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Development of a Biological System for the Treatment of Mill Waste Waters Containing Thiosulfate

B.C. Research

INLAND WATERS DIRECTORATE,
WATER QUALITY BRANCH,
OTTAWA, CANADA, 1973.

Contract No. 02KX.KL327-3-8061

Thorn Press Limited

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B.C. RESEARCH

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Project No. 1525

June 1, 1973

To: Water Quality Branch
Inland Waters Directorate
Water Management Service
Department of the Environment
Ottawa, Ontario K1A 0E7

Subject: DEVELOPMENT OF A BIOLOGICAL SYSTEM FOR THE TREATMENT OF MILL WASTE WATERS CONTAINING THIOSULFATE

A. OBJECT

To develop the engineering design parameters necessary for the operation of a biological system to treat flotation-mill waste waters containing thiosulfate.

B. BACKGROUND

Studies were carried out in New Brunswick in 1968 (1, 2, 3) to determine the cause of an acid problem in the river system receiving waste water from the flotation mill of a mining operation. The mill was treating a lead zinc ore containing pyrite and some pyrrhotite. The mill effluent was discharged to a tailing pond system (4), where solids were separated. Lime and soda ash were used in the mill for pH control and additional lime was added to raise the pH of the tailings pulp to 9.5. After settling, the tailing pond effluent was charged to the river system. Prior to the addition of lime at the discharge weir, the pH of the effluent from the tailings pond varied from about pH 9.5 in winter, to pH 6 in summer. However, regardless of the pH at which it was discharged, acidic conditions (pH 2.5 - 3.5) were produced in the stream within a distance of 13 miles.

The effluent was found to contain high concentrations of thiosulfate, in the order of 500 - 1000 mg/l, as well as higher polythionates, such as trithionate and tetrathionate. It was concluded that the oxidation of these thio-compounds by sulfur-oxidizing bacteria (Thiobacillus species) with the subsequent production of sulfuric acid, was the principal reason for the acidic environment developing in the receiving stream.

The Department of the Environment wants to develop a biological waste treatment system which will oxidize thiosulfate in waste waters of the type emanating from the New Brunswick operation. Ideally, the system should be designed to function at a neutral or alkaline pH, and temperatures down to 2 C. A contract was awarded to B. C. Research for a literature survey covering nutritional and environmental factors influencing the oxidation of thiosulfate by thiobacilli. In addition, laboratory studies were to be carried out to assess the practicality of using Thiobacillus species to oxidize thiosulfate in waste water. Following successful completion of the laboratory studies, experiments involving a bench scale continuous reactor were to to undertaken. The systems selected for evaluation were a simulated activated sludge system with recycle and a fixed film reactor.

This report presents the results of this investigation, and the flow sheet for a pilot plant system designed to operate at 10 gal/min.

C. SUMMARY

1. Literature Survey

The literature concerning the oxidation of thiosulfate by members of the genus Thiobacillus was reviewed. It revealed that such bacteria, especially Thiobacillus thiooxidans and the closely related T. ferrooxidans, were ideally suited for oxidizing thiosulfate in an adverse environment. The requirements for a biological oxidation system would appear to be that there by oxygen, and carbon dioxide available from the air, and that the pH be below 5.5. Ammonium and phosphate ions would probably have to be added.

2. Bacteria in Tailings Water

Examination of a sample of tailings water indicated that at least two Thiobacillus sp. were present. One was definitely T. ferrooxidans and the more prevalent species was probably T. thiooxidans.

3. Batch Laboratory Experiments

a. Batch experiments using the culture isolated from the tailings water showed that thiosulfate oxidation rates were the same when the initial pH was 3, 4 or 5. The final pH was in the range 1.7 - 1.9 regardless of the initial pH. When the initial pH was 6, rapid thiosulfate oxidation sometimes occurred after a long lag. Examination of the pH data indicated that rapid oxidation only occurred if the pH dropped below 5.6.

b. A decrease in the incubation temperature reduced the rate of thiosulfate oxidation. The estimated rate at 2 C was 12 mg/l/hr, which was approximately 1/10 the rate at 23 C and 1/20 the rate at 30 C.

c. The conversion of oxidized thiosulfate to sulfuric acid ranged from 85 - 95%. The conversion was not effected by pH, but it decreased with a decrease in temperature. The missing thiosulfate could not be accounted for.

d. The generation time of the tailings water isolate was calculated to be 9.4 hr at 23 C and 83 hr at 5 C, based on total organic carbon values.

e. The only micro nutrient requirements demonstrated during this study were for nitrogen and phosphate. The indicated requirement in batch studies was much higher than anticipated, based on our experience and on the literature.

f. A pure culture of T. thioparus did not oxidize thiosulfate at higher pH values or more rapidly than did the culture isolated from the mine water.

4. Biodisc Experiments at Room Temperature

a. Continuous flow studies were initiated in a biodisc unit at room temperature using a feed containing 3.6 g/l $S_2O_3^{=}$ and a residence time of 131 min. Thiosulfate oxidation rates of 267 mg/sq.ft/hr were obtained. The effluent pH was 1.7 - 1.9 and the % conversion to sulfuric acid was 91 - 92%.

b. When the feed grade was reduced to 1 g/l $S_2O_3^{=}$ and the residence time decreased to 38 min, the same rates were achieved. The effluent had a pH of 2 - 2.1.

c. Increasing the pH of the feed to 9 from the range 4.0 - 4.5 used above, did not affect the rate nor the effluent pH.

d. Reduction of the added micro nutrients to 18 mg/l of NH_4^+ and 46 mg/l PO_4^{-3} had no adverse effect on the thiosulfate oxidation rate.

5. Biodisc Experiments at 8.5 C

a. When the temperature was reduced to 8.5 ±0.5 C the 1 g/l of thiosulfate in the feed, which contained 18 mg/l NH_4 and 46 mg/l PO_4^{-3}, at pH 4 - 4.5, was oxidized at an average rate of 146 mg/sq.ft/hr. The effluent was at pH 2.1 - 2.2 and

80% of the thiosulfate which had been oxidized could be accounted for as sulfuric acid.

b. When the speed of the biodisc was reduced from 3.8 to 1.3 rpm the thiosulfate oxidation rate dropped to 104 mg/sq.ft/hr. Increasing the speed to 5 rpm did not increase the rate beyond that achieved at 3.8 rpm.

c. Increasing the carbon dioxide content of the ambient air did not increase the thiosulfate oxidation rate.

d. Reduction of the NH_4^+ and PO_4^{-3} levels to 8 and 5 mg/l respectively, had no influence on thiosulfate oxidation rate which remained at 146 mg/sq.ft/hr. When the levels were lowered to 4 mg/l of NH_4^+ and 5 mg/l of PO_4^{-3} the average oxidation rate dropped to 128 mg/sq.ft/hr.

e. Analysis of the thiosulfate oxidation data suggests that the % conversion to sulfuric acid decreased as the nutrient level was decreased, even when the thiosulfate oxidation rate did not change. This suggests formation of some intermediate oxidation product, but no sulfur, tetra- or trithionate was detected.

f. One test indicated that a clear supernatant could be achieved by adjusting the pH to 8 with limestone and lime and adding 5 mg/l of Calgon M-500.

6. Biodisc Treatment of Brunswick Tailings Water at 8.5 C

When a sample of Brunswick tailings water was used as feed it proved to be inhibitory and the biomass began to detach from the discs. After a 24 hr adaptation period, thiosulfate oxidation rates comparable to those achieved in the laboratory medium of similar nutrient composition were achieved. The inhibitory factor in the tailings water should not be a problem in any pilot-plant operation.

7. Busch Fermenter Experiments at Room Temperature

A laboratory scale activated sludge unit was operated at room temperature, using the medium of Bounds and Colmer as feed. The maximum thiosulfate oxidation rate was 60 mg/l/hr compared to rates of 2800 mg/l/hr obtained with the biodisc under similar conditions. The bacteria clung to the walls of the unit preventing the formation of a normal floc. Attempts to create artificial flocs were unsuccessful.

8. Ten gpm Pilot Plant

The design for a pilot plant handling 10 gal/min of tailings water containing 1 g/l of thiosulfate at 8 C is presented. Its capital cost would be approximately $63,200.00 for equipment and $132,000.00 for delivery, site preparation, installation, etc.

D. STAFF

This project was carried out in the Microbiology Group of the Division of Applied Biology, B. C. Research, under the supervision of Dr. D. W. Duncan. The literature was reviewed by Dr. Duncan and the laboratory studies were carried out by Miss H. Kurtz and Miss M. Lewis with assistance from Mr. R. Voss, Mr. E. Vegsund, Mr. R. Hartman and Mr. R. Gawley. Specialized analytical services were provided by the Analytical Section of the Water Quality Group under the supervision of Dr. J. Leach.

Dr. C. C. Walden, Head of the Division, Dr. J. Mueller, Group Leader, Waste Treatment, and Dr. R. O. McElroy, of the Extractive Metallurgy Section, Microbiology Group, provided consulting services.

E. EXPERIMENTAL PROCEDURES

1. Bacteria

a. Native Strains

A 1-litre portion of tailings pond effluent was received from the New Brunswick Mining and Smelting oxidation pond on December 20, 1972. Fifteen ml portions were transferred into the thiosulfate medium of Bounds and Colmer (5) at pH 4.5, and into the medium 9K of Silverman and Lundgren (6) at pH 2.5. Incubation was at 35 C on a gyratory shaker. Growth was monitored by the change in thiosulfate or ferrous iron concentration. The culture growing in the thiosulfate medium was repeatedly transferred and carried as a stock culture.

b. Thiobacillus thioparus

A strain of Thiobacillus thioparus (ATCC No. 8158) was obtained from Dr. Isamu Suzuki, of the Department of Microbiology, University of Manitoba. It was transferred to the medium of Bounds and Colmer at pH 4.5, and carried as a stock culture.

2. Batch Laboratory Experiments

The thiosulfate oxidation experiments at various temperatures and pH's were carried out by placing 100 ml portions of the medium of Bounds and Colmer (5) adjusted to the appropriate pH in 250 ml Erlenmeyer flasks. The flasks were inoculated with 5 ml of an active culture of the test organism, and incubated on a gyratory shaker at the appropriate temperature. The rate of thiosulfate oxidation was monitored by following the changes in pH, total organic carbon, and thiosulfate concentration in the medium. Upon completion of the experiment, the sulfuric acid production was measured.

3. Analytical Procedures

a. Thiosulfate

Thiosulfate was determined by the procedure of Sorbo (7).

b. Polythionates

Mercurous nitrate which forms a black precipitate with thiosulfate and trithionate, and a brilliant yellow precipitate with tetrathionate, was used as a spot test (8). A quantitative procedure involved the conversion of tetrathionate to thiosulfate by boiling for 5 min in an equal volume of 10% KOH (9), and then determining the thiosulfate by the Sorbo method. A chromatographic procedure for the detection of the different polythionates was based on the studies of Skarzynski and Szczepkowski (10). The solvent system used consisted of pyridine: acetone: N-propanol: H_2O: NH_4OH (30%), in the ratio of 35: 20: 30: 15: 2.5 v/v. Whatman No. 31 filter paper was used for ascending chromatograms which were run at room temperature for 1 hr, developed by spraying with 2% silver nitrate in 2% ammonium hydroxide, and dried at 50 C. In this system thiosulfate stayed at the starting line and the Rf of the polythionates increased in the order of increasing polymerisation.

c. Total Carbons

Total organic carbon values were determined by injecting an appropriate aliquot of the test solutions directly into a Beckman model 915 Total Organic Carbon Analyzer.

d. Sulfuric Acid Production

The production of sulfuric acid was determined by titrating to a pH 7 end point with 0.5 N sodium hydroxide using a

Radiometer automatic titrator. Since the pH of the uninoculated controls increased due to the chemical formation of tetrathionate, the original medium was titrated to provide the blank value.

e. Miscellaneous Nutrients

Free ammonium ion levels were determined by distilling aliquots of the fermentation media in a micro-Kjeldahl apparatus into saturated boric acid and back titrating with 0.01 N hydrochloric acid. Inorganic phosphate was determined by the molybdenum-blue procedure, sulfate by barium precipitation, and dissolved metals on an atomic absorption spectrophotometer.

