MICROBIAL REDUCTION OF URANIUM USING CELLULOSIC SUBSTRATES

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ABSTRACT Previous work at the University of New Mexico and elsewhere has shown that sulfate-reducing bacteria are capable of reducing uranium from the soluble +6 oxidation state to the insoluble +4 oxidation state. This chemistry forms the basis of a proposed ground water remediation strategy in which microbial reduction would be used to immobilize soluble uranium. One such system would consist of a subsurface permeable barrier which would stimulate microbial growth resulting in the reduction of sulfate and nitrate and immobilization of metals while permitting the unhindered flow of ground water through it. This research investigated some of the engineering considerations associated with a microbial reducing barrier such as identifying an appropriate biological substrate, estimating the rate of substrate utilization, and identifying the final fate of the contaminants concentrated in the barrier matrix. The performance of batch reactors and column systems that treated simulated plume water was evaluated using cellulose, wheat straw, alfalfa hay, sawdust, and soluble starch as substrates. The concentrations of sulfate, nitrate, and U(VI) were monitored over time. Precipitates from each system were collected, and the precipitated U(IV) was determined to be crystalline $UO_{2(s)}$ by x-ray diffraction. The results of this study support the proposed use of cellulosic substrates as candidate barrier materials.

KEY WORDS: sulfate reduction, uranium reduction, permeable barriers

INTRODUCTION

Uranium-contaminated ground and surface waters near abandoned mill tailings piles are matters of concern in many western United States. Inexpensive and effective remediation techniques are needed which separate uranium and other contaminants from aqueous waste plumes. In nature, uranium generally exists in either the U(VI) or the U(IV) oxidation state. The oxidation-reduction and acid-base chemistry of uranium under equilibrium conditions is conveniently summarized in an Eh-pH diagram (Figure 1) [1]. This diagram shows that under oxidizing conditions U(VI) is present as the uranyl ion UO_2^{2+} which is soluble in water and, therefore, mobile in the aqueous environment. Under reducing conditions insoluble U(IV) is stable and presents a smaller threat to water resources. In recent years, several researchers have discovered that anaerobic bacteria can mediate the reduction of U(VI) to U(IV). Sulfate-reducing bacteria have been found to be capable of reducing U(VI) with subsequent precipitation of U(IV) species [2, 3].

This knowledge of uranium chemistry forms the basis of a proposed remediation strategy for containment and remediation of ground water associated with a Uranium Mill Tailings Remedial Action (UMTRA) site near Shiprock, NM. The proposed system involves the use of a permeable biological reaction barrier.

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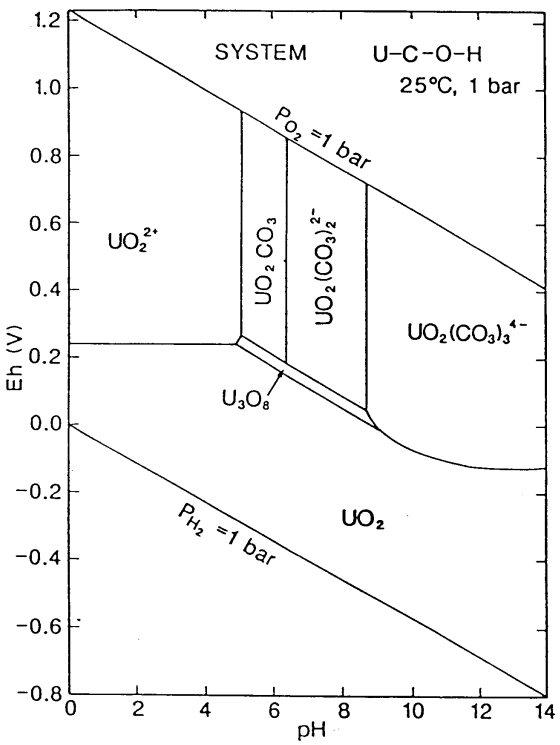

FIGURE 1. Eh-pH DIAGRAM FOR THE U-C-O-H SYSTEM. TOTAL CONCENTRATION OF U = 10^{-6} M, C = 10^{-3} M.

