# ARUM - ACID REDUCTION USING MICROBIOLOGY

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## Abstract

The ability of iron-oxidizing bacteria (e.g., *Thiobacillus* spp) to solubilize metals has been exploited in leaching processes by the mining industry. The same oxidation processes however, are the principal cause of a major environmental problem, the generation of acid rock drainage (ARD) and acid mine drainage (AMD) from pyrite-rich waste materials.

Remineralization of metals in AMD takes place in the sediments of oceans, lakes and wetlands through the activities of other types of bacteria. The main objective of the ARUM process is to optimize this mineralization in sediments created in mining waste management areas.

The most important parameter driving ARUM (or the remineralization process) is the flux of organic carbon from the water column to the microbial communities in the sediment. Various organic materials have been tested as sources of carbon for ARUM in AMD from base metal and coal operations. Carbon availability from materials such as peat, sawdust or **Typha** leaves depends on decomposition which is extremely limited in acidic, anoxic conditions where ARUM can occur. Weight loss from decomposition bags and sequential nutritional analysis were used to assess decomposition of test organic materials after prolonged exposure (up to two years) in AMD in ponds, lake enclosures (limnocorrals) or constructed ARUM test cells. The results indicate that decomposition does occur in the reducing conditions associated with ARUM and that of all the material tested, peat is the most promising and sawdust the least promising ARUM substrate.

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## Introduction

Mining wastes, tailings and waste rock, containing metals and reduced forms of sulfur are subject to oxidation on exposure to air. This leads to generation of sulfuric acid laden with metals and is referred to as acid mine drainage (AMD) or acid rock drainage (ARD). These effluents represent major environmental liabilities, particularly at the time the ore body is exhausted and mining ceases. Treatment through neutralization with lime is required which results in large volumes of hydroxide sludges. Acid generation is essentially a microbially aided weathering process and can be expected to continue for perpetuity.

Waste managements areas of mining operations generally contain large AMD collection ponds, where access of oxygen to the acid generating waste materials is not restricted. It is proposed that through the development of a microbially active sediment in such collection ponds, improvements in the AMD can be achieved. The processes which occur in the microbially active sediment are referred to collectively as ARUM, Acid Reduction Using Microbiology. Key aspects of the studies which were carried out towards the development the ARUM process are summarized in this paper.

In AMD, sulfate and dissolved metal ions, particularly iron, are abundant. In reducing conditions and provided that sources of carbon and other nutrients are available, iron reducing bacteria can reduce Fe(III) to Fe(II) and sulfate reducing bacteria can reduce sulfate to sulfide with the generation of alkalinity. If both substrates (Fe(III) and sulfate) are available, Fe(III) will be used up first (1). Most attention has been directed at sulphate reduction and this is potentially the most important source of alkalinity generation (2). Only recently has iron reduction by bacteria been characterised and the relative importance of these processes is only now being addressed (3). With alkalinity generation, as the pH rises, the sulfide will precipitate divalent metal cations such as $Cu^{2+}$, $Fe^{2+}$, $Ni^{2+}$ and $Zn^{2+}$ as metal sulfides. Thus metal concentrations in AMD can be reduced.

The alkalinity generating processes generally utilize organic acids as a carbon source. These can be provided from the decomposition products of plant materials. AMD itself is generally very low in soluble carbon. Therefore the provision of a continuous supply of decomposable organic material is a key factor in building an ARUM sediment.

Wetlands have been employed as a means to ameliorate AMD. Success of such wetlands is very variable (4,5). Cattails (Typha spp.) can thrive in AMD and wetlands to treat AMD are generally dominated by cattails. However, cattails do not accumulate high concentrations of metals in their biomass (6). The substrates of cattail dominated wetlands can harbour the alkalinity generating activities outlined above. Cattail wetlands remove significant metal loadings through alkalinity generating processes occurring in anaerobic conditions in the sediments. However carbon supplies are needed for the alkalinity generating processes in the ARUM sediment. Floating cattail mats can produce carbon through root exudation of recent photosynthate and from the decomposition of plant materials. Decomposition rates in cattail stands on acid generating tailings in northern Canadian conditions (7) are comparable to those for other temperate bog systems (8). Therefore these weedy acid tolerant species have the potential to provide considerable quantities of

substrate (e.g. volatile fatty acids) for the alkalinity generating bacteria. If configured over the ARUM sediment as a floating mat, further wave action and oxygen penetration to the sediment is reduced.