4. Continuous Fermenter Studies

a. Busch Fermenter

The Busch fermenter (Figure 1) consists of concentrically mounted 2- and 6- litre pyrex cones. Air, monitored through a rotameter, is sparged through the bottom of the fermenter, oxygenating and mixing the liquor. Operational volume was 5.5 1. An open-ended 1-litre cylinder, (ID: 6.8 cm; height 21 cm submerged in the mixed liquor; volume: 0.76 1) mounted inside the top of the 2-1 cone provides a sludge settling zone. Retention time in the settling cone depends upon feed rate to the system. The fermenter was fed by an adjustable, direct displacement Brailsford pump. Treated effluent was aspirated from the top of the settling zone. When necessary, the bacterial growth was dislodged from the sides of the fermenter with a test-tube brush.

b. Biodisc Unit

The biodisc is a novel biological waste treatment system, which is referred to by various names: RBC (Rotating Biological Contacter), RBS (Rotating Biological Surface), or RBD (Rotating Biological Discs). A detailed description of the principle is given by Borchardt (11) and by Antonie (12). Briefly, the unit consists of a series of closely spaced discs anchored to a shaft and supported above a trough which contains the waste to be treated (Figure 2). Thus, an area of biological slime is alternately submerged to absorb food, and then exposed to the air for aeration. The waste is injected into the system perpendicular to the disc faces, but in passing between the discs, flows parallel to the adjacent faces of the disc. The drag forces generated by the slow rotation of the discs imparts a lifting action to

the waste. This causes the waste adjacent to each disc space to flow in a circular pattern over the submerged portion of the disc. Contact between waste and discs is thus not a single pass through adjacent surfaces, but rather a rapid circulation of waste many times over several quadrants of the disc.

The unit used in these studies had a 6.4 l capacity, and the holding trough was divided into three compartments. The shaft supported 99 closely spaced discs, 33 discs in each compartment. The plexiglass discs have a roughened surface to enable easier attachment of the microorganisms, and were 8 inches in diameter, and ½ inch thick. A circle, 1.25 inches in diameter, in the centre of the disc was not wetted so that the total area per disc was 98.1 sq. inches, or 0.681 sq. ft. The discs were driven at 1 - 5 rpm by an adjustable-speed electric motor.

For the initial studies, the unit was placed at room temperature and 6.4 l of the medium of Bounds and Colmer containing 3.8 g/l of thiosulfate was added. The pH was adjusted to 4.6 and the discs were rotated at 3.8 rpm. One hundred and fifty ml of the mixed culture isolated from the Brunswick tailing water was added as an inoculum. Various operating conditions of the biodisc are outlined in the Results section.

F. LITERATURE SURVEY

The oxidation of inorganic sulfur compounds by microorganisms has been extensively reviewed by Trudinger (13), Kelly (14), and in 1970 Roy and Trudinger (15) published a monograph on the biochemistry of inorganic compounds of sulfur, which includes a chapter on this subject. Briefly, these reviews have shown that all thiobacilli are capable of oxidizing thiosulfate; and sulfate, sulfur and tetrathionate are the principal products of bacterial oxidation. The exact mechanism of thiosulfate oxidation and the differences, if any, which exist between various species of the thiobacilli, is not clearly understood at the present time. Both Trudinger and Kelly point out that many of the differences in oxidation rates and metabolic end products observed by various investigators can probably be traced to the experimental conditions employed. Under suitable environmental conditions sulfate is the sole end product of thiosulfate oxidation according to the following equation:

$$S_2O_3^{=} + 2O_2 + H_2O \rightarrow 2SO_4^{=} + 2H^{+} \qquad (1)$$

This equation shows that one mole of thiosulfate produces two moles

of hydrogen ions, and was the basis for the thiosulfate conversion calculations.

One of the goals of this study was to utilize a strain of thiobacillus which would oxidize thiosulfate at a neutral or alkaline pH. Trudinger (13) suggests that Thiobacillus thioparus, T. neopolitanus, T. denitrificans, T. novellus and T. intermedius meet this requirement. The references he cites for this information go as far back as 1902. Hutchinson, Johnstone and White (16) have re-examined the genus Thiobacillus, and show that of the species mentioned, only T. thioparus and T. intermedius oxidize thiosulfate completely. The authors show that when thiosulfate is being oxidized completely, the pH drops significantly, whereas the maintenance of high pH values suggests that thiosulfate is only being oxidized partially to tetrathionate. Since the pH can only be kept high during oxidation of thiosulfate by using a highly buffered solution, or by continuous pH control, this requirement for the system does not seem practical.

It would seem more practical to let the pH drop to its desired level, and then neutralize the effluent after oxidation of the reduced sulfur compounds was complete. If this approach is adopted, the usefulness of T. thioparus is reduced, because this organism has a lower pH limit of 3.5. Once the pH drops below that level, thiosulfate oxidation ceases. Depending on the initial thiosulfate load and on the buffering capacity of the system under investigation, this can occur when there are still significant quantities of thiosulfate left. It thus appears that more acid tolerant strains such as T. thiooxidans and T. ferrooxidans are more useful organisms for the complete oxidation of thiosulfate in a biological waste treatment system.

The literature shows that T. thiooxidans and T. ferrooxidans are able to tolerate very low pH values (17). Trudinger (13) shows that T. thiooxidans produces sulfate as the usual end product from thiosulfate oxidation, and is also capable of oxidizing sulfide, tetrathionate and trithionate should they be present. Landesman, Duncan and Walden (18) and Hutchinson et al. (17) have shown that T. ferrooxidans has very similar properties.

The post-1967 literature on thiosulfate oxidation by thiobacilli, provides little additional information of value to the current study. Lyric and Suzuki (19, 20, 21) have studied the enzymes involved in the metabolism of thiosulfate by T. thioparus. They determined the optimum pH for the activity of these enzymes, but this provides little information regarding the best conditions for growth, because the enzymes react within the cell where the pH can be very different from the external pH. They do suggest, however, that the formation of higher polythionates such as tetrathionate, are not the normal products of the oxidation of thiosulfate. Charles (22) studied the oxidation of thiosulfate by T. intermedius which

was grown autotrophically, and concluded that the normal pathway is direct oxidation of thiosulfate to sulfite rather than polythionate formation. Sulfur does not accumulate.

Studies by Silver and Lundgren (23), Tabita, Silver and Lundgren (24), Silver (25), Vestal and Lundgren (26), and Lundgren (27), have shown that T. ferrooxidans is able to oxidize thiosulfate through a similar, if not identical, enzyme pathway. Barton and Shively (28) have confirmed that T. thiooxidans can also oxidize this substrate.

The majority of the foregoing studies have been concerned with the enzymes and the pathways involved in thiosulfate oxidation, and have done little to suggest optimum growth conditions. Data on this subject are quite limited. The recent study by Bounds and Colmer (5) is probably most relevant to this investigation. They compared the kinetics of thiosulfate oxidation of the iron-sulfur oxidizers. They found that whenever the pH rose above 5 - 5.2, thiosulfate oxidation decreased. The increase in pH was believed to be due to tetrathionate formation. When tetrathionate was used as a substrate, the pH always declined.

The thiobacilli can oxidize reduced sulfur compounds over a wide range of pH values. McGoran, Duncan and Walden (29) found that the pH range for growth of T. ferrooxidans depended on the substrate. The widest range occurred when sulfur was the substrate, although the lower limits of all three substrates were approximately the same, i.e. around pH 1.2. When T. ferrooxidans was oxidizing sulfur the rate of acid production was constant from pH 1.75 to pH 5. A lower rate occurred at pH 1.5, but at pH 1.25 and below and at pH 6, growth could not be initiated. Maximum oxidation of chalcopyrite, a sulfide mineral, occurred when the pH range was between 1.8 and 3.5. Leach rates dropped sharply at pH 1.2, whereas at pH 3.7 and above, no copper release was evident. Torma, Walden and Branion (30) found that the optimum pH for T. ferrooxidans when oxidizing zinc sulfide was approximately 2.3. Rao and Berger (31) present data to indicate that a low pH is essential for growth for T. thiooxidans. They could not achieve any growth at pH 7. T. thiooxidans frequently drives the pH down below 1 when growing on sulfur (32). Hutchinson, Johnstone and White (16, 17) indicate that it is necessary to keep the initial pH in the range 4 - 5.5 to initiate growth of most thiobacilli on thiosulfate. The lower limit of pH would appear to depend on the species present.

The thiobacilli grow over a wide range of temperatures, but the rate of oxidation of various substrates would appear to depend on the species. Optimum temperatures range from 28 to 35 C (6, 30, 32). No data was located suggesting the temperature range over which thiosulfate is successfully oxidized, but in general the thiobacilli

do not grow above 40 C. Marked inhibition of oxidation occurs at 45 C (30, 32, 33). Lundgren (24) has recently isolated a strain which grew over the temperature range 2 - 31 C, and the author has isolated T. ferrooxidans from mine waters at 3 C.

No literature could be found which specifically examined the nutritional requirements necessary for the oxidation of thiosulfate. Lundgren, Andersen, Remsen and Mahoney (34) examined the nutritional requirements of T. ferrooxidans growing on ferrous iron. They found that ammonium sulfate was the preferred inorganic nitrogen source, and that neither nitrate nor nitrite replaced the ammonium requirement. They found that urea in low concentrations was useful as a nitrogen source, as did Brierley and Brierley (35). Initially, high concentrations of nitrates are inhibitory (36).

Tuovinen et al. (36) have recently reviewed the requirements of T. ferrooxidans for mineral nutrients. They showed that ammonium ion and a phosphate source were essential, but that it was extremely difficult to establish a minimum level when oxidizing natural sulfides because of the presence of an unknown quantity of the nutrient on the substrate. Studies in our laboraotry indicated that the only demonstrable requirements when T. ferrooxidans was used to oxidize sulfide bearing ores, were for ammonium ion and phosphate. Usually, 50 - 60 mg/l of ammonium ion and 35 - 40 mg/l of phosphate were adequate. Undoubtedly, other minerals were required, but they were frequently supplied as contaminants of the substrates used. Torma, Walden and Branion (30) reported similar results from their studies on zinc sulfide concentrate.

Chernyak and Mineev (37) found that the addition of 100 mg/l of KH_2PO_4 stimulated the oxidation of ferrous iron by T. ferrooxidans. They also found that magnesium was essential. Watanabe et al. (38) found that 200 - 600 mg/l of nitrogen as ammonium ion was essential for the leaching of copper sulfide by this organism. Bryner and Jones (32) found that 130 mg/l of phosphate ion and 90 mg/l of ammonium chloride were the optimum concentrations. When ammonium nitrate was used, the optimum concentration was 250 mg/l. Only the ammonium portion of this nutrient was utilized. Tomizuka and Takahara (39) reported that 300 mg/l of ammonium sulfate was the only nutrient necessary to support oxidation of sulfide minerals in their experiments.

The limited literature available suggests that thiobacilli growing in natural waters would have a demonstrable requirement only for ammonium and phosphate ions. Our experience with waters from microbiological leaching circuits confirms this. In many environmental situations there may be sufficient phosphate ion naturally present that additional levels would not have to be added. However, Trudinger (13) cites a reference by Santer et al. which reports that polythionates

accumulate during the oxidation of thiosulfate in the absence of inorganic phosphate. Jones and Happold (40) reported that polythionate formation from thiosulfate was suppressed by high phosphate concentrations. Thus, the concentration of this nutrient may be critical for complete thiosulfate oxidation.

From the foregoing it is evident that the thiobacilli are a very adaptable group of microorganisms which can tolerate extremes of the environment. They will grow readily at pH 2 and below, at temperatures as low as 2 C, and in environments containing as much as 56 g/l of copper or 120 g/l of zinc. If the environmental conditions are satisfactory for the strain involved, thiosulfate will be oxidized through to sulfate with a minimum quantity of intermediate products released to the environment. Thus, thiobacilli should be ideal organisms for oxidizing thiosulfate through to sulfuric acid in a hostile environment as represented by tailings effluent emanating from a mining operation. It would appear that the only requirements are that oxygen and carbon dioxide be made available, usually from the air, that ammonium and phosphate be added at a low level, and that the pH of the reactor be at 5.5 or below. The availability of a large surface area should be beneficial because thiobacilli appear to prefer to attach themselves to solid surfaces (29).

G. RESULTS

1. Isolation of Inoculum

Active growth occurred in the thiosulfate medium within 24 hr, whereas it required more than 8 days for active oxidation of ferrous iron to occur. These results indicate that the original mine effluent sample contains organisms capable of oxidizing thiosulfate in relatively large numbers, as well as organisms capable of oxidizing ferrous iron. The organisms capable of oxidizing thiosulfate could be any one of a number of thiobacilli, but the low final pH values observed suggest that they are *Thiobacillus thiooxidans*. The iron oxidizer is *T. ferrooxidans*. This is in agreement with the findings of Schmidt and Conn (1, 3). For the purpose of this report they will be referred to as "a thiobacillus culture". The thiobacillus culture was repeatedly transferred on the medium of Bounds and Colmer every 4 days, to produce an active inoculum for growth experiments. After the second transfer there were still significant numbers of organisms present with iron-oxidizing capability.

Microscopically, the organisms were rod shaped, with the typical appearance of *Thiobacillus sp*. They were motile, and the cells were considerably larger than the strain of *T. ferrooxidans* in normal use in our laboratory.

2. Oxidation of Thiosulfate at Various Temperature and pH Values

Typical thiosulfate oxidation curves are illustrated in Figure 3 and the data obtained at various temperatures and pH values are summarized in Table 1. The rate of thiosulfate oxidation at any one temperature is essentially the same at initial pH values of 3, 4 and 5. At an initial pH value of 6 the results varied. Occasionally rapid thiosulfate oxidation occurred after a long lag (Figure 3). Examination of the data showed that thiosulfate oxidation occurred at a rate significantly different from the uninoculated control only after the pH dropped below 5.6.

