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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 the progress review meeting held at the Biohydrometallurgy Conference in August, **1989** to April, 1990.

2.0 MATERIALS AND METHODS

2.1 Microbiological Media

All media were purchased from Difco Laboratories, Detroit, Michigan, U.S.A.

2.2 Chemicals

All analytical reagents were purchased from BDH, Toronto, Ontario Canada unless otherwise specified. $Na_2MoO_4 \cdot 2H_2O$ was purchased from Allied Chemicals, Morristown, New Jersey, U.S.A. Myacide (2-bromo-2-nitropropane-1,3-diol) (The Boots Co. **PLC**) was purchased from **OSP** Microcheck Inc. (Calgary, Alta.)

2.3 Amendments

Guinea Pig pellets and aquarium gravel were manufactured by Hagen Inc. Montreal, Quebec, Canada. Barley and bran were purchased from a Toronto Bulk Food Store. Alfalfa was purchased from L/M Animal Farms, Pleasant Plain, Ohio, **U.S.A.** Cooked meat medium and Bacto-peptone were purchased from Difco Laboratories, Detroit, Michigan, **U.S.A.** Casein was purchased from Sigma Chemical Co., St.Louis, Missouri, U.S.A. Sawdust was obtained from Asarco Incorporated, Buchans, Newfoundland. Straw and flax were obtained from Boojum Research Ltd.

2.4 Equipment

Wheaton vials (40 mL) were purchased from Wheaton, Millville, New Jersey, U.S.A. Nitex Screen (25 microns) was purchased from B. & S.H. Thompson and Co. Ltd. Scarborough, Ontario, Canada.

2.5 Microbiological Analyses

Microbiological analyses were performed as described earlier (1).

2.6 Treatment of Denison Mine Seepage Water: Reactor Experiments

Three water column reactors as described previously (1) were prepared. They were filled with approximately 1 L of amendment sample obtained from the seepage site. Next, seepage water was added to the 1.5 L level and the top of the reactor was put in place and secured.

2.7 Treatment of Denison Mine Seepage Water: Amendment Screening

The following types of amendments were screened for their ability to induce alkalinity generation in Denison Mine seepage water: flax; flax and iron filings; crop residues and cereal grains (flax, alfalfa, barley, bran and pet food pellets); flax and organic acids (lactic and acetic acid)); flax and protein residues (peptone and casein); flax and sugar (glucose) and; protein (cooked meat media pellets).

All tests were performed in 40 mL Wheaton screw-cap vials. The first component added to every vial was a 2 cm layer of washed aquarium gravel. Next, amendment which had been finely ground in a Waring blender was added to a thickness of 1 cm. Cooked meat and guinea pig feed pellets were unground. Chemicals or iron filings were added dry on top of the amendments. Two series of vials containing amendments were prepared.

Untreated Denison water was added to one series while the other series received Denison water supplemented with BOD mineral nutrients at the concentration applied for a standard BOD test (2). Before addition of Denison water, each vial received 1 mL of sample from the Buchans Oriental East limnocorral site which was known to contain sulphate reducing bacteria.

The test amendments were inoculated with 1 mL of a seed containing sulphate reducing bacteria. The seed was obtained from a sawdust amendment sample taken from the Oriental East Limnocorrals of the Buchans mine site, Newfoundland. The seed was tested for ATP and for numbers of sulphate reducing bacteria, iron reducing bacteria and ammonifiers. Direct microscopic counts of total bacteria, fungi and algae were also performed.

The test amendments were incubated at ambient temperature (22°C). After one week, and weekly thereafter, pH was determined. The vials were further observed for blackening indicating the presence of sulphate reducing bacteria.

28 Treatment of Denison Mine Seepage Water: Acclimation Experiment

To initiate an acclimation experiment, a dilution series of Denison acidic seepage water was prepared. The different dilutions were added to amendment and then inoculated to determine what strength of acidic seepage could be tolerated by alkalinity generating microorganisms.

The tests were set up in 40 mL Wheaton vials by adding 1 cm finely ground flax (ground in a Waring blender for 60 seconds) to each vial, then filling it with diluted acidic seepage water. Mineral nutrients, used for BOD analyses, as described in Standard Methods for Examination of Water and Wastewater 16th Edition (2) were also added. The vials were then inoculated with 1 mL of seed containing sulphate reducing bacteria. The seed was same one used in the amendment screening experiment (Section 2.7).

The vials were incubated at ambient temperature (22°C). After one week and weekly thereafter, pH was determined. The vials were also observed for blackening, indicating the presence of sulphate reducing bacteria.

29 Treatment of Denison Mine Seepage Water; Mechanisms of Alkalinity Generation

An assortment of amendment test conditions were set up in 40 mL Wheaton vials to help identify the mechanisms of alkalinity generation.

The order of addition to the vials was gravel (2 cm), amendment (1.0 cm) then acidic seepage water.

The treatments tested were as follows: (i) flax and iron filings; (ii) flax, iron filings and 1 mL seed; and, (iii) flax, iron filings, 1 mL seed and 1000 ppm $Na_2MoO_4.2H_2O$ (iv) flax, iron filings and 500 ppm of Biomate 750 (a Dearborn biocide containing 19.6% bromonitropropane(BNPD)). A similar set of vials were set up using magnesium ribbon strips (1 cm) to replace the iron filings.

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

Mineral nutrients, used for BOD analysis, as described in Standard Methods for Examination of Water and Wastewater 16th Edition (2) were added to all the vials.

The seed was obtained from the amendment screening experiment from the test condition flax and iron filings (section 2.7).

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

After a two week period, pH was determined.

2.10 Makela: Flow Experiments

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

The amendment portion consisted of layers of rocks, flax and straw which filled two thirds of the reactor (ie. to a level of 1.5 L) Acidic seepage water from the Makela seepage site was then added to cover the amendment.

During the addition of amendment, bags containing straw and cellophane were placed in the reactor. These bags were added as part of an experimental protocol to estimate cellulose degradation in the reactors. The bags were tied to nylon strings so they could be retrieved from the reactor. Four bags were placed in each reactor. Two were secured at the top; the other two were secured at the bottom of the reactor.

The bags had been prepared as follows: Cellophane strips (1 cm x 5 cm) and straw (cut to 1 cm lengths) were stained with remazol brilliant blue as described by Moore et al (3). Nylon screen (25 micron porosity, Nitex, purchased from B. & S. & H. Thompson, Toronto) was then cut into 2.5 cm squares. Into each square was placed a 0.5 g portion of stained straw and one cellophane strip which had been weighed after staining. The corners of the square were tied together to form a bag. The bags were then tied to nylon string as described above. Cellulose degradation of the straw and cellophane can be measured by weight loss and removal of stain **(3).**

The reactors were incubated at ambient temperature (22°C) and were observed for blackening indicating the presence of sulphate reducing bacteria. After two weeks, and weekly thereafter, pH was determined. Before feeding into a reactor, acidic seepage

water was allowed to stand at ambient temperatures for at least 24 h to permit oxidation of ferrous iron and precipitation of ferric iron products. The acidic influent was then pumped intermittently into one of the reactors once every hour and an equal volume was removed. The initial flow rate selected was 20.8 mL per h or approximately 500 mL per day. The process continued until the blackening in the reactor disappeared.

Acidic seepage water from the same source was pumped into the second reactor intermittently at a rate of 10.4 mL every hour or approximately 250 mL per day. This process continued until the pH decreased to below 4. The sulphate concentration of the acidic influent and effluent were determined by ion chromotography. (Dionex, Model 16).

In a third experiment, the acidic seepage water from the same source was pumped at an intermittent rate of 2.1 mL every 30 minutes or approximately 100 mL per day, and an equal volume was removed. This process is presently being maintained.

In addition to measurement of pH, the acidic influent (feed) and effluent were monitored for changes in sulphate, nickel and iron concentrations. Nickel concentrations are determined by atomic adsorption spectrophotometry, while ferrous and ferric concentrations were determined by an EDTA titration procedure (4).

2.11 Evaluation of Selective Media for Sulphate Reducing Bacteria

A number of media were evaluated for the enumeration of sulphate reducing bacteria. All vials and test tubes received 10 mL aliquots of medium. Before dispensing into tubes or vials, each lot of medium was divided in two parts. One part was adjusted to pH 5.0, while the other was adjusted to pH 6.0. Acid pH was chosen for the media as this reflected more closely the environment of the samples. After the vials and tubes of media were capped, they were autoclaved for 15 minutes at 121°C.

Postgate Media B, E and F, were prepared according to Postgate (6). Postgate Media

E and F were dispensed into 15 mL screw cap vials before autoclaving. Cook's modification of Starkey's medium (7) was also prepared in 15 mL screw cap vials. These media were treated to 100°C prior to use to re-melt the agar and to drive out oxygen. They were cooled in 44°C water bath before inoculation to minimize heat shock.

Two sets of Postgate B medium each with subsets of pH 5 and pH 6 were prepared anaerobically in 15 mL serum vials. Before autoclaving, each vial of one set received a de-greased iron finishing nail while each vial of the other set received 3 mm of iron dust. A third set of Postgate B medium was prepared anaerobically in 15 mL screw cap test tubes. After autoclaving, 0.5 cm of magnesium ribbon, which had been sterilized in 70% ethanol, was added anaerobically to each test tube.

Samples used to inoculate the test media were obtained from the top port of a Makela water column reactor (straw and flax amendment - see section 2.10) and from the test condition 'flax and iron filings' in section 2.7 (Denison experiments).

The samples were shaken vigorously for 30 seconds then diluted (10^{0} to 10^{-5}) in anaerobic dilution fluid (8) prior to their addition to each set of medium. The vials and test tubes were incubated at 28°C and observed daily. The day on which blackening was first evident was recorded. Blackening of the media was evidence for bacterial sulphate reduction.

2.12 Isolation of Cellulose Degrading Microorganisms

Attempts were made to isolate both anaerobic and aerobic cellulose degrading microorganisms.

Flasks for isolation of aerobic microorganisms were prepared as follows: Conical shaped, folded cellulose filters (Whatman No. 1) were placed into a 250 mL Erlenmeyer flask and sterilized by autoclaving. Winogradsky's medium (9) was prepared and autoclaved for

15 minutes at 121°C. After the medium had cooled, 50 mL aliquots were poured into the flasks containing the filters.

The flasks were then inoculated with 1 mL of sample from the top port of a Makela reactor and a Pre Bog Acid Creek water column reactor. The flasks were incubated at a temperature of 28°C.

Growth of cellulose degrading bacteria was detected by gradual decomposition of the filter paper.

Vials for the isolation of anaerobic cellulose degraders were prepared as follows: Narrow strips of filter paper and a strip of remazol brilliant blue stained cellophane were placed into 100 mL serum vials and sterilized by autoclaving for 15 minutes at 121°C. Winogradsky's medium was prepared anaerobically and introduced into the sterilized serum vials.

