PROCEEDINGS OF THE NINTH ANNUAL GENERAL MEETING OF BIOMINET

August 26, 1992
Edmonton, Alberta

Edited by W.D Gould, D. W. Koren and S. Lord

COMPTE RENDU DE LA NEUVIÈME RÉUNION GÉNÉRALE ANNUELLE DE BIOMINET

26 août 1992
Edmonton (Alberta)

Révisé par W.D. Gould, D.W. Koren and S. Lord

Available in Canada through

Associated Bookstores
and other booksellers

or by mail from

Canadian Government Publishing Centre
Supply and Services Canada
Ottawa, Canada K1A 0S9

Catalogue No. M38-15/93-1E
ISBN 0-660-14961-3

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FOREWORD

BIOMINET presents in this volume the invited technical papers which were presented by members of BIOMINET at the Ninth Annual General Meeting on August 26, 1992. The meeting was held at the Westin Hotel in Edmonton in conjunction with the 31st Annual Conference of Metallurgists. The morning session consisted of a tour of the Alberta Research Council (ARC) Biotechnology Laboratory and the BIOMIRA Laboratories. The technical sessions were presented during the afternoon.

We would like to thank Mr. Julian Coward of Syncrude Research who organized the tours of ARC and BIOMIRA, Dr. Gerry Bolton of Sherritt Technologies who organized the meeting and Dr. Tom Jack of Nova Husky Research who chaired the afternoon technical sessions. We also appreciate the efforts of those who presented papers during the technical sessions.

COMPTE RENDU DE LA NEUVIÈME RÉUNION GÉNÉRALE ANNUELLE DE BIOMINET

AVANT- PROPOS

Dans ce volume sont publiés les articles techniques sollicités que les membres de BIOMINET ont présenté à la neuvième réunion générale annuelle tenue le 26 août 1992, à l'hôtel Westin d'Edmonton, conjointement avec la 31^{e} réunion annuelle de l'Institut canadien des Mines, de la Métallurgie et du Pétrole. La séance de la matinée comportait une visite au Laboratoire de technologie du Conseil de recherches de l'Alberta (Alberta Research Council, ARC), et aux laboratoires BIOMIRA. Les séances techniques ont eu lieu l'après-midi.

Nous tenons à remercier M. Julian Coward de la Syncrude Research qui a organisé les visites du laboratoire de l'ARC et des laboratoires BIOMIRA, M. (Dr.) Gerry Bolton de la Sherritt Technologies qui a organisé la réunion et M. (Dr.) Tom Jack de la Nova Husky Research qui a présidé les séances techniques de l'après-midi. Nous remercions aussi les personnes qui ont fait des exposés pendant les séances techniques.

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DEVELOPMENT OF THE BIOSULPHIDE PROCESS

D. Warkentin, P.Eng.
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Industrial Biotechnology

ABSTRACT

Environmental considerations are of increasing importance to industry as regulations become more stringent. For mining operations, the cost of effluent treatment can be a significant economic factor. Triton has applied biotechnology to the treatment of solutions containing metals and sulphates, such as acid mine wastes. The result is the Biosulphide process, combining a biological system, which utilizes anaerobic bacteria to reduce sulphate to sulphide and to generate alkalinity, with a chemical treatment system which makes use of these biological products to precipitate metals from solution and to adjust the pH. The process is described briefly and the advantages over other treatment methods, such as lime neutralization, are discussed. The theoretical basis of the process is briefly covered and potential applications are considered. The process is applicable in many situations, and detailed engineering and scale-up work is in progress to develop its potential.

LA MISE AU POINT DU PROCÉDÉ DES BIOSULFURES

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Triton Development Corporation
Industrial Biotechnology

RÉSUMÉ

Les considérations environnementales prennent une importance de plus en plus grande dans l'industrie à mesure que la réglementation est renforcée. Lors des opérations d'exploitation minière, le coût du traitement des effluents peut représenter un facteur économique non négligeable. La société Triton a appliqué la biotechnologie au traitement de solutions contenant des métaux et des sulfates, comme les eaux de mine acides. Elle a ainsi créé le procédé des biosulfures, qui allie un système biologique employant des bactéries anaérobies pour réduire les sulfates en sulfures et générer un degré d'alcalinité, à un traitement chimique employant ces produits biologiques pour précipiter les métaux à partir d'une solution et pour ajuster le pH. On décrit brièvement le procédé et ses avantages par rapport à d'autres méthodes de traitement, comme la neutralisation par la chaux. On traite brièvement de la base théorique du procédé et l'on examine les applications potentielles de celui-ci. Ce procédé est utilisable dans de nombreuses situations, et l'on effectue actuellement des travaux techniques détaillées et de mise à l'échelle, pour exploiter son potentiel.

SUMMARY

Triton Development Corporation has been investigating the application of biotechnology to the remediation of high sulphate waste streams such as acid mine drainage. Considerable progress has been made toward the development of biological sulphate reduction processes using sulphate reducing bacteria (SRB's) to convert sulphate to sulphide for metal precipitation. This work has provided the basis of the Biosulphide process, which is now being actively developed for commercial application.

The Biosulphide process integrates a biological stage and a chemical stage to adjust waste streams to discharge quality with maximum efficiency. The entire stream passes through a contacting stage where pH adjustment and the introduction of bacterially generated H_2S gas are used to precipitate metals from solution as sulphides. In cases where the sulphate content is not excessive, the solution can be discharged, with a fraction being diverted to an anaerobic biological reactor system. In the biological stage, sulphate is reduced to sulphide, which is stripped from solution by an inert carrier gas and transported back to the chemical treatment stage. A by-product of this biologically catalyzed reaction is the production of alkalinity in the form of carbonate, which can be recycled to the chemical stage for pH adjustment. The fraction of solution to be treated biologically will normally be controlled by the amount of H_2S required for sufficient metal removal, but a greater portion may be treated if total sulphate targets must be met or if H_2S is desired for other purposes, such as saleable sulphur co-products.

The important advantages of the Biosulphide process may be summarized in point form:

- Precipitation of metals as stable sulphide compounds, which have high densities and good settling characteristics, rather than as voluminous and unstable hydroxides or in combination with biomass. This eliminates the need for cementation and off-site disposal of sludges which contain toxic elements.

- Reduction of effective retention time by greatly reducing the amount of solution which must pass through the biological reactors.

- Potential recovery of valuable metals as sulphide concentrates.

- Ability to integrate the process with existing lime plants or wetlands to reduce capital costs and to provide long term operating cost reductions.

- Possibility of integration with other biological processes, such as bioleaching, to take advantage of synergistic relationships.

- Ability to produce excess H_2S, if desired, for production of saleable products, such as sulphur or NaHS, or for use elsewhere on site. One example would be selective metal precipitation in a Hydrometallurgical plant.

- Ability of biological systems to continue to adapt to conditions over a long period of time, giving continuing increases in process efficiency over the long term.

- Resilience of the mixed cultures of SRB's in the presence of system upsets, showing very fast recoveries from abnormal conditions.

- Ability to utilize readily available nutrient sources such as sewage, sugars, vegetable matter or combustion off-gases.

- Potential to treat waste streams containing thiosalts, gypsum, sulphites or sulphur dioxide in addition to those containing sulphates.

As these points indicate, the Biosulphide process has considerable potential which, with effective demonstration, will become a valuable alternative to more costly remediation measures for a wide range of contaminated industrial effluents.

INTRODUCTION

The Biosulphide process is an integrated biological/chemical process for the treatment of metal contaminated sulphate waste solutions. The key to the process is the reduction of sulphate to sulphide through the action of anaerobic sulphate reducing bacteria (SRB's) in a bio-reactor. Several researchers have studied these bacteria with the objective of utilizing their capacity to reduce sulphate for the treatment of various industrial effluents. Triton Development Corporation has pioneered the integration of biological sulphate reduction with chemical precipitation and neutralization processes. Triton Development is presently developing a highly applications-oriented development program to demonstrate the economics of the process, evaluate flowsheet alternatives and generate engineering, and scale-up data for large scale treatment plants.

The process has the potential for adaptation to a wide variety of applications, including acid mine waste remediation, thiosalt removal, bioleach effluent treatment, gypsum waste removal for the fertilizer and construction industries, stack-gas scrubber solution disposal, refinery effluent treatment, and the recovery of metals from waste dump seepage and recycle streams.

THE NEED FOR A TREATMENT ALTERNATIVE

There are many industrial effluents containing high levels of sulphates, thiosalts and dissolved metals. Often, treatment of these effluents represents a major expense to the source operations, so that lowering the cost of remediation would be an important economic gain for the companies involved. In the mining industry, the main concern is acid mine waste resulting from the oxidation of sulphide-containing waste rock, tailings and exposed mine openings. In some cases, dealing with these effluents represents a major factor in determining the economic viability of the operation. Often, these discharges remain a concern long after mining operations have been abandoned, and their treatment remains as an on-going cost.

Currently, the most common method of dealing with these wastes involves the use of lime to raise the pH and precipitate metals as hydroxides. There are several drawbacks to this treatment method, the most significant of which is the need to dispose of the resulting metal hydroxide and gypsum sludge. These sludges can be voluminous and the metals are subject to re-dissolution if exposed to a low pH solution at any time in the future. In some jurisdictions in the United States, such sludge is considered to be a hazardous waste when it contains relatively low levels of toxic metals such as cadmium, antimony, or mercury and it must be stabilized and stored at considerable expense. It is likely that future discharge guidelines will continue to become more stringent in all jurisdictions, with a corresponding increase in the costs of complying with environmental regulations.

While many methods are currently being investigated to reduce acid generation and to minimize its impact, these procedures are generally uncertain, and often cannot be applied to existing problem sites. There is a need for an economical method of dealing with these wastes as well as the likelihood of a future need to retreat unstable hydroxide sludge produced by some lime treatment plants.

The main economic advantage of the Biosulphide process arises from the low cost generation of H_2S, which can be used to remove metal contaminants from solution as metal sulphides. Sulphides are easily settled, have a high density, and are more chemically stable than hydroxides. Also, sulphide solubility is generally much less than that of the corresponding hydroxide, making it possible to meet more stringent discharge regulations at a lower pH. Finally, metal sulphides possess a range of precipitation characteristics, allowing separation of various metals either for the recovery of those with value or for the isolation of the most toxic elements.

Artificially created wetlands incorporating the use of SRB's are also being investigated for treating acidic mining effluents. While this may prove to be a successful approach for certain dilute solutions, the problem of the long term build-

up of metal precipitates makes it an unlikely method for more highly contaminated effluents. In some cases, the most economic scenario may involve partial treatment with the Biosulphide process, primarily for metal removal, followed by solution polishing in a passive wetland environment.

A relatively recent development in the mining industry has been the commercial application of biological oxidation in the processing of refractory gold ores. Effluents from bioleaching are highly acidic and highly contaminated with metals. Biosulphide treatment has a major potential application in the treatment of these effluents and in removing metals from internal process streams.

Other situations where there is a potential need for this process exist in the metals processing and refining industries; the treatment of waste solutions from wet scrubbers on smelter and power plant smoke stacks; and in the disposal of waste gypsum occurring primarily as a by-product of phosphate fertilizer production, especially in the southern U.S.

There is presently a need for the development of an economical process which focuses on the removal of metals and/or sulphate from waste water streams. The Biosulphide process offers an efficient and cost effective way to meet this challenge.

THEORETICAL ASPECTS

Biological Sulphate Reduction

Reduction of oxidized sulphur in solution occurs through the action of certain species of bacteria which, under anaerobic conditions, can utilize sulphate as an electron acceptor in their metabolism. The main genera of bacteria capable of sulphate reduction are *Desulphovibrio* and *Desulphotomaculum*. There are about 17 species identified in these two genera. Other Sulphate Reducing Bacteria (SRB's) are members of the genera *Desulphobacter, Desulphobacterium, Desulphobulbus, Desulphococcus, Desulphomonas, Desulphonema* and *Desulphurococcus.*

SRB's are found in a variety of anaerobic environments in nature such as marine sediments, wetlands, mud flats and standing water. They are also common in anaerobic digesters in sewage treatment plants.

SRB's can grow on a variety of substrates, ideally simple organic compounds and fermentation products such as lactate, pyruvate or citrate. Some species can grow autotrophically with CO_2 and H_2. In mixed cultures, SRB's normally exist in a symbiotic relationship with other bacterial species which can convert a wide variety of organic and inorganic substrates into compounds which can in turn be utilized by the SRB's. The nutrients which can be used to sustain sulphate reduction, therefore

include gases such as CO_2/H_2, producer gas or CO, as well as organic sources such as sewage sludge, molasses and other sugars, straw and other humic acid sources, sawdust and other ground cellulose, animal matter such as fishmeal, or pure organic compounds such as organic acids and alcohols. The end products of SRB metabolism are either CO_2 or acetate, depending on the species and the substrate.

The biochemical reactions carried out as a part of the sulphate reduction process can be simplified into the following equations. For systems utilizing an organic substrate, using lactate as an example:

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 4CH_3CH(OH)COOH \quad (1)$$

(fermentation to lactic acid)

$$2CH_3CH(OH)COO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2HCO_3^- + H_2S \quad (2)$$

(reduction of SO_4 to H_2S and oxidation of lactate to acetate, with alkalinity generation)

This may be generalized to:

$$2C + SO_4^{2-} + 2H_2O \rightarrow H_2S + 2HCO_3^- \quad (3)$$

For autotrophic systems using H_2 as an energy source (e.g. from producer gas), the net reaction may be represented as:

$$SO_4^{2-} + 4H_2 \rightarrow 2H_2O + H_2S + 2OH^- \quad (4)$$

The role of the bacteria in these reactions is to act as a catalyst through hydrogen or electron transport occurring as a part of the organism's metabolism.

Researchers studying SRB's have observed a wide variation in reduction rates, depending on such factors as reactor design, substrate used, temperature, and sulphide removal efficiency. A summary of some of these results is given in Table 1.

While all SRB's are anaerobic, most species can survive exposure to oxygen. No sulphate reduction will occur when oxygen is present because the bacteria utilize O_2 in preference to sulphate. They are also reasonably tolerant of pH changes, being most active in the pH range of 5.5 - 8.0, but surviving at pH levels well outside of this range. The optimum pH is 6.5 - 7.0 for most species. The optimum temperature is 30 - 35°C.

TABLE 1

Researcher	Medium	Reduction Rate g SO_4^{2-}/L•day
Middleton et.al.(1979)	Acetic acid	0.29
Middleton et.al.(1980)	Primary sewage sludge	2.25
Pipes (1960)	Waste activated sludge	1.20
Maree et.al.(1990)	Producer Gas	1.60
Rabolini (1971)	Sewage sludge	2.79
Burgess et.al.(1961)	Primary sewage sludge	4.50
Oleszkiewicz et.al.(1986)	Cheese whey	1.50
Maree (1989)	Molasses	6.40
Cork et.al.(1978)	Lactic acid	6.30
Hilton et.al.(1985)	Lactic acid	6.50
Barnes et.al.(1991)	Ethanol	1.92

SRB's tend to be tolerant of moderate concentrations of metals and sulphides in solution. Very high metal concentrations are prevented in active sulphate reducing environments through the precipitation of metal sulphides. Very high levels of sulphide ions in solution will inhibit SRB's so that any reactor design must incorporate adequate sulphide removal. Sulphide may be removed from solution as H_2S gas by stripping with an inert carrier gas such as nitrogen. This must be done at a pH below 7.0 as sulphide solubility is greatly increased at a higher pH. The H_2S gas may be used to remove metals from solution, or to generate elemental sulphur or other sulphur compounds.

Chemical Precipitation

Metal sulphide precipitation occurs when metal rich sulphate solutions are contacted with H_2S gas. Various metals can be precipitated selectively due to differing sulphides concentration requirements for metal sulphide formation. An example of a general equation for this reaction is:

$$M^{2+} + S^{2-} \rightarrow MS \quad (5)$$

(where M^{2+} represents a metal ion having a valence of 2+)

Commonly, the solution to be treated will be at a low pH, meaning that the solubility of sulphide will be low. In order to effectively remove all metals from solution, H_2S solubility must be increased by raising the solution pH or by increasing the partial pressure of H_2S in the system, since the solubility of sulphide increases substantially with relatively low increases in pressure. Catalysts may also be beneficial for increasing reaction rates. At a low pH, Hg, Ag, Cu and Bi are readily removed and Fe^{3+} is reduced to Fe^{2+} by oxidation of sulphide. At higher sulphide concentrations, Cd, Pb and Zn are easily removed. Co, Ni, Fe, Mn and other less common metals can also be removed under appropriate operating conditions.

The solubilities of these sulphides are invariably lower than those of the corresponding metal hydroxides and they will be less likely to redissolve if the pH should decrease. As^{3+} is also precipitated at low pH as As_2S, which is slightly soluble so that a residual As concentration will remain in solution. This amount would, however, be less than that remaining from lime treatment.

