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R.G.L. McCREADY

COMPTE RENDU DE LA DEUXIÈME RÉUNION GÉNÉRALE ANNUELLE DE BIOMINET

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PROCEEDINGS OF THE SECOND ANNUAL GENERAL MEETING OF BIOMINET

FOREWORD

BIOMINET presents in this volume the invited technical papers (with the exception of Paper 17 from Dorr Oliver Ltd., which was unavailable) and the abstracts of the mini-presentations made by BIOMINET members at the Second Annual Meeting held in Mississauga, Ontario on October 24th and 25th, 1985.

The efforts of the many individuals involved in the development of the scientific program and the arrangement of meeting facilities are gratefully acknowledged. A special thanks to Ms. Susan Reid, and Dr. V.I. Lakshmanan and the staff of the Ontario Research Foundation, whose efforts resulted in a most enjoyable annual meeting.

R.G.L. McCready
Editor

COMPTE RENDU DE LA DEUXIÈME RÉUNION GÉNÉRALE ANNUELLE DE BIOMINET

AVANT-PROPOS

Dans ce volume, BIOMINET présente les exposés techniques sollicités (exception faite de l'exposé n° 17 de la Dorr Oliver Ltd. qui n'était pas disponible) et les résumés techniques des mini présentations faites par les membres de BIOMINET lors de la Deuxième réunion annuelle qui a été tenue à Mississauga (Ontario) les 24 et 25 octobre 1985.

Nous apprécions grandement les efforts de toutes les personnes qui ont participé au développement du programme scientifique et organisé la rencontre. Nous désirons remercier de façon particulière Mme Susan Reid et M. V. I. Lakshmanan ainsi que le personnel de la Fondation de recherches de l'Ontario dont les efforts ont contribué à faire de cette réunion annuelle un moment des plus agréable.

R.G.L. McCready
Éditeur

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BY THIOBACILLUS FERROOXIDANS

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ABSTRACT

Bacterial oxidation of museum-grade pyrite and analytically pure ferrous iron was studied in Warburg respirometers, shake flasks, and stirred reactors. The metabolic energy available from these reactions was assessed in terms of rH_2 as a function of time. Using four different kinetic models, the specific oxygen uptake data were evaluated on a comparative basis, and the merits and demerits of these models discussed. The apparent activation energy and frequency factor of pyrite and ferrous ion oxidations were determined, as well as the thermodynamic values associated with the formation of the respective activated complexes. Furthermore, the hydrolysis of ferric ion that may result in a variety of sols, gels, amorphous and/or crystalline precipitates was discussed. The distribution of these precipitates in solution was suggested to reflect the interplay of fast proton transfer, olation, and aggregation reactions.

SESSION I: PRÉSENTATION 1

CINÉTIQUE DE L'OXYDATION DE LA PYRITE ET DU FER FERREUX PAR THIOBACILLUS FERROOXIDANS

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RÉSUMÉ

L'oxydation bactérienne de pyrite de qualité musée et de fer ferreux de pureté analytique a été étudiée dans des respiromètres de Warburg, des flacons et des réacteurs agités. L'énergie métabolique rendue disponible par ces réactions a été évaluée d'après la variation du rH_2 en fonction du temps. Quatre modèles cinétiques différents ont servi à évaluer comparativement l'absorption spécifique d'oxygène ainsi que les avantages et les inconvénients des modèles traités. L'énergie apparente d'activation et le facteur de fréquence de l'oxydation de la pyrite et de l'ion ferreux ont été déterminés ainsi que les constantes thermodynamiques correspondant à la formation des complexes activés respectifs. De plus, on traite de l'hydrolyse de l'ion ferrique qui peut donner divers sols, gels et précipités amorphes et/ou cristallins. On laisse entendre que la distribution de ces précipités en solution reflète les interactions dues à des réactions rapides de transferts protoniques, d'olation et d'agrégation.

KINETICS OF PYRITE AND FERROUS IRON OXIDATION BY *THIOBACILLUS FERROOXIDANS*

INTRODUCTION

Oxidation of pyrite has been studied extensively (1) and its chemistry well documented (2). It plays a very important role in the biohydrometallurgical processes (3), where it provides sulphuric acid and ferric ions (4,5):

$$2FeS_2 + 7\ 1/2\ O_2 + H_2O \xrightarrow{\text{bacteria}} Fe_2(SO_4)_3 + H_2SO_4 \qquad \text{Eq 1}$$

which are needed in the heap- and dump-leaching processes as leaching agent and oxidant (6). The chemical oxidation of metal sulphides by ferric ion can be expressed as follows:

$$MS + 2Fe^{3+} \longrightarrow M^{2+} + 2Fe^{2+} + S^{o} \qquad \text{Eq 2}$$

$$Fe^{2+} \xrightarrow{\text{bacteria}} Fe^{3+} + e^{-} \qquad \text{Eq 3}$$

$$S + 1\ 1/2\ O_2 + H_2O \xrightarrow{\text{bacteria}} H_2SO_4 \qquad \text{Eq 4}$$

As shown in Eq 3 and 4, the ferrous iron and elemental sulphur set free in Eq 2 will be oxidized to ferric ion and sulphuric acid by the bacteria. The process is called "indirect bacterial leaching mechanism" (6). In direct leaching (7), bacteria are converting metal sulphides directly to sulphate:

$$MS + 2O_2 \xrightarrow{\text{bacteria}} MSO_4 \qquad \text{Eq 5}$$

The bacteria participating in the leaching of metal sulphides and oxidation of ferrous iron are classified as chemo-lithotrophs (8), which embrace part of the mesophilic (9) and thermophilic Thiobacilli (10,11). Neither the physiology nor the biochemistry of iron by these bacteria is fully understood (12). However, the oxidation of sulphur to sulphuric acid (13) appears to be a necessary concomitant of iron oxidation. The first step in sulphide oxidation was the dissociation of metal sulphides (14). When the bacteria carry out their metabolic reactions, the electrons derived from the oxidation of the ferrous iron and sulphur species are used to generate energy for the fixation of carbon dioxide (12,15). The iron-oxidizing system, believed to be located in the cell envelope of these bacteria, induces c- and a-type cytochromes (16) as well as an unusual blue copper protein, rusticyanin.

Oxidation of ferrous ion to ferric ion immediately induces hydrolysis unless selective ligands bind ferric ion by forming mononuclear $[Fe(ligand)_n^{3-n}]$. highly stable complexes (17). The iron cycle in living organisms involves the continuous chemical competition of hydroxyl ion with other ligands. There are many problems associated with iron precipitation, especially in hydrometallurgical systems in respect to solution purification (18). During the

bacterial leaching of metal sulphides or ferrous sulphate oxidation, pH of the solution is, sometimes, kept at a desired value by adding sulphuric acid or sodium hydroxide to the leach solution as required. When the base is added to the solution, there is an enormous pH gradient at the interface of the drop and the bulk solution, as illustrated in Figure 1. There will be an equilibrium distribution between the free ferric ion, mononuclear and polynuclear, hydroxo- and sulphato-complex species:

$$Fe^{3+} \rightleftharpoons Fe(OH)_n^{3-n} \rightleftharpoons Fe_pO_r(OH)_s^{3p-s} \rightleftharpoons Fe_n(OH)_p(HSO_4)_q(SO_4)_r^{3n-p-q-2r} \qquad \text{Eq 6}$$

When the mixing is complete, one of three distinctly different situations may exist:

- No polynuclear complexes persist while the equilibria are shifted.
- The acidity of the bulk solution is too low to bring about acid decomposition of the stoichastically formed polynuclear aggregates.
- Nuclei have been generated that induce the growth of crystalline polynuclear aggregates in solution.

The type and composition of the different polynuclear iron precipitates will be dictated by the experimental conditions. Industrial applications of iron precipitation include the hematite (19) and the jarosite processes (20). In the hematite process, the ferric hydroxides are converted by thermal transformation to goethite, FeO(OH), and hematite, Fe_2O_3 (21-25). The principal form of jarosite in sulphuric acid media (26) is hydronium jarosite, $H_3O[Fe(SO_4)_2.2Fe(OH)_3]$. A variety of jarosite-type compounds have been found to exist in the bacterial leach solutions (27,28).

Pyrite is generally associated, in varying amounts, with the sulphide-bearing ores, and serves as a source of iron in the bacterial leach processes. The first patent (29) claiming advantage of iron-oxidizing Thiobacillus ferrooxidans in the extraction of copper from low-grade resources was awarded in 1959. Bacterial leaching has been applied industrially for the extraction of uranium (30,31) from ores and wastes. Vanadium extraction by microbial action in the presence of iron was patented (32). The rate of metal extraction was found to improve in the heap, dump and in situ leach processes if the ore contained small amounts of pyrite.

However, the presence of pyrite in raw materials is not always desirable. For example, coals containing high levels of pyrite cannot be used in the power-generating plants as an energy source without penalties. Biohydrometallurgical processes may help to remove pyrite, either by flotation from coal that has been preconditioned with Thiobacillus ferrooxidans followed by oil agglomeration, (34) or by oxidizing pyrite to ferric sulphate and sulphuric acid (35-37), as shown in Eq 1.

When precious metals are finely disseminated within the pyrite or metal sulphide matrix, their recovery by the conventional cyanidation technique is not economical. Subjecting these precious metal-bearing sulphides to bacterial leaching in order to oxidize pyrite, and at the same time to liberate gold and silver, will improve their recovery either by cyanidation or by thiourea leaching (45,46).

Both pyrite and ferrous iron play a very important role in the metabolic activities of microorganisms and, as a consequence, in the extraction of metals from minerals. The present study will provide comparative kinetic and thermodynamic data on pyrite and ferrous iron oxidation by Thiobacillus ferrooxidans.

MATERIALS AND METHODS

Substrates

Pyrite samples were obtained from the New Mexico Mineral Museum. The samples were ground to a particle size less than 37 μm. Chemical analysis of the pyrite indicated that it contained 46.2% iron, 53.4% sulphur, and 0.4% impurities.

Bacteria

The strain of T. ferrooxidans was originally isolated from acid mine waters (38). It was adapted to ferrous iron and pyrite substrates in a nutrient medium (39). For subculturing and for experiments, an aliquot of a late-log phase culture was transferred into a new medium.

Shake Flask Experiments

This series of experiments was carried out in 250-cm^3 Erlenmeyer flasks containing varying amounts of pyrite, 70 cm^3 of nutrient medium, and 5 cm^3 of bacterial inoculum. The flasks were placed on a 250-rpm gyrotory incubator shaker (Model 26, New Brunswick Scientific Company, Incorporated, N.J.) at temperatures varying from 20 to 45°C. Periodically, distilled water was added to the flasks to compensate for evaporation, and a 1-cm^3 sample was removed from the flasks for analysis. The sample was replaced with 1 cm^3 of liquid medium containing basal salts only. In the sterile control flasks, 5 cm^3 of a 2% solution of thymol in methanol was added instead of inoculum.

Tank-Leaching Experiments

A leach tank reactor of 5 dm^3 capacity used in this study was constructed from plexiglass. It was equipped with three baffles and with electrodes for Eh, pH, and dissolved oxygen measurements, as well as variable speed agitation (40). The reactor was charged either with 300 g of pyrite, 2.7 dm^3 of nutrient medium (iron free) (39), and 0.3 dm^3 of inoculum of T. ferrooxidans, or it was charged with 2.7 dm^3 of 9K medium (39) and 0.3 dm^3 of bacterial inoculum.

Harvesting of Bacteria

The T. ferrooxidans cells free of substrates (pyrite or ferrous iron) were collected from the decanted tank leach solutions by centrifugation (41), using

a high-speed of 13 000 min^{-1} rotational frequency equal to 27 300 x gravitational force (g) and 20 000 min^{-1} rotational frequency equal to 48 246 x g. The solutions were centrifuged at 4080 x g for 10 min, the supernatant fluid containing the bacteria being removed carefully so as not to disturb the deposited colloidal and solid inorganic particles. This fluid was then subjected to centrifugation at 39 100 x g for 20 min. The supernatant fluid was discarded, and the packed cells were resuspended in an iron-free nutrient medium (39) to produce a 10% wet-weight-per-volume (W/V) suspension. Cellular protein content of T. ferrooxidans was liberated by alkaline digestion (0.1N NaOH) of substrate-free suspensions and estimated by spectrophotometric determination (42). These bacterial suspensions were used in the Warburg respirometer experiments within five days.

Warburg Manometry

The conventional manometric technique (43) was employed using 16-cm^3 Warburg flasks equipped with a centre well and a side-arm. Each flask had a total volume of 2.7 cm^3. For the studies with pyrite, the flasks were charged with 200 mg of pyrite, 2.4 cm^3 of iron-free basal salts medium (39), and the side-arm with 0.3 cm^3 of a 10% (W/V) bacterial suspension containing 4.6-4.8 mg of protein. For ferrous iron oxidation, the flasks were charged with 2.4 cm^3 9K of nutrient medium (39), and the side-arm with 0.3 cm^3 of the above bacterial suspension. The centre well contained 0.2 cm^3 of 20% potassium hydroxide. In the sterile controls, heat-killed bacteria were used. The experiments were carried out at pH = 2.3, the temperature varying from 20 to 45°C, and a speed of agitation of 130 strokes per minute for 90 min. After 20 min of equilibration, the reaction was started by tipping the cell suspension from the side-arm into the main compartment of the flask.

Analysis

The oxidation of pyrite gives rise to the formation of insoluble reaction products. Therefore, throughout this study the total dissolved iron will be considered. For this purpose, a 1-cm^3 sample is rapidly removed from the agitated leach suspension and treated with 1 cm^3 of 5N HCl for 30 min at room temperature (35). Then the reaction mixture is diluted 10 times with 0.01N H_2SO_4 and centrifuged at 4080 gravitational force for 20 min. Some of the supernatant clear solution is diluted with 0.01N H_2SO_4 and analyzed on an atomic absorption spectrophotometer (Model 703, Perkin Elmer).

Ferrous iron concentrations were determined by titration with dichromate solution (44).

RESULTS AND DISCUSSION

Effect of Pulp Density

The effect of pulp density (PD) was studied in shake-flask experiments with leach suspensions containing 1.7-26.3% PD. In this study, the pulp density or substrate concentration, S, is defined as:

$$PD = \frac{\text{weight of solid (g) x 100}}{\text{volume of liquid (cm}^3\text{)}} \qquad \text{Eq 7}$$

Figure 2 indicates the total dissolved iron as a function of time and pulp density. As can be seen, the final yield of pyrite oxidation by the bacteria is inversely proportional to the pulp density, while the rate (V), which is being calculated as the slope of the straight line section of the curves, is directly proportional to the increase in the pulp density. The highest yield of 72% of pyrite oxidation was obtained with 1.7% pulp density suspension, while only about 53% extraction was achieved with the 26.3% pulp density suspension. The pyrite oxidation in the corresponding sterile controls varied from 0.2 to 1.3%.

The relationship between the rate (V) of bacterial conversion of substrate (S) is given by Monod (47) as a hyperbolic equation:

$$V = \frac{V_m\ S}{K + S} \qquad \text{Eq 8}$$

where V_m is the theoretical maximum rate of bacterial conversion of substrate, and K is the substrate concentration that yields half of the maximum rate. Equation 8 can be linearized by taking its inverse:

$$\frac{1}{V} = \frac{K}{V_m}\frac{1}{S} + \frac{1}{V_m} \qquad \text{Eq 9}$$

By plotting 1/V versus 1/S, a straight line is produced as shown in Figure 3. A linear least-squares, regression-analysis technique calculates the slope = K/V_m and the intercept of the straight line with the 1/V - axis = $1/V_m$. From these values, $V_m = 2.05 \times 10^{-6}$ mol $dm^{-3}s^{-1}$ and K = 10.33% pulp density have been assessed. The significance of this linear plot is that the maximum rate, V_m, can be determined from data obtained with relatively low substrate concentrations. There exist many alternative forms of the linearized Monod equation (48), for example, the so-called Woolf (49) equation:

$$\frac{V}{S} = \frac{S}{V_m} + \frac{K}{V_m} \qquad \text{Eq 10}$$

By plotting S/V versus S, a straight line is obtained where the intercept with the S/V-axis is equal to K/V_m and the slope corresponds to $1/V_m$, as shown in Figure 4. From this presentation, the maximum rate of pyrite oxidation was calculated to be $V_m = 2.048 \times 10^{-6}$ mol $dm^{-3}s^{-1}$, with the associated K-value equal to 10.33% pulp density. In the case of the linearized Monod equation and the Woolf approach, the half-maximum point ($V_m/2$, K) lies to the left side of the straight lines (see Fig. 3 and 4). Therefore, these approaches overemphasize the importance of low values of V in the application of Eq 10, and of high values in the Woolf plot. This is statistically unsound (50). None of these criticisms holds for the Augustinsson linearization technique (49), which is expressed by the following equation:

$$V = V_m - K\frac{V}{S} \qquad \text{Eq 11}$$

By plotting V versus V/S, a straight line is obtained with a finite positive intercept with both of the coordinates as shown in Figure 5. The kinetic values have been determined to be $V_m = 2.054 \times 10^{-6}$ mol $dm^{-3}s^{-1}$ and K = 10.35% pulp density. The half maximum point ($V_m/2$, K) lies in the middle of the straight line; on both sides of this point, equal emphasis is given for the experimental data.

In Figure 6, the observations are plotted as lines in parameter space, instead of points (51). The kinetic values are estimated directly from Figure 6 to be $V_m = 2.03 \times 10^{-6}$ mol $dm^{-3}s^{-1}$ and K = 10.2% pulp density. This approach has the following advantages over the traditional methods of plotting kinetic results:

- It is very simple to construct.
- It is composed entirely of straight lines and requires no calculation.
- The kinetic constants are read off the plot directly, and it may be used during the course of an experiment to judge the experiment's success.

Large-Scale Experiments

Experiments with pyrite concentrate were carried out in three individual runs with 10% pulp density suspensions. The average rate and final yield of pyrite oxidation were determined to be V = 10.2 ± 0.15 mol $dm^{-3}s^{-1}$ and 64.5 ± 0.36% (as total dissolved iron), respectively. The Eh during the leaching varied between 0.36 and 0.86 volt, while the pH from the initial 2.3 value decreased to 1.65 after five days of treatment. As the bacterial oxidation of pyrite progressed, a large portion of iron was precipitated. For example, the final extraction of 64.5% should have corresponded to a ferric iron concentration in solution of 29.799 g dm^{-3}. However, the direct measurement of iron in the leach solution gave only about 5.854 g dm^{-3}. Therefore, the amount of iron that was precipitated in the form of different mono- and polynuclear hydroxo- and sulphato-complexes was approximately 80.35%.

The ferrous iron oxidation was carried out in triplicate runs with complete 9K nutrient solutions, resulting in 98.3 ± 0.44% conversion in five days of treatment. The pH and Eh of these experiments varied from 2.3 to 1.9 and 0.48 to 0.81 volt, respectively. The decrease in the pH from 2.3 (initial) to 1.9 (final) is an indication that the hydrolysis reactions took place during the bacterial oxidation of ferrous iron. The initial 9.0 g dm^{-3} iron content was decreased to 4.695 g dm^{-3} after five days of biooxidation. This represents an approximately 52.17% of iron precipitation.

The energy available from the oxidation of pyrite (according to Eq 1) and of ferrous ion (according to Eq 3) can be expressed in terms of rH_2, which is the negative logarithm of the partial pressure of gaseous hydrogen (52):

$$1/2H_2 \rightleftharpoons H^+ + e^- \qquad \text{Eq 12}$$

The equilibrium constant is:

$$K_e = \frac{[H^+]}{[H_2]^{1/2}} \qquad \text{Eq 13}$$

from where

$$\log K_e = \log[H^+] - 1/2\log[H_2] \qquad \text{Eq 14}$$

Substituting pH and rH_2:

$$pH = -\log[H^+] \qquad \text{and on this analogy:} \qquad \text{Eq 15}$$

$$rH_2 = -\log[H_2] \qquad \text{Eq 16}$$

into Eq 14 yields:

$$\log K_e = -pH + 1/2rH_2 \qquad \text{Eq 17}$$

Application of the Nernst equation on reaction 12 yields:

$$Eh = E^o + \frac{RT}{nF} \ln K_e \qquad \text{Eq 18}$$

where Eh is the redox potential, E^o is the standard redox potential, R is the universal gas constant, n is the number of electrons exchanged in the reaction, and F is the Faraday constant. Substituting the constant values, $\log K_e$ and $E^o = 0$ into Eq 18, we get:

$$Eh = 2.303 \frac{1.987 \times 298}{23.06} (1/2rH_2 - pH) = 29.6(rH_2 - 2pH) \qquad \text{Eq 19}$$

Expressing now rH_2 from Eq 19:

$$rH_2 = Eh/29.6 + 2pH \qquad \text{Eq 20}$$

Oxidizing conditions exist when $rH_2 = 42.6$ and reducing conditions prevail at $rH_2 = 0$ (53). The rH_2 allows the comparison of the redox level in different systems at any time (54). For any redox reaction, the rH_2 can be determined according to the next equation:

$$rH_2 = \frac{2E^o}{0.059} + \frac{2}{n} \log K_e + 2pH \qquad \text{Eq 21}$$

For example, for ferrous ion oxidation according to Eq 3, the above rH_2 expression is changed to:

$$rH_2 = \frac{2 \times 0.771}{0.059} + \frac{2}{1} \log \frac{S_o - S}{S} + 2pH = 26.13 + 2 \log \frac{S_o - S}{S} + 2pH \qquad \text{Eq 22}$$

where S_o is the initial ferrous ion concentration and S is the actual ferrous ion concentration (in mol dm^{-3}). E^o for Fe^{3+}/Fe^{2+} is 0.771 volt (55).

Ferrous iron oxidation by bacteria was reported to follow the reaction (56):

$$Fe^{2+} + H^+ + 1/4O_2 \rightarrow Fe^{3+} + 1/2H_2O \qquad \text{Eq 23}$$

whose equilibrium constant, K_e, is:

$$K_e = \frac{[Fe^{3+}]}{[Fe^{2+}][H^+][O_2]^{1/4}} = 10^{7.7} \qquad \text{Eq 24}$$

The free energy change (ΔF) of reaction 23 was found to be approximately equal to $-1.3 \times pK_e$:

$$\Delta F = -1.3(7.7 - pH - 0.17) \qquad \text{Eq 25}$$

where $0.17 \simeq 0.2^{1/4}$ (0.2 atm being the normal concentration of oxygen in air) and pH - 0.17 represents the difference between the actual free energy change (ΔF) and the Gibbs free energy change (ΔG). However, Eq 25 is valid only if ferrous and ferric ions are both soluble. The entropy change of Eq 25 at 18°C is about $\Delta S = -13$ kcal mol^{-1}. Further studies are needed on the quantitative description of rH_2 involved in the oxidation of ferrous ion and other metal sulphides, in order to gain a better understanding about the energetics of the bioleaching phenomenon.

Respirometer Studies

The influence of temperature on pyrite and ferrous ion oxidations was studied in Warburg respirometers at temperatures varying from 20 to 45°C. The data are summarized in terms of specific oxygen uptake rate (V_{sp}) in Table 1. The V_{sp}-values are derived in the following manner:

- The rate of oxygen uptake (V) was calculated as the slope of the straight lines, representing the oxygen uptake ($\mu dm^3 O_2$) versus time (min).
- The rates were divided by the bacterial protein content used for each series of experiments, giving the specific rates of oxygen uptake $V_{sp}[\mu dm^3 O_2/(\text{min mg protein})]$.

From Table 1 and Figure 7, it can be seen that the highest specific rate of oxygen uptake was realized at 35°C for both of these substrates. The activation and thermal denaturation energies (ΔE_a and ΔE_d), as well as

the associated frequency factors (A), were derived from the linearized Arrhenius plots representing $\log(V_{sp})$ versus $1/T(K)$, as shown in Figure 8. The original Arrhenius equation was described for the reaction rate constant (k):

$$k = A \exp(-\Delta E_a/RT) \qquad \text{Eq 26}$$

In this study, the specific oxygen rate (V_{sp}) was used instead of k. The temperature coefficient, Q_{10}, has been calculated for the activation and thermal denaturation sides of the Arrhenius plots:

$$Q_{10} = \left[\frac{V_{sp2}}{V_{sp1}}\right]^{\frac{10}{T_2-T_1}} \qquad \text{Eq 27}$$

and the data, together with the ΔE_a and A values, are summarized in Table 2. For a reaction, $Q_{10} = 2$, which signifies that the reaction rate doubles for each 10°C rise in the range of temperature specified. The apparent activation energies higher than about 10 kcal are generally found for reactions that are chemically controlled. The data reported in Table 2 are in good agreement with those published for bacterial leaching of ZnS, Cu_2S, and CuS (57), and for the oxidation of $FeSO_4$ (58).

The bacterial oxidation of pyrite and ferrous ion substrates can be considered to proceed through the formation of an activated complex (59) prior to the release of products:

$$\text{Substrate} + \text{Bacteria} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} (\text{Activated Complex}) \xrightarrow{k_3} \text{Products} + \text{Bacteria} \qquad \text{Eq 28}$$

where k_1, k_2, and k_3 are the reaction rate constants for the formation and for the decomposition of the activated complex, and for the formation of products, respectively. Application of the transition state theory on the experimental data of pyrite and chalcopyrite biooxidation can allow the determination of thermodynamic values ($\Delta G^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$) for the activated complex. It is assumed that $k_1 >> k_2$ and $k_1 = k_3 = k$. Therefore, k can be related to the equilibrium constant, $K^{\ddagger}$, for the formation of the activation complex:

$$k = B \times K^{\ddagger} = \frac{k^* T}{h} K^{\ddagger} \qquad \text{Eq 29}$$

where k* is the Boltzmann constant = 1.381×10^{-23} JK^{-1}, T is the absolute temperature in degree Kelvin, and h is the Planck's constant = 6.626×10^{-34} Js. The standard free energy change of activation, $\Delta G^{\ddagger}$, for the formation of activated complex is related to the equilibrium constant by:

$$\Delta G^{\ddagger} = - RT \ln K^{\ddagger} \qquad \text{Eq 30}$$

or from here

$$K^{\ddagger} = \exp(-\Delta G^{\ddagger}/RT) \qquad \text{Eq 31}$$

Inserting Eq 31 into Eq 19 will result in the following:

$$k = \frac{k^{*}T}{h} \exp(-\Delta G^{\ddagger}/RT) \qquad \text{Eq 32}$$

The Gibbs energy of activation, $\Delta G^{\ddagger}$, is related to the corresponding enthalpy and entropy changes by the next equation:

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger} \qquad \text{Eq 33}$$

Combining Eq 33 and 32 yields:

$$k = \frac{k^{*}T}{h} \exp(\Delta S^{\ddagger}/R) \exp(-\Delta H^{\ddagger}/RT) \qquad \text{Eq 34}$$

The quantity $\Delta H^{\ddagger}$ differs slightly from the experimental energy of activation, ΔE_a, according to:

$$\Delta H^{\ddagger} = \Delta E_a - RT \qquad \text{Eq 35}$$

Inserting $\Delta H^{\ddagger}$ into Eq 34 yields:

$$k = \frac{ek^{*}T}{h} \exp(\Delta S^{\ddagger}/R) \exp(-\Delta E_a/RT) \qquad \text{Eq 36}$$

Comparison of Eq 36 with the Arrhenius Eq 26 shows that the frequency factor, A, is given by:

$$A = \frac{ek^{*}T}{h} \exp(\Delta S^{\ddagger}/R) \qquad \text{Eq 37}$$

Using the experimental ΔE_a and A data from Table 2, the V_{sp} data from Table 1 for 35°C, as well as Eq 32, 37, and 35, the thermodynamic values ($\Delta G^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta H^{\ddagger}$) of the activated complex can be calculated. Note that the assumption in these calculations is that V_{sp} replaces k. The calculated thermodynamic data are summarized in Table 3. The above calculations demonstrate the importance and usefulness of the experimental activation energy, ΔE_a, and frequency factor, A. Furthermore, the thermodynamic values ($\Delta G^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta H^{\ddagger}$) are important in gaining a better knowledge of how biooxidation of pyrite and ferrous ion may occur. For example, when a bacterial enzyme molecule undergoes interaction with a substrate molecule, often there is an electrical interaction involving identical or opposite charges, with a resulting effect on the entropy of activation. When the charges of interacting species are of the same sign, there is a much greater loss in entropy of the system and $\Delta S^{\ddagger}$ is negative, because the activated complex has a larger charge than the reacting ion species alone.