During these experiments the pH of the inoculated flasks had dropped to the range 2.1 - 2.4 by the time the thiosulfate concentration had dropped to 1.5 g/l. When all the thiosulfate had been oxidized the pH was in the range 1.7 - 1.9. The pH in the control flasks always increased. In the pH 5 and 6 systems it increased to pH 7 - 8; in the pH 3 and 4 systems, to 4.5 - 5.0. This suggests that chemical formation of tetrathionate was occurring.

As the temperature decreased the rate of thiosulfate oxidation decreased. When this data was plotted and extrapolated (Figure 4) a thiosulfate oxidation rate of 12 mg/l/hr is predicted for a temperature of 2 C. This was approximately 1/10 the rate at 23 C and 1/20 the rate at 30 C.

Total carbon values were also determine during the oxidation experiments. In general, the total carbon values increased from an initial level of 4 mg/l to between 80 and 90 mg/l due to the oxidation of approximately 3.6 g/l of thiosulfate. These carbon values were used to determine generation times, and the values calculated are shown in Table 1 and plotted in Figure 4. The generation time at 5 C was 83 hr, decreasing to about 9½ hr at 23 C and 7½ hr at 30 C.

3. Recovery of Oxidized Thiosulfate as Sulfuric Acid

The sulfuric acid recovery data presented in Table 2 shows no effect due to pH, but a decrease in the recovery as the temperature decreases. On the average, between 85 and 95% of the thiosulfate was converted to a form titratable with sodium hydroxide, and thus was considered to be sulfuric acid. The fate of the remaining 5 - 15% is unknown, since all procedures used failed to detect the presence of any sulfur, tetra- or trithionate.

4. Nutritional Requirements

Thiobacillus sp. generally have minimal nutritional requirements,

oxygen, carbon dioxide, ammonia nitrogen and phosphate being the normal requirements. Experiments were set up using repeated transfer into the complete medium of Bounds and Colmer, and modifications of that medium resulting from reducing the levels of the four micronutrients. No differences in oxidation rates were observed between the complete medium and a deficient medium which contained only the ammonium and phosphate salts (817 mg/l of ammonium ion and 2094 mg/l of phosphate). When ammonium ion was the only nutrient present, the rate of thiosulfate oxidation was slower, but even after 6 transfers into identical media, complete oxidation was occurring (Figure 5).

Past experience with the thiobacilli has suggested that the ammonium ion influences the extent of the reaction, whereas the phosphate ion influences the rate of the reaction, therefore a series of experiments were set up using medium containing 1.6, 4.1 and 8.2 mg/l of ammonium ion, plus either 2.1, 1.0 or 0.5 mg/l of phosphate. The average results were identical with those shown in Figure 5; the addition of phosphate at the levels used had no effect on the rate of thiosulfate oxidation.

The levels of ammonium and phosphate added were increased. As shown in Figure 6, none of the nutrient levels tested gave results comparable to that obtained with the complete medium of Bounds and Colmer. Eighty-two mg/l of ammonium ion as the sole nutrient, gave an oxidation rate which was approximately 40% of the control rate. When the magnesium sulfate and calcium chloride components of the Bounds and Colmer medium were added, the thiosulfate oxidation rate was the same until approximately the 100 hr mark, at which time oxidation virtually ceased. This same effect was observed at lower ammonium ion concentrations. The addition of 21 mg/l of phosphate along with the 82 mg/l of ammonia ion gave a rate which was only ½ that when the ammonium was used alone, and when the magnesium sulfate and calcium chloride were added to this mixture, there was no effect.

The results of these nutrition experiments indicate that relatively high levels of ammonium and phosphate are necessary for maximum oxidation of thiosulfate under the conditions of our test. This is contrary to our experience with other members of the thiobacilli, and is a matter which requires further investigation. The magnesium sulfate and calcium chloride components of the medium of Colmer and Bounds are not necessary.

5. Thiosulfate Oxidation by Thiobacillus thioparus

A pure culture of Thiobacillus thioparus was used to oxidize thiosulfate at various pH's at a temperature of 30 C. As shown

in Figure 7, the oxidation rate at pH 4 and 5 was 138 mg/l/hr. At pH 3, no significant thiosulfate oxidation occurred until after a 70 - 90 hr lag, and then rapid oxidation occurred. The rate was identical with that which occurred at pH 4 and 5.

At pH 6 there was no significant change in the thiosulfate oxidation level, nor in the organic carbon level for the first 50 hr. At this point the thiosulfate level in one of the flasks began to drop at a rate of 45 mg/l/hr. The final pH was 1.9. After 166 hr the thiosulfate level in the other flask had dropped to 2.8 g/l, and the organic carbon level had increased to approximately 1/3 of the value observed in the flasks at the other pH values. However, the pH had risen to 8.0, implying that tetrathionate was being formed, rather than sulfuric acid during the biological oxidation of the thiosulfate. The experiment was not continued.

The maximum total carbon values obtained ranged from 78 - 81 mg/l, which was the same order of magnitude as obtained with the mixed culture from New Brunswick. The acid titration studies indicated that 89 - 93% of the thiosulfate could be accounted for as sulfuric acid. Again, this was not significantly different from the results obtained from the mixed culture from New Brunswick.

When the experiment was repeated at 5 C no thiosulfate oxidation occurred for 200 hr. Subsequently, there was oxidation at a rate of 2.5 mg/l/hr at pH 3, 4 and 5. This was much slower than the rate obtained with the mixed culture (Table 1).

The above data indicate that a pure strain of Thiobacillus thioparus did not oxidize thiosulfate at a higher pH than did the mixed culture, nor did it oxidize it more rapidly. Suzuki (19) reports that he grows this strain at pH 6.5 - 7.0 by addition of K_2CO_3. In our experiments growth was not initiated as rapidly nor was the oxidation rate as rapid at pH 6 as it was at lower values.

6. Biodisc Studies

a. Room Temperature

The initial biodisc studies were carried out at room temperature (20 - 22 C) using the medium of Bounds and Colmer (5), at pH 4.5, made up in tap water. The $S_2O_3^{=}$ level was 3.6 g/l. After approximately a 3 week conditioning period, experiments were begun on March 8, at a flow rate of 1.25

1/hr for a residence time of 307 min. As shown in Table 3, there was less than 10 mg/1 of thiosulfate remaining at disc 66. The thiosulfate oxidation rate over the first bank of discs (i.e. discs 1 - 33) was calculated to be 160 mg/sq.ft/hr. The flow rate was progressively increased to a maximum of 2.94 1/hr, equivalent to a retention time of 131 min. As shown in Figure 8, the rate of thiosulfate oxidation increased progressively, until on March 14 it was 267 mg/sq.ft/hr (based on discs 1 - 33). The system was still not operating at maximum capacity because there was less than 20 mg/1 thiosulfate remaining in the effluent.

The results indicate that thiosulfate can be sucessfully oxidized by the biodisc system and that bacteria attached themselves to the discs as anticipated. The effluent leaving the system was at pH 1.7 - 1.9 and was water clear. In relation to the feed, total carbon values of the effluent did not increase.

Rather than continue using the abnormally high substrate level, which probably influenced the oxidation rate, it was decided to reduce the feed level to approximately 1 g/1, which was more representative of the mine water which would ultimately be run through such a system. To compensate for the reduced feed levels, the flow rate was increased initially to 6.40 1/hr, equivalent to a 60 min residence time. As shown in Table 3, thiosulfate oxidation rates of between 220 and 240 mg/sq.ft/hr were obtained. Increasing the flow rate so that the residence time was 38 min, resulted in approximately 8 mg/1 of thiosulfate appearing in the effluent, indicating the biodisc was approaching its maximum capacity. The effluent pH was 2 - 2.1. The rate of thiosulfate oxidation calculated over the first 33 discs was 270 mg/sq.ft/hr.

The pH of the feed was raised to 9 to simulate the mine water. The system ran at this level for approximately 48 hr, at which time problems were encountered with the feed pump, but after 24 hr of uninterrupted operation at a flow rate of 11.4 1/hr, (equivalent to a 34 min retention time) a thiosulfate oxidation rate of 279 mg/sq.ft/hr was recorded. Approximately 12 mg/1 of thiosulfate was appearing in the effluent which was at pH 2.1.

Total carbon levels of the effluent from the biodisc on March 19, was 2 mg/1, the same reading as was obtained with the feed. On March 20, this had increased to 8 mg/1, whereas on March 22, the reading was 4 mg/1. The effluent was beginning

to be opaque indicating loss of excess bacterial cells from the discs.

The medium in use up until March 14 was that of Bounds and Colmer. When the flow rates were increased and the thiosulfate concentration reduced, the new medium was a scaled down version of that medium. It consisted of 100 g of $Na_2S_2O_3 \cdot 5H_2O$, 30 g of $(NH_4)_2SO_4$, 30 g of KH_2PO_4, 6 g of $MgSO_4$ and 3 g $CaCl_2 \cdot 5H_2O$ per 10 gal of tap water. Three days later the $MgSO_4$ and $CaCl_2$ were eliminated from the medium. This resulted in approximately 1 g/l $S_2O_3^{=}$, 180 mg/l NH_4^+ and 461 mg/l PO_4^{-3}. The biodisc studies were proceeding concurrently with the batch nutritional studies, and since the biodisc results of March 20 and 22 indicated that rates equivalent to those observed previously could be obtained, it was not considered that this reduction in the nutrient level had any detrimental effect. On March 22 the NH_4^+ and PO_4^{-3} levels were reduced to 18 and 46 mg/l respectively, and there was no detrimental effect evident after approximately 9 residence times. It was concluded that this level of nutrient addition was adequate. However, in retrospect, this may not have been enough time for a depletion of any stored phosphate reserves.

Data concerning the recovery of sulfuric acid, as determined by titration of the feed and effluent samples with sodium hydroxide, is also presented in Table 3. The average percentage conversion was 82% when the feed level was 1 g/l $S_2O_3^{-2}$. The presence of sulfur, tetra- or trithionate, was not detected and it is not possible to say at this time what was the fate of the missing 18%.

b. 8.5 C

Following the 2:00 p.m. sample on March 22, the biodisc was emptied and moved into a temperature controlled room, where it was restarted. During the moving process, the bacterial growth which had accumulated in and on the trough was removed. The biodisc was filled with a feed stock containing approximately 1 g/l thiosulfate, 18 mg/l of ammonium ion and 46 mg/l of phosphate in tap water, at a pH of 4 - 5, and run for 90 hr at a flow rate of 7.80 l/hr before the first samples were taken. The temperature was 10.5 C ±0.5 C. Table 4 presents the results obtained during these studies, and typical curves are shown in Figure 9. With the flow rate set at 7.80 l/hr, the oxidation rate was 155 mg/sq.ft/hr.

The temperature was then lowered to 8.5 ±0.5 C and the flow rate increased to 10.68 1/hr, which was equivalent to a 36 min residence time. The oxidation rate averaged 146 mg/sq.ft/hr over three days. Significant levels of thiosulfate appeared in the effluent, indicating that the system was running at its capacity for this temperature.

The effluent was leaving the system at pH 2.1 - 2.2. Titration data indicated 80% conversion of the thiosulfate to sulfuric acid.

With all other conditions remaining the same, the speed of rotation of the biodisc was reduced to 1.3 revolutions per minute from the 3.8 rpm at which it had been running up to this time. This resulted in a reduction of the thiosulfate oxidation rate to 104 mg/sq.ft/hr (Table 4). The speed of rotation was then increased to 5 rpm and the thiosulfate oxidation rate 64 hr later, was 165 mg/sq.ft/hr. This was an increase in oxidation rate beyond that obtained at 3.8 rpm. Compressed carbon dioxide was added to the atmosphere in the room, so that the concentration of CO_2 was increased to approximately 0.1% by volume. As shown in Table 4, this did not increase the rate of thiosulfate oxidation, and the average obtained after 7 days of running at 5 rpm was 150 mg/sq.ft/hr (which includes the high value of 165). This was not considered significantly different than the rate obtained at 3.8 rpm. The fact that the rate of thiosulfate oxidation per square foot of surface area decreased when the speed of rotation decreased, suggests that the thiosulfate available at a point on the disc surface when it leaves the waste water, is oxidized before the point of surface returns to the water. Thus, for part of the time the point on the disc surface is out of the water, no thiosulfate is available for oxidation. Increasing the rotational speed from 3.8 to 5 rpm did not affect the rate. This implies that the thiosulfate concentration at a point on the disc surface remains at a level which supports the maximum oxidation rate the entire time the surface is out of the water at both speeds.

The thiosulfate oxidation rate at 1.3 rpm was 70% of the rate at the higher rotational speeds, which indicates that the minimum rotational speed should be 1.9 rpm. This is equivalent to peripheral velocities of 35.8 ft/min (0.41 mph), 47.7 ft/min (0.54 mph) and 71.6 (0.81 mph) for 6, 8 and 12 ft discs. These values are less than the current speeds frequently found in lakes.