In order to initiate an ARUM sediment in waste water collection ponds, it is necessary to utilize organic materials which are available in large quantities and in relatively close proximity to the mine sites such as sawdust and peat. A large fraction (40-70 %) of these materials is lignocellulose which decomposes slowly. Decomposition of these highly refractory organic materials under acidic conditions may present the limiting component in the ARUM sediment development.

The present studies commenced following observations on test pools at Levack, Ontario amended with organic matter in a stream of AMD. Pockets of elevated pH were found. Samples were sent to CANMET and found to contain populations of sulfate reducing bacteria. Such observations led to the concept of ARUM in engineered ecosystems to provide a long term treatment of AMD. Further development of ARUM in the laboratory and field are summarised elsewhere (9-11). The work reported here summarises the types of AMD effluents in which development of an ARUM sediment is being tested. The supply of organic carbon measured by decomposition of various organic materials is investigated in AMD where reducing (alkalinity generating) conditions have or have not been established.

## Materials and Methods

Experimental systems have been set up in the field at various locations in Canada (Figure 1) to develop an ARUM sediment. The chemistry of the AMD to be treated at each site is summarised in Table I. Acidities range from 100 to 3080 mg/L equivalents of $CaCO_3$. Organic materials (cattail litter, peat, straw, alfalfa hay and sawdust were either suspended in AMD or placed on organic materials serving as ARUM substrate. At all the experimental sites, changes in water chemistry were monitored. Microbiological methods used are as described elsewhere (9,11). Sulfate reducing bacteria were enumerated with Rapidchek test kits (Conoco).

Experiments on decomposition were set up at Buchans, Newfoundland in two open Pits (A and B) form an inactive lead-copper-zinc mine (Asarco), in seepage from the Copper Cliff, Ontario tailings at the Makela pumping station from Inco's nickel mining and in a seepage in Elliot Lake from the inactive Stanrock operation of Denison Mines.

At Buchans, limnocorrals were constructed in May 1989 in two flooded pits. These experiments are described in more detail elsewhere (10). The limnocorrals are columns of plastic sheeting (Fabrene) of 4 m diameter and 2 to 3.5 m depth were anchored to the pit floor with rocks. Buoyancy was maintained with polystyrene floats around the circumference at the water surface. Either peat or sawdust was added to the iimnocorral water column at set up. The following summer, bags of window-screen containing 1 kg, 3 kg or 5 kg of the cattail litter, alfalfa, straw, sawdust or peat were placed in both peat and sawdust amended limnocorrals and in the open pits. These materials were chosen to test a wide range of organic materials for their suitability as substrates for ARUM. The bags were suspended