A permeable barrier is defined as an *in situ* wall that intercepts a contaminated ground water plume and treats it through various physical, chemical, or biological reactions. Remediated ground water passes through the barrier with minimal impact on the ground water hydrology [4, 5]. A conceptual representation of the proposed permeable reaction barrier is presented in Figure 2.

The tailings pile at Shiprock, NM, was one of the first to be stabilized under the UMTRA project. Stabilization consisted of consolidating the tailings and contaminated soils in a 31 ha pile which is covered with a 2 m thick compacted clay cover to serve as a radon barrier, and a riprap cover for erosion protection. However, no measures were taken to protect or remediate the ground water. A summary of ground water quality

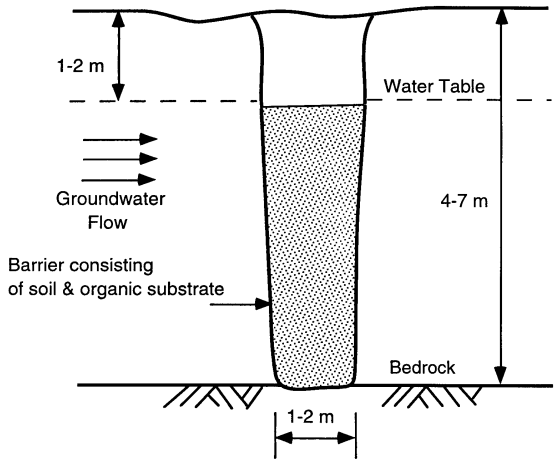

FIGURE 2. CONCEPTUAL REPRESENTATION OF THE PROPOSED PERMEABLE REACTION BARRIER.

characteristics at the Shiprock UMTRA site is presented in a companion paper [5].

The principal contaminants at the Shiprock site include high concentrations of U, SO_4^{-2}, NO_3^-, and selenium (Se). The mechanisms for removal of the contaminants are microbial reduction of NO_3^- and SO_4^{-2} and subsequent precipitation of U. A sequence of reactions is expected to occur which are listed below in the order of decreasing redox potential:

O_2 consumption:

$$\text{Organic Carbon} + O_2 + \text{Nutrients} = \text{Biomass} + H_2O + CO_2 \quad (1)$$

Denitrification:

$$\text{Organic Carbon} + NO_3^- + \text{Nutrients} = \text{Biomass} + N_{2(g)} + H_2O + CO_2 \quad (2)$$

Sulfate reduction:

$$\text{Organic Carbon} + SO_4^{-2} + \text{Nutrients} = \text{Biomass} + HS^- + H_2O + CO_2 \quad (3)$$

Under strongly reducing conditions provided by sulfate reduction, U(VI) has been found

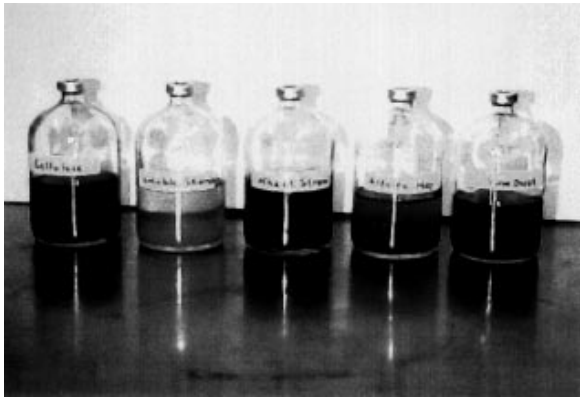

FIGURE 3. BATCH REACTORS.

to be removed as insoluble U(IV) phase $UO_{2(s)}$. However, most U(VI) reduction work to date has focused on pure cultures of sulfate-reducing bacteria using low molecular weight organic acids as substrates. These substrates are not practical for an actual application due to their high cost and the fact that they would be quickly degraded, thus providing a short usable life for a remediation process. The ideal barrier material should satisfy the following criteria

1) support bacterial growth over the design period of the permeable barrier,
2) be relatively inexpensive and readily available, and
3) comprise a manageable volume for use in such a barrier.