H_2S generated in the bio-reactors which is not required for metal removal may be used to form elemental sulphur by any one of several means, the most common being that of the Claus process:

$$2H_2S + SO_2 \rightarrow 3S^0 + 2H_2O, \quad (6)$$

or through the use of Fe^{3+} in solution:

$$2Fe^{3+} + S^{2-} \rightarrow S^0 + 2Fe^{2+}. \quad (7)$$

The excess H_2S could also be used to form other potentially valuable compounds; for example, NaHS:

$$2H_2S + Na_2CO_3 \rightarrow 2NaHS + H_2CO_3. \quad (8)$$

SULPHATE REDUCTION EXPERIENCE AT TRITON

Following is a summary of the work which has lead to development of the Biosulphide process:

A number of continuous 5 litre up-flow column reactor systems were constructed, using packed-beds for bacterial support. Populations of bacteria were established and adapted to the treatment of a wide variety of solutions. Feed types which have been tested successfully include several acid mine drainage solutions

having high metal concentrations and pH as low as 2.0, tailings decant solution containing high levels of thiosalts, neutral gold plant effluent containing high levels of sulphate and thiocyanate, acidic bio-heapleach effluent with very high iron and arsenic concentrations, and neutral calcium sulphate solutions.

With more extreme solutions, considerable adaptation was generally required, but high capacities for metal removal and pH buffering were eventually developed in every case. In some systems, levels of pH and metal content well beyond limits established by other researchers have been routinely treated. The populations have also proven to be highly resilient during system upsets, including the exposure to oxygen. Upset systems generally returned to normal reduction rates quickly once the problem had been corrected.

Laboratory scale results showed high reduction rates and effective removal of metals from solution. Successful operation was facilitated by efficient H_2S removal, which was achieved by solution recycle through external stripping towers sparged with N_2 gas.

In the fixed bed reactors, the bacteria adhered to a porous volcanic rock medium, preventing excessive washout. It is unlikely, however, that such fixed beds will provide adequate biomass concentrations for commercial application. For this reason much of the current work is geared toward the design of a more efficient bio-reactor for the biological sulphate reduction stage.

Stirred reactors have been investigated in several configurations with a mini pilot plant utilizing 50 litre reactors. While sulphate reduction has been established in such systems, lowered biomass stability has kept the performance below that seen with the fixed bed systems. As yet, few of the recently developed design improvements have been incorporated into the stirred tank systems for continuous testing.

As a part of process development, a number of alternate nutrient sources are being investigated. Initial tests were calculated entirely with molasses, but later work has examined producer gas, CO_2/H_2, CO, sewage sludge and sucrose as possible nutrients. At the same time, several medium types have been tested as alternatives to volcanic rock, including activated carbon, non-activated resin buffer beads, polyurethane foam and synthetic sponge.

Results from continuous laboratory pilot operations are shown in Tables 2-5, indicating a range of applications for general acid effluent treatment, selective metal removal (eg. Cu, Zn, Mn, Fe, As), thiosalt removal, and thiocyanate removal.

THE BIOSULPHIDE PROCESS

The design of a new process incorporating key aspects of the work with sulphate reducing bacteria was initiated to enhance the efficiency of sulphate reduction and to make it more flexible as a treatment process. The primary innovation was the separation of metal precipitation in the chemical phase from sulphate reduction in the biological phase. This innovation allows both processes to be operated under optimum conditions for achievement of the treatment objectives of any given application.

Separating the chemical and biological reactions gives the Biosulphide process a major advantage process over ordinary sulphate reduction or wetland treatment methods, since the metals will not be mixed with the resulting biomass.

In previous research, it was well established that it was necessary to remove hydrogen sulphide from solution to prevent inhibition of the SRB's. It was, however, important to retain enough sulphide to chemically precipitate metals from solution. This, together with the high metal content and low pH of the incoming raw feed, tended to reduce bacterial action, giving lowered reduction rates. The Biosulphide process avoids this problem by utilizing the H_2S stripped from the bio-reactors to treat the waste stream, removing metals from solution prior to biological treatment. The pH of the bio-reactor feed solution is also generally much higher after the chemical stage.

Much of the flexibility and efficiency of this process results from the fact that complete sulphate removal is seldom necessary. The primary treatment objectives are normally metal removal and pH adjustment. For many solutions this can be achieved through the biological reduction of a small fraction of the total sulphate, providing enough H_2S and alkalinity for the chemical treatment of the whole stream.

A generalized flowsheet for the Biosulphide process is shown in Figure 1. Briefly, the entire waste stream enters the chemical treatment stage, which consists of one or more mixing vessels and contactors for pH adjustment and metal sulphide precipitation, along with one or more solid/liquid separators for metal sulphide removal and possible recovery. The resulting metal free, neutral solution may be discharged after stripping of residual hydrogen sulphide or treated biologically to reduce sulphate to hydrogen sulphide. Normally, only enough solution will enter the biological phase to provide adequate H_2S for use in the chemical phase. H_2S is removed from the sulphate reduction reactors either by direct stripping, or by the recycle of solution to external stripping vessels where it is transferred to an inert carrier gas for transport to the chemical contactors.

Where highly acidic effluents are to be treated by this process, addition of a reagent such as lime may be the most cost effective method of pH adjustment.

Such a system would still possess the major benefit over conventional lime treatment, which lies in the formation of sulphides rather than hydroxides, giving large savings in sludge disposal costs. This point creates the possibility of integrating Biosulphide systems into existing lime plants, resulting in large savings in capital cost while still obtaining the benefits of sulphide precipitation.

Lime addition is not necessary in all cases, as the discharge from the bio-reactors will be buffered at a pH above 7.0 with high alkalinity in the form of carbonates. Recycle of this effluent, or precipitated $CaCO_3$, will be used to provide some, if not all, of the pH modification in the main waste stream for selective metals precipitation, thus reducing or eliminating the cost of lime addition.

Through control of pH and H_2S concentration, metals may be precipitated individually from solution, making possible the recovery of any contaminants having value. As well, elemental sulphur can easily be produced from H_2S when an excess is generated. There are also other potentially valuable sulphide products, such as NaHS, which could be produced if the economics were favourable.

The difference in effective solution retention time between a system carrying out complete sulphate reduction and one making use of the Biosulphide process is dramatic. Research work on sulphate reduction of whole streams has required retention times of 60 - 200 hours, depending on the conditions. However, the stoichiometric amount of hydrogen sulphide required to remove all necessary metals may be provided by reduction of as little as 10 - 20% of the sulphate in many acidic mine effluents. This results in a reduction in retention time by up to a factor of 10, since as little as 10% of the stream would need to be treated biologically. The chemical stage would have a retention time from a few minutes to 1 or 2 hours in the reaction vessels, depending on the nature of the contaminants and the contactor design. Furthermore, this decrease in bio-reactor retention time does not take into account improvements in efficiency to be expected from the benign nature of the chemically treated bio-reactor feed. As well, more effective bio-reactor designs and biomass development offer further sources of improved process efficiency.

Direction of Process Development

The work conducted thus far by Triton, and others, has clearly demonstrated that it is technically feasible to use SRB's to convert contaminated wastewater streams into environmentally benign discharge water. The next stage of development will require the generation of data which delineates the actual level of improvement gained with the Biosulphide process, and demonstrates its economic feasibility.

A factor which is crucial to process efficiency is the design of the anaerobic bio-reactors. Many conventional designs exist in the sewage treatment field, some of which may be adaptable to this process. A key goal of current activities is to

compare several reactor designs based on the extensive knowledge developed thus far regarding the unique requirements of these bacteria.

An important aspect of Biosulphide process competitiveness is the continued improvement and adaptation of mixed cultures to increase efficiency with differing feed solutions. An important advantage of any biological process is that continued research and adaptation will yield ongoing increases in the efficiency and resilience of the biomass through selective growth and adaptation.

Integration of this system with one or more other treatment steps may improve the economics in some instances. Use of the process to remove metals at existing lime plants would represent a much lower capital outlay than replacement with a completely new facility. Wetlands could also have a role for solution polishing after metals have been removed, avoiding the problems of metal build-up within the wetland area.

One possible scenario could see discharge of biomass from an operating Biosulphide system being used in the build-up of an artificial wetland over a number of years. After mining operations had ceased and the metal content of the waste stream began to decline, the Biosulphide operation could be phased out, leaving a well established wetland as the long term treatment method.

CONCLUSION

The key innovation of the Biosulphide process is the two stage, integrated chemical/biological treatment system. There are a number of potential benefits which arise from such a design and the process aims to take full advantage of these.

Of primary importance is the elimination of hydroxide and gypsum sludge. In any conventional neutralization plant the sludge represents a serious long term concern, especially when highly toxic metals such as mercury or cadmium are present. Production of low volume sulphide sludge is a major advantage of the Biosulphide process. The separation of metal sulphides from the biological sludge is also an important advantage over previous SRB processes, including wetlands treatment. This innovation allows recovery of valuable metal co-products, reduced biological retention time, greater process flexibility, and improved process control. Excess H_2S generated can be converted to saleable sulphur compounds.

The potential for this process is clearly not limited to the treatment of AMD. Thiosalts, for example, are more easily treated than sulphates. As well, integrating the process with a bioleaching system or with biological cyanide/thiocyanate

degradation can yield significant synergistic effects which will further contribute to overall economics.

The flexibility and the synergistic benefits resulting from its innovative design serve to make the Biosulphide process the most realistic waste treatment alternative for many applications. With an intensive program of research and development, Triton is endeavouring to make the process available as an important tool for environmental control within a short time period while focusing on practical applications and engineering aspects of the process.

Figure 1

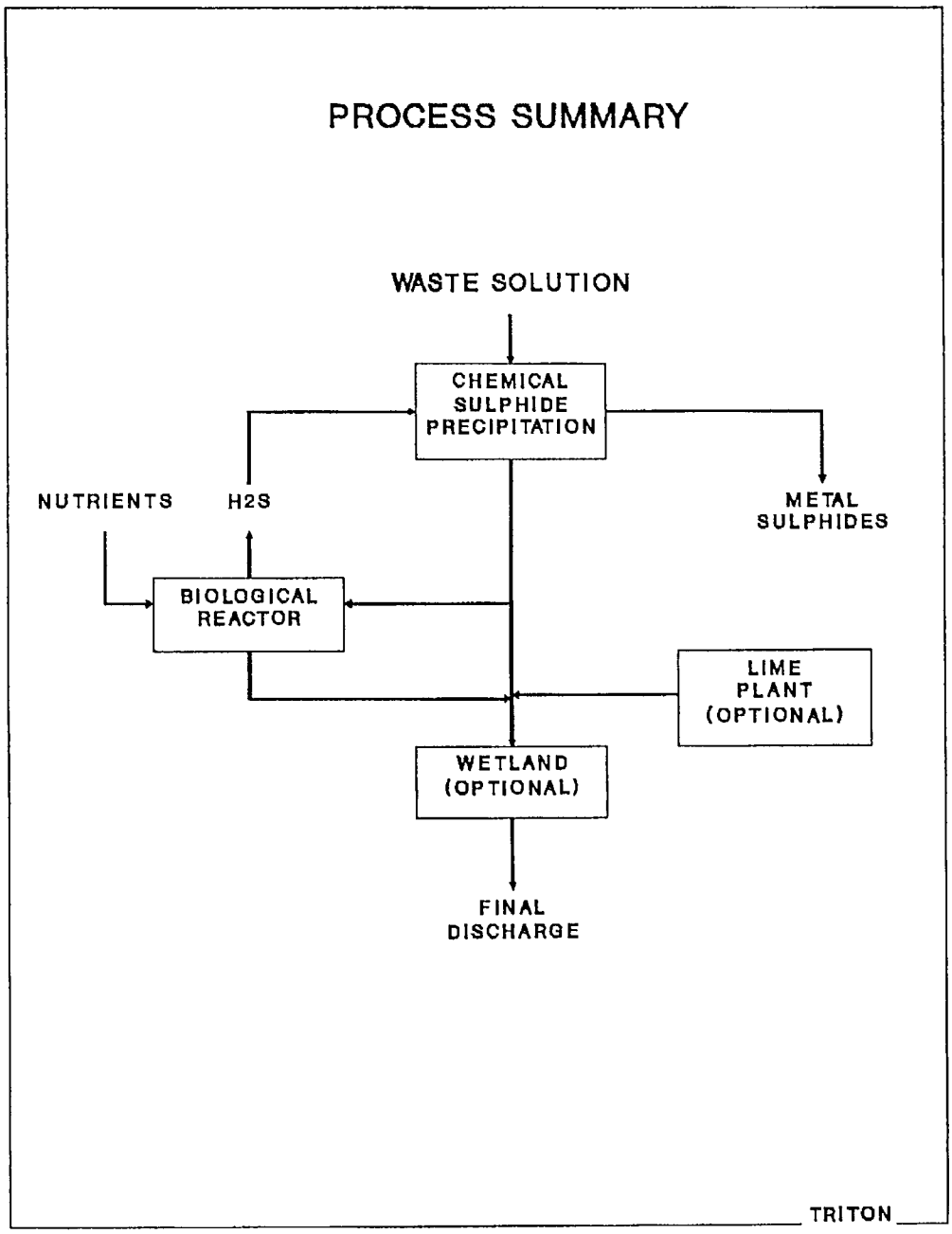
PROCESS SUMMARY
WASTE SOLUTION
CHEMICAL SULPHIDE PRECIPITATION
NUTRIENTS
H2S
METAL SULPHIDES
BIOLOGICAL REACTOR
LIME PLANT (OPTIONAL)
WETLAND (OPTIONAL)
FINAL DISCHARGE
TRITON

TABLE 2

Parameter	Acid Mine Drainage		
	Feed	Discharge	Removal (%)
pH	4.0	7.94	-
SO_4 (mg/l)	7250	60	99.2
Ag (mg/l)	0.06	0.004	93.3
As (mg/l)	2.71	0.77	71.5
Bi (mg/l)	1.33	0.74	44.6
Cd (mg/l)	0.09	0.004	95.4
Co (mg/l)	2.21	0.07	96.7
Cu (mg/l)	0.06	N/D	100.0
Fe (mg/l)	20.50	0.32	98.4
Mn (mg/l)	33.8	2.61	92.3
Mo (mg/l)	0.57	0.18	69.1
Ni (mg/l)	0.10	0.04	66.0
Pb (mg/l)	0.96	0.31	67.9
Sb (mg/l)	2.88	0.63	78.1
Zn (mg/l)	132.0	0.03	100.0

N/D = Not Detected

TABLE 3

	Abandoned Mine Drainage		
Parameter	Feed	Discharge	Removal (%)
pH	2.6	7.2	-
SO_4 (mg/l)	800	10	98.8
As (mg/l)	0.24	0.20	16.7
Bi (mg/l)	0.11	0.08	27.3
Cd (mg/l)	0.79	N/D	100.0
Co (mg/l)	0.17	0.01	94.1
Cu (mg/l)	5.24	0.05	99.0
Fe (mg/l)	3.72	0.15	96.0
Mn (mg/l)	65.2	0.48	99.3
Mo (mg/l)	0.19	0.08	57.9
Ni (mg/l)	0.13	0.03	76.9
Pb (mg/l)	0.45	0.16	64.4
Sb (mg/l)	0.72	0.24	66.7
Zn (mg/l)	127.0	N/D	100.0

N/D = Not Detected

TABLE 4A

Cyanide Tailings Discharge

Parameter	Feed	Discharge	Removal (%)
pH	6.1	7.8	-
SO_4 (mg/l)	1590	10	99.4
SCN (mg/l)	870	N/D	100.0

TABLE 4B

Flotation Tailings Discharge

Parameter	Feed	Discharge	Removal (%)
pH	3.9	8.3	-
SO_4 (mg/l)	2170	10	99.5
S_2O	360	8	97.8
S_2O_3 (mg/l)	374	23	93.9

TABLE 5

	Bio-Heapleach Effluent		
Parameter	Feed	Discharge	Removal (%)
pH	2.0	7.6	-
SO_4 (mg/l)	2500	10	99.6
Ag (mg/l)	0.08	N/D	100.0
As (mg/l)	51.6	0.48	99.1
Bi (mg/l)	0.96	0.31	67.7
Cd (mg/l)	0.026	N/D	100.0
Co (mg/l)	0.75	0.01	98.7
Cu (mg/l)	10.0	N/D	100.0
Fe (mg/l)	736.0	0.22	100.0
Mn (mg/l)	0.18	N/D	100.0
Mo (mg/l)	0.26	0.05	80.8
Ni (mg/l)	2.88	0.01	99.7
Pb (mg/l)	1.24	0.20	83.9
Sb (mg/l)	0.91	0.22	75.8
Zn (mg/l)	1.12	N/D	100.0

N/D = Not Detected

DEVELOPMENT OF AN INDUSTRIAL PROCESS FOR OXALATE BIODEGRADATION

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ABSTRACT

A common problem in the processing of bauxite by the Bayer process for the production of alumina, is the generation and accumulation of oxalate ions. A medium sized Bayer processing plant may generate one to ten tonnes of oxalate daily, thus an industrial oxalate biodegradation process was investigated. Enrichment of bacteria from the rhizosphere of *Rheum rhaponticum* and *Dieffenbachia picta* using a mineral salts oxalate medium, resulted in the isolation of an oxalate degrading bacterium B-1, which degrades 4,500 ppm and 16,000 ppm oxalate in 18 and 50 hours respectively. B-1 could tolerate up to 8 g/L sodium ion, a pH range between 6 and 9 and temperature between 5 and 40°C. Data on B-1 suggests that it belongs to the genus *Pseudomonas*.

A rotating biological contactor (RBC) was employed using the oxalate degrading bacterium in a mixed culture as the inoculum. The RBC was a 12 L RBC with a disk surface area of $3.7m^2$ and operated with a residence time of 5 hours degrading 5,000 ppm oxalate ion from both synthetic medium and industrial effluent medium. Biomass on the solid support medium was unaffected by temporary exposure to high concentrations of oxalate.