On April 9, the nutrient level of the feed was reduced again so that the only salts added to the Vancouver tap water other than the thiosulfate, were 8 mg/l of ammonium ion as ammonium sulfate, and 5 mg/l of phosphate ion as potassium dihydrogen phosphate. The results in Table 4 show the average oxidation rate over 4 days was 146 mg/sq.ft/hr, indicating no effect due to reducing the nutrient levels. The nutrient levels were reduced again on April 16 to 4 mg/l of ammonium ion and 5 mg/l of phosphate, and after 96 hr of operation the average oxidation rate was 128 mg/sq.ft/hr (Table 4), which appears to be a significant reduction in oxidation rate.

To confirm that changing the speed of rotation over the range 3 - 5 rpm does not affect the performance of the system, the speed was reduced to 3.3 rpm. The results in Table 4 show that over 120 hr of operation the rate was 122 mg/sq.ft/hr, showing no significant difference from the oxidation rate at 5 rpm at this low nutrient level.

During the 8.5 C experiments, the feed entering the system was at pH 5.5 - 6.1, which was the natural pH of the salts in the medium. The effluent pH ranged from 2.0 - 2.2 and the thiosulfate conversion to sulfuric acid ranged from 65 - 84% of the thiosulfate oxidized. The data in Table 4 show a definite trend toward lower conversion as the nutrient level drops. No sulfur, tetra- or trithionate were detected by the procedures used. However, it must be noted that the literature predicts increased conversion to tetrathionate at low phosphate levels.

The effluent was moderately opaque and the highest carbon value recorded was 8 mg/l on April 25; 5 mg/l was the more normal reading.

A sample of effluent was collected on April 19 for a brief flocculation study. Its initial pH was 2.1 and it required 0.96 g of $CaCO_3$ to bring the pH to 6.9. Milk of lime was added to bring the pH to 8 and then one portion was stirred slowly for 1 hr and allowed to settle for ½ hr. Good settlement of calcium salts occurred but the solution was still turbid. Other portions received 5 and 10 mg/l of Calgon M-500 flocculant followed by slow stirring for 20 min and ½ hr settling. The 5 mg/l addition was best in that fast flocculation occurred. The floc was slow to settle but a clear supernatant resulted.

c. Operation on Brunswick Mine Water

A supply of Brunswick tailings pond effluent was taken on

February 22, 1973 prior to entering biostabilization pond No. 1. The pH was 9.5 at the time of sampling and 6.7 upon arrival in Vancouver. Chemical analysis of the sample and of Vancouver City tap water used to make up the synthetic medium for the biodisc, is shown in Table 5.

The original experimental plan was to run the mine water through the biodisc system without any added nutrients to determine if the thiosulfate oxidation rate dropped significantly below that which was achieved on the low nutrient synthetic medium. At the time the test was initiated on April 25, the carbon content of the effluent ranged from 5 - 8 mg/l, the thiosulfate content was 90 mg/l, and the flow rate was 7.6 l/hr, equivalent to a residence time of 51 min. The mine water was at pH 3.3 and had a thiosulfate concentration of 900 mg/l. The first sample was taken after 3½ hr, equivalent to approximately 4 residence times. As shown in Figure 10, there was no thiosulfate oxidation in the first stage (33 discs). There was slight oxidation over the next stage, and a maximum oxidation rate of 81 mg/sq.ft/hr over the final stage (Table 6). The effluent was cloudy and had a total carbon value of approximately 55 mg/l. The run was continued for another 3 hr, and as shown in Figure 9, the oxidation curve did not change significantly. The maximum rate in the third chamber was calculated to be 74 mg/sq.ft/hr. The effluent pH was 2.5 - 2.6, down slightly from the 3.3 of the tailings-water feed.

The toxicity of the mine water sample and the fact that it was making the bacteria come off the biodisc was of concern. After taking the 6½ hr sample, the feed was stopped and the biodisc was allowed to run on a batch basis overnight. The next morning (April 26) there was no thiosulfate present at any stage of the biodisc, and pH's of the three stages were 2.3, 1.7 and 1.7 respectively. Samples from the three stages were extremely turbid. The total carbon value in the first stage was 300 mg/l and 700 mg/l in the other two stages. This was far in excess of the amount of carbon which would have been produced from the thiosulfate remaining in the system when it was turned on to batch. The large amount of carbon is attributed to detachment of biomass from the discs. Under microscopic examination the only identifiable material was rod-shaped bacteria typical of Thiobacillus, the remainder was typical cell detritus. There was no evidence of molds or unicellular organisms typical of biodiscs or activated sludge units operating at more normal pH values. The suspended biomass did not settle at a significant rate, and carbon disulfide extraction of the precipitated

pellet (by centrifugation) did not give any measurable amount of material. This was interpreted as meaning that there was no free or adsorbed sulfur in the suspended material.

The pump was then restarted at a rate of 7.60 l/hr, and a sample taken after 4 residence times indicated almost no thiosulfate oxidation in the first stage, but a steady rate equivalent to 103 mg/sq.ft/hr over the last two stages (Figure 10). The total carbon content was 33 mg/l. In view of the level of ammonium and phosphate present in this mine water sample, it is evident that the organisms can recover from the inhibitory factor in the mine water, and oxidize the thiosulfate present, at rates comparable to those obtained for synthetic mine water with equivalent nutrition. A portion of the treated mine water containing 50 mg/l of thiosulfate was adjusted to pH 5.5 with calcium carbonate and then oxidized by the bacteria in a batch culture. The thiosulfate level was reduced to 10 mg/l and the pH dropped to 3.2. This suggests that the maximum allowable concentration of thiosulfate in the effluent must be less than 50 mg/l. This corresponds to greater than 95% oxidation based on 1000 mg/l or greater than 90% oxidation based on 500 mg/l of thiosulfate in the feed.

7. Oxidation of Thiosulfate in a Busch Fermenter

A Busch fermenter is a commercially available laboratory-scale activated sludge unit. The 5.5 l system, maintained at room temperature, was filled with the thiosulfate medium of Bounds and Colmer, inoculated with 250 ml of biodisc effluent, and sampled and observed at convenient intervals. The maximum rate of thiosulfate oxidation observed was 60 mg/l/hr, compared to rates as high as 2800 mg/l/hr in the biodisc. The effluent was water clear and had a total organic carbon content of less than 5 mg/l. Bacterial growth occurred over all the surfaces of the fermenter, confirming our earlier belief that the bacteria preferred to be on a solid surface, and do not form a conventional activated-sludge floc.

An activated sludge floc was created artificially by scraping down the walls of the fermenter. This produced a very heavy sludge but the average thiosulfate oxidation rate was still only 60 mg/l/hr. The floc had a definite tendency to re-attach itself to the walls, and if it was not stirred up at least twice a day, the effluent would be water clear again within 24 hr. An attempt to create an artificial floc by seeding the fermenter with peat as a surface on which the floc would form, was

unsuccessful in that thiosulfate oxidation rates were no different than those obtained when the natural organisms were stirred off the fermenter wall. Eventually the peat settled into the biomass on the walls, and would not stay in suspension.

Due to the poor results, as compared to the biodisc, all further work on this type of a system was abandoned.

H. DISCUSSION

The results of this investigation confirm the original hypothesis that thiobacilli prefer to attach themselves to a solid surface, and thus some sort of a fixed bed reactor was preferable to the conventional sparged or stirred tank aeration system. Using the rotating biological disc fixed bed fermentation system, thiosulfate oxidation rates of 279 mg/sq.ft/hr were obtained at room temperature (20 - 22 C), and 146 mg/sq.ft/hr at 8.5 C.

The nutritional studies performed in shake-flasks were not in agreement with those performed with the biodisc, but based on the latter system, it would appear that approximately 8 mg/l of ammonium ion and 5 mg/l of phosphate are necessary for maximum oxidation rates at thiosulfate levels of 1000 mg/l. That is, the desirable ratio of S_2O_3:NH_4:PO_4 is 1000:8:5. The product of the microbiological oxidation of thiosulfate detected was sulfuric acid, and approximately 80% of the oxidized thiosulfate could be accounted for as this acid. There was no evidence of sulfur, trithionate or tetrathionate being produced during these investigations, and so at the current time, no explanation is available as to the fate of the remaining thiosulfate.

The oxidation of thiosulfate by Thiobacillus sp. in a rotating biological disc system is a potential method of treatment assuming that the biodisc can be constructed from acid resistant materials. There is now a significant body of literature concerning such systems (11, 12, 41, 42, 43, 44, 45, 46), much of it arising from the biodisc manufacturers. However, all the data concerns the treatment of more conventional wastes and is usually expressed on the basis of BOD removed. On the basis of equation 1, 1000 mg/l of $S_2O_3^{=}$ has an oxygen demand of 571 mg/l, assuming 100% conversion to sulfuric acid. This should represent the maximum demand, since the experimental results indicate that only 80 - 90% of the thiosulfate could be accounted for as sulfuric acid.

Briefly, the RBD system requires about the same amount of land as an activated sludge system. The literature suggests hydraulic loadings of 1 - 3 gal/square foot of disc surface/day. As will be shown later, when oxidizing 1 g/l thiosulfate at 8.5 C the hydraulic loading was only 0.77 gal/sq.ft/day. The biodisc system is affected by temperature

in the same manner as an activated sludge system, but it is reportedly more resistant to shock loads. The use of covers or an insulated enclosure is recommended for retaining any waste heat.

The system is characterized by its simplicity, the small number of moving parts and the absence of sludge recycle which minimizes operational and maintenance requirements. It can be operated by untrained personnel because there are no critical operating parameters. The major labour requirement would be periodic maintenance and lubrication. Power requirements have been conservatively estimated at less than one-half those of activated sludge systems.

The capital cost of a biodisc system depends on the material being treated. An average figure for organic waste is $50.00/lb of BOD. Additional investment would be required for nutrient addition, primary clarification and sludge handling if such auxilliary systems are necessary. The system proposed here would require nutrient addition and a neutralization section.

I. DESIGN FOR 10 gpm PILOT PLANT

A flow sheet for a pilot plant designed to handle 10 gpm of tailings water, which is 1/70 of a plant to handle a million gallons a day, is presented in Figure 11. Basically, it consists of a biodisc unit to oxidize thiosulfate to sulfuric acid, a neutralization tank to neutralize the sulfuric acid produced and precipitate metal salts, a lime feeder and pH controller to activate the feeder, and a settling pond to remove the biological solids, metallic hydroxides and calcium sulfate so that a clear effluent can be discharged. The assumptions made for this pilot plant are as follows: The feed will contain 1.0 g/l of thiosulfate ion, contained in tailings water which is available as non-neutralized effluent from the conventional mill tailings pond system. The system will be operating at a temperature of 8 C, and the effluent will be neutralized with lime.

1. Feed System

The pilot plant will require a feed system consisting either of a variable flow pump or a scoop capable of delivering between 5 and 10 gal/min of tailings effluent. In addition, it will be necessary to employ either low flow rate pumps or reagent feeders which will add concentrated ammonium sulfate and potassium phosphate solutions at a rate sufficient to maintain ammonium and phosphate ion concentrations of 8 and 5 mg/l respectively. A scoop feeder is usually an integral part of the biodisc unit. Reagent feeders can be purchased for about $100.00 each.

2. Biodisc Unit

At 8.5 C a biodisc unit is capable of oxidizing 146 mg of thiosulfate ion/sq.ft/hr. Ten gal/min of tailings containing 1 g/l of thiosulfate is the equivalent of 2730 g of thiosulfate per hr. Thus, the system will require 18,700 sq. ft of disc capacity assuming linear reaction kinetics as illustrated in Figure 9. The hydraulic loading is thus 0.77 gal/sq.ft/day. The surface of various disc diameters are listed in Table 7. These make no allowance for the loss of area due to the shaft on which the discs are mounted. Thus, using a 6 ft diameter disc, 331 would be required, whereas only 83 would be required if a 12 ft diameter disc were utilized. If the temperature of the waste was maintained at 20 - 22 C, the equipment size would be reduced by approximately 50%.

This amount of surface will not reduce the thiosulfate concentration of the effluent to zero at a flow rate of 10 gpm, but will result in an effluent containing between 50 and 100 mg/l of thiosulfate. The additional surface area necessary to remove this additional 5 - 10% cannot be estimated accurately from the data available, but it should not exceed 20% of the estimated surface area. By operating the pilot plant at flow rates of less than 10 gpm this figure can be accurately determined.

Another alternative which should be examined is that of using the biodisc to reduce the thiosulfate level to the range 50 - 100 mg/l, followed by chemical treatment to oxidize the remaining material to a safe level. The safe level, assuming no assimilative capacity of the receiving stream, is less than 50 mg/l.

The estimated capital cost would be $60,000.00 if units containing 72-6 ft diameter discs per unit were used. The use of larger diameter discs should reduce this initial cost.