It is postulated that plant residues can meet these criteria.

The purpose of this study was to examine the suitability of using low-cost cellulosic materials as substrates for mixed culture anaerobic bacterial growth. Potential carbon sources chosen in this study were wheat straw, alfalfa hay, sawdust, commercially-available cellulose and soluble starch.

The focus of this paper is to address some fundamental aspects of using a permeable barrier such as:

1) identification of an appropriate substrate for growth of bacteria,
2) estimating substrate utilization rates, and
3) determine the final form of the contaminants concentrated in the barrier matrix.

EXPERIMENTAL SECTION

The suitability of cellulosic substrates for mixed culture sulfate reduction was investigated using two experimental methods:

1) batch studies, and
2) laboratory column studies.

Batch experiments: Materials and methods

Batch experiments were carried out in 250 ml glass bottles acting as batch reactors (Figure 3). Organic substrates and nutrients were added to each bottle. The bottles and caps were autoclaved at 250ºF for 30 minutes. The bottles were then sealed with rubber butyl stoppers and aluminum caps in an aseptic environment. Filter sterilized U(VI) was added from a 10 mM stock solution of uranyl nitrate to obtain a U(VI) concentration of 1 mM. The reactors were then inoculated with a mixed bacterial culture collected from ground water at the Shiprock UMTRA project site. The head space in each reactor was purged with nitrogen gas for 15 minutes to provide an anaerobic environment. A modified minimal salts solution was used as a growth medium. Sodium bicarbonate was incorporated as a buffer in place of phosphate to prevent U(VI)-phosphate precipitation. The growth

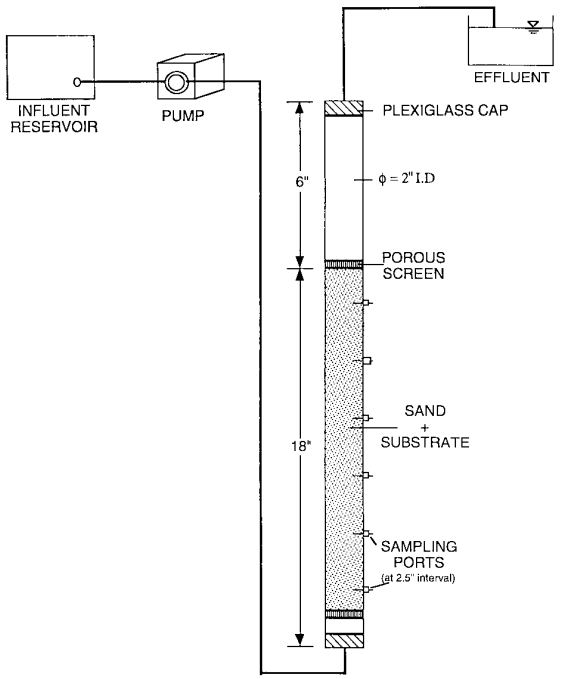

FIGURE 4. SCHEMATIC DIAGRAM OF THE COLUMN SYSTEM.

medium consisted of, per liter: KCl, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $NaHCO_3$, 2.5 g; Na_3-citrate, 14.705 g; K_2HPO_4, 0.005 g; Na_2SO_4, 4.0 g; $FeSO_4 \cdot 7H_2O$, 0.1 g; $NaNO_3$, 1.0 g; L-cysteine hydrochloride monohydrate, 0.15 g; yeast extract, 0.1 g; resazurin, 0.001 g; trace metals solution, 20 ml; carbon source, 5.0 g; the pH was adjusted to 7.0 with 50% NaOH.