CONCLUSIONS

The applicability of different kinetic evaluation techniques to the effect of pulp density on the oxidation of pyrite by T. ferrooxidans has been demonstrated. The formation of iron mono- and polynuclear, hydroxo- and sulphato-complex species during the oxidation of pyrite and ferrous iron was discussed. The possibility of using rH_2 expressions in the energetic description of substrate oxidation by T. ferrooxidans was shown. The effects of temperature on pyrite and ferrous ion oxidation by the bacteria were determined from the Warburg respirometer measurement. The ΔE_a, ΔE_d, A, and Q_{10} values were assessed, and the application of transition state theory to the activated complex demonstrated. The kinetic and thermodynamic approaches developed in this study can be useful for the specialist involved in studies of metal sulphides.

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TABLES

Table 1 - Effect of temperature on the specific rate of oxygen uptake by Thiobacillus ferrooxidans

Temperature °C	Specific rate of oxygen uptake* [μdm^3 O_2/(h mg protein)]			
	Fe^{2+} ion		Pyrite	
	Inoculated	Sterile	Inoculated	Sterile
20	13.81 ± 0.16	1.4 ± 0.08	16.74 ± 0.24	1.8 ± 0.22
25	19.53 ± 0.32	1.8 ± 0.11	21.90 ± 0.19	2.1 ± 0.18
30	29.52 ± 0.24	2.3 ± 0.24	42.78 ± 0.32	2.9 ± 0.37
35	42.48 ± 0.21	3.5 ± 0.27	57.60 ± 0.34	3.7 ± 0.42
40	16.29 ± 0.33	2.7 ± 0.13	18.72 ± 0.28	3.8 ± 0.29
45	6.18 ± 0.09	0.8 ± 0.21	6.30 ± 0.18	3.4 ± 0.26
Endogenous respiration at 35°C	1.50 ± 0.09			1.7 ± 0.10

*Each figure is the mean of three individual experiments (standard error of the mean).

Table 2 - Thermodynamic data derived from the influence of temperature on oxidation of pyrite and ferrous ion by Thiobacillus ferrooxidans

Thermodynamic data	Pyrite	Fe^{2+}
ΔE_a (cal)	17.78	13.34
A[$\mu dm^3 O_2$/(min mg protein)]	0.98	30.18
Q_{10} for 20-30°C	2.55	2.00
ΔE_d (cal)	42.96	37.29
A[$\mu dm^3 O_2$/(min mg protein)]	53.70	5.83
Q_{10} for 35-45°C	0.11	0.15

Table 3 - Thermodynamic values for the formation of the activation complex during the oxidation of pyrite and ferrous ion by Thiobacillus ferrooxidans

Thermodynamic values	Pyrite	Fe^{2+}
$\Delta G^{=}$ (kcal)	15.57	15.76
$\Delta S^{=}$ (cal/degree)	-60.64	-53.82
$\Delta H^{=}$ (kcal)	-3.11	-0.82

FIGURES

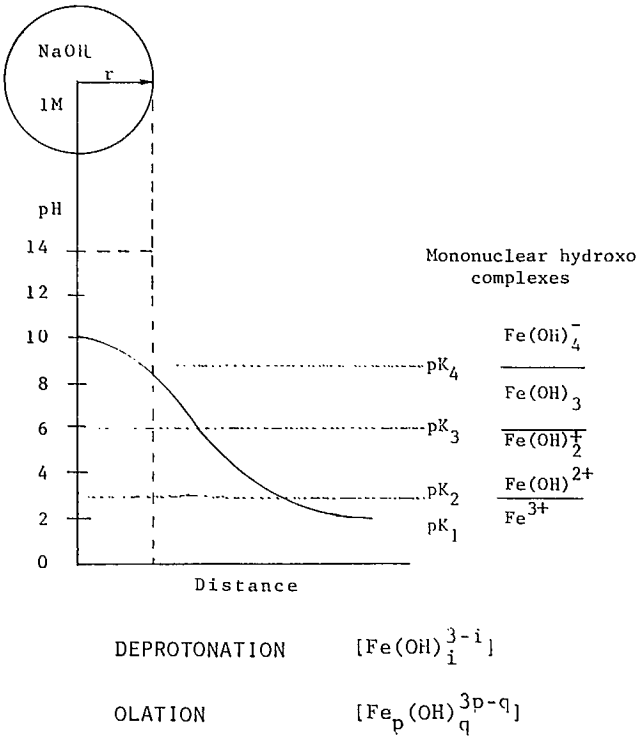


Fig. 1 - pH-profile as a function of time at the addition of NaOH to the leach solution containing ferric ion

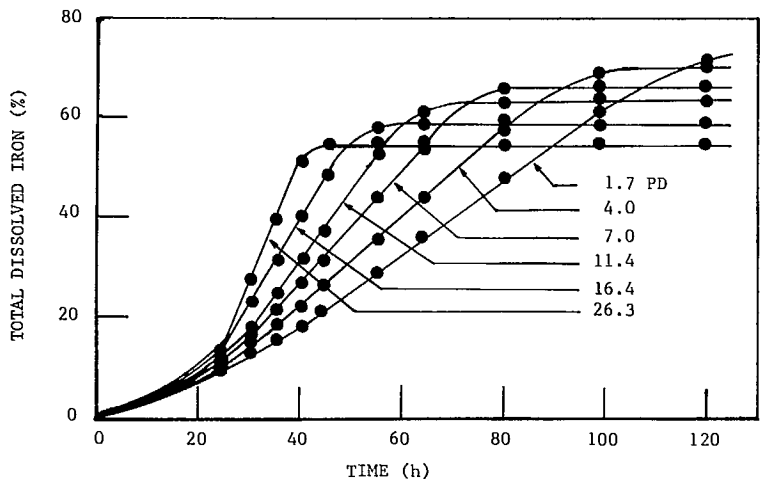


Fig. 2 - Effect of pulp density on pyrite oxidation by Thiobacillus ferrooxidans

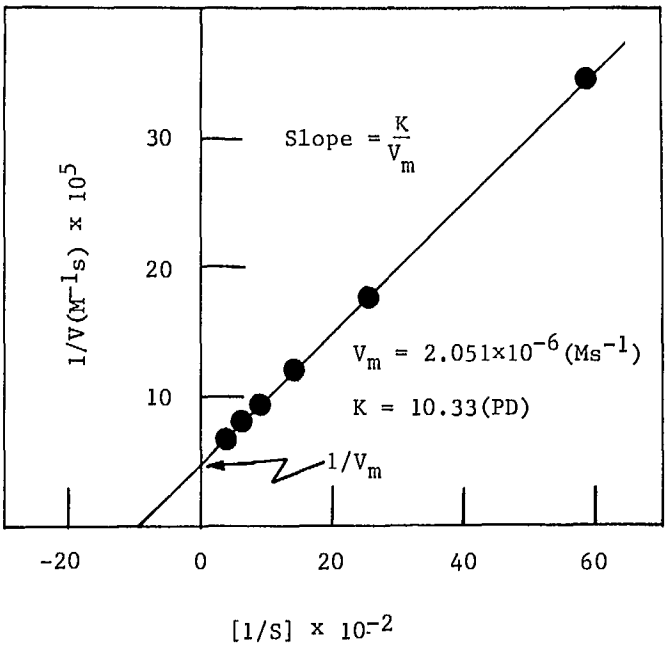


Fig. 3 - Linearized Monod representation of the effect of pulp density

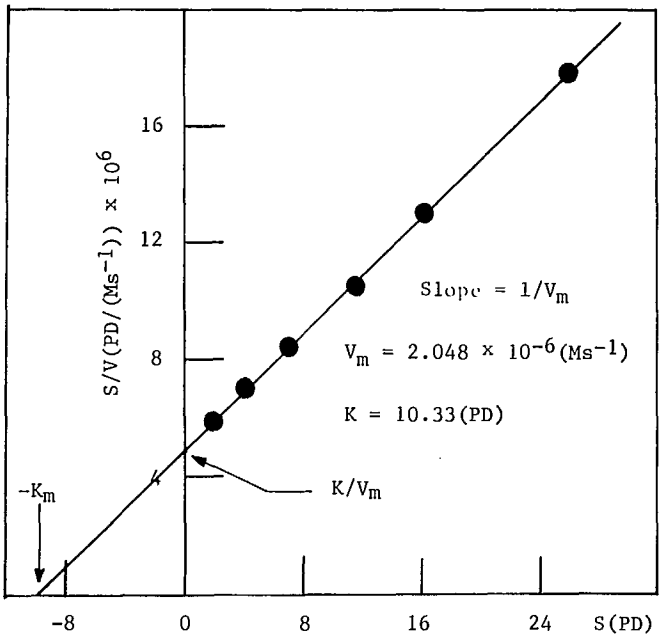


Fig. 4 - Adapted Woolf's linearized approach for the representation of the effect of pulp density

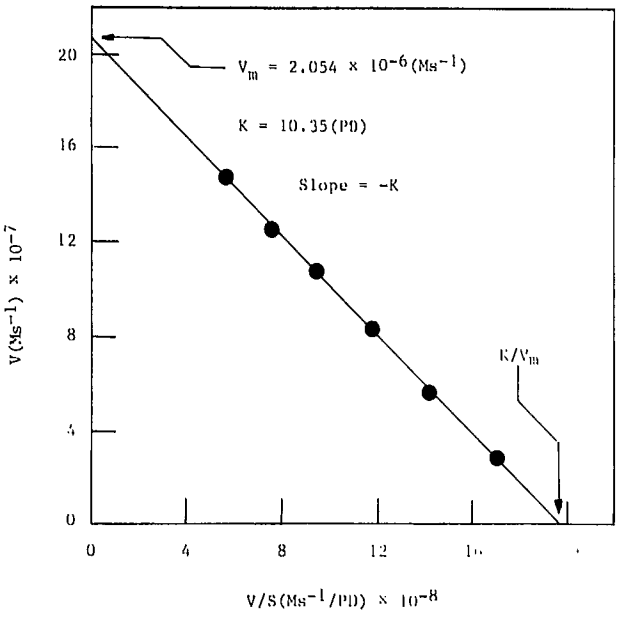


Fig. 5 - Augustinsson linear plot for the effect of pulp density

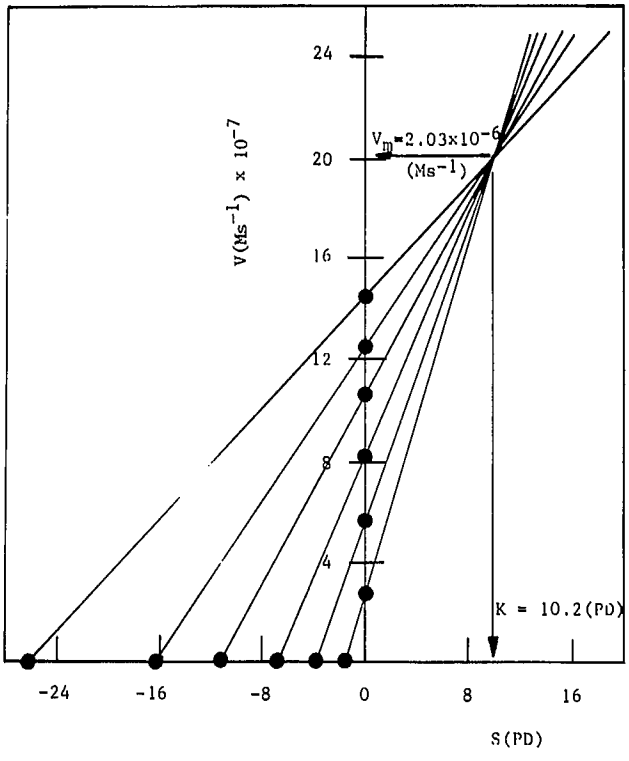


Fig. 6 - Direct linear plot for estimating the kinetic parameters associated with the effect of pulp density

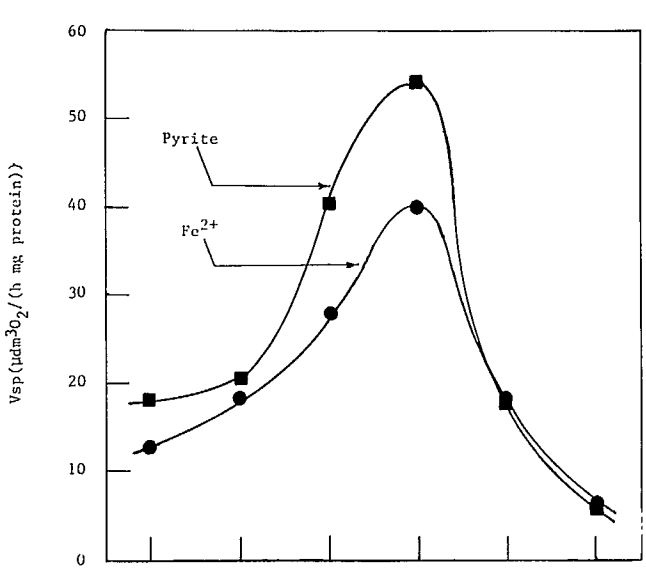
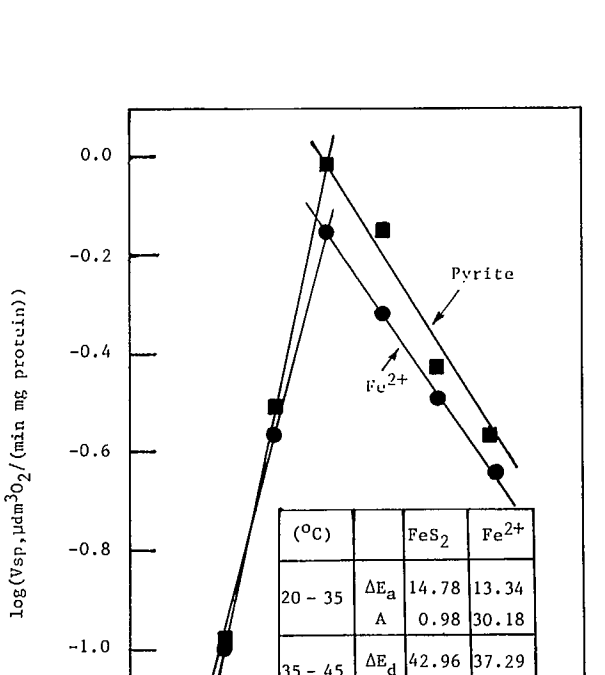


Fig. 7 - Effect of temperature on pyrite and ferrous ion oxidations by Thiobacillus ferrooxidans



(°C)		FeS_2	Fe^{2+}
20 - 35	ΔE_a	14.78	13.34
	A	0.98	30.18
35 - 45	ΔE_d	42.96	37.29
	A	53.70	5.83

Fig. 8 - Arrhenius plots for pyrite and ferrous ion oxidation by Thiobacillus ferrooxidans

SESSION I: PAPER 2

BIOTECHNOLOGICAL CONSIDERATIONS FOR DESIGNING METAL-RECOVERY OPERATIONS

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ABSTRACT

Microorganisms should be an important consideration in designing a semi-enclosed, bioleaching operation such as a stirred-tank reactor. The classical parameters - including particle size, pulp density, residence time, etc. - are typically optimized for different leaching situations. However, very little attention has been given to the type and number of microorganisms present in either pilot-scale or operational metal-recovery systems. The necessity of having a better understanding of the microbiology of bioleaching, with special reference to those applications involving semi-enclosed conditions, is discussed.

SESSION I: PRÉSENTATION 2

CONSIDÉRATIONS D'ORDRE BIOTECHNOLOGIQUE CONCERNANT LA CONCEPTION DES PROCÉDÉS DE RÉCUPÉRATION DE MÉTAUX

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RÉSUMÉ

Il est important, au moment de concevoir un procédé de récupération de métaux, de considérer l'utilisation de micro-organismes. Les paramètres classiques (soit entre autres, la taille des particules, la densité de la pâte, le temps de séjour, etc.) sont optimisés de façon typique pour diverses conditions de lixiviation. On a cependant prêté très peu d'attention au type et à la quantité de biomasse présente, qu'il s'agisse de systèmes de récupération opérationnels ou de systèmes à l'échelle-pilote. On traite d'exemples expliquant pourquoi il est crucial de comprendre et de réguler l'action des micro-organismes lors de la lixiviation en dépotoir, in situ ou en réservoir, ainsi que lors du traitement secondaire.

BIOTECHNOLOGICAL CONSIDERATIONS FOR DESIGNING METAL-RECOVERY OPERATIONS

INTRODUCTION

There are numerous laboratory studies designed to assess the feasibility of using microorganisms for metal recovery from various ores. These studies typically address the effect of parameters such as particle size, pulp density, pH, temperature, aeration, and addition of nutrients on the rate and extent of metal solubilization. These studies include examples of both batch and continuous leaching. They utilize, as inocula, either native microorganisms associated with the ore or laboratory cultures of single or mixed strains of bacteria. However, commercial applications of bioleaching - such as dump and heap leaching - unlike the laboratory studies, utilize only native microorganisms. Since the substrate cannot be sterilized economically, native microorganisms are always going to be present. Thus, any laboratory strains added as an inoculum will have to compete with the native microorganisms.

The benefit of adding inocula of laboratory strains, on either the rate or extent of metal solubilization in leaching dumps, remains to be evaluated. It is possible that a substantial inoculum of suitable microorganisms could significantly reduce the lag phase, which is typically measured in months; however, the expense of this action cannot be justified economically. Moreover, a dump includes a wide range of microbial habitats that vary in temperature, pH, air and nutrient concentrations, mineralogy, etc., and among the initial population it is possible that there will be representative organisms that can grow and solubilize metals from many, if not all, of these various habitats. Therefore, it seems reasonable, at present, to utilize native microorganisms for in situ, dump, and heap leaching.

However, there has been discussion recently on the economic feasibility of utilizing microorganisms, in containers such as semi-enclosed vats and stirred-tanks, for the recovery of metals or for the beneficiation of ores. There are significantly different microbiological and chemical-engineering considerations for these applications compared to dump, heap, or in situ leaching. Chemical-engineering parameters influencing the rate and extent of biooxidation of refractory arsenical sulphide concentrates have been carefully investigated in a continuous, vat-leaching pilot plant at the Equity Silver Mine, British Columbia, Canada (1). In this study and in a similar feasibility study carried out by Gencor, South Africa, it was concluded that scaleup to a plant-size operation was economically feasible. Because of these successes, as well as some additional studies, other mining companies may be encouraged to investigate opportunities for vat and tank bioleaching.

This paper grew out of an attempt to determine the considerations and problems, principally of a microbiological nature, that might arise when microorganisms are utilized in non-sterile, semi-enclosed reactors such as tanks or vats. Many of these problems have been confronted previously by biochemical engineers in other areas of biotechnology. However, there are some additional considerations peculiar to biohydrometallurgical applications. Some of these considerations do not appear to have been investigated sufficiently, or at least articulated adequately, by either the mining and metallurgical community or the research microbiologists. For this reason, it is

worthwhile sharing some thoughts on the subject. For the sake of discussion, these considerations have been divided into the following, somewhat arbitrary, categories:

- continuous culture, non-sterile, semi-enclosed conditions (stirred-tanks, vats, etc.);
- batch culture, non-sterile, semi-enclosed conditions (stirred-tanks, vats, etc.);
- continuous culture, non-sterile, environmental conditions (dumps, heaps, and in situ operations);
- efficiency of native versus laboratory microorganisms;
- additional considerations.

Where possible, laboratory studies needed to address some of these considerations more effectively are identified.

CONTINUOUS CULTURE, NON-STERILE, SEMI-ENCLOSED CONDITIONS (STIRRED-TANKS, VATS, ETC.)

In continuous cultures, microorganisms grow and divide from an initial inoculum until they reach a steady state of growth and activity. At steady state, external parameters such as pH, temperature, and substrate concentration are held as constant as possible. The microorganisms divide at a rate that is controlled largely by substrate concentration. Steady-state behaviour of continuous cultures has been modelled since the pioneering work of Monod (2). However, it has been shown that the Monod model, which was developed for a soluble substrate, may not adequately describe the growth behaviour of T. ferrooxidans on solid zinc concentrates in a laboratory-scale, stirred-tank reactor (3). The most reasonable explanation is that growth and leaching require contact between the bacteria and the substrate surface and are, therefore, affected by available surface area. The significance of bacterial attachment and the production of biofilms in bioleaching operations will be discussed later. The point to be made here is that the considerable experience of biochemical engineers in modelling and designing reactors for other biotechnological applications may not be applicable, at least to a certain extent, to bioleaching operations.

There are several advantages of steady-state culturing versus batch culturing for industrial applications. These include no lag phase (excluding the initial lag phase needed to establish the culture); a uniform set of culture conditions; and certain advantages in loading the substrate and nutrients as well as in harvesting the product. However, with one or two notable exceptions, industrial microbiological processes utilize batch and not continuous culture. The principal reason for this choice is that single-cell cultures of highly selected laboratory strains are generally used in these applications, and it has proved too difficult to keep such cultures free from contamination and from alterations by mutation in continuous culture. Again, it is unfortunate that the mining community cannot draw, at present, on the abundant information available to the biotechnology community involved in batch culturing.

However, there is one good example of an industrial, continuous microbiological process that might serve as a model, to some extent, for certain bio-

leaching operations, namely, biological waste-treatment processes. There are several important similarities between biological waste-treatment processes and projected plans for plant-size, stirred-tank or vat reactors for bio-oxidation of ores. Both processes operate with the continuous introduction of rather variable, ill-defined substrates and both utilize a largely uncharacterized, heterogeneous collection of microorganisms. Both operate under non-sterile conditions subject to fortuitous inoculation from the fluid feeds and from the atmosphere.

In biological waste-treatment processes, the organisms eventually develop elective cultures that are not significantly affected by random inoculation of new organisms or, apparently, by mutation or recombination leading to genetic change among the resident microorganisms. However, there is insufficient experience at the operational level, and insufficient data at the basic research level, to be certain that continuous cultures of bioleaching microorganisms will be similarly insensitive to contamination and genetic change. Because of these uncertainties, one must be aware that incoming microorganisms might destabilize the existing conditions in a bioleaching reactor, or that resident microorganisms might undergo significant genetic change leading to non-uniform and unpredictable biooxidation.

A very real problem encountered in biological waste-treatment processes is the highly dynamic behaviour of the microbial population. Elective populations can undergo remarkably abrupt changes. Such changes can have many causes and they are difficult to control. Also, because such systems are so complex, they are extremely difficult to model and are, therefore, also unpredictable. This instability is considered to be, in part, the manifestation of the complex interactions between organisms in a mixed population. A mixed population in dynamic equilibrium can have members interacting in various positive and negative ways. Some common microbial interactions are listed in Table 1.

In natural populations, it is very likely that a given organism is participating in several interactions, some of which could be oscillatory. These interactions would be expected to be asynchronous and leading to fluctuations, frequently severe, in the population. For example, attacks by bacterial viruses (phages) have been invoked to explain drastic declines in microbial species during waste-treatment processes. More subtle changes have been attributed to other predator-prey relationships such as the production of bacterial toxins by some members of the microbial community, or through predation by protozoa.

Are there real grounds for concern about instability in bioleaching operations? Phages have not been positively identified in the common bioleaching microorganisms such as Thiobacillus ferrooxidans. However, this may be because not enough organisms have been screened yet for phages, or because the phages are in a latent (non-virulent) form. Latent phages are common in many species of bacteria. They remain hidden in the genome of the host for many cell generations but can be induced by environmental perturbations, for example, to enter a virulent state. Also, although acid-tolerant protozoa have been found in mine drainage, the extent to which they prey on bioleaching microorganisms has not been investigated (16).

An additional source of microbial fluctuation in biological waste-treatment processes is the variability of the substrate. The sudden introduction of substances such as antibiotics or toxic chemicals can irreversibly eliminate certain microorganisms. Minor changes in either the substrate or the conditions can set in motion oscillatory fluctuations in the population, which can eventually settle back to its initial state, but abrupt changes can lead to irreversible situations. The substrate utilized in a bioleaching reactor may also vary substantially during the lifetime of the operation. The extent of the physical and chemical diversity of the incoming ore, over a period of time, should be evaluated so that gross inhomogeneities can be reduced, perhaps by bulk mixing. Special attention should be given to the presence of toxic substances such as arsenic and silver.

BATCH CULTURE, NON-STERILE, SEMI-ENCLOSED CONDITIONS (STIRRED-TANKS, VATS, ETC.)

There are many laboratory studies that have investigated the use of microorganisms for metal recovery using batch-fed, stirred-tank reactors. But, there appear to be no examples of commercial applications of this technology for bioleaching.

In principle, batch-fed reactors suffer from many of the potential problems with continuously fed reactors. For example, there could be problems with variability of substrate and inoculum similar to those encountered in a continuous reactor. However, in a batch-fed reactor there are additional important aspects of microbiology that deserve consideration.

In laboratory studies, batch reactors have been optimized for the rate and extent of biooxidation of the minerals, starting with inocula of either native microorganisms or laboratory cultures. The problem is that the culture conditions eventually chosen could represent a compromise between optimal conditions for growth and those for biooxidation. The rate of biooxidation is a function of the concentration of the actively oxidizing cells and of the specific activity of the enzymes involved. Conditions that promote growth and cell division are not necessarily optimal for enzymatic activity. For example, T. ferrooxidans cells generally divide most rapidly at 30°C, but they oxidize iron faster at 35°C. The problem is made more difficult by the non-uniformity of the culture conditions. As biooxidation progresses, the accumulation of metal and ferric ions could be inhibitory. Also, substrate concentrations and the physical characteristics of the substrates change with time.

CONTINUOUS CULTURE, NON-STERILE, ENVIRONMENTAL CONDITIONS (DUMPS, HEAPS AND IN SITU OPERATIONS)

A bioleaching operation such as a dump or a heap requires special consideration from a microbiological point of view. Such an operation represents a non-uniform substrate, physically variable in space and time, and supporting a complex population of microorganisms that is also variable over space and time. Little is known about the species of microorganism present, their re-

lative roles in biooxidation, how they interact with each other, or how the rate and extent of biooxidation are modified by the physical parameters of the operation. The complexity of the situation precludes effective mathematical modelling and makes laboratory scale modelling exceedingly difficult.

Clearly, one of the central issues that deserves increased attention is the development of improved techniques for species identification and enumeration. The introduction of such techniques would greatly expedite efforts to understand the relative roles of the different microorganisms in the bioleaching process. Unfortunately, a number of special problems exist that impede progress in this area. For example, there is a paucity of suitable genetic and biochemical markers to distinguish species. Also, many strains are difficult or impossible to grow on solid media, making it difficult to isolate pure strains. Many species also require long incubation periods. Another significant problem is the attachment of bacteria to rock particles, which makes it awkward to obtain accurate numbers and difficult to interpret the results. Solutions to these problems deserve increased attention.

EFFICIENCY OF NATIVE MICROORGANISMS VERSUS LABORATORY CULTURES

There is a widespread misconception that indigenous microorganisms are necessarily the best-adapted for growth on a given ore and, by implication, that they are also the most efficient for metal recovery. The argument is that they have had a considerable period of time to be selected for that role. It is true that they will have been selected over countless generations for survival in specific ecological niches within a particular orebody. However, a bioleaching operation represents a new and perturbed environment that could be radically different from the natural situation in a number of crucial ways. In a tank reactor, for example, the oxygen, carbon dioxide, and nutrient levels are different, with substrate surfaces having been changed qualitatively and quantitatively. Also, because the rocks are ground-up and mixed, there is an opportunity for microorganisms to come into contact with inhibitory substances such as arsenic that might, in nature, have been sequestered away from an area colonized by microorganisms. Therefore, it does not necessarily follow that, in the new environment of a tank reactor, the most efficient microorganisms for growth or for metal solubilization are associated with the native orebody.