The flasks were incubated at a temperature of 28°C. Growth of cellulose degrading bacteria was evident with gradual decomposition of the filter paper and release of dye from the cellophane.

2.13 Total Soluble Carbohydrate Analyses

Total soluble carbohydrate analyses of seepage water was performed using the phenol reaction Molisch test (8) with glucose as the standard.

Prior to analyses the sample was neutralized with 0.1N NaOH pH 7.0 and then filtered through a 0.22 micron Acrodisc filter unit (Geman Sciences Inc, Montreal).

3.0 RESULTS AND DISCUSSION

3.1 Treatment of Denison Mine Seepage Water

Implementation of the ARUM process with this seepage water both in field and laboratory tests conducted by Boojum Research during 1989 had not been successful. This water is highly acidic with a pH of 2 and a free mineral acidity of almost 100 m equivalents per litre.

Subsequently, several experimental approaches were taken to determine the conditions necessary to initiate the ARUM process in these waters.

The first experiment used laboratory reactors of the same design which had been successful using Pre-Bog Acid Creek samples (1). Three such reactors were prepared. One reactor had only the straw amendment collected from the Denison field site, while in the second reactor 25% of the straw was replaced with alfalfa, and in the third reactor a supplement of 1000 ppm glucose was added.

Although bench scale tests had previously been unsuccessful at Boojum's facilities, the extra depth of the above reactors may permit the development of an anaerobic environment sufficient for alkalinity generation. Addition of alfalfa to one reactor was made to eliminate the possibility of nitrogen deficiency. Glucose was added to the third reactor to ensure that insufficient cellulytic activity was not a problem.

Figure 1 is a photograph of the three reactors taken at the end of the experiment, while the data obtained while monitoring the reactors is shown in Table 1.

By examining the table, it can be seen that no net alkalinity generation occurred over the 85 day test period.

Possible explanations for lack of alkalinity generation include the following:

(1) Anaerobosis has not been successfully achieved.
(2) An essential nutrient for alkalinity generation is missing.
(3) Key microbial species are absent or inhibited.
(4) The environment is too hostile to prevent any significant microbial processes.

The last explanation is apparently not the difficulty as the comprehensive analysis performed on Day 14 indicates (see Table 2). It can be seen that all three reactors had high amounts of viable biomass. ATP levels ranged from 0.50 to 1.12 ng per mL. The high level of biological activity indicated by the ATP assay was also confirmed by the measurement of significant amounts of CO_2 (3,680 to 6400 mg/L) which had accumulated in the reactor head spaces.

However, the comprehensive analysis did not detect any sulphate reducing bacteria. Therefore, on Day 36, the reactors were reinoculated with 5 mL aliquots transferred by syringe from an alkalinity generating reactor containing water and amendment from Pre-Bog Acid Creek. Unfortunately, this inoculation failed to stimulate alkalinity generation.

Two different experiments were conducted in an attempt to overcome the lack of alkalinity generation in the Denison waters. In one of the experiments, a wide variety of amendments were screened for their ability to supply the essential nutrients which may be missing in the straw amendment. In the other experiment, the Denison water was diluted serially in an attempt to reduce the concentration of (a) hostile element(s) and permit growth of alkalinity generating organisms. In this experiment, flax was the nutrient source. In both experiments, the Denison waters were inoculated with a microbial seed obtained from the sawdust amendment sample from the Oriental East Iimnocorral (Buchans Mine site, Newfoundland). Table 3 is a summary of microbial analyses performed on the seed.

The results of the amendment screening experiment are summarized in Table 4. Figure 2 shows examples of the vials with the experimental amendments. Two treatments were successful in generating alkalinity: cooked meat and flax with iron filings.

It is interesting to note that this is the second time when cooked meat was a successful amendment for treating waters which resist development of the ARUM process. The first occasion occurred with a Pre-Bog Acid Creek sample. it is suspected that cooked meat with its high organic nitrogen content stimulates ammonifiers to produce ammonia which neutralizes the acidic water. Contribution of alkalinity by sulphate reducing bacteria activity appeared to be negligible because no blackening of the waters occurred.

On the other hand, sulphate reducing bacteria activity was evident in waters treated with flax and iron. A discussion of the suspected role of iron is presented later in this section of the report.

The results of the second experiment are shown in Table 5. It demonstrates that successful alkalinity generation occurred when the Denison water was diluted by at least **16** fold. These results indicate that it might be possible to gradually "train" the microbial ecosystem to tolerate more concentrated Denison Waters. However, because of the success of the amendment screening experiment described above, this acclimation approach was not continued.

Experiments are currently in progress to define more clearly the mechanisms of alkalinity generation in the flax/iron amendment. A variety of alterations in the amendment composition have been examined in an attempt to ascertain what role the individual components of the treatment played. The results are summarized in Table **6.**

All treatments contain flax which, as shown previously in Table **3,** was incapable by itself of stimulating alkalinity generation. There were two main sets of treatments: one set with iron, and another set in which the iron filings were replaced by pieces of magnesium

ribbon. Magnesium ribbon is a superior reducing agent to iron metal and reports in the literature (5) have indicated that magnesium, used as a sacrificial anode to prevent corrosion in industrial water systems, has stimulated the growth of sulphate reducing bacteria. However, in this experiment, none of the treatments with magnesium successfully generated alkalinity.

Therefore, it appears that the role of iron is not merely to help poise the redox potential. Postgate (6) reports that a redox potential of minus 100 mV or lower is required for growth of sulphate reducing bacteria.

This experiment also examined the role of the biological processes in the treatment. One flax/iron treatment had received no microbial seed while another also had received no seed but was treated with a biocide to prevent the contribution of indigenous microorganisms to the alkalinity generating process. A third flax/iron treatment had received seed but was treated with sodium molybdate (a specific inhibitor of sulphate reducing bacteria). The fourth vial in the test series was a positive control and contained flax/iron, plus microbial seed.

It was found that all four conditions permitted alkalinity generation. These results were initially surprising until the test vials of the experiment were examined more closely. An ATP assay was conducted on the vial containing biocide and it was found that it contained more than 12 ng ATP per mL. The biocide had failed to prevent the growth of a population capable of producing alkalinity. it is interesting to note that sulphate reducing bacteria activity (blackening) had not occurred. This indicates that the biocide had successfully inhibited the sulphate reducing bacteria population. It also indicates that sulphate reducing bacteria activity is not an essential part of this alkalinity generating process.

A high ATP level was also found in the vial without microbial seed. It appears that the Denison and/or flax has an indigenous population of alkalinity generating microorganisms

and that inoculation with a seed is not an essential part of the process.

Finally, the blackening of the vial containing sodium molybdate indicates that in this environment molybdate is not effective. During a discussion of these results with the Dearborn Research Department, it was observed that the molybdate is used as a corrosion inhibitor and reacts with iron. It might be possible that iron serves a similar role in Denison water in reducing metal toxicity to the sulphate reducers.

3.2 Makela: Flow Experiments

Until these experiments were initiated, all laboratory work had been performed in batch reactors.

There are several purposes for studying the ARUM process in the lab under flow conditions:

1. Flow conditions more closely approximate field conditions. Therefore, it is valuable to see how the process performs in this mode under the controlled environment of the laboratory.

2. Lab studies can provide an indication of what acid and metal capacities can be handled by **ARUM** during a flow operation. These values can provide goals for future optimization of **ARUM** under field conditions.

3. It is possible that problems in **ARUM** operations may be revealed during flow conditions that may otherwise be undetected in batch operations. If such problems exist, solutions can be sought under more controlled cost effective conditions of a lab scale operation.

4. Operating reactors under flow conditions will assist studies to improve rate limiting

steps such as cellulose degradation. Flow conditions will maximize the exhaustion of the readily degradable sugars, proteins, and non-cellulosic polysaccharides. These nutrients will be present only at the process start-up and will mask the requirement for effective cellulose degradation. Cellulose is the main degradable organic component of the amendments, and so it is crucial that its degradation occurs effectively for long term ARUM operation.

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

Three such reactors were established. Figure 3 is a graph demonstrating the increase in pH which occurred in one of the reactors following addition of seepage water to the amendment. The location of greatest alkalinity generation is surprising in that the top of the reactor generated more alkalinity than the bottom. Sulphate reduction also followed the same trend and at the end of several weeks of incubation, the top half of the reactor had blackened (presumably with ferrous sulphide).

The expected performance of the reactor was the opposite since alkalinity generation and sulphate reductian are anaerobic processes. Thus, these processes are usually most vigorous in the bottom of a water column away from the air/water interface.

It was noted that on sampling the bottom portion of the reactors that the water had a sour rancid odour typical of volatile fatty acids, while this odour was less pronounced in samples from the top port. it is well known in municipal anaerobic waste treatment systems, that high volatile fatty acid (VFA) levels are inhibitory to their degradation especially at pH ranges below 7.5 (7). Perhaps this phenomenon also occurs during alkalinity generation. Analysis for VFAs in samples taken from the top and bottom ports of the reactor are currently in progress.

Reactors which have generated sufficient alkalinity to neutralize the seepage water to a

pH greater than pH 5 were considered sufficiently established for use in flow experiments, A photograph of the apparatus is shown in Figure 4.

Before feeding acidic seepage into a reactor, it was allowed to stand for at least 24 h in a shallow open tub to permit oxidation and precipitation of ferrous iron. As it can be seen in Figure 5, a titration curve of the Makela acid seepage water, most of the acidity in the samples is free mineral acidity. Thus during the neutralization process, most of the alkalinity is required to raise the pH to pH 4.

Figures 6 to 8 display the results of flow experiments. The initial choice of feeding 500 mL or seepage water per day (ie. a fluid retention time of approximately three days) to one of the reactors (Reactor #3) was arbitrary. Unfortunately, as shown in Figure 6, this flow rate is excessive. The sulphate reducing bacteria were able to produce sufficient hydrogen sulphide to maintain blackening of the reactor for less than 5 days. However, the pH of the effluent was kept above pH 4. The drop in pH at the top of the reactor was expected since this was the region receiving the acidic seepage water.

After the 5 day operation period, the pumps to and from the reactor were stopped to determine whether the sulphate reducing bacteria could recover. A full recovery of sulphate reduction and alkalinity generation was seen after 25 days (see Figure 6).

During the recovery period of this reactor, a second flow experiment (Reactor #1) was started with flow rates set at 250 mL. (ie. fluid retention time was approximately 6 days). As shown in Figure 7, the flow rate was still excessive. The ARUM process held the pH of the effluent above pH 4 for approximately 20 days. Sulphate reduction appeared to be present after for approximately 1 week, but was gradually lost during the following 2 weeks. During the first week of operation, the blackening of the reactor shifted from the top to the bottom. This likely occurred as a result of aeration when influent was pumped into the reactor. Furthermore, if inhibitory levels of volatile fatty acids had accumulated before flow conditions in the bottom of the reactor, they would gradually be transported

into the reactor effluent.