Figure 1 Site locations of ARUM field experiments

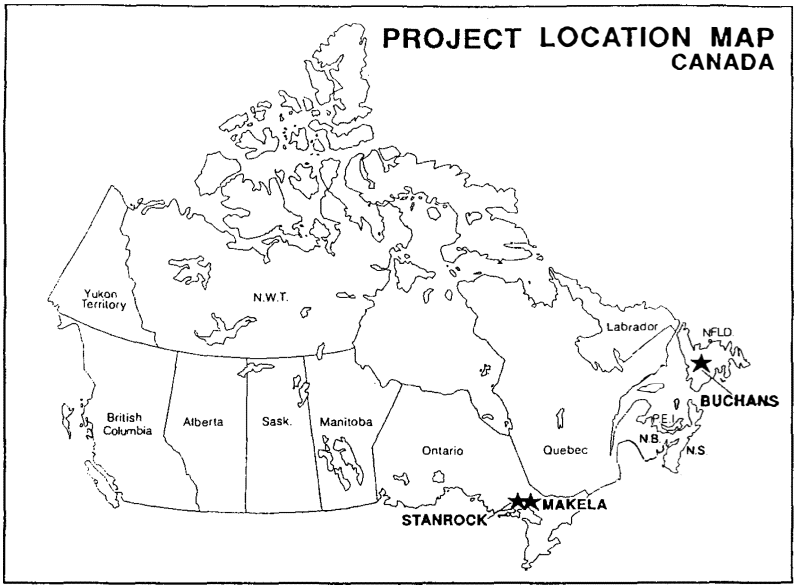


Table 1 ARUM field experiments. Acidity, pockets of elevated pH and microbiology

| Site | Acidity mg/L CaCO3 | pH orig | pH elev | Elevated pH pockets days | Microbiology SR | IRB | AM | DN |
|---|---|---|---|---|---|---|---|---|
| STANROCK | | | | | | | | |
| Straw Pond | 3080 | 2.1 | 5.5 | 365 | + | + | + | ND |
| MAKELA | | | | | | | | |
| Cell 3 | 520 | 3.0 | 3.5 | 350 | + | + | + | + |
| BUCHANS | | | | | | | | |
| Pit A | 120 | 3.5 | | | ND | ND | ND | ND |
| Pit A peat limnocorral | | 3.5 | 4.8 | 290 | + | + | + | ND |
| Pit A sawdust limnocorral | | 3.5 | 4.5 | 140 | + | + | + | ND |
| Pit B | 100 | 6.5 | | | ND | ND | ND | ND |
| Pit B peat limnocorral | | *3.2 | 4.7 | 143 | ND | ND | ND | ND |
| Pit B sawdust limnocorral | | 6.5 | 6.8 | 150 | ND | ND | ND | ND |

ND not determined; SRB sulfate reducing bacteria; IRB iron reducing bacteria
AM ammonifying bacteria; DN denitrifying bacteria
* pH dropped after addition of amendment

from booms suspended from the floating collar of the limnocorral or from Styrofoam floats in the open pits.

At Makela, a series of cells were constructed with flow control. The first cell is used for iron precipitation, the second for complete acidification of the AMD and the third and forth cells for ARUM sediment development. The decomposition bags containing each one of the five organic materials were placed in Cell 3. The Makela system generally experienced a flow rate of 1 l/minute. Floating cattails were established on Cell 3 and Cell 4 to assist the development of the ARUM sediment.

At Stanrock, a complete set of decomposition bags was placed on the surface of straw amendment in a shallow pond (Straw Pond) through which tailings drainage flows.

Between the placement of the decomposition bags and the first sampling date, ARUM was established above the sediment in the Makela cells, some of the limnocorrals but only for a short period in the uranium seepage. By the second sampling date (two years) reducing *conditions and ARUM (populations of sulfate reducing bacteria, $H_2S$ smell) activity were found throughout the water column in Cell 3 and in the active limnocorrals.

Bags were retrieved one year and two years after placement. The materials were air dried, weighed and subsamples were subjected to sequential nutritional analysis by the method of Goering and Van Soest (12). Acetone soluble fractions (waxes, fatty acids) and HCl soluble fractions (sugars, amino acids, hemicellulose, metal ions) were quantified. To determine the inorganic fraction which might have accumulated due to metal precipitation and adsorption, loss on ignition (LOI) was determined by heating one gram of ground organic matter at 480°C for one hour in a muffle furnace and remeasuring the sample dry weight.

## Results and Discussion

The development of ARUM activity at the study sites is summarised in Table I. In general, a period of 1 to two years was required before an increase in pH was registered, which generally occurred only in isolated packets of the organic material. The delay in ARUM is attributable to 1) the long time required to generate reducing conditions (requires the microbial-mediated decomposition of organic materials and the consumption of oxygen due to respiration by these microorganisms) and 2) the development of microbial consortia able to generate alkalinity in these conditions. Following commencement of ARUM (pockets of elevated pH) samples of materials were found to contain populations of sulfate reducers, iron reducers, ammonifiers and denitrifiers, all bacteria associated with alkalinity generating processes. Although a variety of organic materials was used in these studies microbial colonization did not differ with respect to the material. Identification of microbial consortia was restricted to presence /absence determination, which does not reflect metabolic activity.