A key issue, at present, is whether indigenous microorganisms are more effective at bioleaching their native ore than are laboratory strains, added as either mixed or single cultures. There is a real need to investigate this problem more thoroughly.

In those cases where it can be demonstrated that laboratory cultures are as effective at bioleaching as the native microorganisms, a good case can be made for utilizing the laboratory strains. They are more amenable to fundamental studies providing opportunities to improve or to modify their performance. They are less likely to harbour phage. Because their population is less complex, they are less likely to undergo unpredictable fluctuations. Also, the adoption of laboratory strains would permit a certain amount of unification in the application of microorganisms to bioleaching. At present, each operation must evaluate and draw upon the potential of its own microor-

ganism. The adoption of publicly available laboratory strains would permit companies to share results, problems, and research.

Encouraging results for the use of laboratory strains are being achieved. Although T. ferrooxidans and T. thiooxidans rank among the most important organisms in mineral leaching, there are several studies indicating that both iron- and sulphur-oxidizing bacteria, including certain thermophilic species, can play an important role in metal solubilization. Indeed, some of these other microorganisms have higher rates of iron or sulphur oxidation, with better yields on certain recalcitrant ores and at higher pulp densities than T. ferrooxidans (5,6).

When considering the application of laboratory strains, an important question is whether pure cultures of a single bacterial strain can solubilize metals as fast as mixed cultures. An example of the possible benefit of mixed cultures for bioleaching is illustrated by the proposed symbiotic relationship between T. ferrooxidans and associated acidophilic heterotrophs (12,13). It is well established that pyruvate inhibits growth and iron oxidation of T. ferrooxidans (7), and it has been demonstrated that this inhibition can be overcome by associated acidophilic heterotrophs (8) at the laboratory level. However, the significance of this to commercial bioleaching remains to be investigated. As another example, it has been proposed that the oxidation of elemental sulphur may be an important limiting factor in some bioleaching operations, and that T. thiooxidans can promote the dissolution of iron by T. ferrooxidans through oxidizing any elemental sulphur that might accumulate (9). Also, it has been shown that mixtures of pure cultures of Thiobacillus species and acidophilic heterotrophic species exhibit a rate of pyrite oxidation in coal that is superior to the rate achieved by pure cultures of a single strain of Thiobacillus, and that the rate approaches the one exhibited by enrichment cultures (10).

Comparisons between the more thoroughly understood bioleaching microorganisms, such as T. ferrooxidans, and some of the newly discovered microorganisms are only just beginning to be undertaken at the laboratory level. Such comparisons are important, and the mining engineer would profit by following these investigations as they unfold. The principle that is involved here is fundamental to biochemical engineering, namely, a search for alternative catalysts that are more efficient and better understood.

Another matter of considerable importance is the extent of biooxidation carried out by microorganisms attached to ore particles, perhaps as microbial films, versus the activity of microorganisms that remain in solution. It has been demonstrated that several known bioleaching microorganisms are capable of attaching to solid substrates such as rock particles (14). It has also been speculated that a major contribution to the biooxidation of metals in mining dumps is made by bacteria in biofilms (11). This has important implications for the design of bioleaching tank reactors. The similarities between pilot bioleaching vat reactors and biological waste-disposal processes have already been discussed. But there is at least one important difference between the two. In a biological waste-disposal plant, the production of an active biofilm on recyclable particles seems to be the key to effective biological degradation of waste. The biofilm particles have the opportunity to be recycled because it is the liquid phase that is disposed of (harvested) in

the treatment. On the other hand, in a bioleaching reactor it is the solid components that are harvested. Thus, any biofilm that forms on the solid components is lost. The liquid phase, or a portion of it, can be recycled to assist in replenishing acid and microorganisms. The liquid phase is rich in microorganisms, but it is not known whether these microorganisms retain the same ability to adhere to incoming ore particles as do native microorganisms obtained with the original orebody. It is conceivable that recycling the liquid phase encourages the growth of specific organisms that have a reduced ability to adhere to solid particles. These could compete for nutrients with the microorganisms that constitute a resident biofilm. These are issues that have not been investigated very thoroughly.

There has been considerable interest recently in the possible use of classical genetics and genetic engineering for strain improvement of bioleaching microorganisms, especially T. ferrooxidans. Before discussing the possibilities and problems of strain improvement, it should be emphasized that a major objective of classical genetics and genetic engineering, at present, is to assist in the understanding of the biochemistry of bioleaching organisms, especially the biochemistry of chemoautotrophy and acidophilia. Without a thorough understanding of this biochemistry, it would be difficult at best, and impossible at worst, to effectively improve strains for practical bioleaching.

Given that such fundamental investigations into the biochemistry of bioleaching microorganisms will be carried out, the prospects for strain improvement by genetic engineering are exciting to contemplate. Over the past 50 years, since the original discovery by Flemming, penicillin production has been increased by some 60 000 fold per unit of the mold. These strain improvements were accomplished by classical manipulations that, almost certainly, could now be completed in less time using recent genetic engineering technology. What characteristics of say, T. ferrooxidans, might one wish to manipulate in order to enhance its usefulness?

The answer is not simple. It depends on what physical parameters of the leaching operation are limiting, what features of the biochemistry of the organisms are limiting, and what biochemical features are amenable to genetic modification. A discussion of the physical parameters that affect bioleaching such as particle size, mixing, etc., and an analysis of the feasibility of genetic engineering in T. ferrooxidans are outside the scope of this paper. Since biochemical characteristics that affect the rate and extent of bioleaching have not been sufficiently identified by basic research, it is only possible to speculate on what modifications might be desirable for strain improvement. Quite clearly, an enhancement of growth rate would be attractive, as would higher rates of iron and sulphur oxidation. Other characteristics that may prove to be important and worth modifying by genetic engineering might include enhanced ability to oxidize non-ferrous metals; reduced sensitivity to organic compounds; increased or reduced attachment properties; increased metal tolerance; tolerance to cyanide and to chloride ions; the production of surfactants; enhanced activity in the cold; secretion or intracellular production of specific metal-binding proteins; resistance to phage infection; reduced tendency to undergo genetic changes; enhanced ability to fix nitrogen; etc.

Whatever improvements are ultimately made, either directly by genetic manipulation, or indirectly by laboratory selection of naturally occurring strains, the result is a strain of bacteria that must compete and retain the activity of desirable traits in the presence of native microorganisms. The question of the survivability of a laboratory strain in a bioleaching operation, whether in an open dump or a semi-enclosed tank reactor, has not been investigated. This is an area of interest to our laboratory (15). The problem is that there are inadequate techniques for identifying and enumerating most bioleaching organisms. Perhaps useful objectives of genetic engineering will be to introduce suitable genetic markers into a laboratory strain to facilitate strain identification, and to provide a selection mechanism to promote the maintenance of a laboratory strain in a bioleaching operation.

ADDITIONAL CONSIDERATIONS

Two remaining points concern a couple of very tough problems. First, the variability of the substrates being worked on is an impediment to progress. The second point concerns the difficulties encountered in communication between the several disciplines that constitute biohydrometallurgy.

The potential microbiological problems intrinsic to variable substrates within a bioleaching operation have already been discussed. The variations in substrates between operations or mines pose additional problems. It is necessary, apparently, for each mining operation to explore its own bioleaching parameters and to establish its own bioleaching microorganisms. Contrast this to what is happening in other areas of biotechnology around the world. A pharmaceutical company making human insulin from recombinant bacteria can specify precise physical conditions and can utilize a defined microorganism, making it possible to build plants anywhere with reproducible and predictable results. But in bioleaching, the case-by-case evaluation of parameters must necessarily impede the rate of adoption of biological solutions to metal recovery. What is needed to expedite the design of novel metal-recovery operations is to pay special attention to those common operating features. These features could be identified more readily if more attention was given to pinpointing and characterizing the microorganisms involved in a particular bioleaching operation. After all, these microorganisms represent the biggest uncharacterized factor in bioleaching.

A final problem concerns the communication gap between the mining and metallurgy community, on the one hand, and the academic and industrial scientists, on the other hand. Research and practical papers of bioleaching interest are published in many diverse journals and, sometimes, in less accessible proceedings of meetings. The two groups seldom read each other's journals or attend each other's meetings. What can be done to remedy this situation? Obviously, we can increase our efforts in crossing disciplinary boundaries, but it might also be appropriate to form a society to promote biohydrometallurgy and to publish its own journal. Alternatively, scientists and engineers interested in biohydrometallurgy, such as those present at the biannual international meetings on biohydrometallurgy, could form a sub-chapter of some existing organization. Whatever the solution, an attmpt must be made to improve communication.

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TABLE

Table 1 - Common terms for microbial interactions (4)

Neutralism	Lack of interaction
Commensalism	One organism benefits, while the other is unaffected
Mutualism	Each organism benefits from the other
Competition	A race for nutrients and space
Amensalism	One organism adversely changes the environment for the other
Parasitism	One organism steals from another
Predation	One organism ingests another
Synergism	Cooperative metabolism to produce a substance not produced by either alone

SESSION I: PAPER 3

FERROUS ION OXIDATION, NITROGEN FIXATION (ACETYLENE REDUCTION), AND NITRATE REDUCTASE ACTIVITY BY THIOBACILLUS FERROOXIDANS

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ABSTRACT

Five strains of Thiobacillus ferrooxidans were screened for their ability to grow in a nitrogen-free, mineral salts medium containing 120 mM ferrous sulphate as the sole energy substrate. Only one strain was able to grow with Fe^{2+} in the absence of ammonium ion, and the rate was slow in comparison with growth in the ammonium-containing medium. Nitrogenase activity of T. ferrooxidans was determined by using the acetylene reduction technique. Appropriate chemical controls were used to rule out the abiotic reduction of acetylene. The nitrogenase activity, influenced by the headspace gas composition, was dependent on the presence of ferrous ion as the electron donor. Nitrate reductase activity was also shown to be present in T. ferrooxidans.

SESSION I: PRÉSENTATION 3

CROISSANCE ET FIXATION DE L'AZOTE (PAR RÉDUCTION DE L'ACÉTYLÈNE) PAR THIOBACILLUS FERROOXIDANS DANS UN MILIEU EXEMPT D'AZOTE

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RÉSUMÉ

Cinq souches de T. ferrooxidans ont été sélectionnées à cause de leur aptitude à croître dans un milieu de sels minéraux exempt d'azote contenant comme seul substrat énergétique 120 mM de sulfate ferreux. Une seule souche était capable de croître avec du Fe^{2+} en l'absence d'ammonium, et la vitesse de croissance était plus lente que dans un milieu contenant du NH_4^+. L'activité de la nitrogénase a été déterminée chez T. ferrooxidans selon la technique de la réduction de l'acétylène. On a procédé aux contrôles chimiques nécessaires pour éliminer la possibilité de réduction abiotique de l'acétylène. On a montré que la composition du gaz de l'espace de tête et que le pH du milieu influaient sur la capacité de T. ferrooxidans à réduire l'acétylène. La vitesse de réduction biologique de l'acétylène par T. ferrooxidans a également été déterminée.

FERROUS ION OXIDATION, NITROGEN FIXATION (ACETYLENE REDUCTION), AND NITRATE REDUCTASE ACTIVITY BY *THIOBACILLUS FERROOXIDANS*

INTRODUCTION

Ammonium ion is generally included as a source of nitrogen in the mineral salts media for Thiobacillus ferrooxidans. Information on the ability of this bacterium or of other acidophilic thiobacilli to utilize other nitrogen compounds is sparse. An isolate from a copper dump-leaching operation, presumably T. thiooxidans, was described as being able to utilize urea as a source of nitrogen and being incapable of fixing atmospheric N_2 (1), although the presence of urease activity was not sought in this study. Later, patent claims were made (2) on the enhancement of biological leaching due to thiourea (H_2NCSNH_2) and carbamide phosphoric acid ($H_2NCONH_2.H_3PO_4$), but these observations were not further substantiated. Tsuchiya et al. (3), as well as Trivedi and Tsuchiya (4), presented data on copper leaching in mixtures of T. ferrooxidans and Beijerinckia lacticogenes; they also speculated on a mutual interaction whereby B. lacticogenes, as a nitrogen fixer (diazotroph), may provide ammonia for T. ferrooxidans. The latter, as a CO_2 fixer (autotroph), may excrete organic carbon into the medium, which the diazotroph may be able to utilize for growth in an otherwise inorganic medium. These speculations were not validated further, and later attempts to reproduce these effects failed (5). Alternative explanations have been tendered in the literature (6) to suggest that the interplay of the two organisms was not based on nitrogen fixation. Tsuchiya et al. (3) also reported that, based on an acetylene reduction technique which was not given in detail, T. ferrooxidans lacked a nitrogenase activity. Mackintosh (7) demonstrated that a re-purified isolate of T. ferrooxidans grew in the absence of NH_4^+ and was able to produce ethylene from actelene. The diazotrophic character of the culture was confirmed by the demonstration of the enrichment of ^{15}N in cellular protein when the bacteria were incubated with ferrous ion under $^{15}N_2$.

For the present work, the ability of T. ferrooxidans to fix dinitrogen gas was evaluated with the acetylene reduction technique. Evidence was also sought for assimilatory nitrate reductase activity in T. ferrooxidans.

MATERIALS AND METHODS

Bacteria and Growth Conditions

T. ferrooxidans strain TFI-35 was grown in 100-mL cultures in 250-mL shake flasks (125 rpm) at room temperature in a mineral salts medium containing 4 mM of $(NH_4)_2SO_4$, 2 mM of $MgSO_4.7H_2O$, 2.9 mM of K_2HPO_4, 50 mM of H_2SO_4, and 120 mM of $FeSO_4.7H_2O$. Ten per cent inocula were used for subcultures. Ferrous ion oxidation was monitored titrimetrically with 2 mM potassium permanganate. A nitrogen-free medium was modified from the above by omitting ammonium sulphate.

For harvesting T. ferrooxidans biomass, 1-L cultures in the nitrogen-free medium were transferred into 17-L batches in glass carboys and incubated under forced aeration for seven days. Cells were first concentrated to a retentate by tangential filtration (0.5 µm HVLP-OHV filters at 550 kPa) and pelleted by centrifugation. The cell pellet was washed twice in 5 mM H_2SO_4 and resuspended in one litre of the nitrogen-free medium, followed by a 24-h incubation at 125 rpm. The cells were recovered by centrifugation and finally resuspended in 5 mM of H_2SO_4 for use in the acetylene reduction experiments. The cell suspensions were standardized by protein determination (8).

Acetylene Reduction Assay

Nitrogen fixation by T. ferrooxidans was monitored with the use of an acetylene reduction technique. Acetylene, which also is a substrate for the nitrogenase enzyme complex, is reduced to ethylene in the assay. The incubations for the assays were carried out at room temperature in 167-mL serum bottles containing 30 mL of the medium and 1.0 mL of the cell suspension. The bottles were sealed and the headspace was flushed with helium, which was then replaced by the desired amount of air. The reaction was started by the addition of acetylene (450 µmol/assay). Ethylene in headspace samples was determined by gas chromatography (Varian 3700), using a hydrogen flame ionization detector and a stainless steel column (Porapak R) held at 60°C, and nitrogen as a carrier gas at 45 mL/min.

Nitrate Reductase Assay

Assimilatory nitrate reductase was assayed in a washed cell suspension that was incubated for 24 h in the mineral salts medium in which 1 mM of KNO_3 was substituted for ammonium sulphate. The cells were harvested and resuspended in 10 mM of phosphate buffer (pH 7.0) containing 1 mM of magnesium sulphate, washed three times, and broken in a French Press cell at 140 MPa (9). The cell homogenate was centrifuged at 10 000 x g for 20 min to yield a supernatant (S_{10}) and a pellet (P_{10}) fraction. The nitrate reductase assay followed the procedure given by Formden and Robertson (10). The reaction mixture contained 0.8 mL of either S_{10}, P_{10}, or cell homogenate; 0.2 mL of phosphate buffer with 1 mM of Mg^{2+}; 20 µmol of KNO_3; and 0.15 µmol of methyl viologen. The reaction was started with the addition of 200 µL of dithionite reagent (40 mg $Na_2S_2O_4$ and 40 mg of $NaHCO_3$ in 10 mL of H_2O) and stopped after 10 min by vigorous shaking to oxidize the dithionite. The amount of nitrite produced was determined colorimetrically.

RESULTS

Iron Oxidation

Five strains of T. ferrooxidans were initially screened for the ability to grow with ferrous sulphate in ammonium-free media. Three of the strains (TFI-1, TFI-4, and TFI-10) were originally isolated from the leach liquors obtained from the Agnew Lake uranium mine (11); strain TFI-29 was originally

received from Dr. A.E. Torma, and strain TFI-35 was a reference culture maintained in this laboratory for several years (12). Of these, strains TFI-1 and TFI-35 survived four successive subcultures in ammonium-free media with Fe^{2+} as a substrate. Strain TFI-1 later lost the ability to grow without NH_4^+ addition; this loss coincided with the change of ferrous sulphate supply from commercial sources. Ferrous sulphate has variations in impurities depending on the manufacturer and the lot. These impurities may consist of metallic constituents or nitrogenous contaminants that may affect the nitrogenase activity and, therefore, the growth of T. ferrooxidans in ammonium-free media.

Although T. ferrooxidans TFI-35 continued to grow with Fe^{2+} in ammonium-free media upon successive subcultures, the presence of ammonium ion resulted in increased iron oxidation rates (Fig. 1). Substitution of ammonium with nitrite (1 mM) resulted in the cessation of ferrous ion oxidation. Urea (1 mM) was not inhibitory, resulting in a more-or-less similar iron oxidation rate to that determined in the ammonium-free medium. In comparison, nitrate ion (0.5 mM) slightly enhanced iron oxidation rates in ammonium-free media (Fig. 2). At a high concentration (5-50 mM), nitrate was inhibitory to T. ferrooxidans; at a low concentration of 0.05 mM, it reduced the ferrous ion-dependent growth in ammonium-free media (Fig. 2).

Nitrate Reductase Activity

Cells previously incubated in the presence of 1 mM NO_3^- demonstrated a nitrate reductase activity (Table 1). The activity, assayed with reduced methyl viologen as the electron donor, was detected both in the pellet and supernatant fractions of TFI-35.

Nitrogenase Activity

The nitrogenase activity was determined with the use of the acetylene reduction technique. Acetylene reduction assays with intact cells of TFI-35 indicated that the culture was able to produce ethylene at a relatively slow rate. Abiotic acetylene reduction was ruled out by incubating sterile media in the presence of acetylene, with no reduction occurring. In the presence of T. ferrooxidans, the rate of acetylene reduction increased over a period of 96 h, which was subsequently adopted as the standard incubation time in the assay. Ambient headspace conditions completely inhibited the nitrogenase activity. Replacement of 50% of the headspace air with helium restored the activity (9.9×10^{-3} µmol C_2H_4/mg protein/96 h), and a further decrease in the partial tension of oxygen (6% air) in the assay inhibited the activity approximately 50%. The activity was dependent on the presence of Fe^{2+}, as demonstrated in Table 2. Both ammonium ion (8 mM) and nitrate (50 mM) completely repressed the nitrogenase activity. Nitrate at 0.05 mM concentration resulted in >99% inhibition of the nitrogenase activity.

DISCUSSION

Because the ferrous ion oxidation by T. ferrooxidans is an aerobic reaction, the requirement of a reduced atmosphere for N_2 fixation poses a dilemma for

the bacterium. In a confined environment, oxygen depletion due to respiration provides the microaerophilic condition required for the nitrogenase activity but, at the same time, it reduces the amount of energy and reducing equivalents ($NADH + H^+$) that the bacterium can produce during ferrous ion oxidation. Estimates of the energy requirement vary widely in the literature - between 12 and 29 ATP per N_2 fixed (13,14,15,16). In addition, at least three reducing equivalents are required per N_2 fixed by the nitrogenase activity. Although a bacterial cell contains only 10-14% N (dry weight cell material), it is apparent that the diazotrophic mode of growth, coupled with ferrous ion oxidation and carbon dioxide fixation by T. ferrooxidans, is energetically unfavourable, and results in extremely low cell yields.

In acid environments of T. ferrooxidans, such as leach dumps and leach liquors, nitrate as well as ammonium are present due to residuals from the use of explosives; they are also introduced by the leaching from surrounding soils. In general, these two nutrient ions are known to repress the nitrogenase activity in diazotrophic bacteria. The nitrate reductase activity of T. ferrooxidans provides the bacterium with an additional biochemical capability, the ecological advantage of which remains to be determined. The nitrogenase activity demonstrated in the present work warrants further study, since it involves a unique bioenergetic constraint in a chemolithotrophic bacterium in an extreme environment. Because the nif genes in other bacteria are being elucidated in detail, this system may prove to be a useful tool in studying the molecular biology of T. ferrooxidans.

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TABLES

Table 1 - Nitrate reductase activity of *T. ferrooxidans* TFI-35.

Sample	Nitrate reductase activity (μmol NO_2^-/min/mg protein
Cell homogenate	12.25
Pellet (P_{10})	7.52
Supernatant (S_{10})	3.51

Note: The cells were grown with ammonium sulphate for harvest. The washed cell suspension was exposed to 1 mM of KNO_3 for 24 h before cell breakage and assay.

Table 2 - The effect of Fe^{2+} concentration on acetylene reduction by *T. ferrooxidans* TFI-35*

Fe^{2+} concentration (mM)	Acetylene reduction (μmol C_2H_4/mg protein/96 h)	μmol Fe^{2+} oxidized/ mg protein
0	0	0
120	1.2×10^{-3}	93
360	1.1×10^{-4}	75

*Assay headspace composition: 46.3% air, 46.3% He, 7.4% C_2H_2.

FIGURES

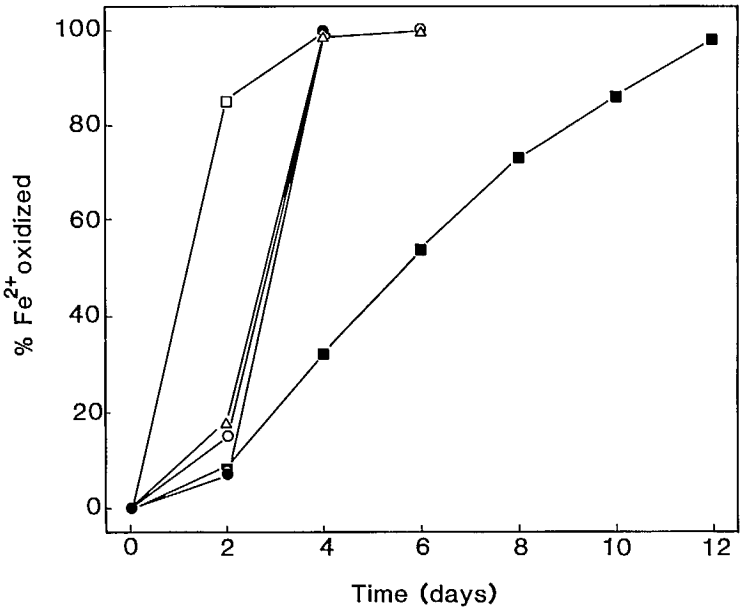

Fig. 1 - The effect of ammonium on ferrous ion oxidation by T. ferrooxidans TFI-35. $(NH_4)_2SO_4$ was added to give final concentrations of either 0 mM (■), 0.05 mM (●), 0.25 mM (○), 0.5 mM (□), or 5.0 mM (△)

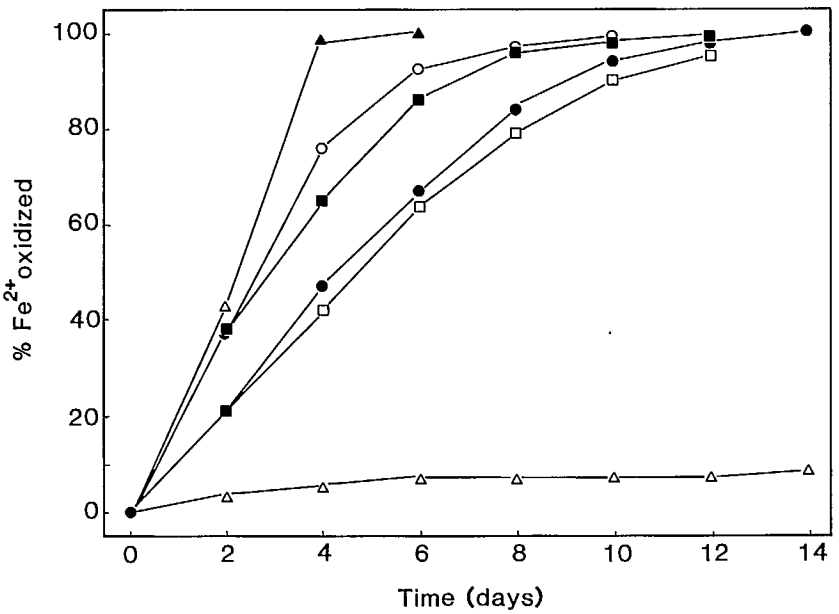

Fig. 2 - The effect of nitrate on ferrous ion oxidation by T. ferrooxidans TFI-35 in ammonium-free medium. KNO_3 was added to give final concentrations of either 0.05 mM (●), 0.5 mM (○), 5.0 mM (□), or 50 mM (△); these are compared to ferrous ion oxidation in nitrogen-free medium (■) and in medium containing 4 mM $(NH_4)_2SO_4$ (△)

SESSION I: PAPER 4

GENETIC MANIPULATIONS OF MINERAL-LEACHING BACTERIA FOR BIOTECHNOLOGY: PERSPECTIVES

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ABSTRACT

Molecular genetic manipulation of microbes involved in metal uptake could contribute significantly to process enhancement. How microbes, their components or derivatives, might be developed for specific biotransformation processes will be reviewed. Included will be a brief discussion of molecular mechanisms of heavy metal resistance, uses of enterochelins and metallothioneins, and microbial transformations.

SESSION I: PRÉSENTATION 4

BIOMINET: PERSPECTIVES EN GÉNÉTIQUE MOLÉCULAIRE

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RÉSUMÉ

La manipulation génétique moléculaire des microbes participant à l'absorption de métaux pourrait contribuer beaucoup à l'amélioration du processus. On revoit les façons possibles de développer des microbes, leurs composantes ou leurs dérivés en vue de biotransformations spécifiques. On discute brièvement, entre autres, des mécanismes moléculaires de résistance aux métaux lourds, de l'utilisation des entérochélines et des métallothionéines, ainsi que des transformations microbiennes.

GENETIC MANIPULATIONS OF MINERAL-LEACHING BACTERIA FOR BIOTECHNOLOGY: PERSPECTIVES

INTRODUCTION

Bio-mining or microbial mineral-leaching is a natural process that has been known for some time, in which microorganisms (mainly the Thiobacilli bacteria) are found to have the intrinsic ability to dissolve or leach metals from ore-bearing rocks. Compared to conventional methods of mining such as smelting and refining, bio-mining has the distinct advantage of being able to process low-grade ores or waste materials. When contained properly, bio-mining also does not produce air pollution or ground pollution. It also requires lower capital investment.

Despite the advantages of bacterial leaching over the conventional processing methods, bio-mining has not attracted the attention of various biotechnological industries that have otherwise focused on producing products related to pharmaceuticals, food, and agriculture via genetic manipulations and recombinant DNA technology. This apparent lack of interest by industry is primarily due to the non-availability of some common recombinant DNA techniques used in Escherichia coli and other genera, but which have not been adopted and developed for mineral-leaching bacteria. It has been suggested that genetic manipulation of bacteria would increase metal-leaching productivity (1). In some cases, metal resistance phenotype has been associated with the presence of a plasmid or plasmids in Thiobacilli. A detailed study of these plasmids, and others as they become available, would form the basis of future manipulation of desirable characteristics in these important bio-mining organisms. A package of techniques to modify these organisms is to be developed. The development of genetic systems for bio-mining bacteria would be of immense importance to the eventual transfer of biotechnology from research laboratories to mining industries, a program recently developed at CANMET (2). This venture will also allow Canada to establish, among a handful of forerunners, an expertise in bio-mining.