The third experiment was conducted at flow rates of 100 mL per day or a fluid retention time of approximately 15 days. The reactor (#3) used in this trial was the one which had recovered from the excessive flow rate of 500 mL per day. At this flow rate, the ARUM process in these reactors appears to be capable of operating continuously. It has now been in operation for 57 days and the bottom region of the reactor still appears black.

Figure 8 is a graphic display of the reactor's performance during the first 26 days of operation. Sulphate and nickel levels were also measured in this experiment. The nickel concentration in the effluent was reduced to non-detectable levels (ie less than 0.2 ppm) on all but 2 sampling periods. On these days, the nickel levels in the effluent were 0.7 ppm and 0.5 ppm compared to influent levels of 18 ppm and 6.8 ppm respectively.

The appearance of detectable amounts of nickel in the reactor effluent coincides not only with increased levels of nickel in the influent, but also increased levels of sulphate. The pH of the effluent was also lower at this time. It appears that the capacity of the ARUM process was overcome by a stronger influent feed. The strength of influent was higher than the previous days probably because this influent was removed from the bottom of the 5 gallon sample of seepage water sent from the Makela site.

In this experiment, the pH stability of the effluent was also monitored. As shown in Figure 9, the sample removed after 5 days of reactor operation did not re-acidify. In fact, the ARUM process appeared to continue in the effluent sample since the pH increased during the following 18 days from pH 5.5 to pH 6.9.

In contrast, effluent samples taken at later dates re-acidified. As shown in the bottom graph on Figure 10, this lack of pH stability was coincidental with an increase in ferrous iron concentration from the reactor effluent. It was also correlated to the change in effluent appearance. The effluent reservoir sample whose pH increased on standing was

brown and changed to black. The effluent reservoir samples which reacidified on standing were yellow/brown with brown particles. The odour of the two types of samples was also different. These experiments show two very important operating principles for ARUM:

1. It is important to reduce the iron content of the influent waters as much as possible. Iron fed to reactors becomes reduced during the ARUM process and thus can contribute to the reacidification of the effluent under aerobic conditions.

2. It is important to assure that sulphate reduction is active during the ARUM process. Sulphate reduction can help stabilize the pH of ARUM effluents by precipitating ferrous iron.

3.3 Enumeration of Sulphate Producing Bacteria (SRBs)

An experimental program to improve enumeration of sulphate reducing bacteria (SRBs) was initiated.

Sulphate reducers are a key part of the ARUM process because:

1. They directly contribute to the alkalinity generation.

2. They produce hydrogen sulphide which can precipitate toxic metals as metal sulphides.

3. They produce hydrogen sulphide which removes ferrous iron as iron sulphide and thus prevents reacidification of treated water as ferrous re-oxidizes.

Hence, effective monitoring tools for these organisms is important. In the early part of the ARUM development research program (1) using Pre-Bog Acid Creek seepage water,

high levels of SRBs were rarely measured by culture techniques. Nevertheless, blackening of the reactors and sample bottles indicated some significant sulphate reduction was occurring.

Part of the reason why SRB numbers appeared low is likely due to the tendency of SRB's to be sessile and therefore not retrievable from water samples. However, there was still some question of whether alternate media might be more effective in recovering SRB's from the experimental samples.

Therefore, a study was conducted where a variety of media were compared for the ability to support the growth of SRB's. Dilutions of a sample suspected to contain high numbers of SRB's were inoculated into each media and the time required for blackening of the media (ie. production of ferrous sulphide as a consequence of sulphate reduction) to be visible was recorded. It is assumed that the media which indicated the highest number of SRB's in the shortest time was superior.

The sample which was used for the experiment contained both water and amendment and was shaken vigorously to suspend as many sulphate reducing bacteria as possible. So far, two sources of samples have been tested: one from a reactor containing Makela water and amendment, the other from a vial containing Denison seepage water amended with iron and flax and inoculated with a seed from a sample of sawdust amendment from the Oriental East Limnocorral at the Buchanan's Mine Site, Newfoundland. The results are summarized in Figure 11 and Figure 12.

From a brief examination of these graphs it becomes apparent that, in contrast to past experiments, it was possible to detect the presence of high numbers of sulphate reducing bacteria. For the Makela SRB's, pH 5 media showed consistently as good growth or better growth than pH 6. However, for the Denison/Oriental East SRB's, pH 6 media appeared more effective. It is interesting to note that the Oriental East water, in contrast to the other waters, is not very acidic.

For the Makela SRB's, the fastest growth occurred in Postgate B medium containing magnesium ribbon, while for Denison/Oriental East SRB's, this media was least effective. The significance of magnesium has been discussed in section 3.1.

On the other hand, the fastest growth for the Denison/Oriental East SRB's was in Postgate B medium, supplemented with iron (either as dust or finishing nails). Postgate B media with iron nails was an effective medium for the Makela SRB's but iron dust was not so effective. An explanation for this was not apparent.

Starkey's medium was ineffective in both case. Copious quantities of gas (probably CO_2) were produced from both samples from this medium. Probably the displacement of agar throughout the culture tube prevented the maintenance of effective anaerobic conditions and thus prevented the growth of SRB's.

The best compromise based on this very limited study is Postdate B medium using a nail to poise the redox potential. It is likely that the SRB's from each geographical location have an optimum medium for growth. However, until future experiments indicate otherwise, the Postgate B/nail medium is recommended in cases where testing a battery of media is not feasible.

3.4 Isolation of Cellulose Degrading Organisms

Experiments to isolate cellulose degrading organisms have been initiated. Such organisms could be used to study the potential of biostimulation (process of changing the environment to encourage the growth and activity of a certain species) and bioaugmentation (direct addition of species with desirable properties to a biological process). It is also useful to have the capability of testing a sample from an ARUM process for the presence of cellulytic organisms because their presence indicates the likelihood that cellulose degradation is occurring.

Figure 13 is a photograph showing the results of an experiment conducted to isolate aerobic cellulose degraders. The collapse of the filter paper indicates that cellulose decomposition has occurred. The cellulytic mould has been isolated from a sample obtained from the Pre Bog Acid Creek site. Recent attempts to grow anaerobic cellulose degraders have so far been unsuccessful.

3.5 Analyses of Total Soluble Carbohydrate

The Molisch test was examined as a simple procedure for measurement of total carbohydrate. Pentoses, hexoses, heptoses and their derivatives are detected, while amino sugars, trioses and tetroses are not.

This test appears to be applicable to analyses of Denison and Makela waters providing the samples are adjusted to pH 7 and filtered prior to analysis. Without this pre-treatment, false positive results are produced. With the pre-treatment included in the procedure, quantitative recovery of 50 mg per L glucose spikes was achieved.

Table 7 shows results of analyses performed on water column reactors. The sample removed from the middle port of the Denison reactor had more than 1000 ppm soluble carbohydrate. Although this represents a high carbohydrate concentration, this reactor had failed to generate alkalinity. This analysis indicates that the difficulty in generating alkalinity in this reactor is not due to a lack of carbon source for volatile fatty acid (VFA) producing bacteria.

Also shown in Table 7, much less carbohydrate was found in the samples taken from the Makela reactors. This is not surprising since this reactor was being operated in the flow mode. The accumulated soluble carbohydrate will be diluted as fresh influent is brought into the reactor. Furthermore, as this reactor has an active ARUM process, soluble carbohydrates in the water will be consumed by the fermentative organisms.

4.0 SUMMARY AND CONCLUSIONS

- Alkalinity generation could not be initiated in water column reactors containing Denison Mine Acid seepage water when the following amendments were applied: 1) straw 2) straw: alfalfa (3:1 v/v) or 3) straw + 1000 ppm glucose.

- Successful initiation of alkalinity generation in 40 mL glass vials was demonstrated with the following amendments: 1) ground flax covered with iron filings 2) cooked meat pellets.

- The function of iron in the initiation of alkalinity generation in Denison water was not merely to poise the redox potential. Magnesium ribbon, a stronger reducing agent than iron was not capable substituting for iron.

- Sulphate reduction was not an essential activity for the initiation of alkalinity generation in Denison water. In some test conditions alkalinity generation occurred without any evidence of sulphate reduction.

- A limited evaluation of media for enumeration of sulphate reducing bacteria was conducted. Postgate B medium with an iron nail in the medium vial appeared to be the best compromise to enumerate these organisms.

- A bench scale flow operation of the ARUM process was successfully achieved using acidic seepage water from the Makela site. The seepage water was pretreated with aeration to precipitate iron.

- A stable flow operation of ARUM was achieved through water column reactors at flow rates of 100 mL Makela seepage water per day. This is equivalent to an hydraulic retention time of 15 days.

- During stable flow operation of ARUM with Makela acid seepage water the pH of the effluent was held above pH 4 and nickel was usually not detected (detection limit = 0.2 mg per L).

- The pH stability of Makela seepage water was examined following ARUM treatment under flow conditions. Maximum stability was achieved when sulphate reduction of ARUM was active and ferrous iron levels in the effluents were lowest.

- The Molisch test was found useful as a simple procedure for measurement of total soluble carbohydrate.

- An aerobic cellulolytic fungus was isolated from a Pre-Bog Acid Creek Sample. This culture will be used in attempts to increase the rate of the ARUM process.

5.0 FUTURE WORK

It is anticipated that goals for the balance of this year's programme will be completed as outlined in December, 1989. This work will include experiments to define the essential components of the ARUM process, to improve alkalinity generation rates in Makela reactors, to assess the process nitrogen requirements, and to further development of field monitoring techniques.

6.0 REFERENCES

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TABLE 1 DENISON REACTOR EXPERIMENTS - pH PROFILES

Days of Incubation	pH					
	Reactor 1		Reactor 2		Reactor 3	
1	2.1		2.1		2.1	
7	2.2		2.25		2.19	
14	2.26		2.24		2.30	
21	2.07		2.15		2.19	
	Added nothing		Added 1000 ppm glucose		Added 20% Alfalfa	
22	2.00		2.10		2.16	
29	2.06		2.05		2.07	
	Top	Bottom	Top	Bottom	Top	Bottom
36*	2.01	2.0	1.96	2.07	2.05	2.04
50	2.37	2.16	2.25	2.31	2.32	2.26
57	2.01	1.78	1.94	1.93	2.02	1.90
54	2.26	2.22	2.23	2.34	2.25	2.27
71	2.15	2.10	2.12	2.24	2.22	2.21
78	2.11	2.04	2.09	2.18	2.15	2.19
85	2.17	2.01	2.07	2.08	2.06	2.05

*Inoculated 5 mL from a Pre-Bog Acid Creek water column reactor.

NOTE: Samples were collected from the bottom port of the water column reactors unless otherwise indicated.

