THIOSULFATE OXIDATION IN A FLUIDIZED BED

by

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To my wife Alice and my daughters

Ricky Michelle and Erica Lorraine

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INTRODUCTION

Technological advances in modern industry have resulted in generating a variety of wastes containing specific materials which sometimes require specialized treatment. One of these materials is a reduced sulfur compound known as thiosulfate. Thiosulfate wastes are not numerous but they do present a problem when considering the preservation and maintenance of a stable environment. Although thiosulfate is not generally considered to be a toxic material, its presence in an aquatic environment can result in adverse effects due to its characteristic feature of being biologically oxidized to sulfuric acid. Of the various treatment schemes which have been used for the removal of thiosulfate, processes utilizing biological methods have proved to be both efficient and economical.

In conjunction with the progress of modern industry, the field of wastewater treatment has also made numerous advances. One of the most recent developments is the application of fluidized bed technology to biological wastewater treatment.

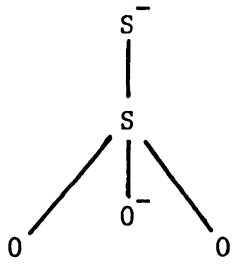
The main purpose of this investigation was to further advance both thiosulfate treatment and fluidization technology by evaluating the possibilities of biologically oxidizing a synthetic thiosulfate waste in a laboratory scale fluidized bed. Other characteristics relevant to thiosulfate oxidation were also investigated.

LITERATURE REVIEW

The following discussion is devoted to a review of the available literature which is considered to be relevant to the objectives of this research. The topics of discussion include: thiosulfate properties, thiosulfate wastes, treatment of thiosulfate wastes, sulfur bacteria, oxidation pathways of thiosulfate, biological films and fluidized beds.

Thiosulfate Properties

General Chemistry. Thiosulfate is a reduced, inorganic sulfur compound with the chemical formula $S_2O_3^{-2}$. The thiosulfate ion may be depicted as having a tetrahedral structure with a hexavalent sulfur atom at the center (1):



The thiosulfate ion may be viewed as being derived from a sulfate ion by substitution of an oxygen atom by a sulfur atom (1,2). In general, any compound in which sulfur in oxidation state -2 is substituted for oxygen is called a thio-compound (2).

Preparation of thiosulfate. Thiosulfate is frequently prepared as a sodium salt by one of the following three methods (3):

1. The reaction of a normal sulfite solution with finely ground sulfur. This process is usually used in commercial production of alkali thiosulfates:

$$Na_2SO_3 + S \rightarrow Na_2S_2O_3. \quad (1)$$

2. The reaction of a sulfide and a sulfite:

$$2NaHS + 4NaHSO_3 \rightarrow 3Na_2S_2O_3 + 3H_2O \quad (2)$$

$$Na_2CO_3 + 4SO_2 + 2Na_2S \rightarrow 3Na_2S_2O_3 + CO_2. \quad (3)$$

3. Careful oxidation of a sulfide:

$$2NaHS + 2O_2 \rightarrow Na_2S_2O_3 + H_2O \quad (4)$$

$$12CaS + 9H_2O + 6O_2 \rightarrow 9Ca(OH)_2 + CaS_2O_3 + 2CaS_5. \quad (5)$$

Uses of thiosulfate. Commercial uses of thiosulfate are not extensive. The largest, industrial applications are as photographic fixers and as dechlorination agents (2,3,4,5).

The thiosulfate ion, $S_2O_3^{-2}$, forms a complex with the silver ion. This complex ion is of particular importance in photography. A solution of sodium thiosulfate, known as hypo, dissolves the unreduced silver ions in an exposed photographic emulsion according to the following reaction (4):

$$AgBr + 3Na_2S_2O_3 \rightarrow Na_5\ [Ag(S_2O_3)_3] + NaBr. \quad (6)$$

The image is then fixed permanently in the film. The fixed image is called a negative.

The oxidation of thiosulfate by chlorine is of technical importance in the use of thiosulfate as a dechlorination agent in the bleaching industry, where excess chlorine is destroyed by $S_2O_3^{-2}$. Sulfate is

the common oxidation product of thiosulfate when a strong oxidizing agent such as chlorine is employed (4):

$$S_2O_3^{-2} + 4Cl_2 + 5H_2O \rightarrow 2HSO_4^- + 8H^+ + 8Cl^-. \quad (7)$$

Various volumetric analyses in water chemistry require thiosulfate reagents as a secondary standard (6,7). Some of these include the determination of concentrations of sulfite, ozone, and dissolved oxygen. The value of thiosulfate in these analyses is its characteristic ability as a relatively strong reducing agent.

The tendency to use concentrated fertilizers to reduce transport costs has led to the development of ammonium thiosulfate as a recent fertilizer (8,9,10). Following application of the fertilizer, the thiosulfate sulfur is made available to plant life through the numerous metabolic pathways of the various sulfur bacteria.

The use of thiosulfate in the medical profession has been reviewed by Senning (4). Sodium thiosulfate has found medical use due to certain pharmacological effects of the thiosulfate ion. The compound is thus used in dermatology as an antimycotic and antiparasitic agent. Sodium thiosulfate is also a well known antidote in cyanide poisoning, its antidotal effect being described as early as 1895. The effect of thiosulfate is due to an enzyme catalyzed reaction between thiosulfate and cyanide, giving the relatively nontoxic thiocyanate and sulfite as products.

The ability of thiosulfate to form complexes with heavy metal ions was earlier explored as a therapy for heavy metal poisoning, but thiosulfate is now considered to be ineffective for this purpose. A related

application of the metal binding properties of thiosulfate is found in gold-sodium thiosulfate treatment of rheumatoid arthritis. Gold compounds have a beneficiary action on this disease but tend to decompose to elemental gold. This reaction is prevented if the gold atom is complex-bound to thiosulfate or to other sulfur containing compounds.

Chemical reactions of thiosulfate. The numerous chemical reactions and characteristics of thiosulfate have been extensively reviewed in various text books devoted to sulfur chemistry (1,3,4,5).

Thiosulfates of alkali metals, ammonia, magnesium, cadmium, zinc, calcium, strontium, nickel, cobalt, iron(II), and manganese(II) are easily soluble in water and form well developed crystals with water of crystallization, e.g., $Na_2S_2O_3 \cdot 5H_2O$ (3,4). Barium, silver, thallium(I) and lead salts are insoluble.

Alkali thiosulfates form soluble complexes with many insoluble salts of the transition metals such as silver, mercury, copper, and gold. As previously noted, this is of considerable practical importance in photography where sodium thiosulfate is used as a fixer.

Metals such as copper, mercury, lead, and silver that form insoluble sulfides are usually precipitated as sulfides when treated with thiosulfate (3). Mercury(I) dissolves in a $Na_2S_2O_3$ solution but precipitates as HgS if warmed. Hg(II) forms a white precipitate which decomposes almost immediately into HgS. The lead and silver complexes turn black rapidly if heated because of the formation of sulfides.

Thiosulfate is relatively stable around pH 7 and can be autoclaved in aqueous solutions with little or no decomposition (1). Decomposition

may, however, be accelerated by metal ions. On boiling, alkaline solutions of thiosulfate slowly decompose to sulfide and sulfate, sulfite, or trithionate depending on the conditions (1,3).

The thiosulfate ion is a reducing agent of moderate strength, as indicated in the following equation (4):

$$2S_2O_3^{-2} \rightarrow S_4O_6^{-2} + 2e^- \quad E^o_{298} = 0.17V. \tag{8}$$

Oxidation of thiosulfate by iodine, forming tetrathionate and iodide, is perhaps the best known reaction of the thiosulfates (4). It is the basis of all the numerous iodometric quantitative determinations of oxidizing agents:

$$2S_2O_3^{-2} + I_2 \rightarrow S_4O_6^{-2} + 2I^-. \tag{9}$$

Despite the extensive use of this reaction in analytical chemistry, its mechanism still remains unexplained in detail. The same oxidation reaction is also reported to take place with other mild oxidants such as Fe(II), Cu(II), Au(III), MnO_4^{-2}, and PbO_2 (3,4).

Strong oxidizing agents such as chlorine and bromine oxidize thiosulfate to sulfate (3). In the oxidation of thiosulfate to sulfate by chlorine, the average oxidation number of sulfur rises from +2 to +6, whereas in the course of the iodometric oxidation of thiosulfate to tetrathionate the average oxidation number of sulfur is brought from +2 to +2.5. Therefore, in the oxidation of a given amount of thiosulfate the consumption of chlorine is eight times that of iodine (4).

Acid decomposition of thiosulfate. The decomposition of acidified thiosulfate solutions has received intensive study (1,3,4,5,11,12,13, 14,15,16,17) and has been important in the development of the physical

chemistry of colloidal solutions (14,17). The products of the reaction have been determined (11,12,13,14,17).

Acid decomposition of thiosulfate produces a complex mixture of various sulfur species (11,15,16):

$$S_2O_3^{-2} + H^+ \rightarrow \begin{matrix} S_8,\ S_6,\ SO_2,\ H_2S \\ H_2SO_4,\ H_2S_xO_6,\ H_2S_{x+1} \end{matrix} \qquad (10)$$

Depending on the conditions, the products of this reaction are: colloidal sulfur, S_8,S_6; sulfur dioxide; hydrogen sulfide; sulfuric acid; polythionic acids, $H_2S_xO_6$; and sulfur oils, H_2S_{x+1}. Zaiser and La Mer (17) have also listed sulfite, SO_3^{-2} as a decomposition product.

The yields of the various decomposition products depend on many factors, but the two most important are the initial concentration of thiosulfate and the concentration of acid. At low acidity the products are sulfur and sulfur dioxide, while at high acidity sulfur, sulfide, sulfate, and polythionic acids are formed, and at medium acidity sulfur containing oils are produced (5,16).

An investigation by Zaiser and La Mer (17) has demonstrated how colloidal sulfur is produced in three distinct stages. The three stages can be summarized as follows:

1. The homogeneous reaction. The reaction begins as soon as the acid and thiosulfate are mixed, and produces molecularly dispersed sulfur. This stage is terminated when the sulfur solution becomes sufficiently super-saturated for stable nuclei to form.

2. The condensation stage. Here the rapid condensation of molecularly dispersed sulfur upon the nuclei relieve the super saturation with

the result that the rate of subsequent nucleation is greatly decreased.

3. The heterogenous stage. The sulfur droplets slowly increase in size by diffusion of dissolved sulfur to the particle surfaces.

The results of this investigation (17) showed that the rate of sulfur appearance in the homogeneous reaction could be expressed as a function of the initial concentration of thiosulfate and the concentration of the acid used in the decomposition. The resulting rate equation can be expressed as follows:

$$\frac{d(S)}{dt} = k\ (Na_2S_2O_3)^{3/2}\ (HCl)^{1/2} \qquad (11)$$

This expression has since been confirmed (12,13).

The homogeneous reaction proposed by Zaiser and La Mer has been further demonstrated (3). The observation has been made that if a thiosulfate solution is acidified and, before opalescence appears, is again made basic, sulfur will nevertheless be precipitated. It appears that the delay of precipitation is due to slow agglomeration of sulfur rather than slow decomposition of thiosulfate.

Toxicity of thiosulfate. Senning (4) has cited extensive evidence showing that sodium thiosulfate and other alkali metal thiosulfates are compounds of a very low toxicity. Experiments on guinea pigs and dogs indicated that sodium thiosulfate became toxic at the very high dose level of 3-4 g/kg body weight. A dose of 1.5 g/kg was, on the other hand, well tolerated. The thiosulfate dose recommended for treatment of cyanide poisoning to a human being is about 0.2 g/kg body weight. Doses of up to 0.4 g/kg body weight produced no sensations in human beings.

The oral toxicity of sodium thiosulfate is very low. Doses of 5-18 grams given to human beings have had only a laxative action.

Evidence of the toxicity of sodium thiosulfate on various aquatic species has also been cited (18,19,20,21,22). The toxic threshold concentration for the flatworm Polycelis nigra was found to be 8400 mg/l (18). The threshold concentration of sodium thiosulfate for the immobilization of Daphnia magna in Lake Erie water was found to be much less than 520 mg/l (19). The minimum lethal concentration for minnows has been reported to be 5 mg/l, for a 120-hour period (20,21). Using highly turbid water at 22-24°C, Wallen et al. (22), found the 96-hour median tolerance limit toward the mosquito-fish, Gambusia affinis, to be 24,000 mg/l.

Thiosulfate Wastes

Industrial wastewaters containing thiosulfate are not numerous. Although photographic wastes have long been known for containing relatively high concentrations of thiosulfate, the available characterization data for these wastewaters are not extensive (23,24). West (23) has reported on a motion-picture film processing waste containing an average thiosulfate concentration of 3480 mg/l. This thiosulfate concentration was cited as having a Biochemical Oxygen Demand (BOD) of 1565 mg/l, representing 70 percent of the total BOD. Dagon (24) has reported characteristics of a photo processing waste with an average thiosulfate concentration of 374 mg/l, noting that this concentration represents 23 percent of the total BOD.

A characterization and treatability study of a sulfur dye waste has cited thiosulfate concentrations of both composite plant wastewater and press cake liquor at 3800 and 105,800 mg/1, respectively (25,26). Since it appeared from the initial characterization of the wastewaters that thiosulfate was the major oxygen consuming constituent of the wastes, the Chemical Oxygen Demand (COD) and BOD equivalent of reagent grade thiosulfate were determined (25). The COD and BOD in the waste were, respectively, approximated to be 0.625 and 0.575 times the concentration of thiosulfate.

Characterization of a flotation-mill waste generated from treating a lead-zinc ore containing pyrite and pyrrhotite showed a thiosulfate concentration ranging from 500-1000 mg/1 (27). Polythionates such as trithionate and tetrathionate were also shown to be present. This wastewater was also completely characterized for various macro and micro nutrients required by biological systems.

Wastewaters from Kraft pulp mill processes (20,21) and gas house wastes (28) have also been reported as containing thiosulfate.

Treatment of Thiosulfate Wastes.

Wastewaters containing thiosulfate have been treated using various methods which include activated sludge, rotating bio-discs, trickling filters, reverse osmosis, and chemical oxidation (23,24,25,26,27,28,29, 30,31,32,33,34,35,36,37,38).

Using a bench scale activated sludge process, an investigation was conducted to determine the treatability of a sulfur dye waste containing thiosulfate (25,26,37). The controlling parameter throughout the

investigation was food to microorganism ratio (F/M) on a thiosulfate basis. The F/M was varied from 0.7 to 26.0.

During the course of this investigation, the influent feed to the biological unit consisted of various combinations of domestic sewage and sulfur dye waste. Upon developing an autotrophic population of bacteria capable of oxidizing thiosulfate, oxidation of the domestic sewage ceased and the effluent pH was reported to drop to a level of 2.0. The influent thiosulfate concentrations ranged from 700 to 2500 mg/l while the F/M ranged from 0.7 to 3.1. Ninety-five to 98 percent removal of thiosulfate was reported.

Using the sulfur dye waste as a sole source of feed, the thiosulfate concentrations varied from 1000 to 4000 mg/l while the F/M ranged between 8.1 and 26.0. Thiosulfate removal varied between 84 and 100 percent and the effluent pH was reported to drop as low as 1.8. It is of interest to note that detention times up to 32 hours were required for complete removal of thiosulfate at the higher loadings.

Throughout this investigation sludge settleability was reported as being a severe problem once an autotrophic population was established. Compared to a typical heterotrophic activated sludge process both oxygen requirements and solids production were very low. Based on the results of the investigation, it was concluded that an autotrophic activated sludge process could be highly effective in removing the oxygen demand associated with a thiosulfate waste, and that a low pH in the process was an excellent indication that a high removal of

thiosulfate was being achieved. Phosphorus was also shown to play an important role in the oxidation of thiosulfate.

In another investigation (29) concerning the oxidation of thiosulfate in an activated sludge process treating domestic sewage, a deterioration in the quality of the sludge was also noted. Analysis of the sludge showed that a large increase in the sulfur content of the sludge had occurred. As the sulfur content of the sludge increased, the ability of the sludge to oxidize thiosulfate decreased. It was assumed that sulfur was replacing active organisms since the sludge concentration remained fairly constant. During the study, the activated sludge unit was acclimated to remove up to 1300 mg/l thiosulfate of which 34 percent was completely oxidized to sulfate. Oxidation of the sewage was satisfactory but a high effluent BOD was reported due to the remaining thiosulfate.

Dagon (24) has reported on a photo processing waste treated by activated sludge. Characterization of the waste showed a thiosulfate concentration of 374 mg/l. Effluent thiosulfate concentrations were not reported.

A study was carried out in New Brunswick (27) to determine the cause of an acid problem in the river system receiving wastewater from a floatation mill of a mining operation. The wastewater was shown to contain thiosulfate at concentrations of 500-1000 mg/l. Prior to discharge of the effluent from a tailing pond the pH was adjusted using lime. However, regardless of the pH at which it was discharged, the pH of the receiving stream dropped to 3.0 within a distance of 13 miles.

A laboratory scale, three stage, rotating bio-disc was evaluated as a possible method of treatment for the mining waste. Continuous flow studies using a synthetic feed demonstrated thiosulfate oxidation rates of 267 mg/ft^2/hr. Influent thiosulfate concentrations ranged from 1000 to 3600 mg/l while the liquid residence time varied between 38 and 131 minutes. The effluent pH throughout the investigation was stable at 2.0 and the conversion of thiosulfate to sulfuric acid was reported as being 92 percent. Reducing the temperature to 8.5°C at an influent thiosulfate concentration of 1000 mg/l brought about thiosulfate oxidation rates of 146 mg/ft^2/hr. The effluent pH remained stable at 2.1, but conversion of thiosulfate to sulfate dropped to 80 percent.

Upon changing over to the actual mining waste at a temperature of 8.5°C, the rotating bio-disc was shown to perform in similar fashion as compared with the synthetic feed. During the course of the entire investigation sloughing of the attached solids was frequently encountered.

During the time that synthetic feed was being used, nutritional studies concerning nitrogen and phosphorus were also conducted. Maximum oxidation rates at thiosulfate concentrations of 1000 mg/l were achieved at a $S_2O_3^{-2}$:N:P ratio of 1000:6.2:1.6.

A laboratory scale activated sludge unit was also operated using the synthetic feed. The maximum thiosulfate oxidation rate was 60 mg/l/hr compared to rates of 2800 mg/l/hr obtained with the rotating bio-disc under similar conditions. The bacteria attached to the walls

of the unit prevented the formation of a normal floc. Attempts to create artificial flocs using peat were unsuccessful.

Trickling filters have been used to remove low concentrations of thiosulfate (28) from gas house wastes. Application of 50 mg/l thiosulfate to the filter resulted in complete oxidation with no deterioration of the quality of effluent from the filter.

Reverse osmosis has also been applied for the recovery of thiosulfate from motion picture film processing wastes (23). Ninety-five percent recovery was achieved at a pressure of 600 lbs/in^2. The waste was concentrated from a thiosulfate concentration of 1500 mg/l to 4200 mg/l with a product water discharge of 200 mg/l.

Thiosulfate has been chemically oxidized by various compounds to remove it from wastewater (30,31,32,33,34,35,36). Some of the chemicals which have been used include ozone, sodium chlorite, chlorine, hydrogen peroxide, and peroxydisulfate. The processes are all very successful in removing the oxygen demand of thiosulfate, but the chemical requirements are very high for each of these possibilities.

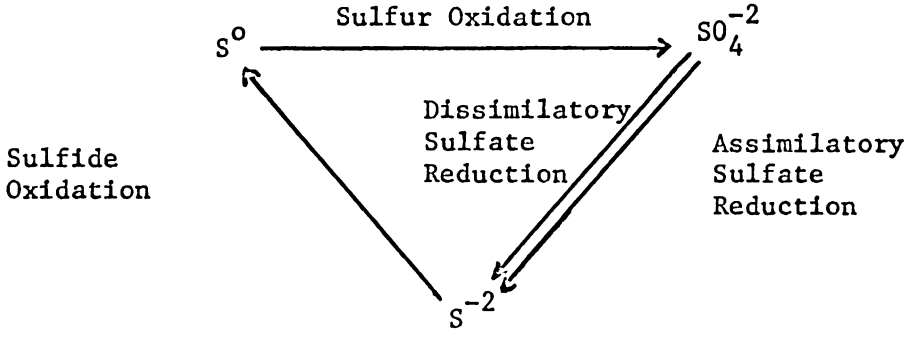
A group of refineries discharging thiosulfate were faced with a serious disinfection problem due to their high thiosulfate concentrations (31). A total refinery discharge of approximately 315,000 pounds of thiosulfate per day indicated that about 800,000 pounds per day of chlorine would be required before any disinfecting chlorine residual would be attained. The cost would have been a minimum of $40,000 per day. In order to provide the maximum economical amount of disinfection, the refineries were required to store their thiosulfate wastewaters

and discharge them only one day per week. No chlorination was attempted on this day.

A waste treatment process, patented by Eccles (38), consisted of establishing an "aqueous oxidative zone" using dilute sodium thiosulfate, ammonium hydroxide and phosphoric acid. The process was reported as being capable of converting thiosulfate to sulfate while maintaining a pH around 1.5. Seven to thirty days were required to establish the "aqueous oxidative zone." In a continuous flow application, a seven day detention time was required to maintain the "aqueous oxidative zone." In many respects this process is similar to an activated sludge process but no mention of this was made in the patent.

Sulfur Bacteria

Microorganisms play a very significant role in the overall sulfur cycle. Soil and water bacteria pass the sulfur atom through the various stages of oxidation illustrated below (5). Thiosulfate is an intermediate product in the oxidation of sulfur to sulfate:



Through assimilatory sulfate reduction, microorganisms produce organic material necessary for growth. The principal organic compounds

synthesized are the sulfur containing amino acids cysteine and methionine (5).

In the dissimilatory sulfate reduction step, inorganic sulfate acts as the terminal electron acceptor during anaerobic respiration. This process is conducted exclusively by certain bacteria, the principal ones belonging to the genera Desulfotomaculum and Desulfovibrio (5).

Sulfide oxidation is carried out by various strains of sulfur-oxidizing bacteria. It has been established that sulfide is oxidized to elemental sulfur and sometimes sulfate, but little is known about the chemistry of the sulfide to sulfur step. The majority of the sulfide oxidizing bacteria are sensitive to high concentrations of sulfide and select a very precise sulfide concentration for growth (5).

Sulfur oxidation is carried out exclusively by microorganisms, the principal ones belonging to the genus Thiobacillus. The total metabolism of Thiobacillus is based on the oxidation of sulfur or reduced sulfur compounds to sulfate.

The genus Thiobacillus has received considerable interest in the past resulting in comprehensive reviews on the general characteristics of these microorganisms (1,10,39,40). The members of this genus are gram negative, non-spore forming, aerobic rods, measuring 0.5 by 1-3 microns, which except for the nonmotile Thiobacillus novellus, are polarly flagellated (39). Most all members of the genus use reduced sulfur compounds such as thiosulfate as electron donors and carbon dioxide as a carbon source. The majority of the Thiobacilli are capable of strictly autotrophic growth by oxidizing elemental sulfur, thiosulfate,

tetrathionate, sulfide, and sulfite (1,5,10,39,40). Typical substrates consist of either elemental sulfur or thiosulfate plus mineral salts. One recently described organism, T. perometabolis, is incapable of strictly autotrophic growth although it derives energy from the oxidation of inorganic sulfur compounds (41,42).

Classification of the Thiobacilli into distinct species has been difficult since the organisms are separated according to their nutritional requirements (1). A more rational approach to the classification of the Thiobacilli based on multivariate analysis has recently been initiated (43,44). By this technique T. thiooxidans, T. ferrooxidans, T. thioparus, T. neapolitanus, and T. denitrificans have been identified as distinct species. This classification is supported to a certain extent by analyses of the DNA base composition of a number of Thiobacilli (1).

The most distinct characteristic of the Thiobacilli is their tolerance to extremely acidic conditions. T. thiooxidans is commonly reported as being the most acid resistant species. Optimum pH for this organism is around 2.0 (1,40,45) but it is capable of lowering the pH to negative values while still being able to survive (39,40). Evidence presented by Rao and Berger (45) has shown that T. thiooxidans ceases to grow above a pH of 5.0, but raising the pH as high as 7.0 does not cause death of the cells. Waksman and Starkey (46) were unable to initiate sulfur oxidation by T. thiooxidans in media containing ten percent sulfuric acid, but their cultures were capable of producing concentrations of sulfuric acid up to ten percent lowering

the pH to zero. This same phenomena had been observed by a previous group of investigators (47). Hence, in the course of development T. thiooxidans adapts itself to high concentrations of acid.

T. thioparus is one species of the Thiobacilli which cannot tolerate extreme acid conditions. The optimum pH for growth of this organism is reported as being near neutrality (1,39,40). T. thioparus is normally inactivated at a pH of 5.0 (40,48) but has been shown to survive in media where the pH was gradually lowered to 2.8 due to sulfur oxidation (40). It seems that under conditions in which the pH is gradually lowered by the growing bacteria, a certain adaptive tolerance to acidity is developed.

Various investigators have reported on the effects of different initial thiosulfate concentrations in growth media (49,50,51,52,53). Using T. thiooxidans, Starkey (49) showed vigorous growth in a three percent thiosulfate solution but observed completely inhibited growth in a ten percent solution.

Vishniac (50) tested different thiosulfate concentrations on T. thioparus. His concentrations ranged from 100 to 9800 mg/1. Up to a concentration of 500 mg/1 thiosulfate, complete oxidation was observed. At concentrations higher than 2000 mg/1, oxidation was less than complete, and sulfur precipitation was reported to be heavy. Each of the cultures was allowed to incubate for 48 hours.

Parker and Prisk (51) cultured eleven different organisms in order to investigate their capabilities of oxidizing reduced sulfur compounds. T. concretivorus and T. thiooxidans were selected for their study on

the effects of varying the initial thiosulfate concentrations. Both organisms were subjected to thiosulfate concentrations ranging from 1800 to 18,000 mg/l. Thiosulfate oxidation by T. concretivorus was not inhibited by 18,000 mg/l, although the time required for complete oxidation increased with an increase in thiosulfate. Thirty-five days were required for complete oxidation of 18,000 mg/l. Thiosulfate oxidation by T. thiooxidans was complete up to 4500 mg/l. At concentrations of 9000 and 18,000 mg/l only about one-tenth of the thiosulfate was oxidized after twenty-five days. Low viable counts indicated that the higher concentrations exerted an inhibitory effect.

Subjecting Thiobacillus X to thiosulfate concentrations up to 900 mg/l did not have adverse effects on this organism (52). At each concentration partially oxidized thiosulfate was accounted for as tetrathionate. Lineweaver-Burk plots of thiosulfate oxidation by T. ferrooxidans have shown maximum oxidation rates at a thiosulfate concentration of 11,200 mg/l (53).

Although most of the Thiobacilli are capable of strictly autotrophic growth, facultative forms are known (41,42). The utilization of organic compounds is in fact more widespread among the Thiobacilli than had been thought. Several of these organisms concentrate amino acids and glycerol from the external medium (1). The incorporation of organic compounds including amino acids, Krebs' cycle intermediates and fructose into the cell material of strictly autotrophic Thiobacilli, has also been demonstrated (1). It has been reported that T. thiooxidans may, under

some circumstances utilize the energy from the oxidation of glucose for growth (1).

Organic substances, added to a media containing elemental sulfur as the source of energy undergo certain changes which do not take place in media devoid of sulfur (54). According to Waksman and Joffe (47), organic substances such as glycerol, in low concentrations, have a slightly favorable effect on the growth of cultures in the presence of a good nitrogen source. However, sulfur oxidation is greatly suppressed by the addition of these substances in higher concentrations. Growth is effectively stopped in media containing ten percent glucose, but concentrations of glucose below five percent do not inhibit growth (55).

In Starkey's opinion (55), glucose in low concentrations might be utilized in the presence of sulfur as a source of energy, a source of carbon, or as a source of both at the same time. Waksman and Starkey (45,56) found that oxidation of sulfur was suppressed by 0.1 percent concentrations of peptone and completely inhibited by one percent concentrations. Dextrose was found to be harmless for T. thiooxidans. The addition of one percent citric acid had no effect, but five percent and higher concentrations were found to inhibit the process of sulfur oxidation (55).

Carboxylic acids such as succinate, fumarate, and malate were found to enhance the respiration rate of T. thiooxidans and T. thioparus (39,57). Growth of T. thioparus is increased 20 to 40 percent in the presence of these acids (39). These added acids have no effect in the absence of either carbon dioxide or thiosulfate (39).

Vishniac and Santer (39) have established the specific effect of phosphates in the oxidation of thiosulfate by T. thioparus. The experiments were conducted in a Warburg apparatus. From the amount of oxygen consumed, it was evident that thiosulfate and tetrathionate were incompletely oxidized by the bacteria, in the absence of phosphates. Upon addition of phosphorus, oxygen consumption reached the expected amounts. Chromatographs of T. thioparus cells, mixed together with labeled thiosulfate and phosphate, revealed compounds containing both labeled elements.

The direct participation of phosphates in the oxidation of thiosulfate was proved experimentally by Santer et al. (48). A suspension of T. thioparus cells completely oxidized thiosulfate in the presence of phosphate labeled in the oxygen atom. The labeled oxygen appeared in the final oxidation product, sulfate. Each sulfate had received its oxygen from phosphate. In the absence of phosphate, oxygen uptake reached only 70 percent of the theoretically possible amount, and by the end of the experiment, tetrathionate had accumulated in the media. BOD experiments by Kreye (25) have also demonstrated the importance of phosphorus in thiosulfate oxidation.

According to Starkey (55), T. thiooxidans tolerates high concentrations of phosphate in the media. Oxidation of sulfur continued at concentrations of three percent potassium or sodium phosphate, but was noticeably suppressed when the concentration of sodium phosphate reached seven percent.

Ammonia nitrogen is most commonly reported as being the best

available source of nitrogen for most of the Thiobacilli (39,40,49,55). T. thioparus has been reported to use nitrates and nitrites but nitrites are toxic in concentrations of 0.1 percent (40). T. thiooxidans does not utilize nitrates or organic nitrogen sources such as peptone, urea, or any of the amino acids and nitrites are toxic (55). Concentrations of $(NH_4)_2SO_4$ as low as 0.01 percent (21 mg/l N) have been shown to be sufficient for complete oxidation of thiosulfate.

Little is known about the requirements of the Thiobacilli for other elements of nutrition. Vishniac (39,50) has emphasized the necessity of adequate amounts of iron and magnesium to achieve good bacterial development of T. thioparus. Sokolova and Karavaiko (40) have reported that oxidation of sulfur is stimulated by several cations in low concentrations such as zinc, cesium and lead. Using a mixed culture of Thiobacilli in Warburg experiments, Kreye (25) demonstrated that copper, lead, zinc and chromium in concentrations up to one mg/l were not toxic to thiosulfate oxidation. Roy and Trudinger (1) have noted that ferrous, nickel or cobalt ions are required by T. thiooxidans for full activity of enzymes important to the metabolism of polythionates. Vishniac (39) has reported that enrichment of T. thioparus and T. thiooxidans for biochemical studies can be enhanced by the addition of trace quantities of zinc, molybdenum, copper, and cobalt. Nutrient requirements for many of the Thiobacilli have also been cited by Stephenson (58).

The rate of sulfur oxidation by T. thiooxidans has been shown to increase with the addition of wetting agents such as Tween 80 and Tergitol 08 (59). Jones and Starkey (60) noted that, during the growth

of T. thiooxidans, sulfur originally floating on the surface of the medium became wet and settled to the bottom of the culture flask. Surface tension measurements and other tests indicated that surface active agents were released by the bacteria during their most active phase of growth. A major component was later identified as phosphatidyl inositol (61) and was shown to wet sulfur and promote its oxidation by T. thiooxidans. Later research confirmed the excretion of phospholipids by T. thiooxidans (1).

Various investigators have reported that Thiobacilli readily attach themselves to solid surfaces while oxidizing various substrates (27,62, 63,64). A mixed culture of attached Thiobacilli on a rotating bio-disc has been used to treat a thiosulfate waste from a mining operation (27). The use of a laboratory scale activated sludge unit during this same investigation, failed to develop a dispersed floc due to the preference of the bacteria to attach themselves to the walls of the vessel. Pure cultures of T. ferrooxidans have demonstrated the ability to attach themselves to metal sheets (62) and sulfide ore particles (64). Electron micrographs of sulfur crystals partially oxidized by T. thiooxidans have provided conclusive evidence that this organism attaches itself during sulfur oxidation (63). Based on this evidence it is apparent that the availability of a large surface area should be beneficial in developing a wastewater treatment system culturing Thiobacilli organisms.

Oxidation Pathways of Thiosulfate

The oxidation of inorganic sulfur compounds has been extensively reviewed (1,4,39,40,65,66). These reviews have shown that all

Thiobacilli are capable of oxidizing thiosulfate; and sulfate, sulfur, and tetrathionate are the principal products of oxidation. Under ideal conditions, sulfate is the ultimate end product.

The exact mechanism of thiosulfate oxidation and the differences which exist between various species of the Thiobacilli is not understood at the present time. It has been pointed 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 (1,65,66).

The following equations represent the general view of the overall oxidation of thiosulfate by the Thiobacilli (67):

$$S_2O_3^{-2} + 2O_2 + H_2O \rightarrow 2SO_4^{-2} + 2H^+ \quad (12)$$

$$3S_2O_3^{-2} + 5/2\ O_2 \rightarrow S_4O_6^{-2} + SO_4^{-2} \quad (13)$$

$$S_2O_3^{-2} + \tfrac{1}{2}O_2 \rightarrow SO_4^{-2} + S \quad (14)$$

$$5S_2O_3 + 4O_2 + H_2O \rightarrow 6SO_4^{-2} + 4S + 2H^+ \quad (15)$$

The oxidation of thiosulfate by Thiobacilli is characterized by the formation of polythionates. The extent of polythionate accumulation, however, varies widely and depends on a number of experimental factors (1,51,52,68). These factors include the species of organism employed, the concentration of bacteria, the concentration of substrate, and the concentration of oxygen. Santer et al. (48) reported that polythionates accumulate only in the absence of inorganic phosphate. Suppression of polythionate formation from thiosulfate by high phosphate concentrations

was also found by Jones and Happhold (69). Nevertheless, phosphate containing media have been used by most investigators to demonstrate polythionate accumulation (1).