ORGANISMS FOR LEACHING

For many years, Thiobacillus ferrooxidans (T.f.) was thought to be the sole organism involved in the leaching of metals from ores. This organism, which is present in acid mine waters, is a motile, flagellated, gram-negative, rod-shaped, non-sporulating bacterium. It is an aerobic, acidophilic autotroph, deriving its energy from oxidation of reduced sulphur and iron compounds, and utilizing carbon dioxide as carbon source. T.f. is used in microbial leaching of metals such as Cu, Ni, Zn, and Co from low-grade sulphide ores. A closely related species, Thiobacillus thiooxidans, also converts elemental sulphur to sulphate under strong acidic conditions but, unlike T.f., is incapable of oxidizing iron. Other microorganisms shown to be involved in mineral leaching are Leptospirillum ferrooxidans, T. organoparus and the thermophilic Sulpholobus sp. T.f. is, however, still recognized as the organism that may play the major role in mineral-leaching operations.

OBJECTIVES

Even though the bio-mining organisms have been used in the mining industry for some time, very little is known about the molecular genetics of these important species. With the advent and success of genetic engineering in E. coli and other genera such as the Bacillus, Rhizobium, or Agrobacterium, it seems desirable to produce mineral-leaching organisms with at least two enhanced qualities, one being an increase in tolerance for toxic metals, and the other a greater ability to generate the oxidant, such as the ferric iron.

The primary objectives of this project would be to develop systems for the genetic manipulation of the Thiobacillus genus. With the development of these techniques, it would be easier to introduce new desirable characteristics, alter the efficiency of leaching, and modify organisms to adapt to the natural environment. The overall 'cryptic' nature of the T.f. plasmids may also be unravelled by direct DNA sequencing of the relevant plasmids and by comparison with the gene banks established from other related species. Such approaches will also help in understanding the biology of the bio-mining organism as a whole.

DETAILED PROPOSAL

Identification of Genetic Marker

A prerequisite for the development of a genetic system is the identification of a selected characteristic that would serve as a marker for the bacterial strain under investigation. One of the problems associated with the acidophilic organisms such as T.f. is the identification of potential markers that could be selected and utilized in inorganic ferrous sulphate medium at pH 1.8. The characteristics that may serve as markers are:

- antibiotic resistance
- heavy metal resistance
- genetic complementation of nutritionally deficient strains.

Antibiotic resistance

Rawlings et al. (3) have demonstrated that the antibiotics gentamicin, kanamycin, streptomycin, tetracycline, vancomycin, tobramycin, and erythromycin are unstable at low pH and high metal ion medium. Nonetheless, they reported the apparent resistances of several T.f. strains to these antibiotics. Davidson and Summers' (4) investigation of the non-acidophilic Thiobacilli reported variable expression of antibiotic resistance markers: kanamycin, gentamicin, streptomycin, and tetracycline were expressed in T. novellus, whereas ampicillin and chloramphenical were poorly expressed. On the other hand, Martin et al. (5) have shown that chloramphenical resistance is a suitable marker for T.f. A systematic study using a wide range of antibiotics to determine which are acid stable and thus useful as marker(s) is to be carried out.

Heavy metal resistance

Variations in resistance to heavy metals have been reported among strains of Thiobacillus spp. Rawlings et al. (6) reported some strains of T.f. resistant to cadmium (>2048 mg/L) and arsenate (1024 mg/L). This resistance may be plasmid-borne since plasmid-containing stains showed more resistance than strains that did not harbour detectable plasmids. In addition to arsenate resistance, some strains of T.f. are sensitive to mercury salts and silver (7,8,9). The coding of genes for resistance to heavy metals has been identified in heterotrophic microorganisms. The resistance can be plasmid-, chromosome-, or transposon-encoded, and may be constitutive or inducible depending on the metal studied (10). These resistance genes could be investigated as to their suitability as genetic markers for the development of genetic systems for Thiobacillus spp. For example, the recovery of gold from arsenopyrite ores, by bacterial leaching with T.f., is hampered by the toxic arsenate concentrations that must be removed by precipitation. Development of arsenate-resistant strains of T.f. would enhance the recovery of gold via bacterial leaching.

Complementation of nutritionally deficient strains

This technique has been used successfully in E. coli, yeast, and many other organisms. As part of this work, mutants would be isolated and such strains could be used as recipients in molecular cloning experiments.

Once suitable phenotypic markers have been identified, the DNA coding for the characteristics will be isolated and subjected to further study by the methodology.

Construction of Suitable Plasmid Vectors for Molecular Cloning in Thiobacillus

In developing cloning vector(s), plasmids that occur naturally in Thiobacillus, or broad-host range plasmids able to replicate in this host system, would play an important role. Plasmids of IncP, IncN, and IncW are known to have a wide host range (able to transfer to, and maintain themselves in, a wide range of bacterial genera), and replication systems of these have been characterized. Construction of shuttle vectors (ones that can be manipulated in at least two different hosts), particularly ones involving E. coli, would allow a relatively easy isolation and manipulation of DNA. Such vectors would allow us to use the clones from the genomic library of Thiobacillus that will also be constructed as a part of this study.

Development of Methods for Introducing Foreign Genes into Thiobacillus Spp

The two commonly used modes of gene transfer in bacteria are "transformation" and "conjugation".

Transformation

Foreign DNA can be introduced in most bacterial strains by alteration of growth conditions or by treatment with various salts such as calcium chloride. However, attempts to introduce DNA in Thiobacillus under various growth conditions have been unsuccessful, except for one isolated case. Yankopky et al. (11) were able to transform DNA into T. thioparus, but even in this case slow transformation kinetics was observed. The National Research Council intends to establish a workable transformation system for the transfer of purified plasmid DNA from both homologous and heterologous origins. Among the other transformation procedures currently available, the 'freeze-thaw' technique that has been shown to be efficient in Rhizobium and Agrobacterium will be investigated.

Conjugation

Conjugation, a process involving cell-to-cell contact, is known to be a more efficient mode of gene transfer than transformation. In most cases, the genes necessary to carry out transfer are known to be carried on plasmids. Some plasmids that are able to transfer to a large variety of bacterial genera are called broad-host range plasmids. Even some of the plasmids that are not self-transmissible contain a region on their DNA such as "mob", which are able to use the machinery of the conjugative plasmid to transfer themselves - a process known as mobilization. The broad-host range plasmids of the IncN (R46), IncP (RP4, RP1), IncW (R388, Sa), and IncQ (R300B) groups can transfer themselves and mobilize other non-self-transmissible plasmids (12). To date, there has been no report in the literature regarding the direct transfer or mobilization of plasmids from the broad-host range heterotrophic bacteria to the acidophilic autotrophic T.f. Studies (4,13) have shown that plasmids could be transferred to non-acidophiles, e.g., T. novellus from E. coli, and from T. novellus to other thiobacilli; also, the RP1 plasmid was transferred from E. coli to T. neopolitanus.

One can therefore approach the conjugation process by initially transferring plasmids from heterotrophic bacteria to the non-iron-oxidizing thiobacilli, which can grow in low pH media, and then mate these thiobacilli with the acidophilic metal-leaching thiobacilli, thus acting as a shuttle system. Because the possibilities with this approach have not received much attention, this area is to be investigated thoroughly, including the DNA modification or restriction systems that may be operating.

Development of a System of Transposon Delivery and Mutagenesis in Thiobacillus Spp.

Transposon mutagenesis offers several advantages over conventional chemical means. Some of these advantages are:

- easily selectable phenotype;
- portable region of homology;
- introduction of new restriction sites not otherwise available on plasmids;
- polar mutations.

These techniques would enable us to define genetic loci by mutations, thereby enhancing our ability to generate a linkage map, or even to determine if transfer of markers occurs from a single origin of transfer in a single direction. Knowledge gained from these studies would aid in the improvement of the Thiobacillus strains used in the metal-leaching operations.

The technique of transposon mutagenesis has been used successfully in a wide range of heterotrophic bacteria (14,15,16).

Transposons may be introduced into the Thiobacillus spp by:

- plasmid incompatability (17);
- use of 'suicide' vectors (18) already available;
- construction of vectors especially to work efficiently in Thiobacilli.

With these vehicles, mutants could be used in various genetic complementation analyses. The mutants could also be screened for one that is 'recombinationally deficient'. Such a Thiobacillus strain would allow us to develop a 'maxi-cell' analogue system in Thiobacillus, since it is known that genes from Thiobacilli are not expressed in vivo in E. coli.

Nucleotide Sequencing of T.f. Plasmids and Related DNA

Thus far, the T.f. plasmids that have been isolated are by and large cryptic or phenotypically silent, except for the glutamine synthetase gene identified recently by Rawlings et al. (unpublished). Once the T.f. plasmids have been isolated, DNA sequencing of selected segments can begin. These primary sequences will provide a fast way of unravelling the basis for the silent phenomena, assuming that there will be sufficient sequence similarity or homology with the gene sequences from other genera already in the various gene databanks. Elucidation of the primary sequences will also provide clues to the various means of activating the cryptic genes to potential functional entities.

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SESSION II

OPEN SESSION

SESSION II: PAPER 5

ECOLOGICAL ENGINEERING: EVIDENCE OF A VIABLE CONCEPT

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ABSTRACT

Ecological engineering involves the use of wastewater and a variety of amendment materials which promote the development of the indigenous populations that will, in turn, improve the site conditions. Biological polishing addresses water quality improvements by introducing populations of specific species that, due to their inherent characteristics, have the ability to remove undesirable substances from the wastewaters.

The methods supporting this new approach have been derived from ecological studies of waste sites and from the identification of those factors inhibiting expansion of tolerant, indigenous plant populations. A procedure has been developed to test the practicality of this approach. Examples are given of ongoing experiments to investigate the promotion of moss covers, which are believed to reduce infiltration of moisture and air. Investigations on algal populations in acid seepages indicate a potentially powerful tool in improving water quality.

A pilot demonstration project of ecological engineering and biological polishing is under joint development, with CANMET and Inco Ltd., in the framework of the Reactive Acid Tailings study.

SESSION II: PRÉSENTATION 5

LE GÉNIE ÉCOLOGIQUE: DONNÉES À L'APPUI DE SA VIABILITÉ

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RÉSUMÉ

Le génie écologique comporte l'utilisation des eaux usées et de diverses substances d'amendement qui permettent aux populations indigènes de mieux se développer et, ainsi, d'améliorer les conditions prévalant à un site. L'épuration finale biologique consiste à améliorer la qualité des eaux en introduisant des populations d'espèces spécifiques qui, en raison de leurs caractéristiques inhérentes, peuvent éliminer des substances indésirables dans les eaux usées.

Les méthodes étayant cette nouvelle approche proviennent d'études écologiques sur des sites incultes et de l'identification des facteurs inhibitant la prolifération de populations végétales indigènes qui sont tolérantes. On a mis au point une technique pour vérifier l'aspect pratique de cette approche. On cite en exemple des expériences en cours visant à étudier les moyens permettant d'obtenir un meilleur développement des muscinées qui, estime-t-on, diminuent l'infiltration de l'humidité et de l'air. Des études portant sur des populations algales dans les eaux d'infiltration acides révèlent qu'il s'agit là d'un outil puissant susceptible d'améliorer la qualité des eaux.

Un projet de démonstration à l'échelle-pilote portant sur le génie écologique et l'épuration finale biologique est actuellement en cours de préparation, dans le cadre d'une étude sur les résidus acides réactifs entreprise conjointement avec le CANMET et l'Inco.

SESSION II: PAPER 6

METHOD FOR THE ENUMERATION OF THIOBACILLUS FERROOXIDANS IN SLURRIES

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ABSTRACT

The enumeration of T. ferrooxidans in the presence of mineral particles is subject to considerable errors due, in part, to the tenacious manner in which the organism associates with the surfaces of sulphide minerals. Methods for the enumeration of T. ferrooxidans will be reviewed, and their feasibility for use by the mining industry to monitor T. ferrooxidan populations in slurries, e.g., bioleach processes or tailings, will be discussed. The adenosine triphosphate (ATP) procedure for the measurement of viable biomass has been investigated as a technique to monitor the efficiency with which viable T. ferrooxidans cells are released from their association with a zinc sulphide ore, by the action of different physical and chemical treatments. The results of these laboratory tests will be discussed, and alternative approaches to the solution of this problem will be considered.

SESSION II: PRÉSENTATION 6

MÉTHODE DE DÉNOMBREMENT DE THIOBACILLUS FERROOXIDANS DANS LES SUSPENSIONS

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RÉSUMÉ

Le dénombrement de T. ferrooxidans en présence de particules minérales est sujet à des erreurs considérables qui proviennent, en partie, de la ténacité avec laquelle le micro-organisme forme des associations avec la surface des minéraux sulfurés. On examinera les méthodes de dénombrement de T. ferrooxidans et on étudiera leur applicabilité dans l'industrie minière pour contrôler les populations de T. ferrooxidans dans les suspensions, p. ex. les procédés de biolixiviation des résidus, etc.. La méthode à l'adénosine-triphosphate (ATP), qui permet de mesurer la biomasse viable, a été étudiée comme moyen pour contrôler l'efficacité de divers traitements physiques ou chimiques à provoquer la rupture de l'association entre des cellules viables de T. ferrooxidans et un minerai de sulfure de zinc. Les résultats de ces essais en laboratoire seront examinés et d'autres approches visant à résoudre ce problème seront envisagées.

SESSION II: PAPER 7

AN ASSESSMENT OF THE USE OF MICROBIAL PROCESSES TO CONTROL NITRATE LEVELS IN PROCESS EFFLUENTS

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ABSTRACT

Published information on the sources and levels of nitrate ion in mining effluents has been documented, together with an appraisal of the significance of nitrate ion as an environmental pollutant for the mining industry.

The feasibility of biological denitrification technologies to reduce nitrate ion concentrations in mining effluents will be discussed. Various types of biological treatment systems will be considered, including suspended growth reactors, fixed film reactors, fluidized-bed reactors, and rotary biological contactors.

The performance characteristics of the respective reactor designs will be assessed, relative to the needs of the mining industry. Information on installed systems treating waste streams of similar composition to mining effluents will be considered.

Although it is difficult to isolate operating costs for denitrification systems, which are often used in conjunction with nitrification processes to effect total nitrogen removal, relative cost data will be compared with those of physical and chemical treatment options. Recommendations for future studies will be presented.

SESSION II: PRÉSENTATION 7

ÉVALUATION DE L'UTILISATION DE PROCÉDÉS MICROBIENS POUR DIMINUER LES CONCENTRATIONS DE NITRATES DANS LES EFFLUENTS INDUSTRIELS

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RÉSUMÉ

On a rassemblé les données publiées sur les sources et les concentrations de nitrates dans les effluents miniers, et on a évalué l'importance pour l'industrie minière de ces espèces ioniques comme polluants de l'environnement.

On examinera s'il est possible d'utiliser des techniques de dénitrification biologique pour diminuer la concentration des nitrates dans les effluents miniers. Divers types de systèmes de traitement biologique seront envisagés, notamment les réacteurs à croissance interrompue, les réacteurs à couche mince fixe, les réacteurs à lit fluidisé et les contacteurs biologiques rotatifs.

Les caractéristiques de rendement de chaque type de réacteurs seront évaluées en fonction des besoins de l'industrie minière. On examinera les données sur les systèmes installés qui traitent les effluents résiduaires de composition semblable à celle des effluents miniers.

Même s'il est difficile de dissocier le coût d'exploitation des systèmes de dénitrification, souvent utilisés conjointement avec des procédés de nitrification en vue d'éliminer complètement l'azote, on comparera les coûts relatifs à ceux des autres méthodes de traitement physique ou chimique. On présentera des recommandations concernant les études ultérieures.

SESSION II: PAPER 8

SYNTHETIC GENES' CODING FOR METAL-BINDING PROTEINS

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ABSTRACT

Metallothioneins are a class of naturally occurring proteins of low molecular weight (25 to 63 amino acids) and a high capacity for metal binding (6-7 atoms Cu per mole). The genes' coding for two metallothioneins has been produced synthetically, one from Neurospora and one from humans, and an attempt is being made to express these genes in E. coli. It is hoped that this expression will lead to bioaccumulation of metals within E. coli and to increased resistance towards the toxic effects of these metals. We are also currently assembling variants of these genes to gain an understanding of structure-specificity relationships among metal-binding proteins. The goal of this work is to create a family of metal-resistant bacteria with the capacity to bioaccumulate specifically valuable metals or to remove traces of toxic metals from solution for pollution control.

SESSION II: PRÉSENTATION 8

GÈNES SYNTHÉTIQUES CODANT DES PROTÉINES FIXATRICES DE MÉTAUX

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RÉSUMÉ

Les métallothionéines constituent une classe de protéines naturelles de faible masse moléculaire (25 à 63 acides aminés) qui ont une grande capacité de former des liaisons avec des métaux (6 - 7 atomes de Cu par mole). Nous avons synthétisé des gènes codant deux métallothionéines, l'un provenant de Neurospora et l'autre d'origine humaine, et nous tentons actuellement de faire exprimer ces gènes chez E. coli. Nous espérons que l'expression de ces gènes conduira ce micro-organisme à bioaccumuler des métaux et à devenir plus résistant à leurs effets toxiques. En outre, nous assemblons actuellement des variantes de ces gènes afin de mieux comprendre les relations structure-spécificité chez les protéines fixatrices de métaux. Cette recherche vise à créer une famille de bactéries résistantes aux métaux et capables de bioaccumuler spécifiquement des métaux de valeur ou de débarrasser les solutions des traces de métaux toxiques qu'elles contiennent, à des fins de dépollution.

SESSION II: PAPER 9

BACTERIALLY ASSISTED LEACHING OF A ZINC ORE

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ABSTRACT

Ore containing 4.7% zinc from Newfoundland Zinc Mines gave encouraging results when tested in shake flasks. However, when about 450 kg of the ore was tested in a column (33 cm in diameter and 5.4 m high), the results were disappointing. The extraction was only 0.3% per month compared to an extraction rate of 1.3% obtained from -15 cm (minus six in.), lower grade (1.3%) chalcopyrite ore from Falconbridge Mines.

Mineralogical analysis of the leached ore clearly showed a coating of calcium sulphate 100-200 μm thick. Presumably, diffusion of the lixiviant through this layer was the limiting factor responsible for low extraction rates. It is also interesting to note that the micrographs of the leached ore show "zones", which support the thesis that the shrinking core model is applicable to leaching under the conditions of the experiment.

SESSION II: PRÉSENTATION 9

LIXIVIATION D'UN MINERAI DE ZINC ASSISTÉE PAR DES BACTÉRIES

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RÉSUMÉ

On a obtenu des résultats encourageants avec un minerai renfermant 4,7 % de zinc provenant de la Newfoundland Zinc Mines, à la suite d'essais en milieu agité. Cependant, on a obtenu des résultats décevants au cours d'essais avec 450 kilogrammes de minerai dans une colonne de 33 centimètres de diamètre et de 5,4 mètres de haut. Le taux d'extraction n'atteignait que 0,3 % par mois, comparativement à un taux de 1,3 % obtenu avec de la chalcopyrite de -15 cm (moins 6 pouces) à teneur plus faible (1,3 %) provenant de la Falconbridge Mines.

L'analyse minéralogique du minerai lixivié a montré clairement qu'il y avait une couche de sulfate de calcium de 100-200 micromètres d'épaisseur. On suppose que la diffusion du liquide de lixiviation à travers cette couche constitue le facteur limitant à l'origine des faibles taux d'extraction. Il est également intéressant de remarquer que les micrographies du minerai lixivié présentent des "zones", ce qui confirme la thèse selon laquelle on peut appliquer le modèle de retrait à la lixiviation dans les conditions de l'expérience.

SESSION II: PAPER 10

BIOLOGICAL DESTRUCTION OF CYANIDE IN CANADIAN MINERAL-PROCESSING EFFLUENTS - A REVIEW

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ABSTRACT

A review of biological treatment of cyanide from several industrial wastewaters is given. The available technology is critically assessed to select a candidate process for application in the mineral-processing industry. Primary considerations of the evaluation are:

- suitability of process to Canadian environment;
- availability of sufficient treatability data in long-term testing of mineral-processing wastewaters;
- process characteristics; and
- economics.

Areas requiring further research are identified.

SESSION II: PRÉSENTATION 10

DESTRUCTION BIOLOGIQUE DES CYANURES DANS LES EFFLUENTS DE TRAITEMENT DES MINÉRAUX AU CANADA - UNE RÉCAPITULATION

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RÉSUMÉ

On examine le traitement biologique des cyanures présents dans différentes eaux usées industrielles. On évalue de façon critique les techniques dont on dispose, afin de choisir un procédé susceptible d'être appliqué par l'industrie du traitement des minéraux. Les principaux aspects de cette évaluation portent sur les points suivants:

- l'applicabilité du procédé à l'environnement canadien;
- la disponibilité de données suffisantes relatives au traitement, au cours d'essais à long terme sur des eaux usées obtenues lors du traitement réel de minéraux;
- les caractéristiques du procédé; et
- les questions économiques.

On identifie les domaines où il y aurait lieu d'effectuer d'autres recherches.

SESSION II: PAPER 11

BIOLOGICAL POLISHING AGENTS FOR MILL WASTEWATER AN EXAMPLE: *CHARA*

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ABSTRACT

Improved treatment of mine and mill wastewater is continuously sought, particularly for abandonment of waste areas. Biological polishing may prove to be a feasible method of augmenting present wastewaster treatment and may possibly lead to a satisfactory close-out procedure.

The aquatic macrophytic alga, *Chara*, has been investigated since 1983 as a biological polishing agent. Reported here are efforts to address the alga's potential for proliferation in mine waste management areas, as well as its capacity to bioaccumulate and filter undesirable components from these waters.

Introduced in uranium wastewater management areas, *Chara* grew, overwintering and regenerating the following spring in several sites. *Chara*, grown in wastewaters from the uranium and nickel industry in the field and laboratory, bioaccumulated significant quantities of uranium, ^{226}radium, nickel, and copper.

At present, techniques of establishing populations beyond mere field tests are being developed, and tolerance ranges to milling reagents are being evaluated. Specific nutrient requirements relating to rhizoid and photosynthetic portions of the plant are being studied. Future work will address the promotion of *Chara* proliferation in wastewater management areas. Achieving this final criterion will permit a feasibility assessment of *Chara* as an effective and economical polishing agent on line.

This work was supported by Denison Mines Ltd., Rio Algom Ltd., Inco Ltd., Alcan International, and CANMET.

SESSION II: PRÉSENTATION 11

AGENTS D'ÉPURATION BIOLOGIQUE DES EAUX USÉES REJETÉES PAR LES USINES UN EXEMPLE: CHARA

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RÉSUMÉ

On cherche continuellement à améliorer le traitement des eaux usées provenant des mines et des usines de préparation en général, notamment en vue de l'abandon des aires de gestion des déchets. L'épuration biologique peut se révéler un moyen d'améliorer le traitement actuel des eaux usées et peut conduire à la mise au point d'une méthode satisfaisante de fermeture.

Depuis 1983, on étudie la possibilité d'utiliser des algues aquatiques macrophytes, p. ex., Chara, comme agents d'épuration biologique. Nous décrivons ici nos travaux sur la possibilité de prolifération de ces algues dans les aires de gestion des résidus miniers, et sur leur capacité de bioaccumuler et de filtrer les composés indésirables présents dans ces eaux.

Les algues Chara, introduites dans des aires de gestion des eaux usées provenant de la préparation de minerais uranifères, se développent, passent l'hiver et se régénèrent dans plusieurs sites le printemps suivant. Chez ces algues cultivées sur le terrain et en laboratoire dans des eaux usées provenant de la préparation de minerais d'uranium et de nickel, on a constaté qu'il y avait bioaccumulation de quantités appréciables d'uranium, de radium-226, de nickel et de cuivre.

On tente actuellement de mettre au point des techniques permettant d'établir des populations, qui sont plus que de simples essais sur le terrain, et on évalue la gamme de concentrations à l'intérieur de laquelle ces organismes tolèrent les réactifs utilisés pour la préparation. On étudie les besoins en éléments nutritifs spécifiques des parties rhizoides et photosynthétiques de la plante. Des recherches ultérieures porteront sur les moyens d'augmenter la prolifération des populations de Chara dans les aires de gestion des eaux usées. Une fois ce dernier critère atteint, on pourra évaluer, à l'échelle - pilote, s'il est possible d'utiliser cette algue comme agent d'épuration de façon efficace et rentable.

Cette recherche a été financée par la Denison Mines Ltd., la Rio Algom Ltd., la Inco Ltd., l'Alcan International et le CANMET.

SESSION II: PAPER 12

APPLICATION OF BIOTECHNOLOGY FOR METHANE CONTROL IN COAL MINES

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ABSTRACT

Effective control of methane during all phases of mining is essential to ensure a safer mining environment. This paper reports on work done, to date, on an unconventional method of methane control based on biotechnology, i.e., the use of microorganisms to oxidize methane into carbon dioxide. Methane-oxidizing bacteria, grown easily under controlled laboratory conditions, have been isolated successfully from some Canadian coal mines. Laboratory tests have shown that these organisms can remain active over an extended period with low-sulphur coals, and that the rate of methane oxidation is quite appreciable. The use of microorganisms is considered to have potential for large-scale application in methane degasification.

SESSION II: PRÉSENTATION 12

UTILISATION DE LA BIOTECHNOLOGIE POUR ÉLIMINER LE MÉTHANE DANS LES MINES DE CHARBON

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RÉSUMÉ

Le contrôle efficace du méthane durant toutes les phases de l'exploitation minière est essentiel afin d'assurer un environnement minier plus sûr. Ce rapport présente un aperçu des travaux effectués à ce jour selon une méthode originale de contrôle du méthane basée sur la biotechnologie, c.-à-d. l'utilisation de micro-organismes pour oxyder le méthane le transformant ainsi en anhydride carbonique. Les bactéries oxydantes du méthane, qui peuvent être cultivées en laboratoire dans des conditions contrôlées, ont été isolées dans certaines mines de charbon canadiennes. Les essais en laboratoire ont démontré que ces organismes peuvent demeurer actifs pendant une période assez longue avec des charbons à faible teneur en soufre, et que la taux d'oxydation du méthane est assez considérable. On considère donc que l'emploi de micro-organismes sur une grande échelle dans le procédé de dégasification du méthane semble prometteur.

SESSION III

TECHNICAL SEMINARS — APPLIED BIOADSORPTION

SESSION III: PAPER 13

A REVIEW OF BIOADSORPTION TECHNIQUES TO RECOVER HEAVY METALS FROM MINERAL-PROCESSING STREAMS

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ABSTRACT

The processing of ores to recover primary metals generates "waste" streams containing low (ppm) concentrations of either the primary or secondary metals. Concentration techniques, which permit the recovery of valuable primary metals or the removal of trace concentrations of toxic secondary metals from mineral process streams or effluents, will benefit many mining companies.

Various microorganisms, including fungi, bacteria, and algae, have been found to concentrate heavy metals effectively from low-concentration aqueous solutions. In this paper, a number of bioadsorption methods for metal recovery are reviewed, with particular emphasis on the process design aspects of the techniques. Finally, the candidate processes are compared and recommendations are made for future test work in the use of biosorbents as metal extractants from mine process streams.

SESSION III: PRÉSENTATION 13

REVUE DES DIFFÉRENTES TECHNIQUES DE BIOADSORPTION UTILISÉES POUR RÉCUPÉRER LES MÉTAUX LOURDS DES EFFLUENTS PROVENANT DES INSTALLATIONS DE TRAITEMENT MINÉRAL

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RÉSUMÉ

Le traitement des minerais en vue de récupérer les métaux primaires et secondaires génère un écoulement d'eaux résiduelles contenant de faibles concentrations (ppm) soit de métaux primaires ou secondaires. Les techniques de concentration qui permettent de récupérer les métaux primaires précieux et d'éliminer les concentrations trace de métaux secondaires toxiques des eaux usées ou effluents provenant des installations de traitement de minerais seront utiles à plusieurs entreprises.

Des micro-organismes divers y compris les champignons, les cultures bactériennes et les algues sont utilisées efficacement pour la concentration des métaux lourds à partir de solutions aqueuses faiblement concentrées. Dans ce document, l'auteur présente un certain nombre de méthodes de biadsorption pour la récupération des métaux en accordant une attention particulière à l'élaboration des procédés de traitement. Enfin, il examine et compare des schémas de traitement hypothétiques et fait des recommandations concernant le travaux de recherche futurs portant sur l'utilisation de biosorbants en tant qu'agents d'extraction du métal se trouvant dans les eaux usées en provenance des installations de traitement.