Sampling and analyses were carried out at 0, 1, 3, 5, 10, 20, 38, 60, and 90 days. Uranium concentration in the samples was measured by a colorimetric method using a hexanol extraction technique [6]. The Lowry method for protein measurement was used to determine the biomass concentration [7]. Sulfate and nitrate concentrations in the samples were analyzed by ion chromatography using a Dionex Model 2110i. Samples were filtered using Gelman Acrodiscs, pore size 0.2 μm. Cellulose was analyzed using the sulfuric acid digestion method [8]. The method involves extraction with an acetic acid/nitric acid reagent to remove lignin, hemicellulose, and xylosans, followed by digestion with 67% sulfuric acid and final determination using the anthrone reagent. Transmission electron microscopy and x-ray diffraction were used to identify the phase of the reduced uranium.

Laboratory column experiments: Materials and methods

Two inch diameter Plexiglass columns, 2 feet long, were used to conduct column tests (Figure 4). Influent and effluent ports were separated from the packing material by 2 stainless steel screens. Sample ports were machined into the column walls at 2 inch intervals consisted of Teflon fittings (Swagelok #7/16-20) to allow sampling using a hypodermic needle attached to a syringe. Each carbon source was dispersed in the sand in the column. Wheat straw and alfalfa hay were ground to less than 2 mm using a coffee and spice mill. Sawdust passing through a #10 sieve was used. The different carbon sources were mixed with treated silica sand in 100 g portions to obtain a fraction of organic carbon by weight of 2.5%. The feed solution had a chemical composition similar to that of ground water at the Shiprock site. The feed solution consisted of, per liter: Na_2SO_4, 22 g; $NaNO_3$, 5.5 g; $NaHCO_3$, 2.5; Na_3-citrate, 14.705 g; K_2HPO_4, 0.005 g; $FeSO_4 \cdot 7H_2O$, 0.1 g; KCl, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; trace metals solution, 20 ml; the pH was adjusted to 7.0 with 50% NaOH.

The simulated plume water was pumped upflow through the columns at a flow rate of 15 ml/hr. Flow rates were adjusted to obtain a hydraulic residence time of one day. About two pore volumes of influent solution were then passed through the column to prepare the column for the introduction of the

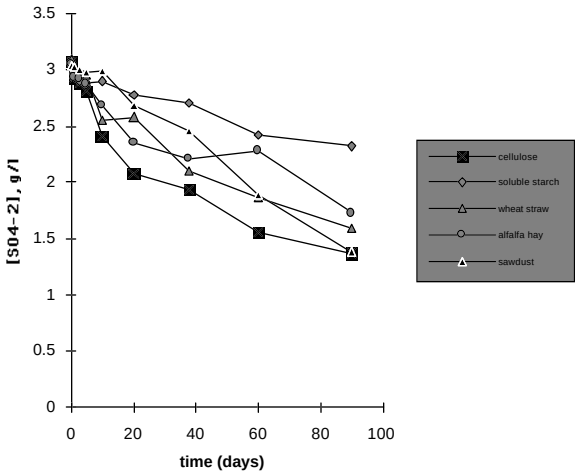

FIGURE 5a. REDUCTION OF SULFATE WITH TIME IN BATCH REACTORS.

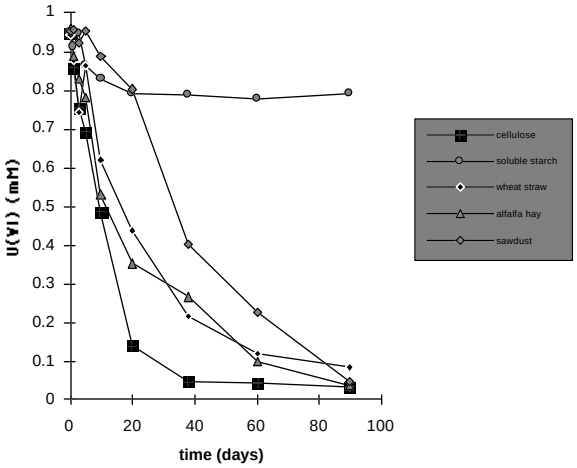

FIGURE 5b. REDUCTION OF SOLUBLE U(VI) WITH TIME IN BATCH REACTORS.

