations were at their lowest between 20 – 26 %.

At the beginning of the experiments, the total uranium concentrations were at around 60 % and after the experiment, it went down drastically except for the NaAc experiment which ended at 100 % of added U(VI). Sawdust and EtOH carbon sources reduced U(VI) levels to zero within the first hour, but after that the data was scattered until they reached zero after 5 hours of incubation. There was a definite trend in terms of carbon sources but in the lower concentrations (35 – 75 mg/L) Sawdust seems to be the carbon source that allows for U(VI) concentrations to reach zero first. For the higher concentrations (200 – 400 mg/L), NaAc is the carbon source where U(VI) levels reached zero first.

At High U(VI) Concentrations

At 400 mg/L of U(VI), mixed culture reduced approximately 93% of added uranium within the first hour for all carbon sources, moreover, 84-99% of U(VI) was also recovered at the end of the experiment for all carbon sources. It has to be noted that the uranium concentration of the batch culture grown in NaAc, reached zero first although the U(VI) rised again after 3 hours only to go back to zero after 5 hours.

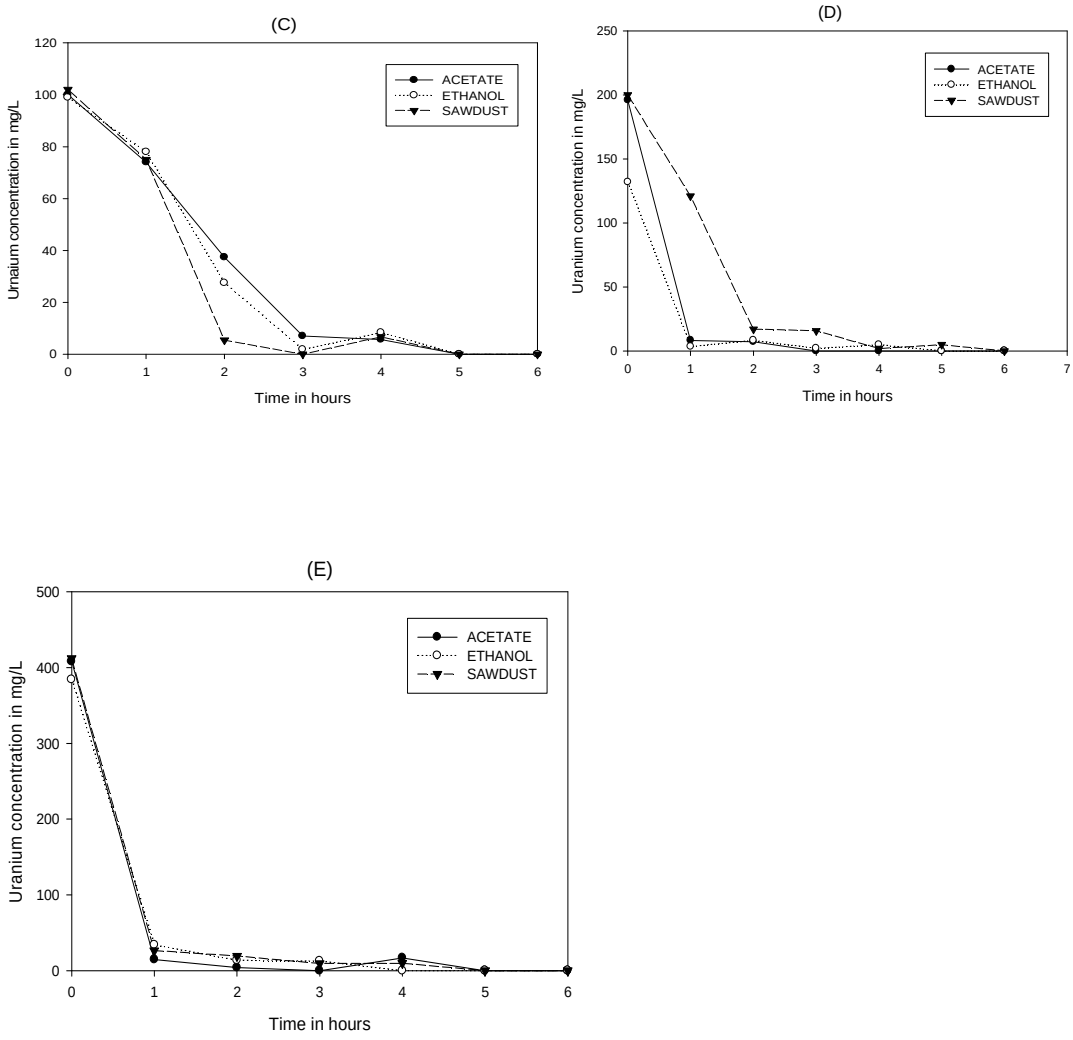

Figure 1.2 Mixed culture under varied carbon sources, C: 100 mg/L, D: 200 mg/L, E: 400 mg/L.