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MISE AU POINT D'UN PROCÉDÉ INDUSTRIEL DE BIODÉGRADATION DES OXALATES

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RÉSUMÉ

Un problème courant lors du traitement de la bauxite par le procédé Bayer de production de l'alumine, est la formation et l'accumulation d'ions oxalate. Une usine de traitement Bayer de taille moyenne peut générer quotidiennement une à dix tonnes métriques d'oxalate, par conséquent on a examiné un processus industriel de biodégradation des oxalates. L'enrichissement en bactéries prélevées dans la rhizosphère de *Rheum rhaponticum* et *Dieffenbachia picta* dans un milieu à oxalates minéraux, a permis d'isoler une bactérie capable de dégrader les oxalates, la bactérie B-1, qui peut décomposer 4 500 ppm et 16 000 ppm d'oxalate en 18 et 50 heures respectivement. B-1 pourrait tolérer jusqu'à 8 g/l d'ion sodium, un intervalle de pH compris entre 6 et 9 et un intervalle de températures compris entre 5 et 40°C. Les données relatives à B-1 suggèrent que celle-ci appartient au genre *Pseudomonas*.

On a employé un contacteur biologique rotatif (RBC), en mettant la bactérie capable de dégrader l'oxalate dans un milieu de culture mixte servant d'inoculum. Le contacteur RBC a une capacité de 12 l et une surface de disque de 3,7 m^2, et avec un temps de séjour de 5 heures, et permet de dégrader 5 000 ppm d'ion oxalate à la fois dans un milieu synthétique et dans un milieu composé d'effluent industriel. La biomasse présente sur le substrat solide n'a pas été modifiée par une exposition temporaire à des concentrations élevées d'oxalate.

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INTRODUCTION

Oxalate is a ubiquitous chemical found throughout the animal and plant kingdom (Kingsbury, 1964; Stephens, 1980; Libert and Franseschi, 1987). Because animals do not produce oxalate, it is reasonable to assume that most of the oxalate found in animals comes from the ingestion of plant material. Oxalate ingested can be poisonous, causing severe gastroenteritis with vomiting, diarrhea and melena. In severe cases it can cause convulsions, coma and even death as a result of cardiovascular collapse (Merck Index, 1987).

Some industrial processes, such as the Bayer process used for the production of alumina, produce a large quantity of oxalates as a by-product. Since oxalate is an environmental hazard, a means of disposing of the chemical both efficiently and economically is required. A method to degrade oxalate would be to isolate oxalate degrading organisms from the root rhizosphere of oxalate producing plants and use these microorganisms in a continuous process. A number of organisms are known to utilize oxalic acid or oxalate as a sole carbon source. Aerobic oxalate degradation can be carried out by certain bacteria such as *Vibrio oxaliticus* (Bhat and Barker, 1948), *Streptomyces* sp. (Khambata and Bhat, 1954), *Pseudomonas oxalaticus* (Quayle and Keech, 1959; Blackmore *et al.*, 1968; Dijkhuizen *et al.*, 1977), *Thiobacillus novellus* (Chandra and Shethna, 1977), a *Spirillum* sp. (Van Der Kooij and Hijnen, 1984), and several *Alcaligenes* and *Pseudomonad* species (Khambata and Bhat, 1954; Chandra and Shethna, 1975b; Friedrich *et al.*, 1979), and a number of fungi (Vaisey et al, 1961; Emiliani and Bekes, 1964; Lillehoj and Smith, 1965; McLung, 1954). In addition anaerobic oxalate degradation has been observed in the rumen (Dawson *et al.*, 1980) and in lake sediments (Smith and Oremland, 1983; Dehning and Schink, 1989).

In order to utilize bacteria to degrade oxalate in a continuous process an apparatus, such as a rotating biological contactor (RBC), would be used. An RBC is a half cylindrical trough in which disks rotate half submersed in the liquid medium. The disks provide a surface area with which the bacteria can adhere, as well as providing the bacteria with air and nutrients for aerobic growth. The trough is divided into sections so that the medium can transverse the disks and move through the trough in a serpentine fashion. An RBC was first described in 1929, however it was not until 1960 that the units became commercially available for municipal waste treatment (Pike *et al.*, 1982). Today, RBC's are being used for municipal waste treatment as well as industrial wastes produced from food processing, leather manufacture, pulp and paper, refinery, petrochemical and textile industries (Borghei, 1981; Lee and Mueller, 1975; Mudder and Whitlock, 1984; Anderson and Duarte, 1981).

The aim of this research is to isolate oxalate degrading bacteria from the environment, and using these bacteria, develop an efficient and economical biological oxalate degrading process using an RBC.

MATERIALS AND METHODS

All of the chemicals used were of reagent grade except for crude sodium oxalate obtained from Alcan International Ltd.. Soil samples were obtained from three different sources: (1) Rhubarb soil from Embrum, Ontario; (2) Dieffenbachia soil from Carleton University, Ottawa, Ontario; and (3) Dieffenbachia soil from Energy, Mines and Resources, Ottawa, Ontario.

Medium and Isolation Procedure

An oxalate medium (Oxa-1) was used to isolate and maintain the oxalate degrading bacteria. The medium contained the following constituents in g/L: $NaH_2PO_4.H_2O$, 0.5; $MgSO_4.7H_2O$, 0.1; $(NH_4)_2SO_4.7H_2O$, 0.5; $FeSO_4.7H_2O$, 0.05; Yeast Extract 0.1 and $(COOH)_2.2H_2O$, 5.0. The pH was adjusted to 7.0 with NaOH. One gram of each soil type was inoculated into 100 mL of the Oxa-1 medium and incubated at 28°C and 150 rpm for 48 hours. After 48 hours the cultures obtained were subcultured into fresh Oxa-1 medium using a 5% inoculum. This was performed two more times. Growth of bacteria was determined by measuring the turbidity of the medium.

To isolate the individual organisms Oxa-1 medium containing 1.5% agar was used. After the third subculture in broth medium, 0.1 mL of culture from a serial dilution series was used to inoculate Oxa-1 agar plates. The plates were incubated for 96 hours at 28°C. Each of the bacterial isolates were subcultured onto Oxa-1 agar medium (with and without oxalate added) to ensure the purity of the culture and to make sure that the isolates were not scavenging trace carbon sources in the medium.

Oxalate Assay (Indole Method)

The assay is a modification of that outlined in Snell and Snell (1961). The sample was dissolved in 0.3N H_2SO_4 such that the sample contained 0.2 - 2.0 mg of oxalic acid (2 mL of sample was added to 1 mL of 1N H_2SO_4). Two mL of 0.1% indole solution in concentrated sulphuric acid was pipetted down the side of the tube to minimize heat development. It was heated at 80-90°C for 45 minutes, cooled, and the absorbance read at 575 nm against a reagent blank. To increase the sensitivity of the assay, readings were taken at 560 nm.

Identification of the Individual Isolates

Each of the isolates were subjected to the Gram stain (Gugol Gram stain kit) and oxidase test. If the organism was Gram negative and oxidase positive the isolate was inoculated into Oxiferm tubes (Hoffman-LaRoche Diagnostics Inc.). If the organism was Gram negative and oxidase negative the isolate was inoculated into Enterotubes (Hoffman-LaRoche Diagnostics Inc.). Each of the tubes were incubated at 37°C for 48 hours and then identified on the basis of their biochemical reactions.

If an organism could not be identified in our laboratory, it was sent to the Microbiology section, Health and Welfare, Canada, and if that proved to be

unsuccessful to the American Type Culture Collection, Maryland, USA. Transmission electron microscopy (TEM) was also used in the identification of unknown organisms. TEM was performed at The Laboratory Center for Disease Control, Health and Welfare, Canada and in Dr. J.W. Costerton's laboratory, University of Calgary, Calgary, Alberta.

Kinetics of Oxalate Degradation

All of the mixed cultures from the various soil types and the pure isolates from two of these cultures (C and F) were assayed for their ability to degrade oxalate in Oxa-1 broth using the oxalate assay procedure. Each culture was inoculated into Oxa-1 broth in triplicate using a 5% inoculum and over time monitored for oxalate degradation and growth (O.D. 540 nm on a Varian DMS 200 UV-Vis spectrophotometer). The cultures were incubated at 28°C and 150 rpm.

Physiological, Biochemical and Toxicity Tests of Isolate B-1 Isolated from Rhubarb Soil

Isolate B-1 was grown on industrial sodium oxalate medium (see section "Industrial Sodium Oxalate used in RBC" for details of medium) and Oxa-1 plates for 48 hours at 28°C and sent to the Laboratory Center for Disease Control, Health and Welfare, Canada, and to the American Type Culture Collection, Maryland, USA.

Tolerance of Isolate B-1 to High Levels of Oxalate

Oxa-1 medium was prepared such that duplicate flasks contained concentrations of 10000, 12000, 14000, 16000, 18000 and 20000 ppm oxalate ion. Each flask was inoculated with a 5% inoculum from a mid exponential phase culture of B-1 and incubated at 28°C and 150 rpm. Oxalate concentrations were determined at regular intervals over a period of time.

Sodium analysis of Oxalic Acid Standards

Oxalic acid standards were prepared such that each volumetric flask contained between 10000 to 20000 ppm oxalate ion adjusted to pH 7.0 with NaOH. Each sample was then analyzed for the sodium concentration using Atomic Absorption Spectrophotometry.

Operation of an RBC using Mixed Culture F from Rhubarb Soil

The RBC, manufactured at CANMET, Energy, Mines and Resources, Ottawa, Ontario, Canada has a volume of 12 liter and a disk surface area of 3.7 m^2. The dimensions are 75 cm x 20 cm x 12 cm (L x W x H) with the RBC divided into three 24 cm long chambers. It was started up in batch mode for 14 days at room temperature (23°C) containing 12 L of Oxa-1 medium and inoculated with 200 mL of mixed culture F (1 x 10^8 cells/mL). Half of the Oxa-1 medium was removed every second day and fresh medium added to replenish the degraded oxalate. Fresh Oxa-1 medium was then added to the RBC at a flow rate of 4.0 mL/min. (50 hour residence time) to flush out the spent medium and to prevent evaporative losses in

the RBC. The RBC was run for 2-4 weeks in this manner until a film of biomass developed on the disks. Once the biomass had developed, the residence time was slowly reduced to 4-5 hours ensuring that complete degradation of oxalate was observed in the effluent.

Industrial Sodium Oxalate used in the RBC

Industrial sodium oxalate was provided by Alcan International Ltd.. Oxa-1 medium was modified due to the high concentration of sodium in the sodium oxalate cake. The medium was modified to contain the following in g/L: $NH_4H_2PO_4$, 0.5; $MgSO_4.7H_2O$, 0.1; Yeast Extract, 0.1; $FeSO_4.7H_2O$, 0.5; and $(COONa)_2.xH_2O$, 10-12. The pH was adjusted to 7.0 with NaOH. An initial shake flask study using the sodium oxalate medium (Na-Ox) with an inoculum from the RBC running with Oxa-1 medium was performed at 28°C and 150 rpm to ensure that the medium would have no detrimental effects on the oxalate degrading culture. Since the oxalate degradation rate was not affected by the Na-Ox medium in shake flask, the new medium was used in the RBC.

Tolerance of Culture F from the RBC to Chemical and Physical Factors

Duplicate sets of 250 mL flasks were set up for each experiment: sodium ion tolerance, pH tolerance, oxalate ion tolerance and temperature tolerance. The sodium ion concentrations were made up to 0, 4, 8 and 12 g/L using $NaHCO_3$ as a sodium source in Oxa-1 medium. A pH range between 2 to 12 was used in Na-Ox medium. Oxalate concentrations between 8 to 24 g/L were set up using Na-Ox medium and a temperature range between 5 and 45 °C was used for culture F in Na-Ox medium. All the flasks except the temperature experiment flasks were incubated at 28°C and 150 rpm.

Shock Loading of the RBC with Sodium Oxalate

When the RBC was running efficiently with 8 g/L Na-Ox (2500 ppm oxalate ion), a 24 g/L sodium oxalate (13130 ppm) shock loading experiment was performed over a 24 hour period. The residence time of medium running through the RBC was 5.5 hours.

Minimization of Sodium Oxalate Medium Constituents

Na-Ox medium was prepared such that the four different individual medium constituents were varied separately (for example, sodium oxalate medium was prepared with three different concentrations of ferrous sulfate 0.05, 0.025 and 0.0 g/L). The flasks were inoculated with a 5% inoculum of the mixed culture in the RBC and the flasks were incubated at 28°C and 150 rpm. Oxalate concentrations were determined at various time intervals.

Once the optimal degradation rates versus lowest constituent concentration were obtained from above, all of the medium constituents were reduced in one experiment and degradation parameters were compared to those observed with the

regular sodium oxalate medium. Again 250 mL flasks were inoculated with a 5% inoculum from the RBC and the flasks incubated at 28°C and 150 rpm.

The final experiment involved running the revised medium through the RBC at a residence time of 5 hours and comparing the degradation parameters to those obtained with the regular medium.

RESULTS AND DISCUSSION

Soil samples were obtained from the rhizosphere of Rhubarb and Dieffenbachia, which are plants known to contain oxalate (Kingsbury, 1964; Stephens, 1980; Libert and Franseschi, 1987). Since the plants contain oxalate , one would have to assume that microorganisms are present either on the plant or in the soil that are capable of degrading oxalate. Enrichment culture techniques have been previously used to isolate oxalate degrading bacteria from soil (Bhat and Barker, 1948; Jayasuriya, 1955; Chandra and Shethna, 1975a). In order to enrich for oxalate degrading microorganisms, a simple low cost medium was developed, Oxa-1, from a complex medium used by Jayasuriya (1955) and Chandra and Shethna (1975a).

Out of six mixed cultures tested for oxalate degradation, only two could degrade oxalate; mixed culture C from Dieffenbachia soil and mixed culture F from Rhubarb soil (Figure 1). Mixed culture C took 48 hours to degrade all of the oxalate where as mixed culture F degraded all of the oxalate in 22 hours. Since mixed culture F had the best degradation rate, it was used to observe the degradation of oxalate over time versed growth (Figure 2). This same culture was used to inoculate the RBC.

Isolation and Identification of Pure Cultures from Mixed Cultures C and F

In the initial isolation of pure isolates from mixed cultures C and F four bacteria were identified, *Pseudomonas stutzeri*, *Moraxella* sp., *Citrobacter freundii* and *Pseudomonas cepacia* using the Oxiferm and Enterotubes. Once the RBC was inoculated with mixed culture F subsequent isolations were performed in 5 month intervals over 15 months. The bacteria that were isolated were *Acinetobacter lwoffii*, *Pseudomonas cepacia*, *Micrococcus* sp, *Flavobacterium odoratum*, *Citrobacter amalonaticus*, *Aeromonas hydrophilia*, *Enterobacter agglomerans*, *Pseudomonas fluorescens* and an unidentified B-1. All of the isolates were tested for their ability to degrade oxalate however the only organism observed to degrade oxalate was the unidentified B-1 (Figure 3). The other organisms must be transient contaminants surviving on the dead biomass of B-1.

TEM thin sections and negative stain of B-1 clearly revealed that it was a Gram negative rod with a single polar flagellum characteristic of *Pseudomonads* (Data not shown). The results obtained from similar tests performed at ATCC and Health and Welfare, Canada are identical, although both institutes had difficulty identifying B-1 on the basis of conventional biochemical and nutritional tests. The

biochemical tests at Health and Welfare, Canada showed the organism to resemble a Pseudomonad. Oxalate degrading organisms similar to B-1 have been isolated before, Jayasuriya (1955) and Chandra and Shethna (1975a) isolated *Pseudomonas* OD1 and *Pseudomonas* YOx respectively, which were rods (0.5 x 1.5 um) with a single polar flagellum which produced a yellow insoluble pigment.

ATCC's biochemical and physiological tests showed that B-1 resembled a *Xylophilus ampelina*, however the growth factors and nutritional spectra are quite different. ATCC stated that *X. ampelina* forms a subbranch of *Pseudomonas acidovorans* based on rRNA (ribosomal ribonucleic acid). However, the nomenclature of strains in this rRNA complex is presently being challenged. The fatty acid analysis performed by ATCC was also equivocal because of the slow growth of the strain (Table 1).

Toxicity tests were performed at Health and Welfare, Canada. The tests were performed using 24 and 48 hour filtrates of cultures from both nutrient broth and oxalate medium. The tissue culture system included Vero (monkey kidney cells), HeLa (human cervical carcinoma cells), and CHO (chinese hamster ovary cells). These cell lines respond to a variety of microbial enterotoxins and cytotoxins. Suckling mouse tests were performed using 3 day old CF1 mice. Weak, non-specific cytoxicity was observed in all cell lines with the 48 hour oxalate filtrate, however these effects were not observed in the nutrient broth filtrates. Intestinal weight/remaining body weight ratios (IB/BW) in the suckling mice after 4 hours were considered negative for microbial enterotoxins reactive in this bioassay.

Oxalate Degradation by Isolate B-1

Complete degradation of oxalate was observed within 18 hours after inoculation with isolate B-1 (Figure 3). This curve is comparable to the kinetics study performed with mixed culture F suggesting that B-1 is the organism responsible for oxalate degradation in the mixed culture.