Although weight loss of organic matter derived from bags with a known weight is a standard method of assessing decomposition rates, results require careful interpretation. Results for the Buchans bags after one and two years of exposure

are shown in Table II. After one year, the peat bags had lost more than 70 % of the original weight. Much of this large weight loss is certainly attributable to loss through the mesh of the bag. With coarser materials (straw, cattails and alfatfa) a smaller fraction is attributable to physical loss. Most bags lost about 50 % of their weight or greater during the first year of exposure. Assuming that physical weight loss will be the same for all bags of each amendment, the weight reductions in various incubation conditions can be compared.

Losses were greater for all materials in the peat limnocorrals which had an active ARUM sediment (reducing) compared to the sawdust limnocorrals which provided mostly oxidizing conditions. This indicates greater decomposition and therefore a potentially greater supply of substrates for ARUM bacteria. Weight losses in bags suspended in the open pit were generally similar to those in the peat limnocorrals. However these values are undoubtedly inflated due to losses of undecomposed material through the mesh by wave action. Weight losses in Pit B were higher than for Pit A for all materials except peat and straw. The greater losses in this pit are certainly related to the higher pH (pH 7) in this pit than in Pit A (pH 3.5) providing conditions more favourable for decomposition. Weight losses at Makela were generally less than at Buchans. In part it is likely due to the AMD characteristics at Makela which has higher acidity and lower pH than the AMD in Buchans (Table I). Very little if any weight loss was apparent after two years at Stanrock. This as attributable to the greater acidity (3080 mg/L equiv. $CaCO_3$). It should be pointed out that the Stanrock bags were covered with a heavy encrustation of iron (III) hydroxide at the time of sampling which may affect decomposition processes. Such precipitate covers may ARUM was very localized at this site.

Sequential analysis data for peat and sawdust sampled from the bag materials are summarised in Table III. The extractions segregate essentially refractory organic pools (remaining after HCl extraction) from those which are biodegradable or available as carbon sources (acetone and HCl extractions).

Acetone extractable materials (waxes, fatty acids) of both peat and sawdust declined on exposure to AMD in both pits at Buchans, at Stanrock and in the peat at Makela. There was no decline between the one and two year samples indicating that some factor, probably plant cuticles (surface waxy deposits) was lost in the first year of exposure. Part of the acetone soluble fraction is undoubtedly fatty acids which are products of decomposition and substrates for alkalinity generating bacteria. The reduction of this fraction was similar for the two Buchans pits. Within each pit there were no clear differences between the three locations. HCl extractable materials for both peat and sawdust exhibited a decline in Pit A and an increase in Pit B and also at Stanrock. A decline indicates that some store of material is being used up. An increase indicates that more materials are entering the pool than leaving. Perhaps greater cellulose decomposition is occurring in Pit B than in Pit A relative to the utilization of products of that decomposition. We established that cellulase activity was present in both pits using a remazol blue-stained cellulose strip assay (13). Unfortunately we were unable to quantify that activity. The LOI data (not shown) indicates that for Pit A most of the HCl extractable fraction is organic material and not metal ions or displaced sediment. However, for Pit B, the LOI values are lower and the apparent increases in HCl extractable material may be due to accumulation of precipitates of metal satts.

Table 3 Sequential nutritional analysis of decomposition bag samples
Figures represent % dry weight of a sample soluble in acetone or HCl