A REVIEW OF BIOADSORPTION TECHNIQUES TO RECOVER HEAVY METALS FROM MINERAL-PROCESSING STREAMS

OVERVIEW

This paper presents a summary of a two-phase study sponsored by CANMET to assess bioadsorption technology in the mining industry. The first phase is a general overview of bioadsorption technology, and the second phase is a specific laboratory testing and evaluation program.

PHASE 1 - LITERATURE INVESTIGATION

Introduction

The processing of ores to recover primary metals generates "waste" streams containing low (ppm) concentrations of either the primary or secondary metals. The daily volumes of such wastes are usually high. The elements are commonly heavy metals such as copper, lead, zinc, cadmium, molybdenum, arsenic, mercury, uranium, radium, thorium, gold, or silver. At the trace concentrations involved, it is not economical to recover primary metals by conventional extraction procedures. However, these elements may represent an environmental hazard, so waste treatment (often expensive) may be required prior to discharge.

Concentration techniques, which permit the recovery of valuable primary metals or the removal of trace concentrations of toxic secondary metals from mineral process streams or effluents, will provide a significant benefit to many mining companies. Biological systems are potential candidates.

Various microorganisms and plants have been shown to concentrate heavy metals effectively from aqueous solutions containing low concentrations of the element in question. Much of the interest in this phenomenon has centred on the potential use of such species as biological monitors of heavy metal pollution. However, from these studies has come the realization that such biological agents can selectively concentrate heavy metals from aqueous solutions by factors as high as several thousandfold. Many species of bacteria, fungi, algae, and aqueous plants have been tested for their ability to concentrate heavy metals.

In this paper, focus will be placed on the process design aspects of the various bioadsorption processes for metal recovery described to date. The technical and economic factors of each system will be evaluated for their suitability to the Canadian mining environment.

This is important to note since many of the bioadsorption processes studied to date have involved relatively clean and well-defined solutions, rather than the variable streams prevalent in mining operations.

Key points in evaluating bioadsorption techniques for the recovery of heavy metals in mining processes include:

- availability of biosorbent;
- reproducibility of biosorbent, especially if it is not produced from a defined microbial culture;
- specificity of method to particular metal;
- reusability of biomass;
- capacity and efficiency of process;
- nutrient costs;
- adaptability of method to existing waste treatment equipment;
- sensitivity of process to operating parameters;
- sensitivity of method to other cations and anions in process streams;
- economic feasibility of more sophisticated bioadsorption processes;
- regeneration of biosorbent.

The assessment of bioadsorption processes requires some understanding of the mechanisms by which microorganisms accumulate and concentrate heavy metals from their immediate environment. A brief overview of the mechanisms of uptake of heavy metals by microbes is warranted.

The Concentration of Metals from Solution by Microorganisms

The ability of certain bacteria, fungi, and algae to accumulate and concentrate metals has long been recognized. Much of the research focuses on the potential use of these organisms as indicators of heavy metal pollution in the environment, where they have been shown to accumulate metal contents as high as 36-40% by dry cell weight. However, the mechanisms enabling these organisms to survive and grow in the presence of metal concentrations, which are toxic to other organisms, have also been studied. Significant variations are noted between the metal uptake ability of different genera, different species, and also different strains within a species.

The uptake of metals by microorganisms is a two-stage process. The first phase occurs very rapidly in most organisms (within 5-30 min of cell contact with the metal-containing solution) and involves the "passive" physiochemical adsorption/retention of metal cations and anions by the constituents of the cell wall. This "passive" process we define as bioadsorption. The second phase involves the "active" physiological absorption of metals via energy-dependent metabolic processes. This latter phase, identified as bioaccumulation, is dependent on the tolerance of the specific organism to relatively high concentrations of "toxic" elements in the intracellular cytoplasm and other subcellular components.

Evaluation of Bioadsorption Processes

The bioadsorption processes evaluated for use in recovering heavy metals from mineral-processing streams may be classified in terms of the nature of the biosorbent, as follows:

- activated sludge
- defined bacterial cultures

- fungi and yeasts
- algae
- microbial derivatives.

There is some overlap of information for these processes, but specific attributes of each type of adsorbent warrant a separate assessment.

At this point, it is necessary to distinguish between bioadsorption and bioaccumulation since this important aspect of process design has been poorly described by many researchers. The two processes have often been combined into the single heading of uptake because the predominant mechanism in effect is not known.

Bioaccumulation, which is the predominant uptake mechanism in many organisms, is less desirable in process design because it requires the cells to be actively metabolizing, and the poor release of heavy metal from the intracellular matrix makes it difficult to reuse the biosorbent.

Bioadsorption, on the other hand, involves the chelation of metals on the cell surface. The uptake is generally rapid, as well as it being easier to recover the metal(s) and reuse the biosorbent. These are important criteria for process design.

Two basic alternatives are available for bioadsorption processes. One approach involves a single-stage procedure, with actively growing, metal-tolerant organisms in the ore-processing stream, fortified with appropriate nutrients. The second approach involves producing the biomass in a separate reactor, and then bringing the isolated microbial biomass into contact with the process liquor in a controlled environment, i.e., a two-stage concept. The biomass may be used as produced; deactivated before use, then treated to improve physical characteristics (e.g., immobilization); or extracted to yield purified components with high complexing ability.

The use of actively growing culture (i.e., combined bioadsorption/bioaccumulation) has hitherto been more compatible with processes based on large storage systems or lagoons exposed to the natural elements. This approach has many limitations. Expensive storage facilities and lack of space at mine sites make it uneconomical. More compact systems that can be operated in controlled environments, with high adsorption of metals and short residence times by biosorbents, are preferable.

The attractive feature of bioadsorption is a certain specificity of the biosorbent for divalent and multivalent heavy metal cations. As noted earlier, the metal uptake may vary widely for different mutant strains within a species and between different genera. The nutrient status of the organism, the age of the cells, the temperature, pH, etc., are all important parameters affecting the performance of a biosorbent. An additional factor is whether enough biomass can be generated at an economical cost to meet the needs of a process.

Activated Sludge Systems

For the purpose of this study, activated sludge (AS) is defined as a biosorbent consisting of a naturally selected, undefined, mixed culture of microorganisms. In addition to bacteria, the biological constituents include algae, fungi, ciliated protozoa, rotifers, and nematodes.

At first glance, AS systems, which are applied primarily to the reduction of the organic content of wastes, do not seem to apply to the mining industry. However, their ability to remove heavy metals from municipal sewage has long been recognized, and AS systems are probably the best characterized of any of the bioadsorption processes. Most of the developments using pure cultures of microorganisms and isolated microbial polymers are, as discussed later, based on the behaviour of microorganisms in AS processes.

Data generated in municipal waste treatment plants have sparked much of the interest in the mechanisms of metal removal by AS systems. A number of studies using synthetic media have been conducted to determine the adsorption of a variety of metals by activated sludge. In general, the data indicate that this method is useful for the uptake of metals such as mercury, cadmium, lead, copper, cobalt, radium, and to a lesser degree zinc, nickel, uranium, and thorium.

Adsorption efficiencies of over 90% have been achieved for cadmium, copper, lead, and mercury. However, it is estimated that as much as 20% of the metal removal in these waste treatment systems is due to the filtration of metal particulates. Depending on the metal in question, actual metal recovery due to AS activity is about 10-80% (1).

Metal uptake by activated sludge was found to be affected significantly by pH (1) and age of the cells. In general, younger cultures were found to be more active adsorbents than older ones. Moderate temperature variations and deactivation of the sludge by heat sterilization did not significantly affect AS activity.

Bioadsorption by AS is a rapid process, generally complete within 10 min. The speed of the reaction indicates that the principal mechanism is "passive" bioadsorption.

As stated earlier, the testwork conducted to date has involved synthetic media. No processes using activated sludge to adsorb metals from ore-processing streams have been identified. However, this approach or its refinement using defined microbial species (discussed in the following section) warrants further investigation.

The following are some of the attractive features of this biosorbent:

- Bioadsorption, rather than bioaccumulation, is the predominant mechanism for the uptake of heavy metals such as mercury, lead, cadmium, copper, and cobalt by activated sludge.
- The bioadsorption is a rapid process.
- Deactivation of the sludge is not necessary.

- The natural selection process of activated sludge systems leads to a predominance of "hardy" strains of organisms.
- The organisms do not require a highly defined culture medium, e.g., sewage from the mining community would serve as one nutrient source.

However, before larger scale applications of AS to the removal of metals from ore process streams are considered, additional information is required, including:

- adaptation/acclimation of activated sludge culture;
- evaluation and optimization of the bioadsorption efficiency/kinetics with process liquor, e.g., effect of pH, temperature, contact time, age of biomass, nutrient requirements, effect of competing cations/anions, effect of chelating agents in the ore process liquor;
- evaluation of the efficiency of biomass recovery (filtration, sedimentation, particle size, etc.);
- evaluation of procedures to recover metals from biomass;
- adsorption capacity of regenerated biomass as well as physical characteristics.

The information in the literature indicates that bacteria, which have capsules or slime layers or which liberate polymers into the surrounding solution, are the more effective biosorbents. They tend to grow attached to the surface of reactors. If metal-laden sludge can be regenerated, the preferred process would be the two-stage one, incorporating a separate reactor to produce the biosorbent.

Defined Bacterial Cultures

This concept is a refinement of the activated sludge process. The major goal is to isolate bacterial species with enhanced capacities to adsorb heavy metals. The most successful sources for such organisms, to date, have been either activated sludge or metal-polluted soils, in which exposure to toxic metals has already induced a natural selection process favouring metal-tolerant strains.

With the exception of one organism, Zooglea ramigera 115 (2), test data have not been published on the behaviour of metal-accumulating organisms in liquors of similar composition to that of mine process streams. Most of the studies to date have used highly defined culture media that are not relevant to the mining situation. Many bacteria and fungi have been screened for their uptake of specific metals, e.g., Cu, Pb, and Cd (3,4,5,6). Bioaccumulation, however, appears to be the predominant mechanism of uptake.

Dugan and Pickrum (2) tested the ability of Z. ramigera 115 to remove cations and anions from several mine waters (pH 3.0). An estimated 25-33% of the cations and 25% of the sulphate was adsorbed by the biomass. As no data were presented for the adsorption of heavy metal cations, the results are inconclusive.

Data from investigations using synthetic heavy metal mixtures may be summarized as follows:

- Iron, cadmium, and mercury were well adsorbed by Z. ramigera 115 (2).
- Sphaerotilus natans was found to adsorb iron, cadmium, copper, and cobalt (19).
- Cadmium was accumulated by Citrobacter sp. (6).
- Lead was concentrated by Azotobacter sp. (7).
- Pseudomonas aeruginosa was found to adsorb uranium very rapidly (complete within one minute) from solution (8,9). However, the recovery of metal from biomass was not efficient.

Until more complete data are available on the rate of uptake of the respective elements, the adsorption isotherms, the rate of production of biomass relative to the total metal adsorption capacity, and the performance of these organisms in more realistic test conditions, it would not be justified to use such organisms directly in mill process streams. The approach of most research groups has been to identify candidate organisms for further laboratory investigations into their usefulness in future bioadsorption processes.

In terms of process design, defined bacterial cultures have the same limitations as outlined for activated sludge.

Fungi and Yeasts

Although both yeasts and fungi have been shown to adsorb metals from solution, from the standpoint of process design only fungi deserve serious consideration. Fungi have the important advantage over other biosorbents in that metals, particularly uranium, can be readily desorbed from the biomass by dilute alkali carbonates. This is a key factor in the design of process systems.

Investigations have shown that uranium, lead, cadmium, and a number of other heavy metals can be adsorbed effectively by spent fungal mycelium.

One of the most promising technologies for the bioadsorption of uranium and other radionuclides is the use of spent fungal mycelia from the fermentation industry (18). The potential exists for the use of deactivated fungal mycelium as an ion exchange material able to be reused many times. At this stage, however, the data on the interaction of heavy metals with mycelia of different fungal species have not been well enough documented to permit direct application to mine process streams.

The growth rate for fungi is generally lower than bacteria. Culture requirements and nutrients are also more fastidious. This poses the question of whether or not adequate amounts of biomass would be available. The physical handling characteristics will also be important but, if the biosorbent can be reused, the supply of biomass becomes less significant. At present, reuse would be limited to elements such as uranium and, possibly, radium.

Algae

Algae concentrate metals from the natural environment and show impressive accumulation factors. Algal cells have been found to adsorb cadmium, copper, and other cations.

Algae have been proposed as bioadsorbents to remove heavy metals from wastewater (10). The most successful applications have involved tailings ponds and meander systems (11), and this combination serves to overcome the major limitation of inadequate light intensity. Shallow water systems, large surface areas, adequate light intensity, and a suitable nutrient source will foster the growth of algal blooms (11). However, due to the large pond area required by the process and the limited yield of algal cultures, the feasibility of using growing algae for direct treatment of processing streams is poor.

Reuse of algal biomass is key to the application of algae to remove heavy metals from large daily volumes of mine-processing liquids. The use of algal derivatives (next section) may improve its value. At this time, however, an economical and practical process cannot be designed using this biosorbent.

Microbial Derivatives

This section will cover two types of products: immobilized cells and metal-chelating compounds (e.g., gelatinous polymers, polysaccharides) extracted from microbial cultures. The common goal of these separate approaches is to prepare a reusable chemical product, of natural origin, with a higher metal-complexing ability than crude biomass preparations.

Immobilized Cells

Bacterial and algal cells have been immobilized in gel supports such as toluene diisocyanate, glutaraldehyde, polyacrylamide, agar, cellulose, and alginate. Most investigations into heavy metal uptake by immobilized cells have been conducted using polyacrylamide because of its prior commercial use in other immobilized systems. However, Nakajima et al. (20) showed that toluene diisocyanate and glutaraldehyde systems gave the highest uranium adsorption.

Most investigations to date have concentrated on the adsorption of uranium by immobilized cells. However, lead has also been found to have limited adsorption characteristics (5). Data on desorption methods are limited.

A variant of the immobilization approach is the use of chemically stiffened fungal mycelium. This type of biosorbent has been patented for use in the recovery of uranium and radionuclides (12). However, there are indications that the adsorbent will also remove lead. No data on desorption techniques are available for this method.

At present, inadequate data are available to design a system that uses these advance bioadsorbents. With free cells/mycelia, major difficulties in a

large-scale system will be encountered in the production and physical recovery of the biomass. Costs of producing the biosorbent may be significant. However, immobilization of cells by entrapment or stiffening is a realistic approach to improving the physical characteristics for use in column reactors and the economics of reusability.

Process factors, which must be defined for this concept before it can be used in mining environments, include:

- the conditions/reagents needed to desorb heavy metals (other than uranium);
- the effect of desorbing agents on the physical characteristics of the adsorbent;
- the stability of the immobilized biosorbents in the presence of high ionic strength solutions and organic compounds/solvents liable to be encountered in mineral-processing streams;
- the effects of temperature and pH variations on the biosorbent;
- the effect of immobilization on the metal adsorption capacity and the kinetics of adsorption;
- the economics of the immobilization procedures.

Microbial Polymers

It has been demonstrated that microbial polymers (usually anionic or cellulosic-type polysaccharides) can efficiently bind heavy metals in synthetic solutions (13,14,2,15). Significant proportions of these complexes remain water-soluble.

A number of microbial polymers have been proposed to recover metals from mine drainage. Cell flocs of Z. ramigera 115 have been found to recover metals from acid mine water (2,16,17), and it is reported that the metals can be recovered from the gel by acid extraction. No studies were conducted on the extracted polymers of these compounds.

Other natural polyelectrolytes have also been proposed for heavy metal removal. However, all of these compounds are very specialized, as well as very limited in supply or expensive to produce.

The use of natural polyelectrolytes from organisms is an advanced process. As with immobilized derivatives, considerable information must still be obtained before these compounds can be used in actual process streams. No data have been published on the adsorption characteristics of these polymers in complex mine-processing liquors or in competition with other cations, e.g., Ca, Mg.

At present, the most practical way to use these polyelectrolytes is in natural association with the cells. However, there is considerable scope for future development. The isolation and culture of microbial strains that produce higher yields of polyelectrolytes, or improvements in technology to prepare polyelectrolytes for use in mining processes, are two areas for development. However, considerable research effort is necessary.

Conclusions

In the current literature, no bioadsorption process or concept has been identified for which adequate data are available to permit the direct design of a system to treat mine process streams. The limitations of available data include:

- lack of information on biosorbent activity in complex mixtures such as actual mine process streams;
- limited data on metal desorption characteristics and biomass regeneration;
- lack of research on the supply or generation of adequate amounts of biomass, and the nutrient requirements;
- no available data on process schematics and mass balance calculations.

These factors all significantly influence the likelihood that bioadsorption processes can be developed to the level of economically attractive routes to recover low concentrations of metals in mine process liquids.

Of the processes reviewed, algae appear to have limited potential in the Canadian mining environment. The economical generation of adequate amounts of algal biomass is an obvious practical limitation. The use of immobilized algal systems, however, may bypass this limitation.

Based on available information, the most promising target metal for further bioadsorption studies is uranium. This selection is influenced strongly by the efficient regeneration procedures noted for this element with several biosorbents (an important criterion for process design).

From this review, the various processes may be ranked in the following manner for their ability to recover uranium:

- fungal mycelium
- microbial derivatives (immobilized cells)
- activated sludge
- yeasts or defined microbial cultures
- algae.

Fungal mycelium have been found to have a high capacity for uranium in aqueous solutions. In addition, simple recovery of the metal from the biomass may be achieved by washing with dilute carbonate salts. Both these attributes are important in designing the bioadsorption process.

For the recovery of metals other than uranium, the process types are ranked in the following order of effectiveness:

- defined bacteria cultures
- activated sludge
- microbial derivatives
- fungi and algae.

The reversal of the ranking, compared with that for uranium, indicates that fungal mycelium may not be feasible for use as a general biosorbent.

The selection of the type of bioadsorption method to be used in mine process waters will probably be site-specific. Several methods have been identified to adsorb a number of heavy metals from solutions. However, the biosorbents can only be evaluated for performance based on the composition of the actual process liquids, so each case must be considered individually. No one method can be applied to all mining situations.

PHASE II - LABORATORY STUDY AND EVALUATION

Introduction

Five biomasses - Rhizopus arrhizus, Streptomyces levoris, a mixed culture sewage sludge, Saccharomyces cerevisiae, and Chlorella vulgaris - were evaluated for potential utilization in a uranium recovery process. These laboratory studies were conducted by the Institute of Biotechnology at the University of Waterloo. The major difference between these studies and many previous investigations was the use of a process-mining solution rather than a synthetic pure solution. This resulted in a major difference in bioadsorption rates and capacity.

Summary of Laboratory Findings

The principal findings of the laboratory study are highlighted below.

Biomass growth rates and growth yields

- The fungus Rhizopus arrhizus and the yeast Saccharomyces cerevisiae, which are very simple to cultivate in axenic culture, produce high growth yields. Rhizopus arrhizus is harvested with ease.
- Streptomyces levoris is also simple to cultivate, but requires a more complex growth medium.
- Chlorella vulgaris is an alga that possesses very low specific growth rate and low cell yield. As such, it is difficult to produce in large quantities and is subject to contamination. These aspects were troublesome in this study.
- Air-lift fermentation in a continuous fed-batch mode, which produces high growth yields with minimal problems, would be an excellent method for large-scale biomass production.

Uranium adsorption

- Rhizopus arrhizus, Streptomyces levoris, and the mixed culture proved to be effective biomasses for uranium adsorption. The data in this study suggest that Rhizopus oligosporous may be equivalent or superior to Rhizopus arrhizus.
- There may be many other organisms that would perform equally well as the organisms tested in this study.

- Immobilization of cultures, coupled with the retention of high bioadsorption capacity, is difficult to achieve and many failures can be expected. This area needs further work.
- Bioadsorption capacity is a strong function of pH. Optimum levels that maximize both adsorption capacity and uranium concentration in process solutions would be in the 3.5 to 4 pH range.
- Thermal inactivation and cell age have only a marginal effect on equilibrium bioadsorption capacity. However, thermal inactivation results in a doubling of the kinetic rate constant for microbes with mycelial growth habits and for sewage flocs. Unicellular microbes such as Chlorella do not appear to be affected.
- Presence of contaminants such as iron, sulphate, and heavy metals can greatly reduce bioadsorption capacity. For example, 15 mg/L of ferric iron added to synthetic solutions resulted in a 64% reduction in Streptomyces levoris bioadsorption capacity. Similarly, 15 mg/L of cobalt, copper, nickel, and zinc resulted in 31%, 25%, 24%, and 26% reductions in bioadsorption capacity, respectively.
- Sulphate levels in process liquor give rise to sulphato-complexes that are not adsorbed by any biomass.
- The adsorption temperature had a marked effect on both viable and thermally inactivated cultures. An increase in temperatures from 4°C to 35°C resulted in a 75% increase in bioadsorption capacity for viable cultures, and a 55% increase for inactivated cultures.
- Contact time is an important variable, especially at lower pH values.
- Process liquor (mine water) is not suited for direct uranium recovery by biomass. The pH level at 2.0 appears to degrade the cell structure, with the associated high contaminant levels (Fe^{3+}, for example) resulting in a marked reduction in bioadsorption capacity.
- Bioadsorption data from process liquors are not amenable to direct comparison with published data on synthetic solutions.

Uranium desorption

- $NaHCO_3$ is a simple and effective stripping agent for all biomasses tested.
- Repeated adsorption/desorption cycles do not greatly affect biomass loading or desorption capacity.
- Weak sulphuric acid was marginally effective as a stripping agent, while sodium EDTA and ammonium sulphate were ineffective.

Mechanisms

- Simple sorption isotherms, such as the Freundlich and Langmuir isotherm, describe the experimental data adequately.
- A kinetic model has been proposed to describe the time-dependent change of the uranium adsorption capacity. The model predictions compare favourably with experimental observations.

General comments

- Data on uranium bioadsorption must be interpreted cautiously. Factors such as temperature, biomass concentration, cell physiology, uranium content in test solutions, contaminant levels, pH, etc., can greatly distort results. Casual observations can lead to erroneous interpretations of the data.
- Although uranium bioadsorption appears to have a good potential for application with process solutions, this study has not delineated all pertinent factors for optimal design.

Potential Process Applications

General

Biomasses are not likely to replace conventional uranium concentration/purification processes, such as ion exchange or solvent extraction. These latter processes are highly efficient, well-developed, and very cost-effective in uranium mill applications. However, these processes are somewhat sophisticated and require rigourous operator attention.

Biomass applications are likely to be directed at low-grade solutions where minimal process control is possible. In addition, where biomass costs are low, one-time applications are certainly a possibility. Perhaps the most likely applications deal with the recovery of uranium from wastewater or bioleach solutions. The primary examples would be:

- acid mine waters
- acid tailings runoff
- underground leaching solutions.

A discussion of these potential applications follows.

Acid mine water

A typical acid mine water was described in the Phase II study as having a uranium content of 127 mg/L and a pH of 2.7. At these pH and uranium levels, most biomasses would have an effective net adsorption capacity of at least 20 mg U/g and, as such, 43 kg of biomass would be required per kg of uranium (U_3O_8) recovered. Biomass purchase cost ranges from nil for waste biomass, such as sewage sludge or brewery waste, to several dollars per kg.

Obviously, if the biomass could be obtained at no cost, one-time utilization and disposal of the biomass is possible if minimal alteration to the as-received biomass is required before use. However, if the biomass is purchased, it must be capable of repetitive use.

For one-time application, we foresee a material such as an inactivated, thickened sewage sludge applied to the inlet of underground sumps in the mine, and allowed to contact with mine-water solutions. Because of the long contact times, the biomass may reach substantially higher bioadsorption levels. Mine

water for use in underground leaching would be recycled from the sumps. As the sumps become full, the fluid-like biomass would be pumped to the surface with the mine water (at about 1% solids). Residual biomass would be mucked out with the sump sludges during routine sump clean-outs. Given a residual mine-water concentration of 100 mg U/L for every 100 000 L of solution with 1% biomass, one would get 11.7 kg of uranium (U_3O_8) in the water, and 23.8 kg of uranium in the biomass. On the surface, the biomass would be eluted with bicarbonate or a strong acid solution, discarded and/or recycled, and the soluble uranium sent to the mill for recovery.

The net effect would be:

- mine waters would be recycled underground
- effective uranium concentration in mine water pumped to the surface is tripled, i.e., three times more uranium goes to the surface in the same volume of mine water.

The major problem would be getting an adequate supply of biomass. For example, a typical municipal sewage plant would produce .5 kg of biomass per kg of BOD_5 applied. A community of 20 000 people could only produce 1000 kg of biomass per day. This would only support the production of 23 kg of U_3O_8 per day. In addition, the sludge would require thickening before pumping underground (10% solids would be reasonable). Using a centrifuge or vacuum filter, it should be possible to thicken and recycle the sludge. For a 10-cycle operation, this would increase the potential production to 230 kg/d or 84 000 kg/a. Total pumping capacity required at 1% solids to support this level of production would be 1 000 000 L/d. Obviously, if the adsorption capacity could be improved, much greater levels of uranium could be produced.

Acid tailings runoff

Acid runoff and seepages from uranium tailings areas contain elevated levels of uranium; however, these levels are in the range of <1-5 mg U/L with pH levels in the 2.2-3.5 range. These runoff and seepage streams require continuous long-term treatment at a substantial cost. With the low uranium values in solution, conventional recovery technologies might be applicable, but costs would be prohibitive. In these situations, biomass could prove to be effective for uranium recovery. The uranium recovered could offset the cost of treatment, with spent biomass being used as a surface soil conditioner for the tailings. This would provide:

- potential revenue from uranium
- a method for disposing of sewage sludge
- improved conditions for tailings area revegetation and rehabilitation.

For this application, sludges could be added upstream of the sedimentation pond, then allowed to settle and contact with the seepage. Once or twice per year, the sludge would be recovered, eluted for its uranium content, and the waste material disposed on the tailings surface.

Bioleaching solutions

One potential application is the recovery of uranium from heap-leaching or underground leaching operations. These operations produce a uranium content typically in the range of 100 to 300 mg/L. For a heap-leaching operation on the surface, ion exchange or solvent extraction technology would likely be the simplest operation, but underground recovery with biomass certainly has some potential. Again, the driving force would be to have an inexpensive adsorber that does not require major process controls. The bioleach process solutions tested in this study had a very high acid content, at pH of 2.0 and a uranium level of approximately 250 mg/L. At the low pH, the biomass was degraded. It will therefore be necessary to perform some pre-treatment to adjust the pH upwards prior to adsorption. This pre-treatment may also have some beneficial side effects, since under the current scheme the leaching solutions are too strong and can actually inhibit bacterial activity.

For this type of application, either a system similar to that discussed for the mine water would be employed, or possibly a contacting system using columns could also be considered.

Again, the benefits are:

- direct underground recycle of leach water
- higher production levels for the same quantity of mine water pumped.

Evaluation of Biomass Solids Contact/Separation Equipment

General

There are two basic types of contacting equipment: stirred reactors and columns. Both have potential application for uranium bioadsorption. Devices for the separation of solids include thickeners, filters, and centrifuges. Given the difficulty in dewatering many biomasses, the thickener and centrifuge systems are probably most applicable; however, filters can, and have, been used. The centrifuge is probably most applicable when wet recycle of the biomass is required.

In order to demonstrate potential requirements for contacting/separation for a uranium recovery system, two sample flowsheets have been developed. The basic design criteria for loading capacity, elution requirement, etc., have been derived from the laboratory testwork. The process solution is mine leach water at 250 mg/L of uranium and pH adjusted to the 2.5-3.5 range.

This section also includes an overall assessment of a range of other contact/separation devices and how amenable these devices are for each biomass tested.

Sample Biomass Recovery Flowsheets

The sample flowsheets were developed by A.H. Ross and Associates.