3. Neutralization System

The effluent will leave the biodisc system at a pH in the range 2 - 2.2, and contain a very low, but unknown load of suspended solids (in laboratory experiments the maximum suspended solids level expressed as total carbon was about 10 mg/l. If the biodisc unit is allowed to run for a longer time, it must eventually reach the point where biomass begins to slough off, hence increasing the suspended solids level. However, based on laboratory experience, this is likely to take months to happen). The effluent from the biodisc system will flow into a tank of approximately 45 gal capacity, which could be adequately stirred by a ½ horsepower motor. Lime would be added as required to

bring the pH to the desired level and the effluent discharged to the tailings pond.

In these investigations the effluent was neutralized to a pH of 7 and the experimental data indicated that the neutralization requirement was 80 - 90% of the amount predicted based on the decrease in thiosulfate concentration. In practice, it may be necessary to raise the pH to 9, due to the fact that heavy metals will not have been precipitated out of the tailings water prior to entering the system. The neutralized effluent will then have to pass to a settling basin where the precipitated metal hydroxides and calcium sulfate will be removed. The necessity of adding flocculating agents to aid this process can best be determined when the pilot plant is operating on plant tailing water. Laboratory tests on synthetic mine water indicated that 5 mg/l of Calgon M-500 was a satisfactory flocculant at pH 8.

The Department of the Environment is currently operating a neutralization plant at the source of the test-water. It may be desirable to send the biodisc effluent to that plant. If not the neutralization arrangement suggested here could be purchased as follows:

Denver Vibratory or Omega Disc Feeder	$1,500.00
Great Lakes pH Controller	1,150.00
Reaction Vessel	100.00
½ HP Lightnin Mixer	250.00
Total	$3,000.00

4. Summary of Capital Costs

Feed System	$ 200.00
Biodisc Unit	60,000.00
Neutralization	3,000.00
Settling Pond	0
	$ 63,200.00
Installation	130,000.00
Total Cost	$193,200.00

This cost estimate is based on the normal chemical engineering bare module cost factor of 2.95 times the F.O.B. capital cost.

J. FULL SCALE TREATMENT PLANT

A full scale plant treating 1 million gallons per day of tailings water containing 1 g/l of thiosulfate must oxidize 10,000 lb of thiosulfate per day. This is equivalent to 5714 lb of BOD. On the basis of the figure of $50.00 per lb of BOD, the capital cost would be $286,000.00. However, the relationship between the treatment of typical organic wastes and an inorganic waste such as thiosulfate is not known. The fact that the hydraulic loadings quoted in the literature are 1.3 to 3.9 times higher than the loadings achieved in our bench scale experiments suggests that the capital cost may increase proportionally. This decreased efficiency is due, at least in part, to the fact that the thiosulfate data is for a temperature of 8.5 C. The economics of heating the waste water must be evaluated against the possible savings in capital cost during any pilot scale evaluation of this process.

The laboratory studies reported here indicate that a full scale plant would require approximately 1,310,000 square feet of disc surface. Since the more conventional means of estimating capital cost based on BOD loading may not be applicable, manufacturers of biodisc equipment were contacted for unit costs based on disc area. Ames Crosta Mills (Canada) Limited, 105 Brisbane Road, Downsview, Ontario, informed us "that company policy at this time is to limit biodisc application to domestic sewage and therefore do not wish to be involved in case of strongly acid waste". Thus, we were unable to get a price from them. The Autotrol Corporation, Bio-systems Division, 5855 North Glen Park Road, Milwaukee, Wisconsin 53209, has failed to provide the requested information. Rather than delay issuing this report, a capital cost estimate based on disc surface will be submitted as an addendum if and when the required information becomes available.

K. CONCLUSION

On a laboratory basis a biodisc unit has proven to be a technologically successful procedure for oxidizing thiosulfate at 8.5 C. A pilot plant installation is required to determine if the system will scale-up at the projected rates and to determine its economic feasibility. Factors which need elucidating under field operating conditions include: anticipated load on the system, (i.e. lb of thiosulfate/day); level of nutritional supplementation necessary; maximum tolerable concentration of thiosulfate in the effluent; economics of chemical vs biological reduction of thiosulfate remaining in the effluent; operating costs, including the cost of heating the waste water; maintenance requirements; long term effect of inhibitory agents used in the concentrator (shock effects) and clarification of the effluent.

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TABLE 1 BIOLOGICAL OXIDATION OF THIOSULFATE IN BATCH CULTURE

Temperature (C)	Thiosulfate ($S_2O_3^{=}$) Oxidation Rate (mg/l/hr) Initial pH				Generation Time (Hours)
	pH 3	pH 4	pH 5	pH 6	
30	240	240	240	4	7.6
23	110	110	105	4	9.4
10	33	33	33	3	22.4
5	16	16	24	1	83.0

TABLE 2 % RECOVERY OF OXIDIZED THIOSULFATE ($S_2O_3^{=}$) AS SULFURIC ACID

Temperature (C)	Initial pH			Total Number of Observations	Average % Recovery
	pH 3	pH 4	pH 5		
30	-	-	94	10	94
23	-	93	96	4	95
10	89	89	88	9	88
5	86	85	80	7	85

TABLE 3 BIOLOGICAL THIOSULFATE OXIDATION AT ROOM TEMPERATURE IN A BIODISC SYSTEM

Date	$S_2O_3^{=}$ Concentration (mg/l)				Biodisc RPM	Flow Rate l/hr	Residence Time (minutes)	Maximum $S_2O_3^{=}$ Oxidation Rate mg/sq ft/hr	% H_2SO_4 Recovery From Oxidized $S_2O_3^{=}$	Remarks
	Feed	Biodisc Stage *								
		1	2	3						
March 8**	3,740	860	<10	<10	3.8	1.25	307	160	82	Increased flow rate.
9	3,650	680	<10	<10	3.8	2.30	167	304	-	Increased flow rate.
12	3,650	2,200	1,000	625	3.8	2.94	131	189	86	3:00 p.m. sample.
13	3,440	1,830	435	30	3.8	2.94	131	211	92	
13	3,640	1,740	415	<30	3.8	2.94	131	249	-	
14	3,600	1,560	225	<20	3.8	2.94	131	267	91	Nutrient level reduced.
15	1,050	270	30	<10	3.8	6.40	60	222	89	
16	1,080	255	22	<10	3.8	6.46	59	238	83	Stopped adding $MgSO_4$ and $CaCl_2$
19	1,110	300	25	<10	3.8	5.76	67	208	82	Increased flow rate.
20	1,020	425	85	8	3.8	10.20	38	270	82	
20	1,020	425	100	6	3.8	10.20	38	270	82	3:00 p.m. sample, pH of feed raised to pH 9.
21										Pump broke down.
22	960	550	195	25	3.8	11.40	34	208	83	Nutrient level reduced to 18 mg/l NH_4^{+} and 46 mg/l $PO_4^{=}$.
22	910	360	90	12	3.8	11.40	34	279	-	2:00 p.m. sample.

* Samples taken at discs 33, 66 and 99.

** Samples taken 8:30 a.m., adjustment made at 9:00 a.m. unless otherwise noted.

TABLE 4 BIOLOGICAL THIOSULFATE OXIDATION AT 8.5 C IN A BIODISC SYSTEM

Date	$S_2O_3^{=}$ Concentration (mg/l)				Biodisc Rotation Speed rpm	Flow Rate l/hr	Residence Time (minutes)	Maximum $S_2O_3^{=}$ Oxidation Rate mg/sq ft/hr	% H_2SO_4 Recovery From Oxidized $S_2O_3^{=}$	Remarks
	Feed	Biodisc Stage*								
		1	2	3						
March 26 **	1,120	645	230	50	3.8	7.80	49	155	79	Running at 10.5 C for 90 hr, 18 mg/l NH_4^+, 46 mg/l $PO_4^{=}$. Temperature to 8.5 ±0.5 C.
27	940	590	320	140	3.8	10.68	36	147	84	
28	900	600	280	120	3.8	10.68	36	147	83	
29	1,160	860	550	320	3.8	10.60	36	145	76	
Average								146	80	rpm reduced.
29	900	730	470	295	1.3	10.60	36	94	-	4:00 p.m. sample.
30	940	700	450	280	1.3	10.44	37	114	83	rpm increased at 4:00 p.m.
Average								104	83	
April 2	1,140	790	400	160	5.0	10.08	38	165	74	CO_2 added to atmosphere at 4:00 p.m.
3	950	590	260	90	5.0	10.08	38	155	77	
5	1,030	750	420	170	5.0	10.32	37	140	-	
6	980	700	345	120	5.0	10.32	37	146	-	
9	980	650	300	90	5.0	9.43	41	143	79	Nutrient levels reduced to 8 mg/l NH_4^+ and 5 mg/l $PO_4^{=}$
Average								150	77	
10	1,190	860	460	170	5.0	9.84	39	149	66	
11	1,190	915	480	175	5.0	9.78	39	147	-	
12	1,160	840	420	140	5.0	9.70	39	147	-	
13	1,100	750	370	110	5.0	9.70	39	142	65	
Average								146	66	

* Samples taken at discs 33, 66 and 99.

** Samples taken 8:30 a.m., adjustment made at 9:00 a.m. unless otherwise noted.

TABLE 4 BIOLOGICAL THIOSULFATE OXIDATION AT 8.5 C IN A BIODISC SYSTEM (CONT'D)

Date	$S_2O_3^{=}$ Concentration (mg/l)				Biodisc Rotation Speed rpm	Flow Rate l/hr	Residence Time (minutes)	Maximum $S_2O_3^{=}$ Oxidation Rate mg/sq ft/hr	.% H_2SO_4 Recovery From Oxidized $S_2O_3^{=}$	Remarks
	Feed	Biodisc Stage*								
		1	2	3						
April 17 **	880	615	280	100	5.0	9.26	41	124	-	Nutrient levels reduced to 4 mg/l NH_4^+ and 5 mg/l $PO_4^{=}$ on April 16.
18	960	610	230	<20	5.0	7.68	50	125	-	
19	985	730	415	180	5.0	11.20	34	134	-	rpm reduced.
Average								128		
24	1,140	830	425	140	3.3	7.58	50	122	-	
25	995	650	270	90	3.3	7.60	51	123	-	
Average								122		

* Samples taken at discs 33, 66 and 99.

** Samples taken 8:30 a.m., adjustment made at 9:00 a.m. unless otherwise noted.

TABLE 5 CHEMICAL ANALYSIS OF BRUNSWICK TAILINGS EFFLUENT AND VANCOUVER TAP WATER

Element	Concentration mg/l	
	Tailings Effluent	Tap Water
Thiosulfate ($S_2O_3^{=}$)	900	-
Free ammonium ion	3.60	0.005
Total Kjeldahl nitrogen	6.02	0.125
Phosphate	0.86	<0.015
Calcium	473	1.68
Magnesium	13.7	0.23
Manganese	0.13	<0.005
Chloride	598	1.08
Sodium	360	0.75
Potassium	10.7	0.30
Sulfate	674	3.1
Copper	<0.05	<0.05
Zinc	0.07	<0.03
Cobalt	<0.05	<0.05
Iron	<0.10	<0.10

TABLE 6 BIOLOGICAL OXIDATION OF THIOSULFATE IN BRUNSWICK TAILING WATER AT 8.5 C IN A BIODISC SYSTEM

Date	$S_2O_3^{=}$ Concentration (mg/l)				Biodisc Rotation Speed rpm	Flow Rate l/hr	Residence Time (minutes)	Maximum $S_2O_3^{=}$ Oxidation Rate mg/sq ft/hr	Remarks
	Feed	Biodisc Stage*							
		1	2	3					
April 25									
1:00 p.m.	890	900	820	580	3.3	7.60	51	81	3 1/2 hours after feed started.
4:00 p.m.	950	950	880	660	3.3	7.60	51	74	6 1/2 hours.
April 26									
8:00 a.m.	-	<10	<10	<10	3.3	0	-	-	On batch overnight. Pump turned on at 8:00 a.m.
9:00 a.m.	900	610	230	<20	3.3	7.60	51	-	
11:45 a.m.	900	830	510	220	3.3	7.60	51	103	

* Samples taken at discs 33, 66 and 99.