TABLE 2 DENISON REACTORS COMPREHENSIVE ANALYSES (DAY 14)

Parameter	1	2	3
pH (units)	2.26	2.24	2.30
FMA (meq/L)	75	85	80
SO_4^{-2}(meq/L)	140	100	140
NO_3^{-1}(ppm)	5	<5	c5
NO_2^{-1}(ppm)	c5	<5	<5
PO_4^{-3}(ppm)	c5	c5	c5
NH_4^+ (ppm)	<1	<1	<1
CO_2 (ppm)	6400	3680	5320
ATP (ng/mL)	0.92	1.12	0.50
SRB Postgate E (organisms/mL)	<1	<1	<1
SRB Postgate F (organisms/mL)	<1	<1	<1
Ammonifiers (organisms/mL)	10^3	10^3	10^3
IRB (organisms/mL)	1	1_{03}	10^2
Bacteria (organisms/mL)	$2x10^7$	$3x10^7$	$2x10^7$
Mould (organisms/mL)	$<10^4$	$<10^4$	$<10^4$
Algae (organisms/mL)	$<10^4$	$<10^4$	$<10^4$

TABLE 3 MICROBIOLOGICAL PROFILE OF MICROBIAL SEED FROM ORIENTAL EAST LIMNOCORRAL

Test Parameters	Results
ATP	12.1 ng/mL
Total Microscopic Count:	
Bacterial (mainly spirochaetes)	10^7 per mL
Moulds	$<10^4$ per mL
Algae	$<10^4$ per mL
Sulphate Reducing Bacteria:	
Postgate E Medium	10^4 per mL
Postgate F Medium	10^5 per mL
Iron Reducing Bacteria	10^4 per mL
Ammonifiers	$\geq 10^6$ per mL

Amendment			pH After 9 Days	pH After 22 Days
1	Flax	without BOD nutrients	2.2	2.2
		with BOD nutrients	2.3	2.2
2	Alfalfa	without BOD nutrients	2.5	2.6
		with BOD nutrients	2.5	2.5
3	Cooked meat pellets	without BOD nutrients	4.4'"	4.3(1)
		with BOD nutrients	4.4'"	4.3'"
4	Flax + 50 mg glucose	without BOD nutrients	2.2	2.2
		with BOD nutrients	2.2	2.2
5	Flax + 20 µg lactic acid	without BOD nutrients	2.2	2.3
		with BOD nutrients	2.2	2.2
6	Flax + 50 mg peptone + 20 µg lactic acid	without BOD nutrients	2.4	2.4
		with BOD nutrients	2.2	2.3
7	Flax + 20 µg acetic acid	without BOD nutrients	2.2	2.0
		with BOD nutrients	2.2	2.1
8	Flax + 50 mg calcium	without BOD nutrients	2.4	2.3
		with BOD nutrients	2.4	2.3
9	Flax + Iron Filings	without BOD nutrients	4.7(2)	5.9(2)
		with BOD nutrients	6.0(2)	5.4(2)
10	Barley	without BOD nutrients	2.2	2.3
		with BOD nutrients	2.2	2.2
11	Guinea pig pellets	without BOD nutrients	2.5	2.7
		with BOD nutrients	2.8	2.9
12	Bran	without BOD nutrients	2.5	2.3
		with BOD nutrients	2.3	2.3

TABLE 4 NOTES:

(1) No evidence of sulphate reducing bacteria activity (blackening) was evident. The liquid had turned orange and fungal growth was evident on the surface.

(2) Both vials had turned black and had a strong sulphide odour.

During the first week of incubation, all vials showed evidence of vigorous microbial activity as indicated in bubbling (i.e. probably CO_2 produced by cell respiration). At the end of the experiment, all waters which remained acid turned an amber colour and a film of green fungal growth occurred at the air-water interface.

TABLE 5 DENISON ACCLIMATION EXPERIMENT

Sample Dilution	pH at Beginning of Test	pH After 21 Days Incubation	Observations after 21 Days Incubation
1/1	2.13	2.22	dark amber coloured liquid with fungus growing on top of liquid and flax.
1/2	2.25	2.57	dark amber liquid with fungus on top of liquid flax.
1/4	2.44	2.58	dark amber liquid with fungus on top of liquid and flax.
1/8	2.58	2.87	dark amber liquid with fungus on top of liquid and flax.
1/16	2.76	5.10	dark green liquid - algae observed. blackening observed throughout amendment and liquid.
1/32	2.90	5.38	dark green liquid - algae observed. blackening observed throughout amendment and liquid.

TABLE 6 EXPERIMENT TO DETERMINE MECHANISM OF ALKALINITY GENERATION IN ACID WATER

Test Conditions	pH		ATP (ng/mL)	Observations
	After 14 Days Incubation	After 18 Days Incubation	After 18 Days Incubation	
Iron	4.38	5.12	20.40	Partially blackened throughout amendment - amber liquid with gas production.
Iron + Microbial *seed*	5.60	5.83		Blackened throughout amendment - amber liquid with gas production.
Iron + Biocide	4.47	4.67	12.40	No blackening observed - amber liquid with gas production.
Iron + Molybdate + Microbial seed	5.38	5.81		Blackened throughout amendment - amber liquid with gas production.
Magnesium	2.08	2.10		Dark amber liquid, white mold on top.
Magnesium + Microbial *seed*	2.08	2.15		Dark amber liquid, white mold on top.
Magnesium + Biocide	2.13	2.13		Dark amber liquid, white mold on top.
Magnesium + Molybdate + Microbial *seed*	2.20	2.17		Dark amber liquid, white mold on top.

Note: 1. Microbial seed consisted of a one mL inoculation of a suspension known to contain sulphate reducing bacteria. The suspension was prepared by shaking a vial of Denison water which had been successfully amended with flax and iron filings.

2. Biocide treatment consisted of an addition of 500 ppm and of Dearborn Biomate 750, a product containing 2-bromo-2-nitropropane-1,3-diol.

TABLE 7 TOTAL SOLUBLE CARBOHYDRATE ANALYSES OF WATERS IN ARUM REACTORS

Sample	Total Carbohydrate (ppm)
Untreated Denison Water	10
Denison #3 Reactor (middle)	1150
Untreated Makela Water	10
Makela #3 Reactor (middle)	275
Makela #3 (spigot)	290

Note: Analyses were performed using the phenol reaction Molisch test with glucose as the standard.

FIGURE 1: DENISON WATER COLUMN REACTORS

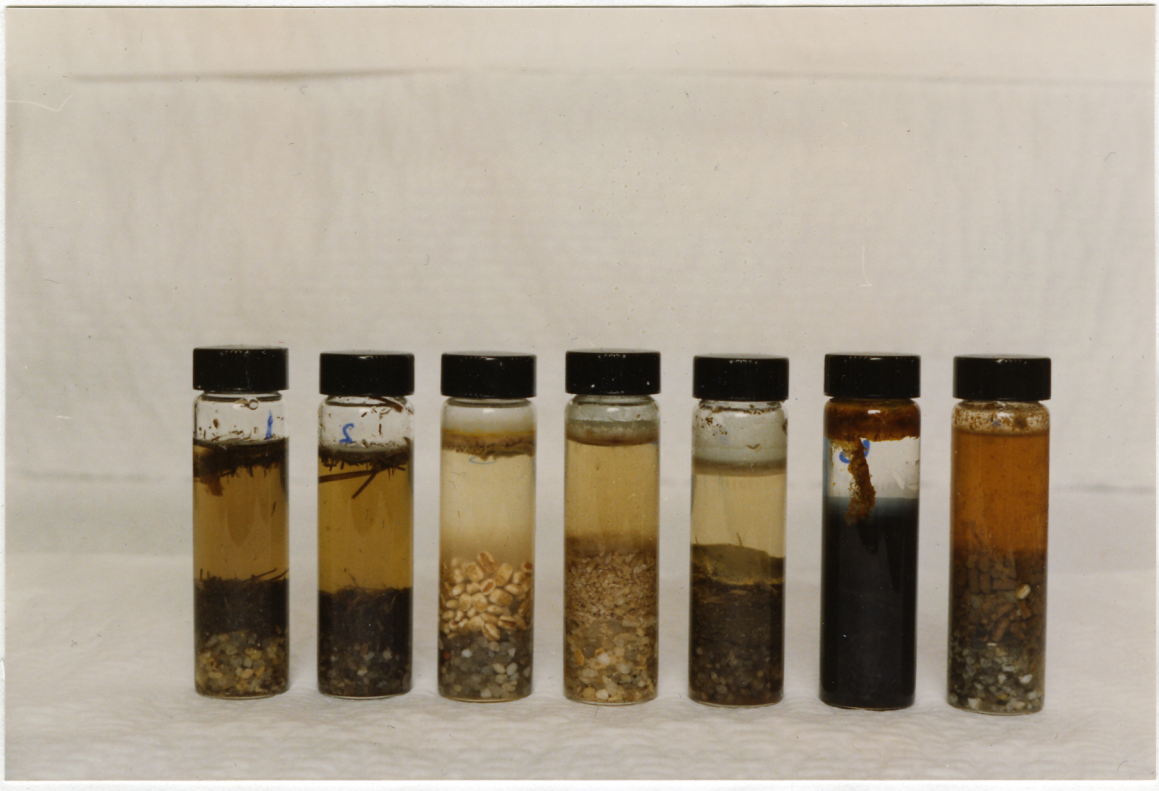

FIGURE 2: DENISON AMENDMENT SCREENING EXPERIMENT
Amendments from left to right are as follows: flax, flax plus lactic acid and peptone; barley; bran; alfalfa; flax plus iron filings; cooked meat pellets.

Alkalinity generation occurred only with the latter two amendments.