Contacting System

The size of the equipment necessary to remove soluble uranium from typical process mine water has been estimated for stirred tanks and gravity downflow column systems. The design parameters are considered reasonably conservative but require confirmation.

Stirred tank system

Design

The flowchart is shown in Figure 1. Mine water and biomass are contacted in a series of three stirred tanks for a total contact time of 30 min. Sufficient biomass is introduced to reduce the uranium content of the water by an average of 0.1 kg/m^3.

The water/biomass pulp gravitates from the final tank to a gravity clarifier. The water overflowing at the top of the vessel is recycled to a leaching stope. The solids settle to the bottom of the clarifier, then are raked to the centre to be pumped out as a 3% solids pulp. Typically, such clarifiers are provided with a deep centre well and with a thick bed of suspended solids that filter the upflowing solution. Progressing cavity pumps are used on biomass pulps to avoid physical degradation of the material. Flocculants are generally not used during clarifications, but they could prove beneficial in improving underflow densities.

The thickened biomass is directed to a solid bowl centrifuge. The extracted water is returned to the clarifier and the biomass, now at 20% solids, is directed to the elution tanks. Uranium is eluted from the biomass with a 0.1 Molar (8.4 kg/m^3) solution of sodium bicarbonate. Contact time of fifteen minutes is provided to transfer uranium from the biomass into the solution.

A minor amount of bicarbonate is destroyed by the acid in the water introduced with the biomass. If proved advantageous, this amount could be reduced by repulping the solids with clean water and separating in a second centrifuge.

Pulp that overflows the second elution vessel is pumped to another centrifuge. It would be located above the contactors so that a screw conveyor could assist in returning the thickened biomass to the contactors. The centrate is directed to a mine sump and pumped to the surface.

The amount of uranium returning with the recycled biomass to the contactor could be reduced by simply repulping the biomass with water and centrifuging the pulp in a third machine. The consumption of sodium bicarbonate would thereby be reduced.

Comments

1. The system appears simple, the equipment of familiar type and size, and the operations straightforward.

2. Replacement of biomass with fresh material is readily accomplished.
3. The sodium bicarbonate consumption depends upon a sufficient per cent solids in the biomass discharge from centrifuges. The moisture in the material may vary with the age and condition of the biomass.

Equipment list

Contactor tanks	3	2.0 m diam x 2.4 m, agitated
Clarifier	1	8 m diam
Slurry pump	2	Moyno type, variable feed drive
Centrifuge	2	420 mm diam x 1675 mm, 37.5 kW
Elution tanks	3	1.0 m x 1.4 m, agitated
Bicarbonate mix tank	2	4.0 m diam x 4 m, agitated
Bicarbonate feed pump	1	centrifugal
Screw conveyor	2	225 mm diam x 3 m
Estimated sodium bicarbonate		11 kg/kg U 1040 kg/d
Estimated connected power		105 kW

Capital cost

A rough estimate of the capital cost would be $1 000 000.

Column system

Design

The flowsheet is shown in Figure 2. Mine water and biomass are contacted by passing the water, under gravity, through a bed of biomass. After the passage of a measured amount, the mine water is stopped and displaced from the bed with a flow of fresh water. The fresh water is followed by a sodium bicarbonate elution and a second water wash. The water washes reduce contact between the acidic mine water and bicarbonate solution, avoiding disruption of the bed that would be caused by the release of carbon dioxide.

The rate of water percolation through the bed, the low, and a large bed area is required to pass the design flow. Mine water would be sprinkled into a shallow pool on the top of the bed. Pressure on the bed is avoided in order not to compact the bed and thereby restrict the flow.

Treated mine water is collected with a bed support system at the bottom and recirculated to leaching areas. Outlet eluant is directed to mine sumps for pumping to the surface.

A spare column to permit normal operation during replacement of a bed is provided.

Comments

The column equipment is simple, with the system being cheaper to install and operate than the stirred tank system. Operation should require little attention, except for periodic replacement of the beds and routine makeup of the sodium bicarbonate eluant. The chances of a successful technical operation, however, appear less certain. Potential problems with high turbidity, resulting in column plugging, are also possible.

Incomplete separation of mine water and eluant, and the resulting CO_2 generation, may disrupt the bed and lead to significant channelling of solution flows. This would reduce adsorption/elution effectiveness and provide uranium recovery lower than expected.

The throughput is critically dependent on percolation into, and through, the bed. Stability of the flowthrough rate, as a function of prior treatment of the biomass and operating conditions, requires confirmation.

Equipment list

Operating cycle operation	Time h	Volume m^3	Rate m^3/h
Adsorption	19.2	240	12.5
Wash	1.44	18	12.5
Elution	1.92	24	12.5
Wash	1.44	18	12.5
TOTAL	24.00	300	12.5

Wash water consumption	1.5 m^3/kg
Sodium bicarbonate consumption	8.4 kg/kg U
	806 kg/d
Biomass bed, 5 @	12 m^3, 5.2 m diam x 0.57 m
Eluant makeup tanks, 2	agitated
Eluant feed pump	centrifugal

Capital cost

A rough estimate of the capital cost for this type of application is $700 000.

Major Economic Hurdles

The primary economic hurdles are the biomass cost, capital cost of equipment, and the operating cost for desorbing agents and labour. A short discussion of each is provided.

Biomass cost

Although the two primary candidates, Rhizopus arrhizus and the mixed culture, are available either as waste by-products or they can be produced from waste materials, factors such as transportation, treatment, dewatering, etc., will still contribute greatly to the cost. A major factor will also be the scale of production. If the demand is small, the cost will be very high. Conversely, if demand is great, the economy of scale will play a major role in reducing costs. Realistically, a cost of $3 to $5/kg would be appropriate to permit culturing, inactivation, and transportation. For immobilization, an additional $1/kg would be appropriate.

From the laboratory study, it was concluded that 10 adsorption/desorption cycles was the best that could be expected for reusing the biomass. For this use level and a net effective 20 mg/g of biomass adsorption capacity, a total of 4.3 kg of biomass would be required per kg of U_3O_8. At $4/kg for biomass and $50/kg for U_3O_8, the biomass represents 34% of the cost of the uranium product or approximately $17/kg of U_3O_8.

Capital cost and equipment

A preliminary estimate for the capital cost of equipment for underground recovery of uranium has been undertaken, using the stirred reactor-type adsorption flowsheet. Approximate costs for this facility would be $1 000 000. Assuming a three-year amortization at 12%, borrowing and capital payback costs represent approximately $9.65/kg of U_3O_8.

Elution agent

Sodium bicarbonate is the most effective agent, with approximately 9 kg of bicarbonate required per kg of U_3O_8. Allowing 40¢/kg for bicarbonate, this represents approximately $3.60/kg of U_3O_8.

Labour

The labour requirement would likely be one full-time operator/labourer, two shifts/day. At $50 000 labour cost per person, this would represent approximately $2.43/kg of U_3O_8.

Miscellaneous

Ongoing maintenance and power costs would be added to the above costs, but are not expected to add substantially to production expenses.

From the preceding discussion, it can be seen that biomass and capital costs are the most significant factors. The potential cost could be in the range of $30-$35/kg of U_3O_8 recovered from a level solution.

Major Technical Problems

The major technical problems affecting the full-scale application of biomass for uranium recovery would be:

- large-scale production, at a reasonable cost, of an immobilized culture that has good bioadsorption characteristics in combination with mechanical strength;
- maintenance of the bioadsorption characteristics and mechanical properties in cyclic adsorption/desorption operations;
- small-scale production of biomass (with limited demand, costs for biomass could be very prohibitive);
- cost-competitive applications as compared to conventional technologies;
- for underground applications, concerns over handling of chemicals and CO_2 generation from bicarbonate;
- contamination and degradation of the biomass by facultative and anaerobic bacteria, thus causing the development of septic conditions and odours;
- instrumentation of the uranium recovery system for optimum performance.

CONCLUSIONS FROM PHASE I AND II STUDIES

- Bioadsorption technology has potential application for removal of metals from waste streams. In addition, potential also exists for the development of economical metal recovery processes.
- The current applications for metal recovery studies in these projects were not economical.

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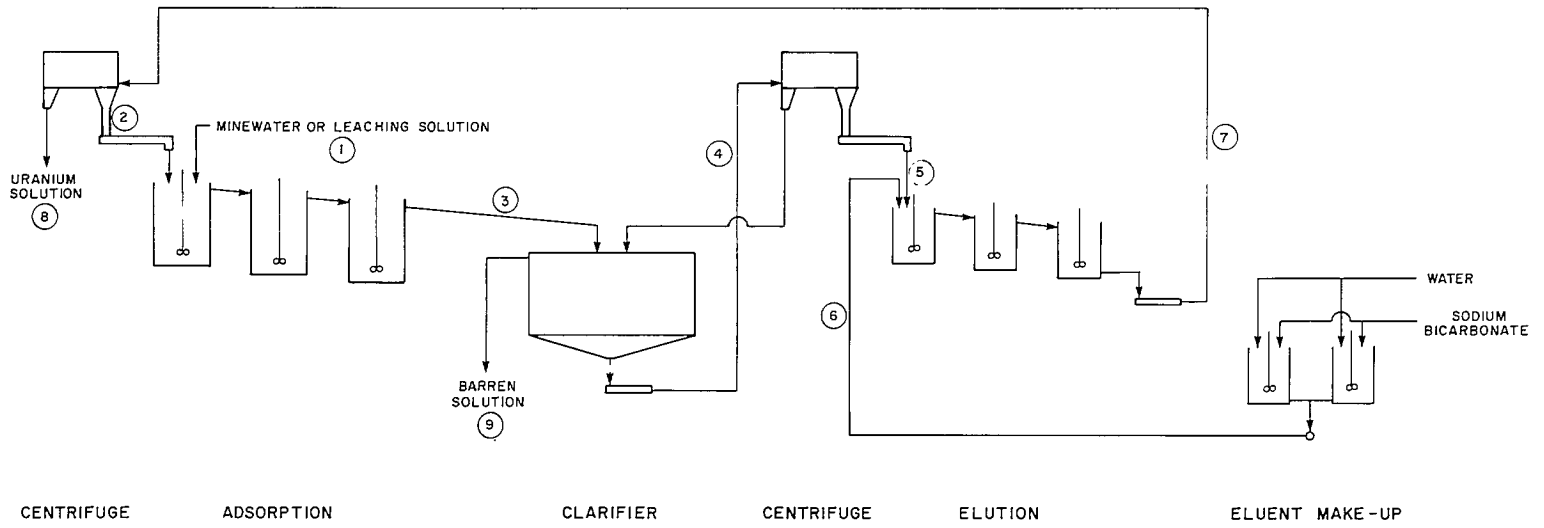
FIGURES



	1	2	3	4	5	6	7	8	9
SOLUTION FLOW (m^3/h)	40	1·0	41	8·08	1·0	4·0	5·0	4·0	40
BIOMASS FLOW (kg/h)	-	250	250	250	250	-	250	-	-
URANIUM CONCENTRATION (kg/m^3)	0·250	-	0·15	-	-	-	1·0	1·0	0·15
URANIUM CONCENTRATION NET (kg/kg)	-	-	0·020	0·020	0·020	-	-	-	-

NOTE:
SEE SECTION 4.2.2. FOR EQUIPMENT SIZING

Fig. 1 - Preliminary flowsheet for uranium recovery from minewater using biomass and stirred reactors

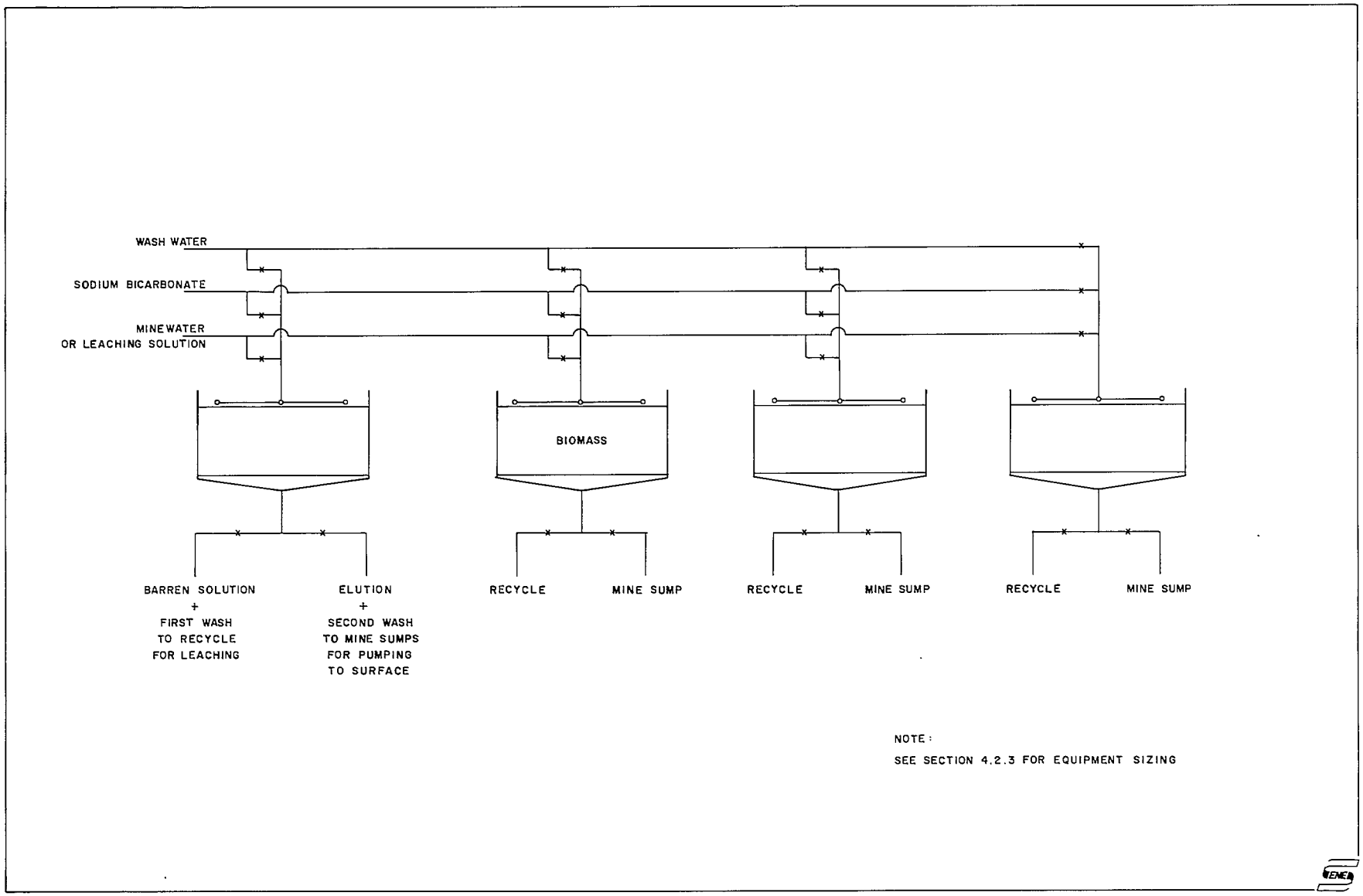


Fig. 2 - Preliminary flowsheet for uranium recovery from minewater using biomass and columns

SESSION III: PAPER 14

LOW-MOLECULAR-WEIGHT, COBALT-BINDING PROTEINS FROM *SACCHAROMYCES CEREVISIAE* AND *BACILLUS SUBTILIS*

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ABSTRACT

Economic and environmental pressures are incentive for improving the technological base associated with metal recovery and refining. Large quantities of valued metals are not available for use because the technology does not exist for separation, concentration, and recovery from relatively dilute solutions.

Research in our laboratory concerns the use of low-molecular-weight proteins, metallothioneins, for divalent metal separation and metal concentration from solution. Initial efforts were directed toward:

- determining if the production of these proteins can be synthesized microbially;
- isolating and purifying the proteins;
- determining if these proteins are inducible with a specific metal and if they are different in structure or binding capacity;
- attempting to mass-produce the metal-binding protein via genetic manipulation.

Future goals are:

- determining if these proteins provide a viable technique for metal recovery and separation;
- determining if binding strengths are changed after modification of the proteins, thus providing additional selectivity.

Thus far, metallothionein-like proteins have been isolated from three strains of microorganisms. The production of a protein has been induced by cobalt. Isolation purification, quantitation of the protein, and cysteine content have been accomplished.

SESSION III: PRÉSENTATION 14

PROTÉINES MÉTALLO-COORDINATRICES D'ORIGINE MICROBIENNE

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RÉSUMÉ

Les pressions économiques et environnementales encouragent les chercheurs à améliorer la base technologique de la récupération et du raffinage des métaux. De grandes quantités de métaux de valeur ne peuvent être utilisées, car il n'existe pas de technologie permettant de séparer, de concentrer et de récupérer ceux qui sont contenus dans les solutions relativement diluées.

Les recherches en cours dans notre laboratoire s'intéressent à l'utilisation de protéines de faible masse moléculaire, les métallothionéines, pour séparer des métaux divalents et les concentrer à partir d'une solution. Nous nous sommes d'abord efforcés de:

- déterminer s'il est possible de produire ces protéines par synthèse microbienne.
- isoler et purifier les protéines.
- déterminer si ces protéines peuvent être induites par un métal donné et si leur structure ou leur capacité séquestrante sont différentes.
- essayer de produire de grandes quantités de protéine métallo-coordinatrice par manipulation génétique.

Voici nos objectifs pour l'avenir:

- déterminer si ces protéines permettent de mettre au point une technique valable de récupération et de séparation des métaux.
- déterminer, après modification des protéines, s'il y a variation de la capacité séquestrante, ce qui les rendrait plus sélectives.

On a, jusqu'ici, isolé des protéines de type métallothionéine à partir de trois souches de micro-organismes. La production d'une protéine a été induite par le cobalt. On a procédé à l'isolation, à la purification et au dosage de la protéine, et on a déterminé la proportion de cystéine qu'elle renferme.

LOW-MOLECULAR-WEIGHT, COBALT-BINDING PROTEINS FROM *SACCHAROMYCES CEREVISIAE* AND *BACILLUS SUBTILIS*

INTRODUCTION

Metal-binding biomolecules may be useful as adsorbents in metal recovery processing and in the treatment of metal-bearing wastewater. The potential use of biomolecules for this purpose is just beginning to be explored. In this paper, preliminary studies to isolate low-molecular-weight, cobalt-binding proteins from Saccharomyces cerevisiae and Bacillus subtilis are described. These proteins could be useful in cobalt recovery processing.

A representative composition of processed leachate (i.e., after copper recovery) from copper mines that utilize dump leaching is shown in Table 1. Leachate streams contain various metals, including cobalt. At present, cobalt is not recovered from mining leachate streams because economic separation operations are not available for the recovery of cobalt from dilute aqueous streams. This valuable resource could be recovered if methods for separating and concentrating cobalt from leachate streams were available. The incentive for developing such an operation will probably continue to increase. The United States is interested in domestic cobalt resources for strategic reasons, and mining companies would certainly benefit if cobalt could also be recovered economically from mining leachate streams.

Biosorption, the use of whole microbial cells or biomolecules for the sorption of metals from solution, may provide an economic method for recovery of metals from leachate and wastewater streams.

The concept of biosorption has, for the most part, been limited to the use of whole cells (1,2). However, whole cell biosorption is relatively nonspecific and may be most useful for wastewater treatment when metal recovery is not the primary goal. The use of biomolecules may be advantageous for metal recovery when a specific metal is to be recovered. Metallothioneins could be used for this purpose.

Metallothioneins are low(approximately 6000)-molecular-weight proteins that have a high metal-binding capacity, especially for cadmium, copper, mercury, and zinc. These proteins are found in many animals, some microorganisms, and may have been isolated from plants. They are characterized by high cysteine content (30 to 35%). The sulphydryl groups of cysteine provide the core of the metal-binding sites. Because of their widespread occurrence in nature, a major function of metallothioneins is thought to be the sequestering of heavy metals to protect organisms from the environment. These proteins may also serve to maintain metal reserves for metabolic needs and to participate in metal transport. Anderson et al. (3) and Webb (4) have written extensive reviews on metallothionein.

Metallothioneins have been isolated from microorganisms. Production of proteins by fermentation with microorganisms will be desirable (as compared to isolation from animal tissue). In studies reported to date, metallothionein and metallothionein-like proteins have been isolated from S. cerevisiae (5,6),

Neurospora crassa (7,8,9,10), Tetrahymena pyriformis (11,12,13), Synechococcus (14,15), Pseudomonas putida (16), and perhaps from various other microorganisms (17). Properties of these proteins are presented in Table 2. Through the use of genetic-engineering techniques, microorganisms can possibly be manipulated to increase microbial production of specific metal-binding proteins.

Because metallothioneins appear to be associated with resistance to heavy metals, perhaps metallothionein-like, cobalt-binding proteins can be isolated from cobalt-resistant microorganisms. Various microorganisms have been reported to be resistant to cobalt (18,19,20,21,22). Norris and Kelly (18,19) reported significant cobalt resistance and uptake by S. cerevisiae and B. subtilis. For this reason, these microorganisms were selected for use in this study.

METHODS AND MATERIALS

The studies described in this paper were performed using Saccharomyces cerevisiae (Red Star) and Bacillus subtilis (ATCC #6051). The S. cerevisiae were grown in a medium consisting of glucose (10.0 g/L), peptone (5.0 g/L), malt extract (3.0 g/L), and yeast extract (3.0 g/L). The B. subtilis were grown in a medium consisting of bacto-beef extract (3.0 g/L) and bacto-peptone (5.0 g/L). Analytical-grade $CuSO_4.5H_2O$ and $CoSO_4.7H_2O$, respectively, were used as the sources of copper and cobalt in these studies. Cells were produced in 6-L volumes at room temperature with slow agitation. Fermentation times and metal concentrations are provided in the results section.

The following procedure was used to recover low-molecular-weight, metal-binding proteins.

The cells were harvested by centrifugation (Beckman Sorvall RC-5B) at 13 000 x g and 4°C. Cells were resuspended at 30 w/v% (or lower) in phosphate buffer (20 mM, pH 7.5), containing dithiothreitol (DTT) at a concentration of 5 mM, and were disrupted with a Biospec Bead Beater (Bartlesville, OK). Cell debris was removed by centrifugation (13 000 x g).

The cell-free supernatant was precipitated with ammonium sulphate (50% saturation) at room temperature. Precipitate was removed by centrifugation at 13 000 x g and 4°C.

The resulting supernatant was then saturated with ammonium sulphate. The remaining protein, including low-molecular-weight, metal-binding protein, was precipitated at 4°C overnight and collected by centrifugation at 13 000 x g and 4°C. The protein was redissolved in a minimum volume of buffer. Initially, proteins were redissolved in phosphate/DTT (20/5 mM, pH 7.5) buffer. However, both phosphate and DTT interact with cobalt ions, and the resulting complexes precipitate. Therefore, in the isolation of cobalt-binding proteins, the precipitated proteins were resuspended in Tris/DTT (20/2 mM, pH 7.5) buffer. (In subsequent studies, not reported herein, DTT was also eliminated from the buffer solutions used in the recovery of cobalt-binding proteins; other steps were taken to prevent formation of disulphide bridges and subsequent protein polymerization/aggregation.)

The redissolved protein solution (generally, about 10 mL) was applied to a Sephadex G-50 gel filtration column (2.5 x 70 cm). For copper-binding proteins, the column was eluted with phosphate/DTT (20/5 mM, pH 7.5) buffer. For cobalt-binding proteins, the column was eluted with either phosphate/DTT (20/5 mM, pH 7.5) buffer or Tris/DTT (20/2 mM, pH 7.5) buffer. For all runs, the flowrates were 20 ± 1 mL/h. Fractions (6 mL) were automatically collected by an ISCO fraction collector (Retriever III) and monitored by an ISCO flow-through UV spectrophotometer (Models UA-5 and 113, Optical Unit Type 6) at 254 and 280 nm. After completion of the gel filtration, samples were stored at 4°C until analyzed for metal content of each fraction. Metal concentrations were determined by atomic absorption spectroscopy (Perkin-Elmer Model 703).

The low-molecular-weight, metal-binding protein fractions were pooled and further purified by DEAE-cellulose (Pharmacia) ion exchange chromatography (2.5 x 25 cm column) using a NaCl gradient (5 mM to 0.5 M). For copper-binding proteins, the column was eluted with phosphate/DTT (20/5 mM, pH 7.5) buffer. For cobalt-binding proteins, Tris/DTT (20/2 mM, pH 7.5) buffer was used. The flowrate was 100 ± 1 mL/h. Fractions were collected, monitored, stored, and analyzed as described for the gel filtration runs.

RESULTS

In initial studies, low-molecular-weight, copper-binding proteins were isolated (as described above) from S. cerevisiae cells, grown for 72 h in a medium containing 25 mg Cu^{2+}/L. Gel filtration yielded a copper-containing peak at an apparent molecular weight of 10 000 ± 1000. The UV-Vis spectrum of these fractions showed a broad shoulder at about 270 nm, which corresponds to the absorption pattern of the copper-thiolate complex and indicates the presence of metallothionein (4). Ion exchange of the pooled fractions around 10 000 daltons produced several overlapping copper-containing peaks, which eluted in the range of 100 to 200 mmho. These findings are consistent with the results reported by Prinz and Weser (5), and Weser et al. (6), who upon additional analysis identified these proteins to be copper metallothioneins. Ion exchange resulted in several overlapping peaks in their studies, also; they attributed this phenomenon to the different metal contents of the same protein molecules.

In subsequent studies, low-molecular-weight, cobalt-binding proteins were isolated (as described above) from S. cerevisiae and B. subtilis grown in the presence of cobalt.

As a control, S. cerevisiae cells were first grown in the absence of cobalt for 48 h. The cells were harvested and the protein isolation procedure was performed. Gel filtration produced three peaks (Fig. 1): the void volume peak, the exclusion volume peak, and a peak at an apparent molecular weight of 3300 ± 300. Interestingly, a very low cobalt concentration (0.1 mg/L) was detected in the four fractions that eluted at an apparent molecular weight of 4000 ± 400. (The uncertainty range of atomic absorption in this study was ± 0.1 mg/L for cobalt.) The OD readings in this molecular weight range were low.

Low-molecular-weight, cobalt-induced, cobalt-binding proteins were then produced, using S. cerevisiae cells, as follows. Cells were grown for 48 h in the absence of cobalt. At 48 h, cobalt was added to the medium to bring the cobalt concentration to 100 mg Co^{2+}/L. After 24 additional hours, the cells were harvested and low-molecular-weight, cobalt-binding proteins were isolated as described above. Gel filtration (Fig. 2) yielded three cobalt-containing peaks. The void volume peak contained about 33% of the cobalt recovered from the column. The other two cobalt-containing peaks eluted at apparent molecular weights of 6000 ± 600 and 5000 ± 500, and contained about 45% of the cobalt recovered from the column. The 5000-dalton peak contained about two-thirds of the cobalt in these two cobalt-containing peaks.

The fractions that eluted at apparent molecule weights of 5000- and 6000-daltons were pooled and run on an ion exchange column (as previously described). Because of low protein and cobalt concentrations, the results were inconclusive. However, most of the protein (95 - 97%) was not bound to DEAE-cellulose and eluted from the column with the initial wash buffer. A single, small peak eluted at a conductivity of 140 to 200 mmho.

Low-molecular-weight, cobalt-binding proteins were also isolated from B. subtilis.

As a control, the protein recovery scheme was performed on B. subtilis cells grown in the absence of cobalt. Upon gel filtration (Fig. 3), proteins were detected over the range of molecular weights eluted from the column. Cobalt was not detected in any fractions.

In order to produce cobalt-binding proteins, B. subtilis cells were grown for 24 h in the absence of cobalt and then cobalt was added (100 mg Co^{+2}/L). The cells were harvested 12 h later and proteins were recovered as previously described. Four cobalt-containing peaks eluted from the gel filtration column (Fig. 4). The void volume peak contained about 40% of the cobalt recovered from the column. The peak that eluted at an apparent molecular weight of 18 000 contained 6% of the cobalt. Two peaks eluted at apparent molecular weights of 6000 ± 600 and 4200 ± 400, containing about 31% and 17% of the cobalt recovered, respectively. This result is similar to the findings with S. cerevisiae.