Tetrathionate is generally the main polythionate which accumulates during thiosulfate oxidation (1). Trithionate and pentathionate are also formed. They generally accumulate in relatively small amounts but, under some conditions, they may be the main polythionates found (68,69). Vishniac (50) assumed that both pentathionate and trithionate are produced by the chemical disproportionation of tetrathionate which is enhanced in the presence of thiosulfate:

$$S_4O_6^{-2} + S_2O_3^{-2} \rightarrow S_5O_6^{-2} + SO_3^{-2} \quad (16)$$

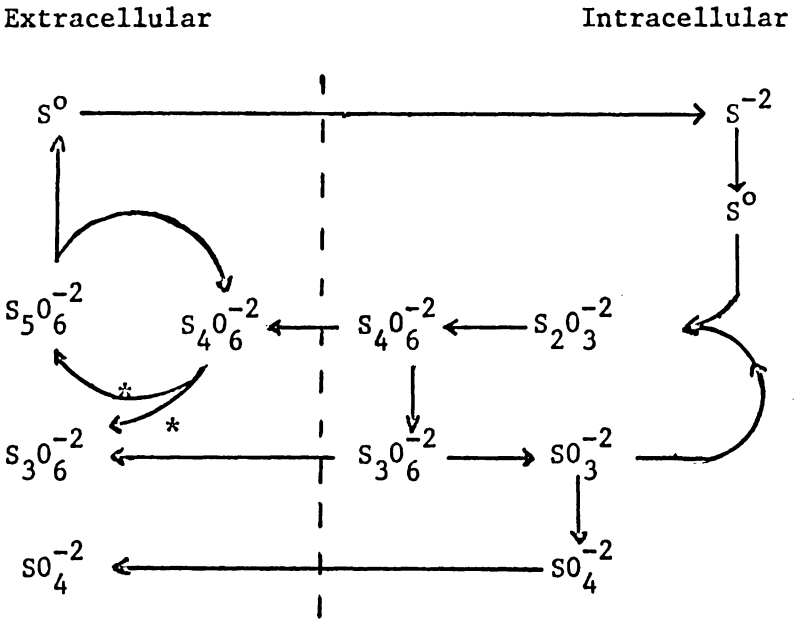
$$S_4O_6^{-2} + SO_3^{-2} \rightarrow S_3O_6^{-2} + S_2O_3^{-2} \quad (17)$$

Elemental sulfur is also a common product of thiosulfate metabolism by Thiobacilli. It accumulates primarily outside the bacterial cell (1). The formation of sulfur is governed by experimental conditions such as increases in substrate concentration, oxygen limitation, and pH (50,52). Vishniac (50) attributed sulfur production to the extracellular decomposition of pentathionate formed from tetrathionate:

$$S_5O_6^{-2} \rightarrow S_4O_6^{-2} + S^o \quad (18)$$

There is now stronger evidence which attributes sulfur formation to specific biological reactions (1).

A summary of the above discussion and a picture of the postulated path of sulfur oxidation proposed by Vishniac and Santer (39) is as follows:



*$S_2O_3^{-2}$ dependent

Biological Films

Continuous flows of a biodegradable substrate in an aqueous phase, over a solid surface leads to the development of a biological film at the solid-liquid interface. Bacterial cells initially become adsorbed as charged particles forming a monolayer. After multiplying over several generations, a biofilm of closely packed bacteria is formed. Over the period of time that the film is developing, the bacteria begin to excrete extracellular polymers which strengthen the attachment between the cells themselves and between the initial layers of cells and the

solid surface. Substrate removal from the aqueous phase occurs through diffusion of all metabolic reactants into the film, metabolism by the organisms, and diffusion of the metabolic end products back through the biofilm into the aqueous phase.

A conceptual illustration of the relationship between substrate concentration and depth within a biofilm has been previously described (70) and is shown in Figure 1. It is assumed that the biofilm is attached to a flat plate and that the rate of reaction is limited by a single substrate S. The concentration of the substrate outside the biofilm in the bulk liquid is S_o; at the biofilm surface, S_s; within the biofilm cellular matrix, S_c; and deep within the biofilm a constant limiting value, S_i. The gradient of S_c at the liquid-film interface where z = o (dS_c/dz) is intermediate between low values which result in a metabolism-limited case and high values which result in a diffusion-limited case.

The incomplete mixing of the liquid phase next to the biofilm surface, coupled with mass transfer into the biofilm results in a stagnant liquid layer and a decrease in substrate concentration between the bulk liquid and the biofilm surface. Williamson and McCarty (71) found that the stagnant liquid layer over an experimental biofilm consisted of two layers termed L_1 and L_2. The outer layer, L_1, can be reduced to zero thickness with adequate mixing. The layer next to the biofilm, L_2, cannot be removed by mixing and is believed to result from the uneven or sponge-like nature of the liquid-biofilm interface. Based on a linear relationship describing the mass transfer of NH_4^+ through nonreactive nitrifying

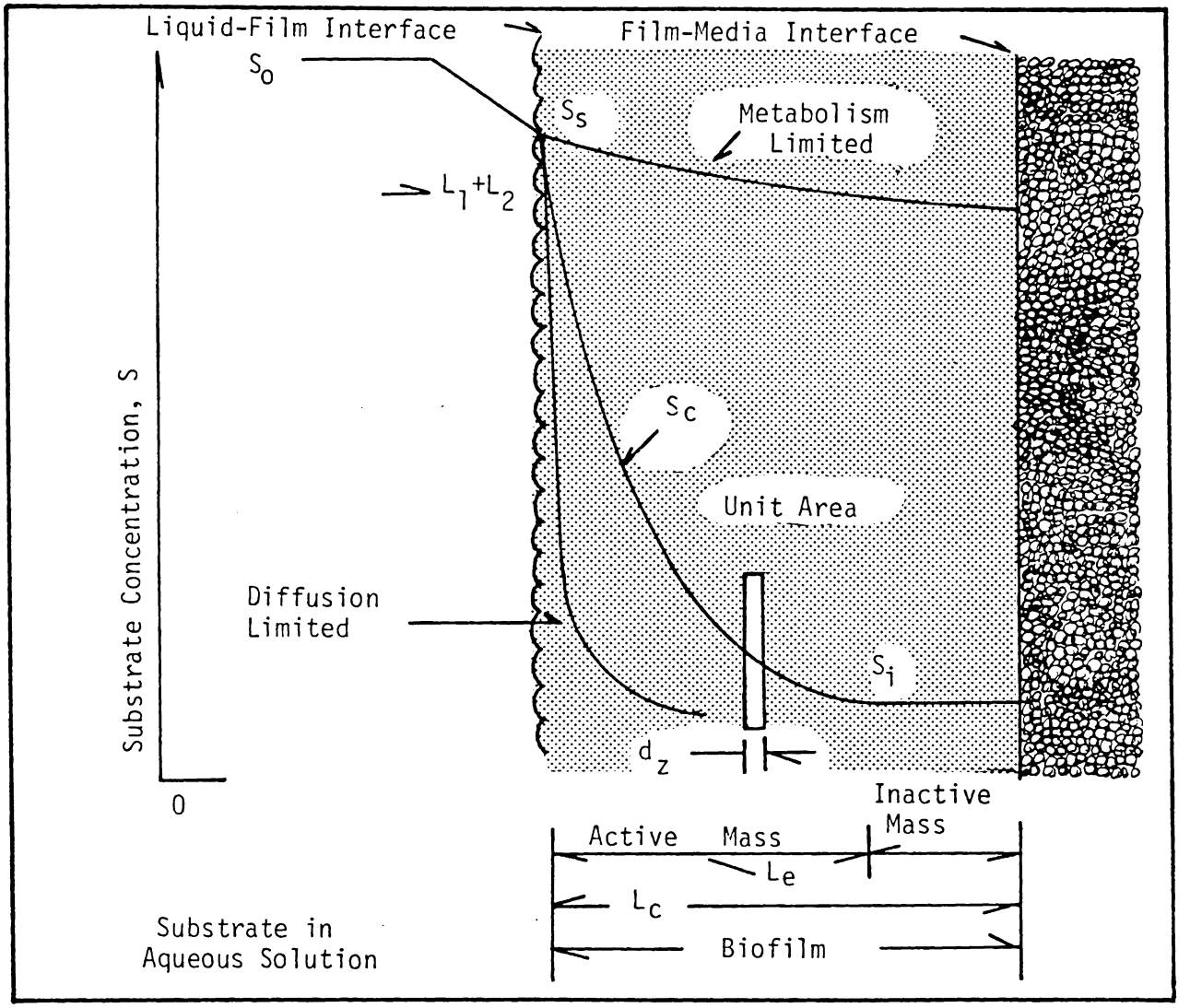


Figure 1. Conceptual Substrate Profile Within a Biofilm. (After Williamson and McCarty (70))

films of thickness, L_c, the depth of L_2 was calculated as 56 microns (71). Whether such a layer exists in all biofilms is currently unknown.

If a bacterial film is not metabolism limited, then the substrate concentration within the depths of the biofilm will reach a minimum value of S_i, at which point bacterial metabolism stops. The depth at which the concentration reaches S_i is termed L_e, the effective depth, and will contain those organisms actively metabolizing the substrate. This situation occurs only in relatively deep biofilms. A deep biofilm is one in which the biofilm depth L_c exceeds L_e. L_e is dependent upon S_o, the substrate concentration outside the biofilm in the bulk liquid (70, 71). If a biofilm depth is restricted by either hydraulic shear or sloughing of the bacterial mass, then metabolism of the substrate may occur throughout the entire layer resulting in a metabolism-limited case.

The nature of biofilms dictates high bacterial densities. The concentration can vary considerably depending on the nature of the bacterial growth, the biofilm depth, the biofilm age, and the presence of other particles in the biofilm matrix. Williamson and McCarty (71) reported bacterial densities of 42 to 109 milligrams total suspended solids per cubic centimeter (mg TSS/cm^3) for biofilm depths ranging from 150 to 550 microns. Owen and Williamson (79) reported bacterial densities of 20 to 60 mg TSS/cm^3 for biofilm depths of 300 to 500 microns. Such concentrations are much larger than what is normally obtained for bacterial flocs in dispersed growth systems in which concentrations up to 10 mg TSS/cm^3 have been reported (70).

Sanitary engineers have taken advantage of biofilm development since 1893 when the first trickling filter was placed in operation in England (72). Until recently, trickling filters were the only sanitary engineering applications of biofilms. The latest developments in biofilm applications include: the rotating bio-disc (27), the anaerobic filter for methane fermentation (73) and denitrification (74), the submerged filter for nitrification (75), the fixed activated sludge process (76), and the biological fluidized bed for denitrification (77).

The recent interest in biofilm applications has prompted several individuals to investigate the kinetics of substrate utilization in biofilms (70,71,78,79). The models proposed to date are based on Fick's Law of diffusion (70,71,78,79) and Monod's relationship for substrate utilization (70,71,79).

Williamson and McCarty (71) have verified their proposed model through nitrification studies but have also extended their implications to include carbonaceous oxidation, denitrification, and anaerobic fermentation (70). To apply their model it must first be determined whether the electron donor or the electron acceptor approaches some near-zero value within the biofilm, an effect which is termed flux limitation. A second condition that must be satisfied is that the flux-limiting species must also be substrate limiting throughout the biofilm. Their biofilm model can be applied to describe the utilization rate of any substrate if the above conditions are met.

Owen and Williamson (79) have extended the model proposed by Williamson and McCarty (70,71) to include those cases where both the

electron donor and electron acceptor simultaneously limit the kinetics of substrate utilization by the biofilm. Their results indicated that heterotrophic biofilm reaction kinetics can be oxygen limited at typical oxygen and organic concentrations found in wastewater treatment reactors.

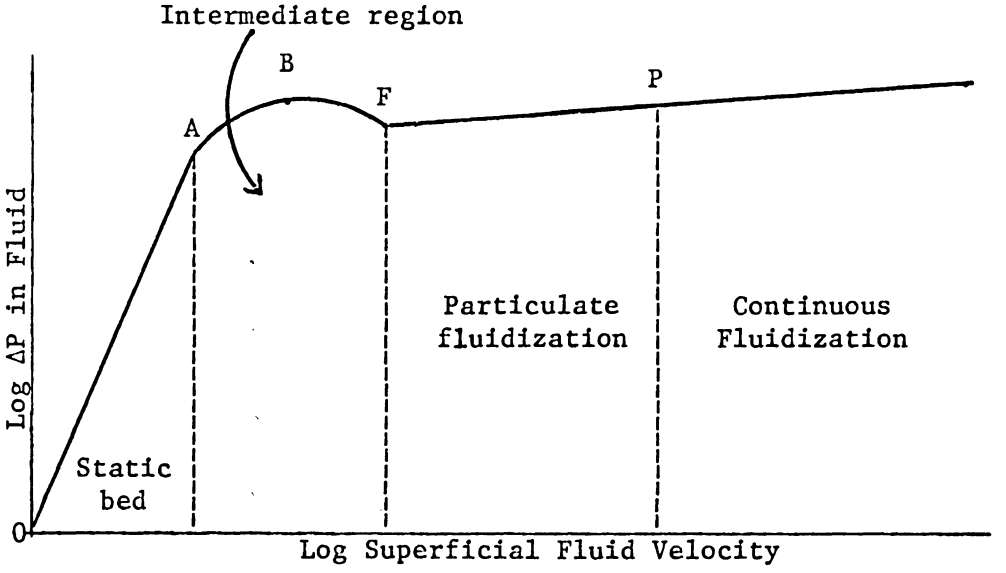
The model proposed by Harremoes (78) was applied to interpret filter denitrification. He has hypothesized that denitrification filter kinetics may be divided into three categories: zero-order, half-order, and first-order. The applicability of the half-order reaction was demonstrated by comparing concentration profiles for various filter denitrification studies.

Fluidized Beds

Transformation of fine solids into a fluidlike state through contact with a liquid is termed fluidization (80). A liquid flowing at low velocities through a porous bed of solid particles does not cause the particles to move. The fluid passes through the small, tortuous channels losing pressure. The pressure drop in a stationary packed bed is large and depends upon, among other things, the porosity of the bed and the superficial velocity of the fluid (81). At higher fluid velocities the particles no longer remain stationary but fluidize under the action of the liquid.

The most convenient way of describing the mechanism of fluidization is to visualize a short, vertical tube partially filled with a uniform size granular material such as sand. As the liquid is flowing at a very low rate through the bottom of the tube it travels upward through the sand without causing the grains to move. As the flow rate

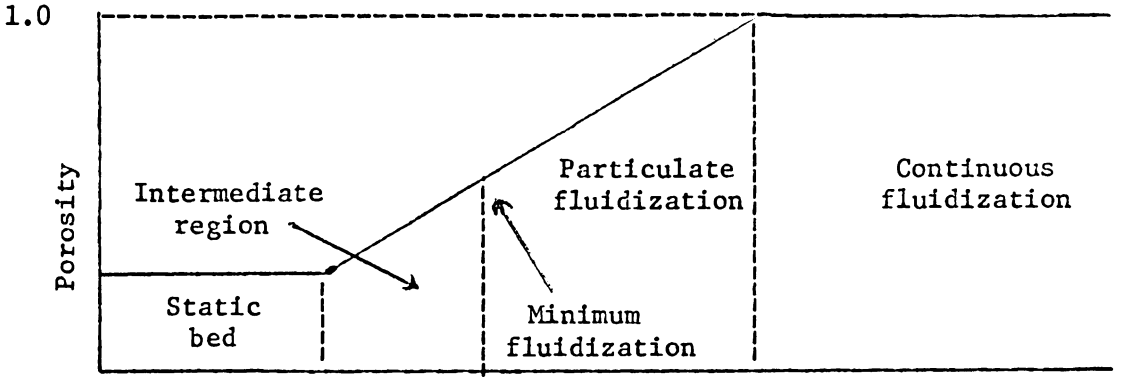
is slowly and progressively increased, the pressure drop in the liquid passing through the bed increases, as is shown by the linear segment OA in the graph below (81).



Eventually the pressure drop equals the force of gravity on the particles, and the grains begin to move. This result is at point A on the graph. Fluidization begins as a gentle rocking or oscillation of the sand particles and the bed expands slightly with the grains still in contact. The porosity increases and the pressure drop rises more slowly than before. When point B is reached, the bed is in the loosest possible condition with the grains still in contact. As the velocity is further increased, the grains separate and true fluidization begins. The pressure drop sometimes decreases slightly from point B to point F (81). In a fully fluidized bed the sand grains move in random directions through all parts of the liquid.

Since the linear velocity of the fluid between the grains is much higher than the velocity in the space above the bed, all of the sand grains drop out of the fluid above the bed. If the fluid velocity is still further increased, the porosity of the bed rises, the bed of sand expands, and its density falls. Entrainment of sand grains becomes appreciable, then severe, then complete. At point P all the grains have been entrained in the fluid, the porosity approaches unity and the bed as such has ceased to exist. From point F to point P and beyond, the pressure drop rises with the fluid velocity but much more slowly than when the bed was stationary. Fluidization without entrainment of the solids is called batch fluidization (81) or particulate fluidization (80). When entrainment is complete, the fluidization is said to be continuous (81).

As noted above, when a bed of fluidized solids expands, the porosity rises, approaching unity when entrainment becomes complete. From the beginning of fluidization to the point of complete entrainment the porosity rises linearly with the logarithm of the superficial fluid velocity as shown below (81). The velocity at the beginning of fluidization is known as the critical velocity (80,81). The velocity at which the porosity becomes unity has been shown to agree well with the terminal settling velocity of the particles (81).



The first large scale, commercially significant use of fluidized beds was by Fritz Winkler for the gasification of powdered coal to produce methane in 1922 (80). Since then, fluidized beds have been used extensively in many catalytic processes such as catalytic-cracking reactors in the petroleum industry (80,81). The metallurgical industries have also applied fluidized bed technology for the preparation of a wide variety of solid materials such as the roasting of sulfide ores (80). Other applications include: transportation, mixing, sizing, and drying of solids; heat exchangers, coating of plastic materials on metal surfaces, adsorption, and activation of charcoal (80).

Sanitary engineering applications of fluidized bed technology to date have not been extensive. The limited applications include: sand filter backwash in water treatment (82), wastewater treatment by activated carbon (72), combustion of sludge solids (72), and denitrification (77).

Periodic backwashing of water treatment plant sand filters is required due to the collection of suspended matter which results in

significant head losses. Once the head loss has reached eight to ten feet, the filter is stopped and the flow is reversed, fluidizing the bed, thus releasing the trapped particles (82).

Activated-carbon treatment of wastewater is usually considered a polishing process for water that has already been treated by normal biological treatment processes. The carbon is used to remove a portion of the dissolved organic matter that remains. Depending on the means of contacting the carbon with water, the particulate matter that is present may also be removed.

In a fixed bed, down-flow column, a provision for backwash and surface wash is necessary because the particulate matter in the influent averages 10 mg/l and is almost completely removed through filtration (72). Backwashing is necessary to keep excessive head loss from building up.

The problem of clogging of the carbon column can be partially overcome if a fluidized bed is used (72). In operation, the influent is introduced at the bottom of the column and the bed is allowed to expand. Spent carbon is displaced continuously with fresh carbon. In such a system head loss does not build up with time after the operating point has been reached.

The Door-Oliver Fluo Solids system utilizes a fluidized bed of sand as a heat reservoir to promote uniform combustion of sludge solids (72). The sludge is dewatered before it enters the fluidized bed reactor. The fluidized bed must be preheated to approximately 1200°F before the sludge is introduced. Inside the reactor, the sludge

is dried and oxidized at a temperature of 1500°F. The resultant combustion gases, ash, and water vapor exit through a wet scrubber, where the ash is removed, and are then exhausted through a stack. The ash is separated from the scrubber water in a cyclone separator. The scrubber water is discharged with the plant effluent.

The most recent application of fluidized bed technology to wastewater treatment is in the area of denitrification. Jeris and Owens (77) have demonstrated biological denitrification of domestic sewage using a pilot scale fluidized bed. The nitrified sewage was supplemented with methanol before entering the bed. The treatment process consistently demonstrated the ability to remove 99 percent of the influent oxidized nitrogen.

The fluidized bed reactor was constructed of ¼ inch thick plexiglass, 1.5 feet in diameter, standing 15.5 feet high. Approximately 600 pounds of white silica sand with an effective size of 0.6 millimeters and a uniformity coefficient of 1.5 was used as the fluidizing media and the nucleus for biological growth.

Under normal operating procedures the hydraulic loading rate was set at 15 gallons per minute per square foot (gpm/ft^2). Although the empty bed detention time at this loading rate was 6.5 minutes, a bed height corresponding to a 3 minutes detention time was shown to achieve 95 percent removal of the influent oxidized nitrogen. The influent nitrate-nitrogen concentrations varied considerably but the effluent concentration was consistently less than 0.5 mg/l. Studies on the effects of various methanol to nitrate-nitrogen ratios indicated that a ratio of

2.9 would provide a removal efficienty of 95 percent or better.

Increasing the loading rate from 15 to 24 gpm/ft^2 reduced the empty bed detention time to about four minutes and indicated no detrimental effect on the effluent quality. Diurnal variation in flow also produced no change in the effluent quality. To determine the effect of power failure on the system, the reactor was shut down for a period of 17 hours. Within 45 minutes after start-up, the system was again achieving 99 percent removal.

During a high nitrogen concentration study, the influent oxidized nitrogen averaged 55 mg/l and the effluent 2.5 mg/l for an overall removal of 95 percent. The influent nitrate-nitrogen concentration of 55 mg/l was equivalent to 850 pounds of nitrate-nitrogen per 1,000 cubic feet of reactor per day (#N/1000 cf/day). The maximum removal capability of the reactor is better described by the loading rates identified at the bottom 38 inches of the reactor where 95 percent removal of the influent nitrate was shown to occur. The loading rate at this level was 1275 #N/1000 cf/day which is equivalent to an influent concentration of 84 mg/l.

The rate of denitrification could also have been expressed in terms of available surface area. By assuming the sand grains to be perfect spheres, the available surface area was approximately 11,000 square feet. Based on the overall results of the high nitrogen study, the rate of denitrification was approximately 27 mg NO_3^--N/ft^2/hr or 120 mg NO_3^-/ft^2/hr.

The remarkable efficiency of this system was attributed to the

concentration of active biomass within the reactor. The small media in the column provided the denitrifying bacteria with an almost unlimited surface area upon which to grow. The average concentration of bacteria, expressed as total volatile solids, in the reactor was between 30,000 and 40,000 mg/l.

MATERIALS AND METHODS

The information contained within this section is a discussion of the experimental materials, equipment, and procedures used during the course of this investigation.

Analytical Procedures

Analytical procedures for the various sulfur species have been well documented (3,5,6). Considering the objectives of this investigation, the principal sulfur compounds monitored were thiosulfate, polythionate, and sulfate. Other parameters which were monitored included volatile solids and oxygen uptake. Standard laboratory glassware and reagent grade chemicals were used at all times.

Thiosulfate. Concentrations of thiosulfate were determined by the method of Rao and Sarma (83). This procedure involves the direct titration of thiosulfate using a standard potassium dichromate solution. The procedures for this method are outlined below.

1. Pipette a 10 ml sample into a 250 ml erlenmeyer flask
2. Add 70 ml of distilled or deionized water
3. Add 10 ml of a 20 percent potassium iodide solution
4. Add 5 ml of 1N oxalic acid
5. Add 2 ml of one percent starch indicator
6. Add 2 ml of 5N sulfuric acid
7. Titrate with standard potassium dichromate to the first signs of a permanent, blue color.

The concentration of thiosulfate is calculated as follows:

$$S_2O_3^{-2}, \text{ mg/l} = \frac{112{,}128\ N_D V_D}{V_s} \qquad (19)$$

where:

N_D = normality of potassium dichromate

V_D = volume of potassium dichromate, ml

V_s = volume of sample, ml

Titrations were performed using a 10 ml burette. This procedure provided conditions for extreme precision and excellent reproducibility at all levels of thiosulfate. The normality of the potassium dichromate was set so that slightly less than 10 ml were required for the highest concentration of thiosulfate being titrated. Detection of the final endpoint was improved by placing a light bulb behind the titration flask and a sheet of white paper between the bulb and the flask.

Sulfite is an interference in this procedure, but it can be eliminated by adding 0.25 ml of a 37 percent formaldehyde solution. Sulfite concentrations can be determined using the above procedure by adding the formaldehyde before the distilled water and taking the difference between titrations, with and without formaldehyde.

Rao and Sarma (83) have failed to point out that a blank correction is necessary due to the impurities of the potassium iodide solution (6). The blank correction should be evaluated daily and must be subtracted for each thiosulfate determination.

Polythionate. At the present time there are no satisfactory quantitative methods for the determination of individual polythionates

in the presence of each other (3). The available quantitative tests measure total polythionate. Qualitative tests for di-, tri-, tetra-, and pentathionate have been described in detail by Karchmer (3). Throughout this investigation no distinction was made between the individual polythionate species. Polythionate concentrations were reported as total polythionate in terms of thiosulfate.

The procedure selected for measuring polythionate concentrations was obtained from a Canadian research team presently conducting investigations on the treatment of thiosulfate wastes (84). Thiosulfate is a direct interference, but can be accounted for if the thiosulfate concentration is known. The procedure is outlined below.

1. Pipette an aliquot of sample into a 100 ml beaker. The aliquot size will be dependent upon the concentration of polythionate plus thiosulfate:
   a. 0-50 mg/l as $S_2O_3^{-2}$, use a 50 ml aliquot
   b. 50-400 mg/l as $S_2O_3^{-2}$, use a 25 ml aliquot
   c. 400-2000 mg/l as $S_2O_3^{-2}$, use a 5 ml aliquot
   d. 2000-5000 mg/l as $S_2O_3^{-2}$, use a 2 ml aliquot
2. Dilute the sample to approximately 25 ml if necessary.
3. Adjust the pH to 4.3 using 0.005N sulfuric acid or 0.005N sodium hydroxide. The normalities of the acid and the caustic solution can be varied in order to prevent excessive dilution. The sample should not be diluted to more than 60 ml. The pH adjustment should be carried out using a pH meter supplemented by magnetic stirring. Varying the rate of stirring can cause

errors; therefore, the magnetic stirrer should be set at the same position for all titrations. A good rate of mixing is furnished just before the sample begins to splash out of the beaker. The pH electrodes and stirring magnet should always be rinsed into the beaker using distilled or deionized water.

4. Add 5 ml of 20 percent mercuric chloride, mix and allow to stand for five minutes.
5. Dilute the sample to 60 ml with distilled or deionized water.
6. Heat rapidly just to boiling.
7. Cool to room temperature.
8. Place the sample on the magnetic stirrer and add 20 percent potassium iodide, drop by drop, until the resulting red precipitate just disappears.
9. Titrate with 0.005N sodium hydroxide to pH 4.3. The sodium hydroxide solution should be prepared weekly and standardized daily. Precautions should be taken to keep the solution stable (6).
10. A blank, using distilled or deionized water, should be run through steps one through eight listed above. The final titration to pH 4.3 is performed using 0.005N sulfuric acid.

The concentration of polythionate is calculated as follows:

$$S_xO_6^{-2}(\text{as } S_2O_3^{-2}),\ \text{mg/l} = \frac{(T_1 + T_2)N}{A} \times 56064 - S_2O_3^{-2} \qquad (20)$$

where:

T_1 = volume of sodium hydroxide from step nine, ml

T_2 = equivalent volume of sodium hydroxide from step ten, ml

N = normality of sodium hydroxide

A = volume of initial sample, ml

The titrations were performed using a 10 ml burette to insure high precision. The normality of the sodium hydroxide solution used in step nine was set so that slightly less than 10 ml were required for the highest concentration of polythionate being titrated.

Potassium iodide is used in the above procedure to eliminate excess mercuric chloride. A blank determination is necessary in order to measure the alkalinity which develops following the addition of potassium iodide.

The resulting mercury solutions were stored in plastic milk containers for ultimate disposal by a private firm contracted by VPI & SU.

Sulfate. Concentrations of sulfate can be determined using various methods (3,5,6). Regardless of the method selected, thiosulfate is an interference (3).

During the preliminary preparation for the investigation, various procedures were evaluated for the assay of sulfate. Interference due to thiosulfate proved difficult to overcome. The method finally selected was provided by a research team in Canada presently involved in the evaluation of various processes for the treatment of thiosulfate wastes (85). The procedure is outlined below. Sulfate concentrations were reported as thiosulfate.

1. Pipette a 25 ml sample into a 100 ml beaker.
2. While stirring, add 1.0N iodine, dropwise, until a pale yellow

color is obtained. An excess of iodine will make it difficult to obtain a good endpoint in the final titration. The normality of the iodine may be reduced or increased depending on the range of thiosulfate present in the samples. It is best to predetermine the thiosulfate concentrations and have a range of iodine solutions of various normalities available so that not more than four to five drops are added.

3. Add one to two grams of strong acid, cation type, ion exchange resin and stir for five minutes. Allow the ion exchange resin to settle.
4. Pipette a 20 ml aliquot into a 150 ml beaker.
5. Add 80 ml of methanol to the 150 ml beaker.
6. Adjust the pH to 3.5 using a pH meter with five percent ammonium hydroxide or five percent hydrochloric acid. Remove the electrodes and wash with distilled or deionized water.
7. Add three drops of 0.2 percent thorin indicator.
8. Titrate with 0.01M barium chloride to the first permanent pink color. The molarity of the titrant can be varied depending on the range of sulfate concentrations being evaluated. The barium chloride solution should be prepared daily from a stock 0.1M solution which has been standardized against 0.1M sodium sulfate following steps four through eight.

The concentration of sulfate is calculated as follows:

$$SO_4^{-2} \text{ (as } S_2O_3^{02}\text{), mg/l} = \frac{96{,}056\ V_B M_B}{1.71 V_A} \qquad (21)$$

where:

V_B = volume of barium chloride titrant, ml

M_B = molarity of barium chloride

V_A = volume of aliquot used for analysis in step four, ml.

Titrations were carried out using a 10 ml burette to insure high precision. Detection of the final endpoint was improved using the same technique as described for thiosulfate determinations.

Thiosulfate and various cations are definite interferences in the above procedure. Sulfite is also a possible interference. Thiosulfate is eliminated by the addition of iodine. Various cations can cause co-precipitation, but this effect is overcome by the addition of the cation exchange resin. If sulfite is present, formaldehyde can be added as a complexing agent. The formaldehyde should be added prior to the addition of iodine. Using 0.25 ml of a 37 percent formaldehyde solution is adequate.

Volatile solids. The procedures for measuring volatile solids were essentially those outlined by Standard Methods (6). The only deviations from these procedures occurred when measuring volatile solids in the fluidized bed reactor. Solids determinations were made by removing several grams of sand from the fluidized bed and placing them into a pre-weighed Gooch crucible which contained a fiber glass filter. When necessary, the remaining liquid was removed by applying a vacuum. The crucibles were then dried for one hour at 103°C, desiccated and weighed, then volatilized at 550°C for 15 minutes. The results were reported as milligrams of volatile solids per gram of sand (mg VS/gm sand). The

remaining sand plus residue was used to calculate the volatile solids concentrations as noted below:

$$\frac{\text{mg } V_S}{\text{gm Sand}} = 1000 \frac{(W_2 - W_3)}{W_3 - W_1} \tag{22}$$

where:

W_1 = tare weight of crucible and filter, gm

W_2 = weight of crucible, filter, and sand sample after drying at 103°C for one hour, gm

W_3 = weight of crucible, filter, and sand sample after volatilization at 550°C for 15 minutes, gm.

To insure nonvolatilization of the sand, triplicate samples of clean sand were analyzed by the above procedures. On the average, a loss of 2.6 milligrams per gram of sand was observed. It was presumed that this slight loss of sand and the inclusion of the residue in the solids analysis would average out these effects.

Oxygen uptake. Oxygen uptake measurements were made using a Yellow Springs Instrument dissolved oxygen meter and a dissolved oxygen probe equipped for stirring. A 70 ml glass bottle fitted with a rubber stopper was used as the containing vessel.

To make the measurements, approximately equal amounts of sand were removed from three levels in the fluidized bed and placed into the glass bottle. The liquid was decanted and the bottle was filled with the influent feed. The dissolved oxygen probe was placed in the bottle and dissolved oxygen concentrations were recorded with time. The rate of oxygen uptake was determined by plotting dissolved oxygen

concentrations against time and measuring the slope of the resulting straight line. Following the measurement of oxygen uptake, the total contents of the glass bottle were analyzed for volatile solids. The final results were expressed as milligrams per liter oxygen per hour per milligram of volatile solids (mg/l O_2/hr·mg/V_S).

Isolation and Enrichment of a Thiobacillus Culture

Thiobacillus bacteria have been isolated from various sources (1,39), one of which includes domestic sewage. The culture used throughout this investigation was originally isolated from primary clarified sewage. Approximately 100 ml of sewage were inoculated into 18 liters of the enrichment broth shown in Table 1 which was aerated using compressed air. The initial pH of the media was 6.5. Calcium and iron salts were not included in the enrichment broth because of their presence in the tap water. Analysis of the tap water showed the concentrations of calcium and iron to be 36 and 0.05 mg/l, respectively.

