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**LABORATORY STUDIES TO DETERMINE OPTIMAL  
CONDITIONS FOR THE ARUM PROCESS AT  
SELBAIE MINING:**

**CURRENT RESULTS AND  
RECOMMENDED LABORATORY PROGRAMME FOR  
1991 - 1994**

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## **1.0 GOALS OF AMENDMENT TESTS**

The purpose of the tests was to determine whether the microbial groups essential for alkalinity generation had been established at the amendment sites. Three amendment samples collected in September, 1990 from sites B2, B4 and C were analyzed, then incubated in closed containers to permit observation of alkalinity generation.

## **2.0 MATERIALS AND METHODS**

ATP and carbohydrate assays, direct microscopic counts, and tests for SRB'S, IRB'S and ammonifiers were performed as described in the April, 1990 ARUM report. Denitrifiers were enumerated as described in Methods of Soil Analyses (American Society of Agronomy, 1982) using the medium of Focht and Joseph (1973).

### **2.1 Observation of Alkalinity Generation**

To observe alkalinity generation, amendment samples were transferred to 1200 mL fleakers so that the headspace in each fleaker was reduced to two inches from the bottom of the fleaker seal. Amendment samples were stored for three weeks at 4°C before they were transferred to the fleakers. All fleakers were incubated at room temperature.

## **3.0 RESULTS AND DISCUSSION**

Results of microbiological profile analyses are summarized in Table I. The analyses revealed that the amendments had strongly stimulated microbial growth. Total bacteria counts were  $10^7$  per mL in every sample and significant levels of mold and algae were also present. Most of these microorganisms were alive as indicated by the high ATP content of each sample. Significant levels of all major alkalinity generating bacteria were present except for denitrifiers. Absence of denitrifiers suggests low nitrate levels in the water. The presence of iron and sulphate reducing bacteria indicates that cellulytic organisms and volatile fatty acid producers must also be present. Soluble carbohydrate was greater than 50 mg/L in all three samples.

Because alkalinity generating bacteria are present it is surprising that the samples pH were low and that after incubation only sample C produced significant alkalinity. The initial low pH of sample C may mean that on the date of sampling, the populations of alkalinity generators had not reached a critical concentration so that the influx of acid at this site was greater than the rate of alkalinity production.

However, additional problems must be occurring, especially with sample B2, since further incubation in a closed chamber did not result in alkalinity production. The water from, this site is a very strong acid mine drainage (AMD). We have found other AMD waters of similar strength required addition of special supplementary amendments to initiate the ARUM process.

#### **4.0 CONCLUSIONS**

Populations of alkalinity generating bacteria were present in all three samples. In sites C and B4, they may have not been adequately established to cope with the acid loading at the time of sampling. However, failure of the population in the sample from site B2 to generate alkalinity after incubation in a closed vessel suggests that a supplementary type of amendment may be required to initiate the ARUM process in this type of AMD.

#### **5.0 RECOMMENDED LABORATORY STUDY TO DEVELOP AN EFFECTIVE ARUM PROCESS FOR THE SELBAIE SITE.**

##### **5.1 Outline of 3 Year Programme**

Three phases each lasting one year are anticipated:

<u>Phase I (Jan. 1991-1992):</u>	Site Specific ARUM Process Optimization.
<u>Phase II (Jan. 1991-1993):</u>	Intensive Monitoring and Trouble Shooting.
<u>Phase III (Jan. 1993-1994):</u>	Maintenance Monitoring.

##### **5.2 Programme Details: Technical and Budget.**

### 5.2.1 Phase I (Jan. 1991-1992)

#### Identification of Amendment Supplements for Process Initiation.

Twenty-five treatments are presently being evaluated as treatments to initiate the ARUM process over the winter. During early 1991, this study will be expanded to include treatments which may stimulate methanogenesis, recently confirmed as another microbial alkalinity generating process. For example, low levels of nickel and cobalt, absent in the Selbaie water, are required for this process. In addition folic acid, which recently has become commercially available as a stimulant for methane production, will also be evaluated. Several of the most successful treatments will be studied under flow conditions to determine which treatment provides the most robust alkalinity generation.

#### Budget Costs:

Scientist:	4 days @ \$590 =	\$ 2,360
Senior Technologist:	20 days @ \$397 =	<u>\$ 7,940</u>
		<u>\$10,300</u>

#### Development of Algae as a High Performance Amendment.

Recent experiments in other AMD waters have indicated that algae may be more rapidly and thoroughly degraded than crop residues. Scientific literature has also been obtained which supports this observation.