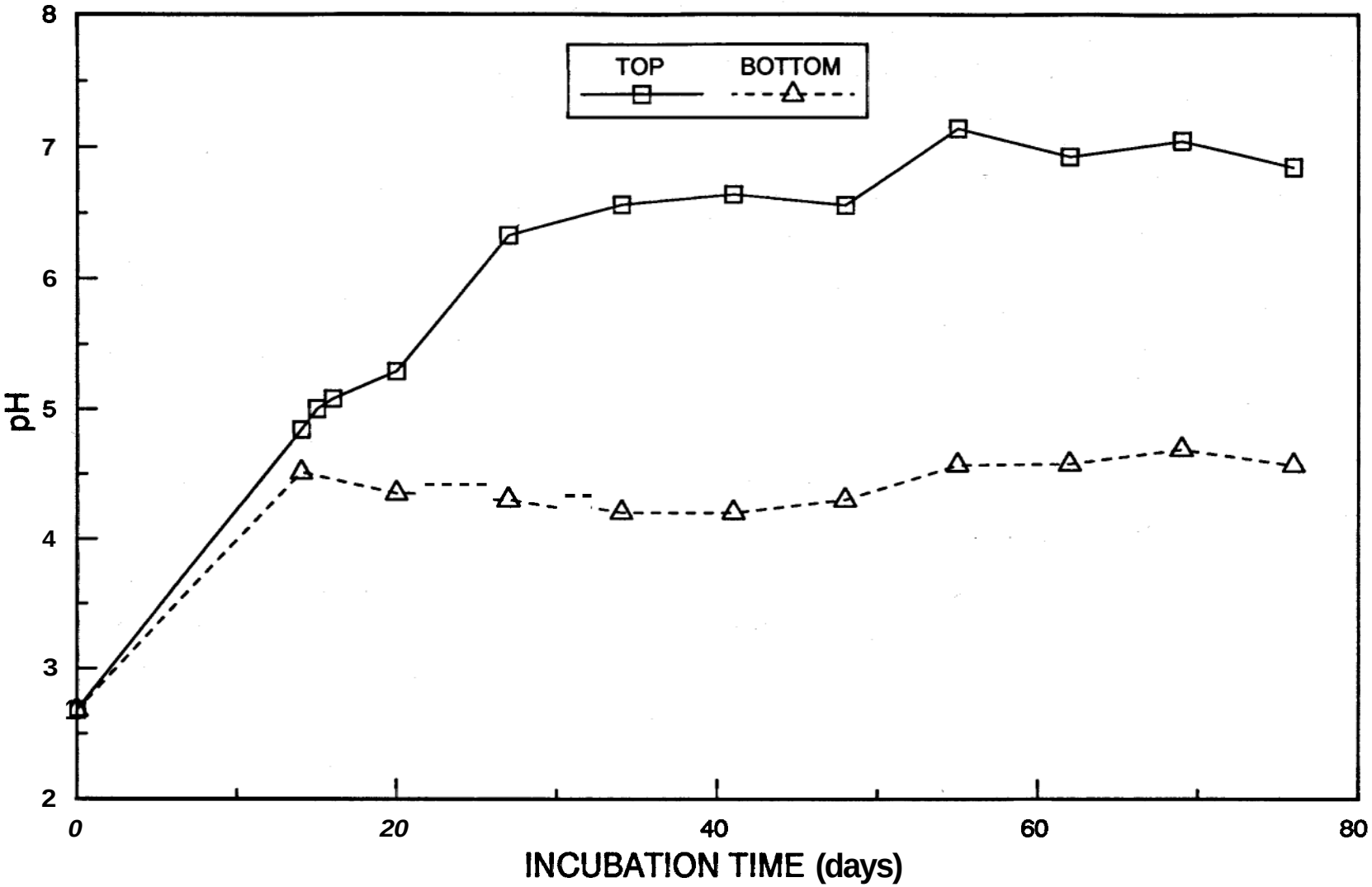

FIGURE **3:** ALKALININ GENERATION **IN MAKELA REACTOR (#2)**

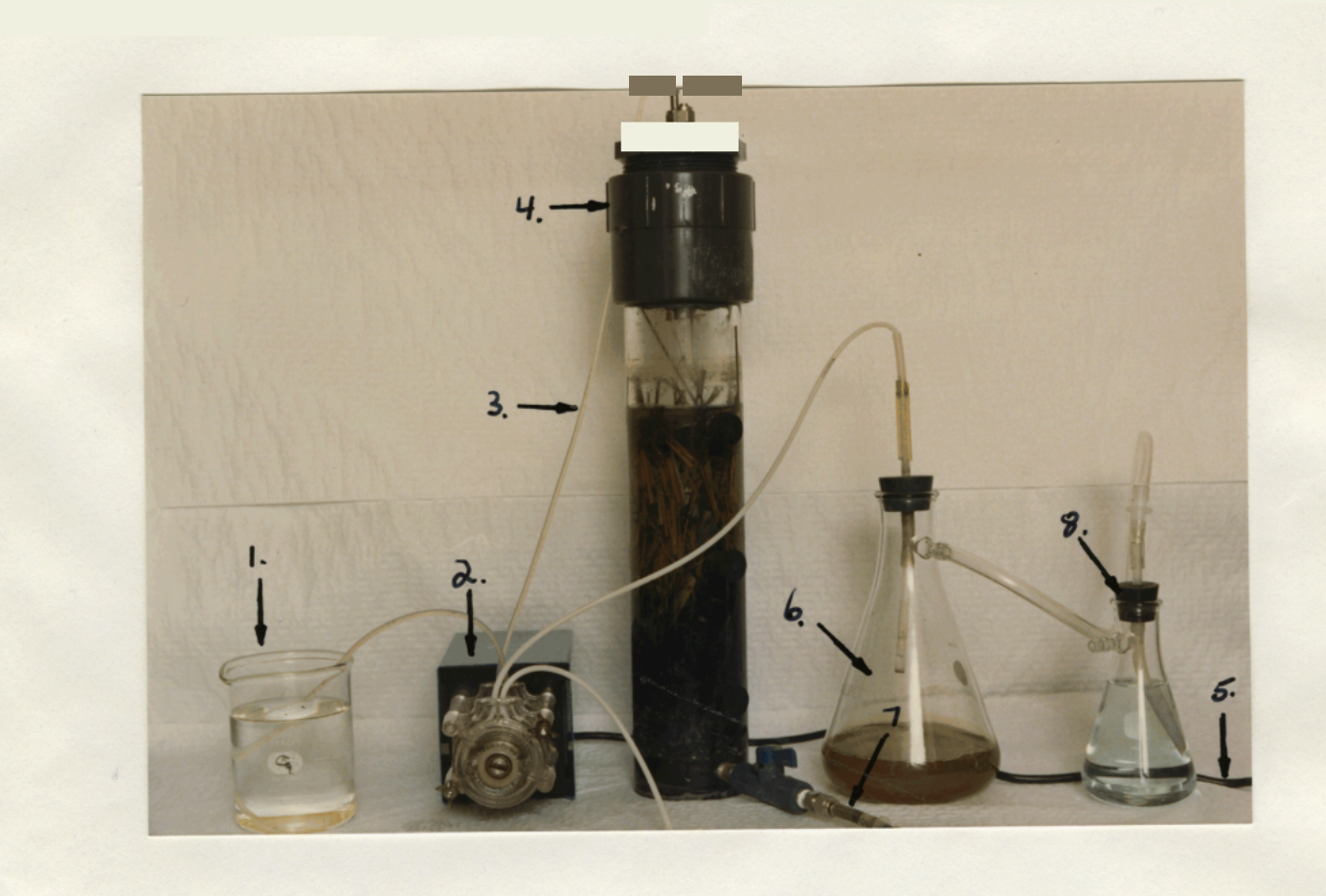

FIGURE 4 EXPERIMENTAL APPARATUS FOR CONDUCTING FLOW STUDIES:

1. Pre-treated Makela seepage water.
2. Peristaltic pump.
3. Influent feed to water column reactor.
4. Water column reactor.
5. Pump's power cord to timer.
6 Effluent reservoir.
7. Effluent line from reactor.
8. H_2S Trap.