After ten days, the pH became stable around 2.5 and the thiosulfate concentration was zero. Aeration was stopped, and the culture was allowed to settle for two hours. The supernatant was decanted and the carboy was again filled with the enrichment broth. The procedure was repeated whenever the pH became stable between 2.0 and 2.5. Gram stains and frequent microscopic examinations revealed short, Gram negative, motile rods.

Nutrient Requirements of the Isolated Thiobacillus Culture

The success of any biological wastewater treatment system is highly

Table 1

Thiobacillus Isolation and Enrichment Broth

Nutrient	Quantity, gm
$Na_2S_2O_3$	25.4
KH_2PO_4	2.4
$(NH_4)_2SO_4$	2.5
$MnSO_4 \cdot H_2O$	0.4
$MgSO_4 \cdot 7H_2O$	1.8
Tap water	18[a]

[a] liters

dependent on the availability of the proper nutrients. Of the many nutrients required by a biological system, nitrogen and phosphorus are particularly important and are most commonly found to be deficient. It has already been established that of these two nutrients, phosphorus could play a significant role in the success of a wastewater treatment system utilizing a culture of Thiobacilli. For these reasons it was considered essential that the nitrogen and phosphorus requirements of the isolated Thiobacillus culture be investigated.

Nitrogen requirements. Since a review of the literature has shown that ammonia-nitrogen is the most readily available source of nitrogen to the majority of the Thiobacilli, ammonium sulfate($(NH_4)_2SO_4$) was selected as the source of nitrogen. The experiment was conducted in a series of batch tests by varying the amount of the nutrient. The concentrations evaluated were: 5, 10, 15, 20, 25, 30, and 35 mg/l nitrogen. Phosphorus was held constant at a concentration of 30 mg/l and the remaining nutrients were added from stock solutions at the concentrations shown in Table 2. A two ml inoculum from the Thiobacillus culture was used as a seed for each test.

The cultures were contained in one liter bottles which were aerated using compressed air and were incubated at a temperature of 20°C. Samples were collected approximately every 24 hours. The pH was checked and thiosulfate and sulfate concentrations were determined.

Phosphorus requirements. The phosphorus requirements of the isolated Thiobacillus culture were evaluated using batch tests and a Warburg respirometer. Potassium phosphate (KH_2PO_4) was used as the source of

Table 2

Nitrogen and Phosphorus Batch Test Nutrients

Nutrient	Concentration mg/l
$S_2O_3^{-2}$	1000
$MnSO_4 \cdot H_2O$	20
$MgSO_4 \cdot 7H_2O$	100
Tap water	1[a]

[a] liter

phosphorus for each experiment.

The batch tests were carried out by varying the amount of phosphorus. Concentrations of 1, 10, 100, and 1000 mg/l phosphorus were evaluated. Nitrogen was held constant at a concentration of 30 mg/l and the remaining nutrients were added from stock solutions at the concentrations shown in Table 2. A two ml inoculum from the Thiobacillus culture was used as a seed for each test.

The cultures were contained in one liter bottles which were aerated using compressed air and were incubated at a temperature of 20°C. Samples were collected approximately every 24 hours. The pH was checked and thiosulfate, polythionate, and sulfate concentrations were determined.

The oxygen uptake experiment with the Warburg respirometer was conducted at a temperature of 20°C using the procedures outlined by Umbriet et al. (86). Large, 125 ml flasks were used and each unit was calibrated by the water method described by McKinney (87). Carbon dioxide was adsorbed by placing 0.5 ml of 20 percent KOH in each center well.

The phosphorus concentrations evaluated were 1, 10, 50, 100, and 1000 mg/l. Nitrogen was held constant at a concentration of 30 mg/l and the remaining nutrients were added at the concentrations shown in Table 2. Each flask contained a 20 ml sample and was seeded with 0.2 ml of the isolated Thiobacillus culture. The final volume of each flask was 20.7 ml. The results were reported as microliters of oxygen uptake.

Characterization of the Fluidized Bed Reactor

The fluidized bed reactor, as shown in Figure 2, was constructed of plexiglass tubing which had a column height of 37 inches and an inside

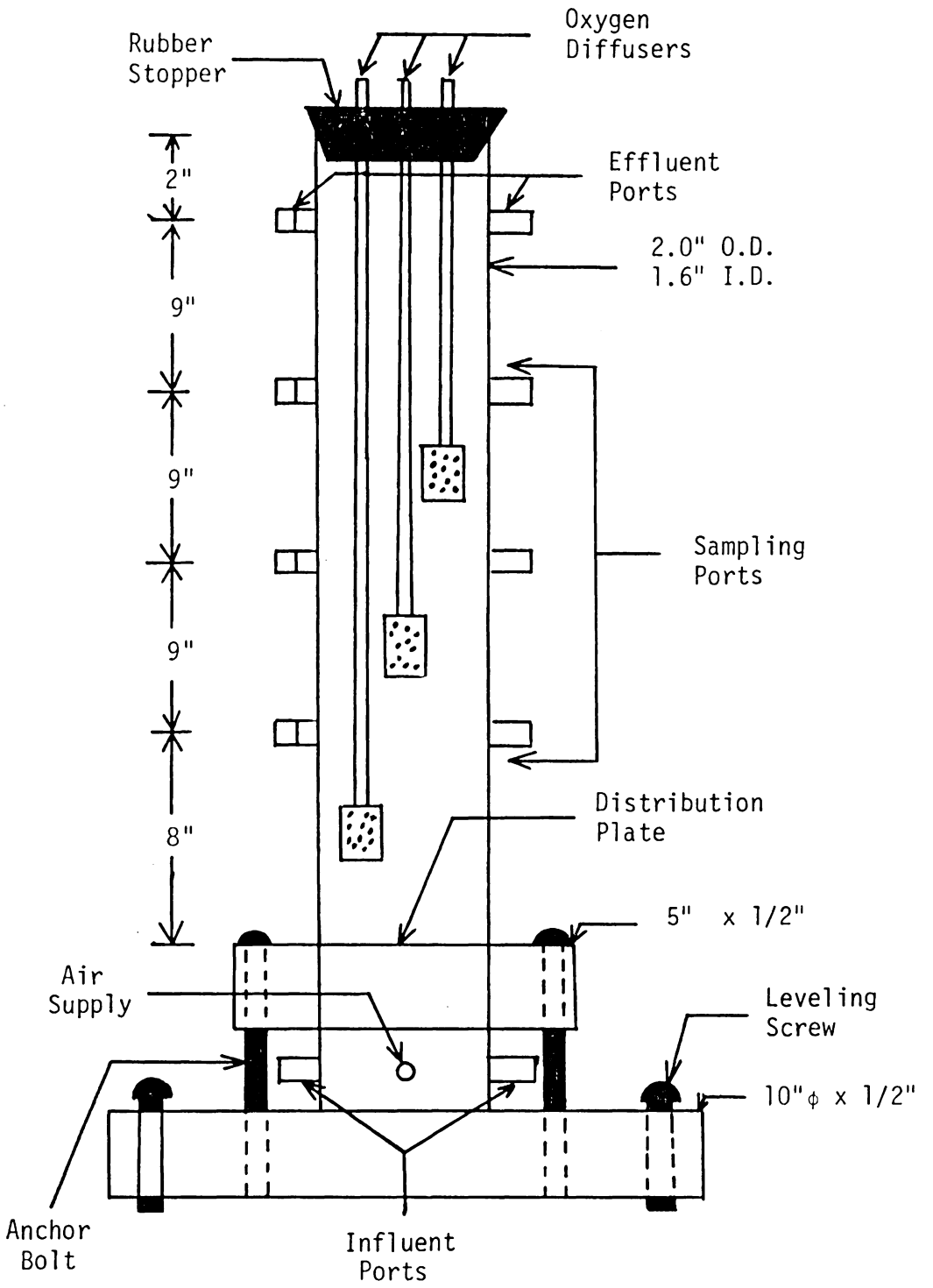


Figure 2. Fluidized Bed Reactor

diameter of 1.6 inches. The first inch above the distributor plate was filled with angular gravel followed by 1200 grams of 30 mesh Ottawa sand. The unexpanded sand occupied 22 inches of bed height. The column section was attached to a circular base plate by four anchor bolts. The reactor as a whole was maintained in a vertical position by four leveling screws. Three sampling ports were placed on each side of the column section. The sampling ports on the left were covered with plastic mesh screen to prevent the loss of sand while collecting samples and the three ports on the right were left unscreened in order to collect samples of sand. Each port was capped with a rubber stopper. An aeration system consisting of three porous stone diffusers was subsequently added and will be further described in the next section.

Before the fluidized bed could be placed into operation it was necessary to establish the hydraulic loading rates which would cause fluidization. This was accomplished by feeding tap water at 20°C through the influent ports. The hydraulic loading rates applied were 5, 7.5, 10, 15, 20, 25, 30, and 35 gpm/ft^2. The bed height at each loading was recorded and the corresponding porosity was determined by the following relationship:

$$\text{porosity} = \frac{V-V_s}{V} \tag{23}$$

where:

V = total volume at the corresponding bed height, ft^3

V_s = volume of sand, ft^3

The volume of the sand was determined by dividing the total weight of sand by a specific gravity of 2.65.

Since hydraulic loading rate, expressed as gpm/ft^2, is equivalent to velocity, a semilog plot of porosity and hydraulic loading rate was made to establish the range of fluidization.

Thiosulfate Oxidation in a Fluidized Bed

Following the characterization of the fluidized bed, the reactor was placed in a batch mode in an effort to seed the system. The reactor was initially seeded by filling the column section with 800 ml of the isolated Thiobacillus culture. After 24 hours had elapsed, the solution was drained from the influent ports. This procedure was repeated on a daily basis for a period of one week. During this time, compressed air was introduced at the bottom of the reactor in order to maintain an aerobic environment.

After the initial seeding, the reactor was placed into continuous operation using the feeding system illustrated in Figure 3. Due to the extremely high volume of water required to maintain continuous fluidization, it was necessary to design a system that would deliver a concentrated synthetic waste to the influent ports of the reactor at the required dilution. This procedure was accomplished by using a constant head system comprised of two-15 gallon plastic containers continuously flowing with oxygen saturated tap water at 20°C. Saturation was achieved by aerating the constant head tanks with compressed air. The concentrated waste was fed into one of the tap water lines by a Sigma Motor finger pump at a rate of 46 liters per day. The concentrated feed shown in Table 3 was prepared daily and was kept in a 15 gallon

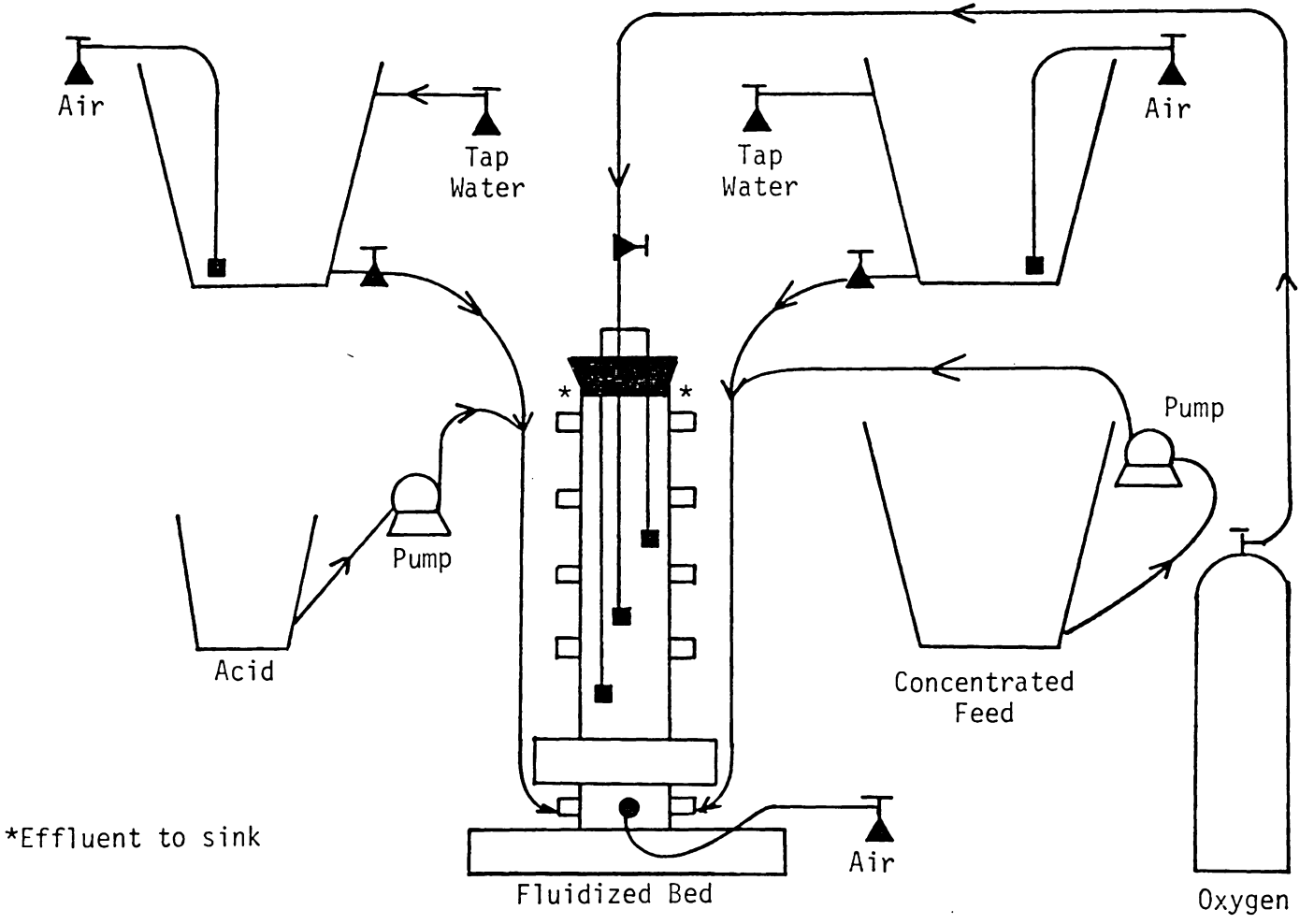


Figure 3. Schematic of Fluidized Bed Feed System

Table 3

Composition of Synthetic Waste

Nutrient	Final concentration mg/l
KH_2PO_4	50[a]
$(NH_4)_2SO_4$	30[b]
$MnSO_4 \cdot H_2O$	20
$MgSO_4 \cdot 7H_2O$	100
$Na_2S_2O_3 \cdot 5H_2O$	1000[c]
	100[d]

[a] As Phosphorus (P)

[b] As Nitrogen (N)

[c] As Thiosulfate ($S_2O_3^{-2}$), first three weeks of continuous operation.

[d] As Thiosulfate ($S_2O_3^{-2}$), remainder of the investigation.

plastic container. Biological growth in the main feed line was retarded by daily chlorination with a strong liquid bleach solution.

During the first three weeks of operation the influent thiosulfate concentration was maintained at 1000 mg/l flowing at a hydraulic loading rate of 10 gpm/ft^2. The effluent was periodically monitored by checking the pH and determining the concentrations of thiosulfate, polythionate, and sulfate. By the end of the three week period, biological growth on the sand became evident. At this time the influent thiosulfate concentration was cut back to 100 mg/l and the aeration system was added to the reactor. The influent concentration of thiosulfate was cut back based on the performance of the reactor up to this time and the performance of other biological film reactors used for thiosulfate oxidation and denitrification (27,77).

The aeration system consisted of three porous stone diffusers which were staggered at various depths in the column. The diffusers were connected to individual glass rods which were held in place by a rubber stopper. High purity oxygen was used as the gas of aeration and was delivered at a rate of 0.1 cubic feet per hour. The flow of oxygen was controlled by an Airco flow regulator, a flow meter, and a series of three aquarium valves. With the addition of the aeration system it was also necessary to increase the hydraulic loading rate to 16.1 gpm/ft^2 in order to prevent the reactor from becoming a slugging bed. This hydraulic loading rate was maintained for the remainder of the investigation.

Following the addition of the aeration system, the reactor was

allowed to operate for one month before the actual testing procedures began. Upon initiation of testing, the parameters monitored were: pH, thiosulfate, polythionate, sulfate, volatile solids and oxygen uptake. Points of sampling for the above analysis included: the influent, the sampling ports along the length of the column, and the effluent.

Since pH is a primary factor in the biological oxidation of thiosulfate, the effects of various acid conditions were investigated by adjusting the pH of the influent feed water. The pH conditions evaluated were: 6.5, 6.0, 5.2, 4.0, and 2.9. Acid requirements to achieve each pH condition were determined by titrating a one liter sample of the diluted feed with a five percent hydrochloric acid solution. The resulting volume was then scaled up based on the total volume of flow through the reactor on a daily basis. The required volume of acid was diluted to 18 liters and was placed in a polyethylene container. Acid was delivered to the second feed line using a Sigma Motor finger pump. Addition of acid was not required at a pH of 6.5 as this was the natural pH of the diluted feed.

Each pH condition was evaluated for a period of five days. During each five day interval, samples were collected on a daily basis for monitoring pH, thiosulfate, polythionate, and sulfate. Volatile solids and oxygen uptake measurements were made only during the last two days.

Acid Decomposition of Thiosulfate

Oxidation of thiosulfate by Thiobacillus bacteria typically results

in the production of an extremely acid environment. Thiosulfate is also known to chemically decompose under acid conditions. Although it is difficult to segregate biological oxidation from chemical decomposition in a combined situation it appeared beneficial to further investigate the acid decomposition of thiosulfate under purely chemical conditions.

Acid decomposition of thiosulfate was evaluated in a series of batch tests by subjecting various concentrations of thiosulfate to different levels of pH. Concentrations of thiosulfate which were evaluated included: 100, 200, 500, 750, and 1000 mg/l. In addition to thiosulfate, the nutrient salts used during the fluidized bed study were also included at the concentrations shown in Table 3. Mercuric chloride at a concentration of 0.1 mg/l mercury was added to eliminate the possible effects of biological oxidation. This concentration of mercury was considered to be sufficient since 0.05 mg/l is known to be toxic to T. thiooxidans (40). A higher concentration of mercury was avoided due to its reactive nature with thiosulfate (3).

The actual test was performed by placing one liter of each solution into glass bottles which were aerated using compressed air. The pH of each original solution was adjusted every 24 hours using hydrochloric acid. Samples were collected prior to each adjustment and were analyzed for thiosulfate, polythionate, and sulfate. Random samples were also selected for microscopic observation. The incremental acidification of each solution resulted in the following sequence of pH levels: 6.5, 6.0, 5.0, 4.0, 3.0, 2.0, and 1.0.

Influence of Trace Nutrients on Oxygen Uptake

Biological systems require trace quantities of various elements such as zinc, iron, molybdenum copper, and cobalt in order to metabolize and function properly. Vishniac and Santer (39) have pointed out that, for biochemical studies, the nutrients listed above can prove to be beneficial in the culturing of Thiobacilli. The following experiment was designed to test the effects of these nutrients on the oxygen uptake of the isolated Thiobacillus culture.

Comparative oxygen uptake rates were made on the influent feed water to the fluidized bed reactor and the influent feed supplemented with the trace nutrients shown in Table 4. Each oxygen uptake measurement was made over a range of pH conditions which included: 6.5, 6.0, 5.0, 4.0, and 3.0. The pH adjustments were made using hydrochloric acid. Prior to measuring oxygen uptake, solids analysis showed the volatile solids concentration of the batch culture to be 580 mg/l VS. The procedures employed during this experiment are outlined below.

1. Transfer 300 ml of the batch culture to a 500 ml graduated cylinder and allow to settle for 15 minutes.
2. Decant the supernatant and replace with the nutrient solution, excluding thiosulfate, which has been pre-adjusted to the appropriate pH level.
3. Aerate with compressed air for 15 minutes then allow to settle for 15 minutes.
4. Repeat steps two and three.
5. Decant the supernatant and replace with the nutrient solution.

Table 4

Supplementary Trace Nutrients

Nutrient	Concentration gm/l	Trace metal concentration mg/l
$ZnSO_4 \cdot 7H_2O$	0.2200	Zn^{+2} - 50
$FeSO_4 \cdot 7H_2O$	0.0499	Fe^{+2} - 10
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.0110	Mo^{+6} - 0.85
$CuSO_4 \cdot 5H_2O$	0.0157	Cu^{+2} - 4
$CoCl_2 \cdot 6H_2O$	0.0161	Co^{+2} - 4
EDTA[a]	0.5000	---

[a]Ethylenediamine tetraacetic acid (complexing agent)

6. Transfer the 300 ml to a standard BOD bottle and measure the endogenous oxygen uptake for 30 minutes using a dissolved oxygen meter and a dissolved oxygen probe equipped for stirring.
7. Remove the probe and allow to settle for two or three minutes.
8. Pipette 1 ml from the BOD bottle supernatant and replace with 1 ml of a thiosulfate solution which will give a final thiosulfate concentration of 100 mg/l.
9. Measure the oxygen uptake of the thiosulfate solution for 60 minutes.

The procedure of washing the sludge twice was used to insure that any traces of thiosulfate from the batch culture were removed. Oxygen uptake rates were reported as mg/l O_2/hr.

Influence of pH on Thiosulfate BOD_{22}

Biological oxidation of thiosulfate by Thiobacillus is influenced by many factors. One of these factors is pH. In the following experiment, the effects of pH on thiosulfate oxidation were evaluated by measuring BOD_{22}.

BOD measurements at 20°C were made using the Hach BOD apparatus. Procedures given in the accompanying instruction manual for reading BOD directly were followed in performing the experiment. The range of pH conditions evaluated included: 6.5, 6.0, 5.0, 4.0, and 3.0. Each bottle contained a thiosulfate concentration of 1000 mg/l and the nutrient salts shown in Tables 3 and 4. Seed material for each bottle was added from the isolated Thiobacillus culture and was based on setting a thiosulfate F/M of 10. The seed material was settled in a test tube and decanted

prior to its addition to each bottle. Thiosulfate was added just before closing each bottle giving a final volume of 157 ml.

Precipitation of Thiosulfate with $BaCO_3$

Analytical procedures for measuring sulfate concentrations in the presence of thiosulfate typically result in a positive interference by thiosulfate. Since these procedures are based on the precipitation of sulfate by barium, it is possible to assume that thiosulfate may also be precipitated by barium. Barium salts of thiosulfate are also reported to be insoluble (3,4). Based on this limited information, and experiment was designed to investigate the possibilities of precipitating thiosulfate with barium carbonate.

Barium carbonate was selected as the precipitating agent because it is highly insoluble and has been used successfully in removing sulfate from sulfuric acid wastes (88). Concentrations of barium carbonate evaluated included: 1, 5, 10, 15, 20, and 25 gm/l. The solution subjected to experimentation was made up from tap water and contained 1000 mg/l thiosulfate and the nutrients shown in Table 3.

The experiment was performed using a Phipps and Bird stirring apparatus and six rectangular mixing jars. A volume of one liter was used for each evaluation. Each jar was flash mixed for 30 minutes at the maximum speed of the apparatus, followed by 30 minutes of flocculation at 20 rpm. Thirty minutes were allowed for settling. One gram of lime was added to each jar prior to flash mixing to aid in the final settling. Thiosulfate concentrations were measured at the end of the experiment.

EXPERIMENTAL RESULTS

The following sections include a presentation of the data and results obtained from the various experiments conducted during this investigation.

Isolation and Enrichment of a Thiobacillus Culture

A culture of Thiobacillus bacteria was successfully isolated from domestic sewage. The culture readily oxidized thiosulfate and produced acid, lowering the pH to between 2.5 and 2.0. Frequent microscopic observations revealed short, Gram negative, motile rods typical of the Thiobacilli. Since attempts were not made to purify the culture, the isolation and enrichment procedures resulted in obtaining a mixed culture of Thiobacilli. It is possible that in subculturing, a dominant strain was obtained but analytical procedures were not employed to establish this possibility.

Nutrient Requirements of the Isolated Thiobacillus Culture

The purpose of this experiment was to investigate the nitrogen and phosphorus requirements of the isolated Thiobacillus culture. Nitrogen requirements were evaluated in a series of batch tests by varying the quantity of ammonia-nitrogen added to each culture. Phosphorus requirements were evaluated in a similar series of batch tests and by measuring the influence of phosphorus on oxygen uptake in a Warburg respirometer.

Each of the tests contained an initial thiosulfate concentration of 1000 mg/l.

Nitrogen requirements. The results of the nitrogen batch tests were evaluated by observing thiosulfate oxidation, sulfate production, and pH. These results are shown in Figures 4, 5 and 6, respectively. Concentrations of nitrogen evaluated were: 5, 10, 15, 20, 25, 30, and 35 mg/l.

Figure 4 shows the influence of ammonia nitrogen on thiosulfate oxidation on a comparative basis. With the exception of 30 mg/l ammonia nitrogen, all of the ammonia nitrogen concentrations evaluated influenced the oxidation of thiosulfate to the same extent. In each case, thiosulfate oxidation was initiated after a 30 hour lag period and was completed within 100 to 120 hours. The culture containing 30 mg/l ammonia nitrogen lagged behind the other cultures but demonstrated a parallel path of thiosulfate oxidation and required 135 hours for completion.

The effects of ammonia nitrogen on sulfate production are shown in Figure 5. All of the nitrogen concentrations evaluated, with the exception of 30 mg/l, did not induce a significant variation in the production of sulfate. Following an initial lag period of 30 hours, sulfate began to appear and increased linearly until the conclusion of the experiment where sulfate reached a concentration of 400 to 450 mg/l. This level was slightly less than half of the total concentration which could have been produced on a theoretical basis. The culture containing 30 mg/l nitrogen lagged behind the other cultures throughout

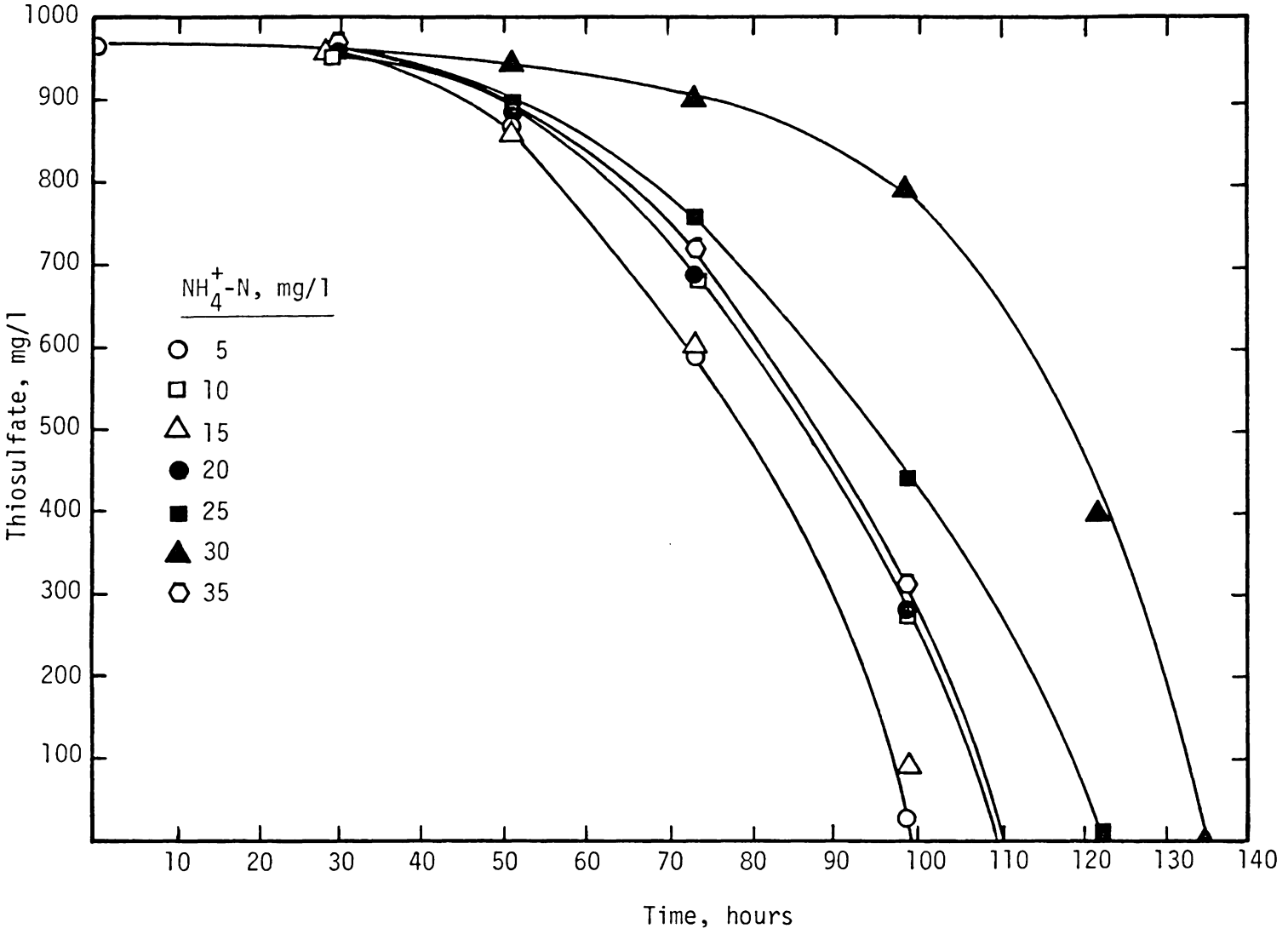


Figure 4. Nitrogen Batch Study, Influence of NH_4^+-N on Thiosulfate Oxidation

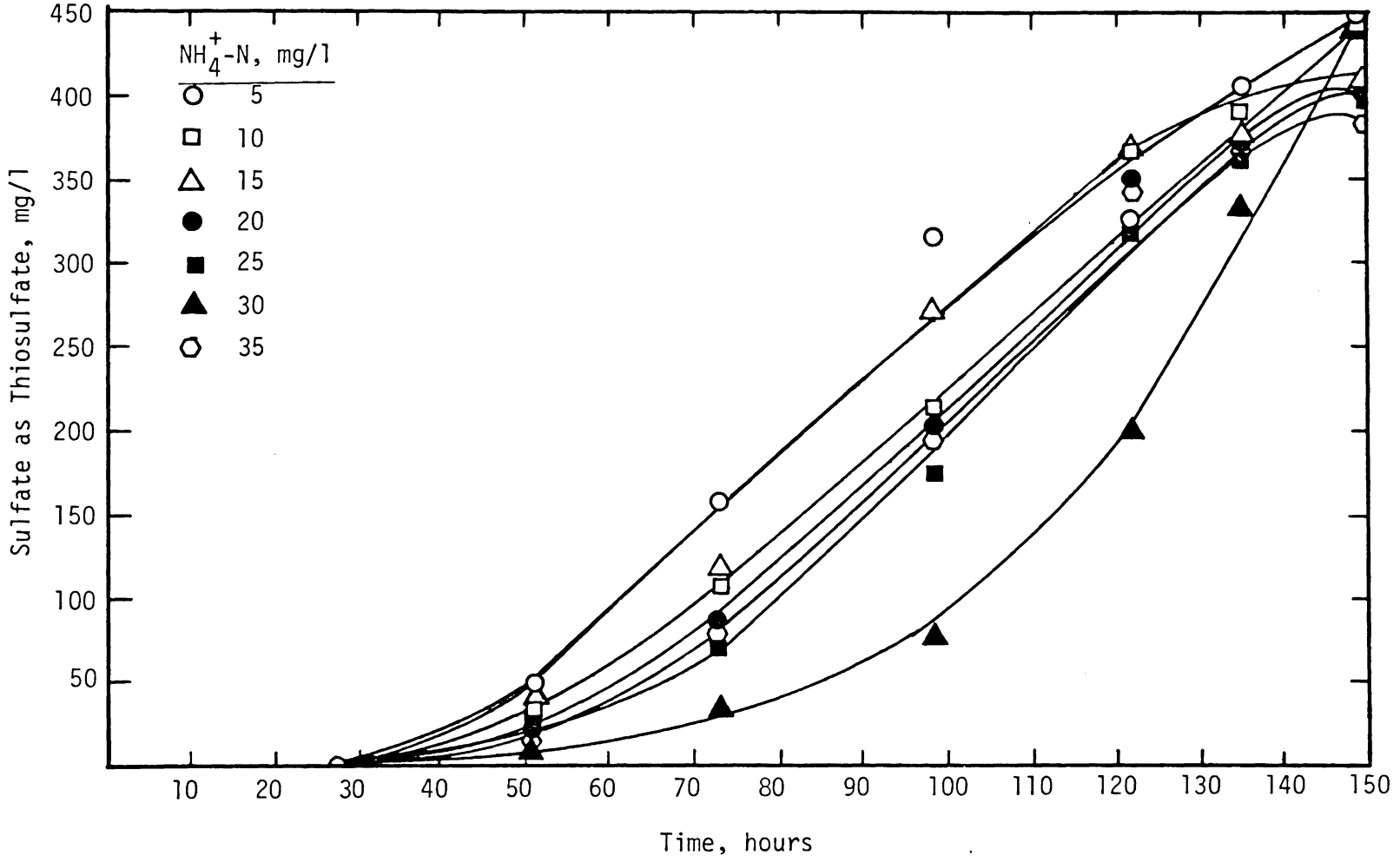


Figure 5. Nitrogen Batch Study, Influence of NH_4^+-N on Sulfate Production

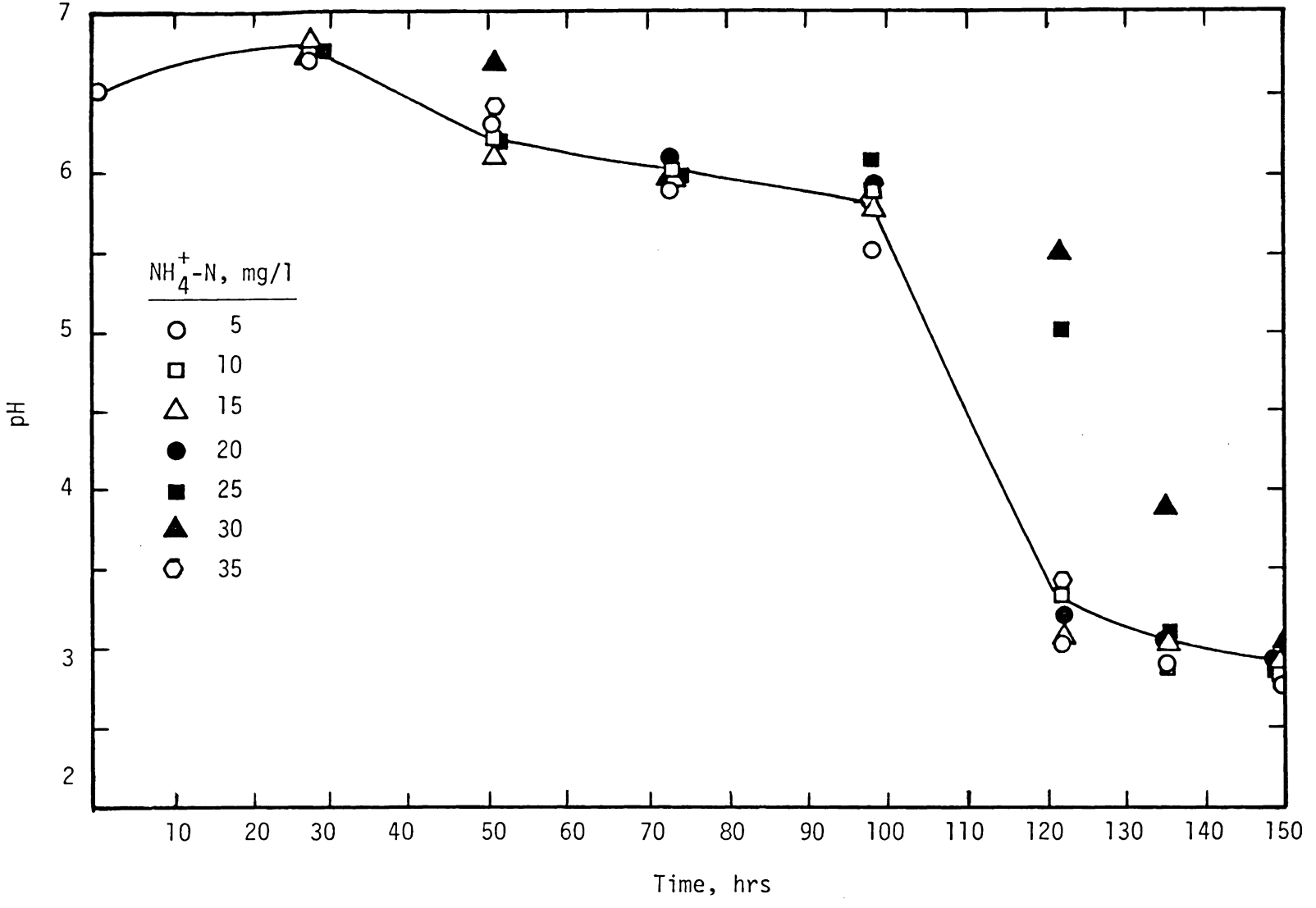


Figure 6. Nitrogen Batch Study, Influence of NH_4^+-N on pH

the test but at the end of the experiment had produced 450 mg/l sulfate.