TABLE 7 SURFACE AREA FOR DISCS OF VARIOUS DIAMETERS

Diameter feet	Area sq.ft/disc	Number of Discs Required for 18,700 sq. ft
5	39.3	476
6	56.5	331
8	100.5	186
10	157.1	119
12	226.2	83

Figure 1

SCHEMATIC DRAWING OF BUSCH FERMENTER
BIO-OXIDATION UNIT

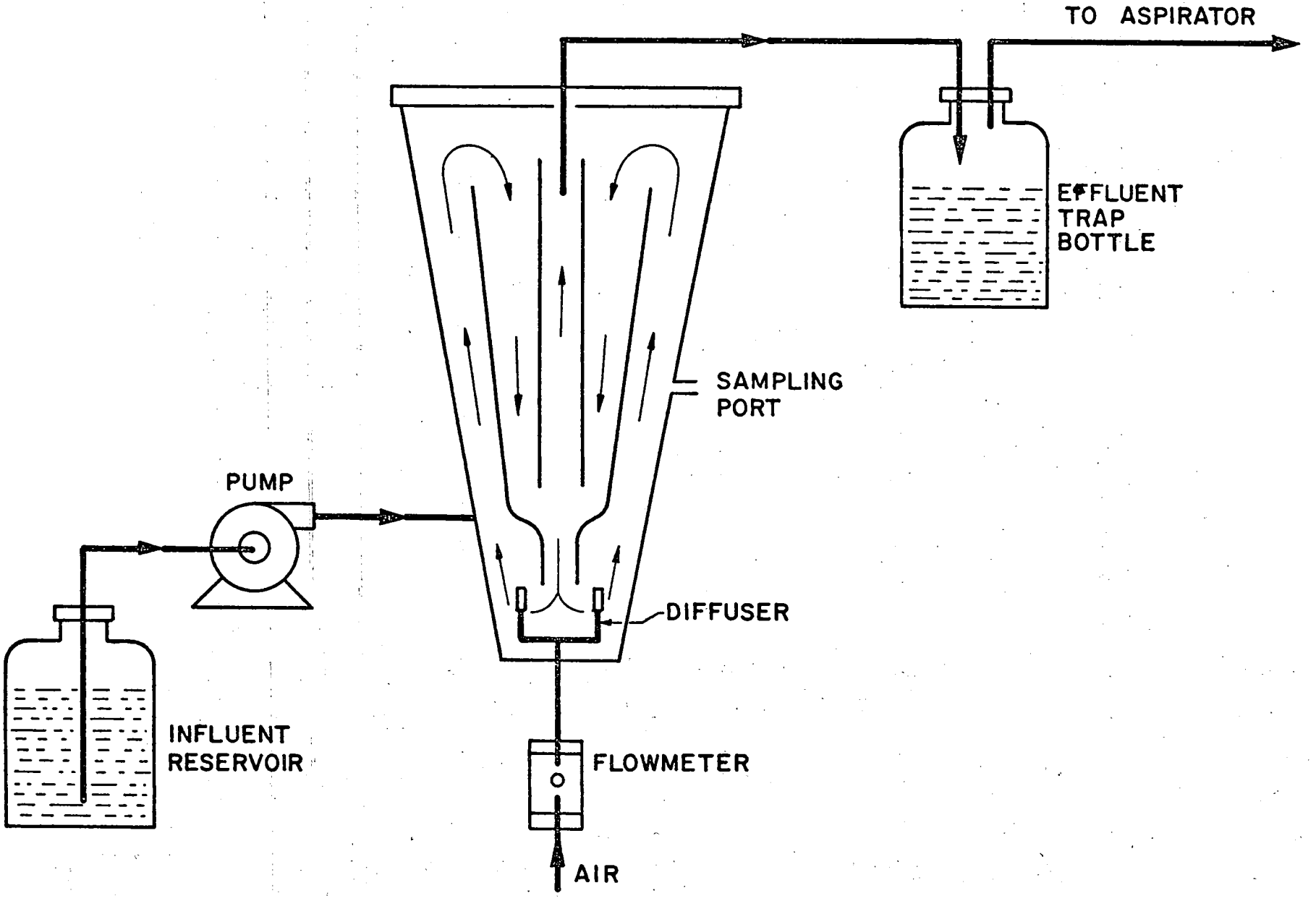

Figure 2

BIO DISC SYSTEM
Schematic Design

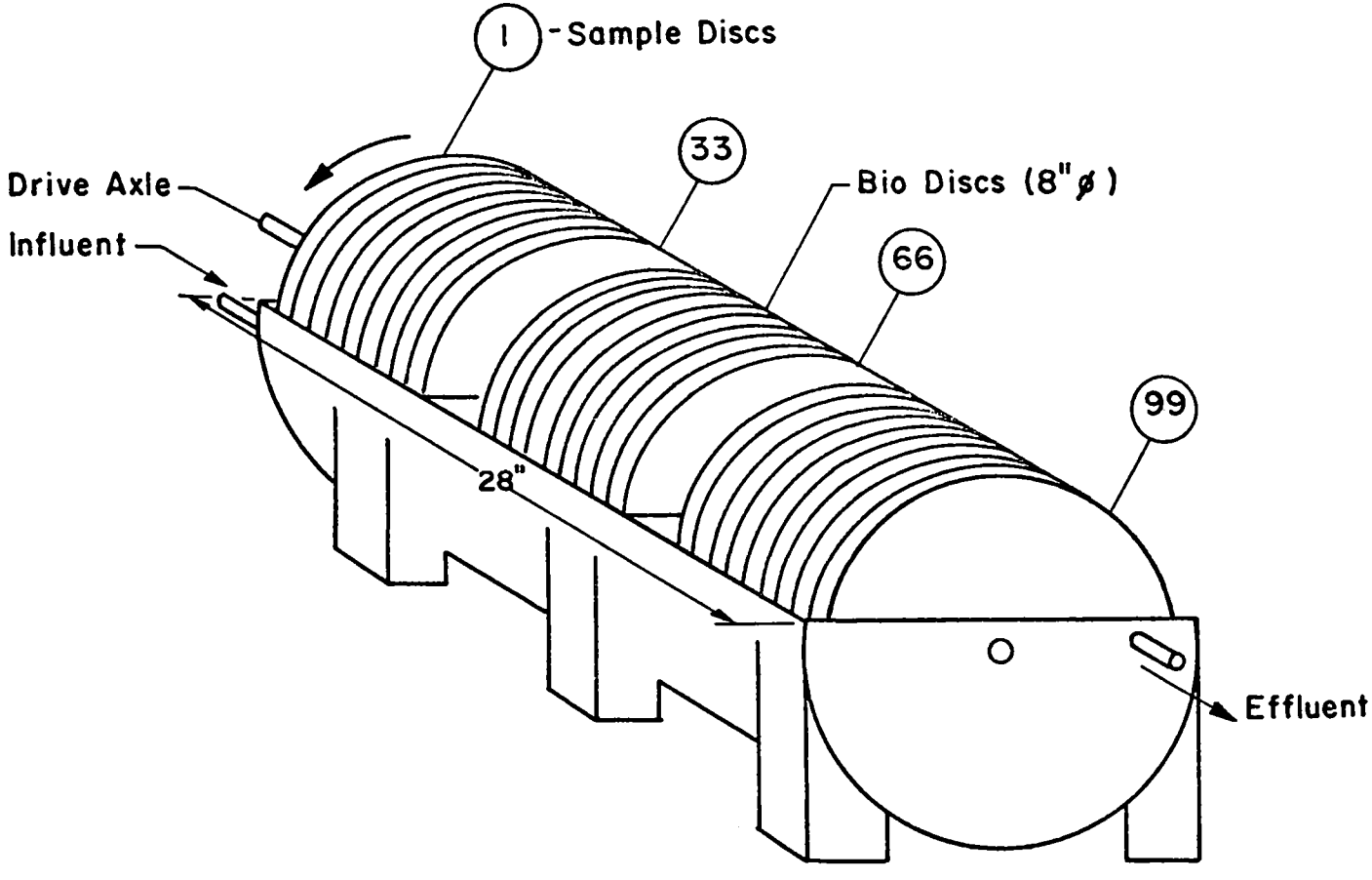

Figure 3

OXIDATION OF THIOSULFATE AT 30°C

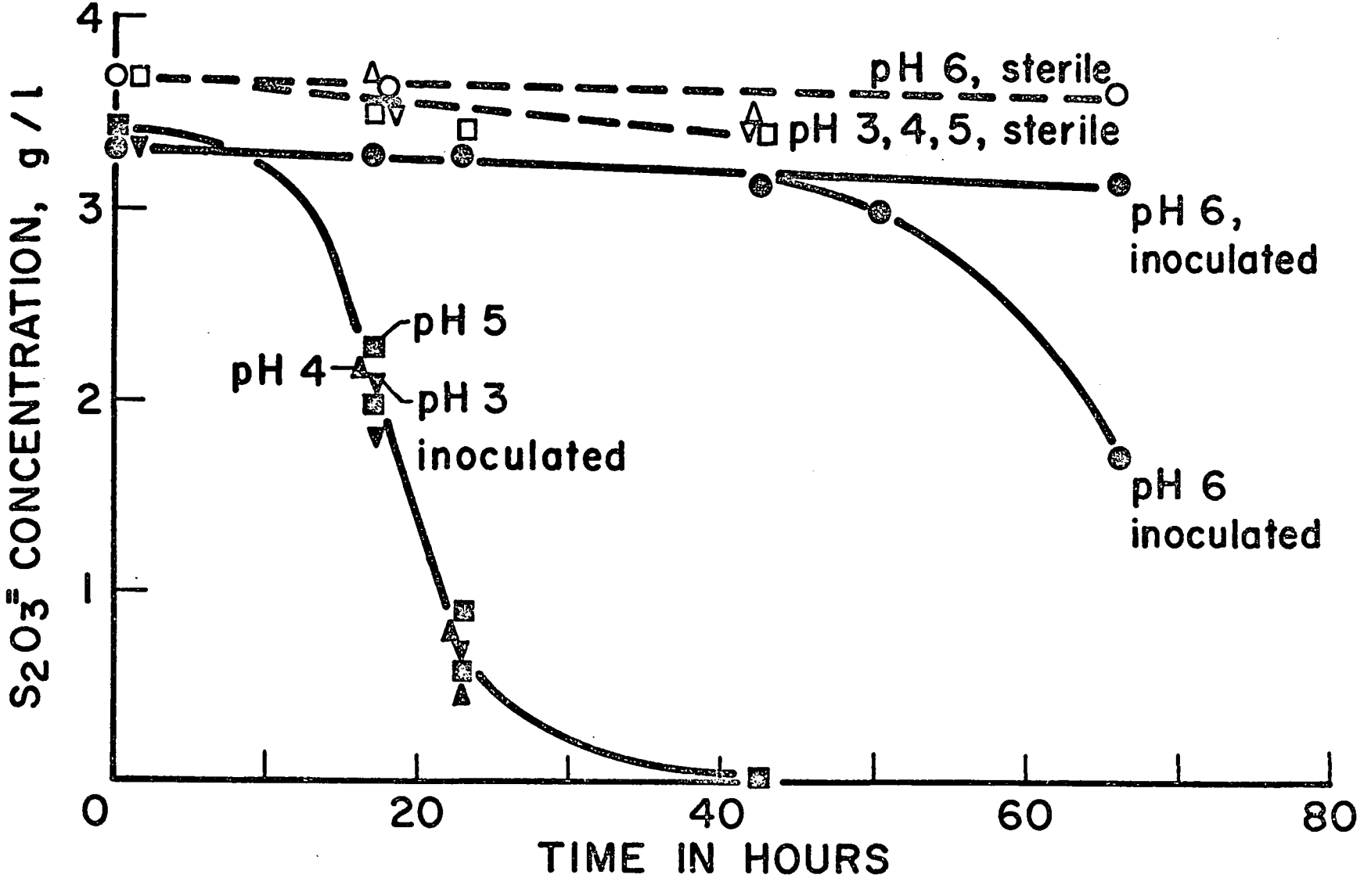

Figure 4

BIOLOGICAL THIOSULFATE OXIDATION RATE AND GENERATION TIME AS A FUNCTION OF TEMPERATURE

