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Accumulations of microorganisms in the in situ leaching environment are believed responsible for plugging of the orebody and thus decreasing uranium extraction. To assess the contribution of microbial growth to this problem, packed columns of ore and core specimens were leached in the laboratory. Samples collected at four in situ uranium mining operations revealed pseudomonads, Xanthomonads, Bacillus sp. and Micrococcus sp. These microorganisms, inoculated into simulated leaching conditions, decreased permeability in two uranium ores by one order of magnitude in 20 days. Hydrogen peroxide alleviated microbial plugging in the laboratory. Periodic injection of hydrogen peroxide through the production well into the orebody may reduce microbial plugging problems in the field.

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Foreword

This report was prepared by the New Mexico Bureau of Mines and Mineral Resources, a division of New Mexico Institute of Mining and Technology, Socorro, New Mexico, under U.S. Department of the Interior, Bureau of Mines Contract number S0211032. The contract was initiated under the Mineral Resources Technology Program. It was administered under the technical direction of Twin Cities Research Center with Steven E. Follin as Technical Project Officer. R.J. Simonich was the Contracting Officer and Dennis E. Maez was the Contract Specialist for the Bureau of Mines. This report is a summary of the work completed for this contract during the period February 23, 1981 to July 15, 1982. This report was submitted by the authors on April 16, 1982. No patentable features are included in this report.

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I. Introduction

In-situ leaching is an economical and environmentally acceptable method for obtaining minerals from low-grade deposits. A critical element in determining the success of an in-situ mining operation is the maintenance of adequate permeability. One factor which influences permeability during leaching is the microbial environment. Several companies involved in in-situ uranium leaching have reported loss of permeability which may have been due to microbial growth in the mineral formation or the production of gas from microbial activity. Microbial growth on submersible pumps and screens at production wells has resulted in decreased flow of leach solution at these sites. Fungal growth on ion exchange resin, used for removal of uranium from leach solution, has greatly decreased the efficiency of these resins (Brierley, 1980). Concern has been expressed by industry that such fungal growth, should it occur in the mineralized zone, could severely reduce permeability.

In cases where microbial growth in production wells has greatly minimized solution flow, temporary improvement has been noted by cleaning the submersible pumps and putting hydrogen peroxide, chlorine bleach, or hypochlorite into the wells. To date, no long-lasting environmentally acceptable techniques have been developed. This contrasts sharply with the petroleum industry which routinely alleviates microbial problems by using antibiotics and bacteriocides. Since leaching is normally done in shallow aquifers, these chemicals are unsuitable for use by in-situ operators.

The bacterial plugging observed in the in-situ uranium leaching industry is expected to be important in any in-situ recovery process involving chemical oxidants. Because microbial problems can result in reduced recovery and production delays, there is a need for an effective means of treatment. This research project identifies some factors which are responsible for producing bacterial plugging and recommends an environmentally sound method for alleviating and preventing recurrence of these problems.

The objectives of this project are: 1)to determine the primary factors in the leaching environment responsible for microbial plugging problems; 2) to identify the microbes responsible for permeability loss, determine their characteristics, and develop a quantitative description of their effects on permeability; and 3) to develop environmentally acceptable treatments to alleviate and prevent recurrence of microbial blockage.

To meet these objectives, samples were collected from four in-situ leaching operations in three states. The numbers and types of microorganisms were defined. Four column experiments involving disaggregated ores and cores were established in the laboratory to simulate field

conditions. The effect of microbial growth on the permeability of the ores was measured with time. Several environmentally acceptable treatments, including pH alteration and hydrogen peroxide addition, were tested for their ability to alleviate microbially-induced permeability loss.

II. Review of the Literature

A. In-Situ Mining

1. Uranium reserves

Interest in uranium mining has increased as research in and development of nuclear weapons and nuclear fuels has increased. As a result, an immense amount of literature has amassed on the subject.

The United States is listed by the International Atomic Energy Agency as having the largest reserves of uranium in the free world, with Canada and Australia following (Technical Insights, Inc., 1980). The Rocky Mountain region is estimated to contain 90 % of the reserves of the United States. More detailed discussions of the United States' uranium reserves are given by the Committee on Accessory Elements (1975, 1979), McLemore (1981), and Technical Insights, Inc. (1981). The geological and hydrological features of the major uranium deposits have been detailed by Kasper et al. (1979), McLemore (1981), and Thompson et al. (1978).

2. Deposition of uranium

Uranium deposits are generally formed when tetravalent uranium, which originated from magma, is oxidized to hexavalent uranium. This is readily soluble in water and is carried along with the groundwater until it encounters a reducing environment, where it precipitates out of solution (Larson et al., 1981; Technical Insights Inc., 1980). The role of microorganisms in this deposition is discussed by Renfro (1979), Misra (1976), and Updegraff and Duros (1972).

3. Uranium chemistry in groundwater

The pH of natural groundwater in the United States generally ranges from 6.0 to 8.5 (Hem, 1970). Under these conditions, uranium is predominantly present in the form of carbonate complexes. These complexes include the bicarbonate complex $UO_2(CO_3)_2(H_2O)_3^{2-}$ and the tricarbonate complex $UO_2(CO_3)_3^{4-}$ (Lisitsin, 1962 and Hostetler and Garrels, 1962).

4. The in-situ mining process

a. physical operation

Mining of uranium by the more conventional methods of underground and open pit mining will not be discussed here. Rather, the focus will be on in-situ leach mining. In this method, a solution, called a lixiviant, is injected into the orebody where it solubilizes the uranium. The "pregnant" solution is then pumped out of the ground and passed through

an ion exchange plant, where the uranium is recovered.

There are several designs which can be used in setting up an in-situ mining operation. Generally, this consists of a series of injection and recovery wells established in a pattern to maximize solution flow through the ore body and enhance the yield of uranium. A detailed discussion of well construction and placement is given by Larson (1978).

b. lixiviants

Selection of a lixiviant is one of the most important aspects of the leaching operation. The lixiviant must not only be effective in solubilizing the uranium from the specific orebody, but consideration must also be given to the effects of the lixiviant on the in-situ environment. Some of the ions in leach solutions are capable of adhering to the host rock; others can mobilize from soil trace elements which are potentially harmful (Kasper et al., 1979). A discussion of the change in composition of the lixiviant as it moves through the orebody is given by Potter et al. (1979).

Briefly, the lixiviant functions as follows: the uranyl ion is obtained by oxidation of tetravalent uranium using either oxygen or hydrogen peroxide as an oxidizing agent; then, depending upon whether the lixiviant is acidic or alkaline, the uranyl ion is complexed either as a sulfate or a carbonate. The reactions describing these processes are given by Larson (1978):

Predominant uranium minerals

uraninite - $(U_{1-x}^{4+}, U_x^{6+})O_{2+x}$, ideally UO_2

coffinite - $U(SiO_4)_{1-x}(OH)_{4x}$

Ammonium carbonate leach reactions

oxidation -

$$UO_2 + H_2O_2 \Longleftrightarrow UO_3 + H_2O$$

leaching -

$$UO_3 + H_2O + 3(NH_4)_2CO_3 \Longleftrightarrow (NH_4)_4UO_2(CO_3)_3 + 2NH_4OH$$

$$UO_3 + 2NH_4HCO_3 \Longleftrightarrow (NH_4)_2UO_2(CO_3)_2 + H_2O$$

$$UO_3 + 2NH_4HCO_3 + (NH_4)_2CO_3 \Longleftrightarrow (NH_4)_4UO_2(CO_3)_3 + H_2O$$

Sodium carbonate leach reactions

oxidation -

$$2UO_2 + O_2 \Longleftrightarrow 2UO_3$$

leaching -

$$UO_3 + Na_2CO_3 + 2NaHCO_3 \Longleftrightarrow Na_4UO_2(CO_3)_3 + H_2O$$

Sulfuric acid leach reactions

oxidation -

$$UO_2 + 2Fe^{3+} \longrightarrow UO_2^{2+} + 2Fe^{2+}$$

leaching -

$$UO_3 + 2H^+ \longrightarrow UO_2 + H_2O$$

$$UO_2^{2+} + SO_4^{2-} \longrightarrow UO_2SO_4$$

$$UO_2SO_4 + SO_4^{2-} \longrightarrow [UO_2(SO_4)_2]^{2-}$$

$$[UO_2(SO_4)_2]^{2-} + SO_4^{2-} \longrightarrow [UO_2(SO_4)_3]^{4-}$$

Another model for the dissolution of uranium by ammonium carbonate leach solutions has been proposed by Hiskey (1979).

Potassium carbonate can also be used as a lixiviant; however, its cost has prevented widespread use. Tweeton (1981) discussed a method whereby use of potassium carbonate is made more economically feasible by first conditioning the orebody with potassium chloride. The advantages and disadvantages of the different lixiviants have been discussed by Kasper et al. (1979) and Tweeton and Peterson (1981).

The placement of monitor wells both in the ore zone and in any adjoining aquifers is important in detecting any excursions of lixiviant (Kasper et al., 1979; Larson, 1978; Thompson et al., 1978). Prior to mining, baseline data such as water chemistry, porosity and permeability of the ore body must be gathered on the area to be mined.

c. restoration

After the ore body has been mined, it must be restored to a level which meets federal and state requirements. Restoration can be a very complicated process, depending upon the type of lixiviant used. The ammonium ion, especially, adheres to clay particles and is very difficult to remove. Johnson and Humenick (1980) investigated the possibility of converting the ammonium ion to either nitrite or nitrate, which would not adhere to the clay minerals. After biological and/or chemical conversion, the nitrite

and/or nitrate- containing groundwater could be pumped out of the aquifer and treated to remove these compounds. There would, however, have to be very close monitoring during this time so that any excursions of the nitrate- or nitrite-bearing groundwater would be immediately detected.

Methods which can be used in restoring the orebody include: 1) pumping at selected wells to draw contaminated water out of the formation and allowing the flow of natural water into the orebody, 2) reinjecting treated water, 3) injecting water with chemicals added to remove trace metals from solution, and 4) allowing the system to restore itself naturally. These are discussed more fully by Thompson et al. (1978).

d. advantages and disadvantages of in-situ mining

There are several advantages to in-situ mining as opposed to conventional mining techniques. Ore bodies which are too deep and/or have uranium concentrations which are too low to be economically feasible to mine by other methods can be mined by this technology. The dangers associated with underground mining, as well as the added danger of exposure to radon gas associated with uranium mining, are avoided. Also, in-situ mining does not alter the environment to the extent that is common with pit mining. In-situ mining also has the advantage of being less expensive than conventional mining. The cost of setting up and operating an in-situ uranium mining operation has been discussed in detail by Toth and Annet (1981).

One problem unique to in-situ mining is that of excursions of the lixiviant from the ore body. An excursion poses hazards with regard to the potential pollution of the adjoining aquifers. As a result, laws explicitly requiring monitoring of groundwater components to detect any changes in the groundwater chemistry have been imposed on mining operations. A list of pertinent federal and state laws as of 1978 has been compiled by Kasper et al. (1979). Buma (1981) has discussed in-situ regulatory requirements in detail.

5. Bacterial leaching

The leaching of uranium can also be accomplished using microorganisms as a part of the process. Thiobacillus ferrooxidans can be used to generate the Fe (III) ion which oxidizes tetravalent uranium to hexavalent uranium. Bacterial leaching of ores has been reviewed by Brierley (1978a) and Lowson (1975).

The use of bacteria in the leaching of oil shale has also been investigated. Sulfuric acid, which is generated from the oxidation of sulfur by thiobacilli causes a dissolution of the oil shale matrix, thus increasing its

porosity and permeability. This, in turn, enhances the recovery of the fuel precursors. In laboratory experiments, the oxidation of sulfur to sulfate by thiobacilli has been combined with the reduction of sulfuric acid in the spent leachate to sulfur by Desulfovibrio, thus creating a cyclic process (Meyer and Yen, 1976).

B. Bacterial and Viral Interactions With Soil

There has been concern for many years regarding the contamination of water by microbial and chemical pollutants. Only recently, however, has this concern been extended to pollution of groundwater, because groundwater is now being relied upon more and more as a source of potable water. In the United States in 1975, 96% of all rural domestic water, 41% of all water used for irrigation, and 37% of all public water supplies was groundwater (Todd, 1980).

The manner in which groundwater becomes polluted is more subtle than that of surface water. If the depth to water is shallow in groundwater recharge areas or the topsoil layer is thin, such as in mountainous terrain, the movement of pollutants through the soil can lead to groundwater contamination. Several studies examined the movement of various pollutants through the soil (Allen and Morrison, 1973; Boyd et al., 1969; Ciravolo et al., 1979; Goyal et al., 1980; Hagedorn et al., 1978; Morrison and Allen, 1972; and Romero, 1970). Field and laboratory studies of bacterial, viral, and bacterial endotoxin contamination of groundwater were reviewed.

