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LABORATORY STUDIES OF THE ARUM PROCESS: PROGRESS REPORT

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1.0 INTRODUCTION

The following is a progress report on the developmental programme of the ARUM process conducted at the Microbiology Services Laboratory of Dearborn Chemical Company Limited. It covers the period from April 1990 to December 1990.

2.0 MATERIALS AND METHODS

All chemicals, media and nutrient supplies were obtained from the sources described in the April, 1990 progress report except where indicated.

Most analytical methods also were described in this progress report. Any new methods are described in the sections below.

2.1 Makela Flow Experiments

Three water column reactors had been prepared as described in April 1990 Progress Report (Section 2.10). Three experiments were conducted using these reactors. Acidic water samped from the same Makela site was used in these experiments.

2.1.1 Makela Flow Experiment I

Acidic seepage water continued to be pumped into column reactor #3 at the intermittent rate of 2.1 mL every 30 minutes or approximately 100 mL per day and an equal volume simultaneously removed. This process continued until the pH decreased to 4.0. (Most of the alkalinity required to raise the pH to 4.0 occurs during this phase). Sulphate, nickel and iron concentrations continued to be monitored.

2.1.2 Makela Flow Experiment II

In the following experiment acidic seepage water was pumped into water column reactor #2 at an intermittent rate of 2.1 mL every 30 minutes or approximately 100 mL per day, and an equal volume was simultaneously removed.

The flow configuration was changed to emulate more closely field conditions, influent or acidic seepage was pumped into the middle port of the reactor and effluent was collected at the top of the water level. This process continued until pH decreased to below 4.

Samples obtained from the top, middle and bottom ports of the reactor were analyzed for the presence of microbiological groups including iron reducing bacteria, sulphate reducing bacteria, ammonifying bacteria and denitrifying bacteria.

2.1.3 Makela Flow Experiment III

The purpose of this experiment was to determine whether reactors previously exposed to excessive flow rates could recover and regenerate sufficient alkalinity to neutralize acidic seepage to above pH 4 under flow conditions.

Acidic seepage water was pumped into two water column reactors at an intermittent rate of 2.1 mL every 30 minutes or approximately 100 mL/day. The process continued until the pH decreased to below 4.0.

2.2 Denison Flow Experiments

Before flow experiments were initiated, the ARUM process was established in three water column reactors as follows:

The test amendments were added to three water column reactors. The order of addition to each reactor was gravel followed by amendment and acidic seepage water from the Denison seepage site.

The amendment portion consisted of a 1 cm layer of iron filings covered by a flax layer which filled two thirds of the reactor (ie. to a level of 1.5 L). Denison acidic seepage was then added to cover the amendment. The reactors then received 10 mL of a microbial seed from the Buchans Oriental East limnocorral site which contained sulphate reducing bacteria.

The acidic seepage water was allowed to stand at ambient temperature (22° C) for at least 24 h prior to addition to the reactors.

The reactors were incubated at ambient temperature (22° C) and were observed for blackening indicating the presence of sulphate reducing bacteria.

After 2 weeks and weekly thereafter, pH was determined.

2.2.1 Denison Flow Experiment I

Once blackening was observed and the pH became >5, acidic seepage water (allowed to stand at ambient temperature (22° C)) was pumped into two reactors (#1 and #3) at an intermittent rate of 2.1 mL every 30 minutes or approximately 100 mL/day and an equal volume was simultaneously removed. This process continued until pH decreased to below 4.0.

Microbiological profiles were performed on samples obtained from Denison reactor #1 and #3 prior to flow.

In addition, measurements of sulphate, nitrate, sulphide and total soluble carbohydrates were also determined prior to flow.

2.2.2 Denison Flow Experiment II

In the next experiment, the acidic seepage water was pumped into a "recovered" reactor (which generated sufficient alkalinity to neutralize the acidic seepage to above pH 5) at an intermittent rate of 2.1 mL every 30 minutes or approximately 100 mL/day. Acidic seepage was pumped into the middle port of the reactor and effluent was collected at the top of the water level. This process continued until pH decreased to below 4 (reactor was unable to maintain ARUM process).

In addition to pH, the influent and effluent were monitored for changes in sulphate, volatile fatty acids and total soluble carbohydrates.

A microbiological profile was performed on a sample obtained from the bottom port of the water column reactor.

2.3 Evaluation of Algae as an ARUM Amendment

This experiment examined the potential of algae to initiate alkalinity. Amendment test conditions were set up in 40 mL Wheaton vials. The order of addition was gravel (2 cm), amendment (1.0 cm) then acidic seepage water.

The treatments tested were as follows:

(i) algae (2 cm) and 2 mL seed;
(ii) 2 cm finely ground flax (ground in a Waring blender for 60 seconds) and 2 mL of seed.

The vial containing algae was covered with aluminum foil.

The seed was obtained from a sawdust amendment sample taken from the Oriental East limnocorral of the Buchans mine site, in Newfoundland. The vials were incubated at ambient temperature and observed for blackening. pH was also monitored.

2.4 Denison Acidic Seepage: Useful Forms of Iron for Process Initiation

A series of 40 mL Wheaton vials were prepared to determine whether rusted iron would be successful in the initiation of alkalinity.

The order of addition to the vials were gravel, amendment and Denison acidic seepage water supplemented with BOD mineral nutrients at the concentration applied for a standard BOD test (1).

The treatments were as follows:

(i) flax and 2 mm of iron filings;
(ii) flax and rusted iron filings;
(iii) flax and 10 de-greased rusted iron finishing nails.

The vials were incubated at ambient temperature and were observed for blackening.

2.5 Treatment of Denison Mine Seepage: Amendment Screening:

An assortment of treatments were utilized to determine whether successful alkalinity generation could be achieved.

The order of addition to 40 mL Wheaton vials was gravel, amendment and acidic seepage water. The treatments were as follows:

(i) flax;
(ii) flax and iron filings and 1 mL of seed;
(iii) flax, 1 mL seed and 200 ppm of $Ca(NO_3)_2$;
(iv) flax, 1 mL seed and 400 ppm $NaNO_3$;
(v) flax, 1 mL of seed and 500 ppm Na_2SO_3.

The flax was ground for 60 seconds in a Waring blender prior to addition.

Acidic acid seepage was allowed to stand at ambient temperature (22° C) for at least 24 h prior to its addition.

Mineral nutrients used for BOD analyses, as described in Standard Methods for the examination of Water and Wastewater 16th edition (1) were added to the vials.
The seed was obtained from the Buchans Oriental East limnocorral site which was

known to contain sulphate reducing bacteria.

The vials were incubated at ambient temperature. After a few weeks, pH was determined. The vials were further observed for blackening indicating the presence of sulphate reducing bacteria.

2.6 Denison Acid Seepage: Contribution of Iron and Amendment to Alkalinity

A number of test conditions were set up to determine whether the test amendment itself contributed to alkalinity generation.

A set of 40 mL Wheaton vials were set up. The order of addition was gravel, amendment and acidic seepage. The treatments were as follows:

(i) flax and iron filings;
(ii) flax;
(iii) iron filings.

The amendment/water vials were sterilized by a Tyndalization Method (8). The vials were incubated at 80° C for 10 minutes. This procedure is repeated three times over several days. The pH was monitored prior to heat shock, and following first and third heat shock.

2.7 Denison Alkalinity Generation Mechanism: Determination of Microbiological Group capable of Independently initiating alkalinity

Forty mL Wheaton vials were prepared to determine which microbiological groups were capable of initiating alkalinity generation.

The order of addition of amendment to the vials were 2 cm gravel, 2 cm finely ground flax, 2mm iron filings and Denison acidic seepage. The vials were then sterilized by the Tyndalization Method as described in Section 2.6.

After the final heat shock, pH-adjusted (pH 2.2) filter sterilized sodium lactate (3 g/L) was added to the vials which would be inoculated with sulphate reducing bacteria and iron reducing bacteria. Sodium lactate was added since it was necessary for the growth of these bacteria. The sodium lactate was also filter sterilized to ensure that only sulphate reducing bacteria or iron reducing bacteria were present in the test environment.

The vials to be inoculated with ammonifiers did not receive sodium lactate since the carbon source necessary for their growth was present in the vial. The vials were then inoculated with pure cultures of sulphate reducing bacteria, iron reducing bacteria and ammonifier bacteria (see Section 2.8 and 2.9 respectively).

The sulphate reducing bacterial cultures and ammonifier cultures were obtained from a sample from the middle port of Denison water column reactor #3. The iron reducing bacterial culture was obtained from a sample from the middle port of Makela water column reactor #3.

The pH of the vials before and 3 weeks followingbacterial inoculation was determined.

2.8 Mechanisms of Alkalinity Generation: Isolation of Pure Cultures of Sulphate Reducing Bacteria

Samples were taken from the top, middle and bottom port of Denison and Makela water column reactors. The samples were inoculated into Postgate B media and incubated at 28° C for 3 weeks. The vials were observed for blackening indicating the presence of sulphate reducing bacteria.

Positive cultures were re-inoculated into Postgate B media and incubated at 28" C for a further 3 weeks.

Postgate E medium was then prepared and cooled to 44.5° C. While molten, oxyrase (30 units/mL) was added to the media. The media was then poured into petri dishes and 1 mL samples of SRB cultures and then dilutions were added. The samples were then incubated in anaerobic pouches (Difco) at 28° C and observed for blackening. After a 3 week incubation period, blackened colonies appeared. Postgate B media was then inoculated with positive colonies and incubated at 28° C for 3 weeks. The cultures are presently being maintained.

2.9 Mechanisms of Alkalinity Generation: Isolation of Pure Cultures of Iron Reducing Bacteria and Ammonifiers

Samples were taken from the top, middle and bottom port of Denison and Makela water column reactors and inoculated into IRB and ammonifier media. The samples were incubated at 28° C for 3 weeks. Samples from positive tubes were plated on TGE agar and incubated at 28" C. Colonies were picked and re-inoculated into IRB and ammonifier media for positive confirmation. These cultures are presently being maintained.

2.10 Determination of Organic Acids

During anaerobic digestion of organic wastes, significant concentrations of volatile fatty acids are formed. They are the carbon sources for a variety of organisms including SRB's and IRB's.

Organic acid concentrations were determined by a colorimetric chemical method using ferric hydroxamate (2). Organic acids react with hydroxylamine to produce hydroxamic acid. The colour of the complex formed by reaction of the hydroxamic acids with ferric chloride is a measure of hydroxamic acid concentrations and thus of the original organic acid concentration. The colour reaction was measured by a spectrophotometric method

(505 nm) relative to reference organic acid standards. Acetic acid was used as the standard. Prior to analysis, samples were neutralized and filtered through 0.2 micron filter unit.

2.11 Preparation of Volatile Fatty Acid Producer Media

Volatile fatty acid (VFA) producer media was a modification of a medium (3) for the growth of Clostridium acetobutylicum (a species capable of producing volatile fatty acids from glucose) with the addition of 100 ppm sodium molybdate ($Na_2MoO_4.H_2O$).

After autoclaving, the media was cooled and oxyrase (enzyme which removes oxygen) was added (30 units/mL). The media was dispensed aseptically into sterile 15 mL serum vials containing a layer of sawdust and then capped.

Sodium molybdate inhibits the growth of sulphate reducing bacteria and iron reducing bacteria which would otherwise use the organic acids produced in the media. Sawdust was added as a substrate for organisms that are able to produce volatile fatty acids directly from cellulose.

At the end of the incubation period tests for volatile fatty acids were conducted as described in Section 2.10, using uninoculated media as the blank. The tests are usually conducted qualitatively.

2.12 Comparison of Sulphate Reducing Bacteria Enumeration Methods

Comparison of the Rapidchek® Sulphate Reducing Bacteria Detection System (Conoco Specialty Products Inc., Houston, Texas) versus conventional cultural test media were made. The assay is based on the fact that all sulphate reducing bacteria possess the enzyme APS reductase. The Rapidchek® SRB Detection System uses purified antibodies specific to this enzyme to detect the presence of sulphate reducing bacteria.

Various ARUM water and amendment samples were analyzed for the presence of

sulphate reducing bacteria. The water samples were filtered through 25 micron nitex screen and tested. The amendment samples were prepared in tap water. The vials were shaken and liquid was filtered though 25 micron nitex screen prior to analyses. In addition, 1 mL aliquots of each sample were inoculated into Postgate B media and serial dilutions were made.

2.13 Cellulose Decomposition

The cellulytic capability of the cellulose degrading population was determined by testing the capability of the microorganisms to degrade cellophane stained by Remazol brilliant blue **(4).**

Nylon screen bags containing Remazol Brilliant Blue (RBB) stained cellophane strips were placed in the top and bottom level of Makela Reactor #2. After a time period of 12 months the bags were removed and analyzed for the percent of cellulose decomposition. This was determined by measuring the stain relative to reference RBB stained cellophane strips taken from the same dye batch as the test strips.

2.14 Sequential Nutritional Analyses of Amendment

Sequential nutritional analyses of amendment following a 12 month ARUM operation in Makela acidic seepage water was performed.

A simplified version of the forage fibre analysis method (5) was used which involved a series of extraction steps.

Initially, the amendment was extracted with acetone which removed lipids and resins. This was followed by an hydrochloric acid (HCI) reflux step which removed soluble sugars, starch, amino acids, and hemicelluloses. Finally, a sulphuric acid digestion was performed on the amendment which removed the remaining cellulose. The samples were dried at 40" C over night and weighed between each extraction step. The percent loss from each treatment was determined.

2.15 Metabolic Activity in ARUM Water Column Reactors as Measured by Carbon Dioxide and Methane

ARUM water column reactors were analyzed for the production of methane and carbon dioxide (CO_2). Following flushing of the reactor headspace CO, metabolic activity was determined. Both CO, and methane in the samples were measured by gas chromatography.