As shown in Figure 6, the concentrations of ammonia nitrogen evaluated had a negligible influence on pH during the oxidation of thiosulfate. In the first 30 hours the pH increased slightly from 6.5 to 6.8 then gradually decreased to 5.8 after 100 hours of incubation. During the next 20 hours the pH decreased sharply from 5.8 to 3.2, then slowly dropped to 2.8 at the end of the experiment. The culture containing 30 mg/l ammonia nitrogen lagged behind the other cultures but ultimately lowered the pH to 3.0.

In general, the concentrations of nitrogen evaluated influenced the overall oxidation of thiosulfate to a very limited extent. The differences in thiosulfate oxidation between initial ammonia nitrogen concentrations of 5 mg/l and 35 mg/l were not appreciable.

Phosphorus requirements. The results of the phosphorus batch tests were evaluated by observing thiosulfate oxidation, polythionate production, sulfate production and pH. These results are shown in Figures 7 through 14. The concentrations of phosphorus evaluated were 1, 10, 100, and 1000 mg/l.

Figures 7, 8, 9, and 10 illustrate the pattern of thiosulfate oxidation at respective phosphorus concentrations of 1, 10, 100, and 1000 mg/l. Each of the cultures, except the one containing 1000 mg/l phosphorus, oxidized thiosulfate in a very similar manner, but to a different extent. The oxidation of thiosulfate was followed by an accumulation of polythionate, a gradual production of sulfate, and a substantial decrease in pH. The cultures containing 1, 10 and 100 mg/l phosphorus

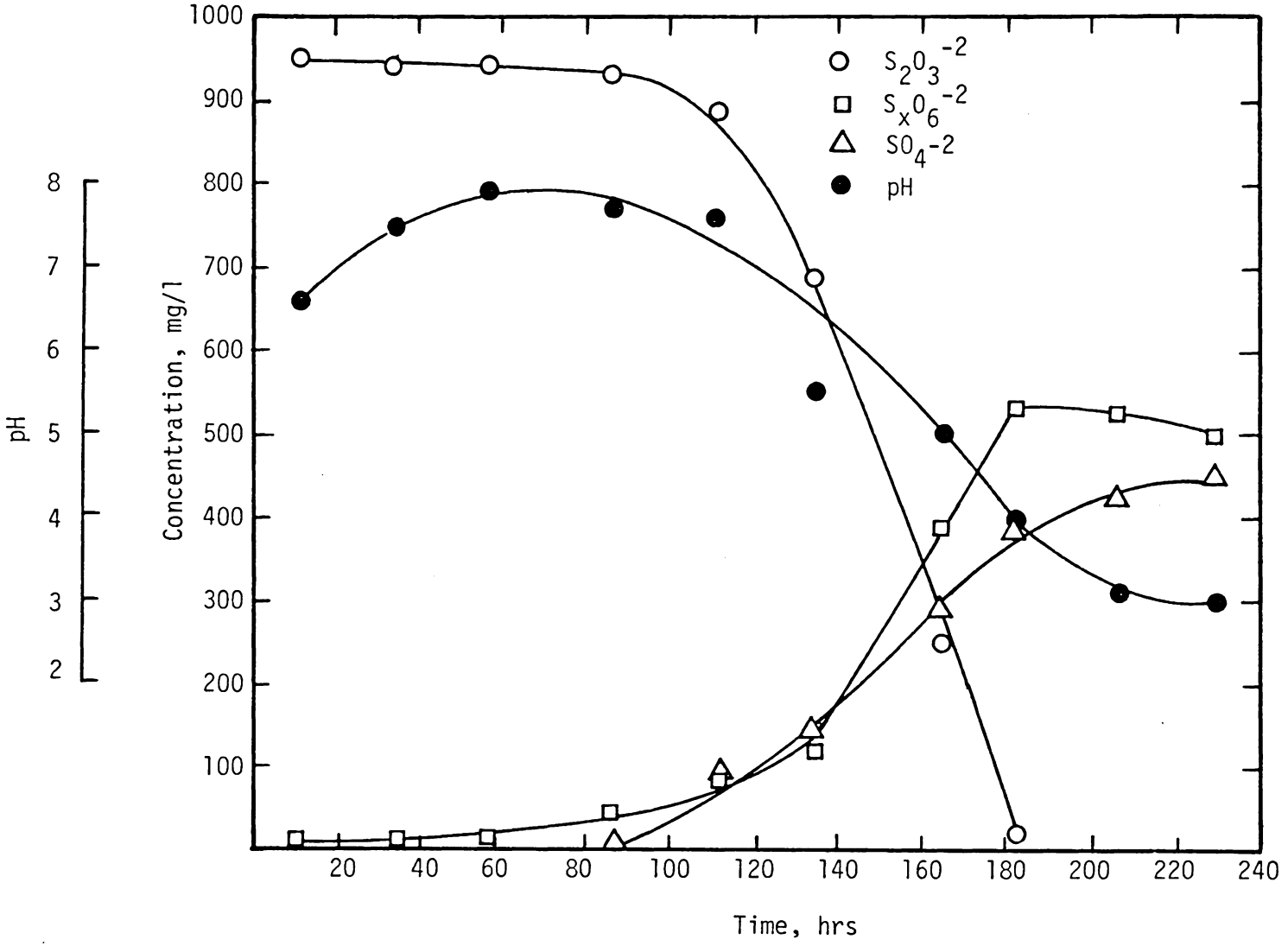


Figure 7. Phosphorus Batch Study, Thiosulfate Oxidation at 1 mg/l Phosphorus

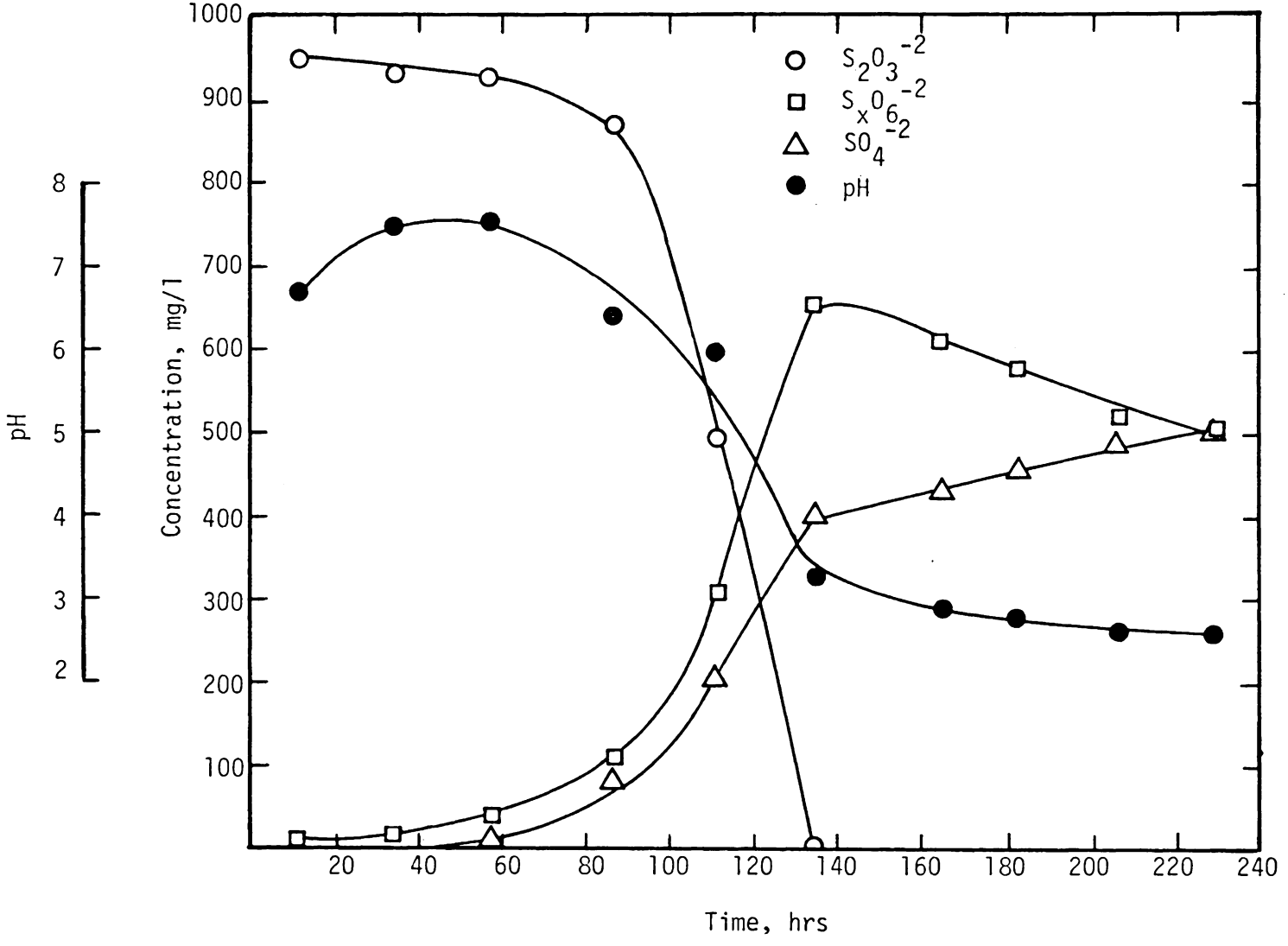


Figure 8. Phosphorus Batch Study, Thiosulfate Oxidation at 10 mg/l Phosphorus

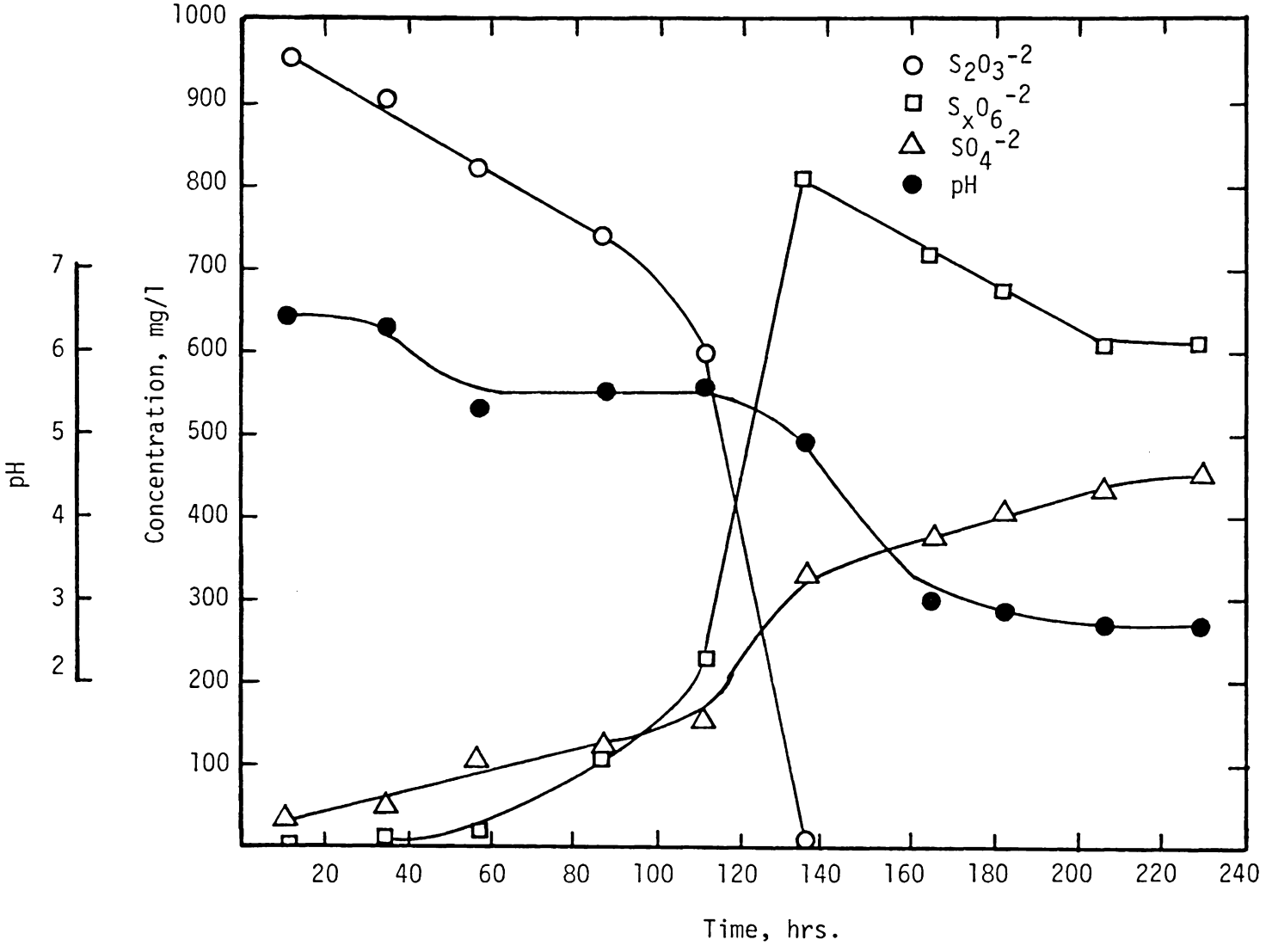


Figure 9. Phosphorus Batch Study, Thiosulfate Oxidation at 100 mg/l Phosphorus

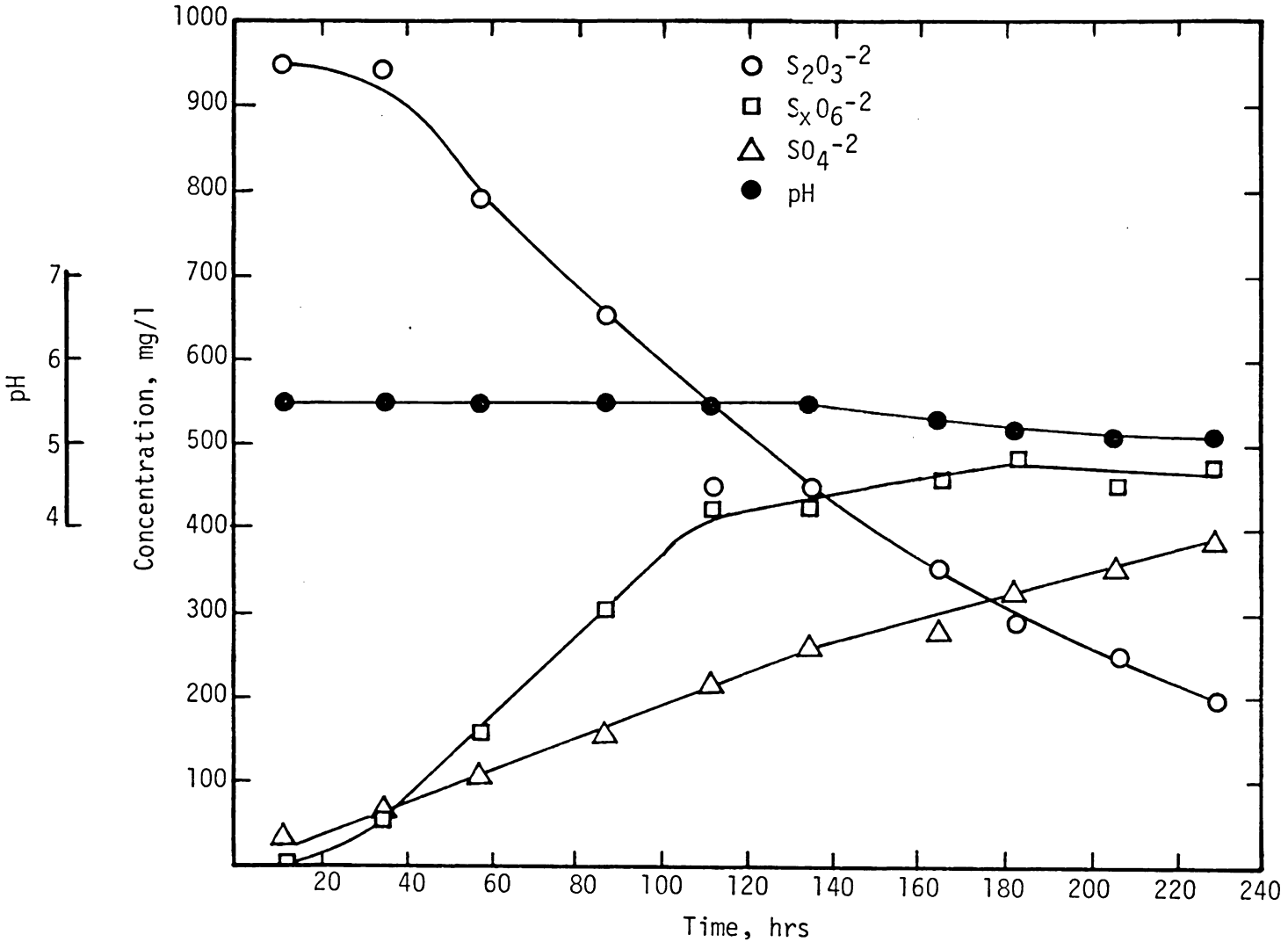


Figure 10. Phosphorus Batch Study, Thiosulfate Oxidation at 1000 mg/l Phosphorus

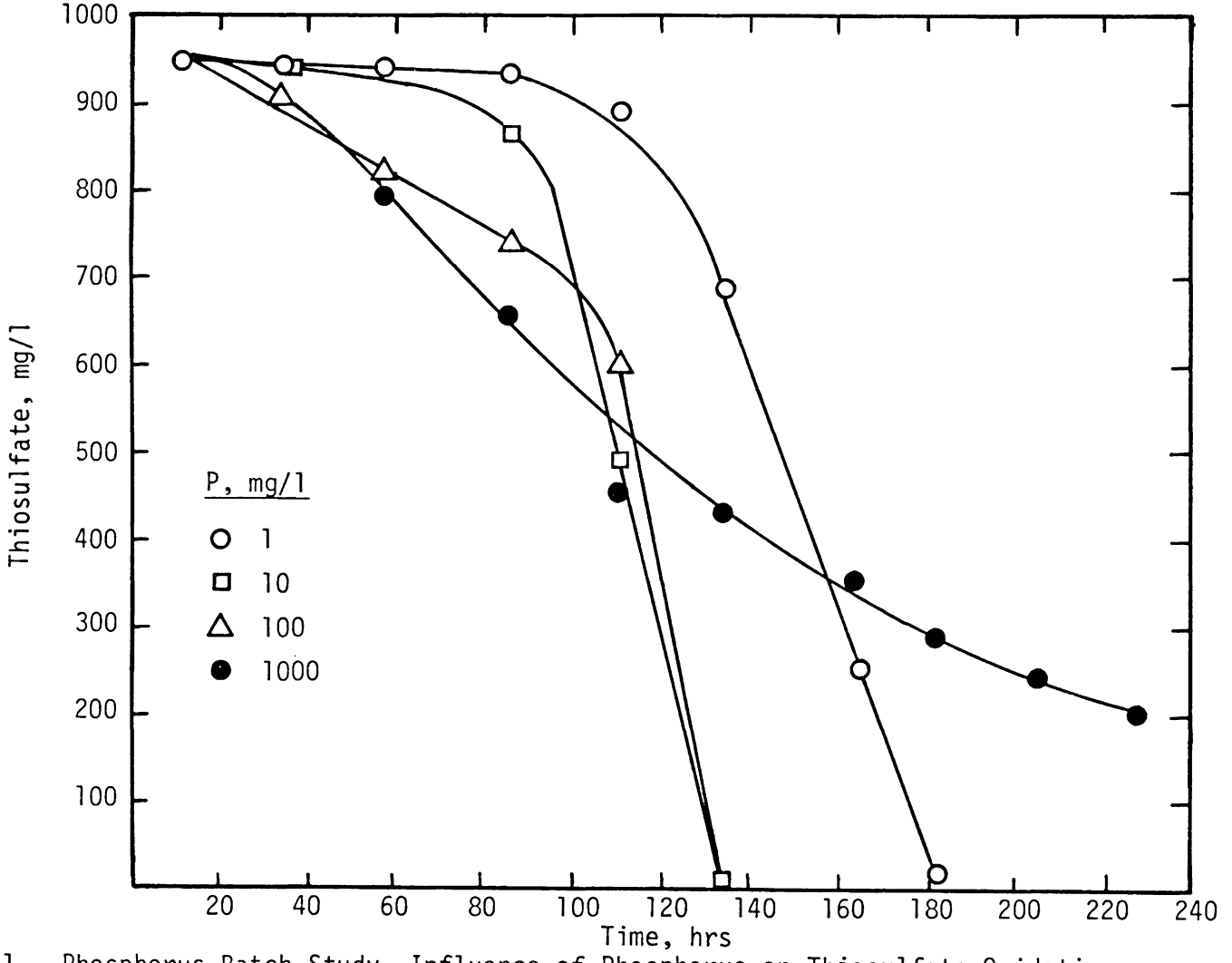


Figure 11. Phosphorus Batch Study, Influence of Phosphorus on Thiosulfate Oxidation

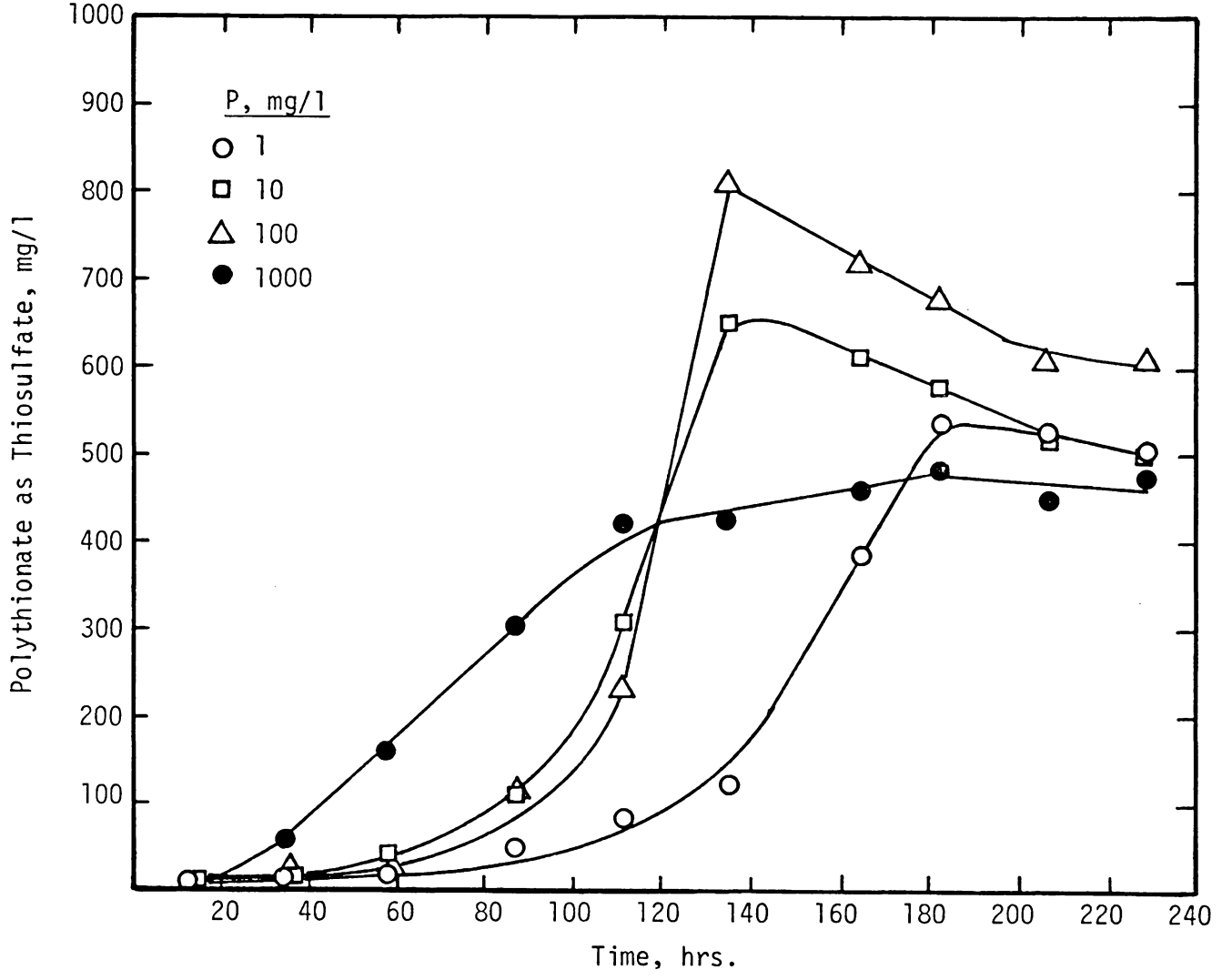


Figure 12. Phosphorus Batch Study, Influence of Phosphorus on Polythionate Production

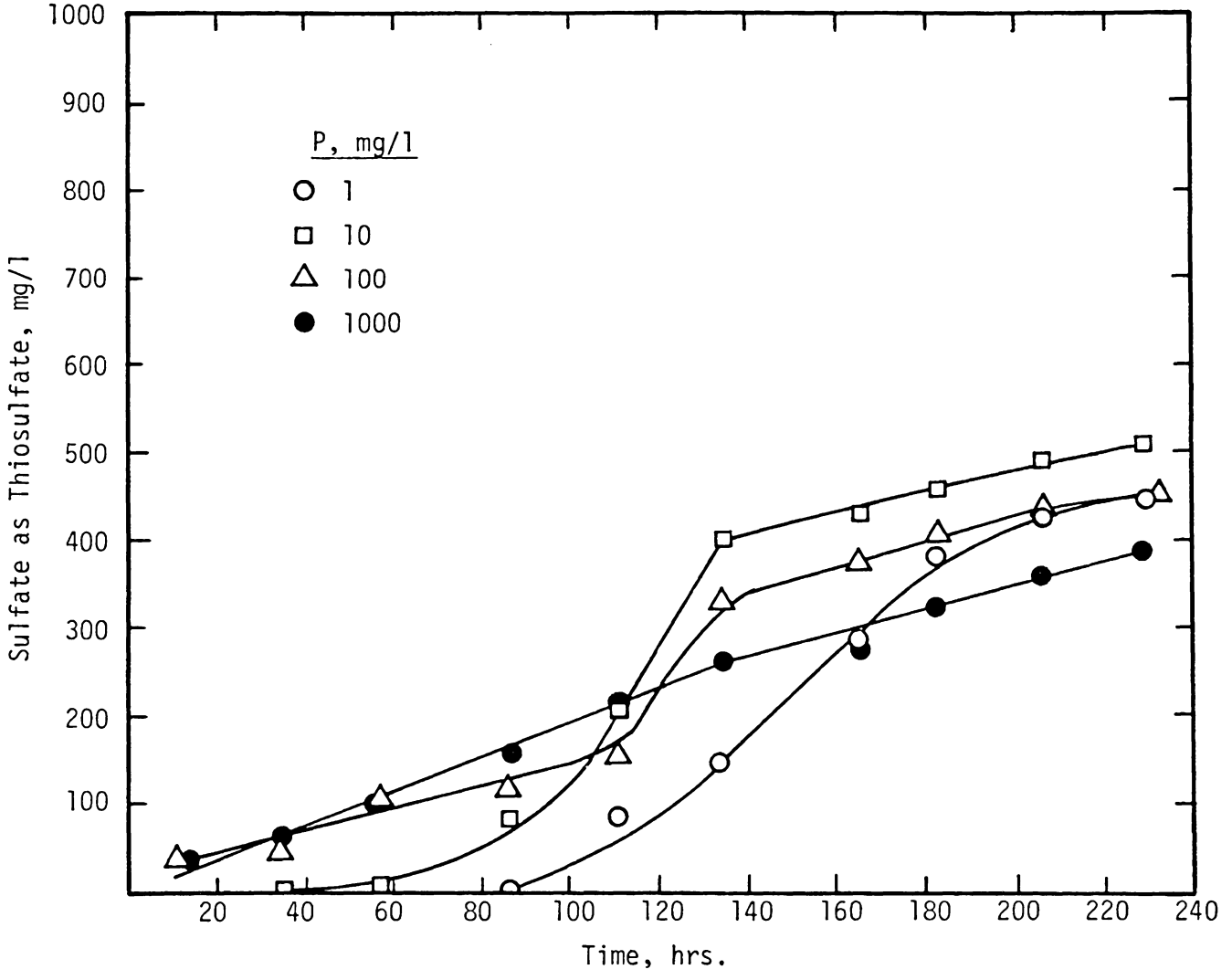


Figure 13. Phosphorus Batch Study, Influence of Phosphorus on Sulfate Production

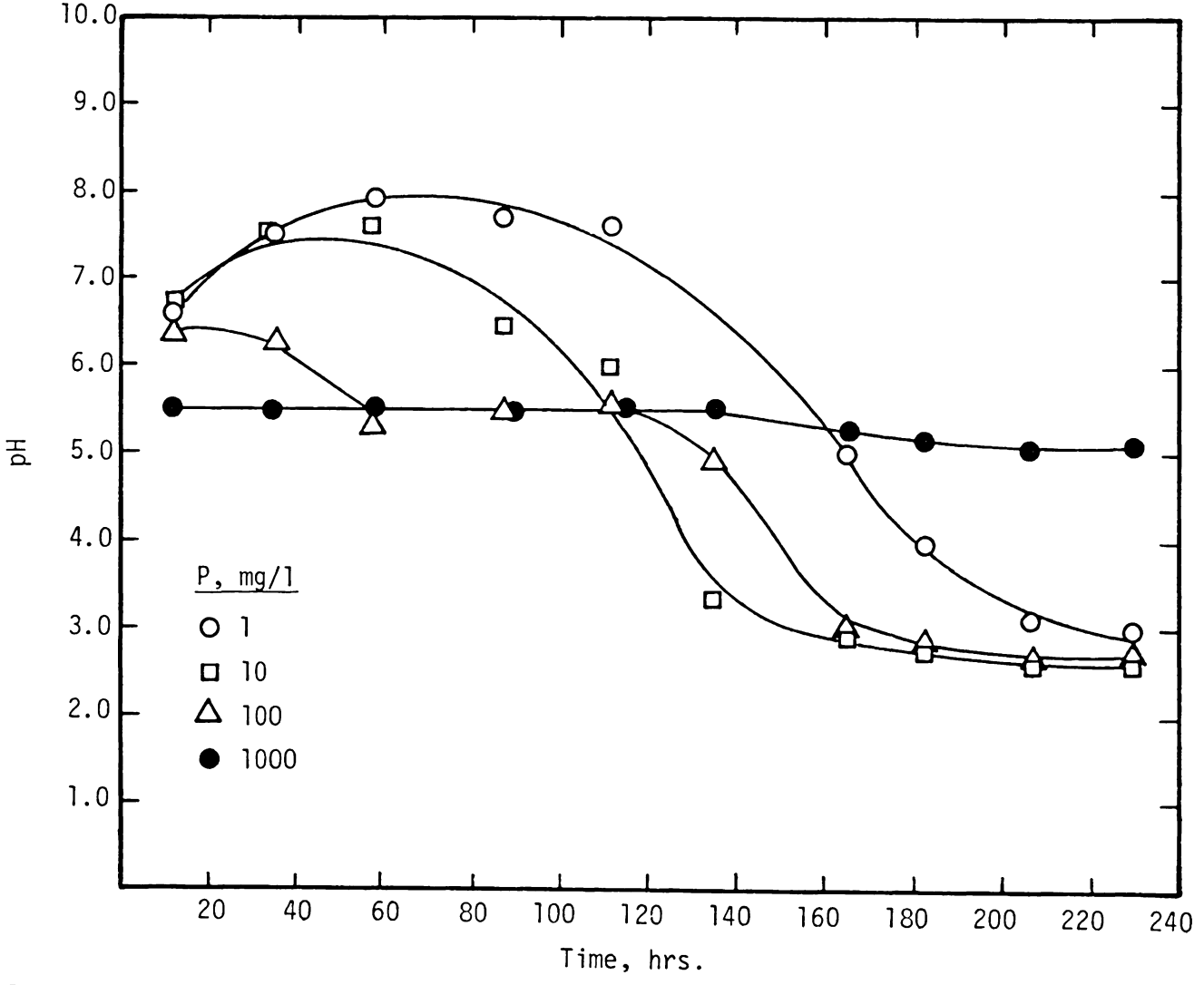


Figure 14. Phosphorus Batch Study, Influence of Phosphorus on pH During Thiosulfate Oxidation

completely exhausted the supply of thiosulfate and showed a maximum accumulation of polythionate at the same time that thiosulfate was depleted. Polythionate decreased in the absence of thiosulfate.