Tolerance of B-1 to High Levels of Oxalate and Sodium Analysis of Oxalic Acid Standards

Isolate B-1 was observed to completely degrade up to 16000 ppm oxalate ion in 50 hours. The highest oxalate ion concentration reported to have been degraded in the literature was 9500 ppm by a *Pseudomonas* KOx (Chandra and Shethna, 1975a). Isolate B-1 did not degrade 18000 and 20000 ppm oxalate ion to completion, the remaining oxalate ion concentrations were 2000 and 4000 ppm respectively (Figure 4). The organism could partially degrade the higher levels of oxalate therefore something other than oxalate must have been preventing the organism from completely degrading the oxalate. It was determined that on adding 16000 ppm oxalate, the sodium levels exceed 8 g/L as a result of the sodium hydroxide required to neutralize the medium (Figure 5). It was observed previously that B-1 could only tolerate up to 8 g/L sodium ion.

Operation of the RBC using Oxalic Acid

When the RBC was started there was not much growth on the disks until the RBC was put into the continuous phase. As the flow rate was increased (residence time decreased), growth increased starting from the disks closest to the influent port. Samples from each of the chambers revealed that at low flow rates, most of the oxalate degradation was taking place in the first chamber, however as the flow rate was increased, bacterial growth on the disks was evenly distributed throughout the RBC and thus oxalate degradation was occurring in all of the chambers (Figure 6). The flow rate was increased to 40 mL/min. (5 hour residence time) resulting in >95% reduction in the oxalate concentration (Figure 7).

Operation of the RBC using Sodium Oxalate

Shake Flask Study using Sodium Oxalate Prior to use in the RBC

Sodium oxalate cake contained 21% sodium and thus substitution of sodium oxalate for oxalic acid in the Oxa-1 medium would increase the sodium ion concentration. To minimize the sodium ion concentration, sodium phosphate and ammonium sulphate were replaced with ammonium phosphate. It was also calculated that to maintain the same concentration of oxalate ion as in Oxa-1 medium, approximately 7.15 g/L sodium oxalate had to be used. The composition of the sodium oxalate cake was inconsistent, therefore the oxalate concentration varied from experiment to experiment due to this industrial substrate.

A shake flask study was set up to test the Na-Ox medium on bacteria from the RBC. In the first set of flasks, 94% of the oxalate was degraded in 24 hours. On subculturing to a second set of flasks, 98% of the oxalate was degraded in 24 hours. This data indicates that the new medium could be used in the RBC without detrimental effects on the bacteria.

Operation of the RBC using Sodium Oxalate Medium

Sodium oxalate medium (Na-OH) was continuously fed into the RBC with a residence time of 5 hours shortly after optimal degradation was achieved using Oxa-1 medium. The addition of industrial sodium oxalate resulted in a heavier film of biomass on the disks. Better growth was observed in the RBC with Na-Ox medium than with the Oxa-1 medium. The reason for the better growth may be the presence of humates in the sodium oxalate cake. This medium also allowed a unique matrix of silicates, polysaccharides, iron and biomass to form on the disks. From Figure 8, complete oxalate degradation occurs within a 5 hour residence time. Growth was so great with the sodium oxalate medium that biomass sloughed off and clogged the bottom of the trough. This was rectified by increasing the disk rotation speed from 0.6 rpm to 3 rpm which produced more turbulence, allowing the wash out of suspended biomass.

Tolerance of Mixed Culture F from the RBC to Chemical and Physical Factors

In a number of shake flask studies, the mixed culture in the RBC running with Na-Ox medium was tested for its tolerance to sodium ion, pH, sodium oxalate and temperature. The results indicated that in separate studies, the culture could

tolerate up to 8 g/L sodium ion, a pH range between 6 and 9, 16 g/L (~5000 ppm) sodium oxalate and temperatures between 5 and 40°C. The pH and temperature range are comparable to oxalate degrading bacteria studied by Bhat and Barker (1948), Jayasuriya (1955) and Chandra and Shethna (1975a). Also the sodium tolerance is normal for a non-halophile (Pelczar and Chan, 1981). It is important in the operation of the RBC to maintain optimum conditions to achieve optimum degradation rates. These results indicate the limits to which parameters can be altered, however the closer to the boundary one gets, the lower the degradation rate.

Shock Loading of the RBC with Sodium Oxalate

The shock loading experiment in the RBC revealed that the mixed culture could not only tolerate high levels of sodium oxalate (13500 ppm oxalate ion), but also degrade approximately 66% of the oxalate in the process (Figure 9). After shock loading, regular sodium oxalate medium was pumped through the RBC with almost complete degradation of the oxalate. No harmful effects were observed on the biomass.

Minimization of Sodium Oxalate Medium Constituents

To minimize operating costs, it was important to reduce the medium constituents to a minimal concentration without affecting the degradation rate. Four graphs were generated by reducing the individual constituents in the sodium oxalate medium (Figure 10).

Each graph showed that at half the constituent concentration, the rate of oxalate degradation was comparable to that at the regular concentration. At less than half the constituent concentration, the rate of oxalate degradation decreased substantially. The results indicated that all of the medium constituents could be decreased by half. Figure 11 shows that half medium constituents medium followed the same rate of oxalate degradation as the regular sodium oxalate medium after inoculation with the mixed culture from the RBC. Complete degradation of oxalate was observed in the RBC when the revised medium was tested (Figure 12).

CONCLUSION

An industrial process was developed using an oxalate degrading bacterium and a rotating biological contactor (RBC). Microorganisms that degrade oxalate are ubiquitous in the environment as there are many natural vegetative sources of oxalate. Enrichment of the organisms proved to be time consuming because both non-oxalate degrading bacteria, metabolizing impurities in the medium, and oxalate degrading bacteria, appear as pin point colonies on the solid oxalate medium. Thus differentiation was difficult. The only oxalate degrading bacterium isolated, B-1, was found to degrade the highest oxalate concentration to date, 16000 ppm. The highest previously reported concentration tolerated by a bacterium was 9500 ppm.

Identification of B-1 has been difficult because it is a new organism and it is fastidious. The data obtained to date on B-1 suggests that it belongs to the genus *Pseudomonas*.

A rotating biological contactor was employed as a continuous aerobic reactor because of its simple design, easy maintenance, low capital cost and low operating cost. The RBC worked very well resulting in the degradation of 5000 ppm oxalate with a 5 hour retention time. For such a system to work efficiently, the residence time should be low with a relatively high organic concentration in the influent to the RBC. The oxalate degradation process met these requirements with oxalic acid, sodium oxalate and the revised sodium oxalate medium. In fact, the biomass on the RBC disks could tolerate very high transient concentrations (shock loading) of oxalate and still maintain efficient oxalate degradation after shock loading.

At present Alcan International Ltd. is working with a pilot scale process to assess the feasibility of a full scale operation.

ACKNOWLEDGEMENTS

The authors would like to thank Alcan International Ltd. for their financial support and the Biotechnology Section, CANMET, Energy, Mines and Resources for their assistance throughout the project. Also thanks is extended to Health and Welfare, Canada for their efforts in identifying some of the bacteria.

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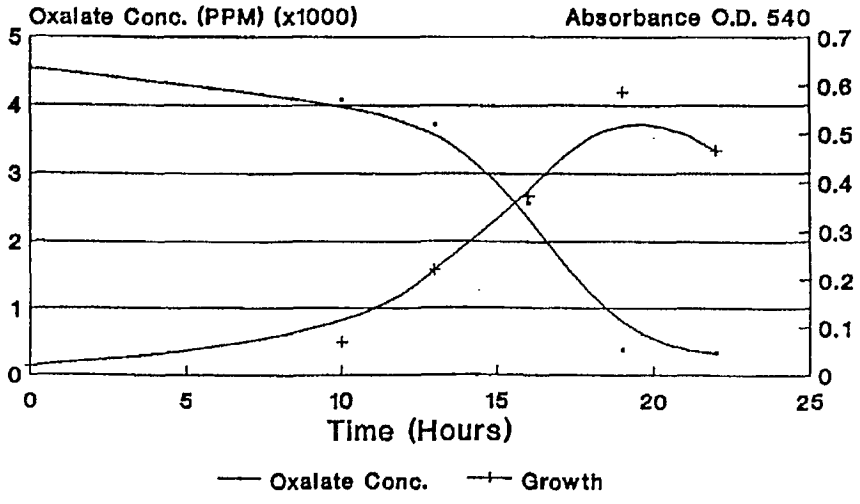


Figure 1. Oxalate degradation of six mixed cultures monitored over a 48 hour time period. A triplicate set of 250 mL flasks containing oxalic acid medium (Oxa-1) were incubated at 28°C and 150 rpm.

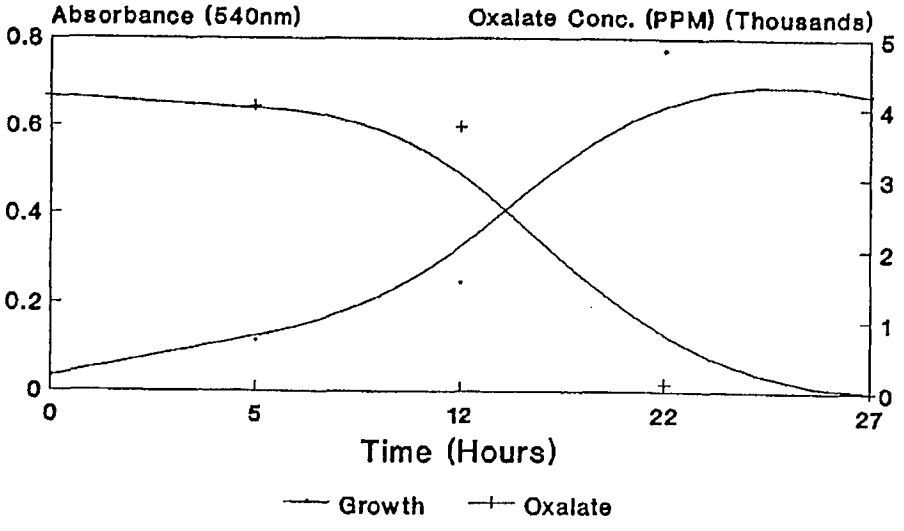


Figure 2. Oxalate degradation and growth of mixed culture F were monitored over a 27 hour time period. A triplicate set of 250 mL flasks containing oxalic acid medium (Oxa-1) were incubated at 28°C and 150 rpm.

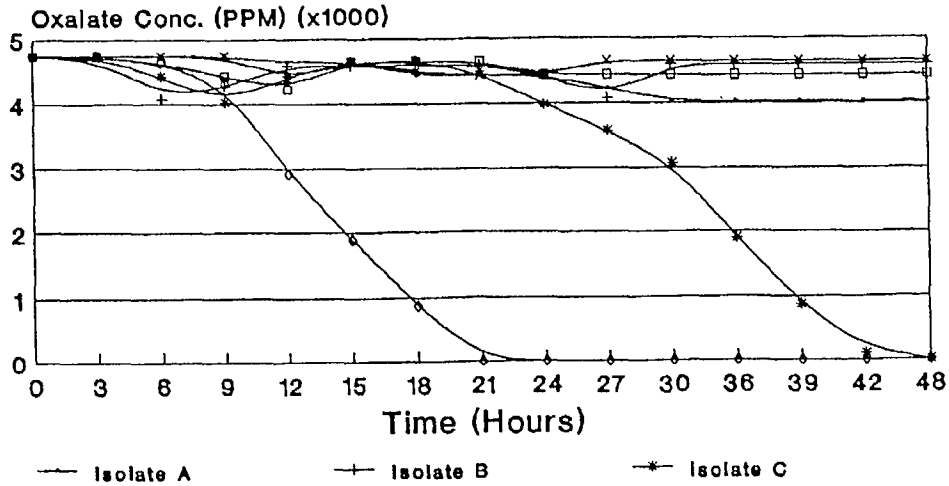


Figure 3. Oxalate degradation and growth of isolate B-1 monitored over a 22 hour time period. A triplicate set of 250 mL flasks containing oxalic acid medium (Oxa-1) were incubated at 28°C and 150 rpm.

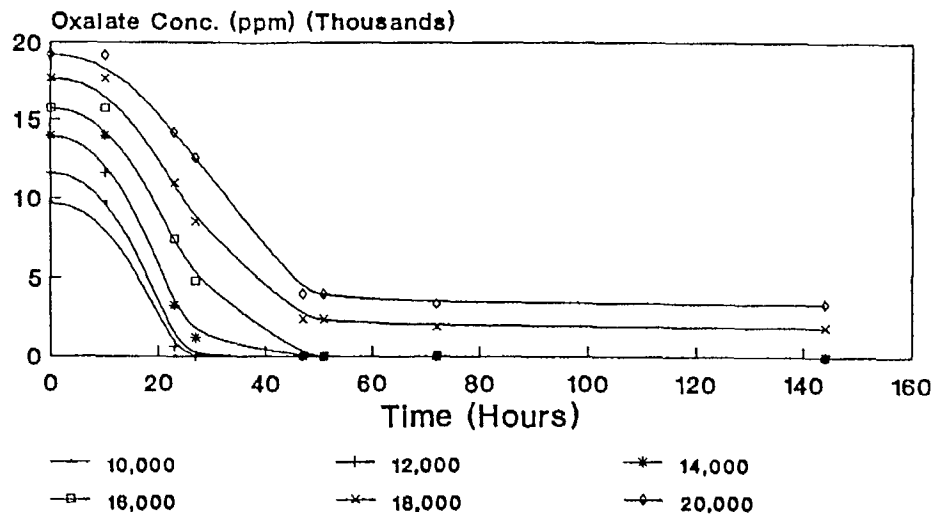


Figure 4. Tolerance of isolate B-1 to high levels of oxalate from oxalic acid (Oxa-1) medium. Oxalate was monitored over a 145 hour period. A duplicate set of 250 mL flasks were incubated at 28°C and 150 rpm.

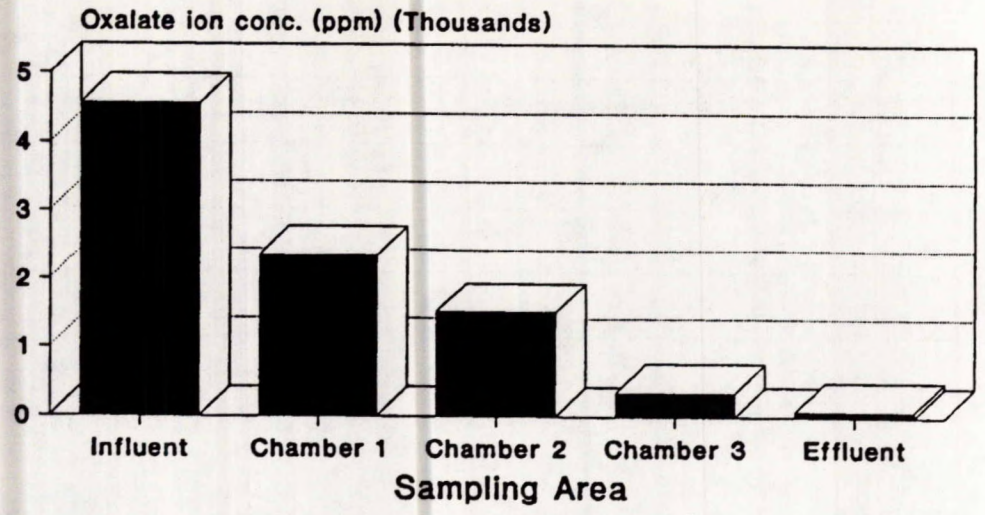


Figure 5. Sodium ion analysis of varying concentrations of oxalic acid medium standards performed on an Atomic Absorption Spectrophotometer.

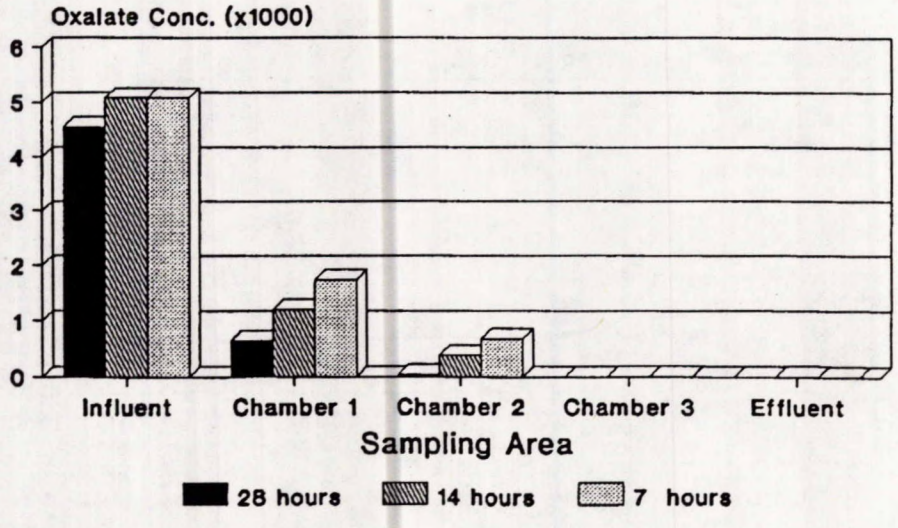


Figure 6. Bar diagram showing the levels of oxalate ion in the influent, RBC chambers and effluent in the RBC. Oxalic acid medium was run through the RBC with residence times of 28, 14 and 7 hours with a disk rotation speed of 0.6 rpm.