3.0 RESULTS AND DISCUSSION

3.1 Makela Flow Experiments

Flow experiments were initiated after placing Makela acidic seepage water and amendment in reactor columns and allowing the ARUM process to raise pH and reduce sulphates.

Reactors which had generated sufficient alkalinity to neutralize seepage water to pH greater than 5 were utilized for flow experiments. Before feeding acidic seepage water into the reactor, it was allowed to stand at ambient temperature (22° C) for at least 24 h to permit oxidation and precipitation of ferrous iron.

A titration curve of Makela acidic seepage water is shown in Figure 1. As it can be seen most of the acidity in the sample is free mineral acidity and therefore during the neutralization process, most of the alkalinity is required to raise pH to pH 4.

3.1.1 Makela Flow Experiment I

Figure 2 shows the results of a flow experiment which was a continuation of an experiment described in the April, 1990 progress report. The experiment was conducted at flow rates of 100 mL per day. The reactor (#3) was the one which had recovered from excessive flow rate of 500 mL per day. At this flow rate, the ARUM process continued for 57 days and the bottom region of the reactor appeared black.

Nickel concentrations in the effluent were reduced to non-detectable limits except for the 4 sampling periods. Substantial reductions in nickel levels were also achieved in these periods. Nickel levels were 0.7 ppm, 0.5 ppm, 0.8 pprn and 2.70 ppm compared to influent levels of 18 ppm, 6.8 ppm, 13.3 pprn and 12.90 ppm. The appearance of detectable amounts of nickel in the reactor coincides with a decrease in pH as well as increased levels

of sulphates. Sulphate levels were also monitored. However, little sulphate reduction was apparent.

It appears that the capacity of the ARUM process was overcome by a stronger influent feed which resulted in failure of the reactor (unable to maintain pH above **4).**

3.1.2 Makela Flow Experiment II

In a similar experiment, flow rates of 100 mL/day were conducted. However, the flow configuration was altered to emulate more closely the field conditions.

The ARUM process continued for 121 days. Ni concentrations in the effluent were initially reduced to non-detectable units. The appearance of detectable amounts of nickel in the effluent not only coincides with increased levels of nickel but also increased level of SO_4^{2-} and acidity (Table 1).

After 82 days of operation, pH levels decreased to below **4** (Figure **3)** 100 ppm of $NaNO_3$ were added to the influent acidic seepage. By day **85,** pH increased to 6.9. It appears that $NaNO_3$ provided a necessary component or nutrient necessary for alkalinity generation to continue in addition to its contribution to alkalinity generation by denitrification. On day **113,** pH decreased to below **4.** 100 ppm of NH_4NO_3 was added to the reactor to stimulate alkalinity generation. Unfortunately, this treatment was unsuccessful.

It is difficult to assess why NH_4NO_3 failed to initiate alkalinity since its addition coincided with the sudden increase in acidity of the influent.

The pH stability of the effluent was also monitored. As shown in Figure

4, the samples removed after 57 days of reactor operation did not re-acidify. In fact, the **ARUM** process appeared to continue in the effluent sample since pH increased and was maintained above pH 4.

Microbiological profiles were performed on samples obtained from the flow system. Tables **2** and **3** are a summary of the results. The concentration of microbial groups responsible for alkalinity generation did not appear to change from one sampling period to another. This suggests that the failure of the reactor (unable to maintain pH>4) was not due to the death or elimination of the microbial groups.

Iron concentrations were also determined for the influent and effluent samples (Table 1). Levels are much lower than the previous experiment (Section 3.1.1). This was due to the fact that the acidic seepage was allowed to stand at ambient temperature for at least 24 h in open tubs to permit oxidation and precipitation of ferrous iron. It was then transferred to a second tub for further precipitation/oxidation.

Initially, iron was effectively reduced in the reactor. However, the appearance of ferric ion in the effluent suggests that incomplete reduction was occurring. This result coincides with detectable amounts of nickel, sulphate and increased acidity in the influent. It appears that the capacity of the **ARUM** process was overcome by **a** stronger influent feed, which may have resulted in failure of the reactor.

Nitrate concentrations were also determined. From Table 1, it appears that nitrate levels decreased in effluent samples suggesting that nitrate was effectively consumed or degraded in the reactor.

Similarly NH_4^+ levels decreased in the effluent when compared to influent

NH_4^+ concentrations (Table 1) suggesting a possible consumption or degradation of NH_4^+. However, NH_4^+ levels of 10.4 and 11.8 ppm appear in the influent acidic seepage (which does not contain NH_4^+), therefore, suggesting that a background level of approximately 10 ppm NH_4^+ was an artifact of the analytical procedure.

3.1.3 Makela Flow Experiment III

In a similar experiment, flow rates of 100 mL/day were conducted in both water column reactors.

Reactor #1 used in this trial was the one which had been exposed to excessive flow rates of 250 mL/day. Reactor #3 had previously been exposed to flow rates of 500 mL/day and 100 mL/day (April 1990 Progress Report, Section **3.2).**

At the flow rate of 100 mL/day the ARUM process continued for **27** and 21 days in Reactor **#1** and **#3** respectively. (Figure **5).**

Tables **4** and **5,** effluent samples removed on day **2** in Reactor #1 and #3 did not re-acidify, but rather pH was maintained for the following 25 days.

In contrast, effluent samples taken at a later date re-acidified. It appears that effluent samples with pH of less than 4.5 reacidify on standing (4.30 to 3.45 for Reactor #1 and 3.0 to 2.68 for Reactor #3). Similar results were observed in April 1990 Progress Report (Section 3.2).

Tables 4 and 5 also demonstrated that effluent samples with pH >5 did not re-acidify on standing. This suggests that a critical pH of approximately 5.0 is necessary for the maintenance and stability of the effluents from the ARUM process.

3.2 Denison Flow Experiments

The purpose of this experiment was to study the ARUM process in Denison acidic seepage under flow conditions. Until this experiment, all laboratory work for Denison had been performed in batch or static conditions.

Laboratory scale water column reactors were established with Denison water. Successful initiation of microbial alkalinity generation in Denison water was achieved (Table *6*).

100 ppm of $NaNO_3$ was added to reactor #1 and pH was monitored. The results are summarized in Table *6*. It appears that addition of the $NaNO_3$ did not change the rate of alkalinity generation in reactor #1 as compared to reactor #2 and #3.

A chemical profile was performed on samples obtained from reactors #1 and #3. Similar results were observed in both reactors (Tables 7 and 8).

A microbiological profile was also performed on samples obtained from the 2 reactors. The results are summarized in Table 9. All microbial groups are present in the reactor, although, some occur only in certain areas of the reactor. Large concentrations of iron reducing bacteria, ammonifiers and sulphate reducing bacteria were present. While the

denitrifier population was low, it was still present and would be capable of utilizing NO, as a nutrient source for growth. It is difficult to explain why the concentration of iron reducing bacteria in reactor #1 bottom port and reactor #3 middle port was very low.

3.2.1 Denison Flow Experiment I

Since the reactors had generated sufficient alkalinity to neutralize the seepage water to pH greater than 5, flow experiments were established on reactors #1 and #3.

Before feeding acidic seepage into the reactor, it was allowed to stand at ambient temperatures for at least 24 h to permit oxidization of ferrous iron and precipitation of ferric iron products.

As it can be seen in Figure ***6,*** a titration curve of the Denison acidic seepage demonstrates that during the neutralization process most of the alkalinity is required to raise the pH to **4.5.**

The acidic seepage was then pumped into both reactors at an intermittent rate of 2.1 mL every 30 minutes or approximately 100 mL/day and an equal volume was simultaneously removed. This process continued until the pH decreased to below pH **4.** The **ARUM** process continued for approximately 21 days (Figure 7).

Following a recovery period of 9 days, the reactor effluent increased to pH **4.5** suggesting that the alkalinity generating process was not irreversibly inhibited and thus the process was able to continue.

In summary, the reactors were unable to maintain the **ARUM** process during flow operation failing after the replacement of approximately one

reactor volume (1.5 L). In addition, pretreatment of the reactor with the low level of nitrate did not prevent failure of the reactor.

3.2.2 Denison Flow Experiment II

The purpose of the experiment was to determine whether a reactor which had previously been exposed to acidic seepage could regenerate alkalinity and maintain flow conditions at rate of 100 mL/day.

The Denison water column reactor #3 had previously been exposed to flow rates of 100 mL/day for 21 days. Following a recovery period of 1.5 months, a flow rate of 100 mL/day was established. The ARUM process continued for 14 days (Figure 8) approximately equal to the fluid retention time of the flow operation (15 days).

A microbiological profile was performed on samples taken before the initiation of the flow experiment and following the failure of the reactor (unable to maintain pH >4.0).

The results are summarized in Table 10. The concentrations of microbial groups remained constant except for the volatile fatty acid (VFA) producer population whose numbers had decreased.

A number of explanations are possible. The VFA producer population may have been very susceptible to the effects of the acidic seepage. Inhibition of growth by acidic mine drainage may explain the low numbers of VFA producers.

Another explanation may be found in Table 11 which demonstrated that carbohydrate (CH_2O) levels in the reactor prior to flow and following failure of the reactor were very low. Soluble carbohydrates may not be

available as a nutrient for the VFA producers either because of low production or as a result of consumption by other microbial populations.

Introduction of acidic seepage through flow conditions and simultaneous removal of effluent may have resulted in the displacement or washing out of bacteria, resulting in low numbers.

A chemical analyses of total soluble carbohydrates was performed (Table 11). There may be a number of reasons which explain the low carbohydrate levels. It may have been due to low production of carbohydrates in the reactor or the rate of consumption was faster than the rate of production of carbohydrates.

It is interesting to note that VFA producer population had decreased following failure of the reactor. Therefore, one would expect that CH_2O levels, would build up since VFA producers are not using the nutrient. But this was not the case. Therefore, this suggests that carbohydrate production in the reactor was low.

Sulphate concentrations in Denison #3 reactor prior to flow conditions and following failure of the reactor are summarized in Table 12. The results suggest that sulphate reduction may have occurred, if one compares influent levels to effluent levels. However, it may also be the case that diffusion of sulphate throughout the reactor was incomplete at the time of sampling.

Table 14 illustrates the metabolic activity in the reactor as measured by CO_2 production. Three days following flushing of the headspace of the reactor, CO, levels were measured. It should be noted that although the reactor had failed (unable to maintain pH >4) the reactor was able to

regenerate CO_2, indicating the presence of biological activity.

3.3 Evaluation of Algae as an *ARUM* Amendment

Benner et **al (6)** proposed that carbon from algae forms a major detrital component in aquatic system and could thus be utilized as a nutrient source.
Furthermore, Schoenberg et **al** (7) have studied the effects of acid stress on the decomposition of algae and found that algae decomposition was less sensitive to low pH than was the decomposition of lignocellulose.

Therefore, algae was evaluated as an alternative amendment and tested for its capability to initiate alkalinity generation in acidic seepage waters.

Successful initiation of alkalinity utilizing algae was achieved (Table 15). Furthermore, the algae amendment stimulated rapid growth of sulphate reducing bacteria within a two week period (blackening of the vial). The vial containing flax alone was also successful in initiating alkalinity within 2 weeks, but there was no evidence of blackening (indicating the presence of sulphate reducing bacteria) until day 21.

3.4 Denison Acidic Seepage: Useful Chemical Forms of Iron for Process Initiation

Experiments were performed to determine whether different chemical forms of iron were useful for the initiation of the alkalinity process.

Successful alkalinity generation was achieved by oxidized and elemental forms of iron. After a **3** week incubation period, vials containing iron filings and rusted iron filings were blackened indicating the presence of sulphate reducing bacteria. One week later, the vials containing rusted iron finishing nails were blackened.

3.5 Denison Acidic Seepage: Amendment Screening

This experiment tested an assortment of treatments for the initiation of alkalinity

generation. The treatments tested were flax alone, flax and iron filings, flax and $Ca(NO_3)_2$, flax and $NaNO_3$ and flax and Na_2SO_3. The results are summarized in Table 16. At the end of the experiment all treatment except for flax alone were capable of stimulating alkalinity generation.

$Ca(NO_3)_2$ and $NaNO_3$ were added as a nutrient in order to stimulate the denitrification process. Na_2SO_3 was added as a reducing agent.

Although alkalinity had been generated in the vial containing $NaNO_3$ by day 28, there was no evidence of sulphate reducing bacteria even by day 74. The addition of $Ca(NO_3)_2$ appears to elicit a stimulatory response resulting in alkalinity generation and growth of sulphate reducing bacteria (blackened vial) was observed by day 74.

The vial containing Na_2SO_3 was blackened by day 42 of incubation, indicating the presence of SRB.

The theoretical chemical contribution of each treatment to alkalinity generation was summarized in Table 26 and compared to the amount of alkalinity production observed in vials according to the titration curve shown in Figure 6.

Two different pH's were used to determine the meq of alkalinity produced. The pH of the acidic seepage water used in the experiment was 2.45. However, addition of flax to the vial raised the pH to 3.08. Therefore, it was important to determine the meq of alkalinity produced to raise the pH from both starting pH's.

The addition of $Ca(NO_3)_2$ contributed 2 meq of alkalinity, that is 8-20% of total alkalinity (based on meq produced to raise pH from 2.45 and 3.08, respectively). Chemical addition of $NaNO_3$ produced 5 meq of alkalinity representing 23% to 71% of total alkalinity generated. Similarly, Na_2SO_3 addition contributed 8 meq of alkalinity, that is, 30% - 73% of total alkalinity produced.

These results suggest that alkalinity generation is not due solely to the chemical addition of the treatments. But, in addition microbial processes are contributing to alkalinity generation.