1. Movement through soil

Romero (1970) summarized studies completed prior to 1970 which examined the extent of bacterial and viral movement through the soil. Some of the characteristics of microbial movement include: 1) the maximum length of travel of biological pollutants with groundwater is 50-100 ft. in an ideal system; 2) in unsaturated soil the maximum travel length is about 10 ft.; and 3) aquifer materials which are best suited for removal of bacteria and viruses are composed of uniform, very fine- to fine- grained sand. These findings have definite implications for site selection of domestic wells. In mountainous terrain other factors which must be considered are the absence of a thick layer of soil to act as a filter for microorganisms, and the presence of fractures along which microbes can easily move (Allen and Morrison, 1973; Wagner et al., 1976; and Waltz, 1972). Allen and Morrison (1972, 1973) found that in the vadose zone the percolation of bacterially contaminated water through fractured bedrock was inadequate in lowering to acceptable levels the number of bacteria which entered the aquifer. They also found that the bacteria were capable of moving more than 100 ft. through bedrock.

Gerba and Lance (1978) found that most viruses were removed from sewage within the first 20 cm. of soil. However, these viruses could be desorbed when deionized water was applied in a manner simulating rainfall. This phenomenon has also been described by other investigators (Wellings et al., 1975). Goyal, Gerba, and Lance (1980) studied the movement of endotoxin through soil columns in the laboratory. They found that endotoxins behave in a manner similar to that of viruses. They are adsorbed onto the first 100 cm. of soil, but can be desorbed by simulated rainfall and will migrate long distances through the soil.

Rainfall, which elevates the water table and thus moves the zone of saturation closer to the surface, was found to be directly correlated with increased numbers of bacteria found in sampling wells (Hagedorn et al., 1978).

2. Removal of bacteria and viruses by soil

Gerba, Wallis, and Melnick (1975) showed that removal of bacteria by soil is due to filtering and adsorption effects, but removal of viruses is due entirely to adsorption. Adsorption of viruses may be governed by electrostatic double layer interactions and van der Waal's forces. Thus, soil and viral surface charge are important to viral adsorption. Due to the charged nature of clays, soils with high clay contents appear to have a high adsorbing capacity for viruses (Gerba et al., 1975). Schiffenbauer and Stotzky (1982) found that the affinity of coliphages T1 and T7 for kaolinite and montmorillonite (clay minerals) differed. The amino acid composition of the phage capsomer proteins may account for this. The adsorption of the phages onto different clays may also be influenced by the anion exchange capacity (AEC), the cation exchange capacity (CEC), and the AEC/CEC ratio of the clay.

3. Survival in soil

The survival time of bacteria in groundwater is variable depending on soil and climactic factors. Under favorable conditions bacteria and viruses can survive up to five years. However, 60-100 days would probably be more typical in temperate climates (Romero, 1970). Morrison and Allen (1972, 1973) found the survival of fecal type bacteria to be several months. Thirty-two days following inoculation into soil, appreciable numbers of bacteria were noted to survive (Hagedorn et al., 1978). Substantially longer survival times were projected in wet, cool soil. The survival of bacteria and viruses in the soil is influenced by three factors: climate, nature of the soil, and nature of the microorganism (Gerba and McNabb, 1981). At temperatures below 4°C, microorganisms can survive in soil for months or even years. Adequate moisture in the soil is also an important factor in the survival of microorganisms. Bacteria can survive longer in alkaline soils (as compared

to soils of pH 3-5) and also in soils with a high organic matter content (Gerba et al., 1975). The survival of viruses appears to be related directly to the degree of adsorption to the soil (Gerba et al., 1975). It has also been suggested that anaerobic soil bacteria may enhance the survival of viruses in the soil.

C. Bacteria and Viruses in Groundwater

1. Potential for hazard

a. fecal coliforms

There have been several studies done on the presence of coliforms in rural domestic wells (Brooks and Cech, 1979; Kudesia et al., 1979; Lamka et al., 1980; LeChevallier and Seidler, 1980). The presence of coliforms is most likely due to migration of these organisms from septic tanks and animal refuse lots, and not to the contamination of the aquifer in which the well is placed. It has been pointed out that routinely performed tests for indication of pollution by fecal coliforms don't necessarily give a true representation of the potential health hazard posed by the water (McFeters et al., 1974; Merrill, 1980; Richard, 1979). More accurate tests must be devised so that the potential for disease can be determined precisely.

b. disease potential

In the period between 1971 and 1977, 192 outbreaks of waterborne disease affecting 36,757 people were reported in the United States (Craun, 1979b). Non-municipal systems were implicated in 70% of the outbreaks, but the majority of the illnesses (67%) resulted from municipal system outbreaks. Untreated or improperly treated groundwater was cited as the cause of almost half of the outbreaks (49%) and 42% of the illnesses (Craun, 1979a). The most common pathogen reported was Giardia lamblia, but etiologic agents were only determined in 43% of the cases (Craun, 1979a). A detailed synopsis of the major outbreaks and their causative agents is presented by Craun (1979a,b).

2. Microbial aspects of recharge

Knowledge of the number and types of bacteria indigenous to the soil and groundwater is an important, but heretofore rarely determined, aspect of many subsurface activities. It is important to have baseline microbiological data so that any increase in the number of organisms will be quickly noticed and possible health problems averted. The increasing practice of artificially recharging aquifers with reclaimed sewage has shown the importance of quantifying and qualifying microbial parameters in groundwater. This is especially true when organics are present in the recharge material, as

degradation of these agents by bacteria may be the only significant way in which organic pollutants, once in the groundwater system, can be transformed (Gerba et al., 1975). Roberts et al. (1980), however, state that there are two main processes involved in removal of organic contaminants: biodegradation and adsorption to the soil matrix. They found that one or the other of these processes is in effect depending on the specific organic compound in question. Champlin and Eichholz (1976) found that trace contaminants may be adsorbed onto the bacteria as well as onto soil particles. Thus, the bacteria as they move can serve to transport these contaminants through the soil.

3. Bacterial and viral populations of groundwater

The microbiological populations of groundwater have not been extensively studied. A review of the literature on the microbiology of groundwater by Geldreich (1976) cites only six studies pertaining to the identification of the microbial flora of groundwater. This paucity of information is due to the expense of sampling groundwater and the difficulty of obtaining uncontaminated and representative samples (Gerba and McNabb, 1981).

Iron bacteria such as Gallionella, Leptothrix, Crenothrix, Siderocapsa, and Siderococcus are present in nearly all groundwater (Hasselbarth and Ludemann, 1973). A method for the identification and cultivation of iron bacteria from groundwater is presented by Cullimore and McCann (1977). Olson et al. (1981) failed to find any iron bacteria in deep aquifers in Montana; however thermophilic sulfate reducers (Desulfotomaculum maculum) and methanogens at concentrations of approximately 1000/ml were noted. No aerobic or any other anaerobic bacteria were detected.

Isolation of microorganisms from saline aquifers by Willis et al. (1975) revealed several genera all commonly found in soil and surface waters. The genera they identified included: Enterobacter, Flavobacterium, Pseudomonas, Micrococcus, Brevibacterium, Bacillus, and Desulfovibrio. These microbes may be native to the formation, but the possibility that they were introduced when the wells were drilled must be considered. Marzouk et al. (1979) identified four viral types occuring in the groundwater in Israel: Echovirus 7, Poliovirus 1, Echovirus 6, and Coxsackie virus B6.

D. Microbial Fouling

1. Artificial recharge

Ehrlich et al. (1972), Ehrlich et al. (1980), Godsy and Ehrlich (1978), Rittmann et al. (1980), and Roberts et al. (1980) investigated the microbiological aspects of groundwater recharge. The general concensus was that a zone

of organically enriched material formed near the injection well creating an optimal environment for bacterial activity. The effects that bacteria inhabiting this zone could have on the aquifer have been summarized by Ehrlich et al. (1972): pores of the aquifer could be blocked by bacterial cells, leading to a decrease in the hydraulic conducivity; particulate organic matter could be solubilized by bacteria and released to the aquifer; and microbial action could degrade groundwater quality by producing undesirable organic compounds.

Another problem encountered during artificial recharge is that of corrosion of well casings and screens. Vecchioli and Giaimo (1972) found that reclaimed water was more corrosive than the natural groundwater. This increased corrosiveness was attributed to the catalytic action of sulfate-reducing bacteria in the treated water.

2. Wells

The decrease in well yields due to the deposition of metal hydroxides by bacteria in groundwater has long been a problem. In Yugoslavia, Barbic et al. (1974) investigated the bacterial population of Ranney wells which had shown a decreased yield over the years. An attempt was made (Barbic et al., 1975) to correlate the amount of oxide deposition (a measure of the qualitative and quantitative characteristics of the bacteria) with the age and yield of the wells. These investigators were only able to state that generally, the older wells had smaller yields and more deposition.

Smith (1980b) reported that the corrosion of well equipment may be due, not to iron bacteria, but to pseudomonads. While reports of contamination of wells by Pseudomonas are rare, this may be due to the scarcity of tests for these organisms. Contamination by Pseudomonas organisms produces water having a foul petroleum taste, a slight odor of gasoline or vanilla, and an oily scum (Smith, 1980b).

3. Drinking water distribution systems

Iron-oxidizing bacteria have also been implicated in the fouling of municipal water systems (Ridgway et al., 1981). Gallionella can attach to pipes and multiply rapidly; this leads to discoloration of the water and eventually to decreased water flow through the pipes due to massive accumulations of insoluble iron salts. The growth of Gallionella provides an environment in which chemoorganotrophic microbes can flourish. The metabolic end products of these microorganisms lead to an unpleasant taste and odor of the water and corrosion of the pipes. Scanning electron microscope evidence for the colonization of drinking water distribution systems by Gallionella is presented by Ridgway and Olson (1981). Corrosion of water

pipes and decreased potability of drinking water has also been attributed to the production of H_2S by sulfate-reducing bacteria present in natural groundwater (Lewis, 1965. Amer. Water Works Assoc. J., 57: 1011-1015, cited by Dockins et al., 1980).

4. Acid mine drainage

Microbes are responsible for the production of iron and acidity in coal mine drainages. The source of this iron and acid is the degradation of pyrite:

$$14Fe^{2+} + 7/2O_2 + 14H^+ \longrightarrow 14Fe^{3+} + 7H_2O$$

$$\underline{FeS_2 + 14Fe^{3+} + 8H_2O \longrightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+}$$

$$FeS_2 + 7/2O_2 + 8H_2O \longrightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$$

The oxidation of Fe (II) to Fe (III) is the rate limiting step of the mechanism; pyrite degradation proceeds rapidly in the presence of Fe (III). Although this reaction proceeds spontaneously, it is dependent on the pH of the solution. Below pH 4.5 the rate of autooxidation is less than 0.25 mg/l/day for an initial Fe (II) concentration of 250 mg/l. (Singer and Stumm, 1970). Above 4.5 the autooxidation proceeds very rapidly. The oxidation of Fe(II) below pH 4.5 is brought about chiefly through bacterial catalysis.

Extensive work has been done in characterizing the numbers and types of bacteria inhabiting acid mine drainage (Belly and Brock, 1973; Dugan et al., 1970; Millar, 1973; Wakao et al., 1977; Walsh, 1980; Walsh and Mitchell, 1972, 1975; Walsh and Engelmann, 1979). Walsh (1980), Walsh and Mitchell (1972, 1975), and Walsh and Engelmann (1979) proposed that a chain of differing microorganisms is responsible for the degradation of pyrite. Mine effluent water having a pH near 5.0 is a suitable environment for Metallogenium which is capable of degrading pyrite. As the pH decreases below 3.5, degradation of pyrite is catalyzed by Thiobacillus ferrooxidans. A proposed mechanism for decreasing the rate of pyrite degradation (Walsh, 1980; Walsh and Engelmann, 1979; Walsh and Mitchell, 1975) involves recycling mine effluent which has a pH of 4.5 and a Fe(II) concentration of at least 100 ppm. This concentration of Fe(II) has been shown to inhibit the growth of Metallogenium thereby diminishing the oxidation of pyrite by T. ferrooxidans. Walsh (1982) reported on the results of this research.

Another possible mechanism for controlling the activity of T. ferrooxidans in acid mine drainage is inhibition of iron oxidation through the use of organic acids. Tuttle and

Dugan (1976) and Tuttle et al. (1977) found that, in the presence of certain organic acids; iron, phosphate, sugars, RNA, and DNA were released from the cells. These researchers suggested that the inhibition of iron oxidation was due to interference with the functioning of the cell envelope.

Heterotrophic bacteria have also been isolated from acid mine drainage (Dugan et al., 1970; Millar, 1973). The metabolic by-products of the autotrophs have been suggested as a source of nutrients for the heterotrophs (Dugan et al., 1970). Growth of heterotrophs in very acidic environments suggests that these streams may support a bacterial population which could aid in the rapid recovery of the water to its normal condition once acid discharge was discontinued.

E. Antimicrobial Agents

When considering antimicrobial agents for alleviating microbial fouling, consideration is given to the following: (Sawyer, 1976)

1) the ability to accomplish acceptable disinfection within a reasonable time or contact period;
2) the range of variations in the physical-chemical characteristics (pH, temperature, organic content, etc.) of the environment over which the disinfectant is effective;
3) the availability and cost of the disinfectant;
4) the safety of handling the disinfectant and its capability of being conveniently applied in a controlled manner; and
5) the production of compounds or reaction products within the wastewater at levels which produce toxic effects in the environment.