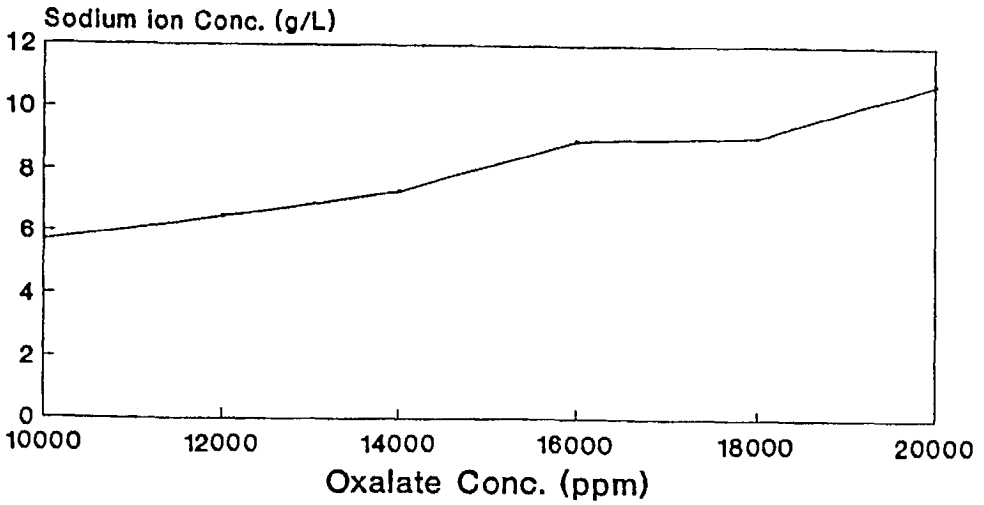


Figure 7. Bar diagram showing the levels of oxalate ion in the influent, RBC chambers and effluent in the RBC. Oxalic acid medium was run through the RBC with a residence time of 5 hours and a disk rotation speed of 0.6 rpm.

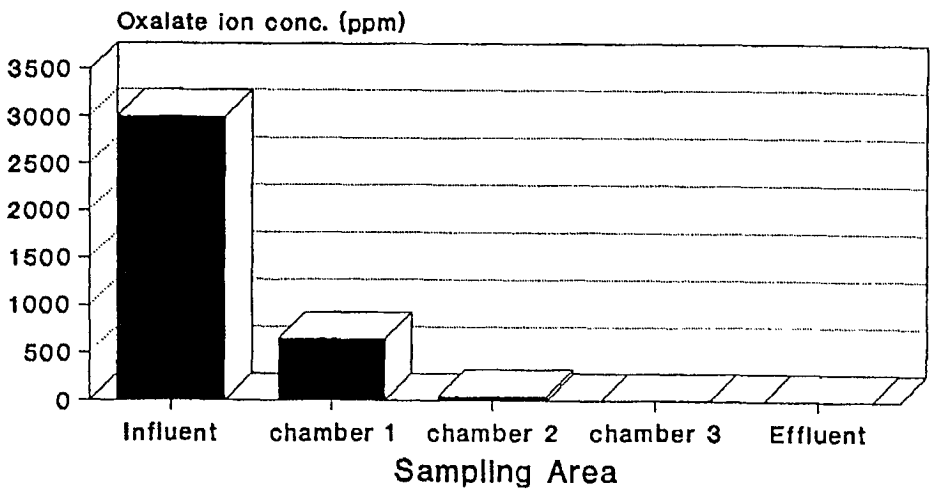


Figure 8. Bar diagram showing the levels of oxalate ion in the influent, RBC chambers and effluent in the RBC. Sodium oxalate medium was run through the RBC with a residence times of 5 hours and a disk rotation speed of 3 rpm.

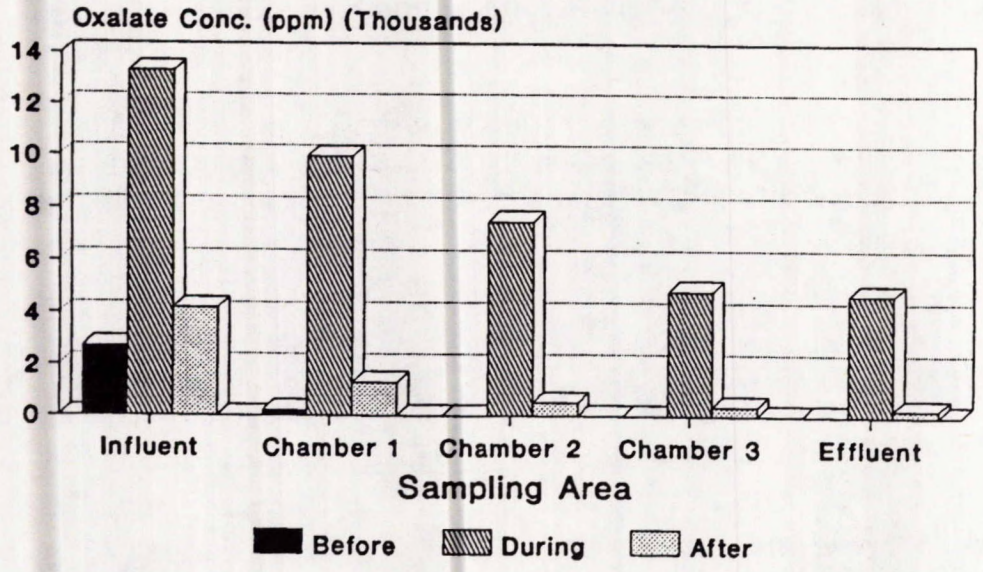
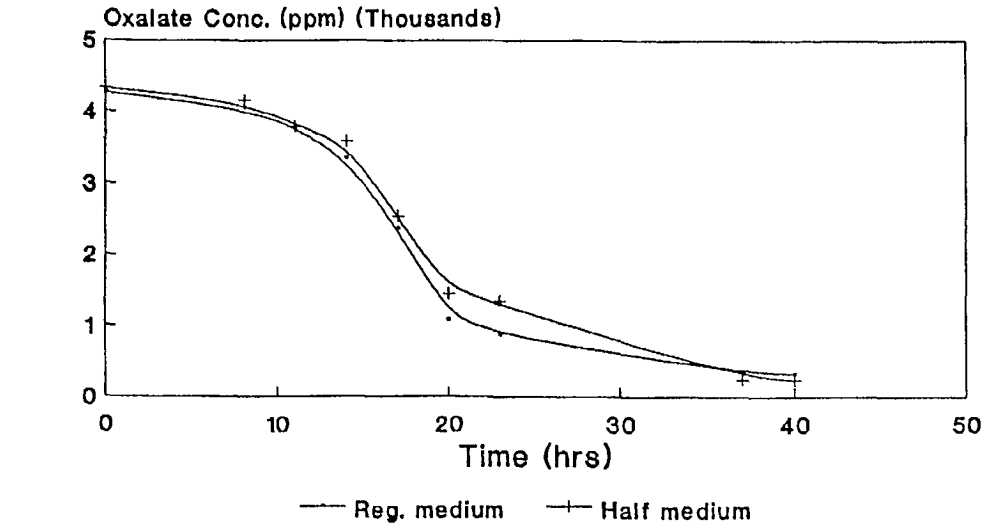


Figure 9. Bar diagram showing the levels of oxalate in the influent, RBC chambers and effluent, before, during and after shock loading. Sodium oxalate medium (Na-Ox) was run through the RBC with a residence time of 5 hours and a disk rotation speed of 3 rpm.



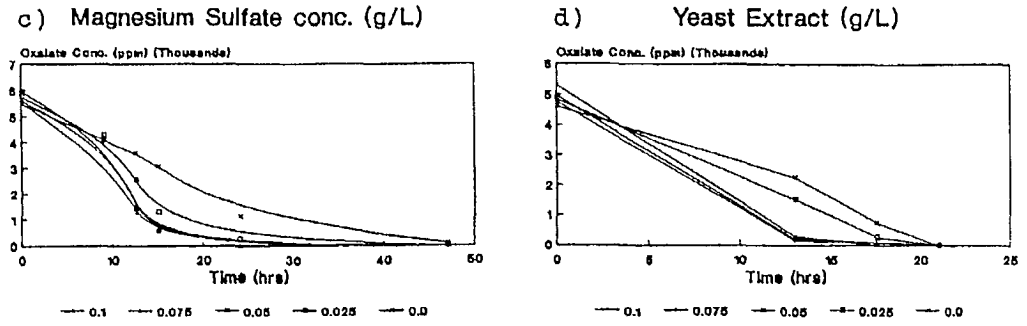


Figure 10. Minimization of sodium oxalate medium (Na-Ox) constituents. Each of the following medium constituents were reduced individually from the medium: (a) Ammonium phosphate, (b) Ferrous sulfate, (c) Magnesium sulfate, and (d) Yeast extract. An inoculum was obtained from the RBC and the duplicate set of 250 mL flasks were incubated at 28°C and 150 rpm. Oxalate degradation was monitored over time.

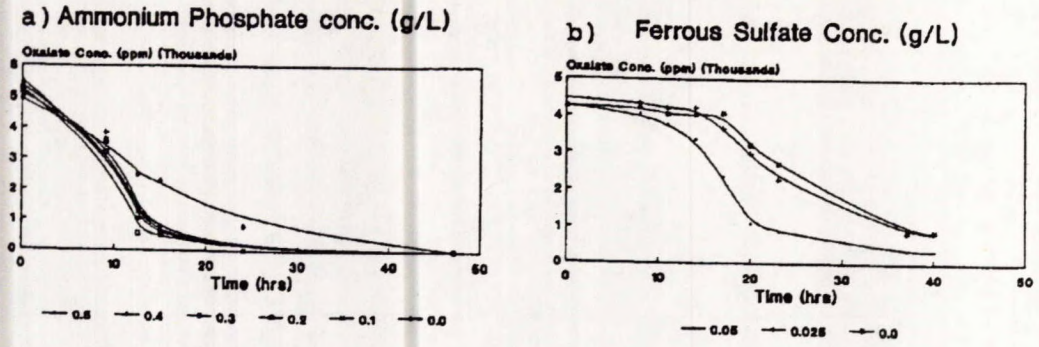


Figure 11. Sodium oxalate (Na-Ox) medium constituents were reduced to half the normal concentration. An inoculum was obtained from the RBC and a duplicate set of 250 mL flasks were incubated at 28°C and 150 rpm. Oxalate degradation was monitored over 40 hours.

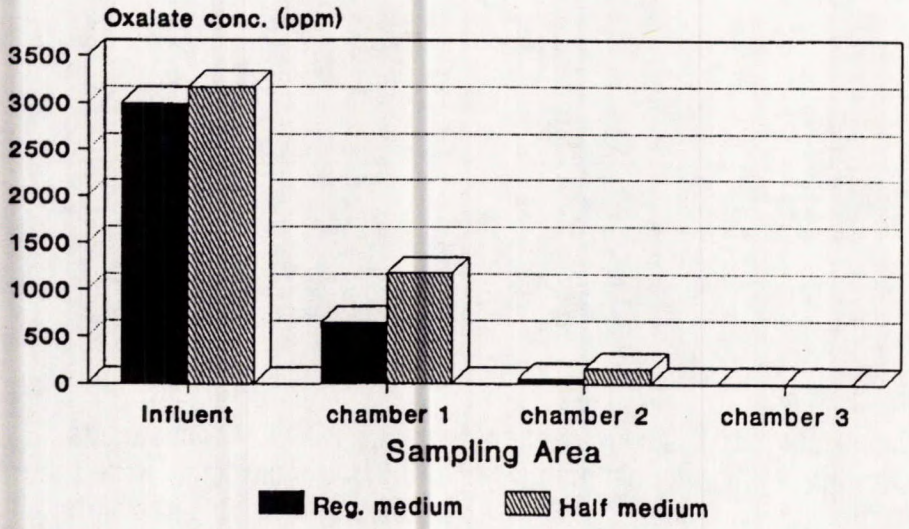


Figure 12. Sodium oxalate medium (Na-Ox) constituents were reduced to half the normal concentration and run through the RBC with a residence time of 5 hours and a disk rotation speed of 3 rpm. The bar diagram shows the levels of oxalate in the influent, RBC and effluent.

Table 1. Results of biochemical tests performed at Health and Welfare, Canada, Ottawa, and the American Type Culture Collection, Maryland, USA
A. Comparison of *Xylophilus ampelina* to isolate B-1

Tests	X.ampelina	Isolate B-1
Flagella monotrichous	+	+
Growth at 4°C, 37°C	-	-
Yellow pigment	+	+
Very slow sparse growth	+	+
Gelatin	-	-
Nitrate reduction	-	-
Phenylalanine deamination	N.G.	N.G.
Catalase	+	+
Oxidase	-	slow & weak
Arginine, ornithine, lysine decarboxylation	-	-
Acid from:		
O-F D-xylose	-	-
D-ribose	-	-
L-rhamnose	-	-
D-glucose	-	-
D-mannose	-	-
D-fructose	-	-
sucrose	-	-
trehalose	-	-
cellobiose	-	-
lactose	-	-
maltose	-	-
dulcitol	-	-
D-mannitol	-	-
D-sorbitol	-	-
Utilization of Sole Carbon Source:		
benzoate	-	-
gluconate	-	-
propionate	-	-
malonate	-	-
DL-malate	+	+
succinate	+	+

N.G. = No growth

B. Significant differences between *X.ampelina* and isolate B-1

Tests	X.ampelina	Isolate B-1
Utilization of oxalate as sole carbon source	-	+
Acid from O-F arabinose	+	-
Urease	+	-
Growth factor requirements	+	-

C. Comparison of isolate B-1 to *Pseudomonas*

Tests	Pseudomonas	Isolate B-1
Gram negative	+	+
Aerobic metabolism	+	+
Polar flagella	+	+
Motility	+	+
Oxidase	+ or -	weak & slow
Catalase	+	+

D. Physiology and biochemistry of B-1

Tests	+/-	Tests	+/-
Gram positive	-	Gelatinase	-
Gram negative	+	Tween 20 hydrolysis	+
Gram variable	-	Tween 80 hydrolysis	-
Motile at RT	+	Indole	-
Flagella peritrichous	-	Simmons citrate growth	W
Flagella lophotrichous	-	Urease	-
Flagella monotrichous	+	Nitrate to nitrite	-
Flagella lateral	-	Nitrite reduction	-
4°C growth	-	Nitrite to nitrogen gas	-
25°C growth	+	Hydrogen sulfide (TSI)	-
30°C growth	+	Lead acetate strip	+
37°C growth	-	Lysine decarboxylase	-
41°C growth	-	Arginine (Mollers)	-
Fluorescien produced	-	Ornithine decarboxylase	-
Pyocyanine produced	-	Phenylalanine deamination	NG
Diffusible orange	-	Phosphate	-
Diffusible yellow	-	Catalase	+
Diffusible purple	-	Oxidase slow and weak	+
Non-diffusible green	-	Gluconate oxidation	-
Other non-diff. pigment	Y	Growth on malonate as SCS	-
Melanin pigment prod.	-	Tyrosine degradation	-
pH 6.0 growth	+	dl-hydroxybutyrate growth	-
3% NaCl growth	+	Growth on 0.05% cetrimide	-
6.5% NaCl growth	-	Growth on acetate as SCS	-
MacConkey agar growth	+	Testosterone degradation	-
Skim milk agar growth	W	Mucoid growth on Glucose	
Aesculin hydrolysis	-	0.1% TTC growth	-
Casein hydrolysis	-	0.02% TTC growth	+
starch hydrolysis	-	Litmus milk acid	-
		Litmus milk peptonized	-

W = weakly positive
Y = yellow
TTC = Triphenyl Tetrozolium chloride

E. Reactions observed when isolate B-1 is grown in Hugh and Liefson O-F medium

Tests	Reaction
Acid from:	
L-arabinose	K
cellobiose	K
ethanol	K
D-fructose	K
D-glucose AO2	K
D-glucose AnO2	-
Alkaline pH in D-glucose	+
Acid from:	
glycerol	K
inositol	K
lactose	K
maltose	K
D-mannitol	K
D-mannose	K
L-rhamnose	K
D-ribose	-
sucrose	K
trehalose	K
D-xylose	K
adonitol	K
dulcitol	K
D-galactose	K
inulin	K
salicin	K
D-sorbitol	K
Control	K

K = alkaline
- = no change
+ = acid

F. Growth of isolate B-1 on sole carbon sources in Stanier's mineral base (Growth factors not required)

Sole Carbon Source	+/-	Sole Carbon Sources	+/-
L-arabinose	-	2-ketogluconate	+
cellobiose	-	DL-lactate	+
D-fructose	+	L-malate	+
D-glucose	-	pelargonate	-
lactose	-	propionate	-
maltose	-	quinate	-
D-mannitol	-	succinate	+
L-rhamnose	-	L-+-tartrate	-
D-ribose	+	valerate	-
D-sorbitol	-	B-alanine	-
sucrose	-	D-A-alanine	+
trehalose	-	betaine	-
D-xylose	-	glycine	-*
adonitol	-	L-histidine	-
erythritol	-	DL-norleucine	-
glycerol	-	L-proline	-
ethanol	-	D-tryptophan	-
geranitol	-	L-valine	-
inositol	-	DL-arginine	-
sebacic acid	-	benzylamine	-
acetamide	-	butylamine	-
adipate	-	putrescine	-
benzoate	-	mesaconate	-
butyrate	-	DL-glycerate	+
citraconate	-	L-tryptophane	-
D-gluconate	-	Methanol	-
M-hydroxybenzoate	-	Oxalate	+

W = weakly positive
* = became positive after 4 weeks

HETEROTROPH LAYERING IN FRESH TAILINGS

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ABSTRACT

The reduction or prevention of AMD in tailings may be accomplished by preventing the movement of water and air through the tailings mass. It is currently believed that this may be accomplished by capping or submerging the tailings. In this paper we describe a third alternative - the incorporation of oxygen-consuming layers within fresh tailings.