Comparative illustrations of thiosulfate oxidation, polythionate production and sulfate production are presented, respectively, in Figures 11, 12, and 13. A comparison of these figures will show that the overall oxidation of thiosulfate was significantly influenced by phosphorus concentrations of 10 and 100 mg/l; thiosulfate was oxidized more rapidly, there was a greater accumulation of polythionate, and sulfate production was higher during the early stages. Although polythionate and sulfate production were high during the early stages of incubation at a phosphorus concentration of 1000 mg/l, the overall oxidation of thiosulfate was greatly suppressed.

The influence of phosphorus on pH during thiosulfate oxidation is shown in Figure 14. At phosphorus concentrations of one and ten mg/l the pH first increased from 6.5 into the alkaline region then gradually decreased to 3.0 and 2.6, respectively. While both cultures ultimately lowered the pH into the acid region, the one containing 10 mg/l phosphorus was more efficient. The culture containing 100 mg/l phosphorus lowered the pH in a more direct manner but appeared to be buffered for several hours at a pH of 5.5. Eventually, the pH reached a level of 2.7. At a phosphorus concentration of 1000 mg/l the pH remained stable at 5.5 for the first 140 hours then gradually decreased to 5.1.

Oxygen uptake in a Warburg respirometer was also used as a means of determining the phosphorus requirements of the isolated Thiobacillus

culture. Phosphorus concentrations evaluated included: 1, 10, 50, 100, and 1000 mg/l. The results, illustrated in Figure 15, show that phosphorus concentrations of 10 mg/l or higher definitely influenced oxygen uptake during thiosulfate oxidation. Although the influence was significant, as compared to a phosphorus concentration of 1 mg/l, the differences between the higher concentrations were not substantial.

The results of the batch and oxygen uptake experiments have demonstrated the influence of phosphorus on the isolated Thiobacillus culture. Generally, phosphorus concentrations between 10 and 100 mg/l improved the overall oxidation of thiosulfate.

Characterization of the Fluidized Bed

Before placing the fluidized bed into continuous operation the range of fluidization velocities was determined by passing tap water through the reactor at various hydraulic loading rates. The applied loading rates ranged from 5 to 35 gpm/ft^2. Corresponding porosities are shown in Figure 16. At hydraulic loading rates between five and nine gpm/ft^2 the bed remained static and had a porosity of 0.38. With a further increase in loading rate the bed became fluidized and the porosity increased to 0.59 at 35 gpm/ft^2. The minimum fluidization hydraulic loading rate appeared to be approximately 10 gpm/ft^2.

Thiosulfate Oxidation in a Fluidized Bed

The objective of this experiment was to investigate the possibilities of biologically oxidizing thiosulfate in a fluidized bed. This result was accomplished by feeding a synthetic waste into the reactor and

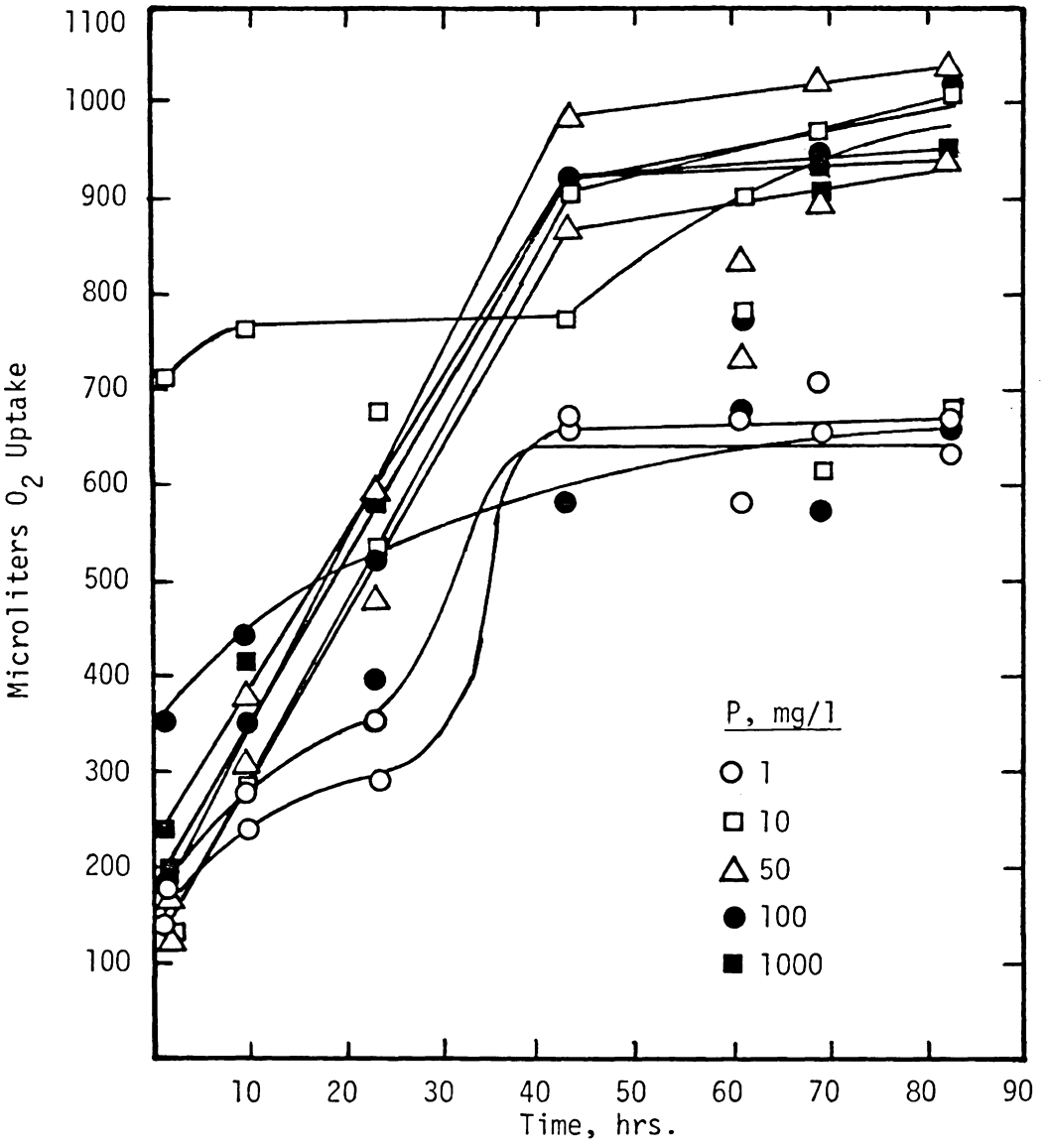


Figure 15. Influence of Phosphorus on Oxygen Uptake

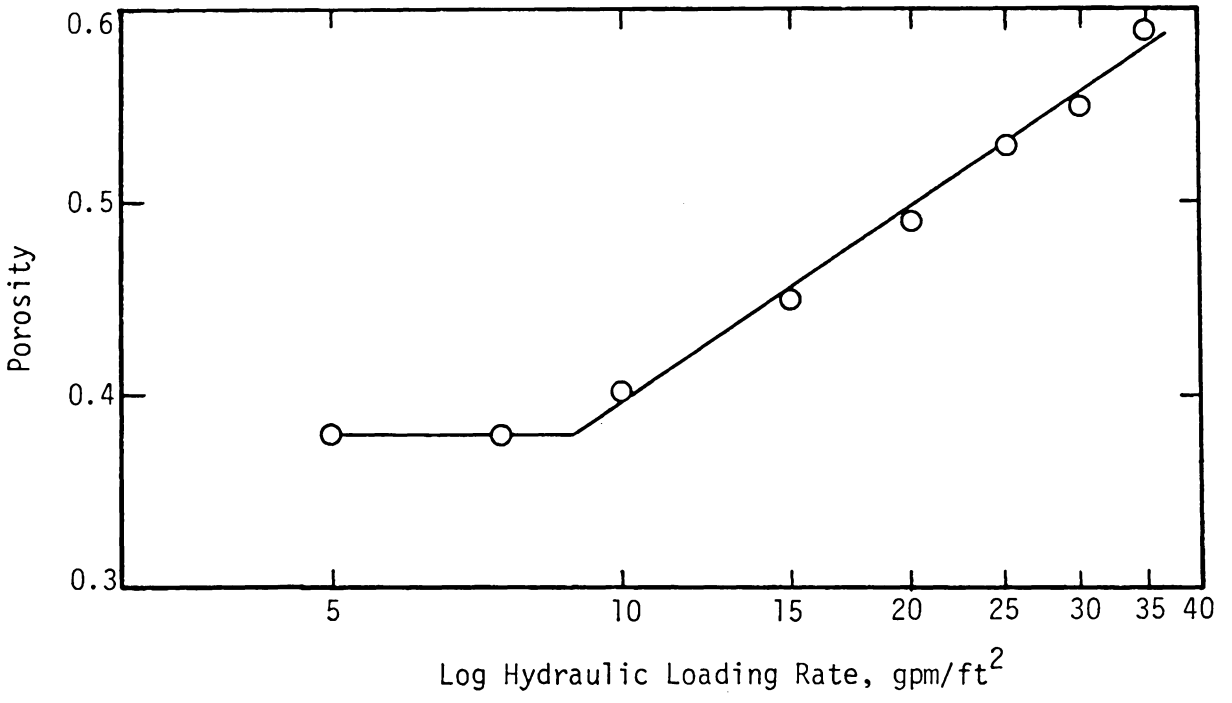


Figure 16. Porosity of the Fluidized Bed

observing the profile of the various sulfur compounds and other related properties characteristic to thiosulfate oxidation in a biological treatment system.

Following the initial seeding procedures, the reactor was placed into continuous operation for a period of three weeks at a hydraulic loading rate of 10 gpm/ft^2 and an influent thiosulfate concentration of 1000 mg/l. During this time biological growth became established on the sand but the overall performance of the reactor was very poor. The effluent thiosulfate concentration was stable at 950 mg/l. The pH was constant at 6.5 and polythionate production accounted for the majority of the oxidized thiosulfate. Sulfate concentrations were normally below 10 mg/l.

Due to the poor performance of the reactor, an estimation of the applied thiosulfate loading rate was made on a basis of available surface area. The hydraulic detention time was also approximated. In estimating the surface area of the fluidized bed, the sand grains were assumed to be perfect spheres. The available surface area was approximately 50 square feet. At a hydraulic loading rate of 10 gpm/ft^2 the void space detention time was estimated to be one minute. Based on the estimated surface area, the thiosulfate loading rate was approximately 640 mg $S_2O_3^{-2}$/ft^2/hr. A comparison of this loading rate to those of previous investigations concerning thiosulfate oxidation in a rotating bio-disc (27) and denitrification in a fluidized bed (77), suggested that the reactor was overloaded.

At this time the influent thiosulfate concentration was reduced to

100 mg/l and the aeration system was added to the reactor. With the addition of the aeration system it was necessary to increase the hydraulic loading rate to 16.1 gpm/ft^2 in order to prevent the reactor from becoming a slugging bed. The applied thiosulfate loading rate became 100 mg $S_2O_3^{-2}/ft^2/hr$ and was maintained at this level for the remainder of the investigation. Increasing the hydraulic loading rate changed the void space detention time to approximately 0.6 minutes.

Following the addition of the aeration system, the reactor was allowed to operate for one month before the actual testing procedures began. An additional month of acclimation was required due to the loss of biological mass which occurred during the design and installation of the aeration system. Several designs were attempted before making the final selection. Each time that a new design was introduced, biological mass tended to slough off from the sand grains. The initial slugging nature of the bed also contributed to the loss of biological mass.

During the first three weeks of reacclimation, the effluent thiosulfate concentration decreased from 80 mg/l to 64 mg/l. Polythionate production increased from 5 mg/l to 9 mg/l while sulfate production increased from 15 mg/l to 27 mg/l. The effluent pH dropped from 6.5 to 6.15. At the end of the fourth week the performance of the reactor was stable.

Figure 17 and Table 5 are a summary of the fluidized bed profile during the fourth week. Thiosulfate gradually decreased through the bed to a final concentration of 64 mg/l for a removal efficiency of 36

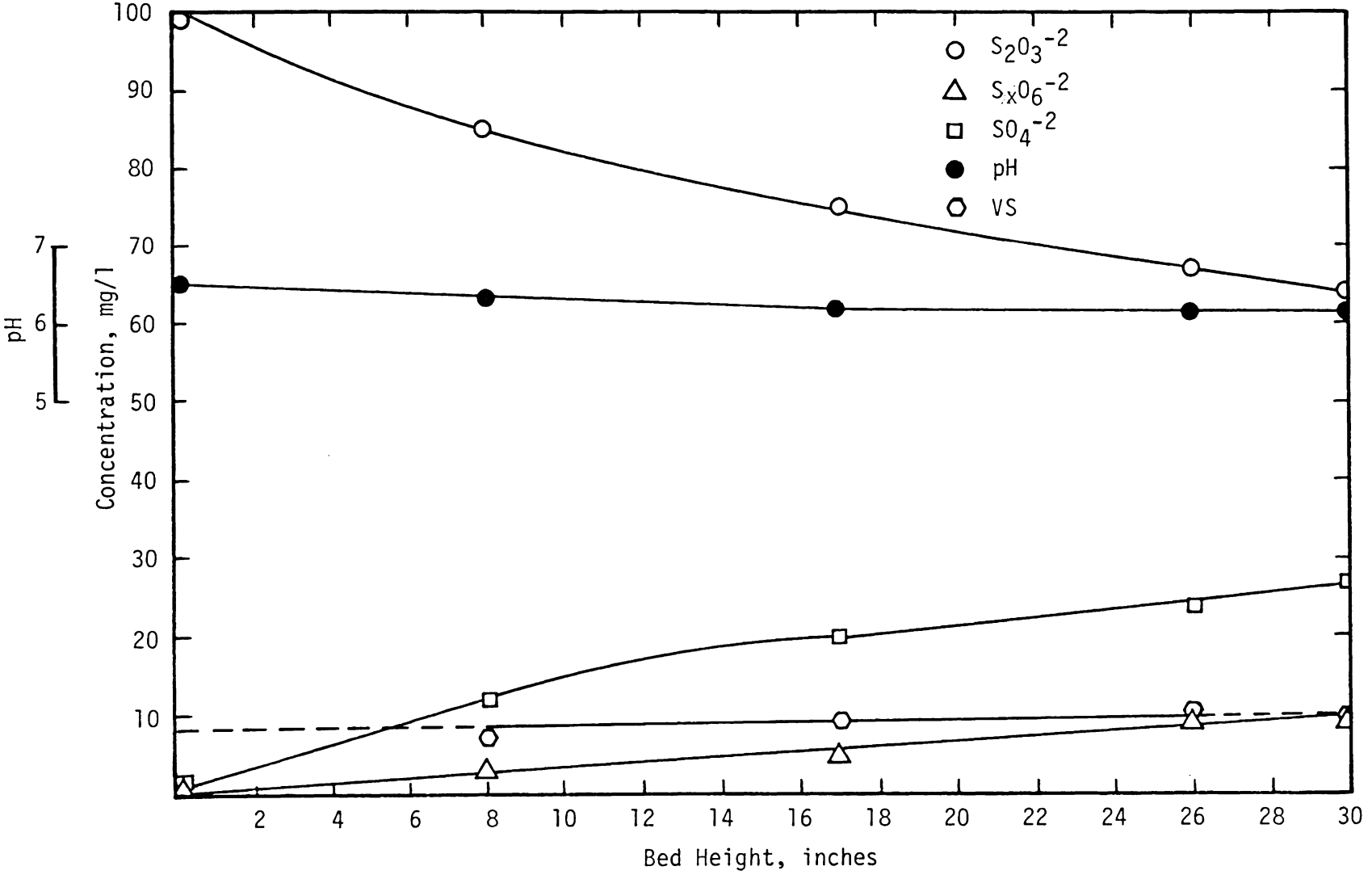


Figure 17. Fluidized Bed Profile at pH 6.5

Table 5

Fluidized Bed Profile at pH 6.5

Sample	pH	$S_2O_3^{-2}$ mg/l	$S_xO_6^{-2}$ mg/l	SO_4^{-2} mg/l	Volatile solids mgVS/gm sand
Influent	6.50	100	0	0	---
8"	6.30	85	3	12	6.8
17"	6.20	75	5	20	9.0
26"	6.15	67	9	24	9.6
Effluent	6.15	64	9	27	---

percent. Sulfate was the major oxidation product, representing 27 percent of the influent thiosulfate. Polythionate production accounted for nine percent. Acid production resulting from the oxidation of thiosulfate lowered the pH from 6.5 to 6.15.

Simple linear regression was used throughout the investigation to fit straight lines to the volatile solids data. The dashed segments at the end of each line indicate that actual data was not obtained within those regions of the bed. While operating at a pH of 6.5, volatile solids in the reactor increased from 7.9 to 10.0 mg VS/gm sand. By summing the area under the volatile solids line the total biological mass was determined to be 10,700 mg VS. Based on this total mass, the fluidized bed was operating at a thiosulfate F/M of 11.4. The oxygen uptake rate at pH 6.5 was 1.07 mg/1 O_2/hr · mg VS.

Since pH is a primary factor in the biological oxidation of thiosulfate, an effort to improve the efficiency of the reactor was made by adjusting the influent pH of the feed water using hydrocloric acid. The pH conditions evaluated were 6.0, 5.2, 4.0, and 2.9. Each pH level was evaluated for a period of five days. Acclimation to each new pH condition was very brief. Usually within 24 hours the performance of the reactor had reached a stable condition. With the exception of pH 2.9 the data presented is an average of the results obtained over a four day period. Throughout this phase of the investigation the effluent dissolved oxygen (DO) concentration fluctuated between two and three mg/1.

Results of the fluidized bed profile at pH 6.0 are presented in Figure 18 and Table 6. These results show that the overall performance of the reactor was still low. Thiosulfate decreased in a nonuniform manner to a final concentration of 52 mg/l for a removal efficiency of 48 percent. Sulfate production was consistent and accounted for 25 percent of the influent thiosulfate. Polythionate on the other hand was essentially constant through the reactor at a concentration of 16 mg/l but ultimately represented 20 percent of the influent thiosulfate. Acid production caused the pH to decrease from 6.0 to 5.3. Volatile solids increased from 7.6 to 11.3 mg VS/gm sand giving a total biological mass of 11,475 mg VS. Based on the total mass, the reactor was operating at a thiosulfate F/M of 10.7. The oxygen uptake rate at pH 6.0 was 0.99 mg/l O_2/hr·mg VS.

Following acidification to pH 6.0 the influent pH was lowered to 5.2 in an effort to further improve the performance of the reactor. Results of the fluidized bed profile at pH 5.2 are summarized in Figure 19 and Table 7. Thiosulfate was consistently oxidized to a final concentration of 40 mg/l for a removal efficiency of 60 percent. Polythionate became the major oxidation product representing 43 percent of the influent thiosulfate. Sulfate production accounted for 17 percent. The pH was lowered from 5.2 to 4.6 due to the production of acid. During this phase of the investigation, a shift in the distribution of biological mass occurred. Volatile solids increased through the reactor from a concentration of 1.3 mg VS/gm sand to 18.5 mg VS/gm sand giving

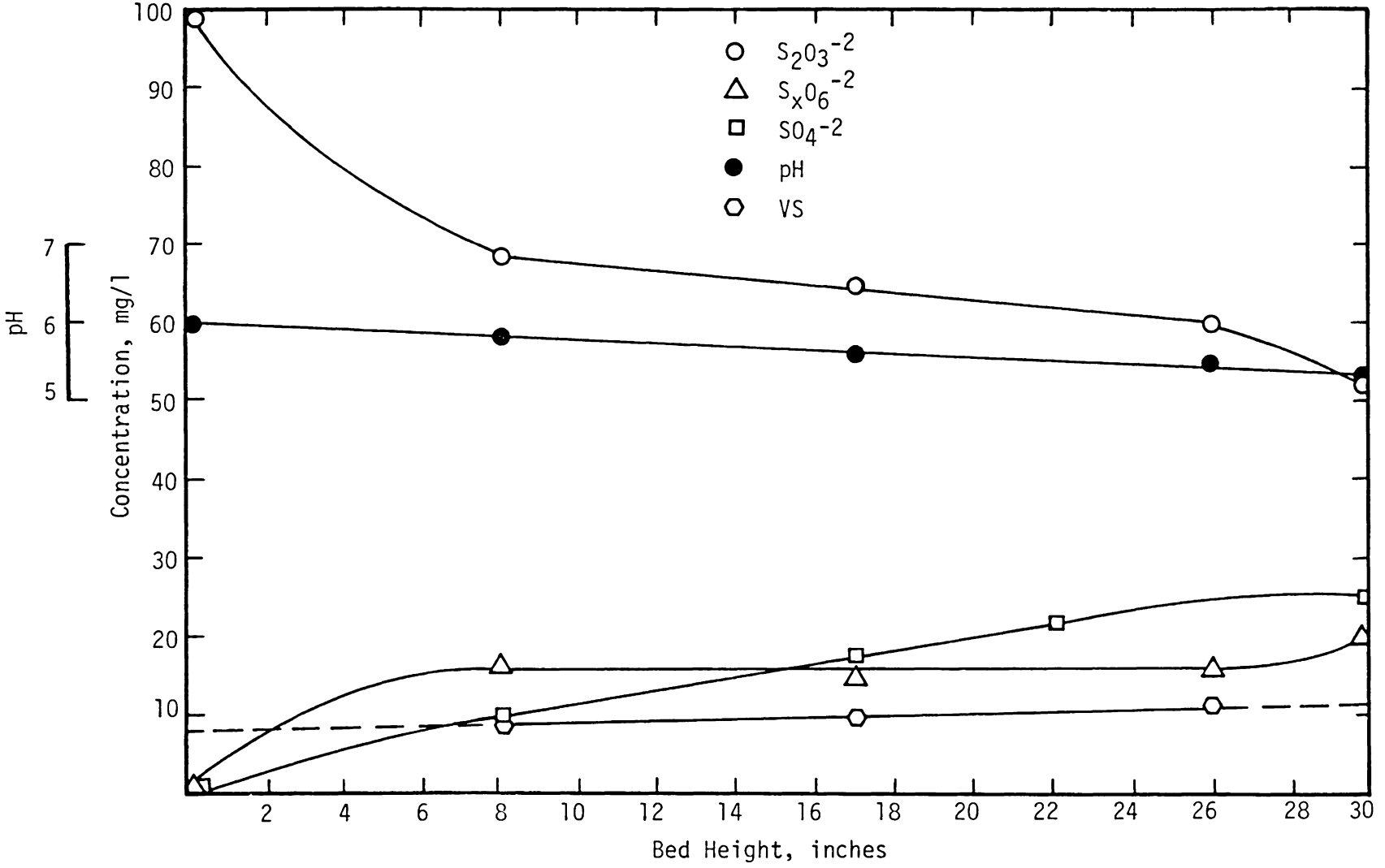


Figure 18. Fluidized Bed Profile at pH 6.0

Table 6

Fluidized Bed Profile at pH 6.0

Sample	pH	$S_2O_3^{-2}$ mg/l	$S_xO_6^{-2}$ mg/l	SO_4^{-2} mg/l	Volatile solids mgVS/gm sand
Influent	6.0	100	1	0	--
8"	5.8	68	16	10	8.8
17"	5.6	65	15	18	9.5
26"	5.5	60	16	22	11.2
Effluent	5.3	52	20	25	---

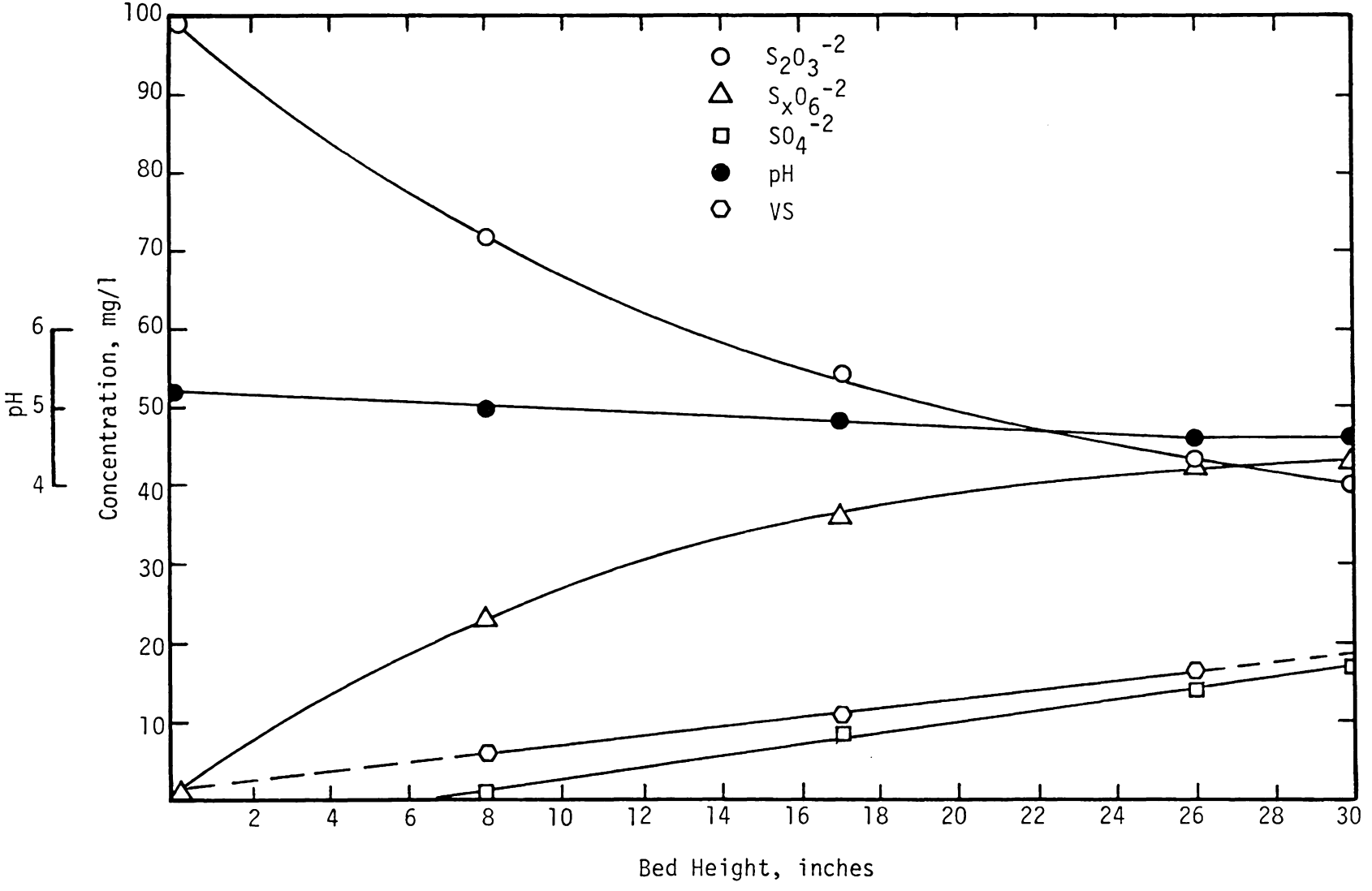


Figure 19. Fluidized Bed Profile at pH 5.2

Table 7

Fluidized Bed Profile at pH 5.2

Sample	pH	$S_2O_3^{-2}$ mg/l	$S_xO_6^{-2}$ mg/l	SO_4^{-2} mg/l	Volatile solids mgVS/gm sand
Influent	5.2	99	1	0	---
8"	5.0	72	23	1	6.1
17"	4.8	54	36	8	10.6
26"	4.6	43	43	14	16.4
Effluent	4.6	40	43	17	---

a total biological mass of 11,840 mg VS. Based on the total mass, the reactor was operating at a thiosulfate F/M of 10.3. The oxygen uptake rate at pH 5.2 was 0.81 mg/l O_2/hr·mg VS.

Up to this time lowering the influent pH had progressively improved the efficiency of thiosulfate removal but the major oxidation product was shifting to polythionate. This shift was indicative of incomplete thiosulfate oxidation. Again the influent pH was lowered another step to 4.0 in anticipation that thiosulfate oxidation would continue to increase and that the major oxidation product would shift back to sulfate.

The results of the fluidized bed profile at pH 4.0 are presented in Figure 20 and Table 8. Thiosulfate removal in the reactor continued to improve and reached an efficiency of 77 percent. Polythionate was still the major oxidation product and represented 57 percent of the influent thiosulfate. Sulfate production was still consistent but only accounted for 23 percent of the influent thiosulfate. In conjunction with the increase in polythionate production, the pH through the fluidized bed also began to increase. A combination of polythionate and sulfate production maintained the pH at 4.5 throughout the length of the reactor. Volatile solids increased from 5.9 to 18.0 mg VS/gm sand giving a total biological mass of 14,435 mg VS. Based on the total mass, the reactor was operating at a thiosulfate F/M of 8.4. The oxygen uptake rate at pH 4.0 was 0.78 mg/l O_2/hr·mg VS.

Following the evaluation of pH 4.0 the acid feed was again increased to lower the influent pH to 2.9. During the first three days of

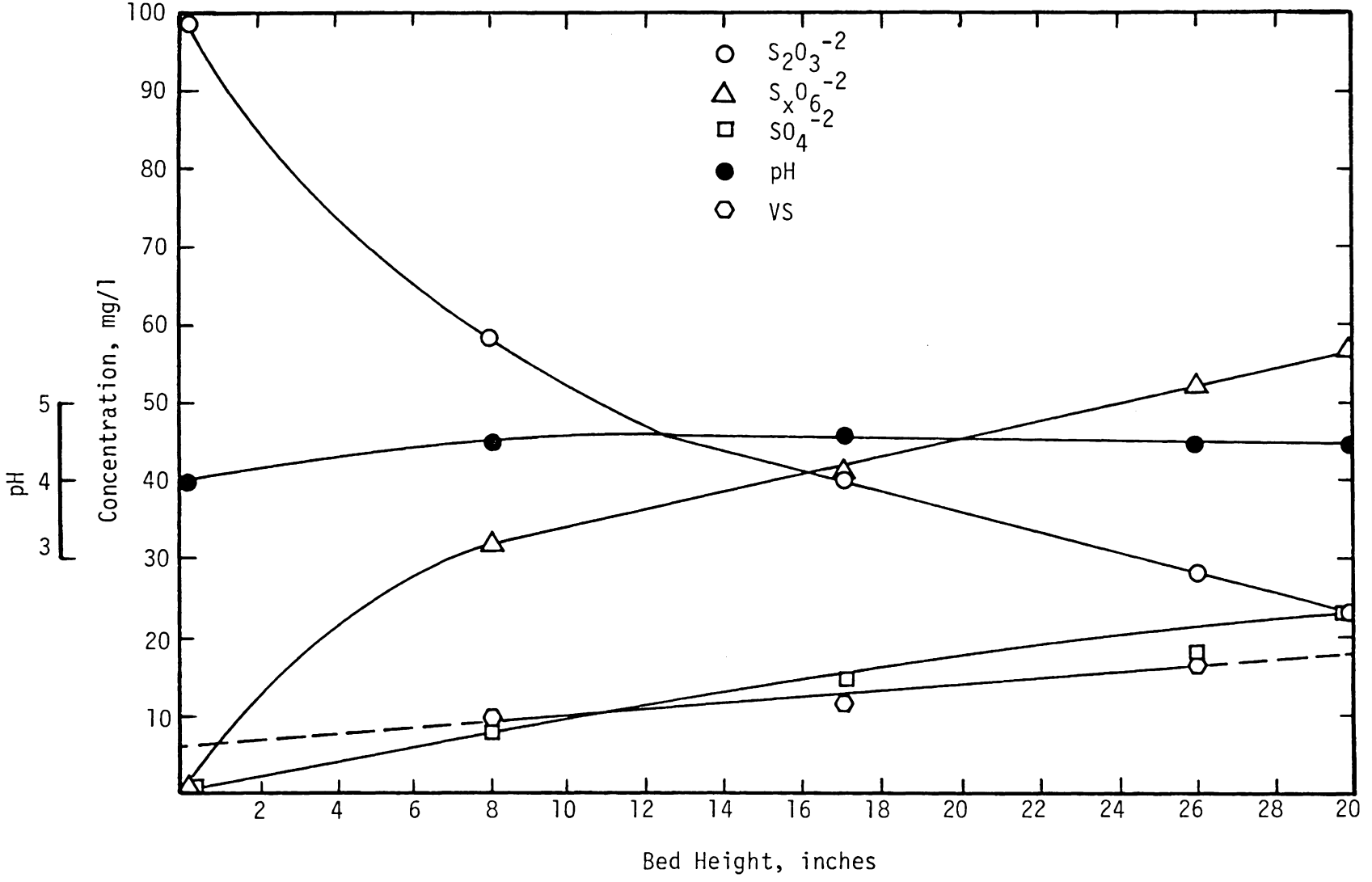


Figure 20. Fluidized Bed Profile at pH 4.0

Table 8

Fluidized Bed Profile at pH 4.0

Sample	pH	$S_2O_3^{-2}$ mg/l	$S_xO_6^{-2}$ mg/l	SO_4^{-2} mg/l	Volatile solids mgVS/gm sand
Influent	4.0	100	0	0	--
8"	4.5	58	32	8	9.7
17"	4.6	40	41	15	11.8
26"	4.5	28	52	18	17.0
Effluent	4.5	23	57	23	---

operation the reactor responded in the usual manner by acclimating to the new acid level within 24 hours and thereafter maintaining a stable condition. For the next three days the fluidized bed operated continuously but all testing procedures were postponed. Figure 21 and Table 9 are a summary of the fluidized bed profile at pH 2.9 during the first three days of operation. Thiosulfate steadily decreased through the reactor to a final concentration of 26 mg/l achieving a removal efficiency of 74 percent. Polythionate was the principal oxidation product and accounted for 72 percent of the influent thiosulfate. Sulfate production was extremely low and represented only four percent of the influent thiosulfate. Polythionate production caused the pH to steadily increase from 2.9 to 3.3. Volatile solids and the oxygen uptake rate were not measured.