The rate of reduction (mg/L/h) at 50 % of added U(VI) for NaAc and Sawdust was very high at 285 mg/L/h, EtOH was just under that at 253 mg/L/h. Looking at the very high concentrations of total U(VI), we can conclude that recovery rates are very good and that the values are remaining relatively constant shows that U(VI) is being reduced to another form and when treated with HNO_3 all these forms are oxidized to U(VI).

U(VI) recovery rates ranging from 75 - 100 % for all carbon sources were recorded. The mixed culture with EtOH and NaAc as electron donors, reached zero first within the first 3 hours of incubation, the experiment carried with Sawdust only reached zero after 4 hours of incubation. The highest rate of reduction was observed in NaAc followed by EtOH then sawdust.

Kinetics of U(VI) Reduction by the Consortium

Kinetic Model Development

To model the system, the reaction scheme, rate equations and kinetic constants for the processes taking place in the batch reactor were chosen from published models on enzymatic (UVI) reduction. Shen and Wang (1997) demonstrated that the rate of U(VI) reduction by enzymes can be expressed as the Monod equation (1) below when enzyme activity is the predominant mechanism of U(VI) reduction in bacterial cells:

$$-\frac{dU}{dt} = \frac{k_u U}{K_u + U}\left(X_0 - \frac{U_0 - U}{T_c}\right) \qquad (1)$$

U (mg/L) is the concentration of U(VI) at time *t* (h); *X* (mg cells/mL) is the density of active bacterial cells at time *t*; k_u (mg U(VI)/mg cells/h) is the specific rate of U(VI)

reduction; and K_u (mg U(VI)/L) is the half velocity constant. However, the active cell concentration, X, may be assumed to decrease in proportion to the amount of U(VI) reduced due to the toxicity of U(VI). U_0 (mg/L) is the initial concentration of U(VI); X_o (mg cells/mL) 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.

Results

The reduction capacity of the mixed culture was up to 234 mg/L with an initial maximum biomass of 11.94 mg cells/mL at an initial U(VI) concentration of 200 mg/L. The infinite reduction capacity of U(VI) reduction was further demonstrated in this culture as a rise in biomass concentration resulted in a rise in reduction capacity $T_C X_O$ in all experiments. 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 subsequent cell growth/death. Therefore the model equation is appropriate to describe the toxic effects of U(VI) on U(VI) reduction. An inverse relationship was observed between the cell concentrations and U(VI) concentration. Statistical comparisons were made between the experimentally determined data and the best fit model defined by the three constants by using coefficients of determination, r 2. In 90% of the experiments, r 2 was 0.91 or better. The comparable kinetic values for both the mixed and pure cell cultures for the reduction of a range of U(VI) concentrations are similar as seen in Table 1.1.

Table 1.1 Kinetic parameters for U(VI) reduction in the consortium consisting of *P. stutzeri*, *P. agglomerans* and *E. cloacae.*

Concentration (mg U(VI)/L)	K_u **(mg U(VI)/L)**	k_u **(mg U(VI)/cell/h)**	T_c **(mgU(VI)/mg cell)**	***Xo*** **(mg cells/mL)**	$\chi 2$
30	178.4	19.2	20.4	14.2	210.3
75	179.1	18	24.9	8.1	1948.7
100	178.7	19.2	21.5	43	113.5
200	179.2	18	19.6	11.9	5029.1
400	168.5	20.9	19.4	20.3	5763
600	160.4	24.9	19	10.1	47930.2
800	160	24.9	19	10	58450.7

Using the obtained parameter values the model simulated U(VI) reduction very well for the upper limit concentration 100, 200, 400 mg/L as shown in Fig. 1.1. The value of T_c decreased slightly with an increase in uranium concentration. The majority of uranium reduction occurs in the first 5 hours of incubation and the model captures that information. Cultures assumed to be in exponential phase at the time of transfer. Other studies by Spear (1999) showed a lag time for uranium removal because of a slower initial uranium removal rate. They proposed a model based on a modified Monod non-growth model that includes a rate-limiting reactant term. The model was fit to experimental data for uranium concentration 100 mg/L fitting the data well with R^2 of 0.9 and yielded values for k_u, K_u and $T_{c,}$ these values were kept constant to simulate the other concentrations. The predicted model of concentrations higher or lower than that did not quite fit the experimental data.