One of the most commonly used antimicrobial agents is chlorine, in the form of gas, hydrochloric acid, or hypochlorite. The antimicrobial activity of chlorine results from the oxidation of the cell's chemical structure, denaturation of cell proteins, and destruction of enzymic processes (Sawyer, 1976). Reportedly, pseudomonads are highly resistant to treatment with hypochlorite; however, the mechanism of this resistance has not been elucidated (Smith, 1980b).

Quaternary ammonium compounds have also been used as a disinfectant. They are relatively non toxic and are therefore suitable for use in wells. Due to their penetrating capability these compounds are especially useful in killing bacteria in slimy deposits on well screens and pipes (Smith, 1980 a). Pseudomonads are reportedly not affected by these compounds and have been found growing in solutions of these compounds (Smith, 1980 b).

The effectiveness of different biocides for use in oil recovery operations has been studied by Boghossian (1980). Of the commercially available biocides used in oil recovery Tretolite X-Cide 215, at concentrations of 50 ppm, was the most effective. However, after three weeks of storage, microbial growth was noted in the solution. Formaldehyde, at concentrations of 400 and 800 ppm, was also found to be effective as a biocide. Both of these biocides have potential for use in the petroleum industry because of the great depths of the wells. However, uranium leaching to date has primarily been conducted at depths of several hundred to several thousand feet. The toxicity of these compounds and the possibility of their entry into potable waters precludes their use by this industry.

Horvath and Elkan (1978) cite a study by DiTommaso and Elkan (in Underground Waste Management and Artificial Recharge, vol. 1, 1973) which showed that, upon injection of wastewater containing 1800 mg/l formaldehyde, the number of bacteria in the water increased by a factor of 1000.

The use of ethylene oxide as an alkylating agent to destroy bacterial endospores has been discussed by Reddish (1957). Kolb and Schneiter (1950) found that the spores of Bacillus anthracis were destroyed after 24-72 hours of exposure to 3.4-3.9 g/l methyl bromide. The continuous chlorination of wells, increasing the pH to above 8.3 to eliminate dissolved CO_2, and the recycling of hypochlorite solutions are a few methods suggested as means of treating problems caused by iron bacteria in groundwater (Cullimore and McCann, 1977).

The toxic effects of hydrogen peroxide on Thiobacillus ferrooxidans and Thiobacillus thiooxidans were studied by Brierley (1979). Oxygen uptake and growth by T. thiooxidans were totally inhibited by 0.82 mM H_2O_2. The inhibition of oxygen uptake by T. ferrooxidans resulted at 8.75 mM H_2O_2, and inhibition of growth was noted after six hours exposure of the organisms to 2.06 mM H_2O_2.

III. Materials and Methods

A. Collection Sites

Samples were collected at four different in-situ mining operations: a Texas leaching operation, a pilot plant in New Mexico, a pilot plant in Wyoming, and a test site in Wyoming.

The pH, dissolved oxygen, conductivity, Eh, and temperature of the wells were measured using a Hydrolab System 8000 probe. The depths of the wells were obtained from company data.

Prior to sampling, the wells were flushed for a period of 5-30 minutes. Samples for chemical analysis were collected at all four operations. Concentrated HNO_3 (15 ml/l) was added to aqueous samples for metals assays; these samples were stored in polyethylene bottles. Samples for analysis of phosphorus and nitrogen species were collected according to standard methods (American Public Health Association, 1976).

Three sets of samples were collected for microbiological analysis on the March, 1981, trip to the Texas leaching operation. One set was stored at 4°C; one set was bubbled with nitrogen to achieve anaerobiosis and frozen; and one set was frozen. At the other sites, and on the January, 1981, trip to the Texas leaching operation, the samples were stored at 4°C. The solid samples were obtained by scraping material from the submersible pump, which had been raised out of the production well. All samples were collected aseptically in sterile containers and stored for 24-72 hours before assaying.

B. Chemical Analyses

The water samples were analyzed for orthophosphate and nitrogen according to standard methods (American Public Health Association, 1976). Determination of selenium was done fluorometrically (Chan, 1976), using a Turner model 110 fluorometer. Analysis of uranium was performed according to the trioctylphosphine oxide method (Yoe et al., 1953), and molybdenum was assayed by a modification (Meglen and Glaze, 1973) of the colorimetric thiocyanate-stannous chloride method (Sandell, 1950) using a Bausch and Lomb Spectronic 20. Solid samples for iron assay were acid digested (Brandvold, 1974); total iron was determined with a Perkin Elmer 303 atomic absorption spectrophotometer. Hydrogen peroxide determination was accomplished by titration with $KMnO_4$ (Appendix A). Dissolved oxygen concentrations were measured in the lab using a D.O. Analyzer (New Brunswick Scientific Company).

C. Microbiology

1. Cultivation of microorganisms

Microbes, isolated from solid samples obtained at the Texas leaching operation and used as inocula, were grown at 25°C in iron peptone broth (Appendix B). For continuous inoculation purposes, the organisms were cultivated in a chemostat at 25°C, with agitation at 400 rpm and an oxygen supply of 0.2 l/min. A 1:2 dilution of modified iron peptone broth (Appendix B) was pumped into the chemostat growth reservoir at 4.2×10^{-2} ml/min.

2. Microbiological analyses

Samples were examined microscopically using a Nikon light microscope. Enumeration of aerobic microbes was performed using duplicate pour plates of iron peptone agar (Appendix B). Anaerobic bacterial counts were made using duplicate pour plates of iron peptone agar and Brewer's thioglycollate agar (BBL); a Gaspak (BBL) system was used to generate hydrogen and carbon dioxide in the jars. Sulfate - reducing bacteria were enumerated by adding 1-ml aliquots of sample to screw-capped test tubes containing 9 ml of Postgate's Medium E (Appendix B). All colony forming unit (cfu) counts were made after incubation of the plates for 5 days at 25°C. Assays for acidophilic microorganisms were made using both acid iron peptone agar and modified Kelly medium (Appendix B). Microbiological assays of the oxidized and unoxidized Wyoming sandstone ore were performed by adding 50 g ore to flasks containing 250 ml K_2CO_3 leach solution. The flasks were placed on a shaker and incubated at 25°C for one month. Ability to utilize epoxy as a source of organics was assessed by inoculating microbes into beakers containing hardened epoxy (Devcon WR) and potassium carbonate leach solution.

Isolation of the microorganisms was accomplished by transferring growth from representative colonies to agar plates of the appropriate medium. These isolates were then identified with the following criteria: cell and colony morphology; Gram reaction; fermentation of lactose, sucrose, glucose, and mannitol; growth on MacConkey agar; and production of oxidase and indol (Appendix C). Identification at the genus level was based on characteristics listed by Gilardi (1975) and Bergey's Manual of Determinative Bacteriology (Buchanon and Gibbons, 1974).

D. Ores

Four uranium ores were used for laboratory studies: one was a core sample, the other three were disaggregated samples. Uranium core was obtained from a Wyoming test site and can be described as a fine- to medium-grained arkosic sandstone, moderately sorted, medium gray with a trace of

calcite, and of the Wasatch formation. The Wyoming sandstone ore was obtained from the U.S. Department of the Interior, Bureau of Mines; petrographic studies revealed that it is composed of quartz, microcline, albite, muscavite, kaolinite, montmorillonite, and chlorite. Both an oxidized and an unoxidized sample of this ore were used; a chemical analysis of the ore is presented in Table 1. The J11 ore was obtained from a uranium mine in New Mexico, and has been described by Brierley (1978b). Particle size analyses of the ores (Figure 1) were made by shaking metal sieves (sizes 1.99, 0.598, 0.417, 0.295, 0.208, 0.149, 0.125, and 0.053 mm) on a Soiltest shaker (Soiltest Engineering Test Equipment, model CL-394B) for 15 minutes. The organic contents of the ores (Appendix D) were 2.4%, 4.3%, and 3.4% for the J11, Wyoming sandstone, and Wyoming core uranium ores, respectively.

E. Lixiviants

The ammonium carbonate-bicarbonate lixiviant was composed of 1.89 g $(NH_4)_2CO_3$ and 3.11 g NH_4HCO_3/l distilled water, with a final pH of 8.9. Panacide (2,2-methylenebis-4-chlorophenol) (50ppm) was added as a biocide to control column lixiviant. The potassium carbonate solution contained 3.0 g/l CO_3^{2-}, with a final pH of 10.8, buffered to 7.0 with HCl when appropriate.

F. Field Simulations

1. General description

For all column experiments Pyrex columns, 22 mm in diameter with a funneled bottom and a 5 mm diameter sidearm, were used. A plug of glass wool was placed at the bottom of each column. Leach solution was contained in a 12 liter reservoir and was fed into the column or core by means of a flow inducer (New Brunswick Scientific Company, model MHRE 2 or 7) at a rate of approximately 10^{-3} ml/sec. A free standing head of solution was maintained at the influent side to allow the flow of solution through the ore to be governed only by the difference in hydraulic head across the sample and the hydraulic conductivity of the ore. The pump speed was adjusted to maintain a constant head of solution at the influent. The effluent from the columns was collected in another reservoir and subsequently discarded. Microorganisms were inoculated into the column or core in known amounts (Appendix E) after stabilization of ore permeability was attained.

The permeability, expressed as hydraulic conductivity, was determined using Darcy's Law (Appendix F). Effluent was collected and measured for a defined period of time, and the flow rate, Q, was calculated. The difference in hydraulic head, which was kept constant throughout the test, was measured. Knowing the dimensions of the sample, the

Table 1

Chemical Analysis of Wyoming Sandstone Ore
(provided by US Department of the Interior, Bureau of Mines)

Chemical	Weight %
total iron	1.1
SiO_2	77.3
Al_2O_3	10.4
CaO	0.40
MgO	0.42
Na_2O	1.5
K_2O	3.3
total carbon	0.4
total sulfur	0.085
Cl	<0.1
U_3O_8	0.25
Mn	0.005

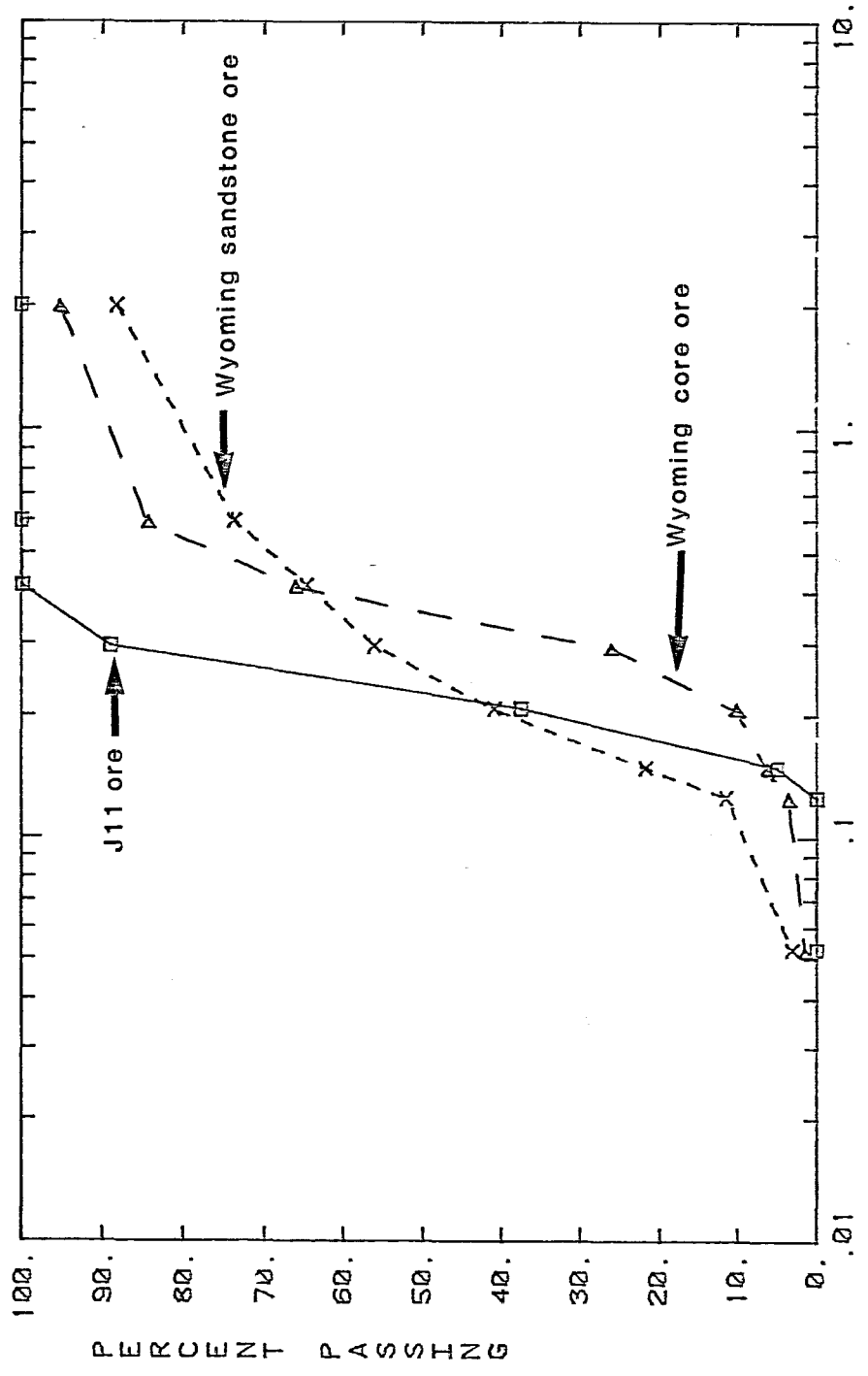

Figure 1. Particle size analyses of J11, Wyoming sandstone, and Wyoming core ores

hydraulic conductivity was calculated.