bacterial inoculum. Each column was inoculated with one pore volume of the mixed bacterial culture from the Shiprock site. Effluent samples were collected on a daily basis. Initial samples were analyzed for SO_4^{-2}, NO_3^- and U. Samples were also collected, at weekly intervals, from the various sampling ports along the length of the columns to generate preliminary vertical concentration profiles. Substrate utilization was estimated by measuring the chemical oxygen demand (COD) of representative soil samples at the beginning and conclusion of the column experiments. The closed-reflux colorimetric method (Standard Method 5220D) was used for the determination of COD [9].

RESULTS AND DISCUSSION

Batch experiments

Concurrent reduction of uranium U(VI) and sulfate

The reduction of sulfate and soluble uranium in the batch reactors was plotted for all the five substrates and is shown in Figures 5a and 5b. As seen from the figures, the batch reactor containing soluble starch as the sole carbon source did not show any appreciable reduction of either sulfate or soluble uranium. Soluble starch is more rapidly degraded than the other solid organic carbon sources tested. Under anaerobic conditions a fermentation occurs with the formation of appreciable amounts of lactic, acetic, and butyric acids [10]. Acidic conditions are unfavorable for the existence of sulfate reducers which explains the limited reduction of sulfate and uranium.

Transmission electron microscopy and energy dispersive x-ray spectroscopy

The black precipitate resulting from the batch reactors was mounted on a copper grid and air-dried overnight. The precipitate was then examined by transmission electron microscopy at the Department of Earth and Planetary Sciences, University of New Mexico, using a JEOL 2010 electron microscope operating at 200 kV. The elemental composition of the precipitate was determined by energy-dispersive X-ray analysis.

Figure 6a shows a transmission electron micrograph of the precipitate. The

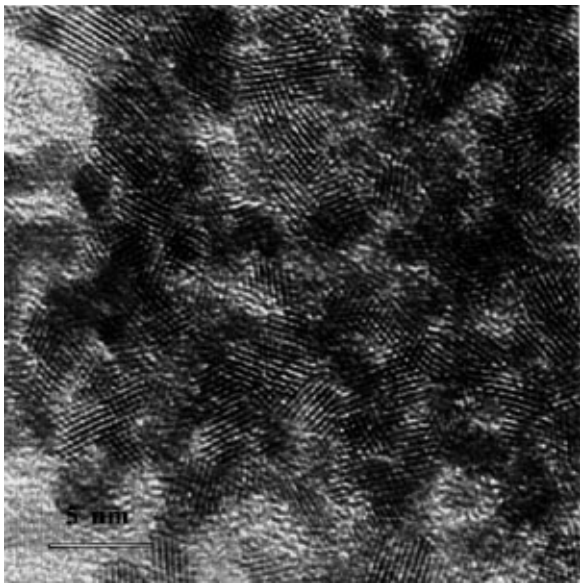

FIGURE 6a. TRANSMISSION ELECTRON MICROGRAPH OF THE ELECTRON DENSE U(IV) PRECIPITATE.

precipitate was entirely extracellular, as confirmed by viewing different locations on the sample. Also, the precipitate appears to be comprised of microcrystals of uraninite (UO_2), which was later confirmed by x-ray diffraction. An energy-dispersive x-ray analysis was carried out to identify the elemental composition of this precipitate (Figure 6b), using a Link EDS system (spot size, 5 nm) attached to a Link light element detector. The copper signal (Cu) was from the sample grid, and the titanium signal (Ti) was from the sample holder.