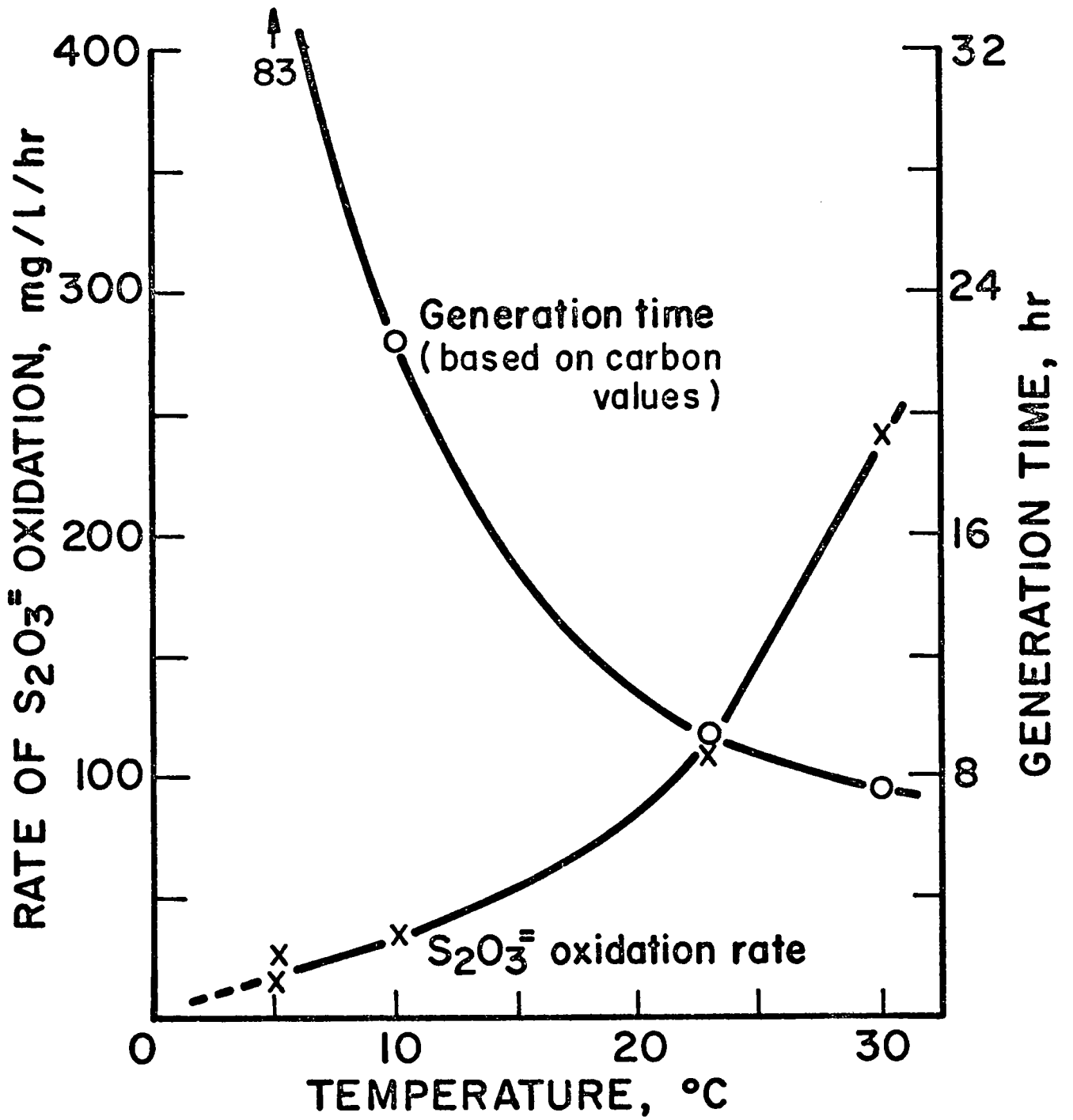

Figure 5

BIOLOGICAL OXIDATION OF THIOSULFATE IN MEDIUM CONTAINING ONLY AMMONIUM SULFATE IN TAP WATER

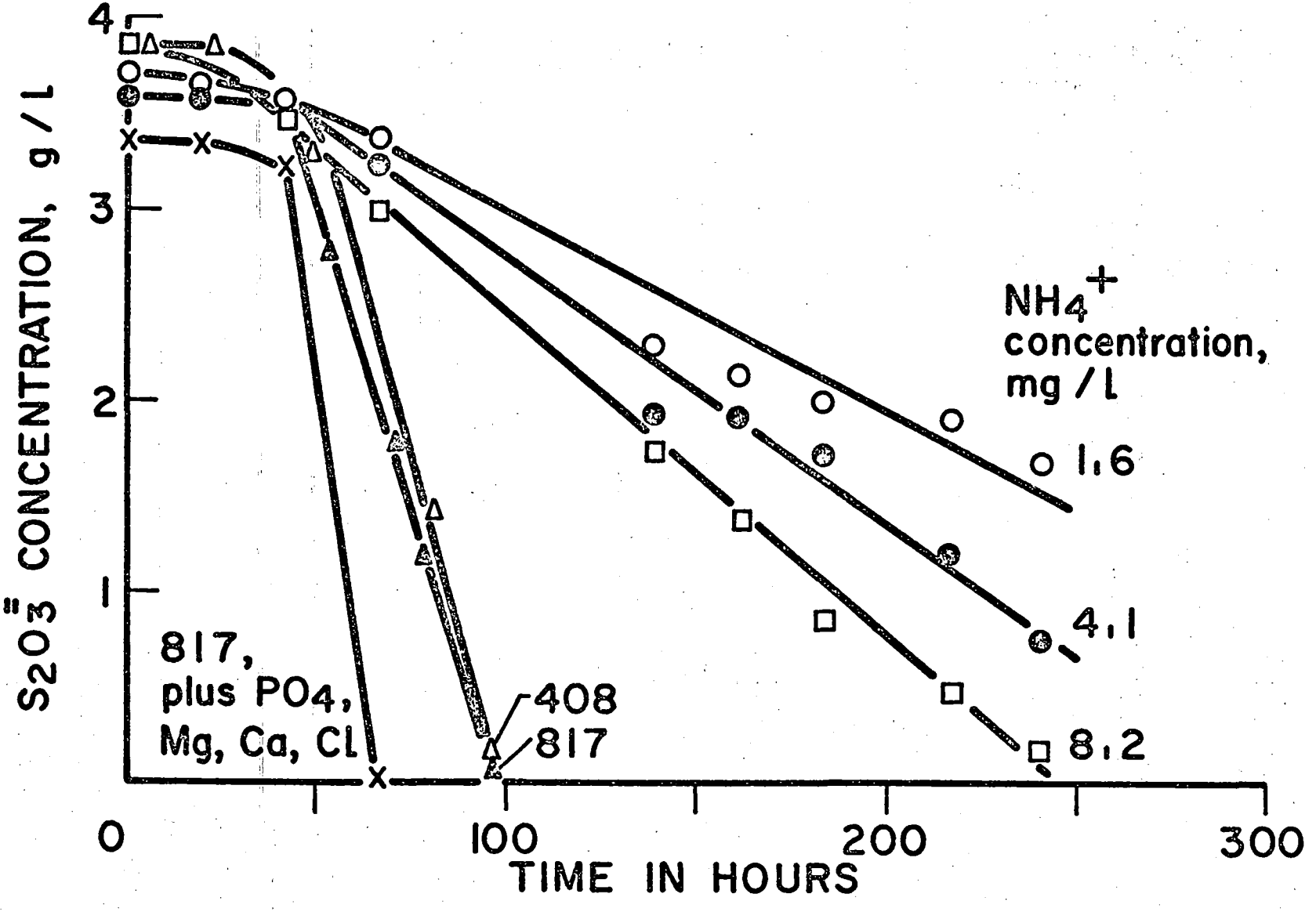

Figure 6

BIOLOGICAL OXIDATION OF THIOSULFATE
IN VARIOUS MODIFICATIONS OF BOUNDS AND COLMER MEDIUM

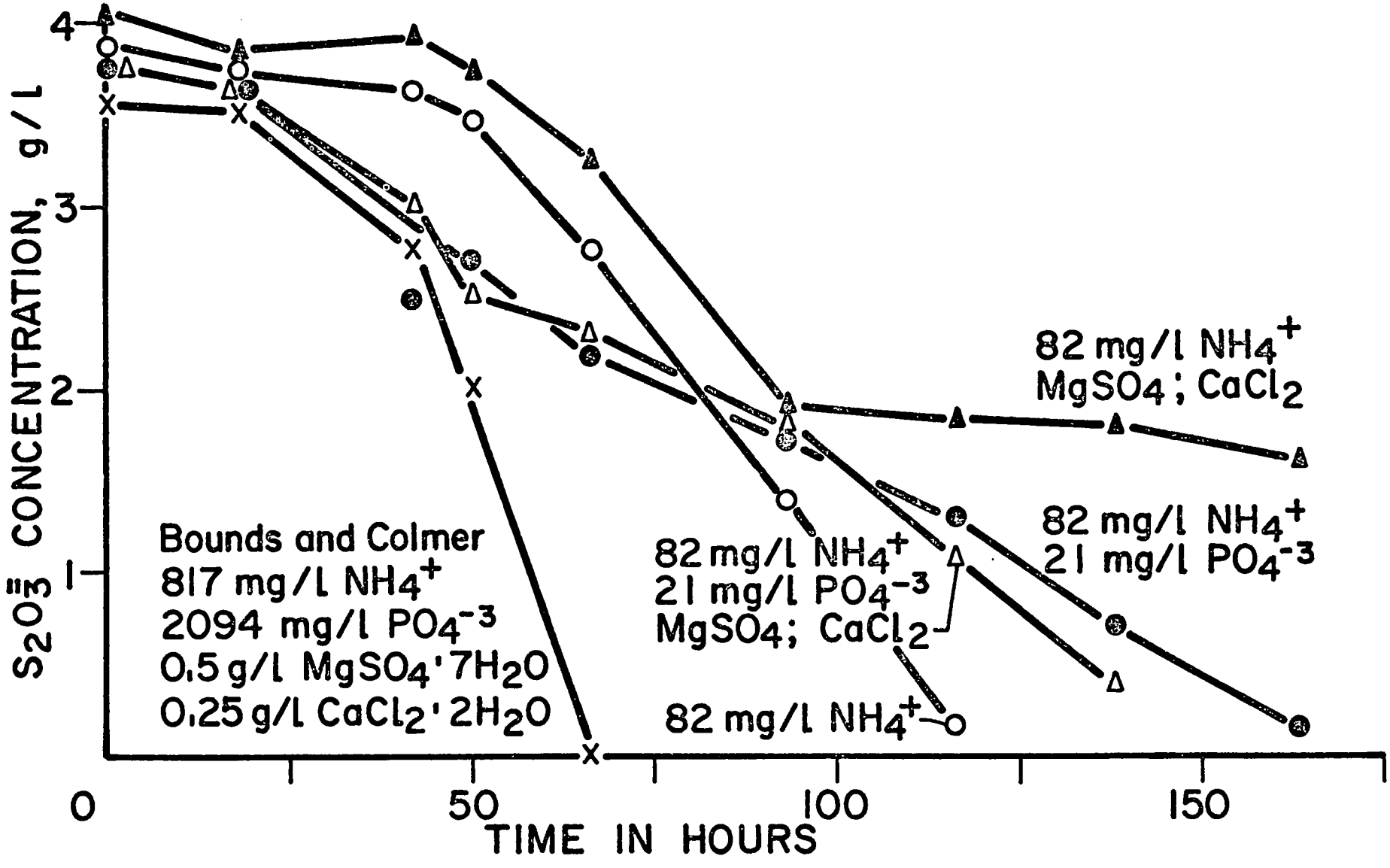

Figure 7

THIOSULFATE OXIDATION BY Thiobacillus thioparus AT DIFFERENT pH LEVELS AT 30°C

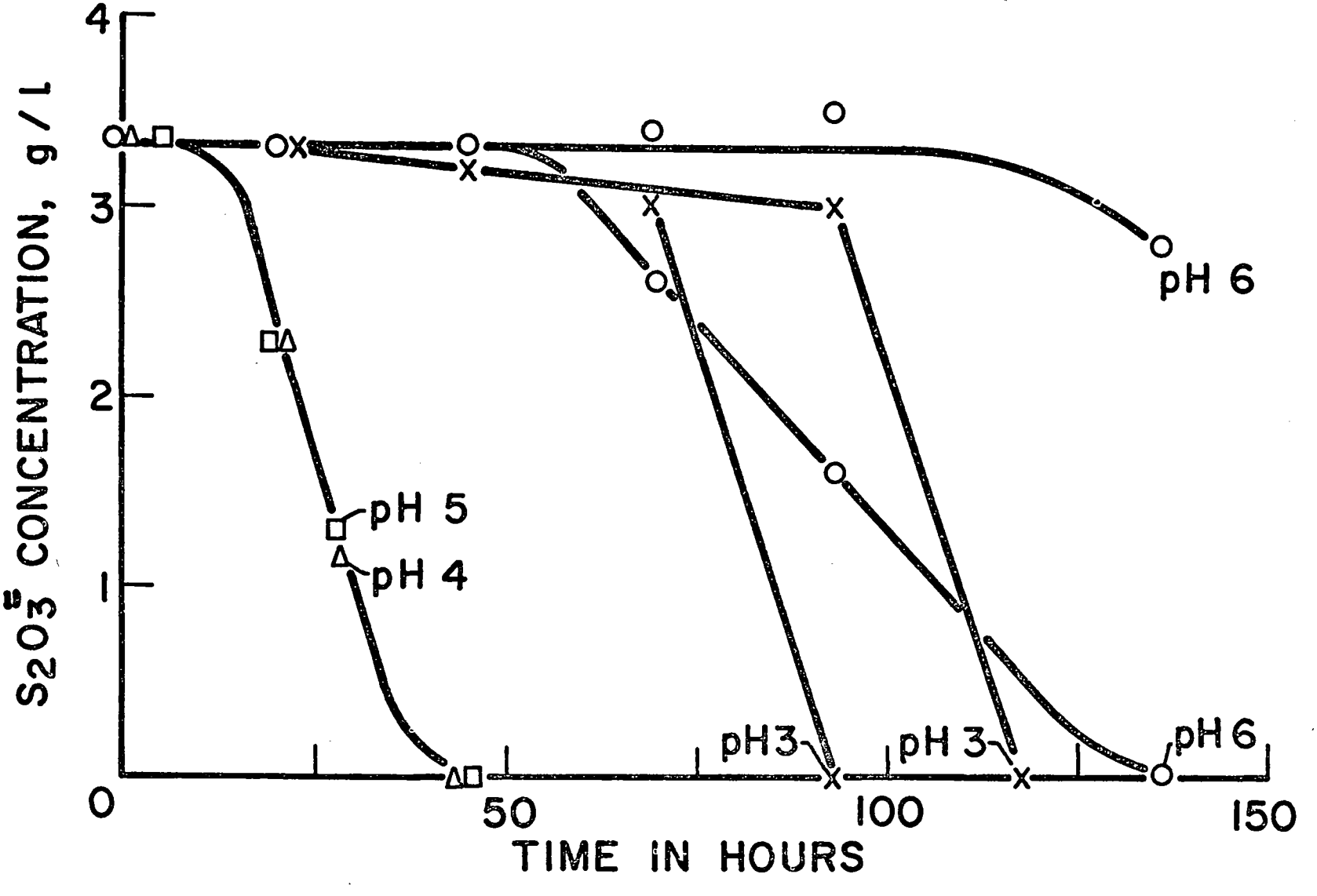

Figure 8

BIOLOGICAL OXIDATION OF THIOSULFATE IN A BIO-DISC

(Room temperature)