These results may have additional significance, since until now, iron had been the only successful supplement for the initiation of alkalinity generation (April 1990 Progress Report, Section **3.1).**

3.6 Denison Acidic Seepage: Contribution of Iron and amendment to Alkalinity

This experiment examined the role of amendment in the initiation process. Amendment/water vials were sterilized by a Tyndalization method (8). Incubation of vials at 80° C for 10 minutes was repeated **3** times over several days allowing for spore germination to occur.

The Tyndalization method was chosen as the sterilization technique since sterilization by autoclaving may have resulted in chemical alteration or decomposition of the amendment.

The results of the experiment are summarized in Table 27 ~~18~~. Following the final heat treatment, the pH of the vial containing iron did not change demonstrating that iron does not contribute to alkalinity. Similarly, the flax alone did not contribute to the initiation of alkalinity. However, the pH of the vial containing flax and iron filings, after the third heat shock, did change. Although the pH did increase, it was still less than pH **3.**

3.7 Denison Alkalinity Generation: Determination of Microbiological Group capable of Independently Initiating Alkalinity

In order to determine which microbiological groups were capable of independently initiating alkalinity generation, vials containing gravel and acidic seepage were inoculated with pure cultures of sulphate reducing bacteria, iron reducing bacteria and

ammonifier bacteria. Results are summarized in Table 19.

Prior to inoculating the vials were sterilized by a Tyndalization method (See Section 3.6). Before the first heat shock, an ATP assay was conducted on the control vials containing iron filings and sodium lactate. It was found that they contained 2.1 ng and 2.6 ng ATP/mL. Following the third heat shock (before addition of the bacteria) the ATP level was found to be 0.41 and 0.46 ng ATP/mL for control vials 1 and 2.

Following a 3 week incubation period, ATP levels of control vials 1 and 2 decreased to 0.27 and 0.24 respectively, suggesting a lack of microbiological activity.

It was suspected that the low levels of ATP in the vials at the beginning of the experiment was due to the presence of residual ATP from stressed cells. The fact that the ATP levels decreased following a 3 week period was consistent with this theory. Although ATP levels remained low, an increase in pH was observed in the control vials. A possible explanation may be due to abiotic hydrolysis of the sodium lactate in the vial.

Blackening of the vial containing sulphate reducing bacterial culture was observed on day 6 following inoculation. Rapid blackening suggests, but does not prove, that sulphate reducing bacteria are capable of initiating alkalinity generation. There is uncertainty in this observation since there was evidence of inconsistent sterilization effectiveness (vial #9 following 3 week incubation period).

The pH of vial #5 containing an iron reducing bacterial culture, after a 3 week incubation period was pH 3.67. This value was similar to that of control values (pH 3.47 and pH 3.60). It appears that the bacterial culture was inactive in this vial. However, vigorous activity (bubbling) was observed in the duplicate vial (#6). Tests for the presence of iron reducing bacteria in vials #5 and #6 are to be confirmed at a later date.

An ATP assay was also conducted on control vials #7 and #8 containing iron filings alone. Before the beginning of the experiment, it was found that they contained 0.25 and 0.18 ng ATP per mL, respectively (comparable to control vials #1 and #2). However, following a 3 week period, **ATP** levels increased to ***0.68*** ng and **3.80** ng per mL respectively, and pH increased to pH 4.65. This suggests that the sterilization technique was ineffective and failed to prevent the growth of a population capable of producing alkalinity.

Inconsistent results were also observed in vials inoculated with ammonifier cultures. Evidence of blackening was observed in one vial indicating the presence of sulphate reducing bacteria. This demonstrated that the sterilization technique that was used was ineffective in preventing growth of alkalinity generating microorganisms.

The pH of vial #10 containing ammonifier culture, after a 3 week incubation period was pH 4.05. This pH value was similar to that of control (pH 4.10). It appears that the bacterial culture was inactive in this environment. Tests for the presence of ammonifier bacteria in vial #10 is to be confirmed at a later date.

In summary, it is unclear from these results which microbiological groups are capable of independently initiating alkalinity. Further experiments are required to define this phenomenon.

3.8 Comparison of SRB Enumeration Methods

Comparisons of the Conoco RapidchekB Sulphate Reducing Bacteria (SRB) Detection System versus conventional cultural media were performed.

The Conoco RapidchekB Detection System uses specific antibodies to detect the presence of SRB. However, the RapidchekB System is able to eliminate chemical interferences by sample pre-treatment steps. In addition, the RapidchekB System is convenient and requires only 20 minutes to obtain results.

It is also able to detect difficult-to-culture strains. Therefore, Rapidchek test results may give different estimated numbers than the cultural method. Results should show higher (or equal) numbers than the media.

Various ARUM water and amendment samples were analyzed for the presence of SRB by both test methods. The results of the comparison of SRB enumeration methods is summarized in Table ~~20~~ 29

The Rapidchek test shows higher or equal numbers of SRB when compared to cultural test method (Postgate B media) in all samples except for sample #4 taken from Buchans Oriental East limnocorral site F sawdust. Since higher or equal numbers of SRB are expected when using the Rapidchek due to its specificity, the reason for the low number by this method compared to cultural test method is unclear. Comparison tests on this sample have been repeated and are currently in progress.

3.9 Cellulose Decomposition

Cellulolytic capability of the decomposer community was determined by the Remazol Brilliant Blue (RBB) dye-assay **(4).**

Stamm et al **(9)** described RBB as a dye which bound to the cellulose molecule and is released quantitatively in proportion to glucose moieties. In the field it is more practicable to measure the dye remaining fast to the residual cellulose which can then be extracted by hot alkali treatment.

Nylon screen bags containing RBB stained cellophane strips were placed in the top and bottom level of the Makela reactors. After a **12** month time period, bags were removed and analyzed for the percent of cellular decomposition by hot alkali treatment. Control cellophane strips taken from he same dye batch as the test strips were also placed in nylon bags and the dye bound to the film was extracted by hot alkali treatment.
The percent of cellulose decomposition was determined by measuring the loss of stain

relative to reference RBB stained cellophane strips (control films). The absorbance reading of the dye extracts from the reference RBB stained cellophane strips were very reproducible (0.177 and 0.167).

The results are summarized in Table 21. The percent of cellulose decomposition estimated by the Remazol Brilliant Blue method in reactors #1 and #3 were 66%, 61%, 67% and **44%** for the top and bottom levels, respectively. However, the percent of cellulose decomposition that occurred in Makela reactor #2 was much lower; **33%** and **34%** at top and bottom levels.

As shown in Table 21, reactor #2 was exposed to a longer period of acidic seepage flow than the other 2 reactors. This long period of exposure to acid may have caused inhibition of the cellulose decomposer community. Although it was static for 2 months before the nylon bags containing RBB stained cellophane were removed, the community of cellulose degraders may have been unable to recover, thus the low percent of cellulose decomposition.

Makela rectors #1 and #3 were also exposed to acidic seepage flow, but for shorter periods of time. Inhibition by the acidic seepage may have also caused stress on the systems, ie. inhibited the cellulose decomposers. However, the static periods between flow, in addition to lower exposure times may have resulted in higher levels of cellulose decomposition.

3.10 Sequential Nutritional Analyses **of** Amendment

Sequential nutritional analyses of amendment following exposure to Makela acidic seepage water were performed. The procedure involved a number of extraction steps which removed the various constituents of the amendment. The results of the analyses are summarized in Table 22.

As the amendment is degraded during the ARUM process, it is expected that the soluble

sugars, starch, amino acids and hemicelluloses (most readily degradable constituents) would be depleted first and thus the percent loss from, the HC1 reflux step should decrease. However, this was not the case. It is surprising that the percent of rapidly degradable material had remained the same **(33%as** compared to **33%** and **38%** from control). In addition, the amount of slowly degrading material (cellulose) had decreased from **17-20%**(control amendment not been previously exposed to Makela acidic seepage water) to 3-4%. **This** may be due to partial degradation of the cellulose component which would result in removal of the partially digested material by the HC1 reflux step. Therefore, it was possible that the percent of rapidly degradable components (soluble sugars, starch, amino acids and hemicelluloses) could have decreased, but the addition of components by partial degradation of cellulose may have caused the percent loss through the HC1 reflux step to remain the same.

As the amendment is degraded by the ARUM process, the amount of non-degradable material, including lignin, cutin, silica and minerals should increase. The results demonstrated that the percent of material resistant to biodegradation following exposure to Makela acidic seepage water did increase from 35-41% (control amendment) to **56-58%.**

3.11 Metabolic activity in ARUM Water Column Reactors as Measured by CO, and Methane

Denison and Makela water column reactors were analyzed for the accumulation of CO, and methane gas. The reactor headspace was then flushed with air. Following a **3** day incubation period, CO_2 levels were determined.

CO, regeneration was used to rapidy demonstrate biological activity in the reactor. The results are summarized in Table **23** which shows that all ARUM water column reactors were able to regenerate CO_2, following the flushing, although to varying levels.

The most active reactor in terms of both methane accumulation and CO, production and regeneration was Denison reactor **#2** which was kept in static conditions for a period of

5 months.

3.12 Alkalinity Producing Reactions of ARUM

Biological alkalinity producing reactions have been listed for lakes (10), sediments (11, 12). Some or all of these potentially occur during the ARUM process. The following is a compilation of these reactions.

R1.1 Iron (as ferric hydroxide) reduction with carbohydrate carbon source (10):

$$(CH_2O)...+4Fe(OH)_3 \rightarrow CO_2+4Fe^{2+}+3H_2O+8OH^-$$

R1.2 Iron (as goethite) reduction with carbohydrate carbon source (11):

$$(CH_2O)...+4FeO(OH)+8H^+ \rightarrow CO_2+4Fe^{2+}+7H_2O$$

R1.3 Iron (as goethite) reduction with glucose as carbon source and lactate production (12):

$$6(CH_2O)+FeO(OH)+H_2O \rightarrow 3CO_2+Fe^{2+}+OH^-+CH_3CHOHCOO^-+3.5\ H_2$$

R1.4 Iron (as goethite) reduction with lactate as carbon source (12):

$$CH_3CHOHCOO^-+FeO(OH)+H_2O \rightarrow CO_2+Fe^{2+}+CH_3COO^-+CO_2+H_2+2OH^-$$

R2.1 Manganese reduction with carbohydrate as carbon source (10):

$$(CH_2O)...+2MnO_2+H_2O \rightarrow CO_2+2Mn^{2+}+4OH^-$$

R2.2 Manganese reduction with glucose as carbon source and lactate production (12):

$$6(CH_2O)+MnO_2 \rightarrow 3CO_2+Mn^{+2}+CH_3CHOHCOO^-+3/2H_2+OH^-+2H_2O$$

R2.3 Manganese reduction with lactate as carbon source (12):

$$CH_3CHOHCOO^-+H_2O+MnO_2 \rightarrow CO_2+Mn^{2+}+CH_3COO^-+2OH^-+(H)$$

R3.1 Ammonia production from ammonification of organic matter with methane production (10):

$(CH_2O)_{106}(NH_3)_{16}+16H_2O \rightarrow 37CO_2+53CH_4+16NH_4^{+}+16HCO_3^{-}$

R3.2 Ammonia production from ammonification of organic matter without methane production (11):

$(CH_2O)_{106}(NH_3)_{16}+106O_2+16H^{+} \rightarrow 106CO_2+16NH_4^{+}+106H_2O$

R3.3 Ammonia production from nitrate ammonification (11):

$2(CH_2O)...+NO_3^{-}+2H^{+} \rightarrow 2CO_2+NH_4^{+}+H_2O$

R3.4 Ammonia production from nitrate ammonification with glucose as carbon source (12):

$6(CH_2O)+3NO_3^{-}+3H^{+} \rightarrow 6CO_2+3NH_4^{+}+3OH^{-}$

R3.5 Ammonia Production from amino acid fermentation (Strickland reaction) (12):

$CH_3CH(NH_2)CO_2H+2H_2NCH_2CO_2H \rightarrow CO_2+3NH_4^{+}+3CH_3COO^{-}$

R3.6 Ammonia production from amino acid fermentation (single species reaction) (12)

$HOCH_2CH(NH_2)COOH \rightarrow CH_3COCOO^{-}+NH_4^{+}$

R4.1 Sulphate reduction using carbohydrate as carbon source with FeS formation (10):

$106(CH_2O)...+48SO_4^{2-}+48Fe(OH)_3 \rightarrow 48FeS+106CO_2+132H_2O+96OH^{-}$

R4.2 Sulphate reduction with carbohvdrate as carbon source (10):

$(CH_2O)...+0.5SO_4^{2-} \rightarrow CO_2+0.5H_2O+0.5HS^{-}+0.5OH^{-}$

R4.3 Sulphate reduction with carbohvdrate as carbon sources (11):

$2(CH_2O)...+SO_4^{2-}+2H^{+} \rightarrow 2CO_2+H_2S+2H_2O$

R3.1 Ammonia production from ammonification of organic matter with methane production (10):

$(CH_2O)_{106}(NH_3)_{16}+16H_2O \rightarrow 37CO_2+53CH_4+16NH_4^{+}+16HCO_3^{-}$

R3.2 Ammonia production from ammonification of organic matter without methane production (11):

$(CH_2O)_{106}(NH_3)_{16}+106O_2+16H^{+} \rightarrow 106CO_2+16NH_4^{+}+106H_2O$

R3.3 Ammonia production from nitrate ammonification (11):

$2(CH_2O)...+NO_3^{-}+2H^{+} \rightarrow 2CO_2+NH_4^{+}+H_2O$

R3.4 Ammonia production from nitrate ammonification with glucose as carbon source (12):

$6(CH_2O)+3NO_3^{-}+3H^{+} \rightarrow 6CO_2+3NH_4^{+}+3OH^{-}$

R3.5 Ammonia Production from amino acid fermentation (Strickland reaction) (12):