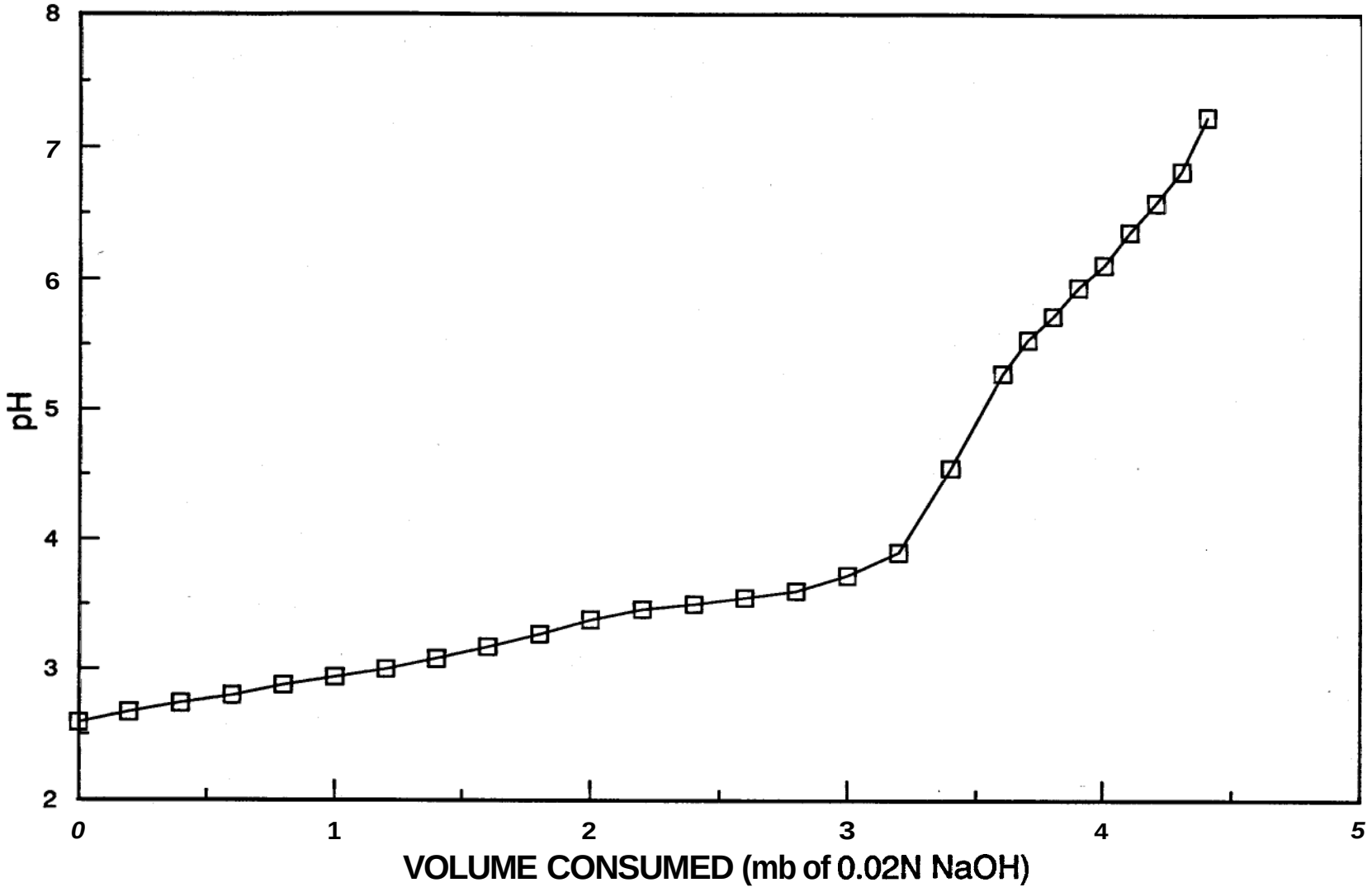

FIGURE **5:** **TITRATION CURVE**
NEUTRALIZATION OF MAKELA SEEPAGE WATER

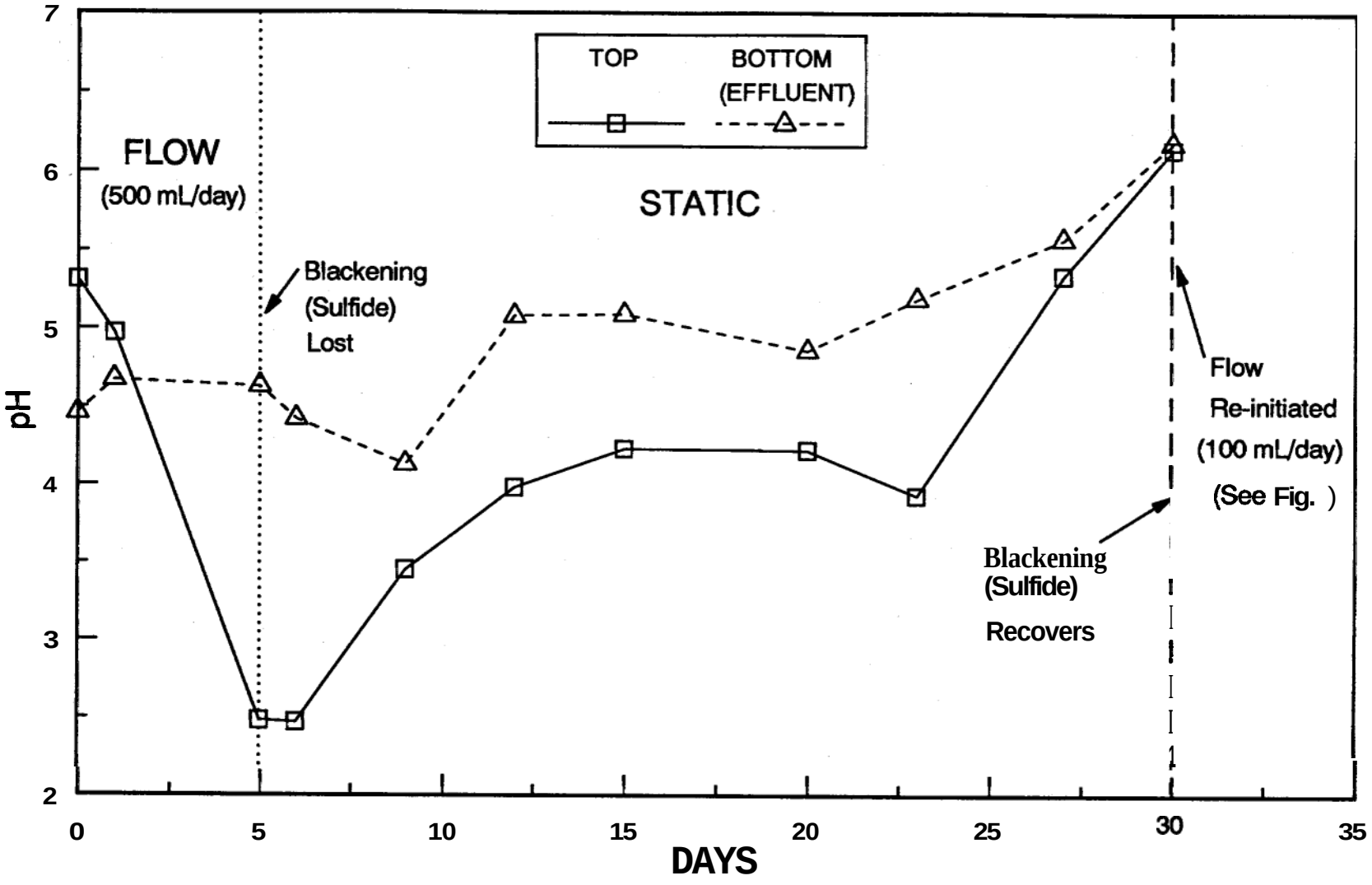

FIGURE 6: RECOVERY OF MAKELA REACTOR #3 FROM EXCESSIVE FLOW RATES

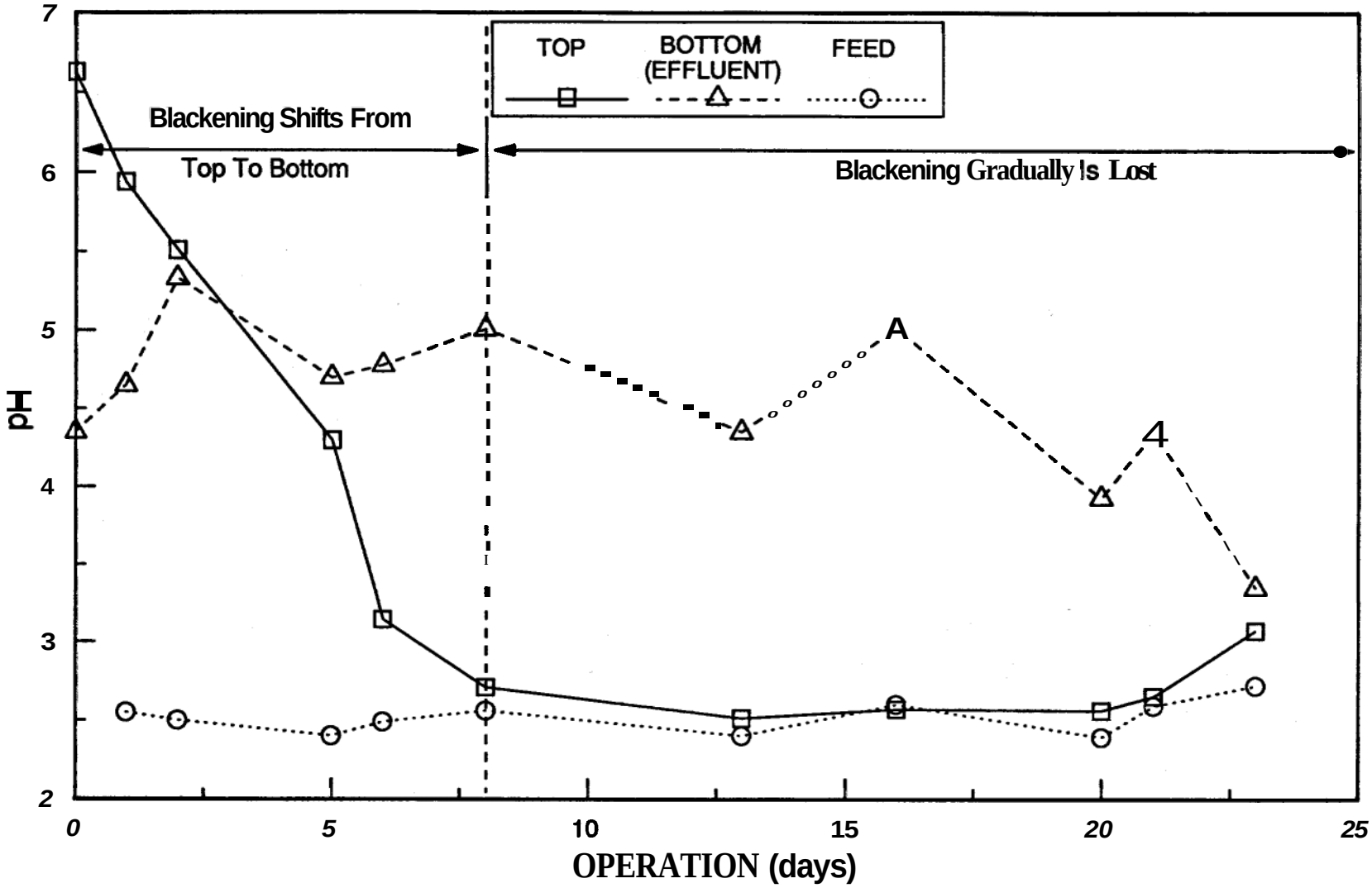

FIGURE *7:* **FLOW EXPERIMENT**
MAKELA REACTOR (#1): 250 mL/DAY

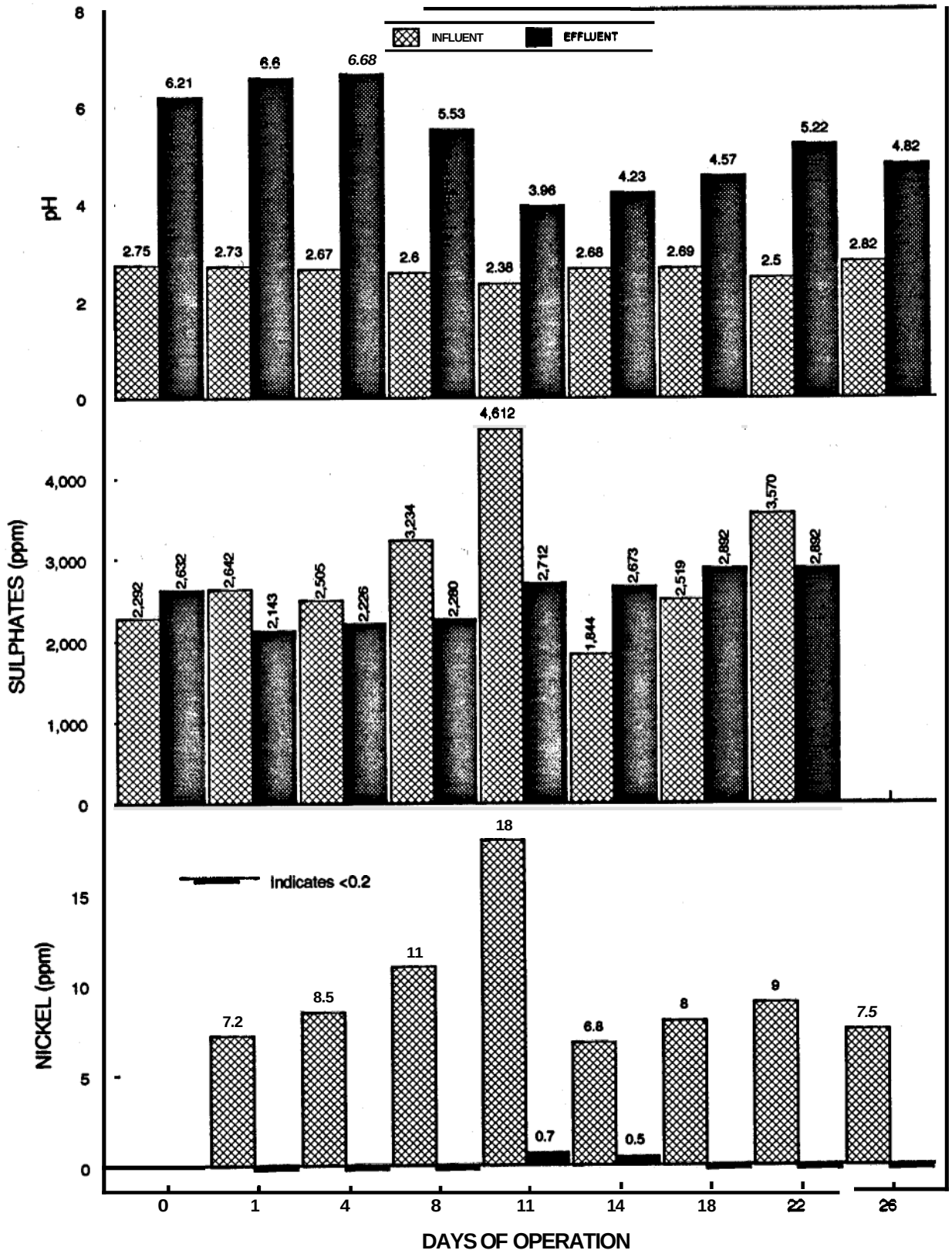

FIGURE 8: FLOW EXPERIMENT: MAKELA REACTOR #3: 100 mL/DAY