The fractions containing the 6000- and 4200-dalton proteins were pooled and run on an ion exchange column as previously described. Again, the results were inconclusive due to low cobalt concentrations. As much as 90 - 95% of the protein loaded onto the column did not bind to the DEAE cellulose, but approximately half of the cobalt appeared to elute in the gradient run. Proteins eluted starting at a conductivity of 120 mmho and continued to elute through the end of the gradient.

In an alternate procedure, B. subtilis cells were grown for 24 h in the absence of cobalt, subjected to 100 mg Co^{2+}/L for 12 h, then harvested and the proteins recovered. In this run, the resuspended protein solution was spiked with cobalt just prior to the gel filtration, and the resulting concentration was 550 mg Co^{2+}/L. The resulting gel filtration chromatogram is shown in Figure 5. One major cobalt-containing peak eluted at an apparent molecular weight of 7000 ± 1000. This peak probably contains the two peaks previously seen in Figure 4.

The fractions contained in the above peak were pooled and run on an ion exchange column (as previously described). This chromatogram is shown in Figure 6. A large fraction of the cobalt-containing proteins were not bound by DEAE-cellulose. However, a cobalt-containing protein did elute in the conductivity range of 120 to 200 mmho (which is similar to the conductivity range in which copper metallothionein elutes). This peak contained about 7% of the cobalt recovered from the column.

DISCUSSION

The cobalt-binding proteins isolated from S. cerevisiae and B. subtilis in these studies are similar to metallothioneins. These cobalt-binding proteins have apparent molecular weights of 4000 to 7000 daltons (i.e., in the range reported for metallothioneins). Some proteins eluted from DEAE-cellulose in the conductivity range between 100 and 200 mmho, which is similar to the conductivity range in which copper-metallothionein from S. cerevisiae elutes. However, a large fraction of the cobalt-binding proteins did not bind to DEAE-cellulose; this phenomenon is not normally observed with metallothioneins.

More detailed characterization of the low-molecular-weight, cobalt-binding proteins is planned. Analysis of metallothionein-like proteins has historically proven to be difficult. Reliable protein and cysteine assays, which are simple to perform, have been difficult to develop (4). To complicate matters further, cobalt has been found to interfere with many of the simple assays attempted in these studies. At this time, the cysteine and cobalt contents (i.e., mg cysteine or cobalt per mg protein) of these proteins have not been determined. Work is underway to develop simple assays for determination of these contents. In addition, analyses of the amino acid composition will be applied to the characterization of these proteins.

CONCLUSIONS

Low-molecular-weight, cobalt-binding proteins have been isolated from S. cerevisiae and B. subtilis. To our knowledge, this study represents the first attempt to isolate cobalt-induced, cobalt-binding, metallothionein-like proteins from microorganisms and the first attempt to isolate any metallothionein-like proteins from the bacterium, B. subtilis. This finding could hold potential industrial significance in metal recovery processing. Further basic research is needed to characterize these proteins and to measure metal-binding capacities and specificities. In addition, extensive process-engineering research will also be needed, if basic studies indicate possible technical and economic feasibility. The isolation of these proteins indicates, at least, the theoretical feasibility of using metal-binding biomolecules in cobalt recovery processing.

The potential for use of biomolecules in industrial practice, and in metals recovery in particular, should not be overlooked. Unique and useful metal-binding biomolecules could well exist in nature and prove economical for use in industrial processing. With additional research, such proteins could be isolated from nature, perhaps improved upon through genetic engineering tech-

niques, and processes could be developed. Applicable research areas include isolating interesting biomolecules, maximizing biomolecule production, measuring metal-binding capacities and selectivities, determining biomolecule stability to processing conditions (temperature, pH, metal sorption and desorption agents, etc.), and performing process development studies. In addition, genetic engineering and the developing field of protein engineering could be applied to actually changing the molecules to enhance their properties. For instance, protein engineering could be applied to manipulate the binding sites and to improve selectivity toward a specific metal. Metallothionein and metallothionein-like proteins may provide the basis for a new type of metal recovery process.

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TABLES

Table 1 - Representative composition of dump-leaching effluent

10 000 GPM Composition		
Element	ppm	Tons/yr
U	7	154
Co	30	660
Ni	35	790
Zn	180	3 960
Mn	280	6 160
Cu	100	2 200
Fe	2 000	-
Al	4 600	93 000

Table 2 - Properties of metallothioneins and metallothionein-like proteins isolated from microorganisms

Microorganism	Metal	Metallothionein	Molecular weight ($x10^3$)	Capacity	Cysteine (%)
Saccharomyces cerevisiae	Cu	Yes	10	10	24
Neurospora crassa	Cu	Yes	2 to 3	6	28
Tetrahymena pyriformis	Cd	Yes	11	4.5	32
	Cu	Yes	n.d.	n.d.	n.d.
	Zn	No	-	-	-
Synechococcus	Cd	Yes	8 to 10	n.d.	14 to 18
	Zn	Yes	n.d.	n.d.	n.d.
	Cu	No	-	-	-
Pseudomonas putida	Cd	Yes	4 to 7	7	10 to 23

FIGURES

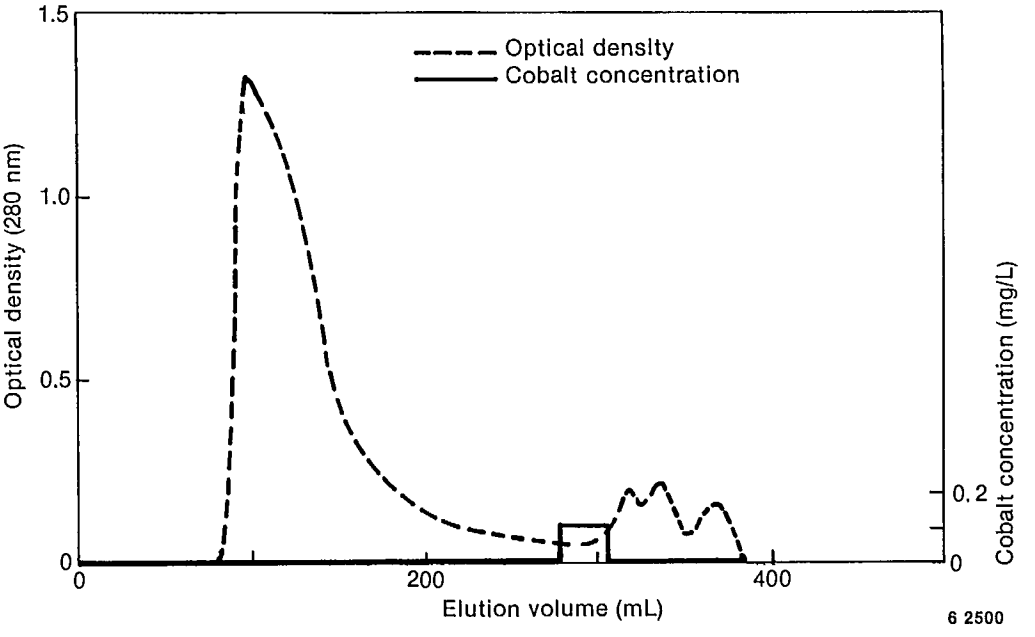

Fig. 1 - Gel filtration of protein solution from S. cerevisiae fermentation, without cobalt. [Sephadex G-50, column -- 2.5 x 70 cm, flowrate -- 20 ± 1 mL/h, fraction volume -- 6.0 mL, eluant -- phosphate/DTT (20/5 mM, pH 7.5) buffer.]

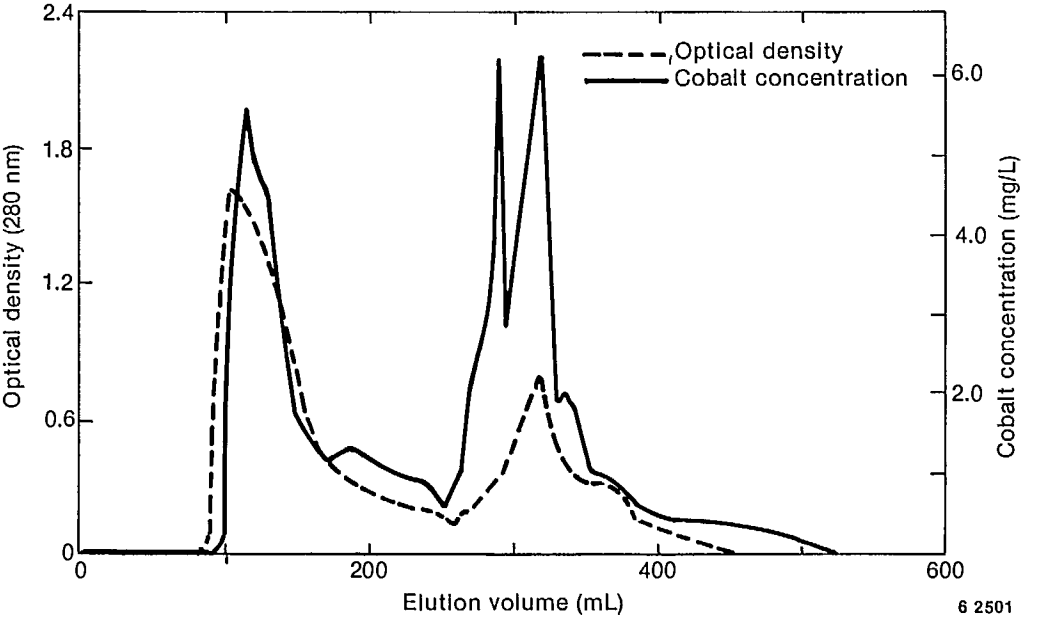

Fig. 2 - Gel filtration of protein solution from S. cerevisiae fermentation, with cobalt. [Sephadex G-50, column -- 2.5 x 70 cm, flowrate -- 20 ± 1 mL/h, fraction volume -- 6.0 mL, eluant -- phosphate/DTT (20/5 mM, pH 7.5) buffer.]

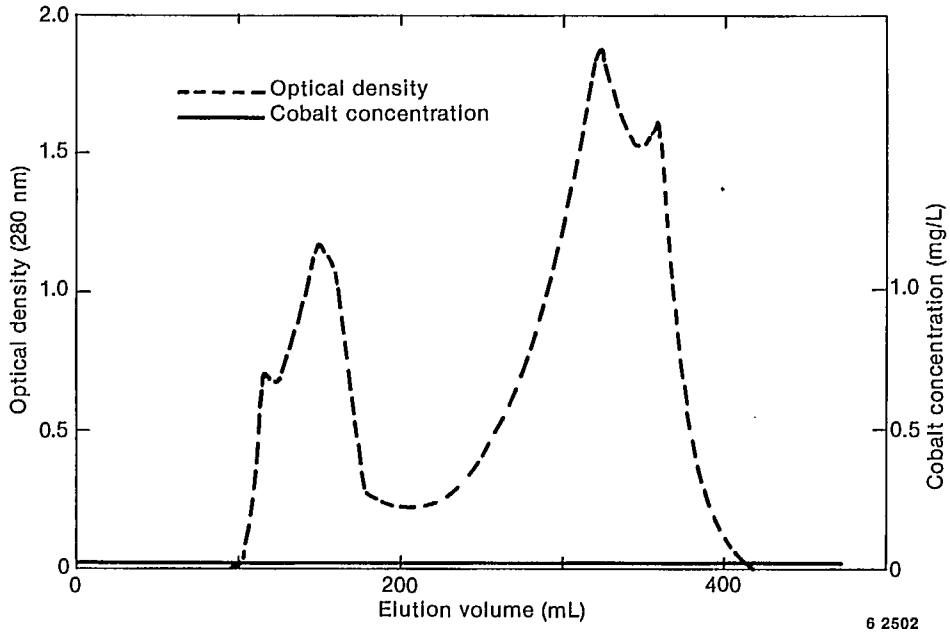

Fig. 3 - Gel filtration of protein solution from B. subtilis fermentation, without cobalt. [Sephadex G-50, column -- 2.5 x 70 cm, flowrate -- 20 ± 1 mL/h, fraction volume -- 6.0 mL, eluant -- Tris/DTT (20/2 mM, pH 7.5) buffer.]

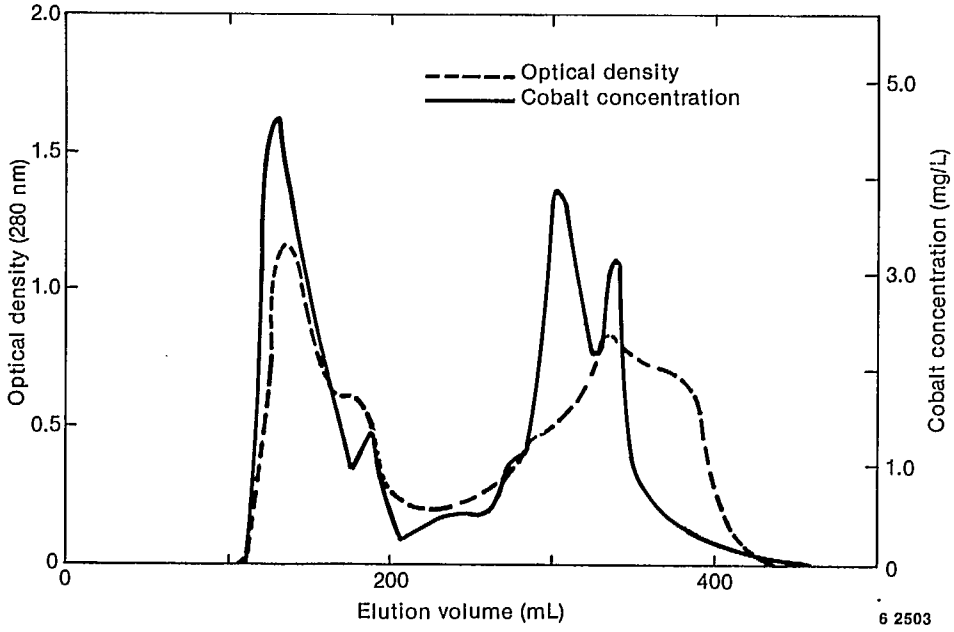

Fig. 4 - Gel filtration of protein solution from B. subtilis fermentation, with cobalt. [Sephadex G-50, column -- 2.5 x 70 cm, flowrate -- 20 ± 1 mL/h, fraction volume -- 6.0 mL, eluant -- Tris/DTT (20/2 mM, pH 7.5) buffer.]

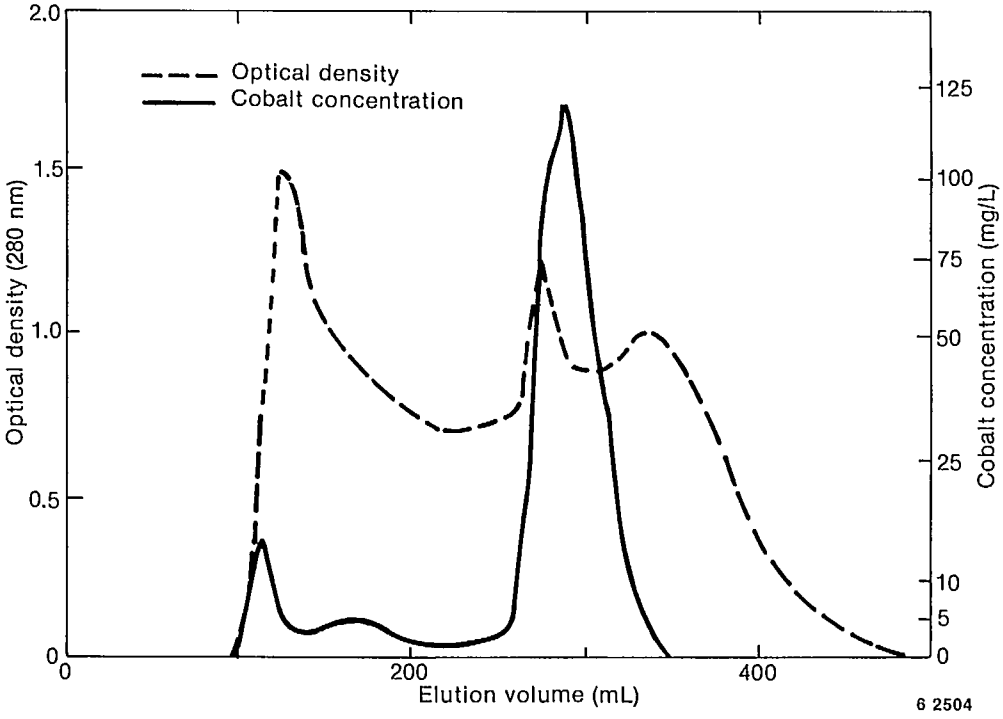

Fig. 5 - Gel filtration of cobalt-spiked (550 mg Co^{2+}/L) protein solution from B. subtilis fermentation, with cobalt. [Sephadex G-50, column -- 2.5 x 70 cm, flowrate -- 20 ± 1 mL/h, fraction volume -- 6.0 mL, eluant -- phosphate/DTT (20/5 mM, pH 7.5) buffer.]

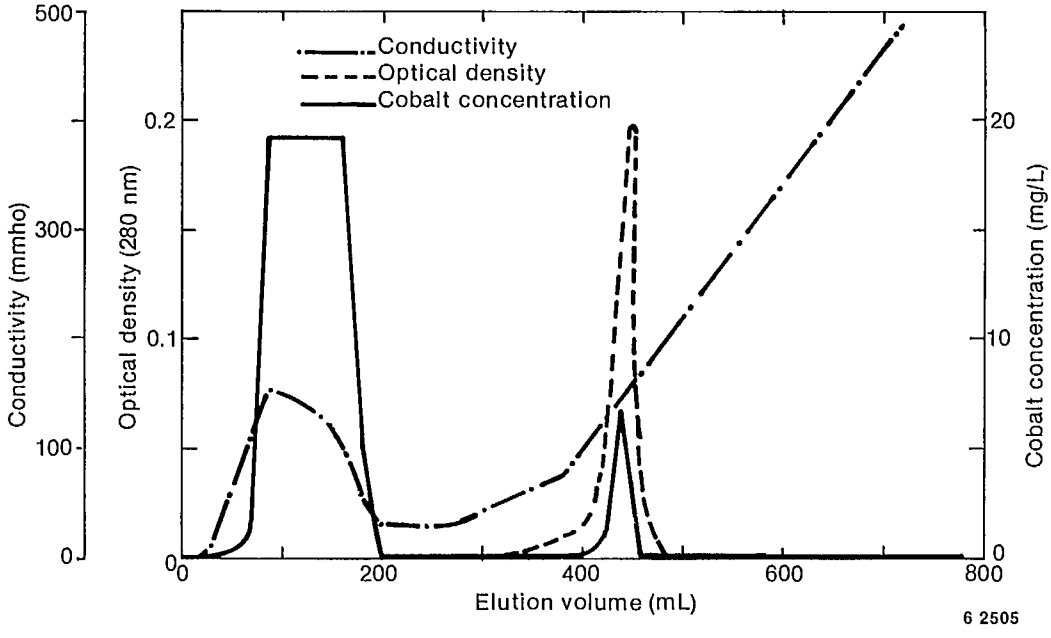

Fig. 6 - Ion exchange of cobalt-binding proteins from B. subtilis fermentation, with cobalt. [DEAE-cellulose, column -- 2.5 x 25 cm, flowrate -- 100 ± 1 mL/h, fraction volume -- 6.0 mL, eluant -- Tris/DTT (20/5 mM, pH 7.5), gradient -- NaCl (5 mM to 0.5 M).]

SESSION III: PAPER 15

METAL SORPTION BY BACTERIAL CELL WALLS

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ABSTRACT

Most bacteria in nature are surrounded by a wall forming the outermost limit to the cell and separating the vital contents of the protoplast from the environment. Many of the homo- and heteropolymers contained within bacterial cell walls are anionic and, therefore, interact strongly with dissolved metals. For example, walls isolated from Gram-positive bacteria, such as Bacillus subtilis, can bind a wide range of metallic ions, including members of the transition and rare earth series. Similarly, the wall of the Gram-negative bacterium, Escherichia coli, is capable of sequestering a variety of metal cations from aqueous solution. The binding of metals to bacterial walls is also strong and resilient, as indicated by results from low-temperature (ca. 100°C), sediment diagenesis experiments. These studies, and recent observations on geothermal sediments, confirm that the walls of bacteria bind metals tenaciously during early diagenesis.

SESSION III: PRÉSENTATION 15

SORPTION DE MÉTAUX PAR LA PAROI CELLULAIRE DES BACTÉRIES

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RÉSUMÉ

Dans la nature, la plupart des bactéries sont entourées d'une paroi formant la limite externe de la cellule et séparant de l'environnement le contenu vital du protoplasme. Bon nombre des homo- et des hétéropolymères contenus à l'intérieur de la paroi cellulaire des bactéries sont anioniques; ils peuvent donc interagir fortement avec les métaux en solution. Par exemple, les parois provenant de bactéries gram-positives (par exemple, Bacillus subtilis) peuvent fixer une vaste gamme d'ions métalliques, dont ceux des métaux alcalins, des terres alcalines, des métaux de transition et des terres rares. De même, la paroi d'Escherichia coli, une bactérie gram-négative, est capable de chélater divers cations métalliques en solution dans l'eau. Comme le montrent les résultats d'expériences simulées de diagenèse des sédimens à base température (environ 100°C), la fixation des métaux à la paroi cellulaire des bactéries est, elle aussi, forte et résistante. Ces études confirment le fait que la paroi des bactéries fixe fermement les métaux au début de la diagenèse et que, dans des conditions géochimiques propices, elle sert de noyau distinct pour la formation des minéraux.

METAL SORPTION BY BACTERIAL CELL WALLS

INTRODUCTION

Bacteria are, without doubt, the most abundant life-form on earth. They are ubiquitous throughout nature, living in almost every stressful environment imaginable. Indeed, bacteria have been isolated from concentrated brines of salt lakes, acid leaching of mine tailings, and boiling hot springs (1,2). However, even though bacteria survive in an unparalleled variety of habitats, most species have settled on a common structure - the cell wall - to reside between their surroundings and the vital contents of the protoplast (3).

The cell wall is of fundamental importance to the bacterium. Besides providing shape and form to the cell, the wall also controls diffusion, to a certain extent, since all soluble material must percolate through the wall fabric before gaining access to the plasma membrane (4). Furthermore, as the first structure to contact the external milieu, the wall is first to react to the environment. Consequently, bacterial walls are necessarily resilient to both mechanical stress and chemical degradation (3). In fact, of all the homo- and heteropolymers of bacteria, the components of walls are the most durable, being recognized as distinct structural or chemical forms in ancient rocks and sediments (5).

THE ANIONIC NATURE OF BACTERIAL WALLS

The walls of bacteria, like most cell surfaces, are anionic. This was established by early microelectrophoresis experiments showing that the isoelectric point of most bacteria varied between pH 2 and 4 (6). Other electrokinetic studies have demonstrated that different bacterial species possess unique negative surface charges related to the various proportions of acidic (anionic) carboxyl and phosphoryl groups within the individual components of the wall (7).

Among bacteria, there are two predominant types of cell walls distinguished not only by their chemistry, but also by their morphology and response to the Gram reaction, a differential staining regimen for light microscopy (8). The wall of Gram-positive bacteria is an amorphous structure that, depending on the organism, may be 20 to 50 nm thick (4). It consists of peptidoglycan, a linear polysaccharide heteropolymer cross-linked in three dimensions by short peptide bridges. A variety of secondary polymers, including teichoic or teichuronic acids and proteins, are interwoven through the peptidoglycan matrix. Together, these components form a highly hydrated sacculus that completely surrounds the cell (9). In contrast, the wall of Gram-negative bacteria contains quantities of lipid and protein arranged to form a bilayer outer membrane; a thin layer of peptidoglycan is usually covalently attached to proteins exposed on the inner face of the outer membrane (10).

In both wall types, carboxyl groups occur within the peptide stems of the peptidoglycan and in proteins (3). The teichuronic acids of Gram-negative walls, and the lipopolysaccharide (a unique glycolipid) of Gram-negative walls, also contain carboxyl groups. Phosphoryl groups are confined to

teichoic acids in the Gram-positive situation (3), whereas they occur as the major acidic moieties in the polar head-groups of lipids in the Gram-negative outer membrane (11).

METAL-BINDING STUDIES

Since their chemistry favours an anionic charge density, bacterial walls can act as potent traps for metallic ions in solution. Some walls interact with soluble metal cations as open, low-density, ion exchange resins (12), whereas others exhibit a certain degree of selectivity with an increased partitioning ability (13,14,15,16). In either case, substantial quantities of metal can be bound, regardless of whether equilibrium or displacement experiments are used.

Results from some of our metal-binding experiments with representative Gram-positive and -negative bacteria are summarized in Table 1. In these experiments, 0.1 to 5.0 mg dry weight of isolated walls were reacted with various aqueous solutions of metallic salts under saturating conditions. Then the walls were washed with high-resistance water until metal could not be detected in the supernatants. The quantity of metal bound by the walls was subsequently determined by either atomic absorption or X-ray fluorescence spectroscopy.

In general, Gram-positive walls tend to bind more metal than Gram-negative walls. This difference is due primarily to the higher anionic charge capacity of the Gram-positive wall (3). However, with metals that exhibit unstable aqueous chemistries, ferric iron for example, the amount of metal bound by both cell-wall types often exceeds the number of available anionic sites. This is probably due to a two-step deposition process (13):

1. A stoichiometric interaction between the soluble metallic species and the wall saturates the negatively charged sites.
2. Then, the initial bound metal serves as nucleation sites for the outgrowth of metal aggregates. In this way, substantial quantities of metallic ions could be removed from solution.

Other experiments have been performed to ascertain the exact chemical sites of metal interactions within bacterial walls. We have extracted teichoic acids and performed metal-binding assays on the remaining peptidoglycan sacculus of Bacillus subtilis, a Gram-positive organism (14). Although the removal of the teichoic acids decreased the amount of metal bound, most of the binding capacity remained with the peptidoglycan. Only after the constituent carboxyl groups were chemically neutralized by glycine ethyl ester was a significant decrease in binding capacity realized.

To examine the site specificity of metal binding in the Gram-negative outer membrane, phosphorus (^{31}P) nuclear magnetic resonance (11) was used. Since the natural abundance of the ^{31}P isotope is 100%, no artificial labelling or chemical modifications are required and, as such, the technique is non-perturbing. An isolated outer membrane from Escherichia coli was titrated with increasing amounts of europium (a paramagnetic lanthanide). As the concentration of Eu^{3+} was increased, the ^{31}P signal from the outer membrane lipids

was broadened until, at a 1:1 mole ratio of europium to phosphorus, it could not be detected. This masking effect of Eu^{3+} on the ^{31}P spectra indicated that the metallic ion interacted directly with the phosphoryl groups of the outer membrane lipids.

GEOCHEMICAL STUDIES

A significant portion of the organic matter in aquatic environments consists of small suspended particles of highly cross-linked, heteropolymeric material (17). At least some of the more durable polymeric networks are from bacteria, the most resilient structure being the cell wall. Eventually, these colloidal elements would come into contact and bind with metal cations, aggregate, then settle to the bottom where they would form part of the sediment. After being immobilized in the sediment, the metal would either be recycled into the overlying water phase, or geochemical mineralization could proceed (17).

To study some of these processes, we simulated low-temperature (*ca.* 100°C) sediment diagenesis in the laboratory by mixing metal-loaded bacteria into a synthetic sediment of quartz and calcite (18). The sediment was monitored during the experiment by electron microscopy, selected area electron diffraction, and energy dispersive X-ray spectroscopy. Most of the metal was associated with the wall and, as the bacteria were aged, elements from the sediment combined with the bound metal to form authigenic microcrystalline phases. In this way, the bacterial cell wall polymers served as distinct nucleation sites for mineral formation (Table 2).

In an effort to extend the results of this laboratory work to a natural environment, we obtained surficial sediment samples from several hot springs in Yellowstone National Park, Wyoming. The geothermal fluids contain elevated levels of dissolved metals, and most of the organic matter in the sediments is of microbial origin, derived from the thermophilic bacteria that are often prolific in such extreme habitats (2).