On the seventh day numerous areas of bare sand were observed along the length of the column indicating that a considerable amount of biological growth had been lost from the reactor. Samples collected on this day showed that thiosulfate removal had dropped to 46 percent. Polythionate and sulfate, respectively, represented 44 and two percent of the influent thiosulfate. The pH increased from 2.9 to 3.1.

By the eighth day a complete washout had occurred. Due to the loss of the biological mass, volatile solids and the oxyten uptake rate were not measured. For the next three weeks the influent feed was maintained at a pH of 2.9 in an effort to reestablish the biological growth. During this time reseeding was attempted by injecting various volumes of the isolated Thiobacillus culture into the lower

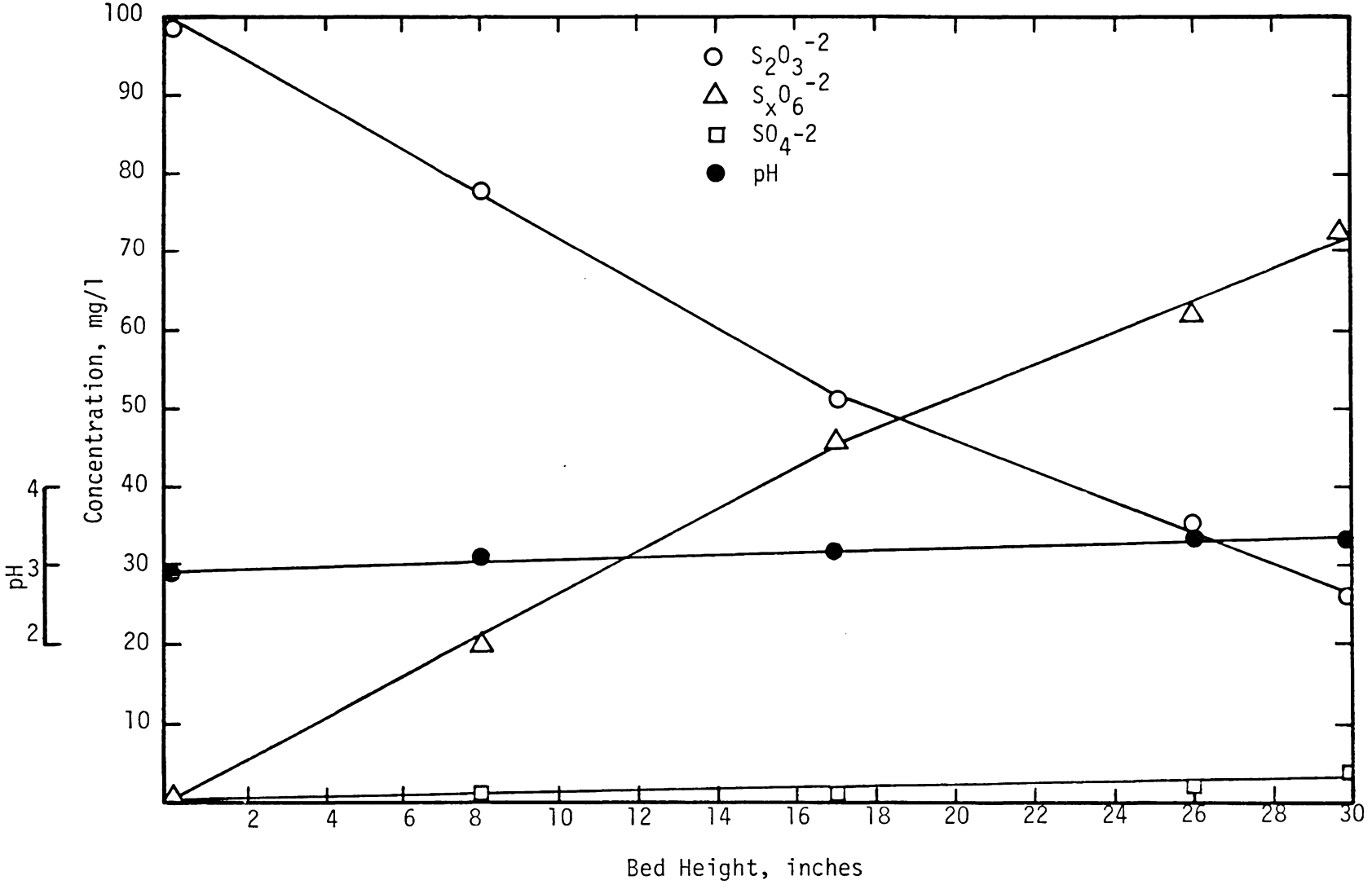


Figure 21. Fluidized Bed Profile at pH 2.9

Table 9

Fluidized Bed Profile at pH 2.9

Sample	pH	$S_2O_3^{-2}$ mg/l	$S_xO_6^{-2}$ mg/l	SO_4^{-2} mg/l
Influent	2.9	100	0	0
8"	3.1	78	20	1
17"	3.2	51	46	1
26"	3.3	35	62	2
Effluent	3.3	26	72	4

end of the main feed line using a hypodermic syringe. The fluidized bed did not recover under this condition.

At this time the acid feed was discontinued, reestablishing an influent pH of 6.5. Aeration within the fluidized bed was also discontinued in an effort to reduce any shearing action brought about by oxygen bubbles moving through the bed. Since aeration had been eliminated, a dissolved oxygen lake probe was placed above the surface of the sand bed in order to monitor the effluent DO. During the next week the effluent DO dropped from 8.5 mg/l to zero indicating an increase in biological activity. As the effluent DO approached zero, the sand began to develop a black coating. In the previous phases of the investigation the outer layer of biological mass was consistently light yellow in color while the inner layer was typically black. Analysis of an effluent sample showed the thiosulfate concentration to be 80 mg/l. Sulfate and polythionate concentrations were 15 mg/l and 4 mg/l, respectively. The effluent pH was 6.3.

Thiosulfate oxidation under anaerobic conditions was briefly evaluated by substituting the nitrogen source, ammonium sulfate, with ammonium nitrate (NH_4NO_3). In an anaerobic environment the nitrate ion is used by T. denitrificans and T. thioparus as the terminal electron acceptor (40, 68). Sufficient ammonium nitrate was added to the concentrated feed tank to give a final influent concentration of 30 mg/l as nitrogen. Analysis of an effluent sample 24 hours later showed the thiosulfate and sulfate concentrations to be 90 and 7 mg/l respectively. Polythionate was not detected. The effluent pH was 6.4 and the DO had increased to

0.5 mg/l. Since ammonium nitrate did not appear to improve the performance of the reactor, ammonium sulfate was reestablished as the nitrogen source and aeration was resumed. The reactor was maintained in continuous operation for an additional two weeks without further improvement. At the end of the two week period, operation of the fluidized bed was discontinued.

A summary of the thiosulfate profiles at the various pH conditions is presented in Figure 22. This illustration shows that down to a pH of 4.0 the incremental decrease of the influent pH consistently improved the removal of thiosulfate. At pH 2.9 thiosulfate removal was slow through the first 17 inches of the bed but ultimately approached the same degree of removal as pH 4.0. Comparing the polythionate profiles at the various acidic conditions shows that polythionate production uniformly increased as the influent pH was lowered. This comparison is shown in Figure 23.

Conversely, sulfate production generally decreased as the influent pH was lowered. The exception to this general trend occurred at pH 4.0 when sulfate production exceeded that of pH 5.2. The sulfate profiles are illustrated in Figure 24. A comparison of Figures 23 and 24 shows that the principal thiosulfate oxidation product shifted from sulfate to polythionate as the influent pH was lowered. At pH 6.5 sulfate production exceeded the production of polythionate but at pH 6.0 the difference was not appreciable. Below a pH of 6.0 polythionate was always the major oxidation product.

A summary of the pH profiles in Figure 25 shows that at the

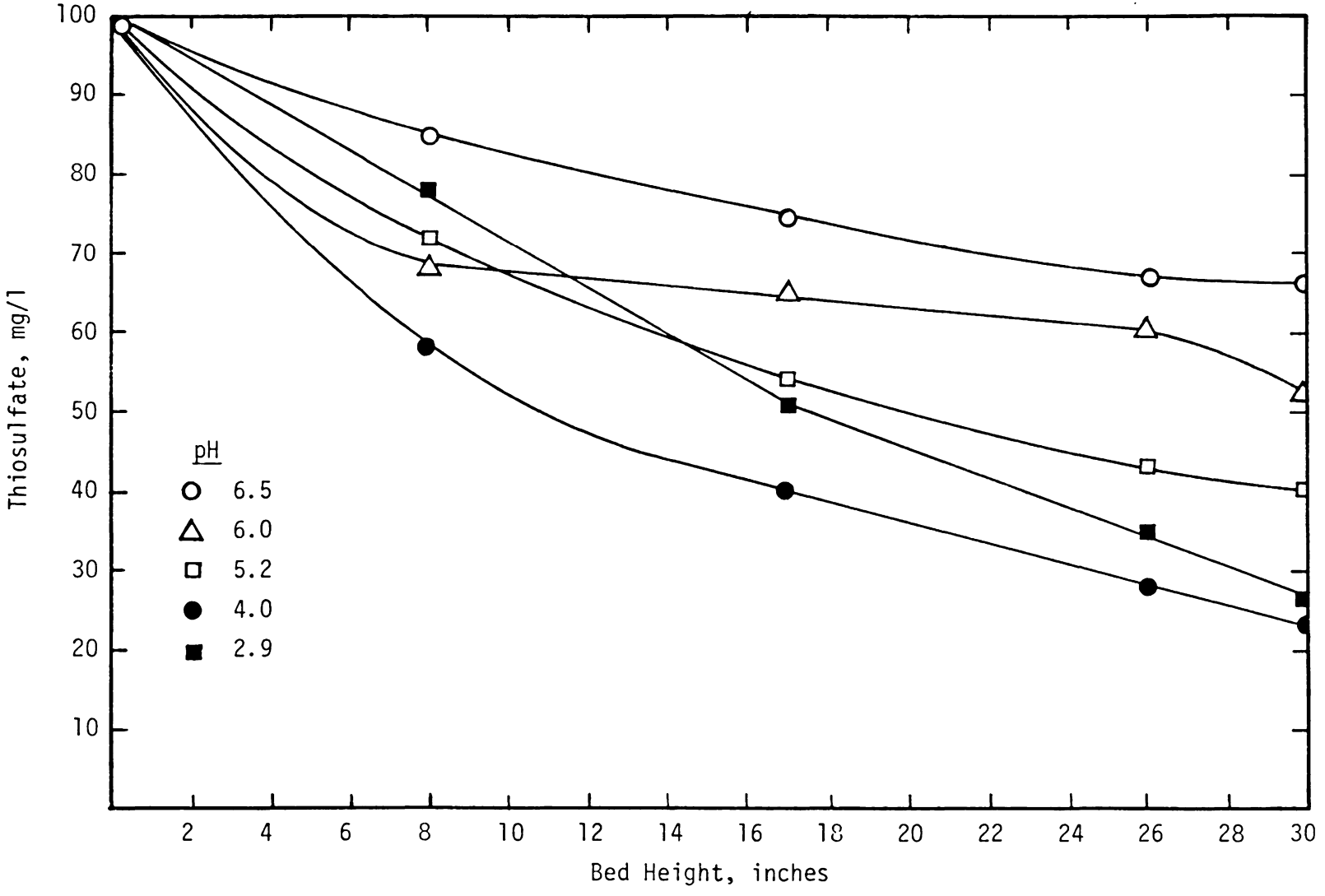


Figure 22. Thiosulfate Profile in the Fluidized Bed at Various pH conditions

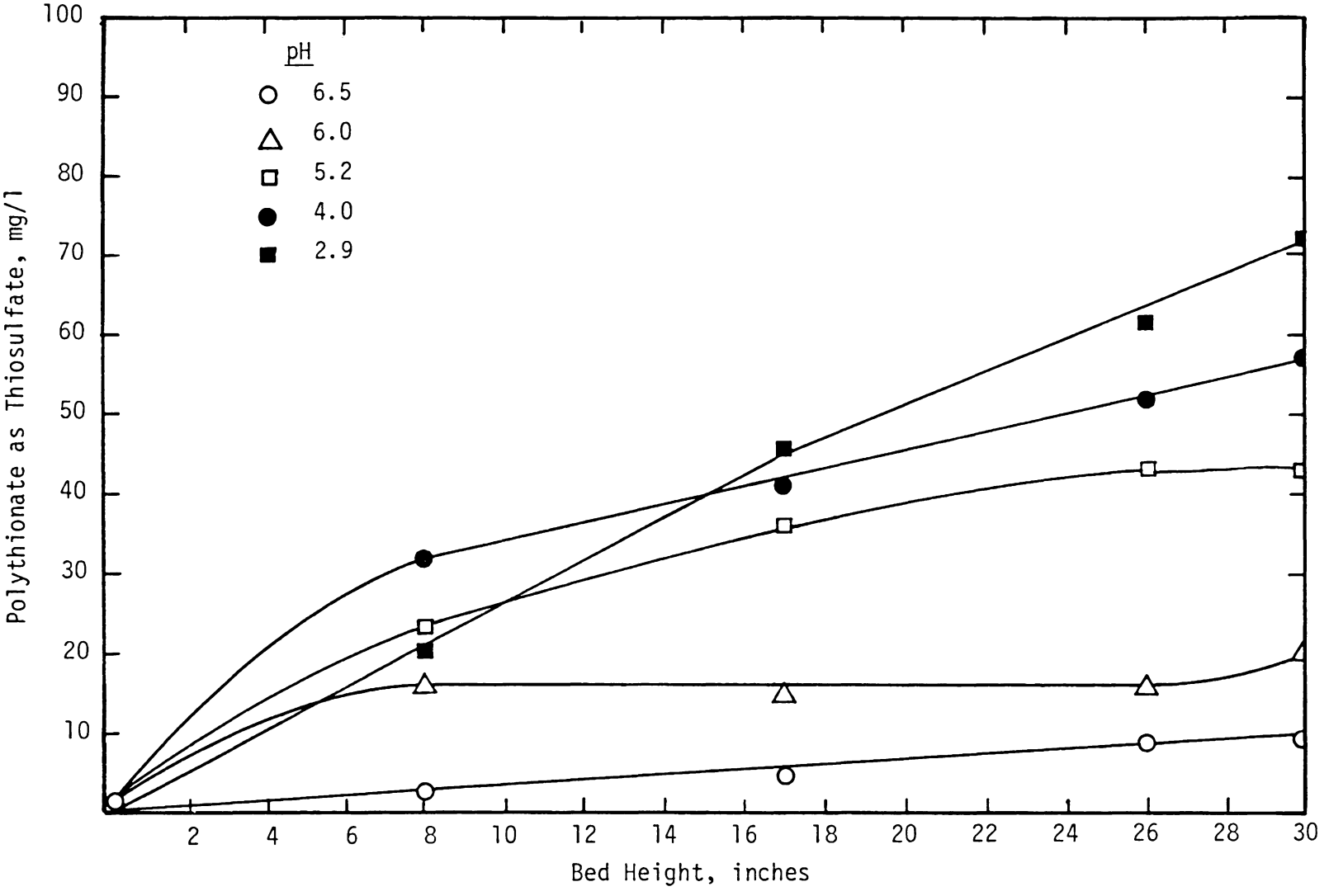


Figure 23. Polythionate Profile in the Fluidized Bed at Various pH conditions

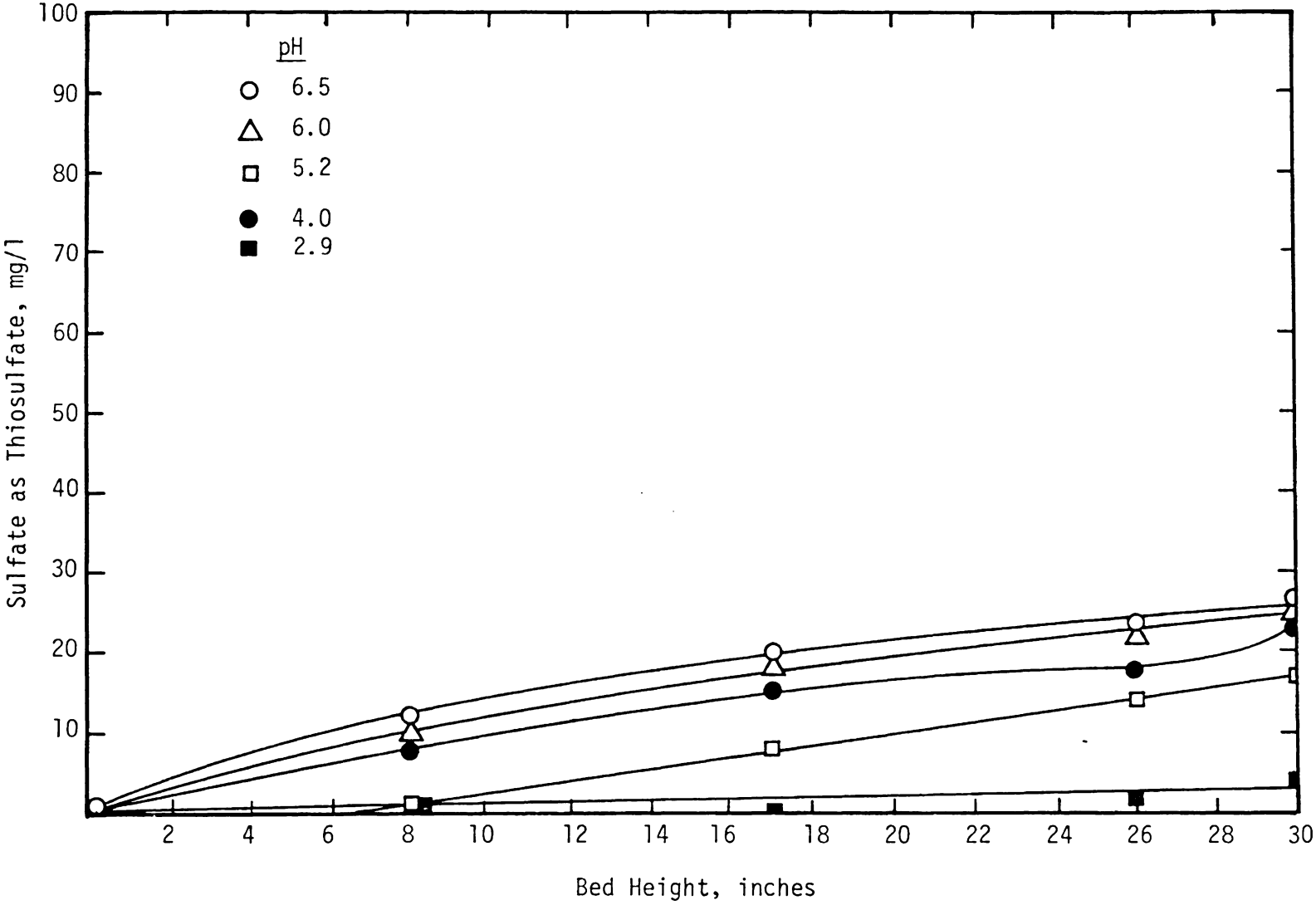


Figure 24. Sulfate Profile in the Fluidized Bed at Various pH Conditions

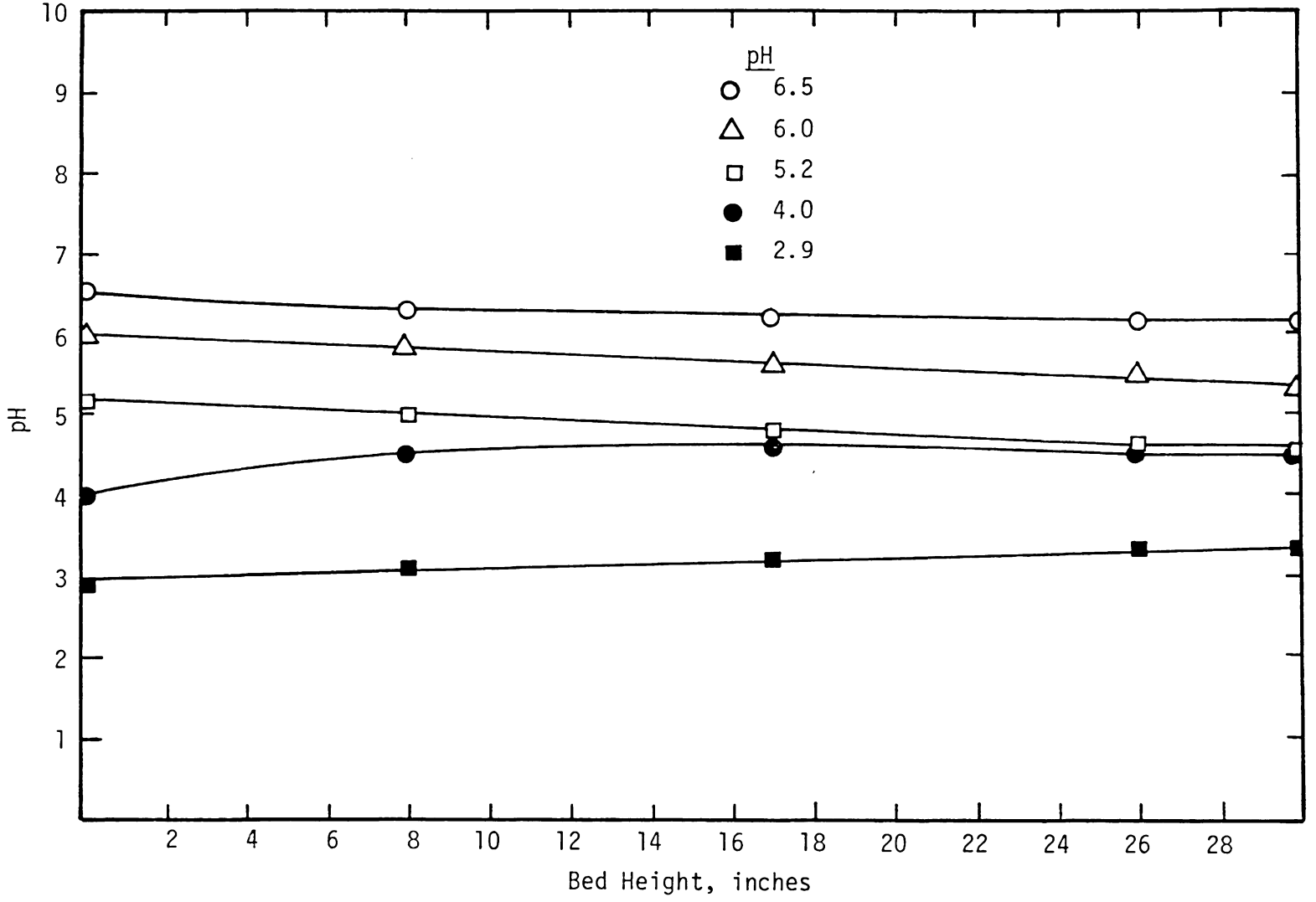


Figure 25. pH Profile in the Fluidized Bed of Various pH Conditions

influent pH levels of 6.5, 6.0, and 5.2, acid was progressively being produced along the length of the bed. At pH levels of 4.0 and 2.9, the pH consistently increased due to the production of polythionate.

The distribution of biomass through the fluidized bed at the various pH conditions is shown in Figure 26 and generally summarized in Table 10. With the exception of lowering the influent pH from 4.0 to 2.9, decreasing the influent pH resulted in an increase of biomass. The largest incremental increase occurred at pH 4.0. At pH levels of 6.5 and 6.0 the distribution of biological mass was very similar but on a comparative basis the total mass at pH 6.0 was greater than that of pH 6.5 by seven percent. At pH 5.2 the distribution shifted. However, the total biological mass was only three percent greater than that of pH 6.0. Decreasing the influent pH from 5.2 to 4.0 resulted in a 22 percent increase in total biomass. Due to the washout which occurred at pH 2.9, volatile solids were not measured. Using a void space volume of 530 ml, the volatile solids concentrations ranged from 20,000 to 27,000 mg/l.

During the time that the fluidized bed was operating continuously, the effluent was discharged into a 1500 ml beaker situated in the laboratory sink. The purpose of the beaker was to prevent sand from being discharged directly into the drain. Very little sand was ever discharged, but as the investigation progressed the sides of the beaker became heavily coated with biological growth. Frequent microscopic observations of samples from the reactor and the beaker revealed short, Gram negative, motile rods typical of the Thiobacilli.

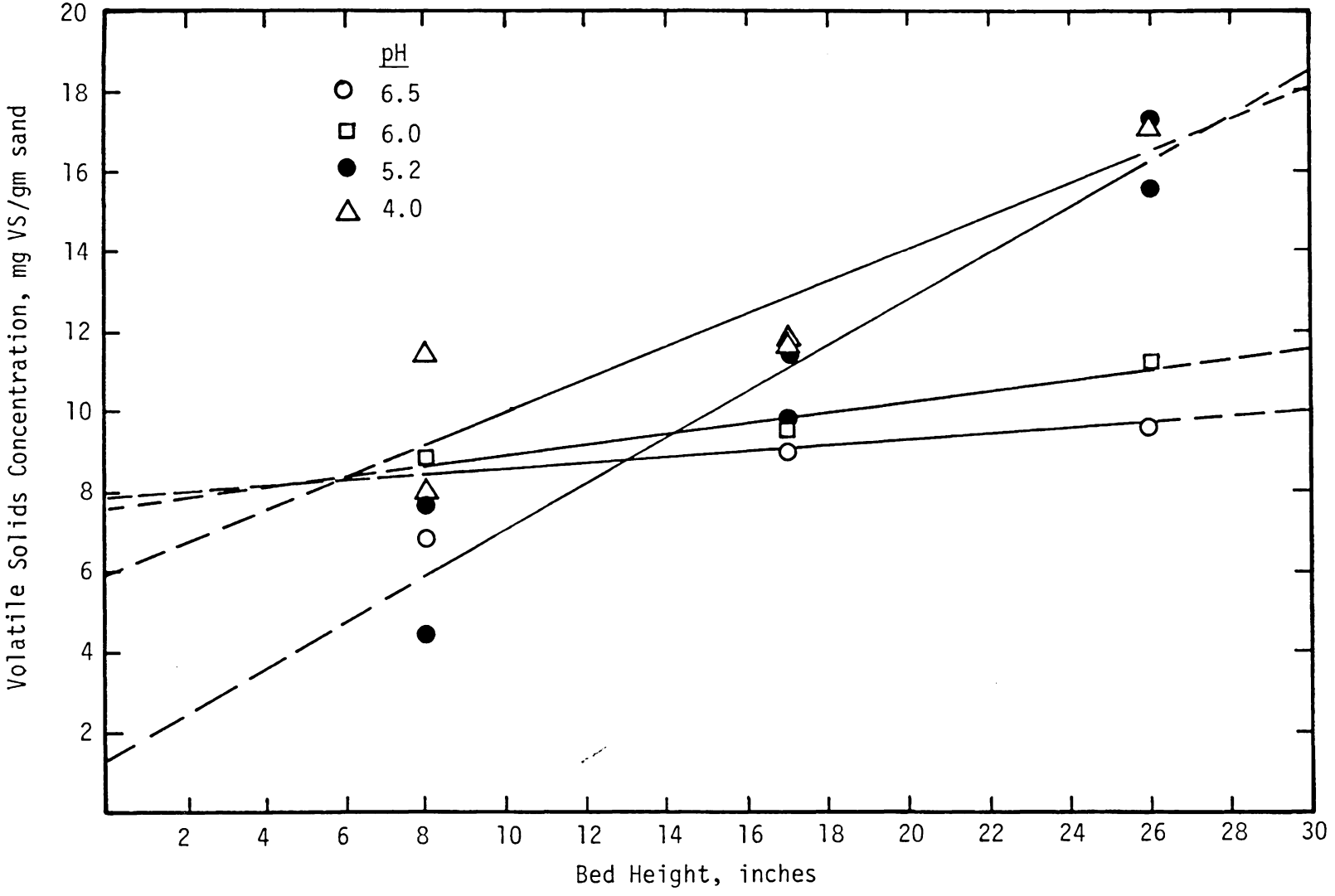


Figure 26. Distribution of Biomass Through the Fluidized Bed

Table 10

Summary of Volatile Solids Data

pH	Total biomass mgVS	Incremental percent increase	Volatile solids concentration mg/l
6.5	10,700	--	20,000
6.0	11,475	7	21,700
5.2	11,840	3	22,300
4.0	14,435	22	27,200
2.9	--	--	--

As shown in Figure 26, biological growth was normally very dense in the lower regions of the bed. At times this feature presented problems because the gravel tended to agglomerate into a solid mass causing the flow to decrease and inducing a slugging action in the bed. Aerating the bottom of the reactor with several short blasts of compressed air usually released the agglomerated mass.

Throughout the investigation, while performing the volatile solids analysis, it was consistently noticed that the odor of burning sulfur was given off when the samples were removed from the drying oven. The biological mass was not analyzed for elemental sulfur.

A summary of the oxygen uptake rates is presented in Figure 27. Simple linear regression was used to fit a straight line to the data. This illustration shows that down to a pH of 4.0 the oxygen uptake rate decreased as the influent pH was lowered. Due to the washout of the biological mass, the oxygen uptake rate was not measured at pH 2.9.