Growing vegetation on fresh tailings, and the incorporation of this vegetation into tailings layers provides a source of carbon for aerobic, heterotrophic bacteria. The growth rate of these bacteria under the proper conditions is several orders of magnitude greater than that of Thiobacillus. Heterotrophic bacteria outcompete Thiobacillus for oxygen, thus, reducing the redox potential and slowing Thiobacillus metabolism and growth.

Growing vegetation on fresh tailings provides a number of unique challenges. This paper describes some of the important parameters and the results of early field trials, both in biomass production and heterotroph numbers.

(Manuscript not available)

STRATIFICATION DES HÉTÉROTROPHES DANS LES STÉRILES NON ALTÉRÉS

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RÉSUMÉ

On peut réduire ou empêcher la production d'eaux de mine acides (AMD) dans les stériles en empêchant la circulation de l'eau et de l'air à travers la masse des stériles. On croit actuellement pouvoir y parvenir en recouvrant à leur sommet les stériles, ou en les submergeant. Dans le présent article, nous décrivons une troisième possibilité - l'incorporation de couches consommatrices d'oxygène aux stériles non altérés.

La croissance de végétaux sur des stériles non altérés et l'incorporation de cette végétation aux couches de stériles fournit une source de carbone aux bactéries hétérotrophes aérobies. Le taux de croissance de ces bactéries dans des conditions appropriées dépasse de plusieurs ordres de grandeur celui de Thiobacillus. Les bactéries hétérotrophes consomment plus d'oxygène que Thiobacillus et par conséquent, réduisent le potentiel rédox de Thiobacillus et ralentissent son métabolisme et sa croissance.

La végétation qui se développe sur les stériles non altérés nous confronte à plusieurs problèmes particuliers. Dans le présent article, figurent quelques-uns des principaux paramètres et les résultats des premiers essais in situ, à la fois du point de vue de la production de biomasse et des nombres d'hétérotrophes.

(L'article n'est pas disponible)

PERIPHYTON GROWTH AND ZINC SEQUESTRATION

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ABSTRACT

Periphyton populations composed mostly of green, filamentous algae have been found in mining waste water, together with large quantities of metal precipitates. These populations, because of the close interaction between precipitate and periphyton, have been termed periphyton-precipitate complexes (PPCs). These complexes can be over 80% precipitate, with zinc concentrations as high as 8%.

While growth of the periphyton and precipitate content of the complex are related to water chemistry, some commonalities have been found between PPCs growing in different waters from different mine sites. The elemental composition of precipitate is also similar to that of PPCs, suggesting that periphyton may play a role as sticky sieves. This paper discusses the relationship between zinc, precipitates, and periphyton growth, primarily from mining sites in northern Ontario and central Newfoundland.

CROISSANCE DU PÉRIPHYTON ET SÉQUESTRATION DU ZINC

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RÉSUMÉ

On a découvert des populations de périphyton, principalement composées d'algues vertes filamenteuses dans les eaux résiduaires des mines, en même temps que de vastes quantités de précipités métalliques. Ces populations, à cause de l'étroite interaction entre les précipités et le périphyton, ont été appelées complexes avec périphyton et précipité (PPC). Ces complexes peuvent contenir plus de 80 % du précipité total, avec des taux de zinc atteignant parfois 8 %.

La croissance du périphyton et la teneur en précipité du complexe sont associés à la chimie de l'eau, et l'on a observé certains traits communs entre des PPC se développant dans des eaux différentes provenant de sites miniers différents. La composition élémentaire des précipités est également semblable à celle des PPC, ce qui semble indiquer que le périphyton peut jouer un rôle de filtre à mailles gluantes. Dans le présent article, on étudie la relation entre le zinc, les précipités et la croissance du périphyton, surtout dans le cas des sites miniers du nord de l'Ontario et du centre de Terre-Neuve.

INTRODUCTION

Attached, or periphytic algae grow in mine effluent ponds and streams, characterized by extremes in pH, elevated metal and suspended solids concentrations (3, 5, 6, 10, 12, 13, 16). Reviews on metal/algae interactions (11, 12, 16) indicate that tolerance is achieved by several different means. Dissolved metals can be either bound to the cell walls, charged carbohydrates, or taken up into the cell and sequestered in specific organelles. All of these processes lead to high concentrations of metals in or on the algal biomass (3, 11).

Metal precipitates present as suspended solids can also attach to periphyton populations either to extracellular carbohydrates or onto cell walls (4, 9, 15) resulting in Periphyton-Precipitate Complexes (PPCs) with high solids content and a small proportion of biomass. Precipitates, especially iron hydroxide, and iron hydroxide coprecipitated with zinc have been found on cell bacterial surfaces (1). This may also correlate with encrustation of iron and manganese found in a number of filamentous algae at sites contaminated by mine water (14).

The periphyton populations inhabiting acidic effluents are multi-species complexes dominated by filamentous, benthic algae, which are associated with mosses and diatoms. Cyanophytes and charophytes dominated periphyton in ponds and ditches containing more alkaline waters.

Growth rates of PPCs have been quantified in the field at two sites by Kalin and Wheeler (7,8). It was found that in Newfoundland, in circumneutral pit water, PPCs "grew" at rates up to 4.6 gdw (sq. m of surface area)$^{-1}$ d^{-1}. In NW Ontario, PPCs in an acidic lake grew at rates of only 1.4 gdw (sq. m of surface area)$^{-1}$ d^{-1}. Quantifying growth of the periphyton portion of the PPC, without accompanying precipitates proved difficult.

This paper describes the relationship between metal precipitates and periphyton growing at two mine sites. Comparisons are made between the composition of PPCs and precipitates, as well as the growth of PPC and deposition rates of precipitates. A basic understanding of these relationships is essential to the development of biological polishing as a water cleansing process.

MATERIALS AND METHODS

Site and Algal Population Description

The first of two, intensively-studied mine sites was located on Confederation Lake in northwest Ontario (Figures 1 and 2). The site included several water bodies which contained extensive periphyton populations. Large amounts of alkaliphyllic cyanobacteria (*Oscillatoria*) occurred near the outflow of Decant Pond. Extensive populations of a *Ulothrix* spp.- dominated community were found on the beach end of Decant Pond and in an acidic lake (Boomerang Lake). Another population of *Ulothrix* spp. was found in Mill Pond (Table 1).

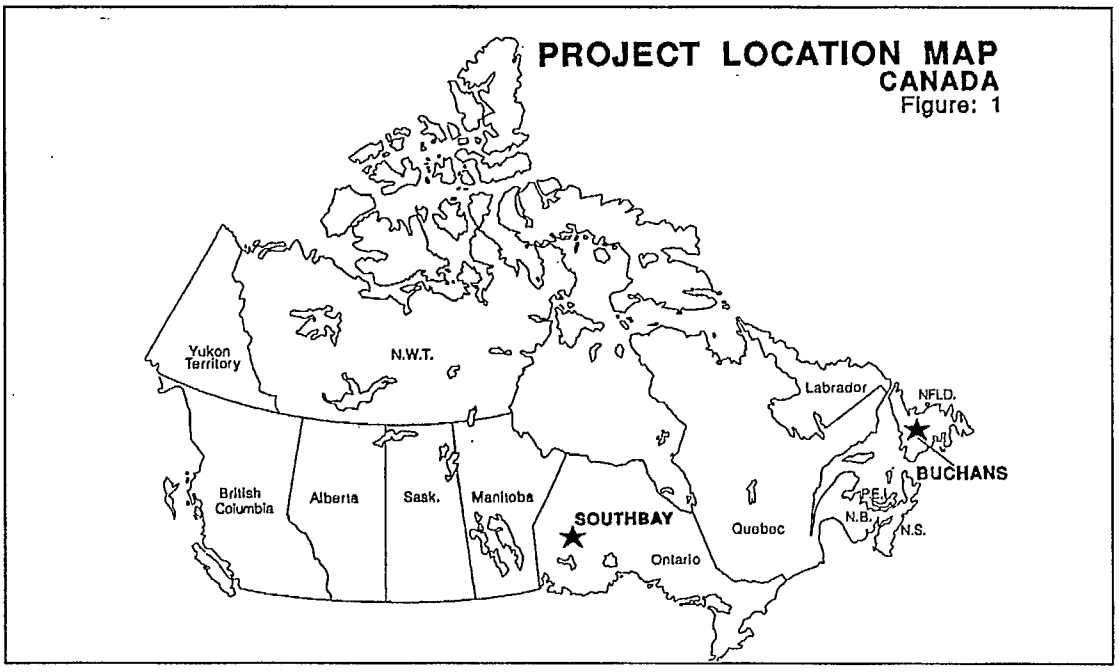

Figure 1: Location of study sites.

The second intensively studied site is the Buchans mine in central Newfoundland (Figures 1 and 3). In the Oriental East Pit and its effluent stream and polishing pond system, a *Microspora*-and moss-dominated community was proliferating (OEP). A *Ulothrix* community dominated the algal flora in the Oriental West Pit (OWP). Populations of a *Ulothrix/Microspora*-dominated community were growing in Second Meadow seepages (MDW), and in the Drainage Tunnel (DT) effluent water, another *Ulothrix/Microspora*-dominated population flourished (Table 1).

In the effluent of the Oriental East Pit, a series of six, serial experimental ponds were constructed in 1988. Each pond had a volume of approximately 40 cubic meters. Alder branches were placed in each pond to act as surface area (appx. 3.8 m^2/m^3) for the growth of PPCs. Flow through the ponds was controlled, to provide an overall residence time between 16 and 79 days.

Field Sampling

PPCs were intensively studied over the summer of 1991 during site visits to the two mines. The habitat, i.e. pond, stream or lake shore was recorded, and pH and electrical conductivity were determined in the field. PPC and water samples were kept cool in plastic bags and bottles until processed in the laboratory.

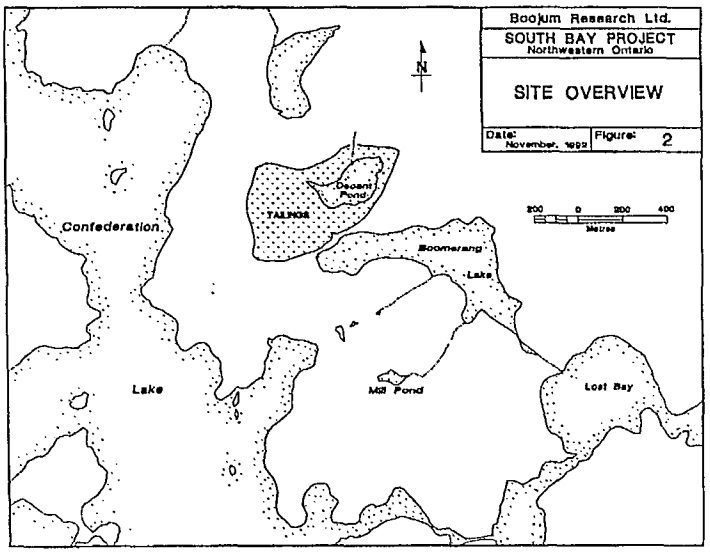

Figure 2: Location of study sites at South Bay.

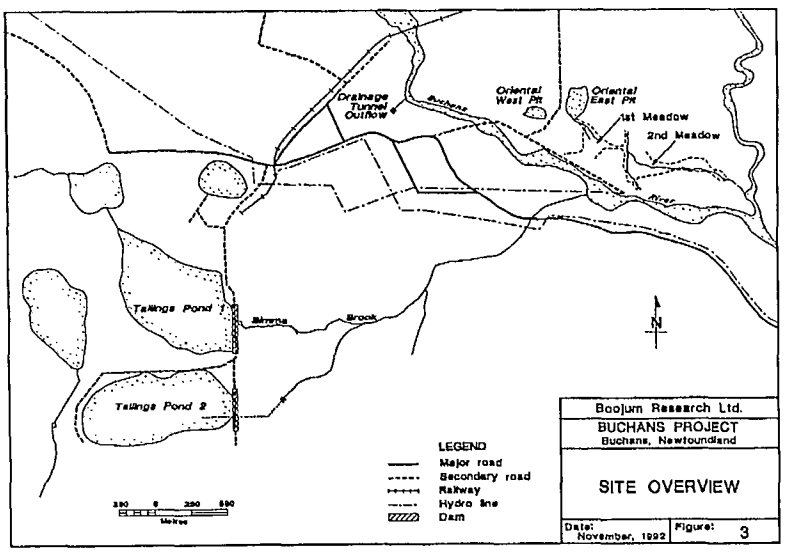

Figure 3: Location of study sites at Buchans.

TABLE 1: Description of Waste Water Sites

MINE SITE	HABITAT	pH	[Zn] mg/L	[Al] mg/L	[Fe] mg/L	[Cu] mg/L
NW Ontario	Boomerang Lake	3.2-3.5	7-11	2.1	4.5	0.1
NW Ontario	Decant Pond	3.2-4.5	1-2	0.1	0.6	0.1
NW Ontario	Decant Pond	5-7	2-6	4	1*	0.04
NW Ontario	Mill Pond	3.2-3.5	150-450	16	32	15.8
Newfoundland	Oriental East Pit	6.1-7.3	20-25	10	1*	1*
Newfoundland	Meadow seepage	6.5	7	4	1*	0.1*
Newfoundland	Drainage Tunnel	6.4-6.7	16-25	1*	1*	0.1*
Newfoundland	Polishing Ponds	6.5-7.5	4-18	4-8	2	1*
Newfoundland	Oriental West Pit	3.3-3.8	26-36	4-6	1	1*

* indicates sample at or below detection limit

PPC growth rates were quantified at both locations using a combination of constructed, artificial substrates and natural, alder or black spruce branches. The artificial structures, called "peritraps" measured both growth and sloughing rates. The traps consisted of an artificial netting structure which housed alder or spruce branches. A plastic bag below the netting collected any PPC material falling from the netting or substrates. PPC growth rates could therefore be determined on both netting and branches. PPC mass was cleaned off the nets and branches, dried and weighed. Total growth was determined by adding PPC weights on nets and branches to that which had fallen into the bag. The cleaned traps were replaced for regrowth three times during the growing season. Peritraps were installed in Boomerang Lake and Decant Pond at South Bay, and in the OWP, OEP, and the polishing pond system at Buchans.

Precipitation rates were quantified at the South Bay site, in Boomerang Lake at 4 locations, including the outfall (2 m depth), the inflow area from Mill Pond (2 m), and in two deeper locations in the lake (4 and 5 m; Figure 2). Precipitation rates were also measured near the outfall in Decant Pond in 2 meters of water. At Buchans, precipitation rates were quantified at 4 locations in the Oriental East Pit, two near the outfall in 2.7 m of water and 2 in the centre of the pit in 20 m of water. The traps were a collection of 5 vertically-mounted tubes, 5 cm diam. and 50 cm in length (2). They were held vertically by a plate and harness, and lowered into the water to specific depths. The traps were hauled to the surface, and allowed to sit for 24 h. The material in the bottom of the tubes was collected, dried, and weighed. Precipitation rates per square meter of lake or pit bottom were calculated from the dry mass collected from the traps over several time intervals.

Laboratory Preparation/Analyses

Water samples from the field were filtered through 0.45 µm cellulose acetate filters within 24 h of collection, and acidified with concentrated nitric acid to a pH less than 1. Samples were subsequently oxidized using a mixture of perchloric and nitric acids, and analyzed for metal content by Inductively Coupled Plasma Spectroscopy (ICP) at a certified laboratory.

Periphyton samples from all sites were brought back to the lab for analysis. There, reference samples for identification of the dominant groups in the algal complexes were preserved in Lugol's fixative. In some cases, material was used to establish cultures in the laboratory to confirm the dominance of the algal group in the PPC.

Sticks and other debris were removed, and the sample was dried in an oven at 40-60 °C for 2 to 3 days. The dry material was powdered in a hand mortar, and stored in glass vials. A subsample was wet oxidized using a mixture of nitric and perchloric acids. Metal concentrations were then determined by ICP at a certified laboratory. Loss on Ignition (LOI) values were derived from another subsample which was combusted for 30 min in a muffle furnace at 500 °C.

To separate periphyton from precipitate, the ash content of "clean periphyton" and "pure precipitates" were determined by LOI. After subtracting the LOI of "clean periphyton", the percentage precipitate was calculated based on a straight line interpolation to 100% at the LOI of the "pure precipitate".

RESULTS

Water and PPC Composition

At South Bay, there were three habitats which contained *Ulothrix* as the dominant taxon, each with differing concentrations of mine contaminants (Table 1). The average composition of these periphyton populations is described in Table 2. The average dry weight to fresh weight ratio for most of these populations was high, ranging between 0.26 and 0.31, suggesting a high inorganics content. Based on LOIs, the average percentage of the PPC, which was calculated as algal, varied between a low of 14 to a high of 93%. Average zinc concentrations ranged from 0.2 to 3.3% of the dry weight for these PPCs. Average aluminum varied between 0.3 and 3.9% of the samples, while iron varied between 3.2 and 28.9% of the dry weight. Other, more minor components of the PPCs are also described in Table 2. Over all of the South Bay samples, about 90% of the dry weight was accounted for, although the missing mass ranged from -10% to 18.9%.