2. Single column experiments

a. solution downflow

Two single columns packed with 100 g each of J11 ore were leached with ammonium carbonate-bicarbonate solution. Both columns, the tubing, and the leachate were sterilized for 15 min at 21 psi and 121°C. Panacide was added to the leachate of column 2. These lixiviants were introduced at the top of the columns (Figure 2). Column 1 was inoculated on days 15, 25, and 31. On day 32, leach solution containing panacide was applied to column 1; solution without panacide was applied to column 2. Column 2 was inoculated on day 34.

b. solution upflow

Four columns were packed with 50 g each of Wyoming sandstone ore: two with the oxidized ore, two with the unoxidized ore. Potassium carbonate solution, pH 7.0, was applied to the ore in an upflow pattern (Figure 3). No microbes were inoculated into any of the columns.

3. Series of columns experiment

Four columns were set up in series by connecting the sidearm of one column with the bottom of the next column (Figure 4); each column was packed with 50 g J11 ore. Two flat pieces of PVC, 0.3 cm thick, 2 cm in diameter, and with 6-3mm holes in each, were placed at the influent end of column 1 and the effluent end of column 4. Solution flow was initiated at the bottom of column 1; when column 1 was filled to the top with leach solution, a rubber stopper was inserted in the top, and solution was allowed to fill the next column in the same manner.

Sterilized tap water was passed through the columns for 8 days to saturate the ore and purge entrapped air. As air displaced solution at the top of the columns, the columns were refilled with sterile tap water. Flow of potassium carbonate leach solution, pH 7, was initiated through the columns on day 9. Microorganisms from the chemostat were continuously inoculated into the columns for 14 days starting on day 15; leach solution inflow continued concurrently. On day 39, hydrogen peroxide (0.2 g/l) was added to the leach solution.

4. Core experiment

Uranium core, obtained from a Wyoming test site, was cut into two blocks, sized 5 x 5 x 6 cm. and 5.9 x 5.3 x 4.6 cm. Lucite plates with a 3/8" hole tapped into the center and a gridwork etched onto one face were glued onto 2

opposite ends of each block. The etched face of the Lucite plate was adjacent to the ore to ensure that solution flow through the cores was uniformly distributed across the face of the ore (Figure 5). The holes in the plates were taped shut, and the cores were placed in disposable, plastic containers and covered with epoxy (Devcon WR, Devcon Corp). The containers were cut away after 48-72 hours, and threaded metal fittings were screwed into the holes; tubing was connected to the fittings (Figure 6).

Solution flow through the cores was directed horizontally across the cores with respect to their orientation in the formation. Sterile tap water was passed through the cores for 9 days to stabilize the permeability; potassium carbonate leaching was initiated in both cores on day 10. Microorganisms were inoculated into core 1 on day 28. The pH of the leach solution was changed from 7.0 to 10.8 on day 44; hydrogen peroxide (0.2 g/l) was added to the leach solution on day 137. Colony counts were made on core 1 effluent on days 73, 110, 137, and for 23 days thereafter until the experiment was terminated on day 160. Core 2 effluent was analyzed periodically for microbial contamination.