As a final summary, the overall efficiencies of the fluidized bed are illustrated in Figure 28 in terms of thiosulfate removal, polythionate production, and sulfate production. Simple linear regression was used to fit a straight line to the sulfate data. This figure shows that thiosulfate removal reached a maximum efficiency of 75 percent at pH 4.0. Polythionate production continuously increased from nine percent to 72 percent as the pH was made more acidic while sulfate production decreased from 27 percent to four percent. It is interesting to note the similarity between sulfate production in Figure 28 and oxygen uptake

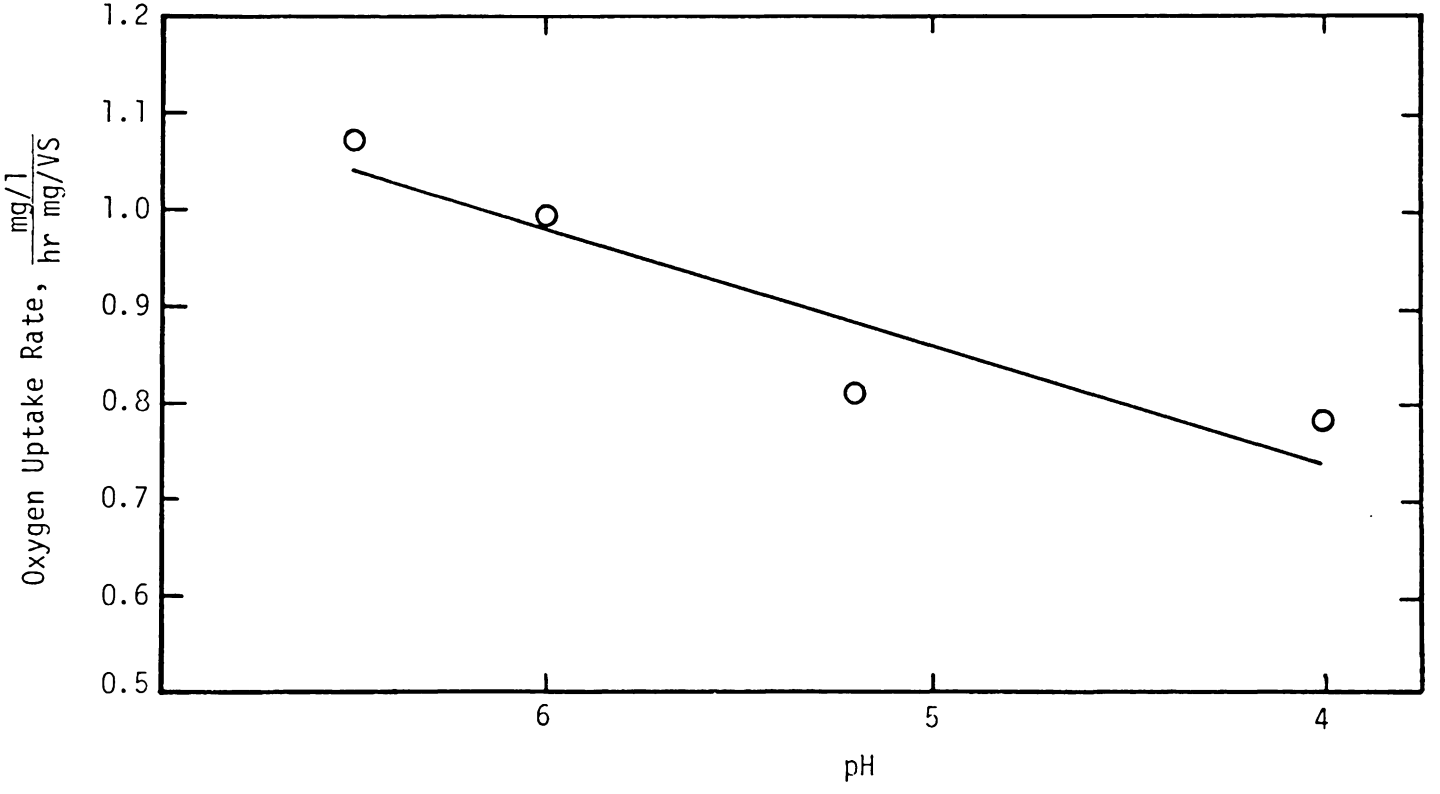


Figure 27. Influence of pH on Oxygen Uptake Rate

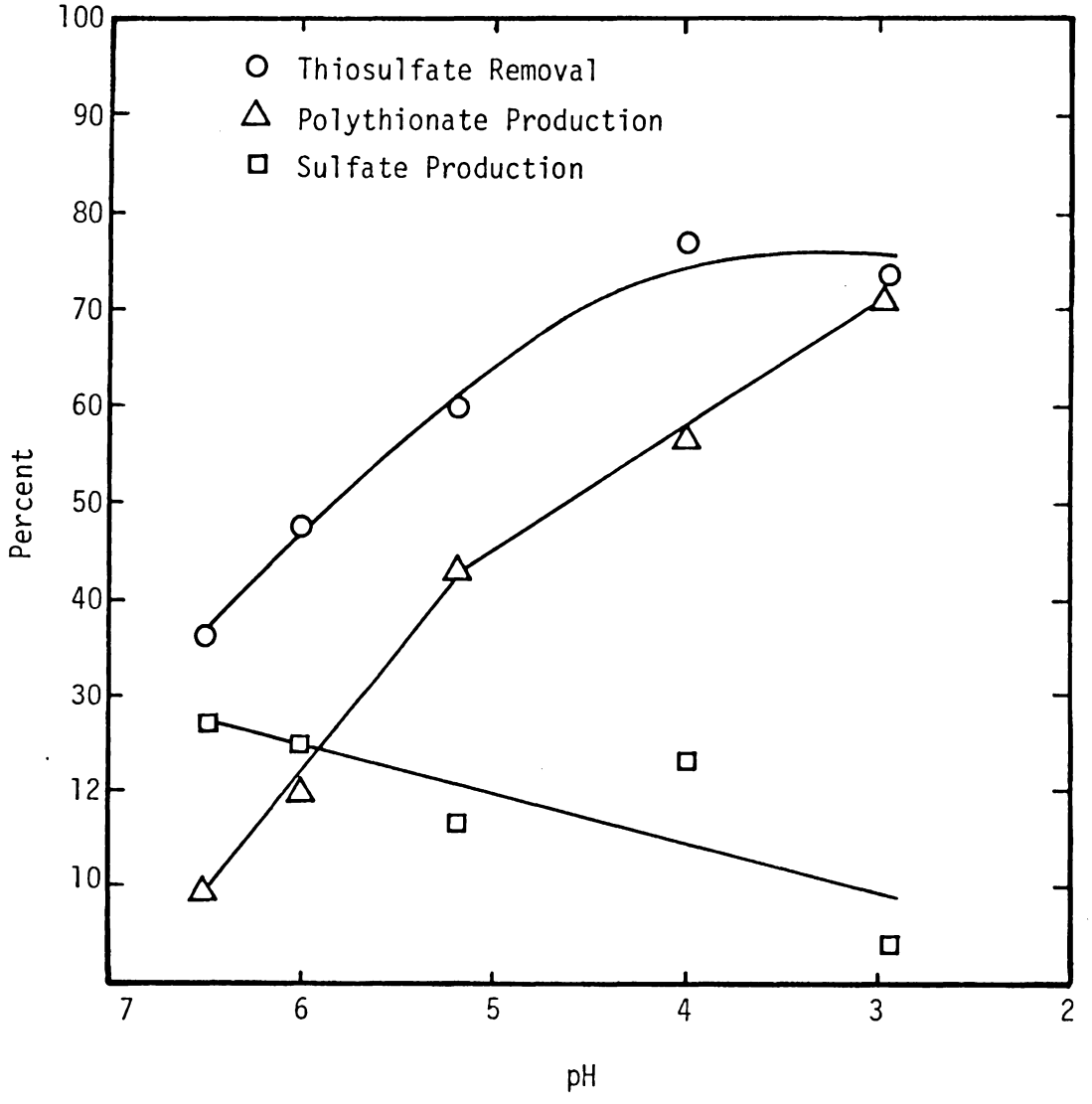


Figure 28. Fluidized Bed Efficiency

rates in Figure 27. Sulfate production and the oxygen uptake rate both decreased as the influent pH became more acidic.

Acid Decomposition of Thiosulfate

Acid decomposition of thiosulfate under purely chemical conditions was investigated with the intent of evaluating the effects of pH on various concentrations of thiosulfate. The actual experiment was conducted in a series of batch tests by subjecting various concentrations of thiosulfate to different levels of pH. Concentrations of thiosulfate which were evaluated included 100, 200, 500, 750, and 1000 mg/l. Each test was examined by monitoring thiosulfate, polythionate, and sulfate. Results of the experiment are summarized in Table 11.

The effects of pH on thiosulfate are shown in Figure 29 through 33. In all cases lowering the pH resulted in thiosulfate decomposition. With the exception of 1000 mg/l, each thiosulfate concentration produced polythionate following acidification. Throughout the experiment sulfate was never detected as a decomposition product.

Between pH levels of 6.5 and 3.0, thiosulfate decomposition proceeded gradually and normally averaged 30 percent. The exception to this occurred at a thiosulfate concentration of 1000 mg/l where lowering the pH down to 3.0 only destroyed six percent of the initial thiosulfate. Below a pH of 3.0 decomposition increased very sharply. At thiosulfate concentrations of 100 and 200 mg/l, acidification to pH 2.0 resulted in 50 percent destruction of the initial thiosulfate. Thiosulfate concentrations of 500, 750, and 1000 mg/l showed 75 percent decomposition at

Table 11

Acid Decomposition of Thiosulfate

pH	$S_2O_3^{-2}$ mg/l	$S_2O_3^{-2}$ Decomposition %	$S_xO_6^{-2}$ mg/l	$S_2O_3^{-2}$ Decomposed to $S_xO_6^{-2}$ %	$S_2O_3^{-2}$ Balance mg/l
6.9	98	--	0	--	98
5.8	92	6	6	6	98
5.0	84	14	14	14	98
4.0	74	24	26	26	100
3.0	66	33	34	34	100
2.0	48	51	35	35	83
1.0	9	91	49	49	58
7.0	196	--	0	--	196
6.0	188	4	4	2	192
4.8	184	6	6	3	190
4.0	178	9	13	7	191
3.0	161	18	28	14	189
2.0	98	50	15	--	113
1.0	10	95	55	28	65
6.4	510	--	0	--	510
5.9	469	8	0	0	469
4.7	443	13	31	6	474
4.0	434	15	38	7	472
3.0	362	29	63	12	425
2.0	141	72	65	13	206
1.0	11	98	119	24	130
6.4	738	--	0	--	738
6.0	672	9	31	4	708
4.8	601	19	111	15	712
4.0	594	20	110	15	704
3.0	510	31	163	22	675
2.0	177	76	209	28	404
1.0	12	98	209	28	394
6.5	1000	--	0	0	1000
6.0	973	3	0	0	973
4.7	992	1	0	0	992
3.9	990	1	0	0	990
3.0	938	6	0	0	938
2.0	249	75	0	0	249
1.0	30	97	0	0	97

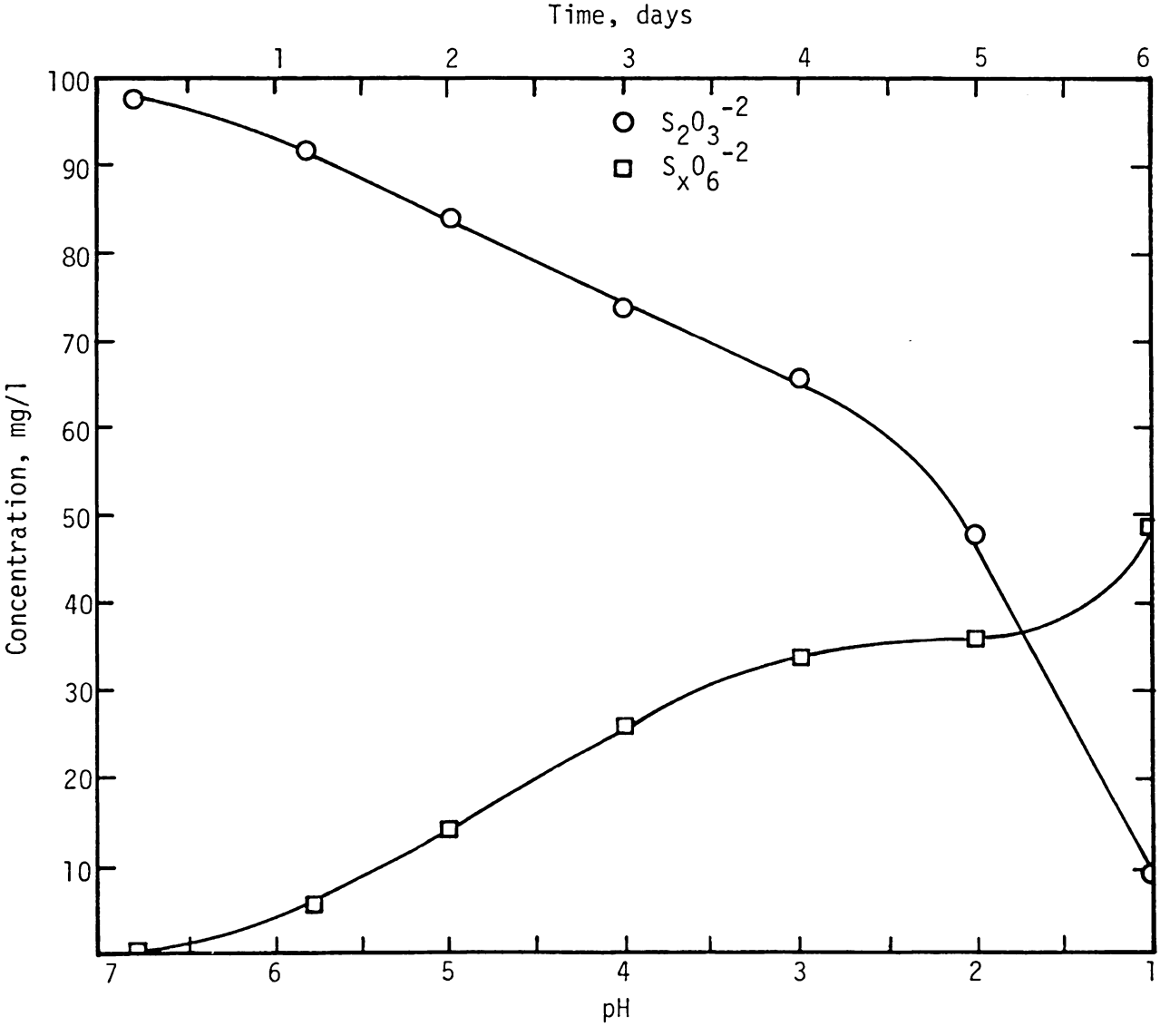


Figure 29. Effect of pH on 100 mg/l Thiosulfte

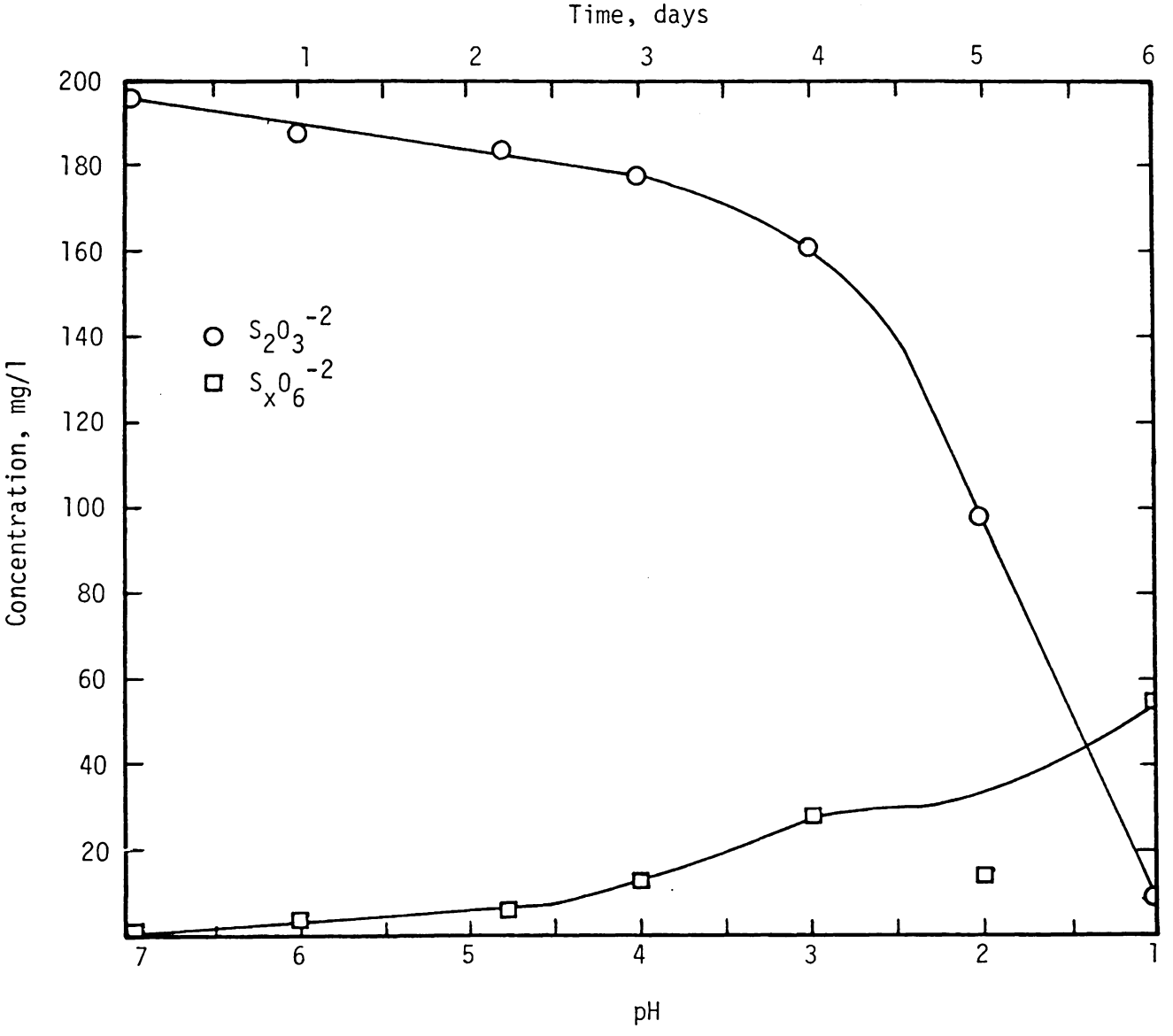


Figure 30. Effect of pH on 200 mg/l Thiosulfate

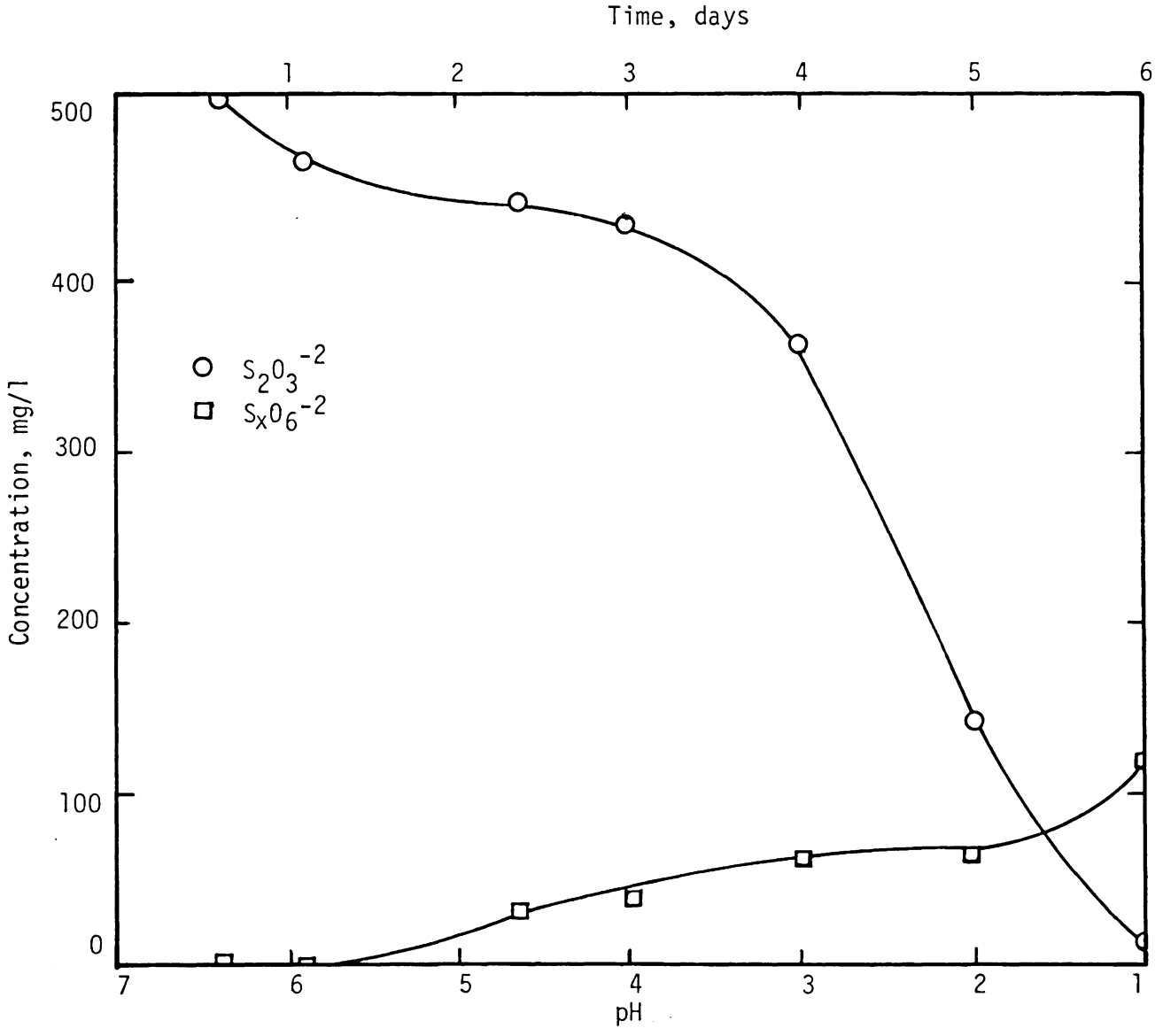


Figure 31. Effect of pH on 500 mg/l Thiosulfate

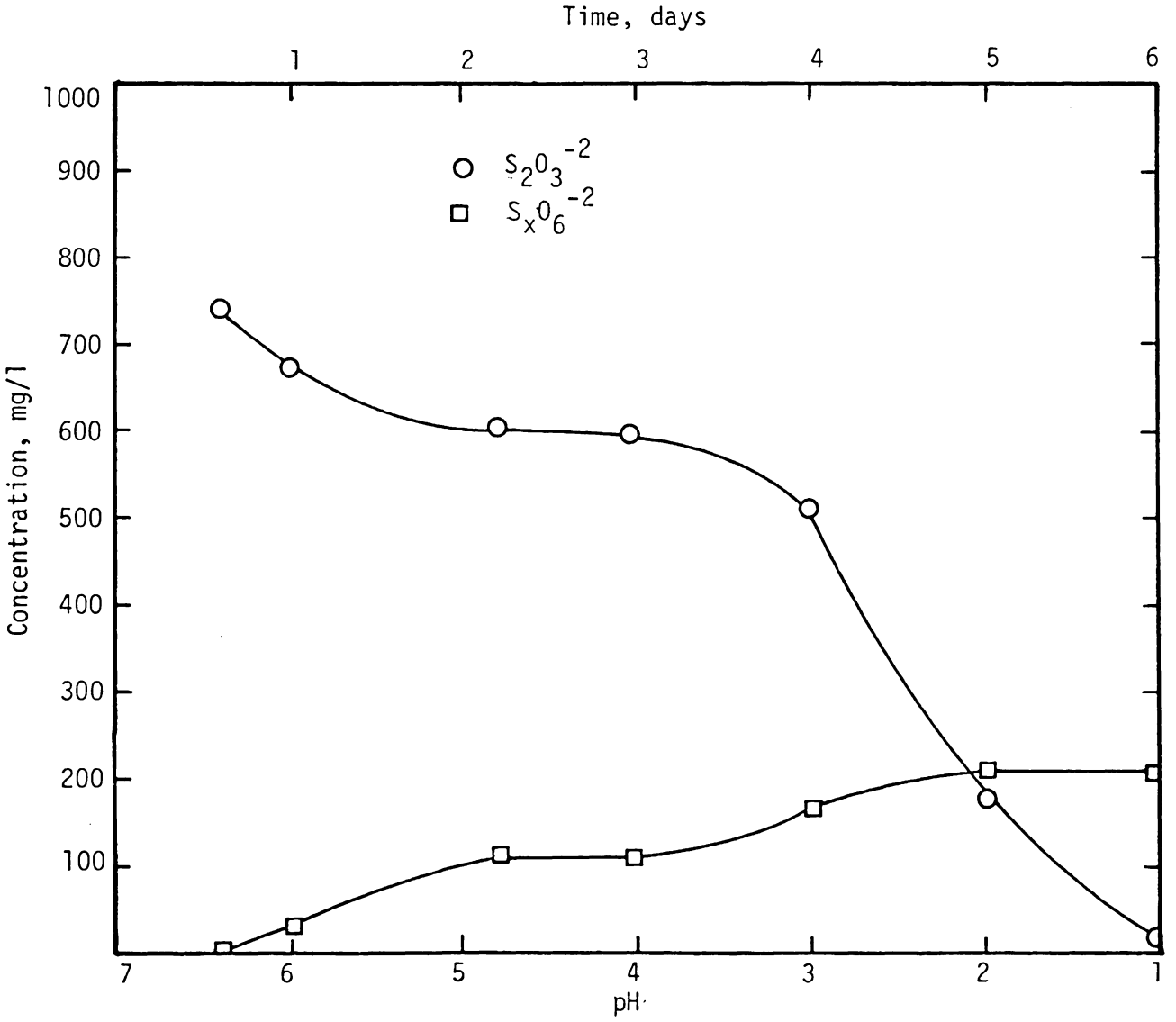


Figure 32. Effect of pH on 750 mg/l Thiosulfate

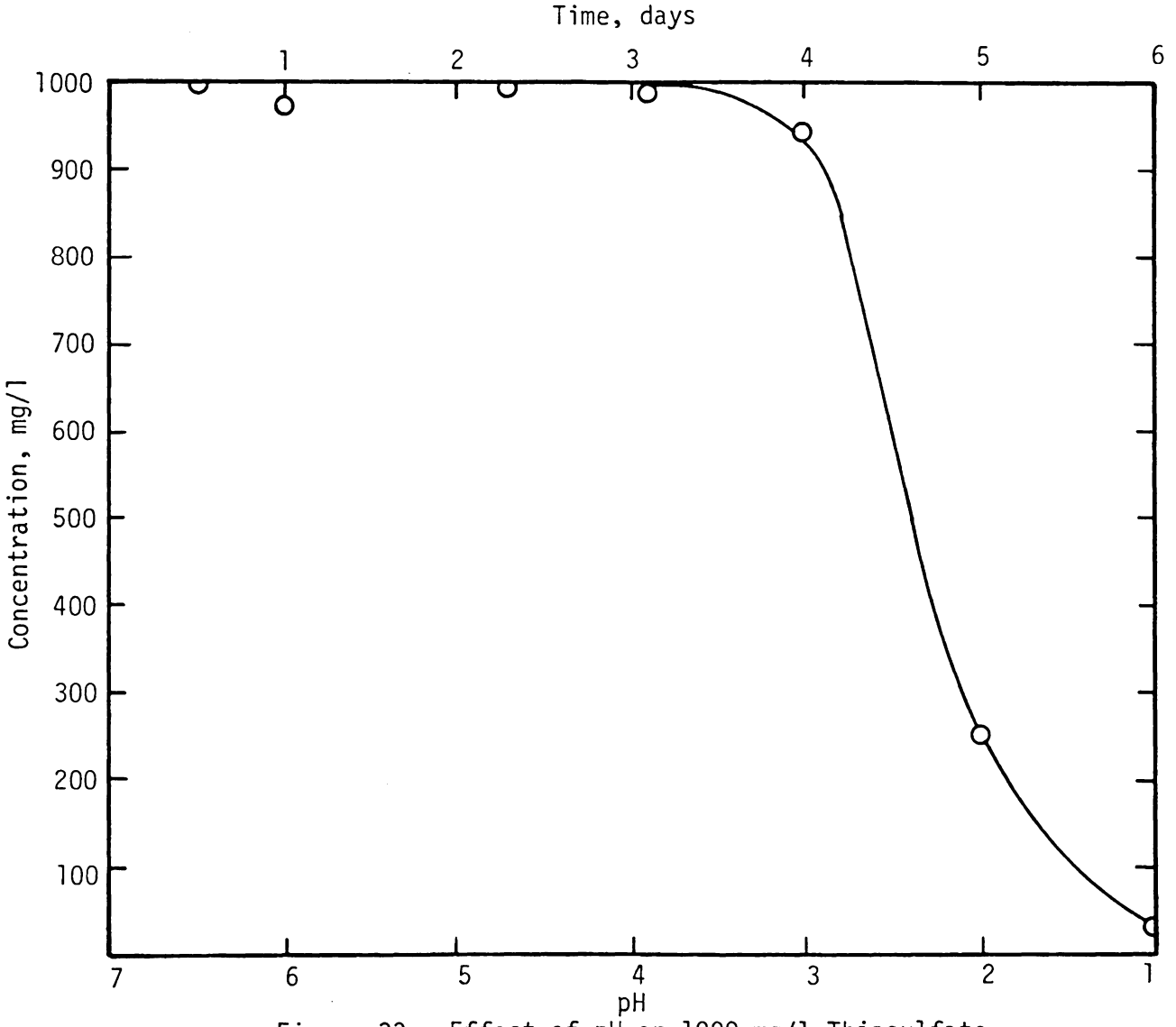


Figure 33. Effect of pH on 1000 mg/l Thiosulfate

a pH of 2.0. Reducing the pH to 1.0 resulted in better than 90 percent decomposition at all thiosulfate levels.

As thiosulfate decomposed by gradually lowering the pH, the polythionate concentration typically increased. However, on a percentage basis, less thiosulfate was converted to polythionate as the initial thiosulfate concentration increased. At a concentration of 100 mg/l, 49 percent of the initial thiosulfate was converted to polythionate by lowering the pH to 1.0. At concentrations of 200, 500, and 750 mg/l, approximately 25 percent of the initial thiosulfate was converted to polythionate at a pH level of 1.0. Polythionate was not detected at a thiosulfate concentration of 1000 mg/l.

A summary of the acid decomposition experiment is shown in Figure 34. The most striking feature of this illustration is that, regardless of the initial thiosulfate concentration, once the pH was lowered below 3.0, acid decomposition proceeded very rapidly.

As shown in Table 11, the thiosulfate balance down to pH 3.0 generally accounted for most of the initial thiosulfate as polythionate and thiosulfate. At pH levels of 2.0 and 1.0, polythionate and thiosulfate did not provide a complete balance. While performing the experiment it was consistently observed that acidification to a pH of 2.0 caused the solutions to become very turbid. Microscopic observations of samples acidified to pH 2.0 and 1.0 revealed the presence of particulate matter which resembled elemental sulfur crystals. Based on the incomplete thiosulfate balance and the results of previous investigations (3,11,17)

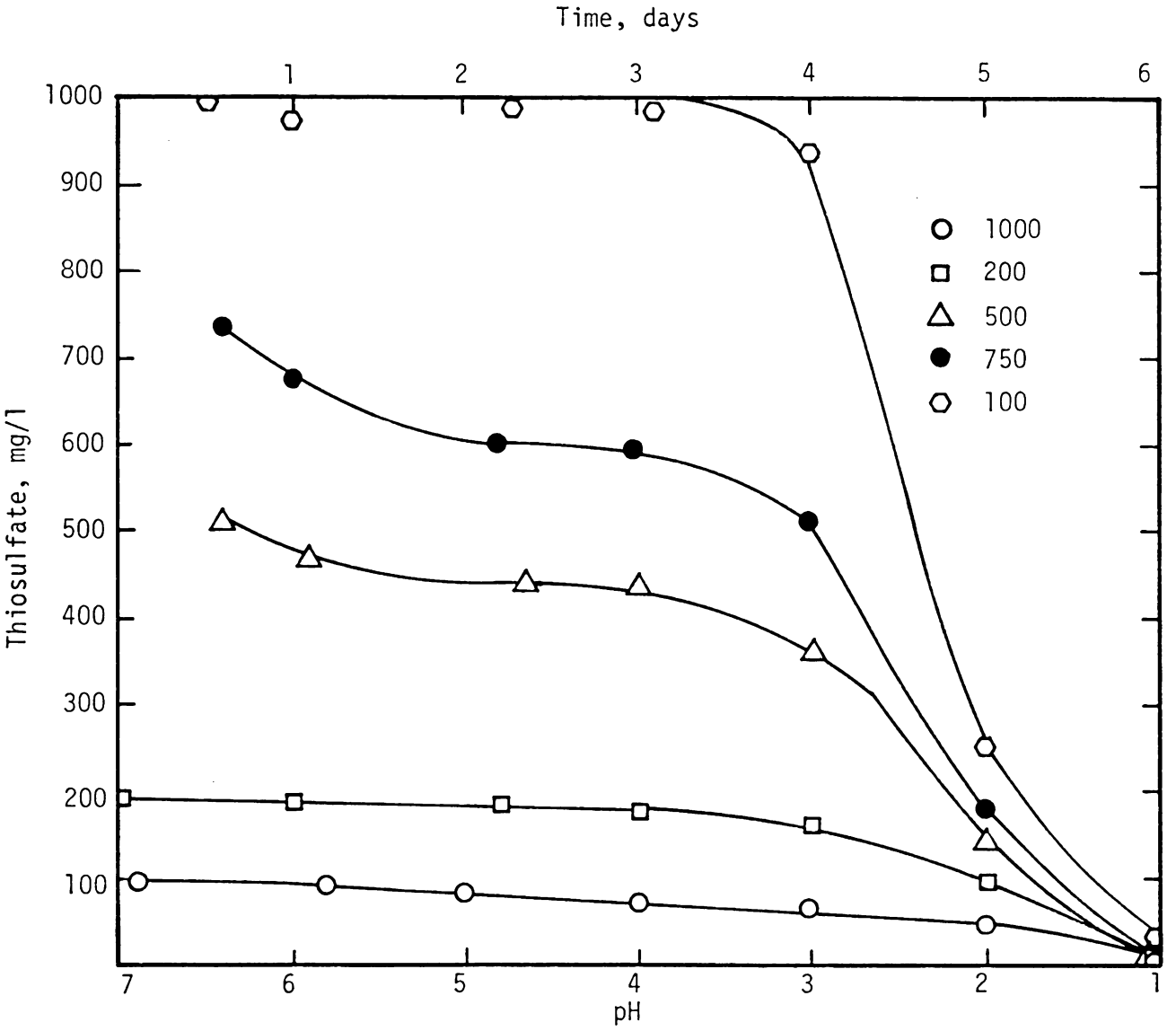


Figure 34. Acid Decomposition of Thiosulfate

the particulate matter was assumed to be crystals of elemental sulfur. A sketch of a typical sulfur crystal is shown in Figure 35. Although the inclusion of elemental sulfur would undoubtedly aid in completing the thiosulfate balance, the possibility exists that other sulfur compounds such as sulfur dioxide, hydrogen sulfide, and sulfur oils were also produced.

Influence of Trace Nutrients on Oxygen Uptake

The influence of trace nutrients on oxygen uptake was evaluated by comparing the oxygen uptake rates of the isolated Thiobacillus culture using the fluidized bed synthetic feed and the synthetic feed supplemented with trace nutrients. The supplementary trace nutrients consisted of the following elements: zinc, iron, molybdenum, copper, and cobalt. Oxygen uptake rates under various pH conditions and a thiosulfate concentrations of 100 mg/l were measured at 20°C. The pH conditions ranged between 6.5 and 3.0. Endogenous oxygen uptake rates were also measured.