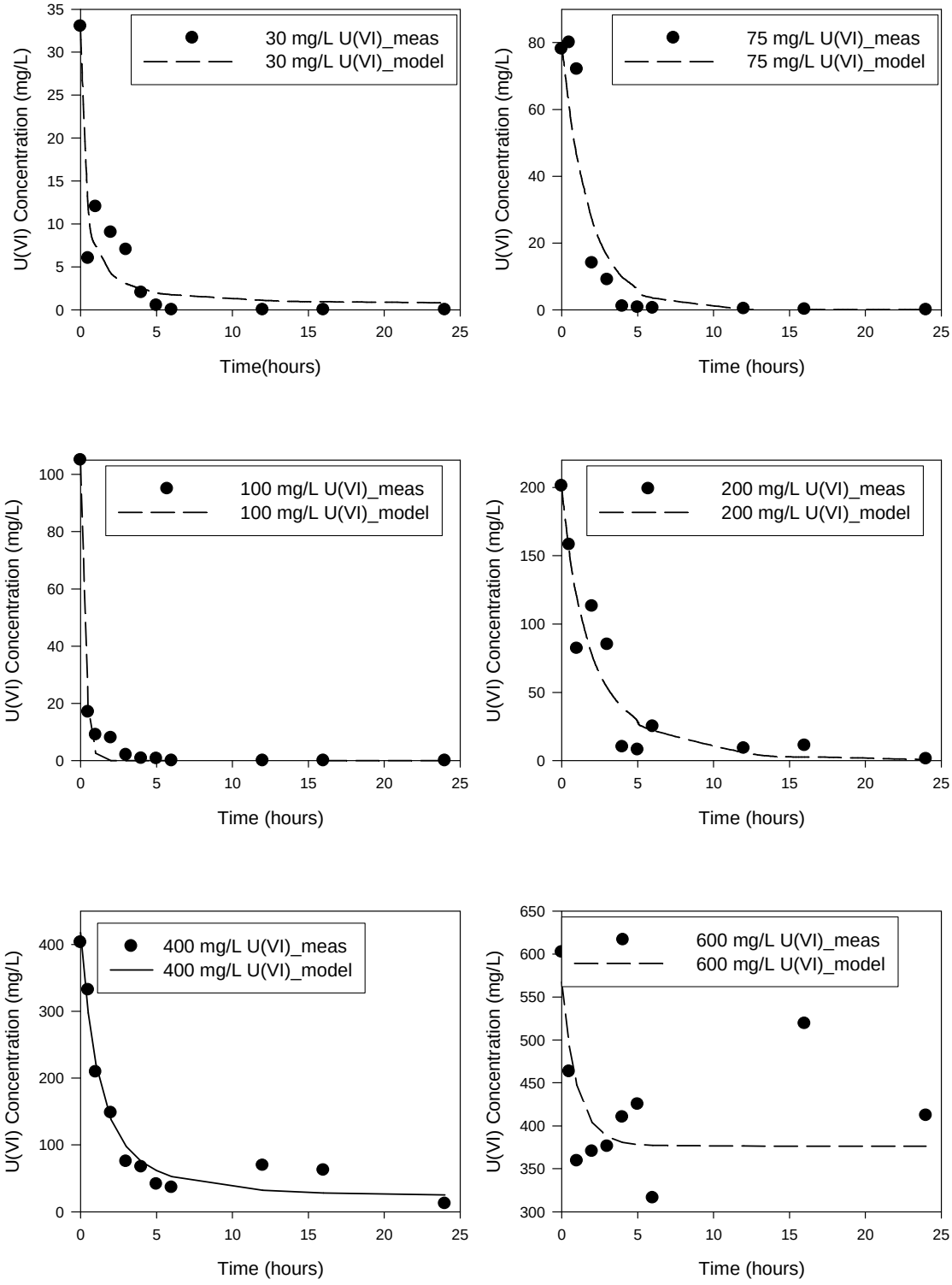

Figure 1.3 U(VI) reduction in batch cultures of the mixed culture for concentrations ranging from 30 to 400 mg/L.

Conclusion

Sawdust is the superior carbon source to ethanol and sodium acetate because of the high carbon content for the micro-organisms to utilize. But also, it is inferior to glucose because the measured values of total organic carbon in the heterogeneous carbon source were found to be 32.5 mg carbon/L of medium, whereas glucose was calculated to be a high value of 1.8 g carbon /L glucose. Nevertheless, sawdust as a carbon source is a viable alternative as it is available in abundance and is cheap to obtain. Ethanol was expected to increase the rate of reduction of the cultures as it provides both the carbon source and the electron donor in the forms of ethanol, acetate (metabolic intermediate), and methanol (an impurity in industrial ethanol) but it performed poorly (Cardenas et al., 2008). In this study, the low reduction rates recorded for acetate can be attributed to the fact that the U(VI) may have been unavailable for reduction due to U(VI)-acetate complexation and/or poor growth of anaerobic microorganisms capable of degrading acetate (Duff et al., 1999).

All carbon sources tested promoted consortium activity and stimulated the reduction and immobilization of aqueous uranium by the indigenous microbial community. It has been reported that the particular electron donor chosen affects not only the rate of uranium removal from solution, but also the extent of U^{6+} conversion to U^{4+}.

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 hours.

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