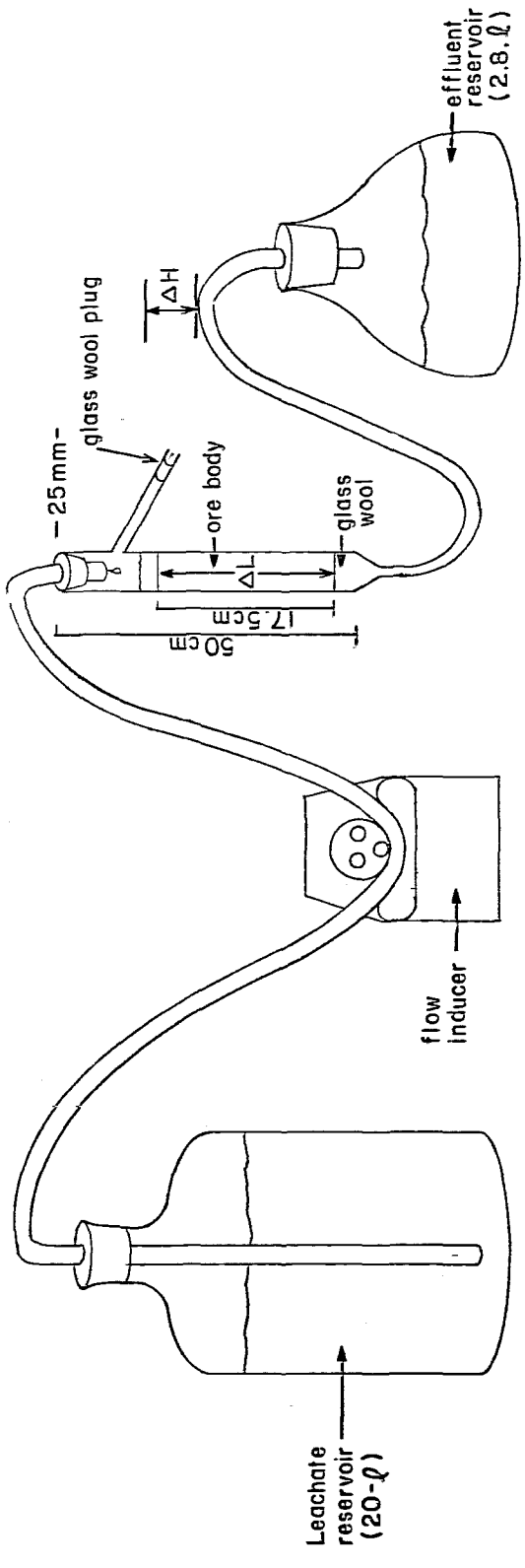

Figure 2. Schematic representation of solution downflow experiment

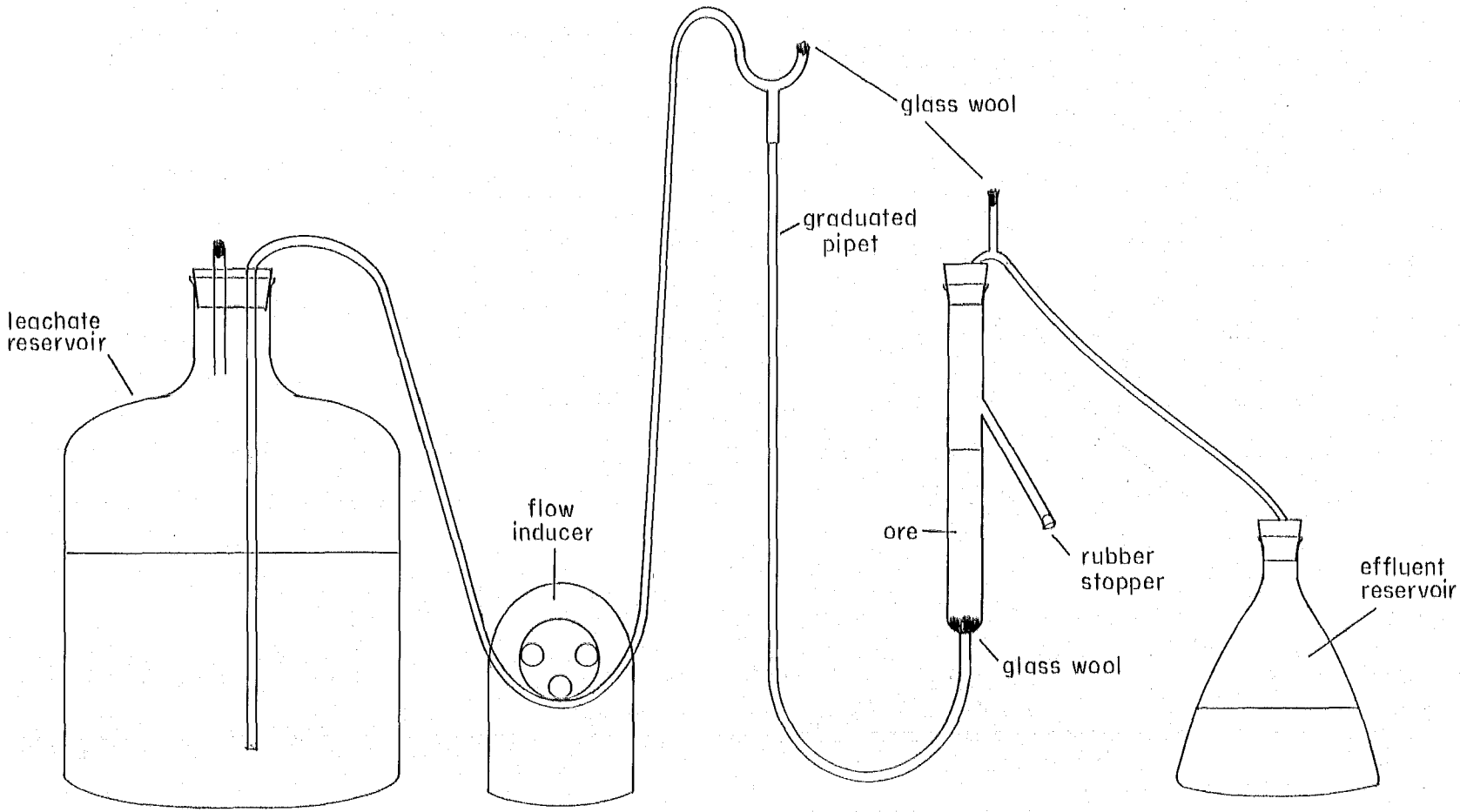

Figure 3. Schematic representation of Wyoming sandstone ore column experiment

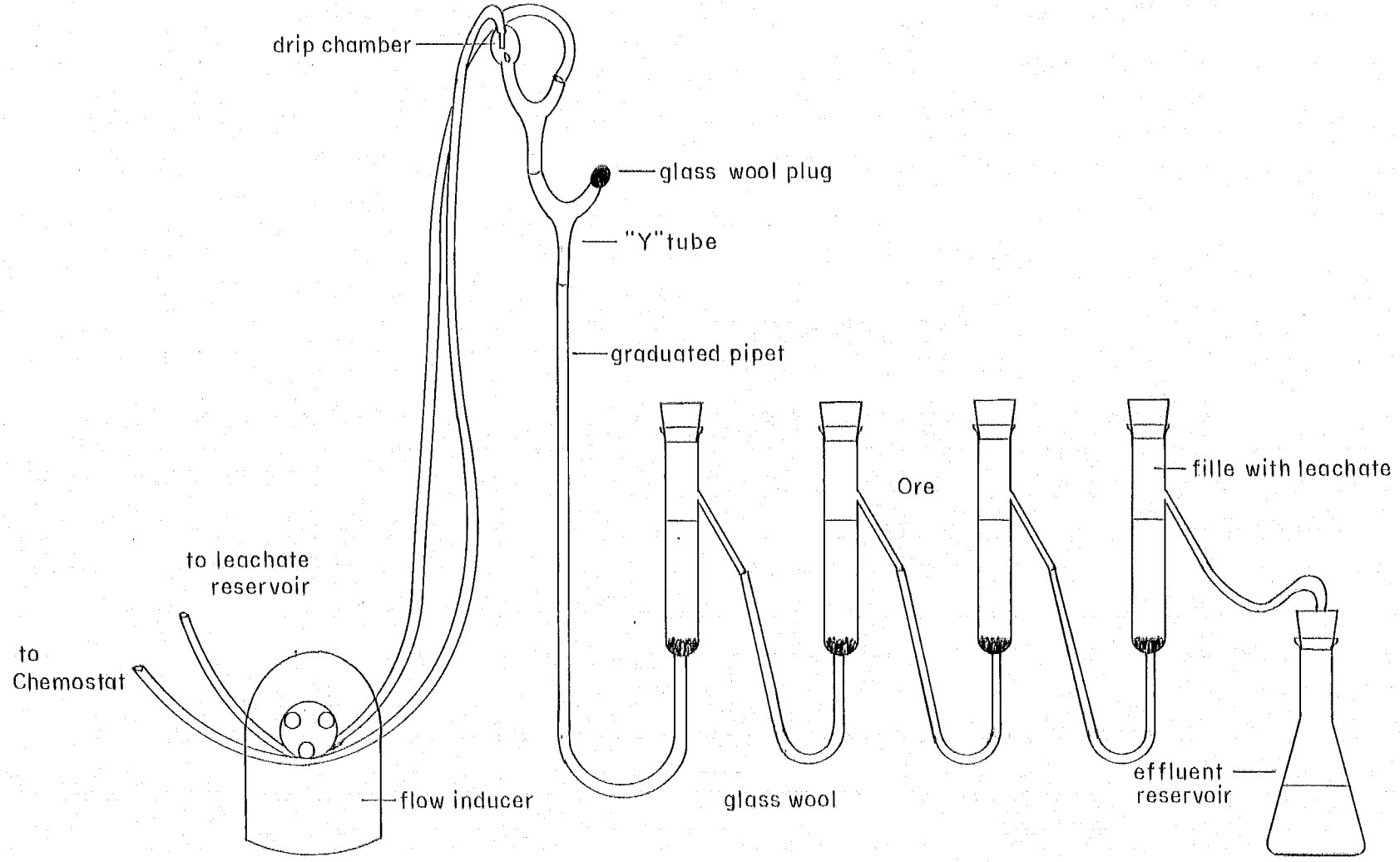

Figure 4. Schematic representation of series of column experiments

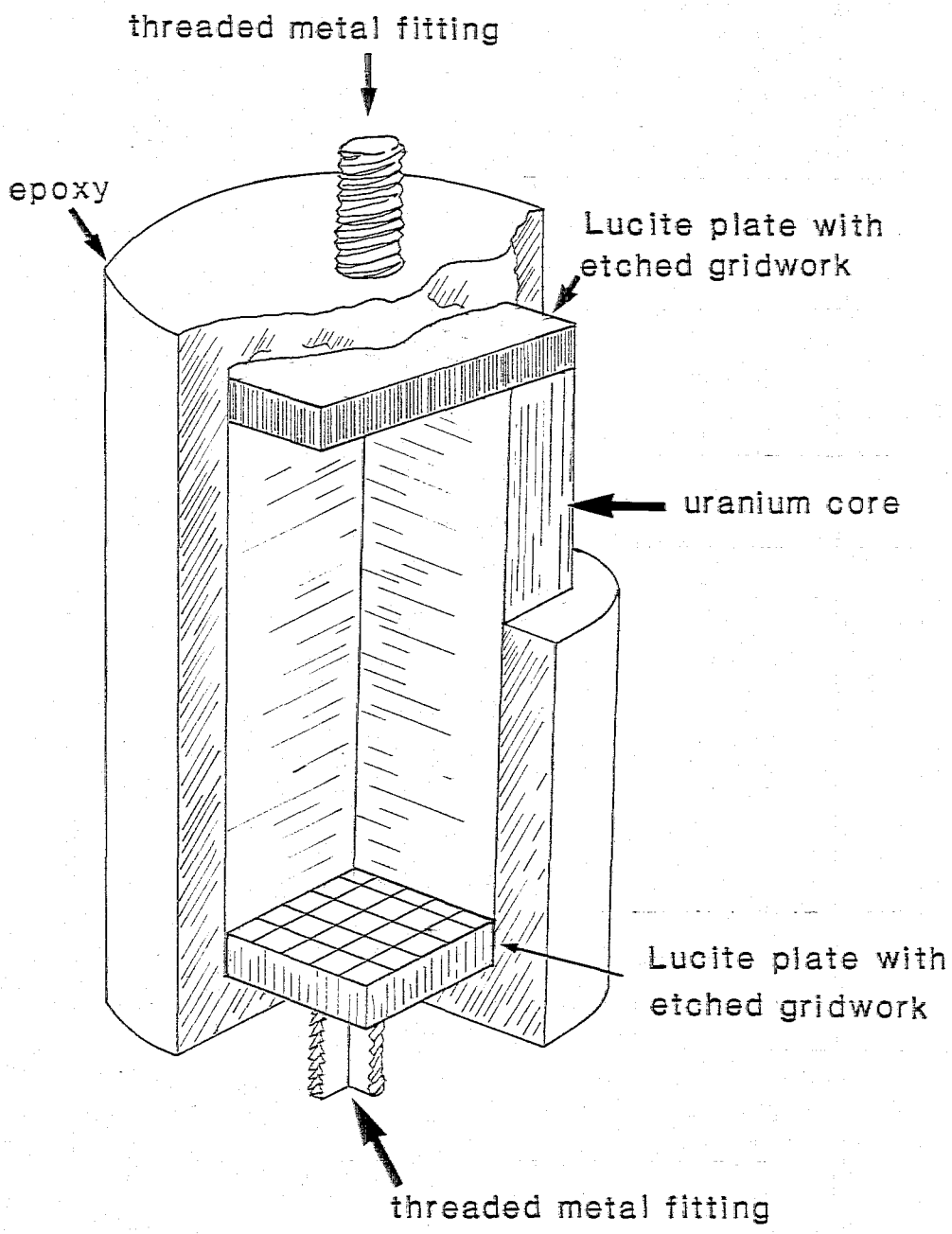

Figure 5. Cutaway schematic of epoxied uranium core

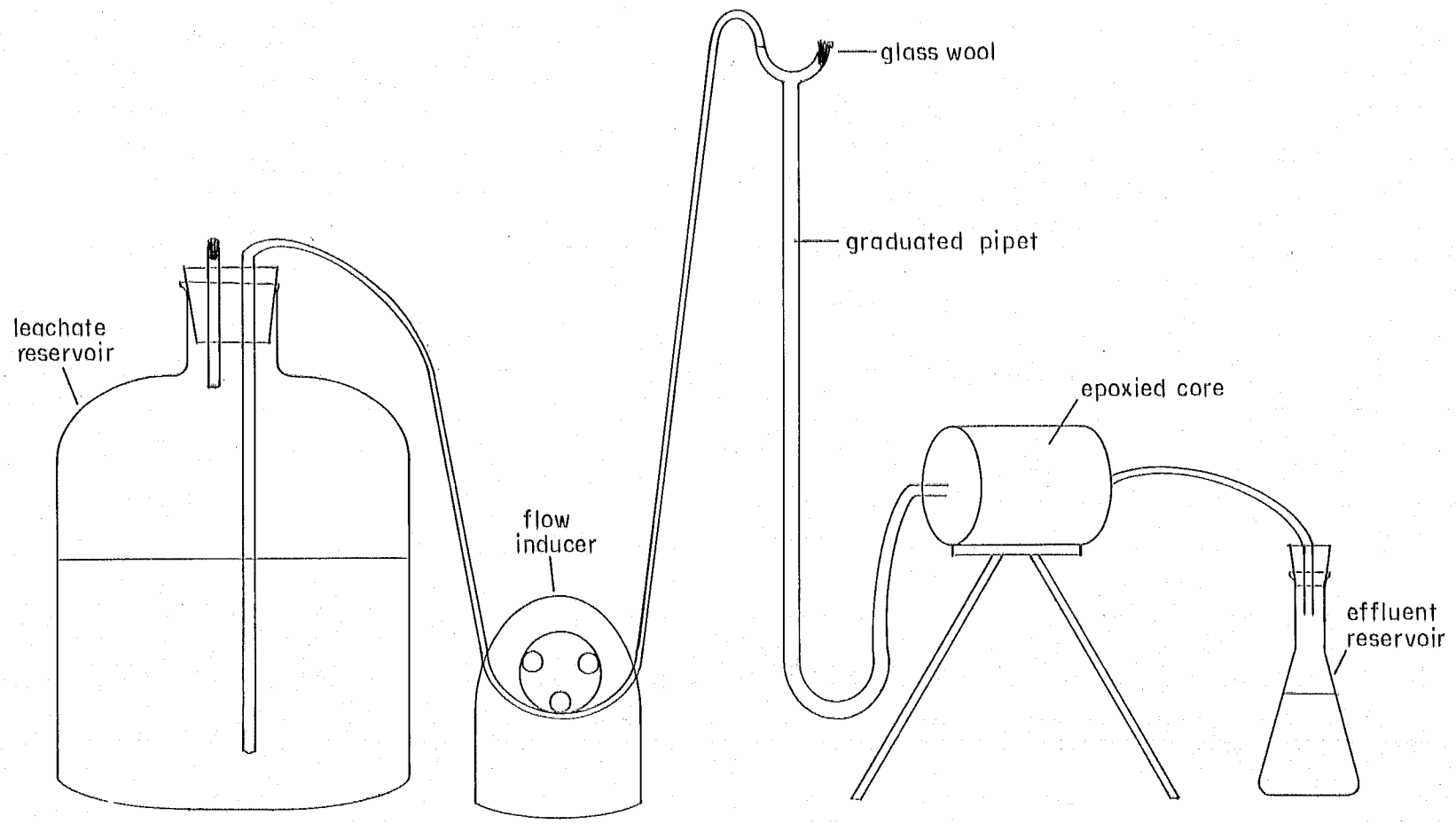

Figure 6. Schematic representation of core experiment

IV. Results and Discussion

A. Collection Sites

The well data and descriptions of the samples collected are presented in Tables 2, 3, and 4.

B. Chemical Analyses of Field Samples

The results of the chemical analyses of the field samples are presented in Table 5. The concentrations of selenium, molybdenum, and uranium, although high in some samples, did not inhibit microbial growth. The solid sample obtained from the submersible pump at the Texas leaching operation contained 40.8% iron.

C. Microbiological Analyses of Field Samples

Direct microscopic examination of the field samples revealed no microorganisms. The numbers of aerobic and anaerobic microbes present in the samples collected at the Texas leaching operation are presented in Table 6. The number of microorganisms was essentially the same in the samples from both monitor wells, the ion exchange site, and the production well. Table 7 shows the the number of aerobic and sulfate-reducing microbes present in the samples collected at the test site in Wyoming, the New Mexico pilot plant, and the Wyoming pilot plant. Except for those samples collected at the New Mexico pilot plant, the microbial counts were similar to those found at the Texas leaching operation. The low microbial counts of some of the samples may be a reflection of the organic content of the orebody; the organisms isolated require organic matter for growth, thus low organic matter contents may limit the number of organisms able to grow in the formation. Other factors which may control microbial numbers in the ore are oxygen availability and the ability of the organisms to move within the formation. Absence of microorganisms in Sample 13 may be a reflection of the alkaline pH (10.4) of the sample.

The genera of the microbes identified and the relative percentage of each type in all field samples are presented in Table 8. The majority of the microbes were identified as either pseudomonads or xanthomonads, both common soil microbes and ubiquitously distributed in nature. Analyses of the anaerobically cultivated microbes revealed microbes of the same genera as those cultivated aerobically.

Table 2

Samples Obtained on Collection Trips to Texas Leaching Operation in January and March, 1981.

Sample	Location	Date
1 (solid)	Production well, P243; orange colored accumulation on PVC pipe	January
2 (solid)	Production well, P243; orange colored accumulation on submersible pump	January
3 (solid)	Production well, P243; orange colored accumulation inside submersible pump	January
4	Outflow from ion exchange plant	January
5	Monitor well, M9	January
6	Production well, P216	January
7	Shallow monitor well, MS-1	January
8 (solid)	Production well, P243, orange colored accumulation on and in submersible pump	March
9	Outflow from ion exchange plant	March
10	Monitor well, M9	March
11	Production well, P243	March
12	Shallow monitor well, MS-2	March

Table 3

Sample Data Obtained on August, 1981, Collection Trips to New Mexico Pilot Plant, Wyoming Test Site, and Wyoming Pilot Plant.

Sample	Site	Location	pH
13	Texas	injection well inflow	10.2
14	Texas	production well,P209, outflow	6.7
15	Texas	production well,P215, outflow	6.5
16	Wyoming1	stagnant test well, 5M20	7.6
17	Wyoming1	5M20 after pumping	7.6
18	Wyoming2	production well, P62, outflow	6.4
19	Wyoming2	pumphouse injection	4.5
20	Wyoming2	injection well	6.5

Table 4

Well Data Obtained at Texas Leaching Operation, January, 1981, and Wyoming Pilot Plant, August, 1981.

No.	Temp. (°C)	pH	DO ($mg\ l^{-1}$)	Eh (mv)	Depth (ft)	Conductivity ($\mu mho\ cm^{-1}$)
3	30.7	6.8	4.66	+294	202	0.694
18	14.3	6.8	3.04	+256	95	0.216
19	14.0	6.4	1.26	-140	164	0.425
20	16.0	7.2	3.38	+161	131	0.112

Table 5

Chemical Analyses of Samples Collected at Texas Leaching Operation, March, 1981; New Mexico Pilot Plant, August, 1981; Wyoming Test Site, August, 1981; and Wyoming Pilot Plant, August, 1981.