PPCs from Buchans had a similar composition. Average dry weight to fresh weight ratios varied between 0.21 and 0.38. The calculated average percentage of algae in the PPCs varied between 18.7 and 56% of the PPC. Average zinc concentrations ranged between 0.3 and 8.2% of dry weight. Average aluminum concentrations in the PPCs ranged from 0.5 to 2.2%, and iron concentrations ranged from 3.1 to 25.8%. Over all the Buchans populations, about 68% of the

mass was accounted for, although the missing mass varied between 3.3 and 60.1% of the total.

Specific comparisons between PPC composition and surrounding water can only be made using PPC populations of a similar biotic composition. Thus, *Ulothrix*-PPCs and surrounding water (Decant Pond, Mill Pond, and Boomerang Lake) were

Table 2: PPC Composition

LOCATION	TAXA	DW/FW	Algae %	Fe %	Fe(OH)3	S %	(SO4)	Zn %	Zn(OH)2	Mn %	Al %	Ca %	CaCO3	Cu %	Other %
Decant Pond	Oscillatoria	0.28	29.5	17.8	34.9	2.5	7.5	3.3	5.1	1.1	3.9	1.4	3.6	1.1	13.3
Decant Pond	Ulothrix	0.309	93.0	3.2	6.3	1.0	3	1.8	2.6	2.1	0.3	1.0	2.6	0.1	-10.1
Mill Pond	Ulothrix	0.264	14.0	28.9	56.6	2.6	7.8	0.2	0.3	-	0.6	0.6	1.5	0.2	16.9
Boom. Lake	Ulothrix	n.d.	35.4	20.1	39.4	1.8	5.4	0.3	0.5	-	0.4	0.5	1.3	-	17.7
Drainage Tunnel	Ulothrix/Microspora	0.376	34.0	3.1	6.1	0.6	1.8	0.7	1.1	-	2.1	0.5	1.3	0.2	53.5
Meadow	Ulothrix/Microspora	0.262	70.9	4.4	8.6	1.4	4.2	2.2	3.4	0.2	0.6	2.1	5.4	-	6.7
Oriental West Pit	Ulothrix/Microspora	n.d.	20.5	5.6	11.0	0.8	2.4	0.3	0.5	-	1.2	1.7	4.3	-	60.1
Polishing Ponds	Microspora	n.d.	18.7	10.9	21.4	0.5	1.5	8.2	12.7	1.4	1.2	1.6	4.1	-	39.0
Oriental East Pit	Microspora	0.206	35.3	25.8	50.6	0.4	1.2	3.6	5.6	0.2	0.5	1.3	3.3	-	3.3

- denotes percentage smaller than 0.1
n.d. - not determined

compared without precipitate correction with respect to their elemental composition (Figure 4a). For these comparisons, individual PPC samples were compared.

The distribution of elements and their concentrations in the waste water were similar to those found in PPCs, with the exception of iron, copper, and zinc. This was expected as the origin of the metals was either tailings or mill site. Sulphur, calcium, and zinc (Mill Pond only) were present in concentrations >100 mg L^{-1}. Those elements with concentrations greater than 10 mg L^{-1} were magnesium, aluminum, silicon, iron (except Boomerang Lake), and copper (Mill Pond only).

In PPCs growing in the waters, the most accumulated element was iron (> 100,000 µg gdw^{-1}), but in the next highest concentration range (>10,000 µg gdw^{-1}) sulphur, manganese (Decant Pond only), and zinc (Decant Pond only) were present. The next highest concentration range (>1,000 µg gdw^{-1}) contained the elements calcium, aluminum, copper (Decant Pond only) and phosphorus. Potassium and sodium, as essential plant nutrients, can be expected to be present in high concentrations.

At Buchans, there were also 3 sites which contained similar periphyton populations composed of *Ulothrix* and *Microspora* (Table 2). Elemental distributions are shown for specific samples of populations in the Drainage Tunnel, the Oriental West Pit, and the Meadow seepages.

Figure 4b compares the concentrations of major elements in Buchans water with those found in corresponding PPCs. Only calcium and sulphur in the OWP and Meadow sites were found in concentrations greater than 100 mg L^{-1}. Of the elements, present in concentrations greater than 10 mg L^{-1}, only zinc was of concern. Iron concentrations at these sites were low, < 10 mg L^{-1}.

The PPCs found growing at these locations had somewhat different elemental distributions. Those elements found in concentrations greater than 10,000 µg gdw^{-1}, were calcium, sulphur, iron, and zinc (only for the Meadow Seep). For the Drainage Tunnel and OWP, only aluminum and iron greater than 10,000 µg gdw^{-1}. Zinc concentrations in the Drainage Tunnel and OWP PPCs were 2000 to 5000 µg gdw^{-1}.

Precipitation Rates

The calculated average precipitate deposition rate in Boomerang Lake was around 2.2 gdw m^{-2} d^{-1} over the summer 1991 (Figure 5a). In Decant Pond, the average metal precipitation rate was 0.6 gdw m^{-2} d^{-1}. In the Oriental East Pit at Buchans, one of the shallow traps caught 2.0 gdw m^{-2} d^{-1} over the winter, and 1.9 gdw m^{-2} d^{-1} over the following summer (Figure 5b). The deeper traps caught just over twice that amount, averaging about 5.0 g m^{-2} d^{-1} over the winter, and 5.3 gdw m^{-2} d^{-1} over the summer. Since the deepest traps in Boomerang Lake were in 4-5 m of water, precipitation rates were probably more comparable to the shallow traps in Buchans (2.7 m).

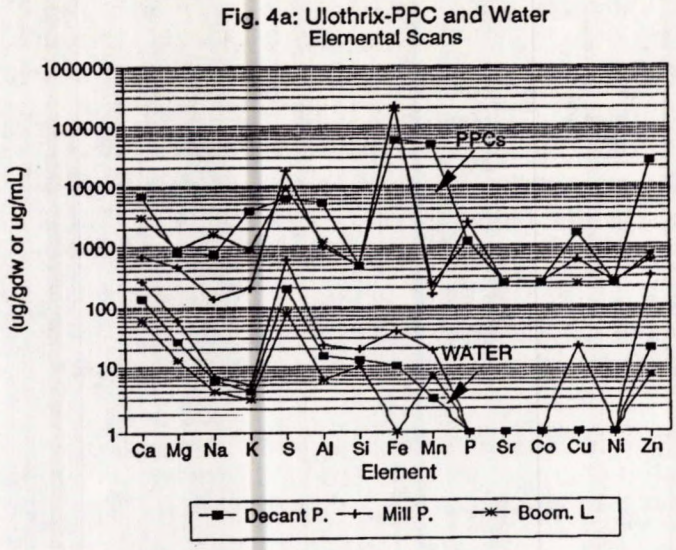

Figure 4a: Elemental scans of *Ulothrix* PPCs and surrounding water.

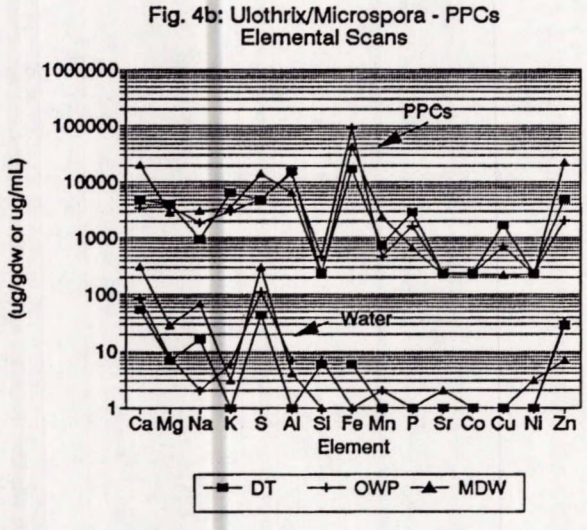

Figure 4b: Elemental scans of Ulothrix/Microspora PPCs and surrounding water.

PPC Growth Rates

At the South Bay site, 13 peritraps were set up at each of two locations in Boomerang Lake in early May. At Buchans, 5 peritraps were placed in each polishing pond at the end of May, with 6 in each of the pits. PPC growth rates over 3 periods in the summer were compared within mine sites (Boomerang Lake, and Decant Pond; OWP, OEP, PP1, and PP6) and between sites (Buchans, South Bay).

Growth rates of PPCs at South Bay and Buchans were calculated based on linear growth, i.e. the mass collected after a given submergence time per unit surface area. Growth rates of PPCs varied depending on site and time of year. In Newfoundland, in the OEP, for example, PPCs grew at rates up to 4.6 gdw (sq. m of surface area)$^{-1}$ d^{-1}. In NW Ontario, PPCs in Boomerang Lake grew at rates of only 1.4 gdw (sq m of surface area)$^{-1}$ d^{-1}. However, in order to compare algal growth at different sites, the precipitate content of the PPCs at each site had to be taken into account. Using the LOI correction for biomass described in the methods, the mean LOI-corrected periphyton growth rates for the summer of 1991 are shown in Figure 6. Due to the large precipitate component, the maximum periphyton growth rates are only about 0.75 gdw (sq. m of surface area)$^{-1}$ d^{-1}. With the exception of periphyton growth in the Oriental West Pit, and June samples from the polishing ponds, most of the growth rates are similar. The peritraps in Decant Pond were set up near the outflow and experienced water with a relatively high pH. Thus, the water bodies with high pHs (Decant Pond, OEP, PP1, and PP6) showed relatively high growth rates, with the exception of June and July data for PP1, and June data for PP6. However, among the acidic sites (all had pHs around 3.5), Boomerang Lake samples showed consistently higher growth rates than those in OWP.

PPC Growth and Precipitate Deposition

The growth of PPCs in South Bay and Buchans were compared to precipitate "growth" rates as measured by precipitate deposition rates. Comparisons were made for those periods when precipitate rates and PPC growth rates overlapped, and where precipitation traps were in close proximity to peritraps. The peritrap data were analyzed as if they were precipitate traps, i.e. growth rates were calculated based on the area of lake (or pit) bottom, rather than on a surface area basis. Data are presented in Table 3.

During peak growing periods, PPC mass "outgrew" precipitate deposition rates (Boomerang Lake July - 2.75 gdw m^{-2} d-1 PPC growth vs. 1.65 gdw m^{-2} d^{-1} precipitate deposition rates), but at other times, rates were generally equal. The percentage precipitate was calculated to indicate which process dominated the "growth rate" of the PPC. Thus, in Boomerang Lake in June, 105% of the "growth" rate could be accounted for by precipitate deposition.

Another way of analyzing the contribution of the periphyton to the precipitate deposition process was to compare the composition of precipitate collected in precipitate traps and the composition of the PPCs collected in peritraps. These

data are shown in Table 4. At South Bay, the LOI of PPCs was nearly identical with those of the precipitates (45.8 vs. 42.5%, respectively). The composition of the PPCs in Boomerang Lake were also nearly identical to the composition of the precipitate, for at least iron, aluminum, and zinc. At Buchans, however, Oriental East Pit precipitates appeared to be significantly enriched with metals over PPCs.

As an example of biological polishing capacity, zinc removal rates in the Buchans polishing ponds were calculated for selected days during the summer of 1991. Data on PPC mass, zinc concentrations in water and PPCs, and flow rates on Aug 25, 1991 were analyzed (Figure 7). Under conditions of: 1) 16 day residence time; 2) 15.5 mg L^{-1} Zn in the inflow stream, and 3) 6.75 pH, 88% of the dissolved zinc was removed from the incoming water stream.

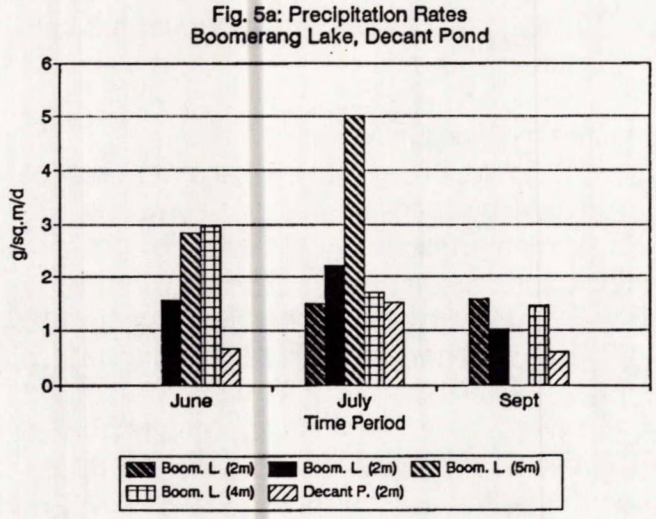

Figure 5a: Precipitation rates in Boomerang Lake and Decant Pond, 1991.

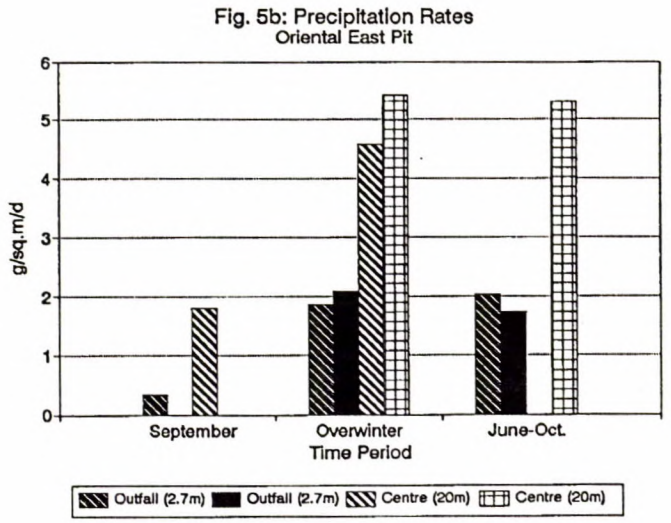

Figure 5b: Precipitation rates in the Oriental East Pit, 1990/1991.

DISCUSSION

Our data suggest that algae can grow well in mine seepages, ponds, and lakes over a range of pHs, and metal concentrations. Metal precipitates, and dissolved metals can be found in association with algae at these sites. During peak growing periods, PPC mass can "outgrow" precipitate deposition rates, but at other times, rates are generally equal. This suggests that precipitate deposition rates dominate the PPC growth rates, and that already formed precipitates are simply being sieved from the water. This is further confirmed by analyzing the composition of the PPCs and precipitates.

Newman et al. (9) correlated the composition of *aufwuchs* with the composition of precipitate. Their finding, that negative correlations between cell density and elemental concentrations indicated that the role of metal hydroxides was more important than periphyton accumulation in explaining the metal concentrations found in *aufwuchs*.

Periphyton surfaces and associated polysaccharides are providing a "sticky" surface which appears to "sieve" precipitates from the water. Where precipitates are not in high concentrations, periphyton appear to be providing surface area for the direct binding of zinc, and other metals onto algal surfaces. Thus, at high concentrations of precipitate, the composition of the precipitate and PPC are nearly identical, and the PPC is a "sticky sieve". At low concentrations of precipitate or with only dissolved metals present, the periphyton are providing a surface on which metals can be bound.

Regardless of the role that periphyton plays, a significant fraction of the zinc, and other metal loadings can be removed from waste streams, especially at higher pHs.

ACKNOWLEDGEMENTS

We wish to acknowledge partial support for this work by the joint venture group at Buchans, Newfoundland - ASARCO Inc. and Abitibi-Price Inc. Particular thanks are given to the General Manager/Consultant for ASARCO at Buchans, Mr. G. Neary. BP Resources, Development Corp. of Cape Breton, and CANMET also provided financial support, through CANMET contracts, DSS File #034SQ.23440-1-9009, DSS File # 034SQ.23440-1-9011, and DSS File #034SQ.23440-1-9010.

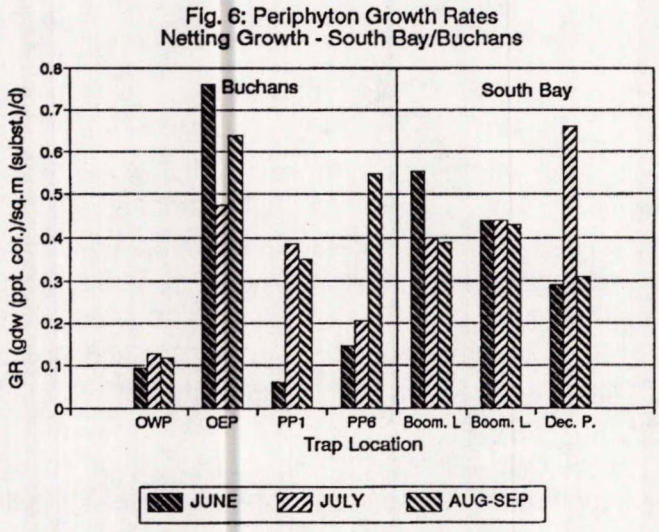

Figure 6: Periphyton growth rates on Peritraps in South Bay and Buchans waste waters.