$CH_3CH(NH_2)CO_2H+2H_2NCH_2CO_2H \rightarrow CO_2+3NH_4^{+}+3CH_3COO^{-}$

R3.6 Ammonia production from amino acid fermentation (single species reaction) (12):

$HOCH_2CH(NH_2)COOH \rightarrow CH_3COCOO^{-}+NH_4^{+}$

R4.1 Sulphate reduction using carbohydrate as carbon source with FeS formation (10):

$106(CH_2O)...+48SO_4^{2-}+48Fe(OH)_3 \rightarrow 48FeS+106CO_2+132H_2O+96OH^{-}$

R4.2 Sulphate reduction with carbohydrate as carbon source (10):

$(CH_2O)...+0.5SO_4^{2-} \rightarrow CO_2+0.5H_2O+0.5HS^{-}+0.5OH^{-}$

R4.3 Sulphate reduction with carbohydrate as carbon sources (11):

$2(CH_2O)...+SO_4^{2-}+2H^{+} \rightarrow 2CO_2+H_2S+2H_2O$

R4.4 Sulphate reduction from lactate (12):

$$CH_3CHOHCOO^- + H_2 + SO_4^{2-} \rightarrow H_2S + CO_2 + CH_3COO^- + 2OH^-$$

5.1 Denitrification with organic matter as carbon source (10):

$$(CH_2O)_{106}(NH_3)_{16}... + 94.4NO_3^- + 94.4H^+ \rightarrow 55.2N_2 + 106CO_2 + 177.2H_2O$$

R5.2 Denitrification with carbohydrate as carbon source (11):

$$5(CH_2O)... + 4NO_3 + 4H^+ \rightarrow 5CO_2 + 2N_2 + 7H_2O$$

R5.3 Denitrification with glucose as carbon source (12):

$$6(CH_2O) + 4.8NO_3^- + 4.8H^+ \rightarrow 6CO_2 + 2.4N_2 + 8.4H_2O$$

R6.1 Photosynthetic production with the assimilation of nitrate (10):

$$106CO_2 + 16NO_3^- + HPO_4^{2-} + SO_4^{2-} + 106H_2O + 20H^+ \rightarrow (CH_2O)_{106}(NH_3)_{16}(H_3PO_4)H_2S + 140O_2$$

R6.2 Photosynthetic production with the assimilation of nitrate (12):

$$106CO_2 + 16NO_3^- + HPO_4^{2-} + 122H_2O + 18H^+ \rightarrow C_{106}H_{263}O_{110}N_{16}P + 138O_2$$

3.13 Contribution of Reactions to Alkalinity

The contributions of the various microbial reactions to alkalinity generation per weight of nutrient component consumed are listed in Table **24.** These values were calculated from the reactions listed in section **3.12.**

Using this table, one can calculate the contribution of microbial processes to alkalinity generation in Denison acidic seepage water. The amount of alkalinity produced by each process was first calculated by measuring the amount of sulphate and iron that had been reduced and ammonia produced at the end of the **4** month operation. Next the range of meq of alkalinity produced were calculated from Table **24.** The results are

summarized in Table **25.** Sulphate reduction generated **12** to **22** meq accounting for the production of **31** - 56.5% of total alkalinity. Iron reduction contributed **41%** of total alkalinity. Ammonia production generated **2.5** - **4.0%** of total alkalinity produced. Other microbial processes also listed in Table **24** contributed 0 - 10 meq of alkalinity, representing 0 - *26%* of alkalinity.

In addition, estimates of carbohydrate requirements for alkalinity generation can be determined from Table **24.**

Using this table one can calculate the amount of carbohydrate required for the generation of alkalinity in Makela acidic seepage. The amount of carbohydrate required for the generation of alkalinity was first calculated by measuring the amount of alkalinity produced during the experiment. Next the range of carbohydrate requirements were calculated from Table **24.**

The amount of alkalinity produced during Makela reactor **#2** flow experiment was **13.7** meq (Table **26).**

Therefore, if iron reduction (Table **24, R1.1, R1.2)** accounted for total alkalinity generated, 0.05 g of carbohydrates would be required. Similarly, if manganese reduction, ammonia production and denitrification reactions (Table **24, R2.1, R3.3, R5.2** and **R5.3)** contributed to alkalinity, 0.10 g to **0.51 g** of carbohydrates would need to be consumed for alkalinity generation to occur. Carbohydrate requirements for sulphate reduction reactions (Table **24, R4.1** - **R4.3)** would be 0.46 g to **1.24** g.

One can also determine whether the amount of amendment (carbohydrate) that was added was sufficient to generate alkalinity by the various microbial processes.

If we take for example, Makela water column reactor **#2** (Section **2.2)** approximately **30** g of straw/flax amendment was added to the reactor.

A sequential analysis of the amendment following a **12** month **ARUM** operation was determined. The results demonstrated that approximately **40%** of the amendment was degradable (Table **22).** Therefore **12** g of the amendment was available for degradation. Since **12** g is far in excess than the amount of carbohydrate requirements (calculated in the previous paragraph), one can assume that the amount of amendment added to the reactor was sufficient to generate alkalinity.

One can also calculate the amount of carbohydrate required for the generation of alkalinity in Denison acidic seepage. Since the amount of alkalinity produced by Denison reactor **#3** experiment was greater than Makela flow experiment, the carbohydrate requirements would be greater. It was calculated that 63.6 meq of alkalinity was produced by Denison reactor #3 flow experiment (Table **27).**

Therefore, if iron reduction (Table **24, R1.1, R1.2)** represented total alkalinity produced, **0.23** g of carbohydrate would be required. **0.49** g to **2.12** g of carbohydrate would need to be consumed if the reaction of manganese reduction, ammonia production and denitrification contributed to alkalinity (Table **24,** R2.1, R3.3, R5.2, and **R5.3).** Sulphate reduction reactions required **2.12** g to **3.70** g of carbohydrates (Table **24, R4.1 - R4.3)** to generate alkalinity. Again, the ammendment requirement was easily satisfied by the amount which had been added to the reactor.

4.0 SUMMARY AND CONCLUSIONS

- A stable flow operation of ARUM was achieved through water column reactors at flow rates of 100 mL Makela acidic seepage water per day. The longest Makela flow operation continued for 121 days.

- During stable Makela flow operation of ARUM the pH of the effluent was held above pH **4,** nickel was usually not detected (detection limit = **0.2** mg per L), sulphate reduction of ARUM was occurring and ferrous iron levels in the effluent were lowest.

- Introduction of sodium nitrate to Makela water colour reactor was able to stimulate alkalinity generation and further maintain successful operation of ARUM.

- Alkalinity generation could be initiated in water column reactors containing Denison mine acidic seepage water with amendments of iron metal and flax.

- A bench scale flow operation of the ARUM process was unsuccessful using Denison acidic seepage water at flow rates of 100 mL per day.

- During flow operation with Denison acidic seepage water, the ARUM process continued for a **2** to **3** week period (approximately equal to the fluid retention time of the flow operation) before the pH fell below pH 4.0.

- Following Denison flow operations, concentration of microbial populations remained constant except for the VFA producer population whose numbers had decreased. It is possible that the VFA producer population was susceptible to the effects of acidic seepage water.

- Both Makela and Denison water column reactors which had previously been exposed to acidic seepage during flow operation and failed were able to regenerate alkalinity during a static period.

- Successful initiation of alkalinity generation in 40 mL glass vials containing acidic seepage from Denison was demonstrated with the following amendments

  (i) algae
  (ii) 200 ppm $Ca(NO_3)_2$
  (iii) 500 ppm Na_2SO_3
  (iv) 400 ppm $NaNO_3$
  (v) Oxidized iron metal.

- Iron alone and flax, alone, did not significantly contribute to alkalinity generation through abiotic reactions.

- Pure cultures of alkalinity generating microorganisms including sulphate reducing bacteria, iron reducing bacteria and ammonifier bacteria were isolated and are presently being maintained.

- Evidence suggests that sulphate reducing bacteria are able to raise the pH of highly acidic seepage water (Denison) from less than 3.0 to pH 5.91, without the assistance of other bacteria.

- A colourmetric chemical method using ferric hydroxamate was found useful as a simple procedure for the determination of total organic acids.

- A volatile fatty acid producer media was developed.

- An evaluation of sulphate reducing bacteria enumeration methods was conducted.

Comparisons of the Conoco Rapidchek® SRB Detection System versus conventional test media were made. The Rapidchek® System is a simple, effective method which gave comparable semi-quantitative results.

- The cellulolytic capability of the cellulose degrading population was determined by testing the capability of the microorganisms to degrade cellophane stained by Remazol brilliant blue (RBB). The percent of cellulose decomposition that occurred in Makela water column reactor #2 following a 12 month **ARUM** operation was approximately **33%.**

- **A** simplified version of a forage fibre analysis method was used to determine the percent of degradable material available for biodegradation. Sequential nutritional analysis of amendment by this technique appears promising but requires validation by further experiments.

- Biological alkalinity producing reactions were summarized from the literature. Carbon requirements for each of these reactions were calculated. The percent contribution of these microbial processes to alkalinity generation was then calculated for an experiment in which Denison acidic seepage water was treated. In addition, examples of the range of total carbohydrate requirements for alkalinity generation were also calculated in both Denison and Makela acidic seepage water treatment experiments.

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TABLE 1 FLOW EXPERIMENT: MAKELA #2 REACTOR 100 mL/DAY

	Days of Operation													
	1	8	15	22	29	36	43	57	64	72*	%**	105	113***	119
pH influent	3	2.9	2.95	2.87	2.75	2.94	2.95	2.91	2.82	2.93	2.65	2.65	2.60	2.55
pH effluent	6.8	6.62	6.3	5.85	5.77	6.0	5.83	4.86	4.20	4.25	5.52	4.76	3.65	3.85
SO_4^{2-} influent (ppm)	1403	1471	1318	1220	1452	1141	1000	1227	1095	1019	4534	2900	3664	6254
SO_4^{2-} effluent (ppm)	1685	1351	1258	1244	1213	1042	1136	1017	1061	978	3986	2340	3132	6761
Ni influent (ppm)	12	13.6	13	11.4	12.7	10.2	10.2	11.4	10.0	83.2	36.2	51.6	56.6	57.1
Ni effluent (ppm)	3.0	0.7	0.7	0.5	<0.1	<0.1	0.2	0.20	0.70	11.7	<0.2	14.3	2.81	15.4
Fe^{3+}/Fe^{2+} influent	35/0	7/0	7/0	5.3/0	5.3/0	5.3/0	<4/0	<4/0	<4/0	<4/0	17.5/35	35/44	14/88	140/123
Fe^{3+}/Fe^{2+} effluent	0/123	0/<4	0/<4.0	0/8.8	0/7	0/7	0/7	0/8.8	0/7	0/7	0/44	9/61	0/193	0/230
NO_3 influent (ppm)							-			-	64	11.0	16.7	87
NO_3 effluent (ppm)		-	-							-	4.2	0.9	1.05	2.5
NH_4^+ influent	-			-			-				-	10.4	11.8	80
NH_4^+ effluent										-	-	9.7	12.2	20
S^{2-} influent			N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
S^{2-} effluent			N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.20	0.10

Note: N.D. = not detected
*Add 100 ppm of $NaNO_3$ on Day 82
**Stop addition of $NaNO_3$ on Day 99
***Add 100 ppm NH_4NO_3

TABLE 2 MICROBIOLOGICAL PROFILE OF SAMPLES OBTAINED FROM MAKELA #2 REACTOR ON DAY 99 FOLLOWING TERMINATION OF $NaNO_3$ TREATMENT

	Iron Reducing Bacteria per mL	Ammonifiers per mL	Sulphate Reducing Bacteria per mL (Postgate B media)	Sulphate Reducing Bacteria per mL (Postgate F media)	Denitrifers per mL
Top Port	$\geq 10^5$	2×10^5	$\geq 10^4$	10^3	10^4
Middle Port	$\geq 10^5$	2×10^5	$\geq 10^4$	10^2	10^4
Bottom Port	$\geq 10^5$	2×10^5	$\geq 10^4$	10^2	10^3

TABLE 3 MICROBIOLOGICAL PROFILE OF SAMPLES OBTAINED FROM MAKELA #2 REACTOR ON DAY 113 PRIOR TO NH_4NO_3 ADDITION

	Iron Reducing Bacteria per mL	Ammonifiers per mL	Sulphate Reducing Bacteria per mL (Postgate B media)	Sulphate Reducing Bacteria per mL (Postgate F media)	Denitrifers per mL
Top Port	10	$\geq 10^4$	$\geq 10^4$	10^2	10^4
Middle Port	$\geq 10^4$	2×10^4	$\geq 10^4$	10^3	10^2
Bottom Port	$\geq 10^4$	$\geq 10^4$	$\geq 10^4$	10^2	10^3

TABLE 4 STABILITY OF EFFLUENT pH: MAKELA REACTOR #1 (SITE FLOW CONFIGURATION)

	Effluent Sample No.		
Days of Operation	1	2	3
2	*6.40	-	-
8	6.58	-	-
15	6.61	*5.45	-
22	6.55	5.75	*4.30
27	6.60	5.80	3.45

TABLE 5 STABILITY OF EFFLUENT pH: MAKELA REACTOR #3 (SITE FLOW CONFIGURATION)

	Effluent Sample No.		
Days of Operation	1	2	3
2	*6.22	-	-
8	6.00	-	-
15	-	*3.00	-
22	-	2.72	*2.81
27	6.00	2.68	2.67

TABLE 6 DENISON REACTOR EXPERIMENTS - pH PROFILES

Days of Incubation	Reactor #1	Reactor #2	Reactor #3
0	2.70	2.70	2.70
14	3.85	4.48	3.93
	Added 100 ppm $NaNO_3$ to Middle Port	Added Nothing	Added Nothing
21	*4.75	*4.95	*4.81
26	*5.08	*5.16	*5.12
33	*5.77	-	*5.68
40	*5.55	-	*6.10