Sample No.	N (ppm)	Orthophosphate (ppm)	Se (ppm)	Mo (ppm)	U_3O_8 (ppm)
9	-	-	-	23.75	-
10	0.40	0.08	-	0.04	0.09
11	0.16	0.17	-	22.50	0.39
12	0.30	0.21	-	0.06	0.12
13	0.16	0.14	0.0	0.125	-
14	0.24	0.62	2.0	45.00	9.0
15	0.30	0.28	0.2	47.50	21.4
16	-	-	-	-	-
17	-	0.40	-	0.055	0.0
18	-	0.52	-	0.035	0.14
19	-	0.24	-	0.015	0.28
20	-	0.08	-	0.02	0.04

Table 6

Numbers of Aerobic and Anaerobic Organisms in Samples Collected at Texas Leaching Operation.

Sample No.	Number of organisms (cfu ml^{-1}) aerobic	anaerobic
1	$1.4 \times 10^{7*}$	-
2	$2.4 \times 10^{7*}$	-
3	$1.4 \times 10^{7*}$	-
4	5.6×10^{5}	-
5	4.7×10^{5}	-
6	9.5×10^{4}	-
7	1.5×10^{5}	-
8	$7.4 \times 10^{8*}$	$5.0 \times 10^{3*}$
9	2.2×10^{2}	3.1×10^{1}
10	5.6×10^{3}	3
11	2.1×10^{4}	7
12	1.1×10^{3}	4

- no analysis

* solid sample

Table 7

Numbers of Organisms in Samples Collected in August, 1981, at New Mexico Pilot Plant, Wyoming Test Site, and Wyoming Pilot Plant.

Sample No.	Number of organisms (cfu ml^{-1})	
	aerobic	sulfate reducing
13	0	0
14	6.5×10^{1}	2
15	1.6×10^{1}	2
16	3.2×10^{5}	5
17	6.7×10^{4}	0
18	7.6×10^{4}	20
19	3.0×10^{2}	0
20	7.2×10^{5}	2

Table 8

Microbes Identified in Samples Collected From All In-Situ Uranium Mining Operations Sampled

	Relative Percentage of Each Type Present				
No.	Pseudomonas	Xanthomonas	Micrococcus	Bacillus	V-E*
1	55.6	22.2	0	0	11.1
2	70.0	20.0	10.0	0	0
3	55.6	33.3	0	0	11.1
4	50.0	30.0	10.0	0	10.0
5	50.0	50.0	0	0	0
6	33.3	55.6	0	0	11.1
7	28.6	42.9	0	0	28.6
8	60.0	40.0	0	0	0
9	37.5	12.5	12.5	37.5	0
10	27.3	63.6	0	9.1	0
11	70.0	20.0	10.0	0	0
12	33.3	66.6	0	0	0
13	0	0	0	0	0
14	33.3	50.0	8.3	8.3	0
15	18.2	72.7	0	0	9.1
16	50.0	50.0	0	0	0
17	41.7	58.3	0	0	0
18	60.0	30.0	0	0	10.0
19	33.3	66.7	0	0	0
20	66.7	33.3	0	0	0

* Biogroup VE is a designation applied to groups of oxidative bacilli which resemble Xanthomonas in some respects and Pseudomonas and Chromobacterium in other respects.

D. Field Simulations

1. Single column experiments

a. solution downflow

The change in hydraulic conductivity in the two columns packed with J11 ore is illustrated in Figure 7. While the permeability of column 2 remained relatively stable, that of column 1 increased after inoculation with microbes. This increase in permeability was attributed to microorganisms. It is probable that all of the air entrapped in the pores of the column was not allowed to escape because the flow of the leachate was downwards. When the microbes were introduced, they utilized this air, thus opening the pores to solution flow. The increase in permeability of column 2 after inoculation with microbes (day 34) and the decrease in permeability on addition of panacide to the leachate (day 32) support this hypothesis.

b. solution upflow

After leaching with potassium carbonate for 23 days, the columns packed with the oxidized Wyoming sandstone ore developed an orange precipitate at the top surface. The precipitate first developed in column 2 and several days later formed in column 1. When this observation was made, the pH values were 4.7 and 3.0 for column 1 and column 2 effluents, respectively; the total iron concentrations were 24.8 ppm for column 1 and 34.8 ppm for column 2. Column 1 effluent contained 4.6×10^5 cfu/ml and column 2 effluent contained 8.2×10^5 cfu/ml. Microbial analyses of these isolates revealed pseudomonads and xanthomonads; no acidophilic or iron-oxidizing microbes were found in the effluent or orange material. No orange material appeared in the columns packed with the unoxidized Wyoming sandstone ore. Attempts to cultivate microbes from the ore in shaker flasks were unsuccessful. The permeability, which was approximately 5×10^{-2} cm/sec, decreased to 4×10^{-3} cm/sec at the appearance of the orange material. After one week, the permeability stabilized at 2×10^{-3} cm/sec, and no further accumulation of the orange material was noted. It is probable that the orange material, which was found to be ferric hydroxide, resulted from the autooxidation of ferrous hydroxide at the near neutral pH of the lixiviant. The mechanism by which the pH was reduced from 7 to 4.7 or 3.0 may have been the result of the hydration of the ferric ion with the concomitant generation of hydrogen ions (Brierley, 1978):

$$Fe^{3+} + 3/2H_2O \longrightarrow 3H^+ + 1/2Fe_2O_3$$

Another possible explanation for the reduction in pH in the oxidized ore is the hydrolysis of the ferric ion, in the presence of the sulfate ion, to jarosite (Brierley, 1978):

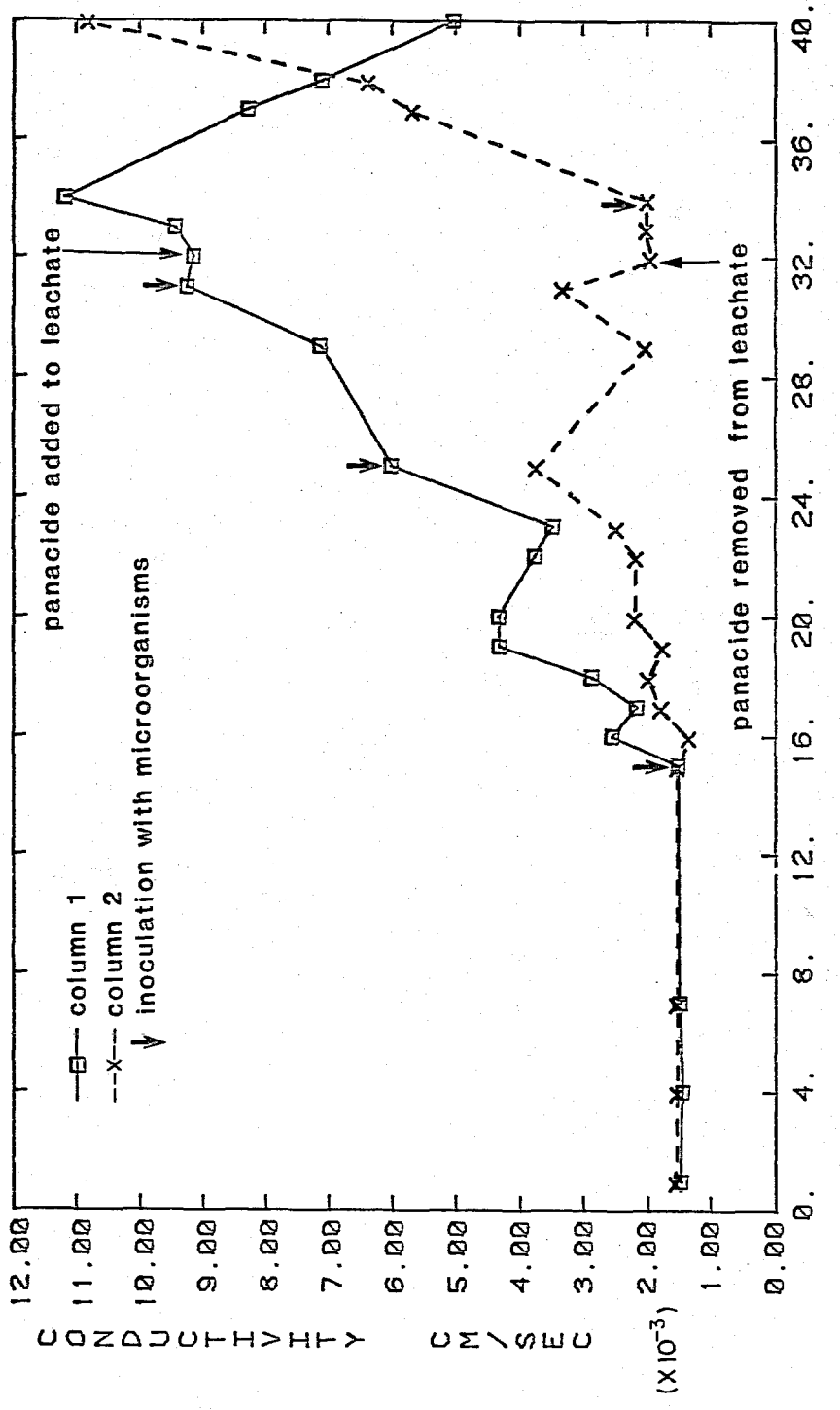

Figure 7. Change in hydraulic conductivity with time in solution downflow, J11 ore packed columns.

$$Fe^{3+} + 7/3H_2O + 2/3SO_4^{2-} \longrightarrow$$

$$5/3H^+ + 1/3Fe_3(SO_4)_2(OH)_5 \cdot 2H_2O$$

2. Series of columns experiment

In the series of columns experiments, entrapped air was eliminated by leaching the columns from the bottom upward. No permeability data were collected for a three week period, between days 20 and 41, because a constant head could not be maintained for a sufficient length of time (Figure 8). Because the volumetric flow rate is proportional to the cross-sectional area perpendicular to the flow, the small area of these columns caused the Darcian flux (q) to be proportionately small, thus the flow rate into the columns was low. This caused the microorganisms in the tubing from the chemostat to aggregate; therefore, the influx of microbes into the columns was not constant, resulting in an unsteady head level at the influent.

When hydrogen peroxide was added to the leach solution (day 39), the permeability continued to decrease for another 24 days. On day 70 the permeability slowly started to increase. With the addition of hydrogen peroxide, bubbles of oxygen began to displace the liquid at the top of the columns, suggesting microbial degradation or chemical instability of the hydrogen peroxide. The formation of oxygen caused blockage of the pores, decreasing the permeability. Only when the oxygen was depleted from the pores by microbial activity or was dissipated as a gas did the permeability increase.

The distribution of microorganisms through the series of columns is presented in Table 9. The organisms were dispersed uniformly; there was no increased growth in the proximity of the PVC, suggesting that the microbes were not using this as a source of nutrients or a site of attachment.

3. Core experiment

The changes in permeability with time in the epoxied uranium cores are shown in Figure 9. Increasing the pH from 7.0 to 10.8 (day 44) in an attempt to inhibit microbial growth had no effect on permeability. However, the addition of hydrogen peroxide (0.2 g/l) to the leach solution on day 137 did result in increased permeability. Microbes, which had been present at approximately 10^5 cfu/ml in core 1 effluent, decreased to zero after 20 days of exposure to the hydrogen peroxide (Figure 10). Core 2 effluent was consistently free of microorganisms.

With the addition of hydrogen peroxide to the leachate, bubbles appeared in the effluent tubing of core 1 but not core 2. The increase in permeability, the sharp drop in viable bacterial counts, and the cessation of the appearance of bubbles in the effluent tubing of core 1 indicated that the hydrogen peroxide eventually proved toxic to the microbes. Subsequent studies on microbe-free cores showed that, while the permeability remained stable and no bubbles appeared in the effluent tubing, only 70% of the hydrogen peroxide entering the cores was present in the effluent. This indicated that 30% of the hydrogen peroxide was degraded as it passed through the ore. After the addition of microbes to one of the cores, 65% of the hydrogen peroxide in the influent was detected in the effluent, while 70% of the hydrogen peroxide was present in the effluent of the uninoculated core. Dissolved oxygen concentrations in the influent and effluent solutions were 7.9 and 8.0 µg/ml.

Microorganisms were unable to grow when provided with only leach solution and epoxy; this indicated that they were incapable of extracting organic matter from the epoxy for growth.

