

## Project 90258

Work has been performed in two areas; the reduction of uranium by bacteria and the characterization of cellulose degraded by bacteria. The uranium reduction study utilized *Shewanella oneidensis*, a widely distributed species of bacteria known to utilize several elements such as iron, manganese and sulfur as electron acceptors. *Shewanella oneidensis* was grown aerobically approximately 24 hours at room temperature in Tryptic Soy Broth (TSB). Cells are then concentrated by centrifugation and washed thoroughly with NaHCO<sub>3</sub> (2.5g/L) buffer. A small sub-sample of cells are stained with DAPI and counted under a microscope for accurate determination of cell number. The cells are then diluted in NaHCO<sub>3</sub> to the appropriate concentration of 2\*10<sup>8</sup> cells/mL. No cells were added as a control. A 5mL solution of cells is then transferred into 100mL of sterile anaerobic bicarbonate buffered freshwater medium with the phosphate removed and 5mM lactate as the carbon source. At several time points three 1mL samples are removed from each of the batch experiments and the control into 1.5mL eppendorf tubes containing 0.1mL formaldehyde. The samples are then removed from the anaerobic environment and frozen until analysis. Individual samples are filtered (0.2 micron pore size) to remove any uraninite and cellular material and diluted with 4mL 0.1M HNO<sub>3</sub>. The concentration of uranyl present in each sample was measured using UV-Visible spectroscopy. Data are fit to a first order model as follows:  $[U]_t = [U]_0 e^{-kt} + [U]_{eq}$  where  $[U]_0$  is the initial uranyl solution concentration,  $[U]_t$  is the uranyl solution concentration at time  $t$ , and  $[U]_{eq}$  is the uranyl concentration remaining in solution at equilibrium. The use of the first order fit was based on previous studies and publications. The reduction of U(VI) to U(IV) was evident by a reduction in the uranyl present in the media over time and the accumulation of a brown-black precipitate determined from previous experiments to be uraninite by XRD. Results showed that the reduction reaction occurs only in live cells, those killed with heat or formaldehyde or inhibited by cyanide and molybdate did not exhibit significant uranyl reduction, only an initial sorption indicated by an immediate drop in uranyl concentration without further reduction over time. The resulting reaction constant was found to be 0.155 +/- 0.027 hr<sup>-1</sup>. This value is consistent with our previous studies on reduction rate as a function of cell number. The kinetics of the reduction reaction by *S. oneidensis* also show that the process fits well to first order, however, without the initial lag period observed by Spear et al for *Desulfovibrio* and *Clostridium*. Our analysis shows an immediate decrease in solution uranyl concentration most likely due to sorption to the cells. This implies that the mechanism for the reduction may include an initial sorption of the uranyl ions to the cells or some cofactor followed by an enzyme mediated conversion of uranyl to uraninite, and also gives support for the difference in reductive capacity with cell density. While adsorption occurs regardless of whether the cells are alive or not, significant uranyl reduction occurs only in the presence of live cells, an indication that the process is not just the result of an abiotic interaction between cellular material and uranyl ions. What remains to be determined is, whether this reaction consists of a series of processes occurring in sequence with a first order reaction being rate-limiting under optimal conditions, or of some other complex mechanism which emulates a first order process. The characterization of the degraded cellulose was performed in conjunction with our project partners at Brookhaven National Laboratory. The Brookhaven team provided cellulose samples degraded by bacteria that were prepared 29 January 1992. The cellulose material was filter paper and paper towels. Some variation in the sample preparation was performed. The resulting samples were unamended (U); unamended/inoculated (UI); amended/inoculated (AI), and amended/inoculated/excess nitrate (AINO<sub>3</sub>). The same exact treatments were also prepared with 5 g bentonite per sample (at the time, a potential backfill material) and labeled BU (unamended), BUI (unamended/inoculated), BAI (amended/inoculated), and BAINO<sub>3</sub> (amended/inoculated/excess nitrate). The samples were incubated at 30 C and sampled periodically for gas production and aqueous chemical characteristics to quantify the rate and extent of microbial degradation of cellulose for WIPP Performance Assessment (PA) models. The studies at UNLV will evaluate the complexation of actinides (U, Pu) with the degraded material in both the solid and solution phase. Quantification of the actinide complexation will be based on similar studies with humic acid [J.I. Kim and K.R. Czerwinski: Complexation of Metal Ions with Humic Acid: Charge Neutralization Model. *Radiochimica Acta* 73, 5 (1996)]. The initial step in this experiment is the determination of the proton exchange capacity. The degraded cellulose is separated from the supernate brine solution prior to preparation of both phases. The cellulose is thoroughly washed with 0.1 M hydrochloric acid, rinsed with water and dried prior to analysis, insuring protonation and the removal of salt. The titrations are performed under argon atmosphere in a 50 mL jacketed titration vessel at room

temperature with a magnetic stir bar to ensure mixture. The samples are analyzed by an Epson 736GP Titrino system with a base titrant, 0.1000 +/- 0.0002 N sodium hydroxide (VWR). Between 0.2 and 0.8 grams of degraded cellulose are added to the titration vessel along with 10 mL of DI water. The sample is stirred for 30 minutes prior to titration. The titrant is added in 0.05 mL increments every 5 minutes. The average proton exchange capacities (PEC) and standard deviations (1 sigma) are listed below. The pH titrations were performed in order to determine the proton exchange capacities of the degraded cellulose samples using the equation:  $PEC = [OH^-]V_{eq}/m$  where PEC is in eq/g,  $[OH^-]$  is the concentration of hydroxide in mol/L, the equivalence volume in L and m the mass of dried degraded cellulose in g. The PEC values will be used for complexation studies with europium uranium, and plutonium. Proton exchange capacities for degraded cellulose samples. Sample Avg PEC (meq/g) Standard deviation AIC 2.228E-01 0.0114 AINO3C 1.710E-01 0.0246 UC 1.824E-01 0.0323 UIC 2.132E-01 0.0450