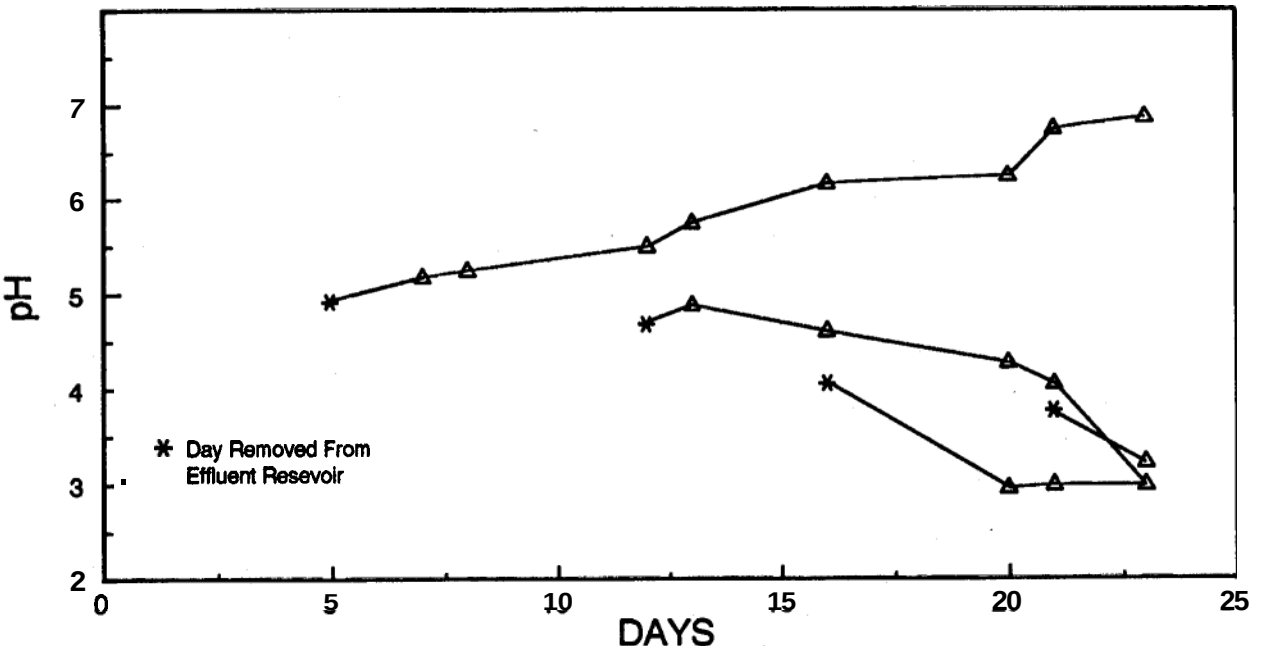

FIGURE 9: STABILITY OF EFFLUENT pH MAKELA REACTOR #1

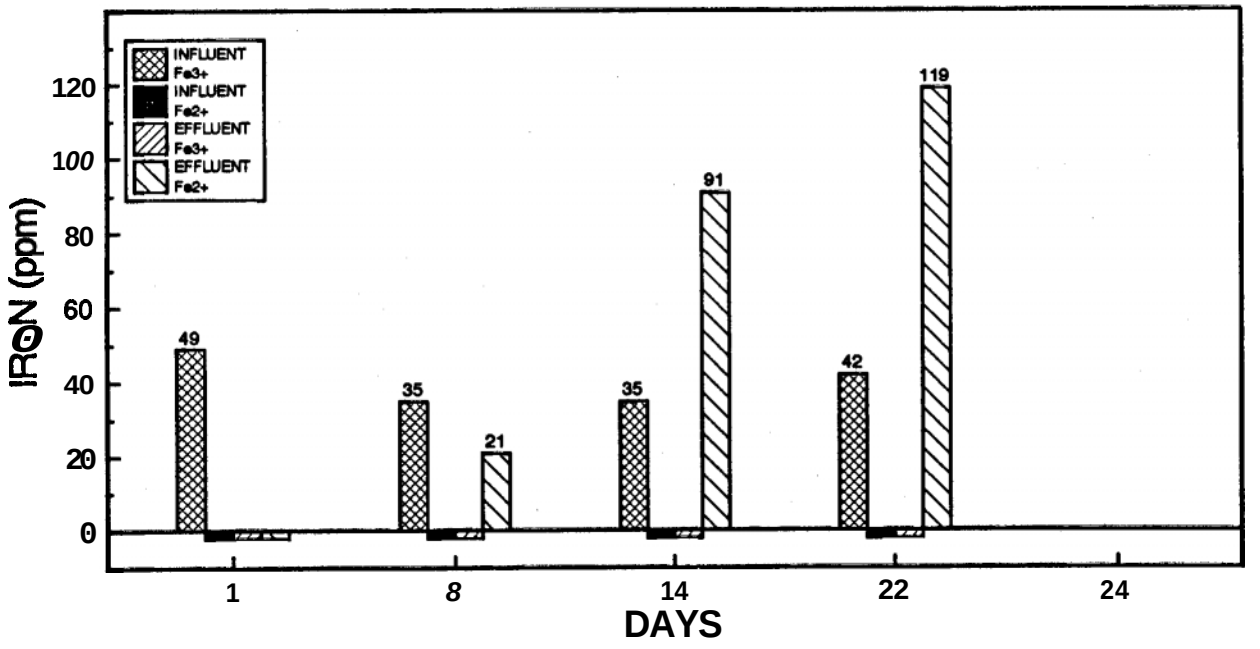

FIGURE 10: INFLUENT AND EFFLUENT IRON LEVELS: MAKELA #1

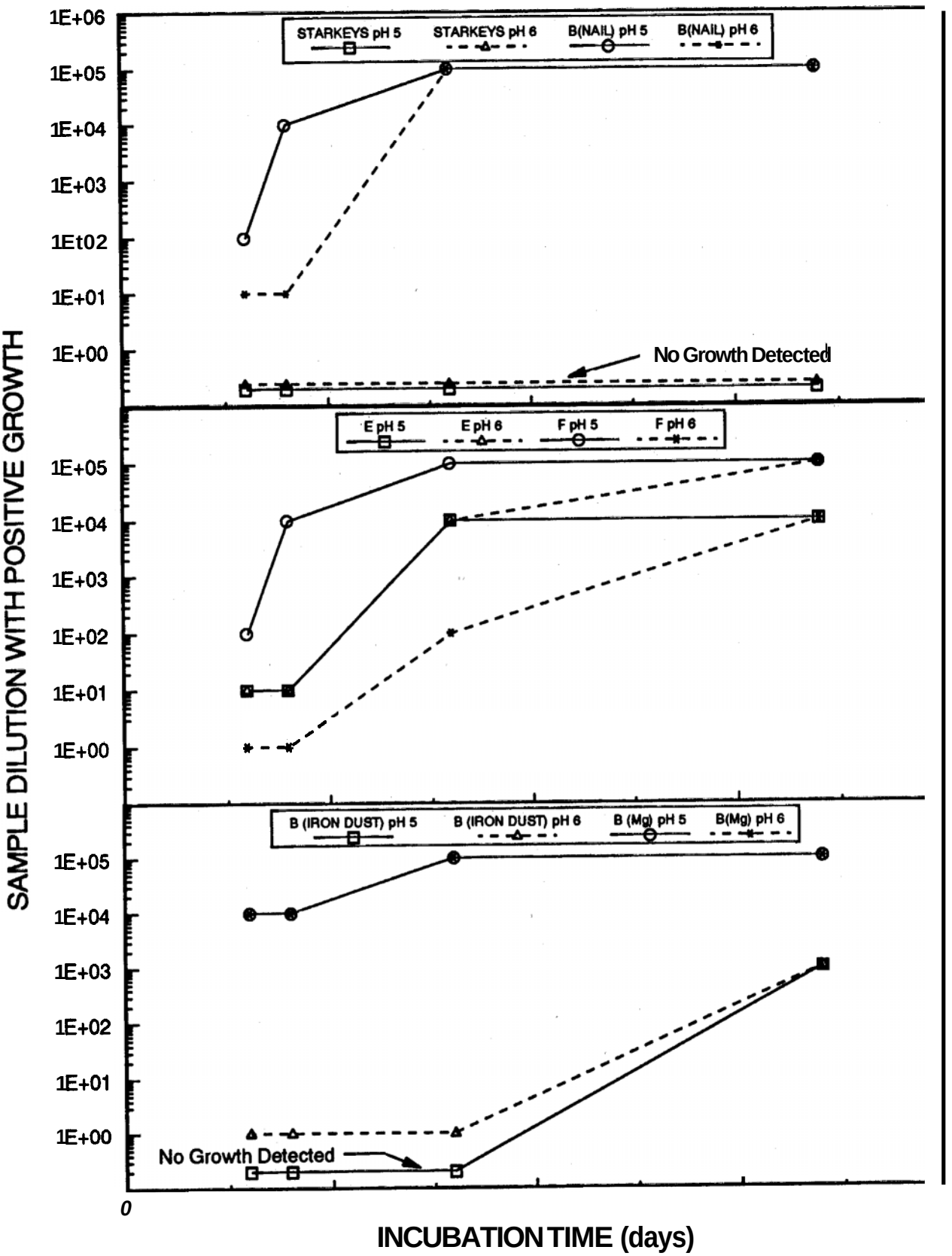

FIGURE 11: **MEDIA TEST FOR ENUMERATION OF SULPHATE REDUCING BACTERIA: MAKELA SAMPLES**

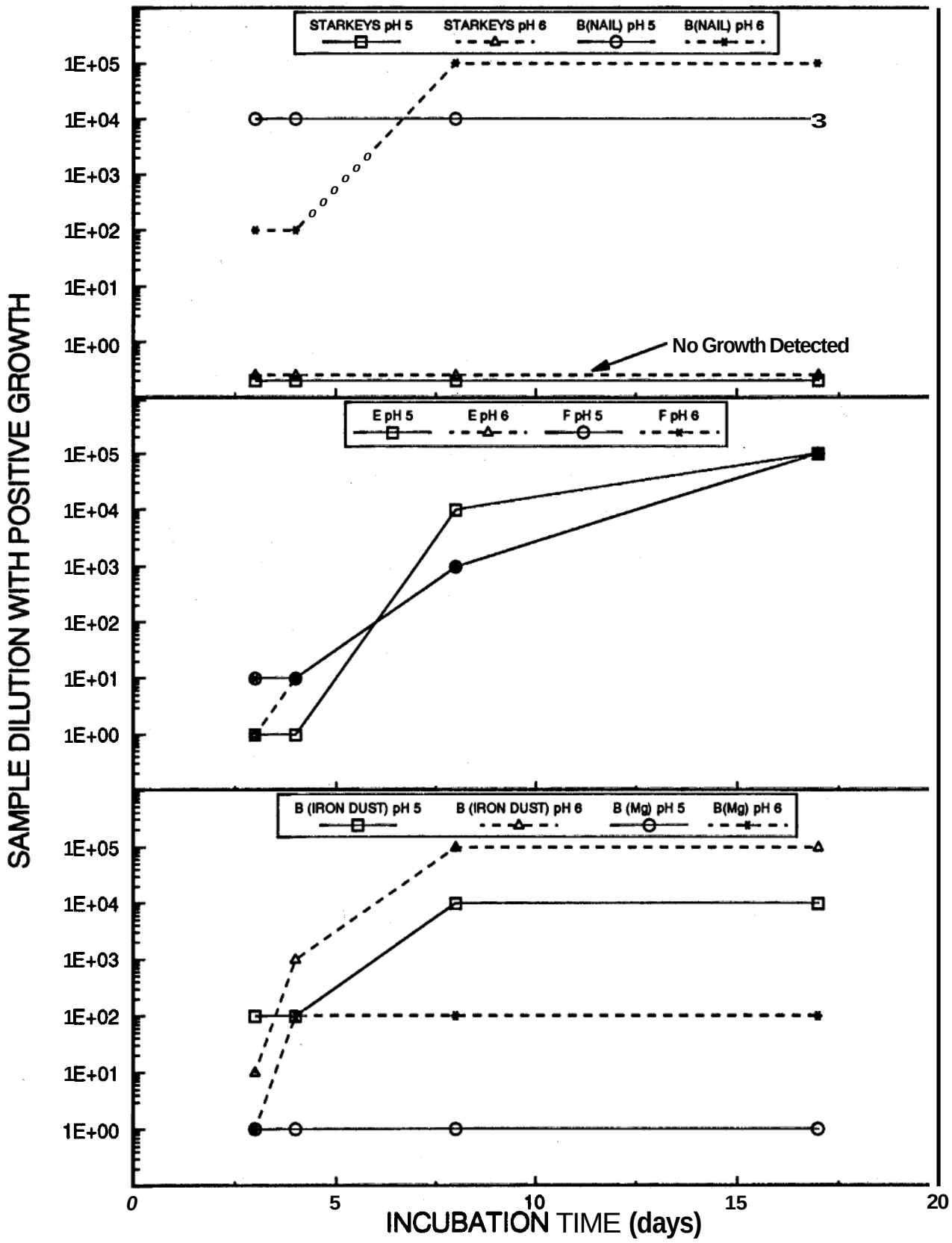