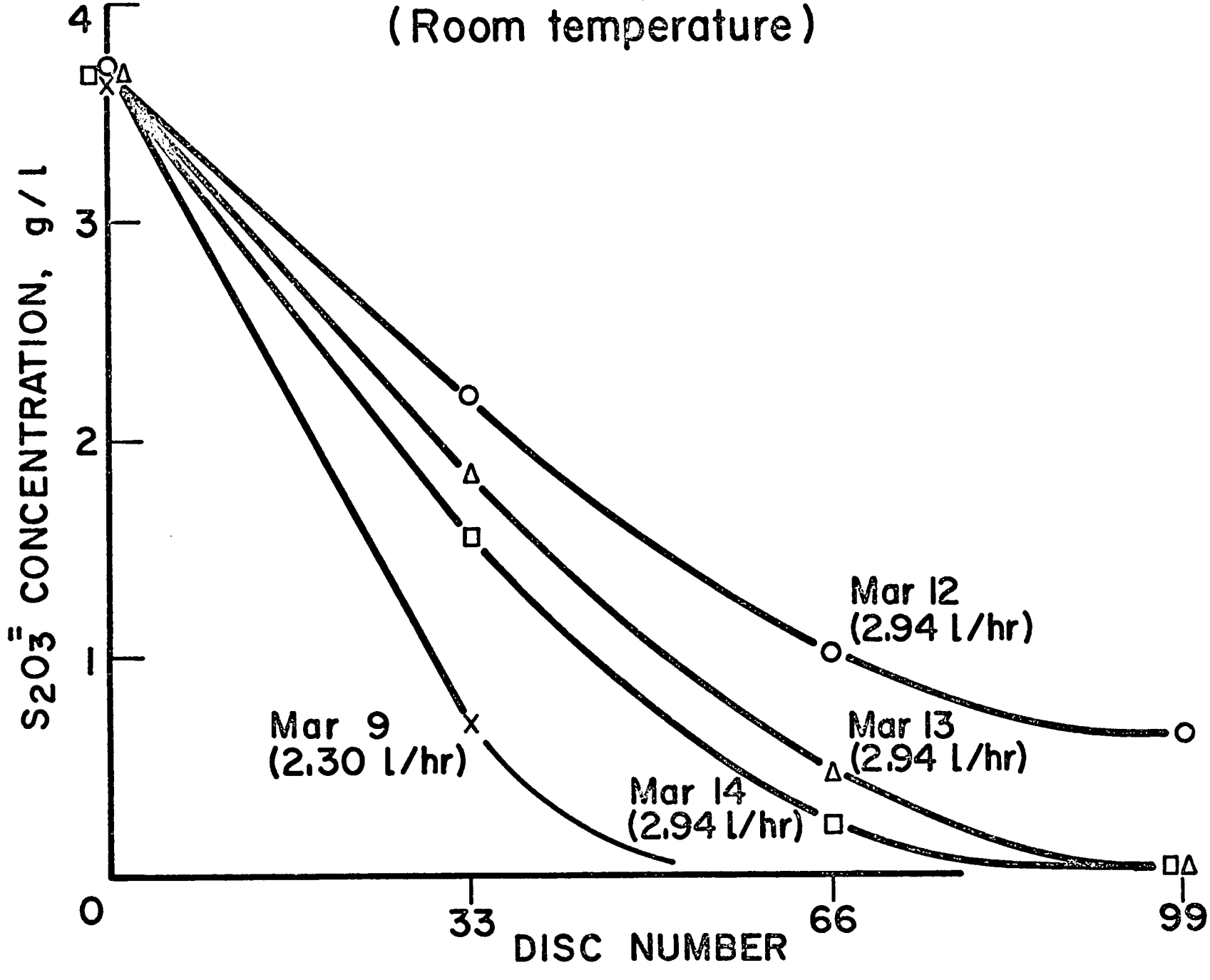

Figure 9

BIOLOGICAL OXIDATION OF THIOSULFATE IN A BIO-DISC (8.5°C)

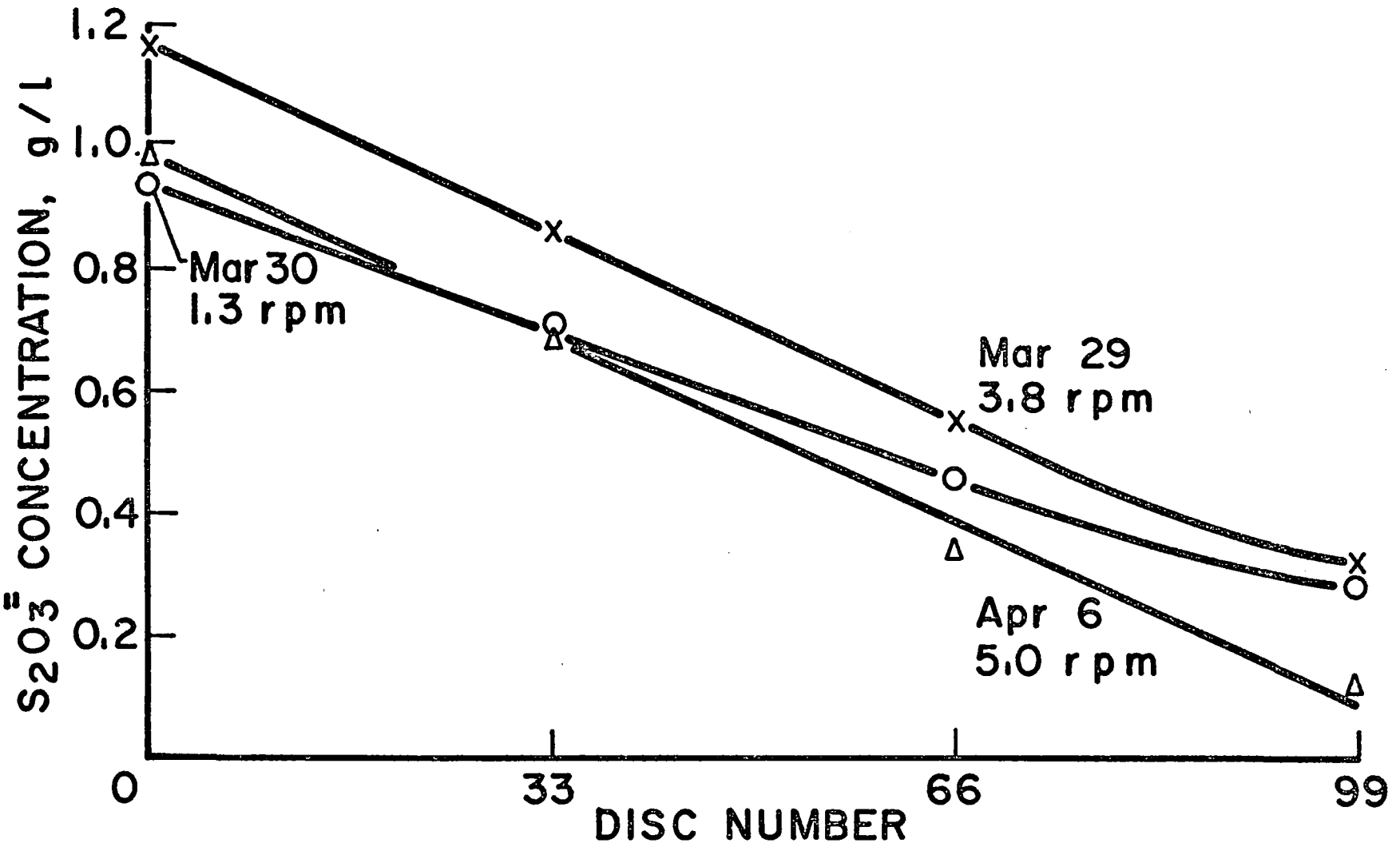

Figure 10

BIOLOGICAL OXIDATION OF THIOSULFATE
IN BRUNSWICK TAILING EFFLUENT IN A BIO-DISC UNIT

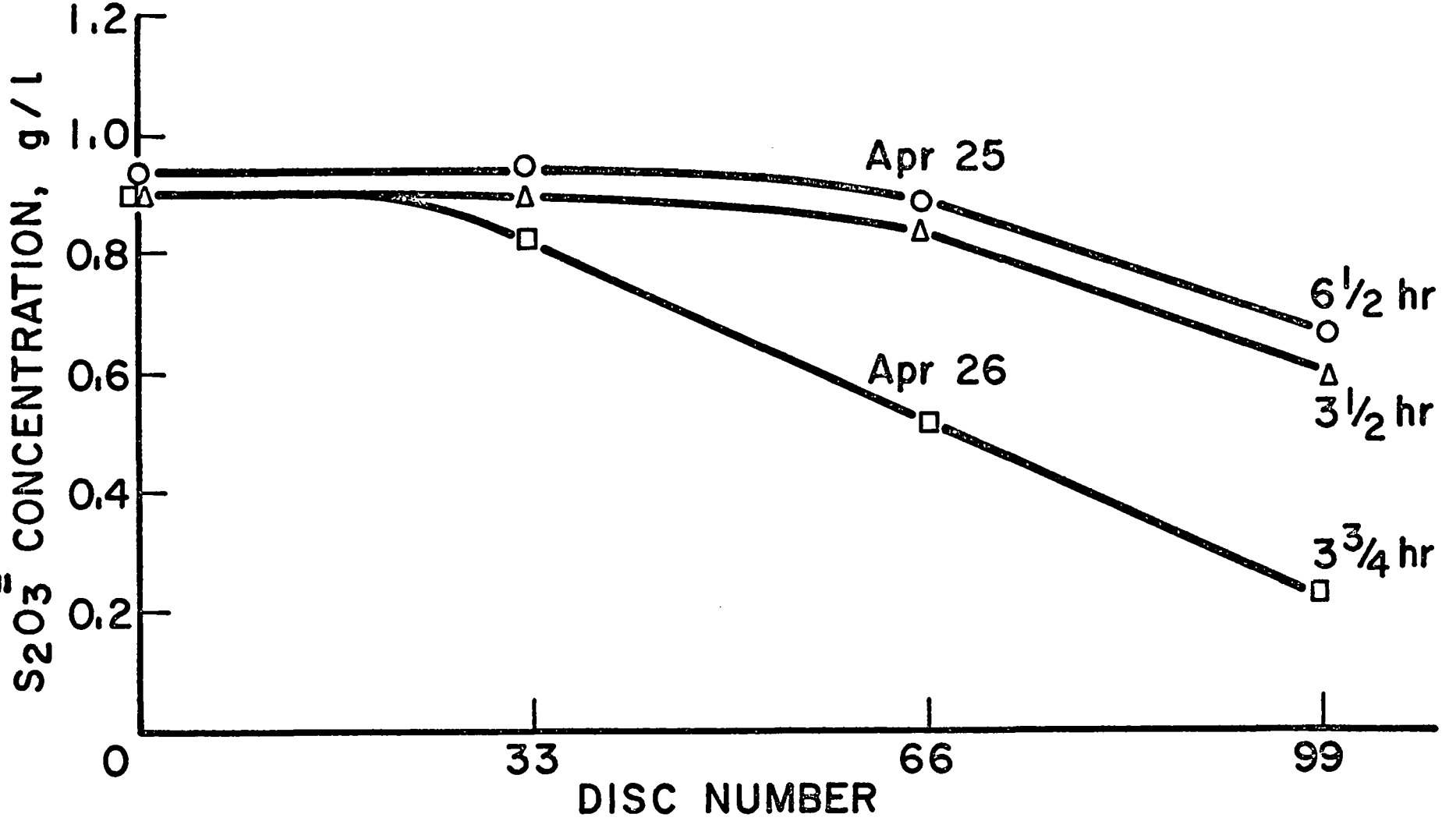

Figure 11

FLOW SHEET FOR THIOSULFATE OXIDATION PILOT PLANT