X-ray diffraction

X-ray diffraction analysis was performed to confirm the phase of the uranium precipitate. The analysis was carried out in the x-ray diffraction laboratory at the Department of Earth and Planetary Sciences, University of New Mexico. The precipitate resulting from the microcosm containing cellulose as the substrate was collected in a glass vial and freeze-dried. The dried precipitate was ground to a fine powder using a mortar and pestle. The powder was mounted on a glass slide with acetone. The x-ray diffractometer was equipped with a graphite monochromater and a nickel filter and used CuKα radiation with a wavelength of 1.54060 Aº. The X-ray diffraction pattern is shown in Figure 7. Table 1 compares the measured x-ray diffraction pattern with the Lattice spacing values for uraninite ($UO_{2(s)}$) obtained from the Joint Committee on Powder Diffraction Studies (JCPDS) data card 5-550.

The pattern closely matches with the JCPDS pattern to within 1.5% error confirming that the uranium phase is 'uraninite' (UO_2).

Bacterial growth

Biomass concentration in the batch reactors was estimated by measuring the amount of protein in a fixed volume of sample. Since the bacterial cell consists of a known percentage of protein, it was possible to calculate biomass concentration in the reactors. Figure 8 shows the bacterial growth curve for each substrate. With the exception of the reactor containing starch as the substrate, the growth curves indicate an exponential growth. The objective of these batch experiments was to identify a substrate which was slowly degradable and supported bacterial growth over a long period of time. As seen from Figure 8, soluble starch is more rapidly consumed and, hence, not practical for an actual remedial application. Due to these reasons, starch was not considered as a candidate substrate in the column experiments.

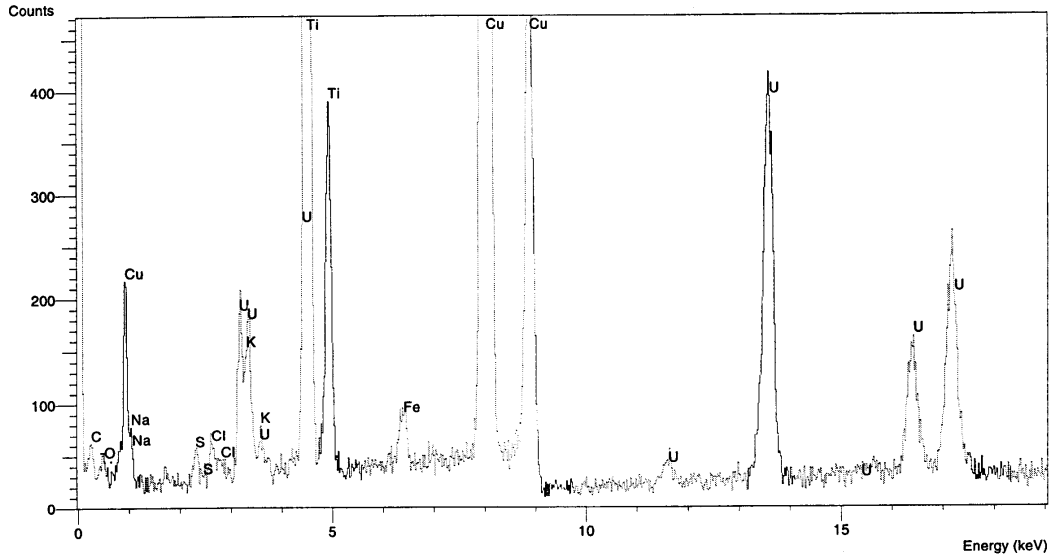

FIGURE 6b. RESULTS OF THE ENERGY DISPERSIVE X-RAY ANALYSIS. THE Cu SIGNAL WAS FROM THE SAMPLE GRID AND THE Ti SIGNAL FROM THE SAMPLE HOLDER.