| Source | Peat | | | | | | Sawdust | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | At placement | | 1 year | | 2 years | | At placement | | 1 year | | 2 years | |
| | Acetone | HCl | Acetone | HCl | Acetone | HCl | Acetone | HCl | Acetone | HCl | Acetone | HCl |
| | % | % | % | % | % | % | % | % | % | 0 | % | % |
| BUCHANS | | | | | | | | | | | | |
| Pit A | 3.4 | 40 | 0.4 | 43 | 2.1 | 32 | 3.2 | 28 | 3.1 | 27 | 1.9 | 27 |
| Pit A-peat limnocorral | 3.4 | 40 | 3.0 | 38 | 2.0 | 37 | 3.2 | 28 | 2.7 | 26 | ND | ND |
| Pit A-sawdust limnocorral | 3.4 | 40 | 1.9 | 40 | 3.4 | 32 | 3.2 | 28 | 3.0 | 25 | 1.9 | 23 |
| Pit B | 3.4 | 40 | 2.0 | 47 | 1.7 | 44 | 3.2 | 28 | 2.9 | 28 | 1.7 | 30 |
| Pit B-peat limnocorral | 3.4 | 40 | 1.8 | 50 | 3.6 | 44 | 3.2 | 28 | 4.9 | 27 | ND | ND |
| Pit B-sawdust limnocorral | 3.4 | 40 | 1.0 | 44 | 3.4 | 44 | 3.2 | 28 | 4.2 | 27 | 2.9 | 31 |
| MAKELA | | | | | | | | | | | | |
| Cell 3 | 3.4 | 40 | 5.6 | 41 | ND | ND | 3.2 | 28 | 2.0 | 23 | ND | ND |
| STANROCK | | | | | | | | | | | | |
| Straw Pond | 3.4 | 40 | ND | ND | 2.6 | 50 | 3.2 | 28 | ND | ND | 1.5 | 29 |

ND not determined

Table 2 Weight loss of materials in decomposition bags after one and two years of exposure to AMD Losses are corrected for LOI values of materials before and after exposure

| | Weight loss (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Peat | | Sawdust | | Straw | | Cattail | | Alfalfa | |
| Site | 1 y | 2 y | 1 y | 2 y | 1 y | 2 y | 1 y | 2 y | 1 y | 2 y |
| BUCHANS | | | | | | | | | | |
| Pit A | **92** | ND | **74** | ND | **64** | ND | **79** | **80** | **75** | **72** |
| Pit A peat limnocorral | **78** | ND | **49** | ND | **67** | ND | **70** | **84** | **76** | **61** |
| Pit A sawdust limnocorral | **75** | ND | **45** | ND | **59** | ND | **70** | **64** | **58** | **81** |
| Pit B | **94** | ND | **76** | ND | **88** | ND | **59** | **47** | **67** | **68** |
| Pit B peat limnocorral | **78** | ND | **57** | ND | **69** | ND | **79** | **66** | **69** | **72** |
| Pit B sawdust limnocorral | **77** | ND | **38** | ND | **36** | ND | **68** | **58** | **63** | **69** |
| MAKELA | | | | | | | | | | |
| Cell 3 | **72** | **87** | **16** | **26** | **21** | ND | **49** | **42** | **32** | **31** |
| STANROCK | | | | | | | | | | |
| Straw Pond | ND | ND | ND | ND | ND | ND | ND | 15 | ND | **-13** |

ND not determined

In the Buchans peat limnocorrals and the Makela test cell, the decomposition bags were located in reducing conditions at the time of sampling. Decomposition rates decline rapidly as oxygen is consumed. Until recently it has been assumed that lignocellulose which comprises 40-70 % of the plant materials used in this study is not decomposed (14). However recent studies with long term incubations of 14-C labelled lignocellulose containing materials indicate that decomposition does occur albeit at low rates in anaerobic lake sediments (15). The weight loss and sequential analysis data clearly indicate that decomposition was occurring in the test conditions. The greater rates apparent in conditions where ARUM was active (Buchans peat limnocorrals and Makela test cells) suggest that even in reducing conditions, the materials can supply substrates for alkalinity generating bacteria. At Stanrock, where the conditions are extreme, low rates of decomposition may be limiting ARUM. Peat is the most promising material as it clearly supports ARUM in the limnocorrals and also accumulates substantial amounts of metals. Sawdust by contrast decomposes slowly (little weight loss) and accumulates little metal precipitate. This is confirmed by the poor performance, with respect to ARUM, of the sawdust limnocorrals.

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