*Blackening observed in reactor indicating the presence of sulphate reducing bacteria

TABLE7 CHEMICAL PROFILE OF SAMPLES OBTAINED FROM DENISON REACTOR #1 PRIOR TO FLOW

Sample	Total Soluble Carbohydrate (ppm)	Nitrate (ppm)	Sulphate (ppm)	Sulphide (ppm
Top Port	47	9.28	947	N.D.
Middle Port	48	7.55	921	<0.10
Bottom Port	30	12.91	941	0.95

TABLE8 CHEMICAL PROFILE OF SAMPLES OBTAINED FROM DENISON REACTOR #3 PRIOR TO FLOW

Sample	Total Soluble Carbohydrate (ppm)	Nitrate (ppm)	Sulphate (ppm)	Sulphide (ppm)
Top Port	53	13.4	915	<0.10
Middle Port	**38**	14.2	875	<0.10
Bottom Port	**15**	17.1	1095	0.92

N.D. = Not detected

TABLE 9 MICROBIOLOGICAL PROFILE OF SAMPLES OBTAINED FROM DENISON REACTORS 1 AND 3 PRIOR TO FLOW

Sample	Iron Reducing Bacteria per mL	Ammonifier per mL	Sulphate Reducing Bacteria per mL (Postgate B media)	Sulphate Reducing Bacteria per mL (Postgate F Media)	Denitrifiers per mL
Denison #1 Top Port	2×10^4	2×10^4	2×10^4	10^2	<1
Denison #1 Middle Port	$\geq 10^4$	2×10^4	2×10^4	10^2	10
Denison #1 Bottom Port	<1	$\geq 1 \times 10^4$	2×10^4	10^3	10
Denison #3 Top Port	2×10^4	$\geq 10^4$	2×10^4	10^2	<1
Denison #3 Middle Port	<1	2×10^4	2×10^4	10	10
Denison #3 Bottom Port	2×10^4	2×10^4	2×10^4	10^3	<1

TABLE 10 MICROBIOLOGICAL PROFILE OF SAMPLES OBTAINED FROM DENISON RECTOR #3

Sample	Iron Reducing Bacteria Per mL	Ammonifiers Per mL	Sulphate Reducing Bacteria Per mL (Postgate B Media)	Sulphate Reducing Bacteria Per mL (Postgate F Media)	Denitrifiers Per mL	Volatile Fatty Acid Producers Per mL	ATP (ng/mL)
Bottom Port (before initiation of flow)	10^3	10^2	10^4	10^2	<1	$\geq 10^5$	2.1
Bottom Port (at failure of reactor)*	10	10^2	10^4	10^3	<1	<1	0.40

*Flow to reactor was turned off on failure (unable to maintain pH >4.0)

TABLE 11 TOTAL SOLUBLE CARBOHYDRATE ANALYSES IN DENISON REACTOR #3

	Total Soluble Carbohydrate (ppm)		
Sample	Before Initiation Of Flow	At Failure Of Reactor*	10 Days After Failure Of Reactor
Top Port	N.A.	N.A.	N.A.
Middle Port	<10	<10	<10
Bottom Port	<10	<10	<10

TABLE 12 SULPHATE ANALYSES IN DENISON REACTOR #3

	Total Sulphates (ppm)		
Sample	Before Initiation Of Flow	At Failure Of Reactor*	10 Days After Failure of Reactor*
Top Port	1282	2227	1828
Middle Port	1225	2545	1916
Bottom Port	N.A.	N.A.	2035
Influent	1919	3114	N.A.

TABLE 13 TOTAL VOLATILE FATTY ACIDS IN DENISON REACTOR #3

	Total Volatile Fatty Acids (ppm)		
Sample	Before Initiation Of Flow	At Failure of Reactor*	10 Days After Failure of Reactor*
Top Port	N.A.	N.A.	N.A.
Middle Port	N.A.	N.A.	N.A.
Bottom Port	270	<200	<200

Note: N.A. = not analyzed.
*Flow to Reactor was turned off on failure (unable to maintain pH >4.0)

TABLE 14 METABOLIC ACTIVITY IN DENISON REACTOR #3 MEASURED BY CO_2 PRODUCTION

	CO_2 (ppm)
10 days after reactor failure*	1900
Following flushing of reactor headspace	380
3 days following flush**	1720

*Flow to reactor was turned off on failure (unable to maintain pH >4.0)
**Reactor pH at top port = 2.8 (ie. still <4.0)

TABLE 15 PRELIMINARY EVALUATION OF ALGAE AS AN AMENDMENT FOR THE ARUM PROCESS

Amendment		pH at beginning of test	pH after 14 days	pH after 21 days
1	Flax	3.50	5.13	*6.22
2	Algae	3.50	*6.52	*6.93

TABLE 16 **RESULTS** OF SCREENING TESTS TO DEVELOP ALKALINITY GENERATION IN DENISON WATER

Amendment		pH after 28 days	pH after 42 days	pH after 74 days
1	Flax	3.08	2.97	3.01
2	Flax and Iron Filings	*6.52	*6.65	-
3	**Flax** and Iron Filings	*6.42	*6.45	-
4	Flax and 400 ppm $NaNO_3$	3.27	3.41	3.76
5	Flax and 200 ppm $Ca(NO_3)_2$	3.81	4.05	*6.75
6	Flax and 500 ppm Na_2SO_3	4.23	*5.45	*6.52

*Blackening of vial observed indicating the presence of sulphate reducing bacteria

Note: pH of water added to vial = 2.45

TABLE 17 TOTAL VS. THEORETICAL CHEMICAL CONTRIBUTION TO ALKALINITY FROM ARUM TREATMENTS WITH DENISON WATER

Treatment	pH After 28 Days	Meq of Alkalinity Produced to Raise pH from		Theoretical Alkalinity (meq) Contributed by Added Cations*
		2.45	3.08	
200 ppm $Ca(NO_3)_2$	3.81	25	10	2
400 ppm $NaNO_3$	3.27	22	7	5
500 ppm Na_2SO_3	4.23	27	11	8

*This is the alkalinity which would be produced if the anion of the treatment was consumed or lost by non-alkalinity generating abiotic processes (eg. volatization)

TABLE18 TREATMENT OF DENISON ACIDIC SEEPAGE: MECHANISMS OF ALKALINITY GENERATION, EXPERIMENTAL CONTROLS

		pH		
No.	Amendment	Before Heat Shock*	Following First Heat Shock*	Following Third Heat Shock*
1	Flax and iron filings	2.23	2.46	2.81
2	Flax	2.22	2.23	2.35
3	Iron filings	2.25	2.15	2.24

Note: All vials contained gravel and Denison acidic seepage water.
* Heat shock was 10 minutes at 80° C. Intervals between heat shock was 2 days.

TABLE 19 DETERMINATION OF MICROBIOLOGICAL GROUPS CAPABLE OF INDEPENDENTLY INITIATING ALKALINITY

Amendment	Vial #	ATP (ng/mL) Before Addition Of Bacteria	pH Before Addition of Bacteria	pH 3 Weeks After Addition Of Bacteria	ATP (ng/mL) 3 Weeks After Addition Of Bacteria
Iron filings and sodium lactate	1	0.41	<3.0	3.47	0.27
Iron filings and sodium lactate (Control)	2	0.46	<3.0	3.60	0.24
Iron filings, sodium lactate and 1 mL of sulphate reducing bacterial culture	3	0.33	<3.0	**5.91	-
	4	0.45	<3.0	*5.64	-
Iron filings, sodium lactate and 1 mL of iron reducing bacterial culture	5	0.41	<3.0	3.67	-
	6	0.33	<3.0	4.78	-
Iron filings	7	0.25	<3.0	4.10	0.68
(Control)	8	0.18	<3.0	4.65	3.80
Iron filings and 1 mL of ammonifier bacterial culture	9	0.16	<3.0	*5.30	-
	10	0.41	<3.0	4.05	-

* Vials were blackened indicating the presence of sulphate reducing bacteria
** Vial was blackened 6 days after addition of bacteria

Notes:

1. All vials contained gravel, flax and Denison acidic seepage water.
2. Test for the presence of iron reducing bacteria in vials #5 and #6 to be confirmed.
3. Tests for the presence of ammonifier bacteria in vial #10 to be confirmed.

TABLE 20 COMPARISON OF SULPHATE REDUCING BACTERIA ENUMERATION METHODS

Sample Description		Rapidchek® SRB Detection System (Sulphate Reducing Bacteria per mL)	Postgate B Media (Sulphate Reducing Bacteria per mL)
1	Denison water column reactor #3 (blackening observed)	10^4	10^4
2	Denison water column reactor (no evidence of blackening and no alkalinity generation produced for **1.5** years)	$<10^3$	10^2
3	Selbaie C amendment	10^5	10^4
4	Buchans Oriental East limnocorral site F sawdust	10^3	10^5
	Buchans Oriental East limnocorral site F sawdust diluted 10X	$<10^3$	(10^4)*
5	Buchans Oriental East P. amendment behind curtain	10^3	10^3
	Buchans Oriental East P. amendment behind curtain diluted 10X	$<10^3$	(10^2)*
6	Makela arumator site **3** amendment	$<10^3$	10^2

*Calculated from results of tests on undiluted sample

TABLE 21 CELLULOSE DECOMPOSITION IN MAKELA WATER COLUMN REACTORS ESTIMATED BY REMAZOL BRILLIANT BLUE METHOD

	% Of Cellulose Decomp-osition	Profile Of Reactor Treatment
Reactor	66	(i) Static for 2 months (ii) Acidic seepage pumped into reactor at rate of 250 mL/day for 23 days (iii) Static for 6 months (iv) Acidic seepage pumped at rate of 100 mL/day for 27 days (v) Static for 2 months
Makela Reactor #1 Top Port		
Makela Reactor #1 Bottom Port	61	
Makela Reactor #2 Top Port	33	(i) Static for 4.0 months (ii) Acidic seepage pumped into reactor at rate of 100 mL/day for 121 days (iii) Static for 3.5 months
Makela Reactor #2 Bottom Port	34	
Makela Reactor #3 Top Port	67	(i) Acidic seepage pumped into reactor at rate of 500 mL/day for 5 days (ii) Static for 1.5 months (iii) Acidic seepage pumped into reactor at rate of 100 mL/day for 57 days (iv) Static for 5 months (v) Acidic seepage at rate of 100 mL/day for 21 days (vi) Static for 2 months
Makela Reactor #3 Bottom Port	44	

Note: Nylon screen bags containing Remazol Brilliant Blue (RBB) stained cellophane strips (1 cm x 5 cm) were placed in the top and bottom level of the reactor. After a time period of 12 months, the bags were analyzed for the percent of cellulose decomposition. This was determined by measuring the loss of stain relative to reference RBB stained cellophane strips taken from the same dye batch as the test strips. The absorbance reading of the dye extracts from duplicate reference RBB stained cellophane strips were 0.177 and 0.167.

TABLE 22 SEQUENTIAL NUTRITIONAL ANALYSES OF AMENDMENT FOLLOWING A 12 MONTH ARUM OPERATION IN MAKELA ACIDIC SEEPAGE WATER

	Composition of Amendment				
Amendment	% Loss From Acetone Extraction (Includes Lipids And Resins)	% Loss From HC1 Reflux (Includes Soluble Sugars, Starch, Amino Acids And Hemicellulose)	% Loss From H_2SO_4 Digestion (Includes Cellulose)	% Remaining as Lignin, Cutin, Silica And Minerals	% Total Degradables
Control: Straw (analysis #1)	6	33	20	41	59
Control: Straw (analysis #2)	6	33	20	41	59
Control: Flax (analysis #1)	8	38	17	37	63
Control: Flax (analysis #2)	11	38	27	35	65
Straw/flax amendment from Makela reactor #2 (analysis #1)	5	33	4	58	42
Straw/flax amendment from Makela Reactor #2 (analysis #2)	4	33	3	56	40

TABLE 23 METABOLIC ACTIVITY IN ARUM WATER COLUMN REACTORS AS MEASURED BY CARBON DIOXIDE AND METHANE

Reactor	Profile Of Reactor Treatment	Total Volume of Acidic Seepage Treated (L)	Accumulated Methane (ppm)	Accumulated CO, (ppm)	CO, (ppm) Following Flushing Of Reactor Headspace	CO, (ppm) 3 Days Following Flush
Makela #1	(i) Static for 2 months (ii) Aadic seepage pumped into reactor at rate of 250 mL/day for 23 days (iii) Static for 6 months (iv) Acidic seepage at rate of 100 mL/day for 27 days (v) Static for 2 months	10.9	0	3220	340	600
Makela #2	(i) Static for 4 months (ii) Acidic Seepage pumped into reactor at rate of 100 mL/day for 125 days (iii) Static for 3.5 months	14.0	7	1680	400	1300
Makela #3	(i) Acidic seepage pumped into reactor at rate of 500 mL/day for 5 days (ii) Static for 1.5 months (iii) Aadic seepage at rate of 100 mL/day for 57 days (iv) Static for 5 months (v) Aadic seepage at rate of 100 mL/day for 21 days (vi) Static for 2 months	9.9	15	1100	340	760
Denison #1	(i) Static for 1 month (ii) Aadic seepage pumped into reactor at a rate of 100 mL/day for 21 days (iii) Static for 3 months	3.6	28	1140	400	800
Denison #2	(i) Static for 5 months	1.5	4300	5500	340	3025
Denison #3	(i) Static for 1.0 month (ii) Aadic seepage pumped into reactor at rate of 100 mL/day for 21 days (iii) Static for 1.5 months (iv) Acidic seepage at rate of 100 mL/day for 14 days (v) Static for 1 month	7.1	7	1900	380	1720