Fig. 7: Biological Polishing
Buchans - Polishing Ponds

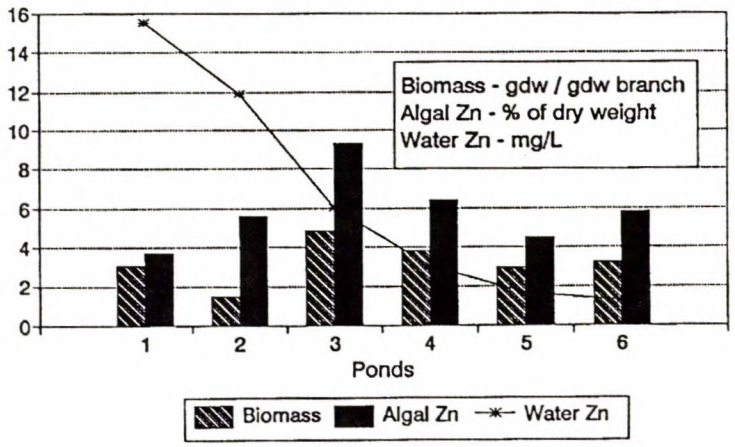

Figure 7: Biological polishing of zinc in Buchans polishing ponds, August 25, 1990.

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REMOVAL OF AMMONIUM AND NITRATE FROM MINE EFFLUENTS BY SEQUENTIAL NITRIFICATION/DENITRIFICATION

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ABSTRACT

Although nitrification/denitrification processes have been used to remove nitrogen from municipal and industrial wastewaters there are no reported systems for the removal of nitrogenous compounds from mine and mill effluents. It is anticipated that the discharge of both ammonium and nitrate by the mining industry will be regulated in the future. The objectives of this study were to determine the effects of various parameters on the removal of nitrate from a simulated mine effluent. A packed bed denitrification reactor operated in the upflow mode was used. Methanol was added as a carbon and energy source. Up to 99% removal of nitrogen from a solution containing 120 ppm nitrate was obtained in the packed bed reactor operated with a retention time of 1.5 h. Work is continuing to evaluate the effects of various parameters with the objective of scaling the reactor up to pilot plant size.

ÉLIMINATION DE L'AMMONIUM ET DU NITRATES RÉTROUVÉS DANS L'EFFLUENT DES MINES PAR UN PROCÉDÉS CONTINU DE NITRIFICATION-DENITRIFICATION

W.D. Gould, P. Bédard, D.W. Koren et D. Liang

RÉSUMÉ

Le procédé de nitrification-dénitrification est utilisé pour éliminer l'azote des eaux usées d'origine municipales et industrielles. Cependant, ce procèdé n'a jamais été appliqué aux effluents miniers. Il est anticipé que le rejet dans les eaux de l'ammonium et du nitrate par l'industrie minière sera réglementé. Les objectifs de cette étude, étaient de déterminer l'influence de différents paramètres sur l'élimination du nitrate d'un effluent minier synthétique. Un réacteur de type lit fixe à flux ascendant est utilisé pour la dénitrification. Du méthanol a été ajouté comme source de carbone et d'énergie pour les bactéries. Une élimination de plus de 99% a été obtenue pour une solution contenant 120 ppm de nitrate dans un réacteur à lit fixe avec un temps de rétention de 1.5h. Les recherches se poursuivent afin d'évaluer l'effet de différents paramétres avec comme objectif de pouvoir passer à échelle pilote.

INTRODUCTION AND LITERATURE REVIEW

Sequential ammonia oxidation and nitrate reduction reactions have been used (Grady and Lim, 1980) to remove nitrogen from both municipal and industrial wastewaters. Other applications (Grady and Lim, 1980) for nitrification/denitrification include the treatment of groundwater, synthetic fuel production wastewater, and mine and mill effluents. Ammonium nitrogen is oxidized to nitrate aerobically by nitrifying bacteria and then anaerobically reduced to nitrogen gas by the denitrifying bacteria and thus removed from the effluent.

Ammonium is oxidized to nitrate primarily by chemolithotrophic bacteria which require only inorganic compounds for growth (Schmidt, 1982). The chemolithotroph, Nitrosomonas sp oxidizes ammonium to nitrite and Nitrobacter sp oxidizes nitrite to nitrate.

$$NH_4^+ \longrightarrow NO_2^- \longrightarrow NO_3^- \qquad (1)$$

The chemolithotrophic nitrifying bacteria can obtain all of their carbon by fixation of CO_2. Nitrosomonas has been shown to be inhibited by low concentrations of various organic compounds (Winogradsky, 1949). However, others (Clark and Schmidt, 1966, 1967) have shown that Nitrosomonas sp. can assimilate trace amounts of various organic compounds and are also not inhibited by them. The nitrifiers also have very slow growth rates (Belser, 1977; Bock, 1978).

Denitrification, which is also referred to as nitrate respiration is mediated by a number of bacterial species (Payne, 1981). Pseudomonas, Paracoccus, Flavobacterium, Alcaligenes and Bacillus spp. are among the genera known to denitrify. The nitrate ion is reduced to dinitrogen gas by the following pathway (Payne, 1981).

$$2NO_3^- \longrightarrow 2NO_2^- \longrightarrow 2NO \longrightarrow N_2O \longrightarrow N_2 \qquad (2)$$

Actively denitrifying cultures are frequently a mixture in which the overall denitrifying activity is the result of several species, each of which mediates one or more steps in the reaction sequence. Although the denitrifying bacteria are aerobic microorganisms, they can utilize oxidized nitrogen compounds as terminal electron acceptors in place of oxygen. Low oxygen concentrations or the absence of oxygen are required for denitrification to occur (Nelson and Knowles, 1978; Terai and Mori, 1975). Denitrifiers are generally nutritionally very versatile and can utilize a variety of carbon substrates (Buchanan and Gibbons, 1974). Both nitrification and denitrification are optimal at pH values near neutrality.

Denitrification systems have been designed for industrial and municipal wastewater. Two possible sequences for a nitrification/denitrification plant can be used (Furun et al., 1989). The first sequence, nitrification followed by denitrification usually requires the addition of an external carbon source to act as an electron donor for denitrification (Narkis et al., 1979). Methanol is the most frequently chosen carbon source due to its low cost and the low biomass yield from growth on this substrate. The other sequence is denitrification followed by nitrification in which a large portion of the nitrified effluent is recycled back to the denitrifying reactor has several advantages: (a) organics in the effluent can be utilized as a carbon source for denitrification which would reduce reagent costs; and (b) organics which might be toxic to the nitrifiers are removed in the first stage. The denitrification/nitrification sequence has the disadvantage of producing a final effluent containing appreciable nitrate concentrations and may not even be feasible if the waste stream is low in metabolizable organic compounds.

The most popular systems for large scale wastewater nitrification are: the continuously stirred tank reactor (CSTR)(Neytzell-DeWilde 1977a,b); packed towers, trickling filter (Grady and Lim, 1980); and the rotating biological contactor (RBC) (Grady and Lim, 1980). Other reactors that can be used for nitrification and denitrification are: the fluidized bed (Turner, 1988; Melcer and Nutt, 1988); and the suspended sludge system (Bridle et al., 1979). The CSTR provides the lowest ammonium concentration in the effluent but is somewhat more difficult and expensive to operate than other systems. The packed tower and RBC produce higher ammonium concentrations in the effluent (1-3 mg N/L) but are relatively simple to control and operate.

Both CSTRs and packed towers are used for denitrification but the packed tower is preferable due to its simplicity of operation. An innovative system that only requires one reactor has been developed for the removal of nitrogen from wastewater. The LINPOR™ process uses an activated sludge reactor containing open pore plastic foam cubes. Nitrification occurs on the surface of the cubes and denitrification occurs within the pores in the interior of the cubes (Reimann, 1990).

Due to the widespread use of ammonium nitrate-fuel oil (ANFO) as a blasting agent and nitrogen containing reagents in the mill, mine and mill effluents can contain appreciable quantities of ammonium and nitrate. The concentrations of both ions in these effluents can vary from 10-50 ppm for ammonia and between 25-300 ppm for nitrate. The concentration of both of these ions varies widely and is site specific. The discharge of both ammonium and nitrate will eventually be regulated. The objective of this study was to develop a system for the removal of both ammonium and nitrate from simulated mine and mill effluents. The studies reported in this paper focused primarily on denitrification because it was found to be more rapid and easier to control than nitrification. Also, many mine effluents contain only the nitrate ion.

MATERIALS AND METHODS

A synthetic feed, equivalent to the average composition of a number of selected tailings effluents were chosen for the study. The feed solution that was used had the following composition, g/L: KNO_3, 0.04-0.2; methanol 0.4; KH_2PO_4, 1.0; NaCl, 0.5; $MgSO_4$, 0.5. The feed composition was changed periodically with the objective of determining the effect of the influent N concentration and optimizing the carbon source. The pH of the solution was initially adjusted to 7.8 with CaOH.

Shake flask experiments were performed in 250 mL Erlenmeyer flasks with 200 mL of feed solution. These were placed in an incubator shaking at 150 rpm and controlled at 20°C. Inoculum for these flasks was 20 mL of effluent from the denitrification columns.

The denitrification columns (5.63 cm diameter x 74 cm long) were packed with fine gravel (+0.635-0.953 cm) and had a total volume of 1494 mL and a liquid volume of 950 mL. In order to determine concentration profiles, seven sample ports were located along the length of the column. The columns were operated in the upflow mode in order to maintain anaerobic conditions. The feed for the denitrification column was initially supplemented with glucose and methanol and once denitrification was established the glucose was removed.

The pH was measured on samples from the shake flasks and columns using a Fisher Accumet pH meter. Optical density was obtained by measuring the absorbance of the solution at 660 nm using a spectrophotometer. Nitrate analysis was performed with a High Performance Liquid Chromatograph (HPLC) on the samples after they were passed through 0.2 μm filters. The nitrate results are reported as nitrate and not total nitrogen as some other methods report. A gas chromatograph was used for methanol analysis. Residence times were calculated using the liquid volume of the reactor (i.e., 950 mL) as the basis.

RESULTS AND DISCUSSION

The preliminary shake flask studies showed a proportional decrease in both nitrate and methanol with time (Figure 1). The methanol to nitrate ratio is an important parameter because insufficient methanol will result in excess nitrate being discharged and excess methanol will produce an effluent with an unacceptably high BOD. The pH was observed to first decrease and then increase with time which is in agreement with theory. Nitrate reduction results in the production of hydroxyl ions which will increase the pH after the culture is established. Although flask studies are a rapid way of evaluating the effects of different parameters, the stoichiometry and kinetics of nitrate reduction will be different during continuous growth in a reactor containing established biomass than in batch culture.

In the packed bed reactor an exponential decrease in nitrate with height was observed (Figure 2). The pH also increased with height. Most of the nitrate was removed in the first half of the column, thus under the operating conditions of the column (1.5 h retention time, 120 ppm nitrate) the column was operating below capacity. However at higher nitrate concentrations only 70% of the nitrate was being removed (Figure 3). Other studies have shown 400-450 ppm is the upper limit for efficient operation of the column reactor. Even at high nitrate concentrations the column will adapt quite rapidly to changes in flow rate (Figure 4). Similar trends are observed at 4°C (Figure 5). The denitrification rates at 4°C are four to five times lower than rates at 23°C under comparable conditions which is typical for temperature effects on most chemical and biological reactions.

CONCLUSIONS

A packed bed reactor operated in the upflow mode was found to be suitable for the removal of nitrate from simulated mine effluents. An effluent low in nitrogen with very short retention times could be obtained with this system. The critical parameters for denitrification are nitrate concentration, temperature and flowrate (retention time).

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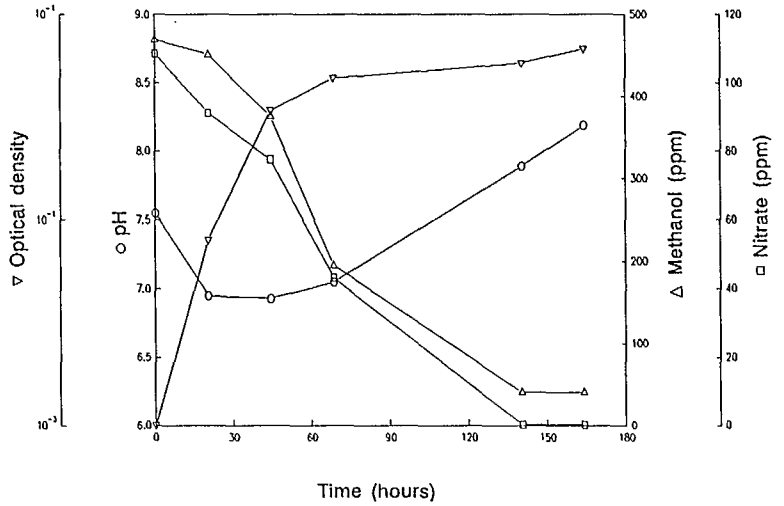

Figure 1: Batch denitrification of a synthetic nitrate solution at room temperature.

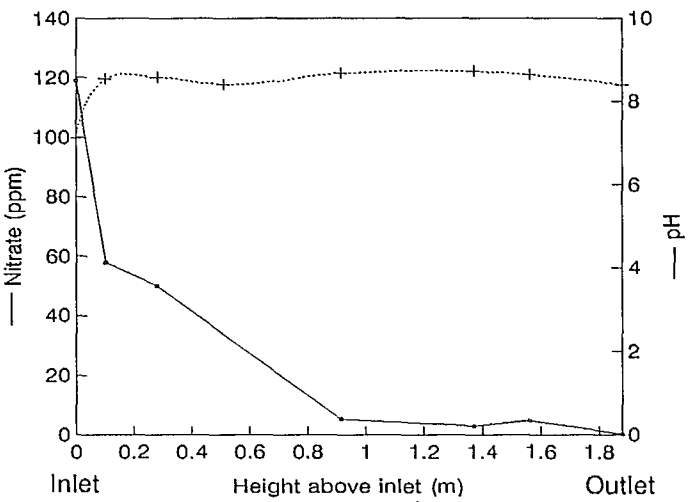

Figure 2: Denitrification in a packed bed reactor at room temperature.

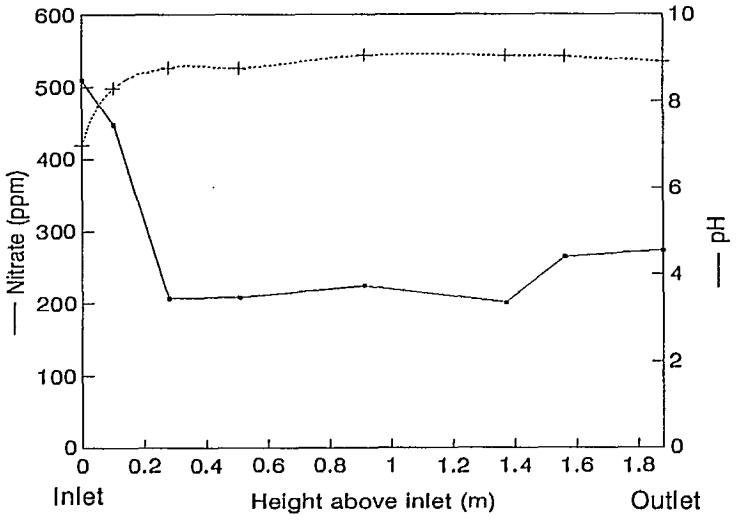

Figure 3: Denitrification in a packed bed reactor at room temperature.

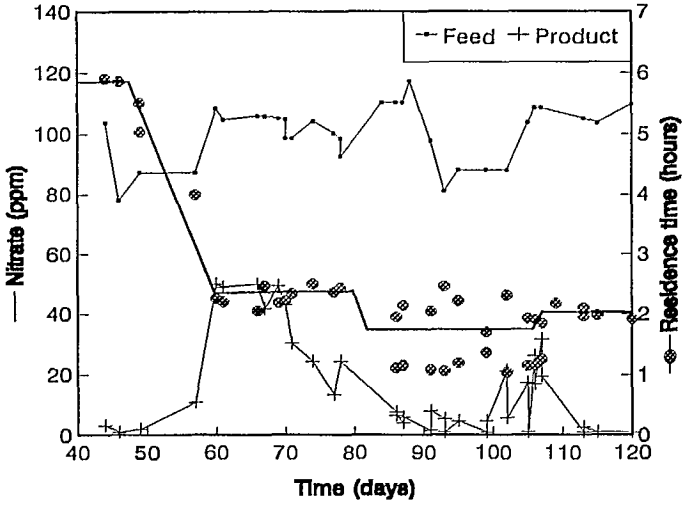

Figure 4: Denitrification in a packed bed reactor at 4^0C.

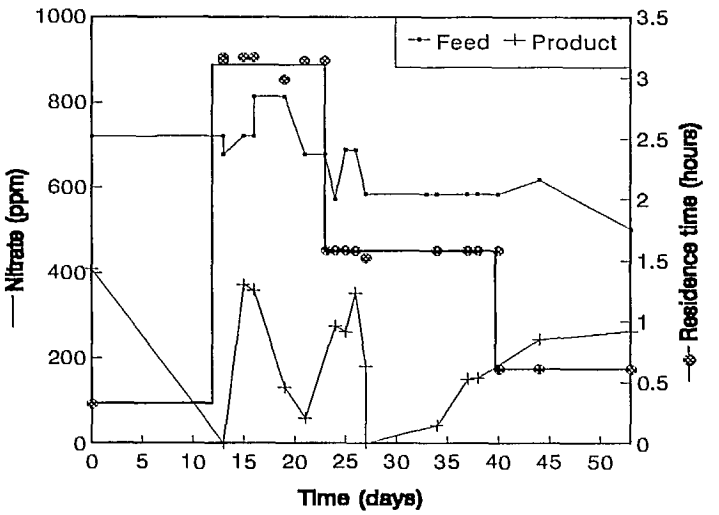

Figure 5: Denitrification in a packed bed reactor at room temperature.