FIGURE **12: MEDIA TEST FOR ENUMERATION OF SULPHATE REDUCING BACTERIA: DENISON/BUCHANS SAMPLES**

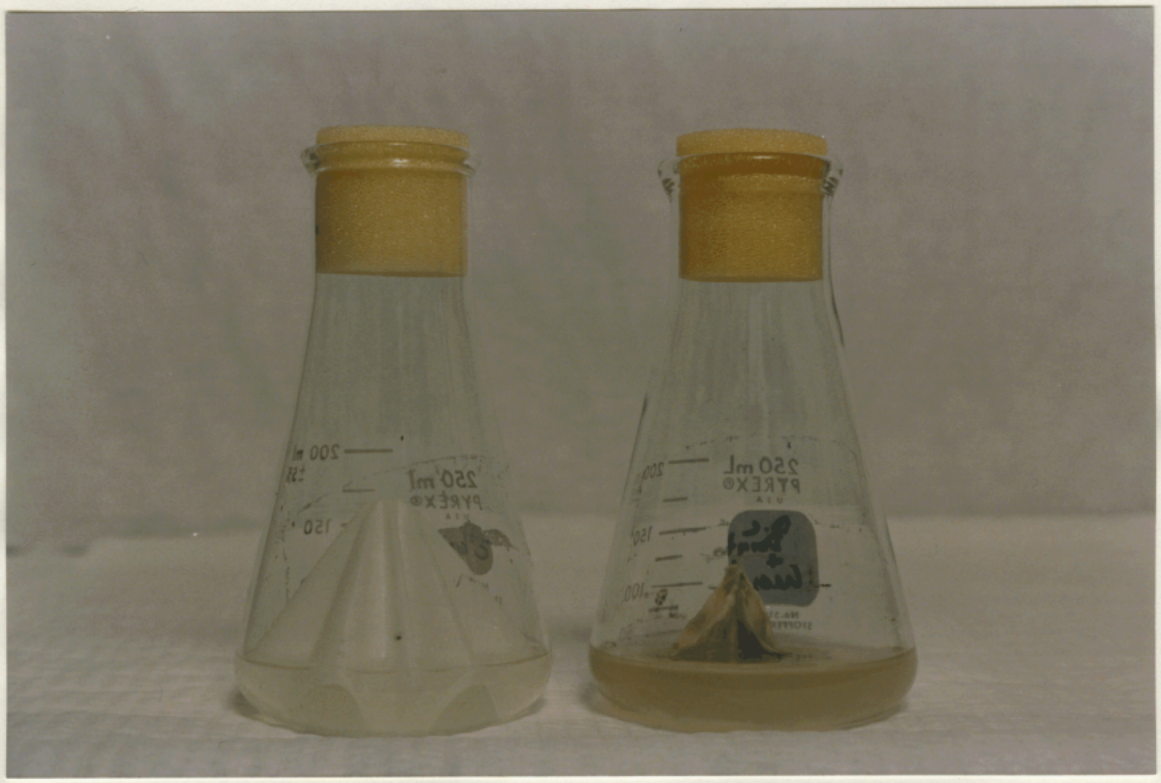

FIGURE 13. CULTURE OF CELLULOSE DEGRADING MICROORGANISMS.

The flask on the left is an un-inoculated control. The filter paper in the flask on the right has been degraded by a cellulytic fungus.

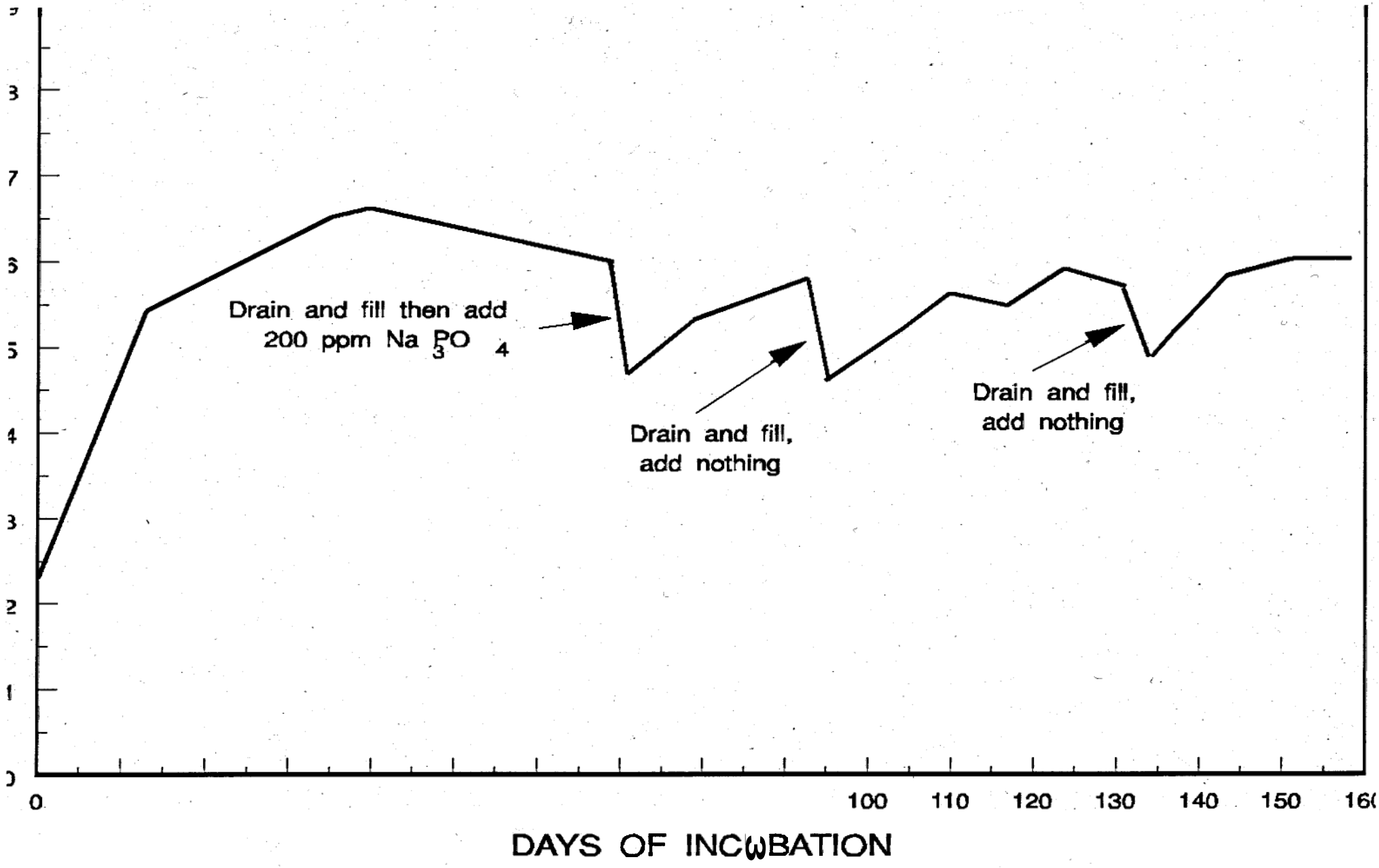

FIGURE 1: EFFECT OF ACID SHOCK ON THE ARUM PROCESS