Column experiments

Nitrate and nitrite concentrations measured in the sawdust column effluent during the course of the experiment are shown in Figure 9a. The initial persistence of nitrate in the effluent indicated that a time lag of about 4 days occurred before conditions necessary for denitrification of influent nitrate were established. The observed nitrate removal was slow and incomplete. As seen from Figure 9a, significant nitrite concentrations were observed in the effluent indicating incomplete denitrification. Therefore, on day 41, the hydraulic residence time was increased to 3 days as it was apparent that slow microbial kinetics limited reduction of these contaminants in the columns. The effluent nitrate and nitrite concentrations rapidly decreased and, by day 55, complete denitrification was observed. Sulfate-reducing conditions, which were manifested as a grayish-black region in the sand column resulting from $FeS_{(s)}$ precipitation, were subsequently established,. A plot of sulfate concentration with time is presented in Figure 9b. It is important to recognize that little or no sulfate reduction occurred in the columns until all the nitrate was reduced. This provides confirmation of sequential reduction of dissolved oxygen, nitrate, and, sulfate. This preferential reduction occurs due to the decreasing amounts of energy released by microbial reduction of each electron acceptor [11].

Effluent U(VI) concentrations were monitored weekly. Complete U(VI) removal (99.94%) was observed on day 60 when the effluent soluble U(VI) concentration was measured as 5.04 ppb. No U(VI) reduction was seen prior to day 60, which is consistent with the concept of sequential reduction.

Chemical results obtained during the wheat straw and alfalfa hay column experiments were similar to those of the sawdust column. However, contaminant removal rates in the sawdust column were slower compared to the wheat straw and alfalfa hay columns due

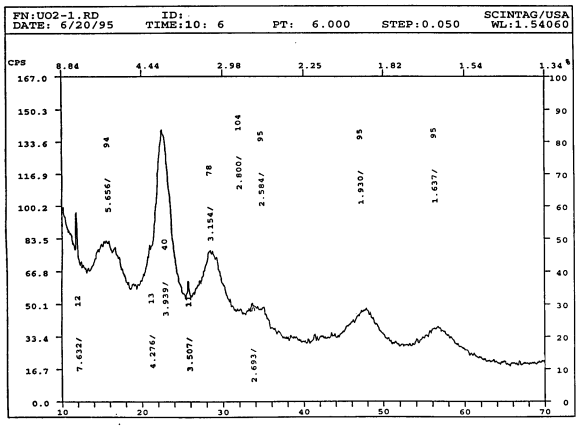

FIGURE 7. RESULTS OF THE X-RAY DIFFRACTION ANALYSIS.

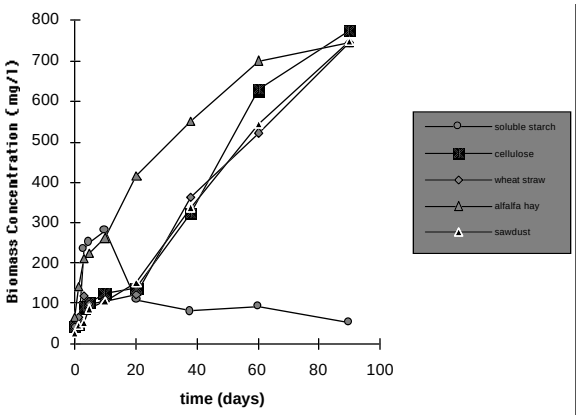

FIGURE 8. BACTERIAL GROWTH MEASURED WITH TIME FOR THE DIFFERENT CARBON SOURCES IN THE BATCH REACTORS.

to the relative percentages of cellulose and lignin in the three materials.

The precipitates formed in the column were collected and subjected to an elemental composition using a transmission electron microscope. The precipitate was found to be extracellular and comprised of micro-crystals of $UO_{2(s)}$ which is consistent with the findings from the batch experiments.

The rate of substrate utilization is an important variable which will influence the design of the permeable barrier. Knowing the rate of substrate utilization, it is possible to estimate the mass of substrate required. Table 2 lists substrate consumption over a period of 70 days for the different columns.