TABLE 24 CONTRIBUTION OF REACTIONS TO ALKALINITY

Reaction Type	Reaction #1 Test sec. 3.5		Alkalinity Contribution.
1. Iron reduction		(a)	For carbon source consumed
	R1.1		270 equivalent alkalinity per kg of carbohydrate
	R1.2		270 equivalent alkalinity per kg of carbohydrate
	R1.3		11 equivalent alkalinity per kg of glucose
	R1.4		22 equivalent alkalinity per kg of lactate
		(b)	For ferric iron consumed
	R1.1		36 equivalent alkalinity per kg of Fe^{3+}
	R1.2		36 equivalent alkalinity per kg of Fe^{3+}
	R1.3		36 equivalent alkalinity per kg of Fe^{3+}
	R1.4		36 equivalent alkalinity per kg of Fe^{3+}
2. Manganese reduction		(a)	For carbon source consumed
	R2.1		130 equivalent alkalinity per kg of carbohydrate
	R2.2		11 equivalent alkalinity per kg of glucose
	R2.3		22 equivalent alkalinity per kg of lactate
		(b)	For *each* MnO_2 reduced
	R2.1		23 equivalent alkalinity per kg of MnO_2
	R2.2		23 equivalent alkalinity per kg of MnO_2
	R2.3		23 equivalent alkalinity per kg of MnO_2
3. Ammonia production		(a)	For carbon source consumed
	R3.1		9.3 equivalent alkalinity per kg organic matter (methane produced)
	R3.2		9 3 equivalent alkalinity per kg organic matter
	R3.3		100 equivalent alkalinity per kg carbohydrate (nitrate ammonification)
	R3.4		50 equivalent alkalinity per kg glucose (nitrate ammonification)
	R3.5		37 equivalent alkalinity per kg amino adds (Strickland)
	R3.6		20 equivalent alkalinity per kg (single amino adds)
		(b)	For ammonia produced
	R3.1		120 equivalent alkalinity per kg ammonia produced
	R3.2		120 equivalent alkalinity per kg ammonia produced
	R3.3		180 equivalent alkalinity per kg ammonia produced
	R3.4		180 equivalent alkalinity per kg ammonia produced
	R3.5		120 equivalent alkalinity per kg ammonia produced
	R3.6		120 equivalent alkalinity per kg ammonia produced
4. Sulphate reduction		(a)	For carbon source consumed
	R4.1		30 equivalent alkalinity per kg carbohydrate (FeS formation)
	R4.2		17 equivalent alkalinity per kg carbohydrate (FeS formation)
	R4.3		30 equivalent alkalinity per kg carbohydrate (FeS formation)
	R4.4		34 equivalent alkalinity per kg lactate (FeS formation)
		(b)	For sulphate consumed
	R4.1		20 equivalent alkalinity per kg SO_4^{2-}
	R4.2		10 equivalent alkalinity per kg SO_4^{2-}
	R4.3		20 equivalent alkalinity per kg SO_4^{2-}
	R4.4		30 equivalent alkalinity per kg SO_4^{2-}

5. Denitrification		(a)	For carbon source consumed
	R5.1		27 equivalent alkalinity per kg organic matter
	R5.2		27 equivalent alkalinity per kg carbohydrate
	R5.3		27 equivalent alkalinity per kg carbohydrate
		(b)	For nitrate consumed
	R5.1		16 equivalent alkalinity per kg NO_3^-
	R5.2		16 equivalent alkalinity per kg NO_3^-
	R5.3		16 equivalent alkalinity per kg NO_3^-
6. Photosynthesis	R6.1		12 equivalent alkalinity per kg plant produced
	R6.2		5.8 equivalent alkalinity per kg plant produced

*Alkalinity = $[HCO_3^-]+2[CO_3^{2-}]+[RCOO^-]+[OH^-]-[H^+]$ from (3)

TABLE 25 CONTRIBUTION OF MICROBIAL PROCESSES TO ALKALINITY GENERATION IN DENISON WATER

Microbial Processes	Number Of Milliequivalents Alkalinity Produced	% Of Total
Sulphate reduction (Table 24, (b) R4.1 - R4.4)	12 - 22	31 - 56.5
Iron reduction (Table 24, (b) R1.1 - R1.4)	16	41
Ammonia production (Table 24, (b) R3.1 - R3.6)	1 -1.5	2.5 - 4.0
Other Processes (Table 24)	0 -10	0 - 26

Notes:

1. This analysis was conducted on Denison water column reactor #2 after 4 months of ARUM initiation in static operation.

2. Number of milli equivalents generated during neutralization process was equal to 39.

3. Methane levels of 4300 ppm was measured in the headspace of the reactor.

4. The amount of alkalinity produced by each process was calculated first by measuring the amount of sulphate and iron reduced and ammonia produced at the end of the 4 month operation. Next, the range of milli equivalents of alkalinity produced was calculated from Table 24 which had been derived from the alkalinity reactions previously listed in Section 3.12.

TABLE 26 CUMULATIVE ALKALINITY GENERATION DURING MAKELA REACTOR #2 FLOW EXPERIMENT

Days of Operation	pH	Volume Treated (mL)	Meq of Alkalinity Produced	Cumulative Volume Treated (mL)	Cumulative Meq Produced
0	7.53	0	0.0	0	0
1	6.80	100	0.210	100	0.210
2	6.85	100	0.20	200	0.41
5	6.77	300	0.610	500	1.02
8	6.62	300	0.59	800	1.61
12	6.62	400	0.79	1200	2.40
15	6.30	300	0.58	1500	2.98
19	6.02	400	0.73	1900	3.71
22	5.85	300	0.54	2200	4.25
27	5.30	500	0.86	2700	5.11
29	5.77	200	0.34	2900	5.45
33	5.84	400	0.70	3300	6.15
36	6.00	300	0.54	3600	6.69
40	5.87	400	0.71	4000	7.40
43	5.83	300	0.53	4300	7.93
47	5.40	400	0.69	4700	8.62
57	4.86	1000	1.60	5700	10.22
61	4.32	400	0.58	6100	10.80
64	4.20	300	0.41	6400	11.21
72	4.25	800	1.08	7200	12.29
78	4.34	600	0.83	7800	13.12
82	3.76	400	0.53	8200	13.65

TABLE 27 CUMULATIVE ALKALINITY GENERATION DURING DENISON REACTOR #3 FLOW EXPERIMENT

Days of Operation	pH	Volume Treated (mL)	Meq of Alkalinity Produced	Cumulative Volume Treated	Cumulative Meq Produced
0	6.00	0	0.0	0	0.0
2	5.93	200	7.40	200	7.4
6	5.78	400	14.40	600	48.2
14	5.28	800	26.40	1400	
21	**3.35**	700	15.40	2100	**63.6**