Electron microscopy showed that bacteria prominent in samples from Cyanidium Creek, an acidic (pH 3.1) stream fed by three small hot springs, were completely encrusted by an electron opaque matrix of granular and spheroidal crystallites (19). The outgrowth of these crystallites was found to be associated with both the cell surface and the wall of the bacteria. Energy dispersive X-ray spectroscopy showed that silicon was the most abundant element, suggesting that the bacteria promoted the deposition of dissolved silica by serving as sites for crystallite nucleation. Iron was also present in the matrix. Presumably, the metal was bound from the water phase by the constituent polymers of the bacteria. This was suggested by the iron signals detected by energy dispersive X-ray spectroscopy in cells where silica crystallite formation was not evident. These observations parallel our earlier efforts to simulate sediment diagenesis in the laboratory (18), and confirm that bacteria can serve as distinct nucleation sites for mineralization in situ.

SUMMARY

From our discussion, it can be concluded that the bacterial cell wall is not just an inert corset surrounding the protoplast. Instead, the wall possesses reactive anionic sites that can bind and exchange environmental counterions. Of course, it is difficult to escape the implications of this reactivity in an applied sense. Indeed, bacterial walls could be used to cleanse industrial effluents of toxic metal contaminants, and real possibilities exist for the secondary recovery of the metals in such situations.

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TABLES

Table 1 - Metal binding by bacterial walls*

Metal	*Bacillus subtilis* Native wall**	*Bacillus licheniformis* Native wall***	*Escherichia coli* Outer membrane****
Na	2.697	0.910	0.200
K	1.944	0.560	0.025
Mg	8.226	0.400	0.084
Ca	0.399	0.590	0.185
Mn	0.801	0.662	0.355
Fe	3.581	0.760	0.541
Ni	0.107	0.520	0.019
Cu	2.990	0.490	n.d.
Au	0.363	0.031	n.d.

*Micromoles of metal bound per milligram dry weight of walls.
**Beveridge and Murray, 1976, 1980.
***Beveridge et al., 1982.
****Ferris, F.G., 1985, Ph.D. Thesis, University of Guelph.
Note: *B. subtilis* and *B. licheniformis* are Gram-positive organisms, whereas *E. coli* is Gram-negative.
n.d.:not determined.

Table 2 - Mineralization during simulated sediment diagenesis experiments with metal-loaded bacteria*

Metal sorbed to bacteria	Sediment mixture	Redox buffer	Time (days)	Initial pH	Final pH	Mineralization
Iron	Absent	M	100	7.0	4.5	Iron phosphate microcrysts
Copper	Q + C	M	100	9.0	6.8	Phosphate microcrysts
	Q + C	S	100	9.0	6.8	Copper sulphides
Zinc	Absent	M	200	7.0	4.5	Phosphate microcrysts
	Absent	S	200	7.0	4.8	Zinc sulphides
Uranium	Q	M	200	7.0	5.0	Uranium phosphate microcrysts
	Absent	S	200	7.0	4.8	Uranium phosphate microcrysts and organic sulphide residues

Q: quartz; C: calcite; S: elemental sulphur; M: magnetite.
*Beveridge et al., 1983.

SESSION III: PAPER 16

AMT-BIOCLAIM™ PROCESS FOR TREATMENT OF METALLIFEROUS WASTEWATER FROM ELECTROPLATING AND OTHER INDUSTRIES

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ABSTRACT

A granulated, microbial biomass product has been developed for the recovery of metals from wastewater. The metal recovery agent (MRA) has a high capacity for accumulating metals: 2.9 mM Pb/g, 2.4 mM Cu/g, 2.1 mM Zn/g, and 1.9 mM Cd/g from solutions containing 10 to 100 mg metal/L. The MRA can achieve near 100% removal of metals over a broad range of solution pH values (from 3 to 9). Removal of some complexed metals, such as gold in the form of gold cyanide, is possible by the MRA granules. The MRA technology is adaptable for wastewater treatment or metal recovery using either a pulsed-bed recovery system of fixed-bed canister units.

SESSION III: PRÉSENTATION 16

UTILISATION DU PROCÉDÉ AMT-BIOCLAIM™ POUR LE TRAITEMENT DES EAUX MÉTALLIFÈRES REJETÉES PAR LES USINES D'ÉLECTROPLACAGE ET PAR D'AUTRES INDUSTRIES

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RÉSUMÉ

Un biomasse microbienne granulaire a été mise au point pour récupérer des métaux dans les eaux usées. L'agent d'extraction (MRA) possède une grande affinité pour les métaux: un gramme de ce produit permet d'extraire 2,9 mM de Pb, 2,4 mM de Cu, 2,1 mM de Zn et 1,9 mM de Cd dans des solutions contenant de 10 à 100 mg de métal par litre. Le MRA permet d'extraire presque 100 % des métaux sur une vaste plage de pH (3-9) et ainsi d'éliminer efficacement les espèces cationiques. Le MRA peut aussi éliminer les chromates et certains complexes métalliques (par exemple, l'or sous forme de cyanure d'or). Cette technologie peut s'adapter au traitement des eaux usées, soit dans des systèmes de récupération à lit pulsé, soit dans des boîtes à lit fixe.

AMT-BIOCLAIM™ PROCESS FOR TREATMENT OF METALLIFEROUS WASTEWATER FROM ELECTROPLATING AND OTHER INDUSTRIES

INTRODUCTION

Microbial processes have potential for use in recovery of heavy metals from dilute solutions. The organisms function by either accumulation of dissolved and particulate metals, or by production of by-products that render the metals insoluble. Two mechanisms have been identified that enable microorganisms to concentrate metals: binding of metals to cell surface components such as cellular ligands and biopolymers (biosorption); and metabolism-dependent intracellular accumulation (1).

Different microbes have been studied for their ability to accumulate metals, as well as their use in processes for recovery of metals and wastewater treatment. Shumate et al. (2), and Strandberg et al. (3), tested Saccharomyces cerevisiae and Pseudomonas aeruginosa as biosorbants for uranium. Each type of microorganism accumulated from 10 to 15% of its dry weight of uranium. The mechanism of accumulation differed for the two microbes. The cells of S. cerevisiae accumulated the uranium on the cell surface in a layer 0.2 μm thick. Ps. aeruginosa accumulated uranium internally, metabolism not being required for uranium to cross the cell membrane. The rate at which the bacterial and yeast cells reached an equilibrium concentration of uranium varied. S. cerevisiae were "slow", reaching equilibrium after one hour; Ps. aeruginosa were "fast", with equilibrium achieved within 10 min after contact with uranium. Rhizopus arrhizus biomass, produced as a by-product of industrial fermentations, has potential for use as a biosorbant of uranium and other metal cations (4,5). These fungal cells have a uranium and thorium uptake capacity of over 180 mg g^{-1} dry weight. Recovery of metals from mine drainage water may also be accomplished by using Zoogloea ramigera flocs in a biosorption process (6). These flocs rapidly establish equilibrium with heavy metals in solution by a process of adsorption.

AMT researchers, aware of the natural capability of microorganisms to accumulate metals, sought development of a more stable material with greatly enhanced metal recovery capability. A granulated, non-living biomass product, having high capacity for soluble metals, was produced and tested. This material is suitable for use as a metal recovery agent for both pollution control and resource recovery. This paper describes some of the characteristics of the product, as well as its use for removal of metals and recovery from wastewaters using the AMT-BIOCLAIM™ process. A previous report (7) (in press) provides information comparing this process with conventional wastewater treatment methods.

MATERIALS AND METHODS

Metal Recovery Agent

The metal recovery agent (MRA) was prepared from biomass and activated by a proprietary process. The MRA was then dried, ground, and segregated to provide granules of given sizes for testing.

Standard Column Tests

Efficacy of metal recovery by the MRA was determined using packed columns. Glass columns (16 mm I.D. x 20 cm length) were packed with 6.5 g of granulated MRA, usually of -35 + 60 mesh standard sieve fraction. Solutions containing soluble metals were pumped at a rate of 10 mL/min upflow through the column; Cole-Parmer Instruments "Masterflex" peristaltic pumps were used. Columns were operated until "breakthrough", i.e., less than 90% of the metal was removed from solution. The column granules were separated into a bottom fraction (2.5 cm length) and the remainder (≈7.6 cm) for determination of metal content. The bottom fraction was considered to represent a maximum for practical metal loading. The following metal salts were used for determination of respective metals' uptake: $AgNO_3$, $CdCl_2.2\ 1/2H_2O$, $CuSO_4.5H_2O$, $Pb(NO_3)_2$, and $ZnSO_4.7H_2O$.

Analyses

Solution pH values were measured using a Beckman phi 21 pH meter. Metal content of solutions was determined with a Perkin Elmer model 380 atomic absorption spectrophotometer. MRA granules were analyzed for metal content following digestion. Two procedures were used. For silver, copper, or lead, the granules (up to 2 g) were digested by boiling in concentrated nitric acid (5 mL) and water (15 mL). Granules containing gold, zinc, or cadmium were digested using the following procedure: a sample (up to 1 g) was heated in concentrated nitric acid (2 mL) and water (5 mL) until fuming ceased; concentrated hydrochloric acid (6 mL) was added and heating continued to incipient dryness, followed by additions of concentrated hydrochloric acid (5 mL) and water (10 mL), which were brought to a boil. Digested materials were brought to volume using deionized water.

RESULTS AND DISCUSSION

The MRA is an amorphous granule with an irregular surface structure that provides a large area for metal accumulation. The granulated nature of the MRA facilitates use in either a fixed-bed column or a fluidized-bed reactor for metal recovery. The granules can be recycled following the stripping of metals in an electrolyte.

The "standard column tests" were used to determine metal-loading capacities of the MRA. Columns were selected because they more closely simulate the manner of utilization of the MRA for metal recovery from wastewaters; thus, the loading capacities determined in the laboratory have predictive value for field use. The MRA effectively removes soluble heavy metals from dilute wastewater; the MRA is refractory to the innocuous ions calcium, potassium, sodium, and magnesium. Heavy metal cations are efficiently removed (>99%) from dilute solutions over a broad pH range of 3 to 9. Examples of metal loading are listed in Table 1 for metals at 10 mg/L and at 100 mg/L concentration. Silver loading by the MRA follows typical isotherm response, i.e., silver accumulation is concentration-dependent. The MRA achieves similar loadings, but independent of the concentrations tested, for copper and zinc. The apparent greater capacity of the MRA for cadmium at 10 mg Cd/L, compared to 100 mg CD/L, may have been an artifact caused by solution channelling in

the small test columns. The MRA achieves excellent loading for lead; the MRA accumulated slightly more lead from the 10 mg Pb/L solution.

The AMT-BIOCLAIM™ MRA granules can be used for recovery of precious metals (e.g., gold and silver) from jewelry, plating, photographic wastewaters in which the metals are complexed with cyanide and other agents such as thiosulphate and acetate. The system has been tested using a jewelry manufacturer's wastewater, which contained cyanide-complexed gold (Table 2). This wastewater is unusual because of the high gold concentration; such a high value is not characteristic of most wastewaters, which would contain much less gold. A column containing 20 g of the MRA removed more than 99% of the metals for the first two litres of solution processed. After treating four litres, only 63% of the gold was removed; the gold retention decreased because of the large amount of gold in solution. The bottom one-fourth of the column loaded with gold to 1.06 mM/g. The MRA is also effective in recovering gold from acid gold cyanide solutions typical of gold-plating processes. Gold was efficiently recovered from a simulated gold drag-out wastewater supplied by a precious metals dealer (Table 3). These results illustrate the effectiveness of the MRA for recovery of cyanide-complexed gold from wastewaters.

Silver from photographic processing wastewater can also be recovered using a "standard column test" containing the MRA (Table 4). The loading capacity was not achieved during the experiment because of insufficient quantity of wastewater available for testing. However, good silver recovery and loading (0.87 mM Ag/g) were attained.

Field-testing of the AMT-BIOCLAIM™ metal recovery granules has been conducted using fluidized pulsed-bed units. The pilot units, which are 15 ft high and 1.5 ft in diameter, treat waste streams of 1-8 gpm or approximately 600-3800 gal/8 h shift. A pilot unit operates in the manner described below.

Initially, the fluidized-bed contactor is filled with approximately 175 lb, or 11 cu ft, of the granular MRA. This yields a bed depth of about 6-7 ft, leaving freeboard for up to a 50% expansion of the bed during fluidization.

The wastewater enters an equalization tank and is then pumped through a control loop to the fluidized-bed contactor. As the wastewater passes upflow through the contactor, the granular MRA is fluidized. This fluidization causes the larger, heavier granules to settle and the smaller, lighter granules to migrate upward, yielding a relatively stable, segregated bed that provides optimum removal of metal ions from the wastewater.

During operation, the granules nearest the bottom of the contactor become most heavily loaded with metal. As the wastewater passes upward through the bed, it contacts material that is less spent until, at some point, approximately 2-3 ft up in the bed, it contacts fresh material. The surge volume of fresh MRA in the vessel acts to "polish" the wastewater, providing maximum removal of metal ions. The large volume of fresh material also acts as a buffer to accommodate any large fluctuations in metal concentration that are characteristic in industrial wastewater.

As the metal recovery agent becomes loaded with metal, it gets heavier and sinks to the bottom of the fluidized bed. This unique property allows accumulation of the most loaded material at the base of the contactor, where

it can be removed by a process called "slugging". Slugging the fluidized-bed contactor involves removing a portion of the MRA in the contactor (approximately 1/7 to 1/10 of the total vessel volume) and replacing the agent with an equal volume of fresh material.

Operation of the system is simple, requiring only periodic monitoring of the reaction zone in the contactor. The feed rate is adjusted to maintain a 15-40% fluidization of the MRA. No other control is necessary.

A 39-day campaign was run to determine the effectiveness of the fluidized-bed AMT-BIOCLAIM™ unit for removal of lead from an industrial effluent. The unit was set up at a secondary lead-refining operation. The pilot unit contained 230 lb of MRA, with lead-containing wastewater processed at a flow of 1 to 4 gal/min. The plant effluent was either previously treated by lime precipitation, the AMT-BIOCLAIM™ system acting as a polishing unit, or raw influent was directly treated by AMT-BIOCLAIM™. In both cases, excellent removal of lead was achieved from lead solutions varying from 0.01 mg Pb/L to 4.30 mg Pb/L (Table 5). This influent pH varied from 4.6 to 9.7, depending on whether or not lime was used. The variation of pH did not influence lead removal by the MRA. The effluent pH remained higher than pH 6.0. When upstream caustic precipitation was employed, the influent to the AMT-BIOCLAIM pilot plant contained 20% (W/V) of lime sludge. This did not cause plugging or deleteriously affect the AMT-BIOCLAIM™ process. No mechanical problems were encountered.

A second field test is in progress at a decorative plating facility with lead and zinc in the wastewater. The reactor was similar to the unit described above. In this plant, the wastewater is not treated prior to passing through the unit. Results, typical of this operation, are presented in Table 6. One problem was the complexing of the metals by detergent used for cleaning the products to be plated. This resulted in higher content of metals in the effluents. The problem was solved by changing to a detergent that had less ability to complex the lead and zinc. The system continues to function, removing the metals to meet regulatory standards. The MRA has now functioned for a period of four months, treating about 3000 gal/d, without granule change. The granule product has good metal recovery and stability characteristics in field conditions.

An important aspect of the AMT-BIOCLAIM™ system is the conversion of pollutant metals to a product of value. The AMT-BIOCLAIM™ technology removes metals from metal-laden MRA, resulting in:

- a product of value
- regenerated MRA, which can be recycled.

An important benefit is that users of AMT-BIOCLAIM™ technology are not burdened with the liability and cost of toxic sludge disposal.

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TABLES

Table 1 - Metals loading (mM/g) by the MRA

Metal	Solution (10 mg/L)	Concentration (100 mg/L)
Ag	0.25	0.85
Cd	1.95	0.94
Cu	2.20	2.41
Pb	3.19	2.94
Zn	2.03	2.11

Table 2 - Removal of copper and gold from a jewelry manufacturer's cyanide waste (pH 10.19, 4.49 mM Au/L)

Volume processed (L)	pH	Effluent Au (mM/L)	Removal (%)
1	8.2	0.001	>99
2	10.1	0.002	>99
3	10.2	0.48	89
4	10.2	1.67	63

Solids' analysis: 1.06 Au/g

Table 3 - Recovery of gold from a gold, drag-out wastewater (pH 4.08, 0.34 mM Au/L)

Volume processed (L)	pH	Effluent Au (mM/L x 10^{-3})	Removal (%)
0.35	3.08	<0.5	>99
1.0	3.72	13	96
2.0	3.60	1	99
3.0	3.70	0.5	99
4.0	3.70	2	99

Solids' analysis: 0.72 mM Au/g

Table 4 - Removal of silver from a photographic processing waste (fixer + developer, 2.27 mM Ag/L, pH 8.39)

Volume processed (L)	pH	Effluent Au (mM/L x 10^{-2})	Removal (%)
1	7.75	0.09	99
2	8.07	0.46	99
3	8.21	0.65	99
4	8.31	4.72	98
5	8.38	14.64	93

Solids' analysis: 0.87 mM Ag/g

Table 5 - Pilot-plant test for removal of lead from a secondary lead-refining plant effluent

Time (days)	Influent pH	Pb (mg/L)	Effluent pH	Pb (mg/L)	Removal (%)
1	n.d.	2.10	6.3	0.003	99.8
3	n.d.	0.33	n.d.	0.006	98.2
12	7.7	0.01	6.4	0.004	60.0
17	6.5	2.70	6.7	0.040	98.6
25	9.7	0.16	8.1	0.022	86.3
27	6.6	1.48	7.2	0.011	99.3
33	4.6	1.80	6.7	0.029	98.4
36	5.4	2.90	6.6	0.030	98.9
39	4.6	4.30	6.2	0.040	99.1

Table 6 - Pilot-plant test for removal of lead and zinc from a decorative plating shop

Time (days)	Influent (mg/L)	Lead effluent (mg/L)	Removal (%)	Influent (mg/L)	Zinc effluent (mg/L)	Removal (%)
0	2.50	0.10	96	2.00	0.19	90
14	6.15	0.12	98	2.34	0.02	99
20	0.90	0.10	89	1.88	0.02	99
25	0.30	0.10	67	0.85	0.02	98
46	1.95	0.23	88	0.47	0.14	70
46	10.20	1.03	90	3.50	0.24	93
63	4.53	0.31	93	0.61	0.15	75
M		0.35			0.12	
s.d.		0.48			0.12	
Number of samples		19			19	

SESSION III: PAPER 17

BIOTECHNOLOGY IN THE MINERAL INDUSTRY: AN ENGINEER'S PERSPECTIVE

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ABSTRACT

The mineral industry has recognized a genuine need for the development of bioprocesses that offer economic feasibility and operational simplicity. Rapid depletion of easily accessible, high-grade reserves and stringent environmental legislation will sustain this considerable interest in biotechnology for mineral processing. However, the enormous gap between the microbiological development and corresponding engineering efforts, which translate the research findings to commercial processes, is hindering the rapid growth of biotechnology in the mineral industry. An important area of development towards the commercialization of bioprocesses is the availability of engineered systems for carrying out the biochemical transformation at its optimum efficiency. The paper identifies engineering and process parameters that are critical to system development, and the engineering guidelines for bioprocesses. Some of the commercially available systems are discussed, with their relative advantages and potential for bioprocessing of mineral ores.

SESSION III: PRÉSENTATION 17

LA BIOTECHNOLOGIE DANS L'INDUSTRIE MINÉRAUX - ASPECTS TECHNIQUES

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RÉSUMÉ

L'industrie des minéraux a reconnu le besoin réel de développer des bioprocédés économiquement faisables et de fonctionnement simple. L'épuisement rapide de réserves à teneur élevée et facilement accessibles et la promulgation de lois strictes sur l'environnement continueront de susciter un intérêt considérable pour la biotechnologie. Par contre, il n'y a eu aucun effort coordonné d'ingénierie pour mettre en valeur la recherche microbiologique et en tirer des procédés commercialement viables. Un domaine important à développer pour atteindre ce but est la conception d'équipement permettant d'effectuer des transformations biochimiques dans un environnement contrôlé. Cet article traitera des procédés unitaires nécessaires et des problèmes associés à l'élaboration de directives d'ingénierie à partir du point de vue des fabricants d'équipement, ainsi que des solutions possibles. On examine aussi les avantages relatifs et les possibilités de la biotechnologie.

SESSION IV

OPEN SESSION

SESSION IV: PAPER 18

ASPECTS OF URANIUM BIOADSORPTION FROM PROCESS LIQUORS

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ABSTRACT

Of the biomasses tested, Rhizopus arrhizus, Streptomyces levoris, and mixed culture (sewage sludge) appear to have some promise for uranium recovery from process (bioleach) solutions. The experimental program included dynamic and equilibrium studies. Both Langmuir- and Freundlich-type isotherms adequately described equilibrium data. Kinetic expressions based upon the type of adsorption mechanism suitably modelled the time dependence of the adsorption.

Thermal inactivation and physiological age had only a marginal effect on the bioadsorption equilibrium. The increase of the adsorption temperature from 4°C to 35°C, however, markedly enhanced the bioadsorption (60-90% increase) by both viable and inactivated biomass. Besides the adsorption temperature, the sulphate concentration, the solution pH, and metal ion contaminants (Ni, Al, Zn, Cu, Co, Fe, Mn) have been identified as the primary parameters affecting the bioadsorption capacity. Of the metal ions tested, the ferric ion competed most effectively with the uranyl ion for the adsorption sites. As a consequence, the bioadsorption capacities in process solutions were significantly lower than in synthetic solutions of uranyl nitrate.

SESSION IV: PRÉSENTATION 18

CERTAINS ASPECTS DE LA BIOADSORPTION DE L'URANIUM CONTENU DANS LES LIQUIDES INDUSTRIELS

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RÉSUMÉ

Parmi les types de biomasse étudiés, Rhizopus arrhizus, Streptomyces levoris et des cultures mixtes (boue d'eaux usées) semblaient être quelque peu prometteuses pour récupérer l'uranium contenu dans les solutions industrielles (biolixiviation). Le programme expérimental comportait des études en conditions dynamiques et en conditions d'équilibre. Les isothermes de type Langmuir et de type Freundlich décrivent de façon satisfaisante les données à l'équilibre. Les expressions cinétiques basées sur le type de mécanisme d'absorption permettent de modéliser correctement la chronodépendance de l'adsorption.

L'inactivation thermique et l'âge physiologique n'ont qu'un effet marginal sur l'équilibre de la bioadsoption. Cependant, un accroissement de la température d'adsorption de 4°C à 35°C améliore nettement la bioadsorption (de 60 à 90 p. cent) par la biomasse viable et par la biomasse inactivée. Outre la température d'adsorption , on a identifié la concentration de sulfates, le pH de la solution et les ions métalliques (Ni, Al, Zn, Cu, Co, Fe, Mn) comme les principaux paramètres influant sur la capacité de bioadsorption. Parmi les ions métalliques étudiés, l'ion ferrique est celui qui fait beaucoup compétition à l'ion uranyle pour les sites d'adsorption. Par conséquent, la capacité de bioadsorption dans des solutions industrielles était bien inférieure à celle dans des solutions synthétiques de nitrate d'uranyle.

SESSION IV: PAPER 19

RETARDATION OF HEAVY METAL DISPERSION IN TAILINGS SEEPAGE WATERS FROM ELLIOT LAKE, VIA METAL UPTAKE BY ALGAE

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ABSTRACT

Examination of uranium mine-tailings waters (Elliot Lake district) revealed solute U at 30 to 600 ppb along with elevated concentrations of Fe, Al, Zn, Mn, Ti, Ni, Pb, Cr, Mo, Co, Th, Cu, and Zr at 10 to 1000 times average world river levels where the seepage pH is 2 to 3. Spectacular growths of Euglena sp. (algae) thriving in tailings waters contain an average 3×10^5 ppb U, and in addition 40-70% Fe by dry weight, as well as average Al 28 000 ppm, Sr 150, Ba 40, Zn 150, Mn 250, Ni 120, Pb 1600, Th 70, Cu 200. Concentration factors for Fe, Ba, Zn, Mn, Ti, V, Ni, Pb, Cr, Ag, Co, and Cu in algae referenced to average world river waters are $\geq 10^6$. Hence, natural cultures of acid-tolerant microorganisms act to extract toxic metals from tailings discharge, retarding their dispersion into the natural environment.

Abundant intracellular and cell wall microcrystalline lepidocrocite [γ-FeO(OH)] has been identified in an Euglena sp. by TEM and electron diffraction imagery. These results collectively endorse the premise that microorganisms mediate transfer of many solutes between the hydrosphere and sedimentary regime.

SESSION IV: PRÉSENTATION 19

L'ABSORPTION DE MÉTAUX PAR LES ALGUES: UN MOYEN DE RETARDER LA DISPERSION DES MÉTAUX LOURDS DANS LES EAUX D'INFILTRATION DES BASSINS À RÉSIDUS D'ELLIOT LAKE

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RÉSUMÉ

L'étude des eaux des bassins à résidus de minerais uranifères (district d'Elliot Lake) a révélé une concentration d'uranium dissous de 30 à 600 ppb (parties par milliard), ainsi que des concentrations élevées de Fe, Al, Zn, Mn, Ti, Ni, Pb, Cr, Mo, Co, Th, Cu et Zr de 10 à 1000 fois supérieures aux concentrations moyennes mesurées dans les cours d'eau du monde où le pH des eaux d'infiltration est de 2 à 3. L'algue Euglena sp., qui prolifère de façon spectaculaire dans les eaux des bassins à résidus, contient en moyenne 3×10^5 ppb d'U, ainsi que 40-70 % de fer (masse sèche) et des concentrations moyennes exprimées, en ppm, des métaux suivants: Al 28 000, Sr 150, Ba 40, Zn 150, Mn 250, Ni 120, Pb 1 600, Th 70 et Cu 200. Les facteurs de concentration du Fe, Ba, Zn, Mn, Ti, V, Ni, Pb, Cr, Ag, Co et Cu pour les algues, par comparaison aux valeurs moyennes dans les cours d'eau du monde, sont $\geq 10^6$. C'est pourquoi des cultures naturelles de micro-organismes tolérants les acides peuvent extraire des métaux toxiques contenus dans des eaux usées déversées et ainsi retarder la dispersion de ces métaux dans l'environnement naturel.

Chez une Euglena sp., on a identifié une importante quantité de lépidocrocite [γ-FeO(OH)] par MET et par imagerie par diffraction électronique. Tous ces résultats confirment les prémisses selon lesquelles les micro-organismes assurent le transport de l'hydrosphère vers les sédiments de nombreux produits dissous.

SESSION IV: PAPER 20

BIOMASS IMMOBILIZED ADSORPTIVE APPLICATIONS IN THE MINERALS INDUSTRY

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ABSTRACT

Inactive microbial biomass has been confirmed as a good adsorbent for various metal ions. Both the potential and the interest for the technical application of this property exist. The limiting factor, so far, has been the very fine particle size of the biomass. A proprietary technology has been developed at McMaster University that produces particles of immobilized biomass of desired particle size, particles with mechanical strength similar to that of activated carbon while maintaining the adsorptive properties of the biomass used. The paper provides information on the application of this new generation of adsorbents in the case of uranium and radium adsorption.

SESSION IV: PRÉSENTATION 20

UTILISATION DE BIOMASSE IMMOBILISÉE COMME ADSORBANT DANS L'INDUSTRIE DU TRAITEMENT DES MINÉRAUX

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RÉSUMÉ

On a établi que la biomasse microbienne inactive constitue un bon adsorbant pour divers ions métalliques. L'application technique de cette propriété possède un certain potentiel et suscite l'intérêt. La très faible taille des particules de biomasse constitue jusqu'ici le facteur limitatif. Une technique brevetée, mise au point à l'Université McMaster, permet d'obtenir des particules de biomasse immobilisée de taille voulue, possédant une résistance mécanique semblable à celle du charbon activé sans perte de propriétés d'adsorption. On fournira au cours de la présentation des informations sur l'application de cette nouvelle génération d'adsorbants à l'adsorption de l'uranium et du radium.