Since lignin compounds are known to be relatively resistant to microbial attack, it can be concluded that substrate consumption values from Table 2 represent the utilization of cellulose for each of the substrates. As expected, wheat straw and alfalfa hay were degraded more than sawdust, which has much higher lignin content.

CONCLUSIONS

Based on the results of this study, the following conclusions were reached:

1) A mixed culture of bacteria was able to utilize cellulose as a substrate to achieve reduction of NO_3^- and SO_4^{-2}.
2) More than 95% removal of soluble

TABLE 1. COMPARISON OF MEASURED X-RAY DIFFRACTION PATTERN WITH JCPDS DATA FILE CARD FOR URANINITE (5-550).

l k h	2-theta	Relative intensity	Measured d-spacings	JCPDS d-spacings	Error (%)
1 1 1	28.245	100	3.154	3.16	0.18
0 0 2	32.717	48	2.693	2.73	1.35
0 2 2	46.943	49	1.930	1.925	0.25
1 1 3	55.696	47	1.637	1.648	0.66

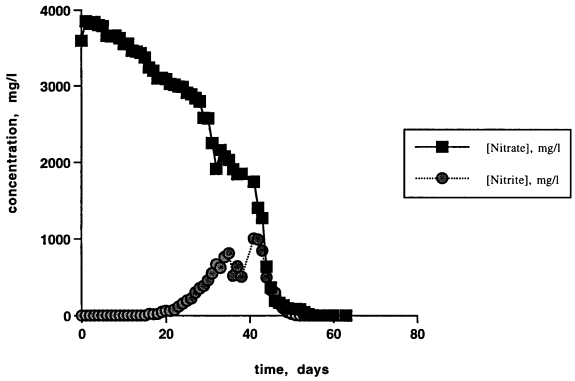

FIGURE 9a. EFFLUENT NITRATE AND NITRITE, SAWDUST COLUMN.

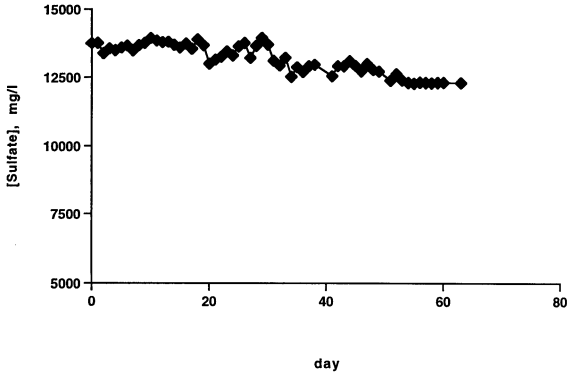

FIGURE 9b. EFFLUENT SULFATE CONCENTRATIONS, SAWDUST COLUMN.

U(VI) was observed in batch and column experiments. X-ray diffraction studies indicate that U(VI) reduction results in the formation of an insoluble uraninite ($UO_{2(s)}$).

3) Approximately 16% utilization of cellulose was reported over a period of 90 days in the batch experiments. The reaction was first order with a rate constant, $k = 2.0 \times 10^{-3}$/day.

4) Removal efficiency for U(VI) in the soil columns was dependent on the hydraulic residence time. Longer residence times provided greater removal efficiency.

5) Substrate utilization for the sawdust column was slower as compared to the wheat straw and alfalfa hay columns. This may be related to the relative percentages of cellulose in the three materials.

6) The results obtained during this research support the concept of a permeable biological reaction barrier for removal of ground water contaminants such as nitrate, sulfate, and U(VI).

TABLE 2. COMPARISON OF SUBSTRATE UTILIZATION IN COLUMN EXPERIMENTS.

Column Type	% substrate utilization in 70 days
Sawdust	6.8
Wheat Straw	11.3
Alfalfa Hay	11.9

ACKNOWLEDGMENTS

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