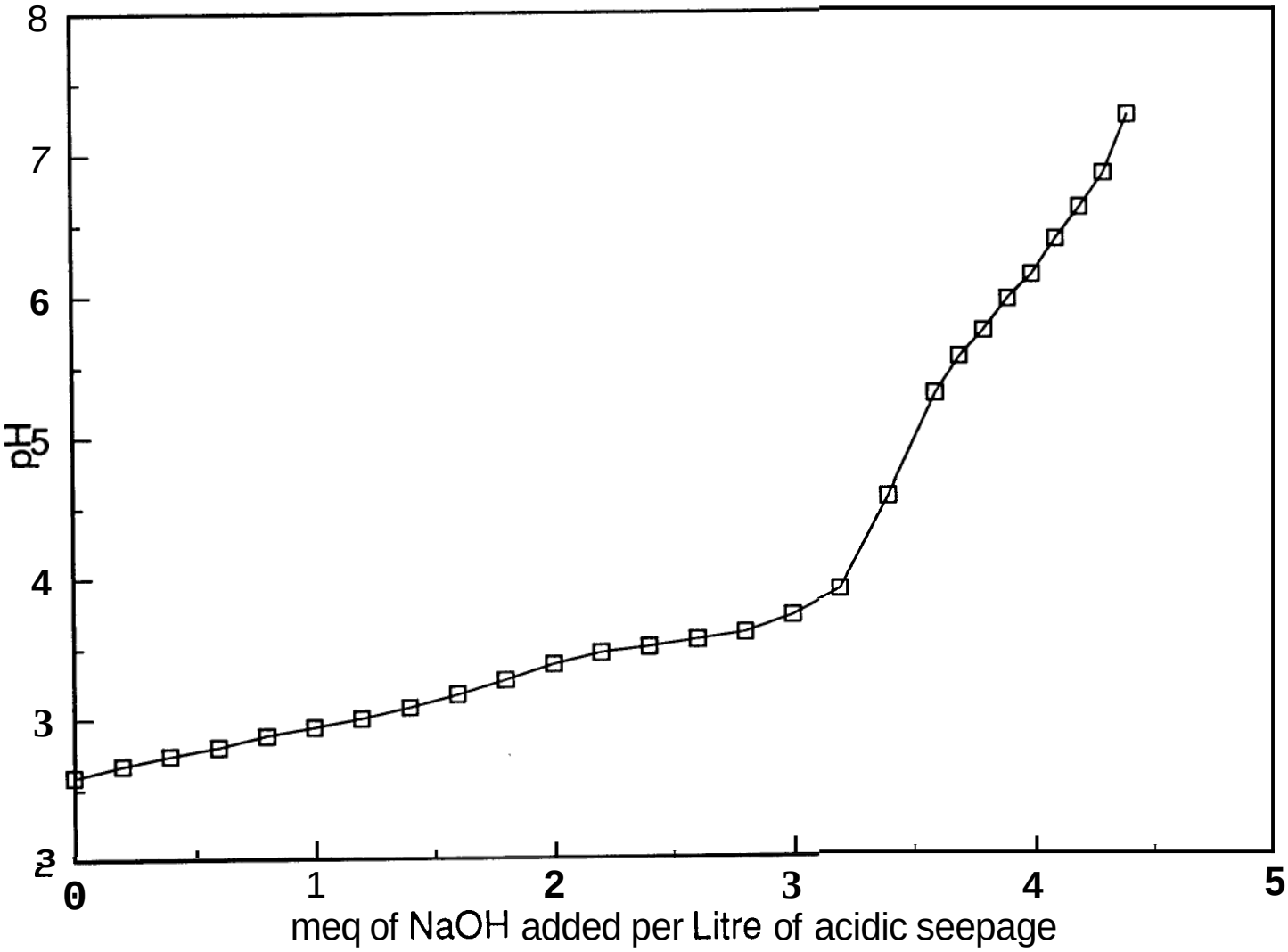


FIGURE 1: NEUTRALIZATION OF MAKELA SEEPAGE WATER TITRATION CURVE

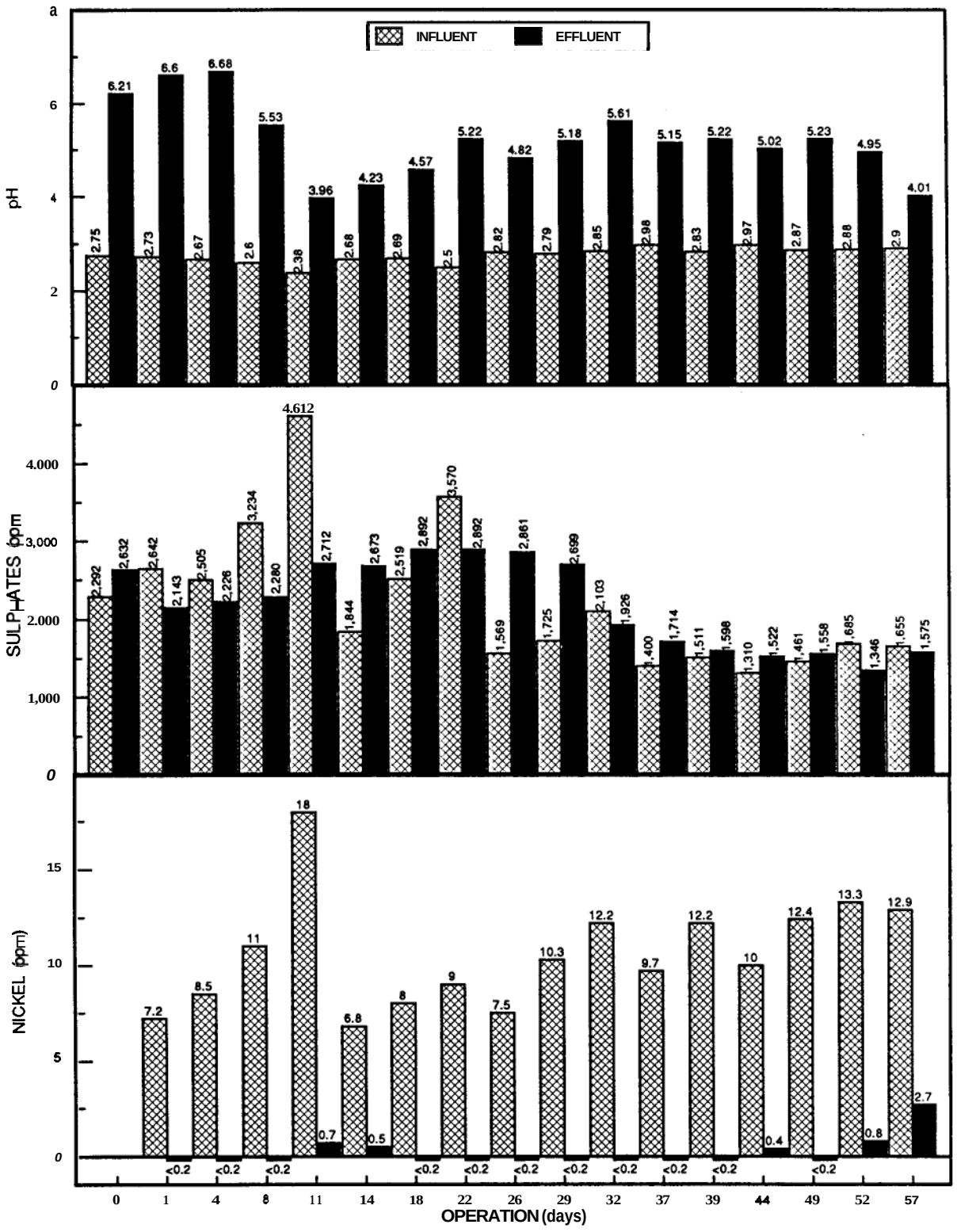
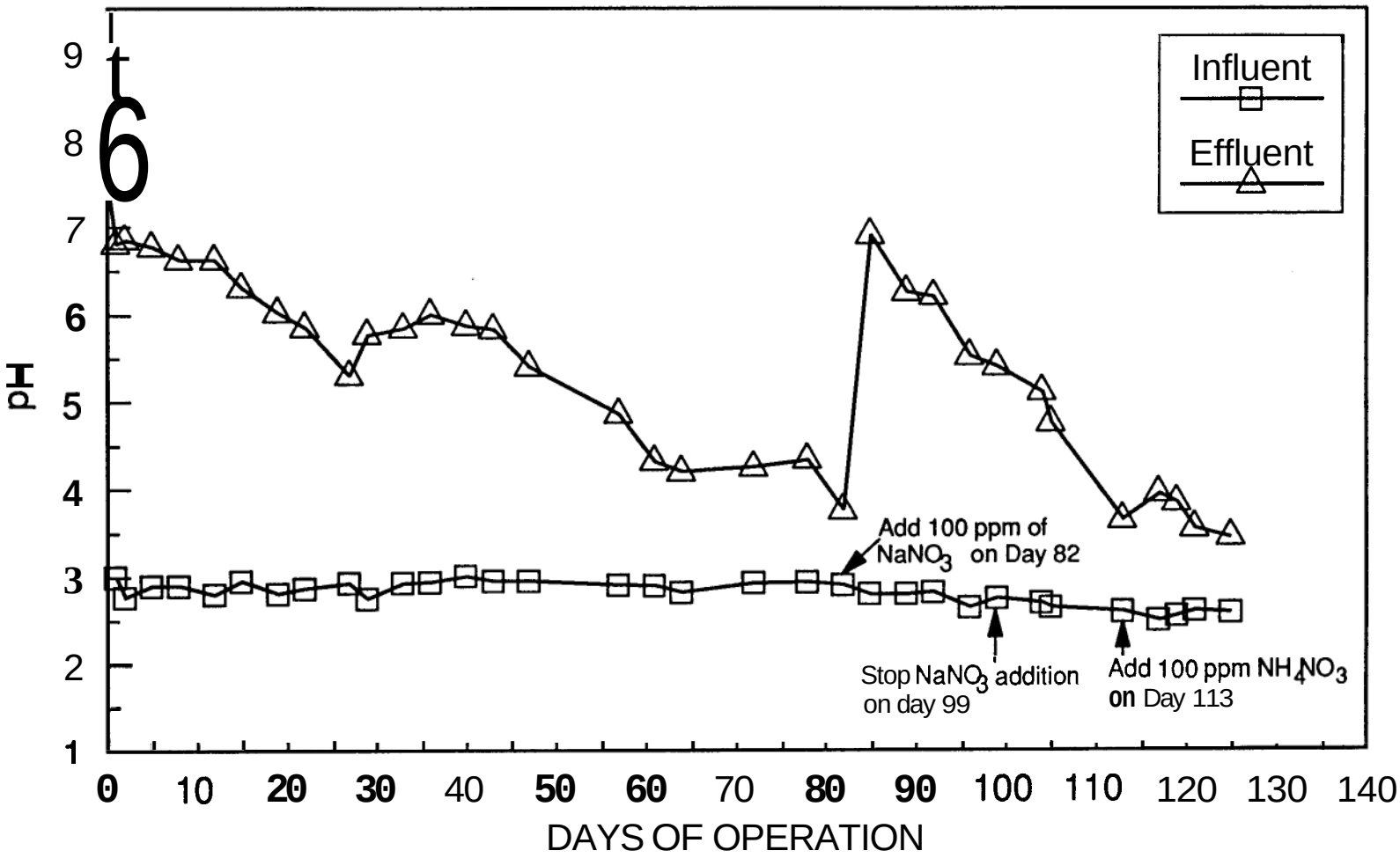
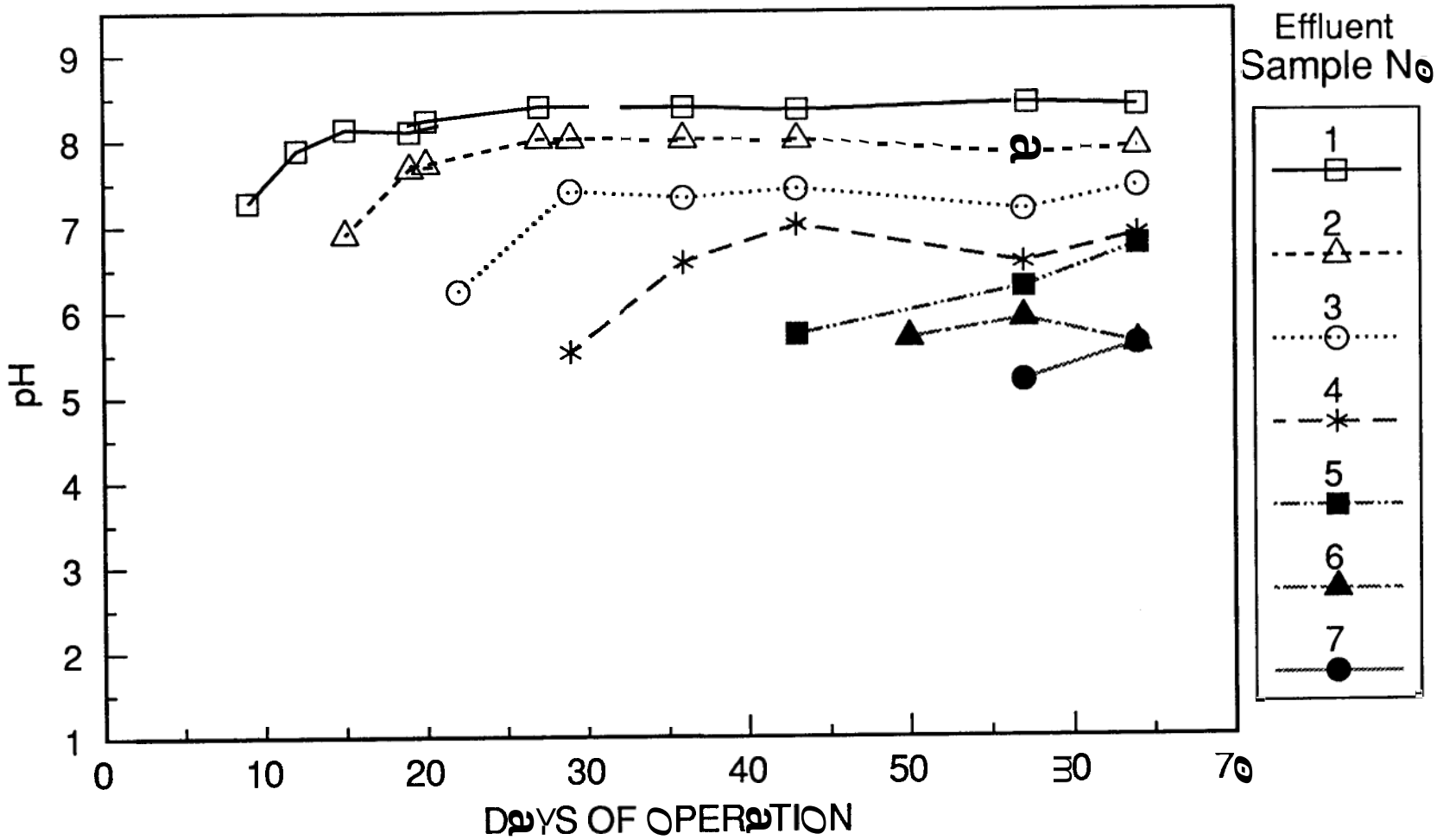


FIGURE 2: FLOW EXPERIMENT: MAKELA REACTOR #3: 100 mL/DAY



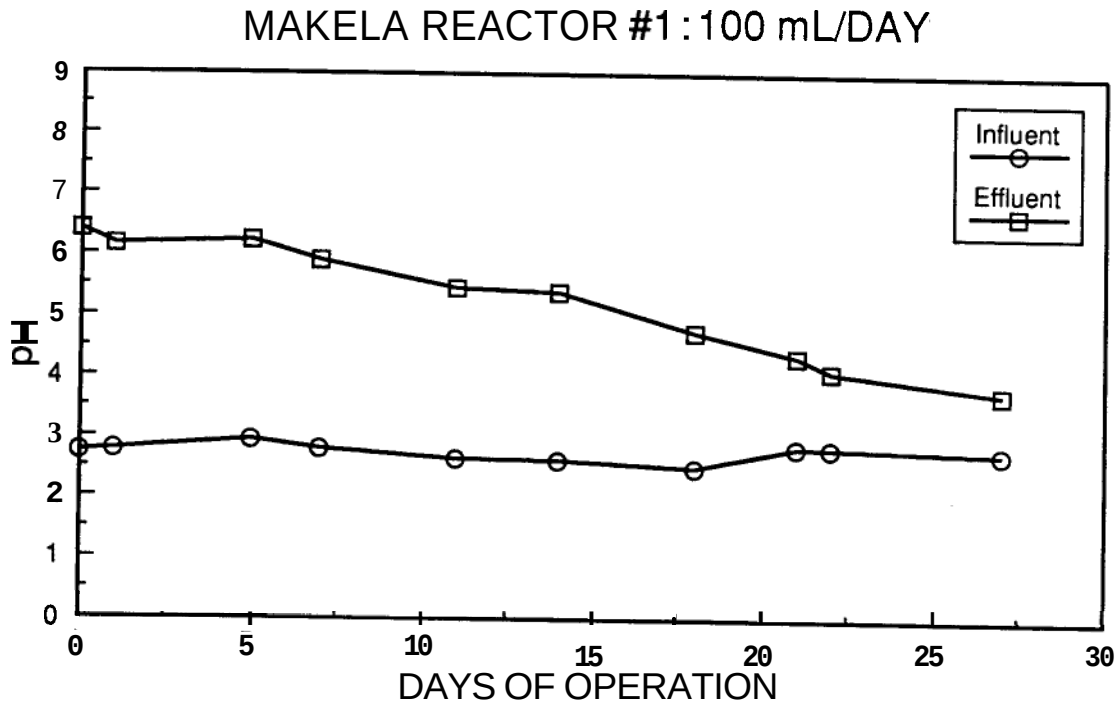
Note: Acidic seepage pumped into reactor at **flow** rate of 100 mL/day

FIGURE 3: ALKALINITY GENERATION IN MAKELA REACTOR #2
(Site Flow Configuration)



NOTE: First data point indicates day removed from effluent reservoir.

FIGURE 4: STABILITY OF EFFLUENT pH: MAKELA REACTOR #2
(Site Flow Configuration)



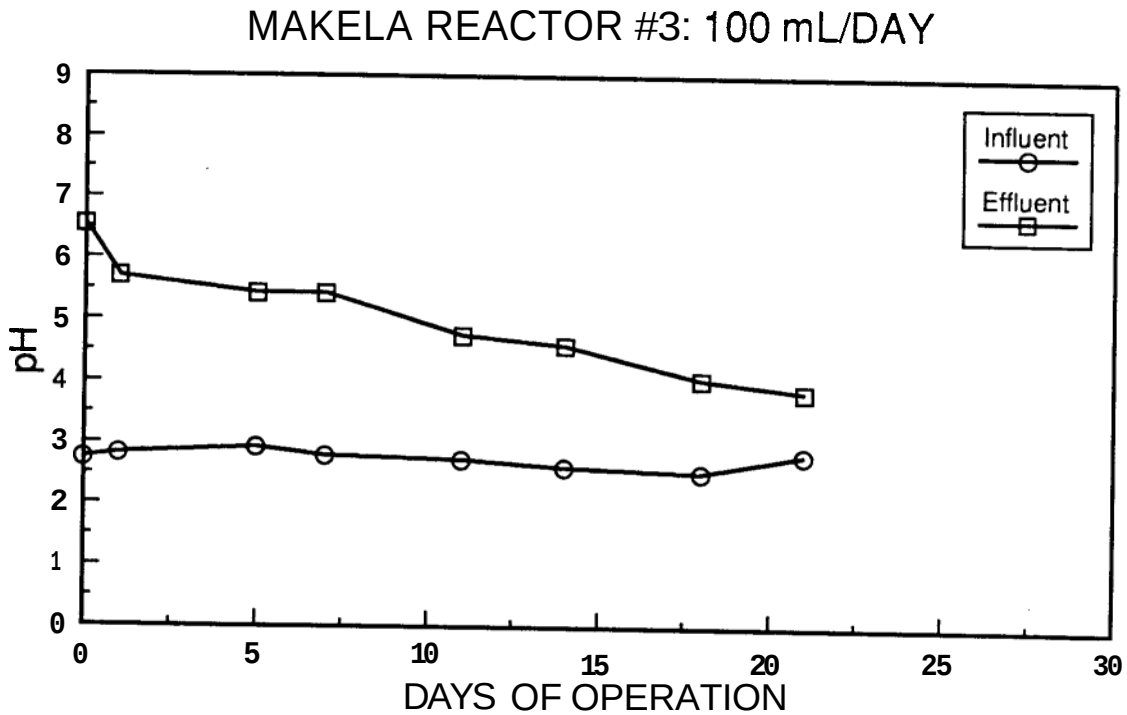


FIGURE 5: FLOW EXPERIMENT
(Site Flow Configuration)