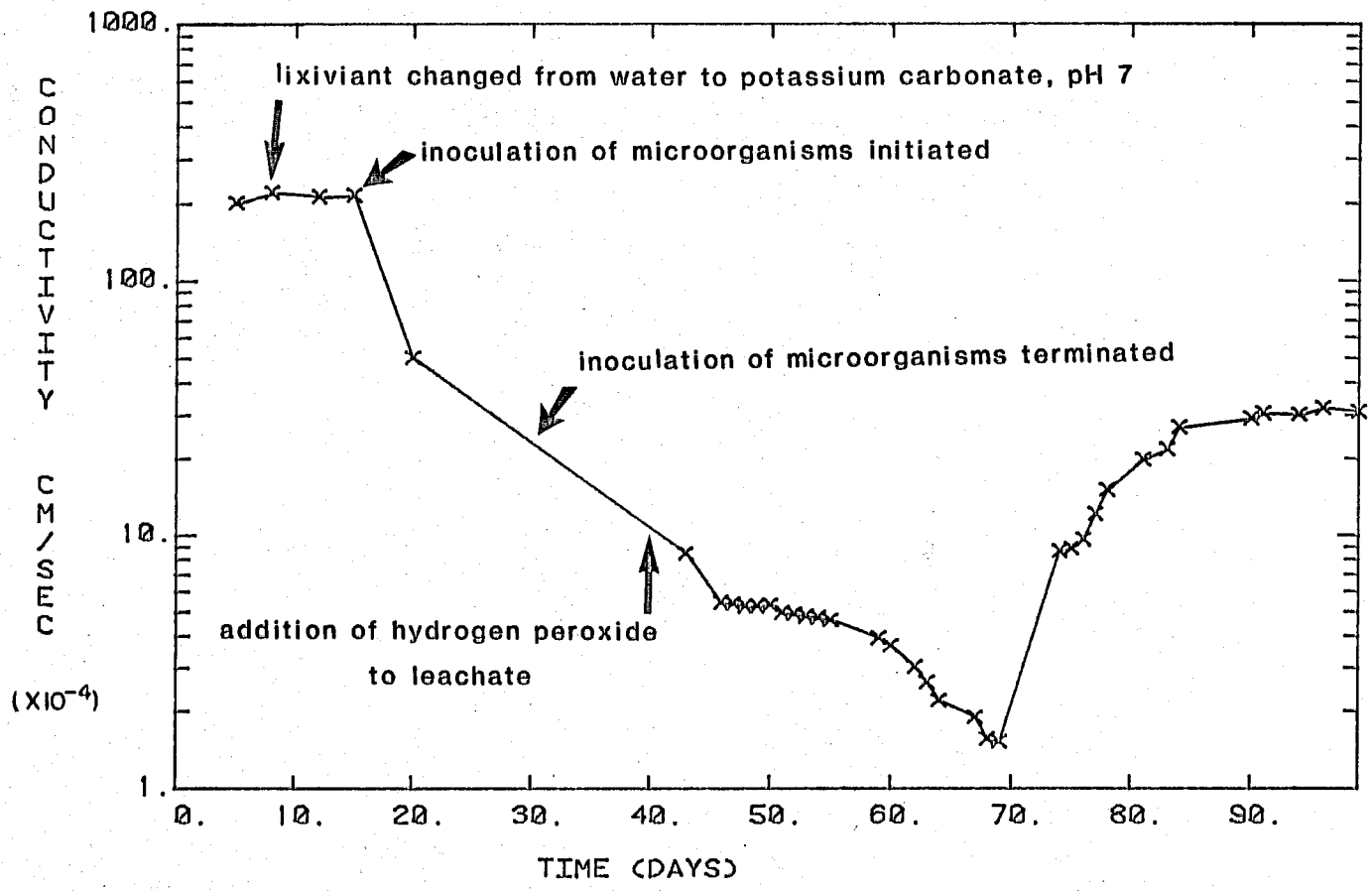

Figure 8. Change in hydraulic conductivity with time in series of columns packed with J11 ore.

Table 9

Distribution of Microbes in Series of Columns Study

Sample location	Number of organisms (cfu g^{-1})
bottom of column 1	6.8×10^6
middle of column 1	2.8×10^5
top of column 1	4.0×10^5
bottom of column 2	4.2×10^5
middle of column 2	1.9×10^6
top of column 2	3.7×10^5
bottom of column 3	3.5×10^5
middle of column 3	2.8×10^5
top of column 3	3.4×10^6
bottom of column 4	8.4×10^5
middle of column 4	3.7×10^5
top of column 4	3.1×10^5

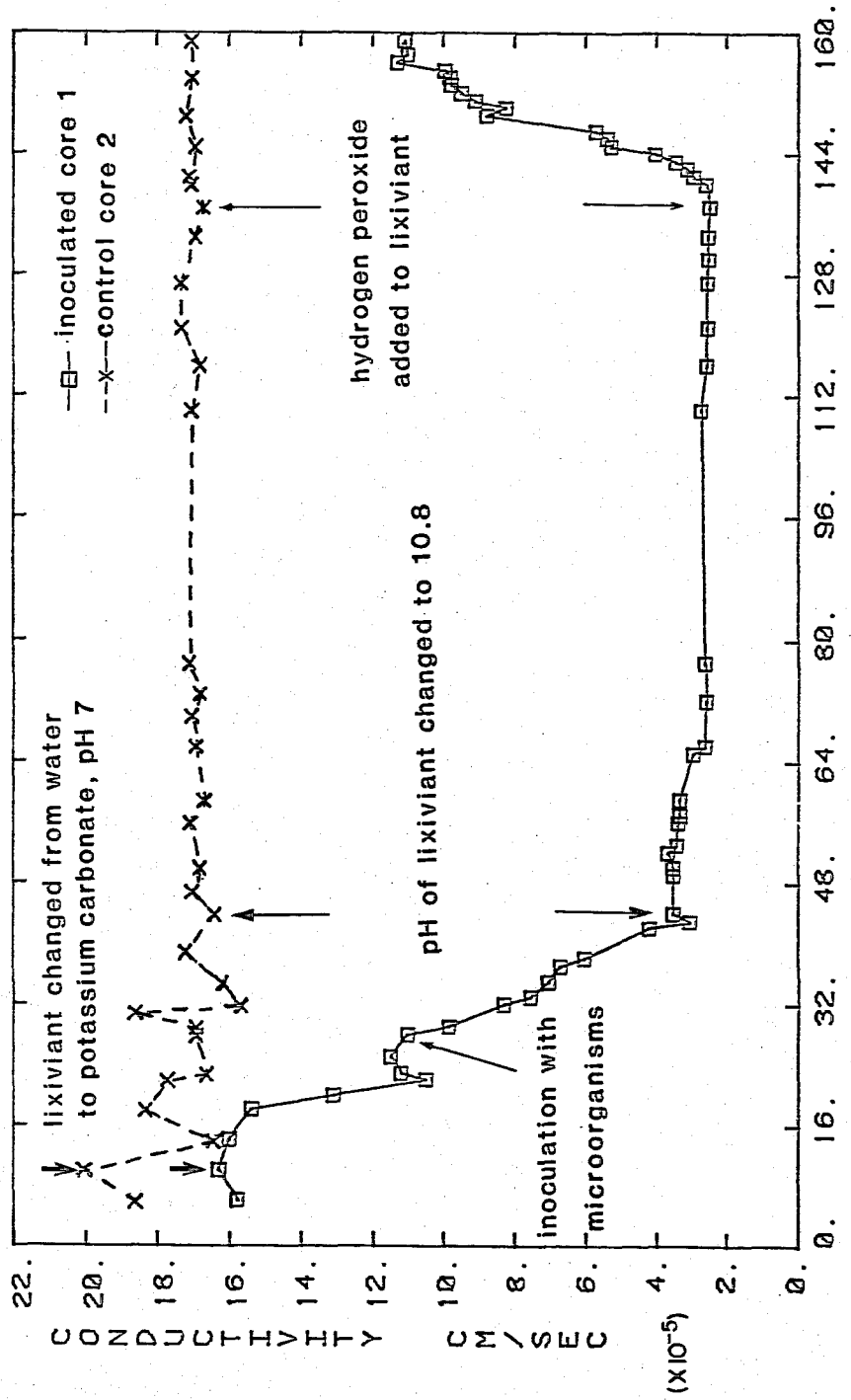

Figure 9. Change in hydraulic conductivity with time in epoxied uranium cores.

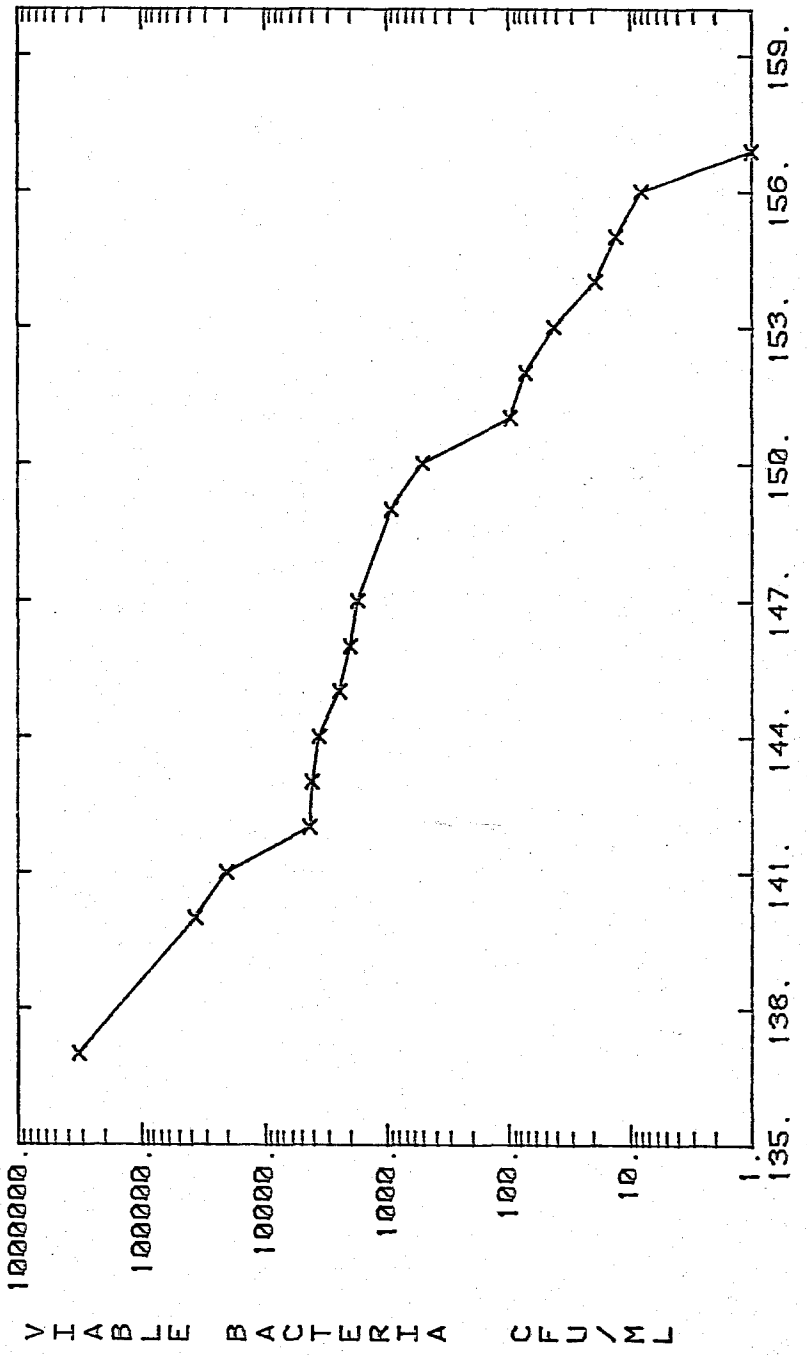

Figure 10. Change in viable bacterial counts with time in epoxied core 1 effluent on addition of hydrogen peroxide to the lixiviant.

V. Conclusions

Microorganisms in samples collected from in-situ leaching operations were present in concentrations up to 10^5 /ml. These organisms were identified as Pseudomonas, Xanthomonas, Bacillus, Micrococcus, and Biogroup VE organisms. All of these organisms are heterotrophic (obtain energy from the oxidation of organic matter), aerobic (require oxygen for growth), and common soil microorganisms. The in-situ leaching of uranium predisposes this environment for the proliferation of the microorganisms, which are ubiquitous in the soils overlying the orebody (Brierley, 1980). The drilling of wells and injection of solutions introduce the organisms into the orebody. Microbes, adsorbed onto soil particles under conditions of natural groundwater flow, could be desorbed by the increased flow rates associated with leaching and subsequently be trapped in the pores of fine-grained materials or on well screens. The trapped organisms proliferate as oxygen and nutrients become available. Oxygen, either used directly as an oxidizing agent for uranium solubilization or formed by the degradation of hydrogen peroxide, is utilized by the aerobic organisms. Most of the western and southwestern United States' uranium orebodies, which are amenable to in-situ leaching, contain organic matter. Although this complex organic matter has not been precisely defined chemically, it is known to be of a humic acid nature and very recalcitrant (Leventhal, 1980). When leaching commences in the orebody, the addition of leach solutions and oxidants undoubtably solubilizes components of organic matter which can serve as nutrients for the heterotrophic soil microorganisms.