After consultation with Boojum's algologist, six species of algae will be selected for evaluation. Acid sensitive and acid resistant species will be included. Acid sensitive species are likely to be degraded more rapidly when introduced into an acid environment but have the disadvantage of requiring an isolated ditch for cultivation. The acid resistant species are likely to have the opposite benefits and disadvantages.

The algae will be cultivated in site water then tested for their ability to provide improved alkalinity generation rates in Selbaie AMD. In addition sequential nutritional extraction analyses will be performed to evaluate this test for future field monitoring.

Budget Costs:

Scientist:	12 days @ \$590 =	\$7,080
Senior Technologist	45 days @ \$397 =	<u>\$17,865</u>
		<u>\$24,945</u>

Development of High Performance ARUM Microbial Consortium.

This component of the 1991-1992 programme will be coupled with the above components. A successfully operating flow reactor will be gradually subjected to increased acid loadings. In addition, the reactor will be regularly inoculated with microbial populations obtained from successful batch reactors. It is anticipated that this procedure will create the evolution of a high performance ARUM microbial consortium. This component of the programme will include an examination of volatile fatty acid species. This will determine if certain organic acids, such as propionate, play a detrimental role as observed in ecosystems of other related anaerobic wastewater treatment processes.

If this component of the programme is successful, a strategy for field scale-up strategy will be outlined.

Budget Cost:

Scientist:	6 days @ \$590 =	\$ 3,540
Senior Technologist:	30 days @ \$397 =	<u>\$11,910</u>
		<u>\$15,450</u>

## Effect of Thiobacillus Inhibitors on the ARUM Process.

It may be beneficial to reduce the amount of acid to be treated by ARUM by inhibiting its formation. However, it is unlikely that all sources of acid generation are accessible and therefore, only a partial reduction will be practical. Since a functioning ARUM process will still be necessary, the success of this approach requires that the Thiobacillus inhibitors not also inhibit the ARUM process. To evaluate the feasibility of this concept the effect of four Thiobacillus inhibitors on the ARUM process will be studied.

### Budget Costs:

Scientist:	4 days @ \$590 =	\$ 2,360
Senior Technologist:	15 days @ \$397 =	<u>\$ 5,955</u>
		<u>\$ 8,315</u>

### TOTAL BUDGET COSTS: - (1991-1992)

Scientist:	26 days @ \$590 =	\$15,340
Senior Technologist:	110 days @ \$397 =	<u>\$43,670</u>
<u>TOTAL:</u>		<u>\$50,010</u>

### **Phase II (Jan. 1992-1993).**

#### Monitoring and Trouble Shooting.

Scientist:	12 days @ \$590 =	\$ 7,080
Senior Technologist:	60 days @ \$397 =	<u>\$23,820</u>
		<u>\$30,900</u>

### **Phase III ((Jan. 1993-1994).**

#### Maintenance Monitoring

Scientist:	6 days @ \$590 =	\$ 3,540
Senior Technologist:	30 days @ \$397 =	<u>\$11,910</u>
		<u>\$15,450</u>

TABLE I

Microbiological Profiles of Selbaie ARUM  
Amendment Samples

	Sample Source		
	B2	B4	C
pH	2.3	2.9	2.8
ATP (ng/mL)	27	7.9	49
Total Bacteria Count (per mL)	$10^7$	$10^7$	$10^7$
Total Mold Count (per m/L)	$10^5$	$10^5$	$10^5$
Total Algae Count (per m/L)	$10^5$	$10^5$	$10^6$
SRB (Postgate B Medium) (per m/L)	$10^3$	$10^4$	$10^3$
SRB (Postgate F Medium) (per m/L)	$10^3$	$10^3$	$10^4$
IRB (per m/L)	$10^4$	$\geq 10^5$	$10^4$
Ammonifiers (per m/L)	$\geq 10^5$	$\geq 10^5$	$\geq 10^5$
Denitrifiers (per m/L)	<1	<1	<1
Soluble Carbohydrate (mg per L)	120	60	70

TABLE II

Selbaie:

Amendments for Water Columns

Sample Source	Initial pH	pH after incubation at ambient temperature		
		7 Days Incubation	14 Days Incubation	20 Days Incubation
B2	2.3	2.3	2.5	2.3
B4	2.9	3.1	3.7	3.4
C	2.8	3.5	5.0	5.3

Samples were stored for three weeks at 4°C before incubation at room temperature.