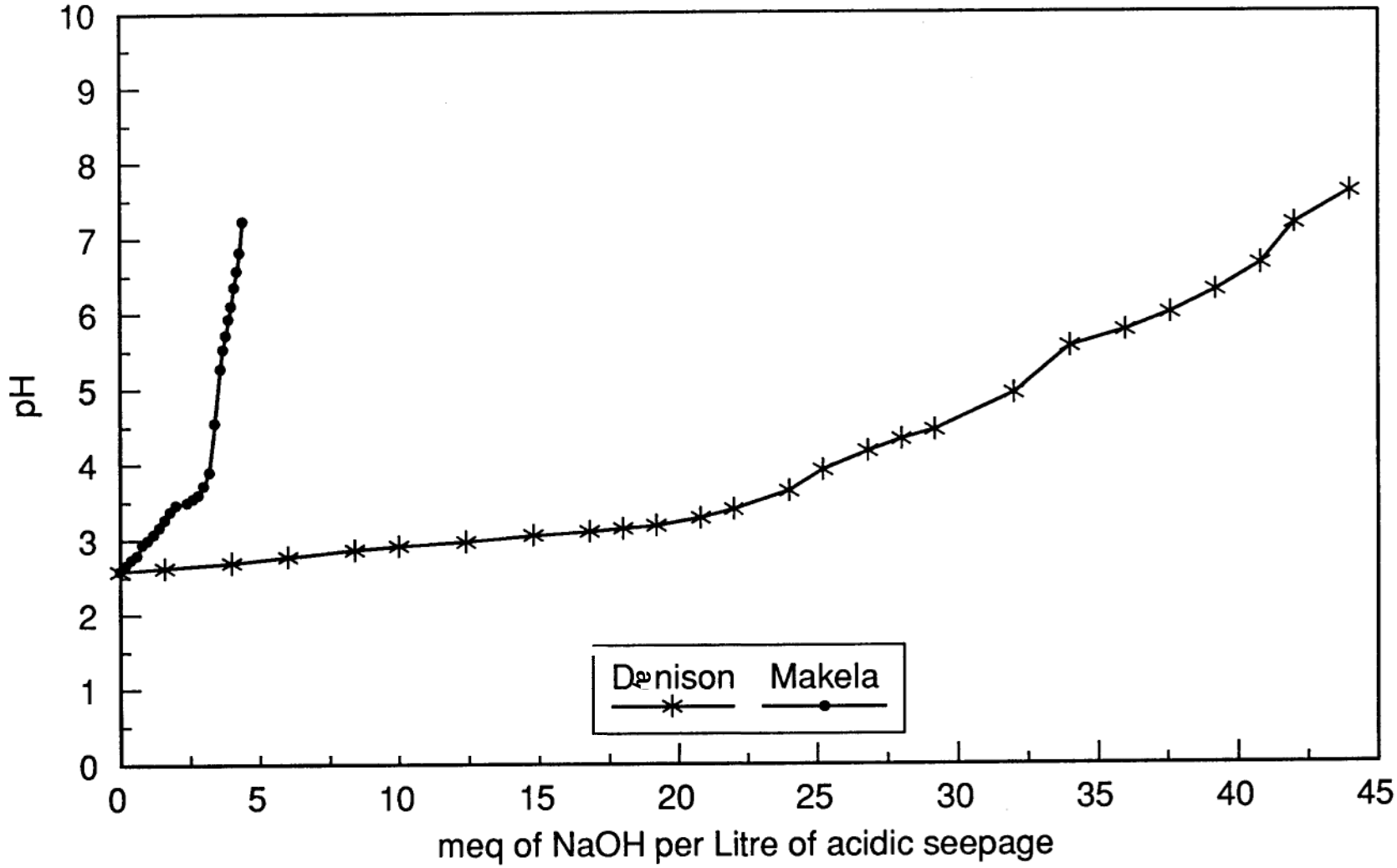
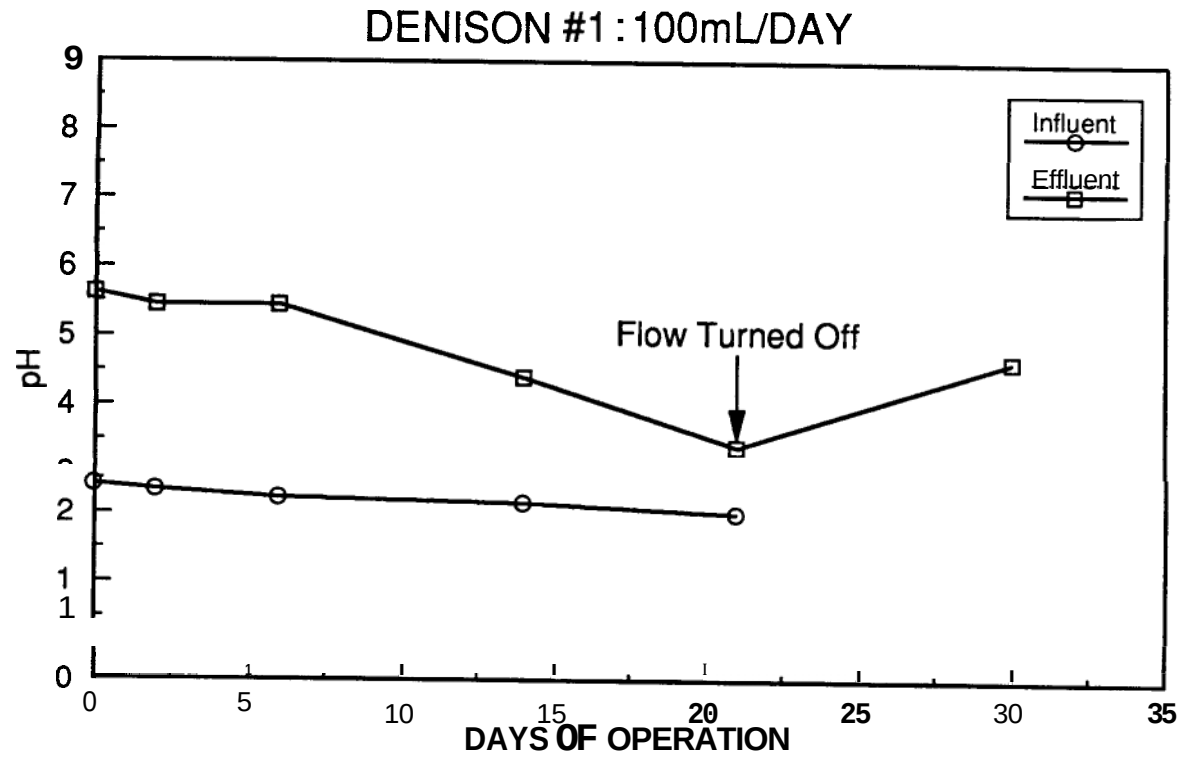


FIGURE 6: NEUTRALIZATION OF SEEPAGE WATER TITRATION CURVES



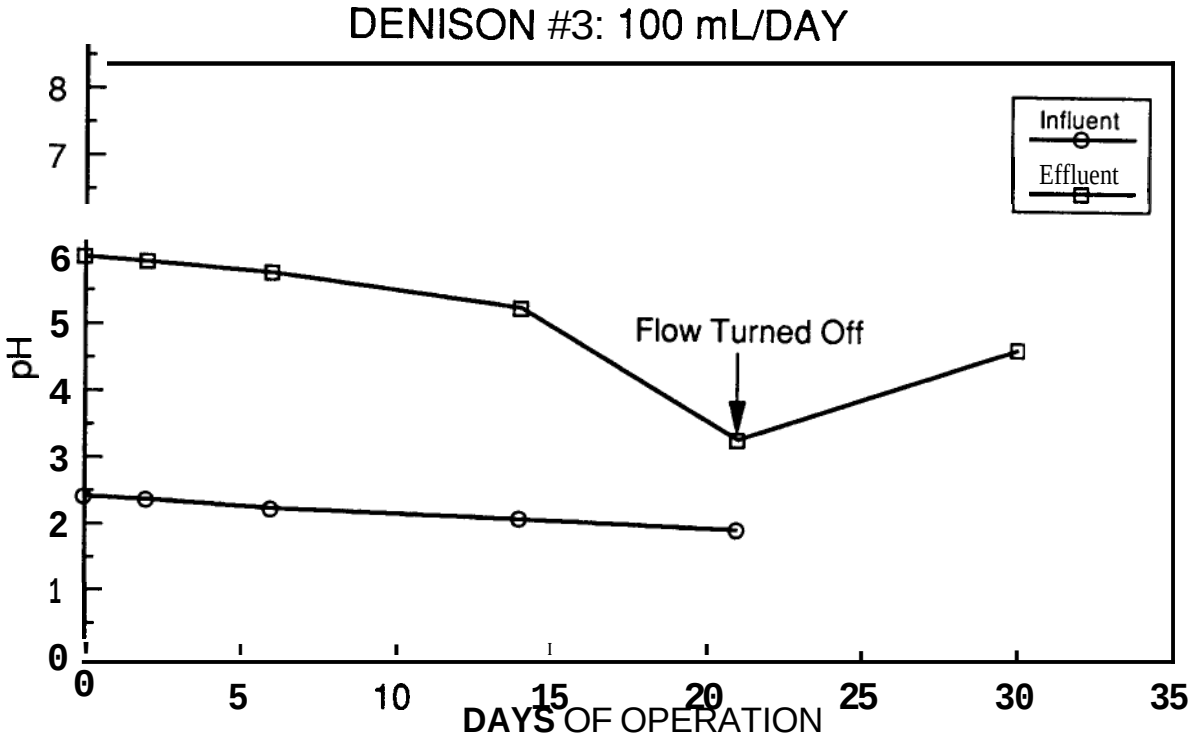


FIGURE 7: FLOW EXPERIMENT
(Site Flow Configuration)

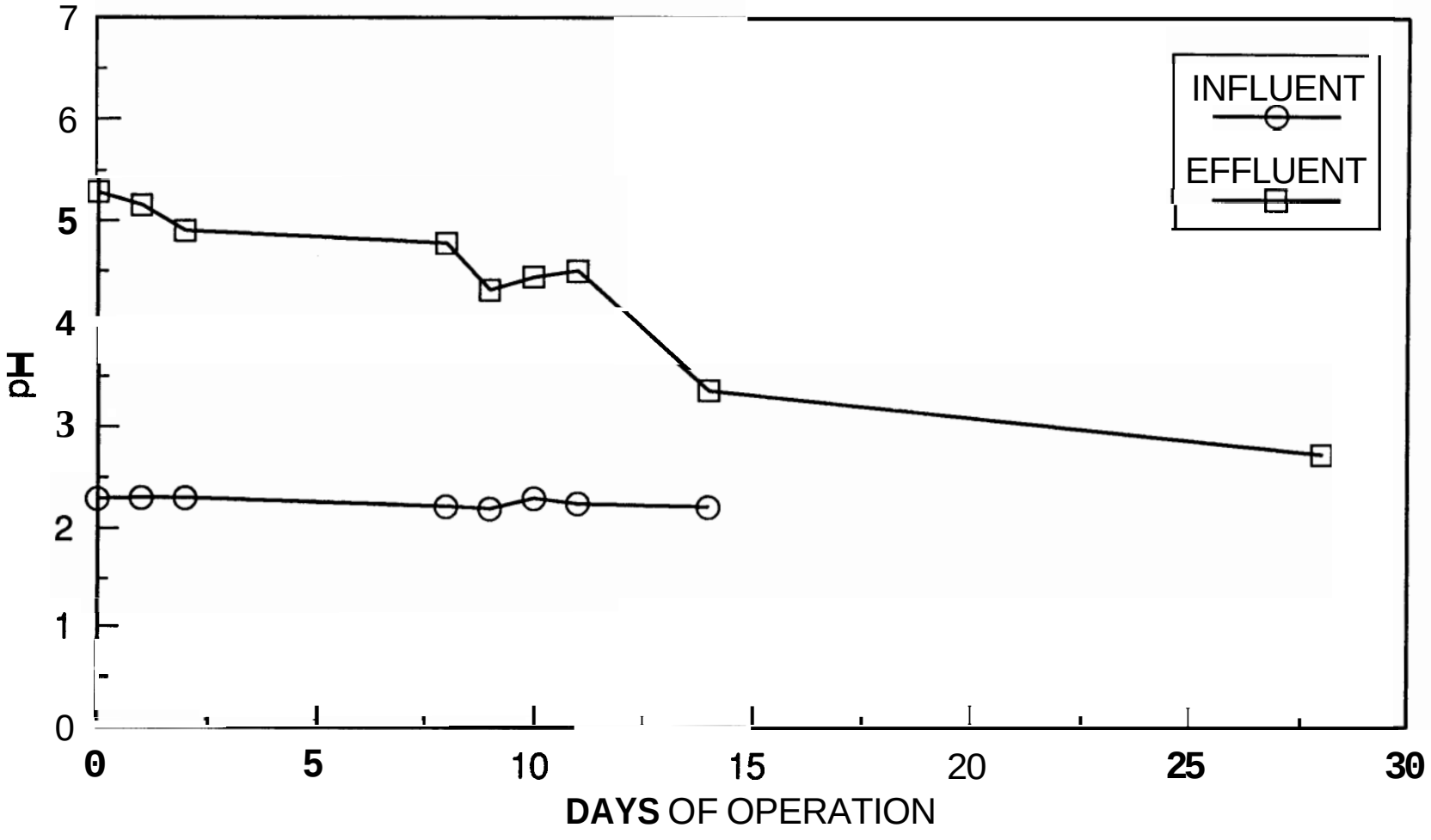


FIGURE 8: FLOW EXPERIMENT: DENISON REACTOR #3: 100mL/DAY
(Site Flow Configuration)