In the laboratory in-situ leaching was simulated using columns packed with disaggregated ores and cores. Columns were leached in both upflow and downflow patterns. Cores and columns were inoculated with microorganisms isolated from field operations. Experiments confirmed that PVC and epoxy were not utilizable by these organisms; therefore, the microbial growth observed in the laboratory cores and columns was attributable to the organic matter shown to be present in the ores used. Analysis of effluents from the inoculated cores and columns confirmed the proliferation of the organisms in the field simulations. Ore samples taken from the inoculated packed columns substantiated that growth had occurred in the columns (Table 9). Microbial growth effected permeability losses of approximately one order of magnitude in epoxied cores. The number of organisms (10^5 cfu/ml) which produced this permeability loss was comparable to the number found in leach solutions from in-situ operations experiencing plugging problems. Permeability loss was also observed when gases were entrapped in the ore; this problem was most acute when downflow leaching was performed. Upflow leaching alleviated permeability loss due to gas entrapment, however, the use of disaggregated ores in upflow columns were found unsuitable for simulation of

in-situ leaching. Cores were found to be most satisfactory for studies of microbially-induced permeability changes.

The development of environmentally acceptable treatments to alleviate microbial problems encountered for in-situ mining is more difficult than in the petroleum industry. The use of antibiotics and organic agents is not acceptable in in-situ mining due to the shallow depths at which the process is carried out; possible contamination of adjacent aquifers used for potable water precludes their use. Increasing the pH of the lixiviant from neutrality to 10.8 did not increase permeability or decrease viable bacterial counts in laboratory experiments. Hydrogen peroxide (0.2 g/l) decreased the numbers of viable bacteria and increased permeability in a simulated field situation. Similar concentrations of hydrogen peroxide have been found to inhibit growth of thiobacilli (Brierley, 1979). Although hydrogen peroxide does degrade to produce oxygen, the concentration of dissolved oxygen in effluents from the laboratory experiments was 8 µg/ml. This concentration would not be inhibitory, as Pseudomonas and Bacillus can grow at oxygen concentrations of 35 µg/ml (ZoBell and Hittle, 1967). However, at increased pressures (5 to 25 atm) such as those which would be expected in the in-situ environment, hyperbaric oxygen concentrations may be high enough to inhibit microbial growth. ZoBell and Hittle (1967) found that 35 to 70 µg/ml dissolved oxygen was toxic to several microorganisms, including Pseudomonas and Bacillus. Laboratory studies to alleviate microbial plugging problems suggest that injecting hydrogen peroxide through production wells into the orebody may diminish microbial growth at the production well and in the nearby ore. The concentration of hydrogen peroxide necessary to kill the microorganisms, the distance through which the reagent can travel without losing its effectiveness, and the potential problem of decreased permeability due to pores blocked by oxygen from hydrogen peroxide degradation must be investigated before this method can be efficiently used in in-situ mining operations.

VI. Recommendations for Application to Mining Industry

Operators of in-situ leaching operations have observed the growth of microorganisms on submersible pumps and on ion-exchange resins used for removal of uranium from the pregnant solution. Although gas formation and precipitation of ferric iron in in-situ leaching operations have been attributed to microbial activity, no specific studies have identified microorganisms as causative agents of these problems.

The objectives of this study were 1) to identify factors in the leaching environment which were responsible for allowing microbial growth to develop in in-situ operations; 2) to identify the organisms which contributed to microbial plugging problems and quantify their effect on permeability, and 3) to test environmentally acceptable methods for alleviating microbial plugging in in-situ uranium leaching operations.

Microorganisms in concentrations of up to 10^5/ml were found at the four uranium in-situ leaching sites examined. The types of organisms identified were all common soil organisms and were basically the same types at all sites.

Columns of disaggregated uranium ore and uranium cores, simulating in-situ leaching conditions, were used to study microbially-induced permeability loss. Microorganisms collected at four in-situ leaching sites were used in the laboratory experiments.

Downflow leaching in the laboratory resulted in gas entrapment which reduced permeability, and microbial usage of the gas actually increased permeability in such leaching systems. Gas production in one industrial in-situ leaching operation reportedly diminished lixiviant flow through the formation; however, the role of microorganisms in either production of the gas or utilization of the gas was never established.

Upflow leaching experiments in the laboratory eliminated gas entrapment but resulted in precipitation of ferric iron in one oxidized ore. A decrease in pH occurred concomitant with the iron accumulation; this may have resulted from the hydration of ferric iron and the formation of jarosite, both of which are acid-producing reactions. Neither the precipitation of ferric iron nor the decrease in pH is believed to be microbially mediated. The precipitation of ferric iron at production wells has been reported by several industrial in-situ operations, and such precipitation may have resulted in permeability loss in the ore formation and production wells.

Microbial growth in an epoxied core of uranium ore reduced permeability by one order of magnitude. The laboratory experiments using this epoxied uranium core suggested that periodic injection of hydrogen peroxide into the orebody through production wells may reduce microbial growth in the well and nearby ore. By injecting hydrogen peroxide through the production well, microbes in the nearby orebody could be killed, reducing their potential to cause plugging problems. Periodic injection of hydrogen peroxide would be necessary, as the microbial population would eventually increase to an unsatisfactory level. The amount of hydrogen peroxide injected would have to be sufficient to kill the microorganisms, but not be so great that the pores of the orebody would be filled with oxygen from the degradation of the hydrogen peroxide. Hydrogen peroxide has reportedly been used by industry for removal of material in production wells which reduced lixiviant flow. Such usage of hydrogen peroxide has been a temporary solution to the problem since microbial growth and other materials re-accumulate at the well sites.

Appendix A

Permanganate Method for Determination of H_2O_2 (Furman, 1946)

The titration depends upon the reaction:

$$5H_2O_2 + 2KMnO_4 + 4H_2SO_4 \longleftrightarrow 2KHSO_4 + 2MnSO_4 + 5O_2 + 8H_2O$$

Procedure:

Ten ml of sample are diluted in 400 ml distilled water. Dilute H_2SO_4 (1:4 in distilled water) (approximately 10 ml) are added. This is titrated with N/100 $KMnO_4$ to a pink endpoint.

The amount of peroxide present in the sample is determined by the stoichiometry of the reaction:

$$\frac{(1/100)\ (1\ KMnO_4\ \text{used})}{2} = \frac{x}{5}$$

x = moles H_2O_2 in sample

Appendix B

Media Used in Cultivation of Microorganisms

1) Iron Peptone Medium (1/2 strength modification of original) (Ferrer, et al., 1963)

Ingredient	Amount
Proteose peptone	2.5 g
$FeSO_4 \cdot 7H_2O$	92 mg
Calcium gluconate	50 mg
$(NH_4)_2SO_4$	50 mg
Agar (for plates)	15 g
Tap water	1 L

2) Postgate's Medium E (Postgate, 1979)

Ingredient	Amount
KH_2PO_4	0.5 g
NH_4Cl	1.0 g
Na_2SO_4	1.0 g
$CaCl_2 \cdot 6H_2O$	1.0 g
$MgCl_2 \cdot 7H_2O$	2.0 g
Sodium lactate	3.5 g
Yeast extract	1.0 g
Ascorbic acid	0.1 g
Thioglycollic acid	0.1 ml
$FeSO_4 \cdot 7H_2O$	0.5 g
Agar	15 g
Tap water	1 L

Adjust pH to 7.6 with NaOH after boiling to dissolve agar. Add extra NaCl for salt water strains.

3) Modified Kelly Medium (Mackintosh, 1978)

Ingredient	Amount
K_2HPO_4	0.04 g
$MgSO_4 \cdot 7H_2O$	0.4 g
$(NH_4)_2SO_4$	0.4 g
$FeSO_4 \cdot 7H_2O$	33.3 g
Yeast extract	0.2 g
pH 1.4-1.6	

Sterilize at 10 psi for 10 min or at 15 psi for 5 min.

4)Modified Iron Peptone Medium

Ingredient	Amount
Proteose peptone	2.5 g
Calcium gluconate	50 mg
$(NH_4)_2SO_4$	50 mg
Tap water	1.0 L
Agar (for plates)	15 g

5)Acid Iron Peptone Medium

The ingredients used are the same as those in the iron peptone medium described in 1). The medium is made in the following way:

a)All of the ingredients except the agar are added to tap water in double the normal amount.
b)The pH of this solution is adjusted to 2.0 using concentrated H_2SO_4.
c)Double the normal amount of agar is dissolved in tap water in another flask.
d)The flasks are autoclaved separately, and allowed to cool to 50°C.
e)The contents of one flask are added to the other aseptically and mixed.

Appendix C

Biochemical Tests Used in Identification of Microbes

1) Gram Stain

a) A 12-18 h pure culture is used. A small amount of bacterial growth is heat fixed to a microscope slide.
b) Cover the smear with a 1% solution of crystal violet and allow to stain for 1-2 min.
c) Wash the excess stain off with water.
d) Cover the smear with a 2% solution of iodine and allow it to act for 2-3 min.
e) Wash with water.
f) Destain with 95% ethanol for 15-30 sec.
g) Wash well with water.
h) Cover the smear with a 2.5% solution of safranin (in a 9:1 water:ethanol mixture), and allow it to act for 30-45 sec.
i) Wash with water.

Gram positive microorganisms stain purple, Gram negative microorganisms stain pink.

2) Fermentation of simple sugars

The medium used consists of:
nutrient broth 8 g/l
1% sugar
10 ml/l brom cresol purple

The medium is dispensed into test tubes in approximately 10 ml aliquots. A small tube (Durham tube) is placed, inverted, into the test tube. The test tubes are autoclaved at 121°C for 15 min and allowed to cool. Each test tube is inoculated with microorganisms, and incubated at 25°C for 7 days.
A yellow coloration of the broth indicates production of acid (fermentation of the sugar). Displacement of the medium at the top of the Durham tube indicates the formation of gas.

3) Indol production

The microbe to be tested is grown for 24 hours in tryptone broth (or other medium rich in tryptone). After incubation, several drops (0.5 ml) of Kovac's reagent (para-dimethyl-amino-benzaldehyde:amyl alcohol:conc. HCl, 1:15:5) is added to the tube (without mixing). Development of a red ring at the broth-reagent interface is a positive test for indol.

4) Oxidase test

This is a test for the production of the enzyme indophenol oxidase.

To perform the test, a drop of oxidase reagent (0.5% tetra-methyl-para-phenylenediamine-dihydrochloride in distilled water) is added to an isolated colony on an agar plate. Oxidase positive microbes cause a dark purple color to develop within 30 seconds.

Appendix D

Determination of Organic Matter in Soil (Pramer and Schmidt, 1964)

Procedure:

1) For each sample to be tested, place a crucible in a triangle supported on a ring stand. Heat to a red glow with a Bunsen burner, and continue to heat for 10 min.
2) Place the crucibles in a dessicator to cool to room temperature and weigh.
3) To each crucible, add oven dry sample until about half full, and determine the total weight of crucible and soil.
4) Place the crucible and contents on a triangle and heat with a moderate flame. Increase the temperature until a red glow is evident within the crucible. Stir the contents periodically. Maintain a glowing red color for 30 minutes.
5) Permit the crucible to cool in a dessicator to room temperature and weigh. Calculate the percent organic matter in the sample by dividing the difference in weight before and after ignition by the original weight.

Appendix E

Method for Determining Inoculum Quantity

The number of microorganisms inoculated into the column or core was determined by multiplying the void volume (V_V) of the sample by 10^5 , the average number of microbes found in 1 ml of samples collected in the field. The V_V can be calculated from the porosity, n, and the total volume of the sample, V_t:

$$V_v = n \times V_t$$

The porosity can be determined using the following equation:

$$n = 1 - (\text{bulk density}/\text{particle density})$$

where:

bulk density = dry weight of sample/V_t

particle density = 2.65 g/cm3

Appendix F

Darcy's Law

Permeability was calculated as hydraulic conductivity (K), and was determined using Darcy's Law:

$$Q = \frac{(-K)\ (H_2 - H_1)\ (A)}{(L_2 - L_1)}$$

where:

Q = flow rate (cm^3/sec)
K = hydraulic conductivity (cm/sec)
$H_2 - H_1$ = difference in hydraulic head (cm)
$L_2 - L_1$ = length of sample (cm)
A = cross-sectional area of sample perpendicular to the flow (cm^2)
The negative sign indicates that the flow is in the direction of decreasing hydraulic head.

The validity of Darcy's Law for these experiments was established by calculating a Reynold's number (R):

$$R = \frac{(q)\ (d_x)}{v}$$

where:

q = Darcian flux (Q/A, cm/sec)
d_x = grain size at x% of the sample (cm)
v = kinematic viscosity (cm /sec)

The value of x is usually taken to be either 10 or 50 (Freeze and Cherry, 1979). The value of d is read from a sieve analysis graph (Figure 1). Darcy's Law is said to be valid for R < 1, and possibly for 1 < R < 10 (Todd, 1980).

Actual calculation for one of the samples:

$$q = \frac{Q}{A} = \frac{1.65 \times 10^{-4}\ cm^3/sec}{31.27\ cm^2} = 5.28 \times 10^{-6}\ cm/sec$$

$v = 0.00897\ cm^2/sec$
$d_{50} = 0.017$ cm

$$R = \frac{(5.28 \times 10^{-6}\ cm/sec)\ (0.017\ cm)}{0.00897\ cm^2/sec} = 1.00 \times 10^{-5}$$

Thus, it was concluded that Darcy's Law was valid for the purposes of the experiments.

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