The results of this experiment are summarized in Figure 36 and Table 12. Generally, endogenous oxygen uptake rates were lower when the isolated Thiobacillus culture was supplemented with the trace nutrients. However, at pH levels of 6.5 and 3.0, the endogenous oxygen uptake rates were essentially the same under both conditions. Figure 36 shows that pH also influenced the rate of endogenous oxygen uptake. Normally, as the pH decreased the endogenous oxygen uptake rate also decreased.

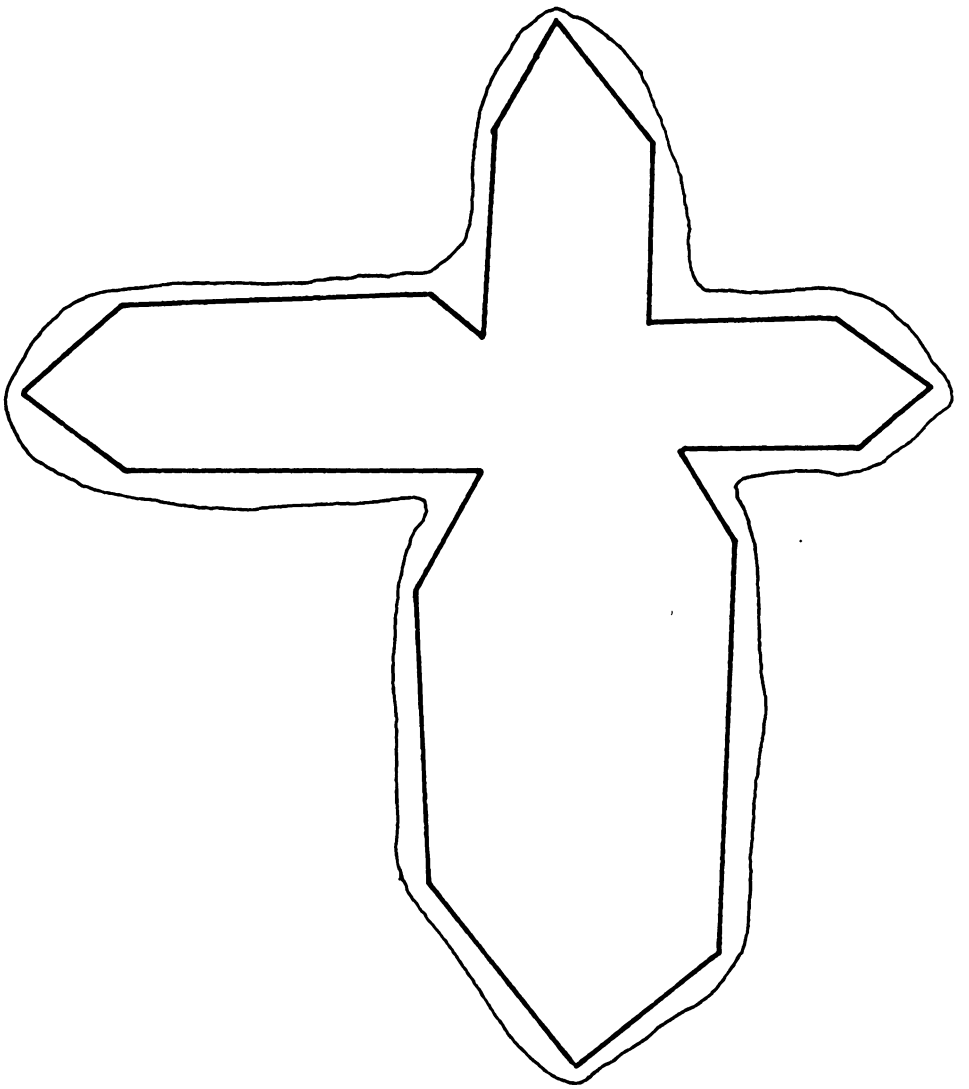


Figure 35. Typical Sulfur Crystals From Acid Decomposition of Thiosulfate (not to scale)

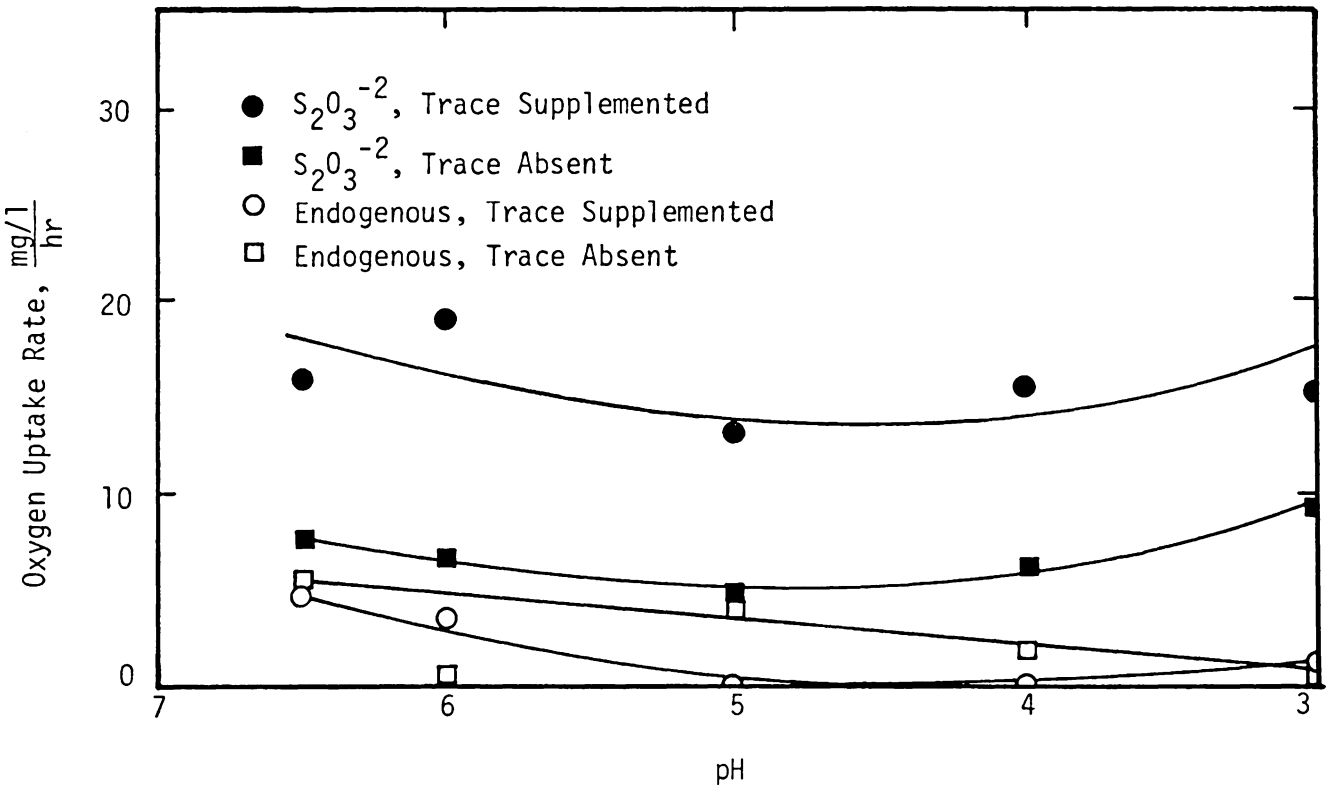


Figure 36. Influence of Trace Nutrients on Oxygen Uptake

Table 12

Influence of Trace Nutrients on Oxygen Uptake

pH	Endogenous O_2 uptake rate mg/l O_2/hr	Trace-Endogenous O_2 uptake rate mg/l O_2/hr	$S_2O_3^{-2}$ O_2 uptake rate mg/l O_2/hr	Trace $S_2O_3^{-2}$ O_2 uptake rate mg/l O_2/hr	$\frac{\text{Trace}-S_2O_3^{-2}}{S_2O_3^{-2}}$
6.5	0.54	0.47	0.77	1.60	2.08
6.0	0.05	0.34	0.67	1.89	2.82
5.0	0.40	0	0.49	1.31	2.67
4.0	0.18	0	0.62	1.54	2.48
3.0	0.05	0.11	0.93	1.52	1.63

Figure 36 shows that the trace nutrients significantly influenced the rate of oxygen uptake during thiosulfate oxidation. Oxygen uptake rates at a thiosulfate concentration of 100 mg/l were higher when the culture was supplemented with the trace nutrients. A comparison of the two rates, as listed in Table 12, shows that normally, the trace-supplemented oxygen uptake rate was 2.5 times higher. The rate of oxygen uptake was also influenced by pH. Under both conditions, lowering the pH from 6.5 to 3.0 resulted first in a decrease of the oxygen uptake rates followed by an increase as the pH was lowered below 5.0.

Influence of pH on Thiosulfate BOD_{22}

The influence of pH on thiosulfate BOD_{22} was evaluated by using the Hach BOD apparatus. BOD_{22} measurements were made on a thiosulfate concentration of 1000 mg/l at pH conditions ranging from 6.5 to 3.0. Each test was seeded with the isolated Thiobacillus culture at a thiosulfate F/M of ten.

The results of this experiment, which are presented in Figure 37, show that pH significantly influenced measurable BOD_{22}. Decreasing the pH from 6.5 to 3.0 progressively inhibited BOD exertion. The most significant effect occurred at pH levels of 4.0 and 3.0 where the BOD_{22} was almost completely suppressed. As seen from these results, five days was an insufficient amount of time to conduct the test. Each BOD test was incubated for 22 days in order to measure the maximum effect of pH on BOD.

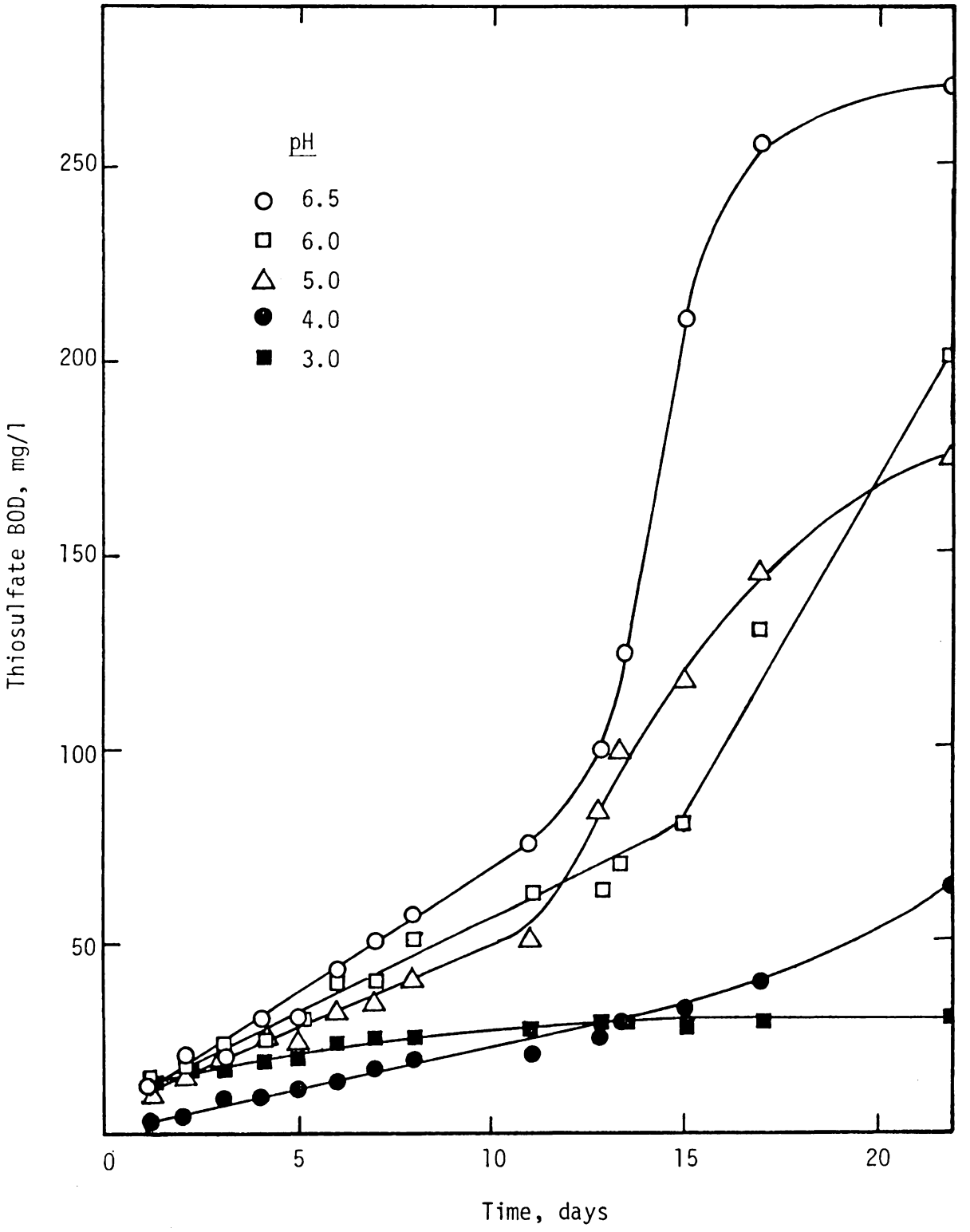


Figure 37. Influence of pH on Thiosulfate BOD

Precipitation of Thiosulfate with $BaCO_3$

Thiosulfate precipitation with barium carbonate was investigated by performing a jar test similar to those used in water softening experiments. Concentrations of barium carbonate evaluated included: 1, 5, 10, 15, 20, and 25 gm/l. Thiosulfate was set at an initial concentration of 1000 mg/l.

Measuring the thiosulfate concentrations at the end of the experiment showed that thiosulfate remaining in solution averaged 960 mg/l. Barium carbonate failed to precipitate thiosulfate.

DISCUSSION OF RESULTS

The following section is a discussion of the experimental results obtained during this investigation.

Nutrient Requirements of the Isolated Thiobacillus Culture

The nutrients which were studied in detail in terms of bacterial requirement were nitrogen and phosphorus.

Nitrogen requirements. Using a series of batch tests, the ammonia nitrogen requirements of the isolated Thiobacillus culture were shown to be relatively low. This conclusion is based on the manner in which ammonia nitrogen concentrations ranging from five to 35 mg/l, influenced the oxidation of thiosulfate. At each ammonia nitrogen concentration, thiosulfate oxidation was influenced to the same extent.

Based on the results of this experiment it appears than an ammonia nitrogen concentration of 5 mg/l should be sufficient for successful oxidation of thiosulfate at a concentration of 1000 mg/l. Expressed as the ratio of $S_2O_3^{-2}$ to N, the desirable ratio for a thiosulfate waste treatment system would be 1000 to 5. This value is in excellent agreement with the ratio extablished by Duncan (27) for a bio-disc system treating a thiosulfate waste. The established ratio was 1000 to 6.2. A previous investigation by Kreye (25) also established that the nitrogen requirements of a Thiobacillus activated sludge system were relatively low. However, a precise numerical relationship between thiosulfate and

nitrogen was not defined in that study.

Phosphorus requirements. Batch test evaluations on the phosphorus requirements of the isolated Thiobacillus culture have demonstrated that phosphorus can either limit, improve, or suppress the oxidation of thiosulfate. At a concentration of one mg/l, phosphorus was inadequately supplied and limited the oxidation of thiosulfate. Increasing the concentration to ten and 100 mg/l provided sufficient phosphorus to improve thiosulfate oxidation. A further increase to 1000 mg/l caused the phosphorus level to be excessive to the point that thiosulfate oxidation was suppressed. Polythionate and sulfate production responded in a similar manner.

Typically thiosulfate oxidation results in decreasing the pH of the culture media to an extremely acid condition. This result was observed in each batch test, except at a phosphorus concentration of 1000 mg/l where the pH was firmly buffered at 5.5. The inability of the isolated Thiobacillus culture to lower the pH at this phosphorus level suggests that the suppressive action associated with the high phosphorus concentration was due to the buffering capacity.

Suppressed buffering at a phosphorus concentration of 1000 mg/l is demonstrated by following the manner in which thiosulfate oxidation and polythionate and sulfate production progressed throughout the experiment. During the first 120 hours of incubation, the above reactions at a phosphorus concentration of 1000 mg/l, proceeded at a faster rate than those at the lower phosphorus levels. Beyond 120 hours, the overall oxidation of thiosulfate at 1000 mg/l phosphorus began to lag behind the lower phosphorus concentrations, where the overall oxidation continued

to progress as did the lowering of the pH. The inability to lower the pH, due to strong buffering, resulted in suppressed thiosulfate oxidation at a phosphorus concentration of 1000 mg/l.

In the Warburg oxygen uptake experiment, the limiting nature of phosphorus was again established at a concentration of one mg/l but a suppressive effect at a concentration of 1000 mg/l was not demonstrated. Phosphorus concentrations ranging between ten and 1000 mg/l significantly improved the oxygen uptake but the differences in the final results were not appreciable.

Based on the results of the batch and oxygen uptake experiments, it appears that a phosphorus concentration between ten and 50 mg/l should be sufficient for the successful biological treatment of thiosulfate at a concentration of 1000 mg/l. This level is in excellent agreement with the phosphorus requirements established by Kreye (25). While conducting an activated sludge treatability study on a sulfur dye waste, increasing the phosphorus concentration from 5.5 to 14 mg/l was reported to result in excellent thiosulfate removal.

Considering the results of this experiment and the phosphorus requirements established by Kreye (25), operating a thiosulfate waste treatment system at a range of $S_2O_3^{-2}$:P ratios between 1000:10 and 1000:50 should provide an adequate supply of phosphorus. This ratio differs considerably with the ratio established by Duncan (27) for a bio-disc system. His established ratio was 1000:1.6.

Unlike nitrogen, phosphorus was demonstrated to be an influential nutrient in the oxidation of thiosulfate. Providing phosphorus in

either an inadequate or excessive amount could prove to be detrimental to a biological system treating thiosulfate.

By combining the results of the nitrogen and phosphorus experiments it is possible to establish the range of the desirable $S_2O_3^{-2}$:N:P ratios for successful biological treatment of a thiosulfate waste. The ratios should range between 1000:5:10 and 1000:5:50.

Thiosulfate Oxidation in a Fluidized Bed

A laboratory scale, autotrophic fluidized bed was developed for the oxidation of thiosulfate. The reactor was maintained at a hydraulic loading rate of 16.1 gpm/ft^2 and an influent thiosulfate concentration of 100 mg/l. The void space detention time at this loading rate was approximately 0.6 minutes. Through a series of influent pH adjustments, the fluidized bed demonstrated the ability to remove up to 75 percent of the applied thiosulfate, but the intermediate thiosulfate metabolic product, polythionate, proved difficult to oxidize. Due to the high oxygen demand of thiosulfate and the high concentration of volatile solids in the reactor, aeration with high purity oxygen was required to maintain an aerobic environment. Continuous operation of the fluidized bed also verified the preference of Thiobacillus bacteria to attach to a solid surface.

During the initial period of continuous operation the fluidized bed was set at a hydraulic loading rate of 10 gpm/ft^2 and an influent thiosulfate concentration of 1000 mg/l. The void space detention time was approximately one minute. Based on the estimated surface area,

the applied thiosulfate loading rate was approximately 640 mg $S_2O_3^{-2}/ft^2/$ hr. Due to the poor performance of the reactor during this time, a comparison was made between the applied thiosulfate loading rate and the loading rates reported for a bio-disc system oxidizing thiosulfate (27) and a fluidized bed denitrifying domestic sewage (77). The bio-disc unit had a detention time of 34 minutes and was operated at an applied thiosulfate loading rate of 170 mg $S_2O_3^{-2}/ft^2/hr$. The fluidized bed had a void space detention time of approximately five minutes and was operated at an applied nitrate loading rate of approximately 125 mg $NO_3^-/ft^2/hr$. This comparison suggested that the fluidized bed was overloaded, so the applied thiosulfate loading rate was lowered to approximately 100 mg $S_2O_3^{-2}/ft^2/hr$.

Following the decrease in the thiosulfate loading rate the reactor was still not performing satisfactorily. Since an acidic pH is generally known to enhance the biological oxidation of thiosulfate, the fluidized bed was subjected to a series of decreasing pH conditions in an effort to improve thiosulfate oxidation.

The overall efficiency of the fluidized bed was found to be significantly influenced by lowering the influent pH from 6.5 to 2.9. Thiosulfate removal responded favorably by increasing from an efficiency of 36 percent to one of 75 percent. Although not considered a favorable response, polythionate production increased from nine to 72 percent. Sulfate production responded adversely by decreasing from 27 to four percent. Considering that sulfate is the desirable, ultimate end product of thiosulfate oxidation, it is apparent that lowering the influent pH

had an overall, adverse effect on the performance of the fluidized bed.

During the course of lowering the influent pH, the major thiosulfate oxidation product consistently changed from sulfate to polythionate. At a pH of 2.9 the oxidized thiosulfate was almost completely converted to polythionate. This shift indicated an incomplete oxidation of thiosulfate and implied that a high oxygen demand still remained.

Since tetrathionate ($S_4O_6^{-2}$)is known to be the principal polythionate produced during thiosulfate oxidation (1) it is assumed that such was the condition in the fluidized bed. In oxidizing thiosulfate to tetrathionate, the oxidation state of sulfur changes from +2 to +2.5, while a complete oxidation to sulfate changes the sulfur oxidation state from +2 to +6. Thus the conversion of thiosulfate to tetrathionate in the fluidized bed satisfied only a fraction of the total oxygen demand. This result is further suggested by the manner in which the oxygen uptake rate and ultimate sulfate production decreased as the influent pH was lowered. The decreasing oxygen uptake rate indicates less oxygen was being used due to the decrease in sulfate production.

Profiles of pH through the fluidized bed generally followed the pattern established with the shift in the major thiosulfate oxidation product. As the oxidation product changed from the production of sulfate to polythionate, the pH profile shifted from acid production to alkalinity production. The increase in alkalinity was a result of the production of hydroxide ions as shown in the following equation:

$$S_2O_3^{-2} + 0.25\ O_2 + 0.5H_2O \rightarrow 0.5S_4O_6^{-2} + OH^- . \qquad (24)$$

Throughout the investigation lowering the influent pH had the effect of decreasing the oxygen uptake rate and the production of sulfate. Both of these results are evidence that increasing acidity had an adverse effect on biological activity within the fluidized bed. These results also suggest that the isolated Thiobacillus culture consisted predominantly of a strain or strains of Thiobacilli which were suppressed by low acid conditions. The final washout and the inability of the fluidized bed to recover at a pH of 2.9 further indicates that the isolated Thiobacillus culture was not acid tolerant.

Various strains of the Thiobacilli are known to be incapable of tolerating extremely acid conditions. Two of the more commonly found species include T. thioparus and T. denitrificans. Both of these organisms are capable of oxidizing thiosulfate and have an optimum pH near 7.0 (39). These organisms can also be found in domestic sewage (1). Of these two, T. thioparus is the most acid resistant. This organism has been reported to drop the pH as low as 2.8 and still survive (40). It seems that under conditions in which the pH is gradually lowered, a certain adaptive tolerance to an extremely acid condition is developed. The brief evaluation of anaerobic thiosulfate oxidation also suggests the presence of T. thioparus or T. denitrificans, since both of these organisms are known to anaerobically oxidize thiosulfate (40,68).

Although the limited performance of the fluidized bed was most

likely due to the isolation of a nonacid tolerant Thiobacilli, the possibility exists that the ultimate electron acceptor for either aerobic or anaerobic oxidation of thiosulfate could have been limiting. If the ultimate electron acceptor was limiting, this would explain the suppressed performance of the fluidized bed at a pH of 6.5.

Observations that the internal layer of the attached biomass was black, as was the sand coating during the brief evaluation of anaerobic oxidation of thiosulfate, indicate that an anaerobic layer did exist within the biofilm. Considering the requirement for nitrate as the ultimate electron acceptor in anaerobic oxidation of thiosulfate, and the fact that nitrate was not supplied, suggests that the lack of nitrate could have limited the complete oxidation of thiosulfate.

Oxygen could also have been the limiting species. Owen and Williamson (79) have verified the prediction by Williamson and McCarty (70) that oxygen at typical dispersed growth concentrations can limit the kinetics of aerobic biofilms. Considering the DO concentration in the fluidizing bed, which ranged between two and three mg/l, oxygen could also have limited the complete oxidation of thiosulfate.

Determination of the distribution of the biological mass through the fluidized bed showed that the solids concentrations were higher in the upper regions of the bed. This result was caused by the sand grains rising due to becoming less dense as the biological growth developed. Although the sand grains rose to different levels of the bed as they became less dense, a heavy growth was always present in the bottom regions of the bed.

Total biomass concentrations in the fluidized bed, ranging from 20,000 to 27,200 mg/l VS, generally agreed with the fluidized bed concentrations reported by Jeris and Owens (77). Jeris and Owens have reported concentrations of 30,000 to 40,000 mg/l VS for a fluidized bed denitrifying domestic sewage.

Based on the total biological mass within the reactor and the influent thiosulfate concentration of 100 mg/l, the fluidized bed was operated at thiosulfate F/M ratios ranging between 8.4 and 11.4. These ratios were well within the range of F/M ratios reported by Kreye (25) for an autotrophic activated sludge process treating thiosulfate. The reported thiosulfate F/M ratios ranged between 8.1 and 26.

Solids production within the fluidized bed was generally low. The largest incremental increase occurred at pH 4.0 and was measured at 22 percent. Solids production in an activated sludge process treating thiosulfate was also reported to be low (25). Generally solids production in an autotrophic system is expected to be low due to the high energy requirements for converting CO_2 to cell intermediates.

Although the biological mass was not analyzed for elemental sulfur, it was consistently noticed that the odor of burning sulfur was given off while determining the biological solids concentrations. This observation suggests that the sulfur content of the biological mass was increasing due to ability of the Thiobacilli to accumulate extracellular elemental sulfur. This same result has been previously reported on a more quantitative basis, in a case where the sulfur content of activated

sludge was shown to increase while oxidizing thiosulfate (29).

Based on the results of this investigation, it is evident that further research is necessary in order to firmly establish whether or not a fluidized bed process can be used as a means of treating thiosulfate wastes. Potentially, the process is sound and demands further research.

Acid Decomposition of Thiosulfate

Using a series of batch tests, acidification with hydrochloric acid was shown to have the effect of decomposing thiosulfate. One of the products of decomposition was identified as polythionate while a second decomposition product was assumed to be elemental sulfur based on the following criteria: microscopic observations, an incomplete thiosulfate balance at the lower levels of acidity, and the results of previous investigations (3,11,17).

The extent of thiosulfate decomposition was found to be highly dependent upon pH and to a slight extent dependent on the initial thiosulfate concentration. Acidification to a pH of 3.0 resulted in 30 percent decomposition at all thiosulfate concentrations except 1000 mg/l. Below a pH of 3.0, acid decomposition increased very sharply with 50 to 75 percent decomposition occurring at a pH of 2.0 and better than 90 percent decomposition occurring down to a pH of 1.0.

Polythionate production was found to be dependent upon the initial thiosulfate concentration. As the initial thiosulfate concentration increased, a smaller percentage was converted to polythionate by lowering the pH to 1.0. At a concentration of 100 mg/l, 49 percent

of the initial thiosulfate was converted to polythionate while at concentrations of 200, 500, and 750 mg/l only 25 percent of the initial thiosulfate was converted in this manner. Polythionate was not detected in the system containing an initial thiosulfate concentration of 1000 mg/l.

Down to a pH of 3.0, the thiosulfate balance accounted for most of the initial thiosulfate as polythionate and thiosulfate. Below a pH of 3.0 the thiosulfate balance was incomplete due to the precipitation of elemental sulfur and the possible formation of other compounds such as sulfur dioxide, hydrogen sulfide, and sulfur oils. The latter sulfur compounds have been previously detected and their production is dependent upon pH (5,16).

Although acid decomposition is an excellent means of removing thiosulfate, its potential as a possible treatment method is limited. As previously noted, chemical costs would be the prohibiting factor (25).

The manner in which pH levels below 3.0 effect thiosulfate, suggests the consideration of a second mechanism of thiosulfate removal in a biological system treating thiosulfate wastes. Typically, pH conditions in a thiosulfate waste treatment system are at a level of 2.0 or lower. Operating at this pH level undoubtedly brings about the precipitation of elemental sulfur along with biological oxidation of thiosulfate.

Zaiser and La Mer have outlined three stages which account for the precipitation of sulfur from thiosulfate under purely chemical conditions (17). The first two stages can be shown to apply to a biological system treating thiosulfate as well as to a purely chemical system.

In the first stage, molecularly dispersed sulfur is immediately produced following the initial contact between thiosulfate and the acid media. In the second stage the molecularly dispersed sulfur condenses on any available nuclei, such as the biomass of a biological treatment system. Once the elemental sulfur is adsorbed it can be oxidized by the Thiobacilli bacteria.

This mechanism would provide a partial explanation for the increasing sulfur content of the activated sludge previously reported by Aulenbach and Heukelekian (29). The remainder of the explanation is provided by the fact that the Thiobacilli have the ability to extracellularly produce elemental sulfur. (1).

Influence of Trace Nutrients on Oxygen Uptake

Supplementing the fluidized bed synthetic feed with trace nutrients resulted in significantly influencing the oxygen uptake rate during thiosulfate oxidation. The oxygen uptake rate was normally 2.5 times higher when the isolated Thiobacillus culture was supplemented with the trace nutrients. Conversely, the endogenous oxygen uptake rate was lower when supplemented with the trace nutrients. This result is probably the effect of the trace nutrients increasing metabolic activity which caused a more efficient depletion of the cellular reserves during the washing of the sludge.

Although Vishniac and Santer have pointed out that these nutrients influence the metabolism of the Thiobacilli, the specific function of each nutrient is not explained (39). Presumably, the nutrients are

important factors in the enzyme systems of these bacteria.

Normally trace nutrients such as the ones used in this experiment are assumed to be supplied in sufficient quantities by tap water. Since tap water was used in this experiment, it appears that some of these nutrients were not adequately supplied by the laboratory tap water.

The influence of pH on the rate of oxygen uptake was also demonstrated in this experiment. By lowering the pH the oxygen uptake rate generally decreased. This result is a further indication that the isolated Thiobacillus culture was not acid tolerant.

Influence of pH on Thiosulfate BOD_{22}

Thiosulfate BOD_{22} measurements further demonstrated the inability of the isolated Thiobacillus culture to tolerate extremely acid conditions. Due to this inability, BOD_{22} exertions were progressively suppressed as the pH was lowered from 6.5 to 3.0.

In general, the shapes of the BOD_{22} curves at pH levels 6.5 to 5.0 tend to indicate the progressive oxidation of thiosulfate and polythionate. The first 11 days represent the oxidation of thiosulfate to polythionate and some sulfate, while the remaining 11 days represent the oxidation of polythionate to sulfate. This progressive oxidation is in excellent agreement with the previous discussion on the incomplete oxidation of thiosulfate in the fluidized bed. At pH levels of 4.0 and 3.0, thiosulfate oxidation was almost completely suppressed due to the extremely acid conditions.

Based on the results of this experiment and the performance of the fluidized bed, it is concluded that the isolated Thiobacillus culture

was not suitable for thiosulfate oxidation in a thiosulfate waste treatment system. The ideal culture would be one that could optimally oxidize thiosulfate to sulfate under extremely acid conditions. One such organism is T. thiooxidans.

Precipitation of Thiosulfate with $BaCO_3$

Jar test evaluations of thiosulfate precipitation by barium carbonate failed to demonstrate any significant removal of thiosulfate. Considering the results of this experiment it is evident that this method is not suitable for thiosulfate treatment. Although the evaluation was very brief it is possible that other factors such as pH could have been important to the experiment.

Justification for investigating thiosulfate precipitation by barium was based on the interference of thiosulfate in sulfate analysis. Although the amount of thiosulfate that was precipitated was not extensive from the standpoint of thiosulfate treatment, it could be a significant interference in the analysis of sulfate.

CONCLUSIONS

The major conclusions drawn from this investigation are the following:

1. Thiobacilli have relatively low ammonia nitrogen requirements for the oxidation of thiosulfate.
2. High phosphorus concentrations have the potential of suppressing thiosulfate oxidation due to a buffering effect.
3. The desirable $S_2O_3^{-2}$: N : P ratios for successful biological treatment of a thiosulfate waste should range between 1000:5:10 and 1000:5:50.
4. The isolated Thiobacillus culture was not suitable for thiosulfate oxidation in a fluidized bed due to its inability to tolerate extremely acid conditions.
5. The fluidized bed process has sound potential for the treatment of thiosulfate wastes and deserves further research.
6. Acid decomposition of thiosulfate occurs primarily below a pH of 3.0 and results in the precipitation of elemental sulfur.
7. Acid decomposition of thiosulfate provides a second mechanism for thiosulfate removal in a biological system treating thiosulfate.
8. Trace nutrients such as zinc, iron, molybdenum, copper, and cobalt can increase the metabolic activity of Thiobacilli oxidizing thiosulfate.

9. Thiosulfate precipitation with barium carbonate is not a practical method of thiosulfate treatment.

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THIOSULFATE OXIDATION IN A FLUIDIZED BED

by

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(ABSTRACT)

This study was conducted with the purpose of evaluating the possibilities of biologically oxidizing thiosulfate in a fluidized bed. A laboratory scale, autotrophic fluidized bed was continuously operated using a synthetic thiosulfate waste. The reactor was maintained at a hydraulic loading rate of 16.1 gpm/ft^2 and an influent thiosulfate concentration of 100 mg/l. The void space detention time at this loading rate was approximately 0.6 minutes. Through a series of influent pH adjustments, the fluidized bed demonstrated the ability to remove up to 75 percent of the applied thiosulfate, but the intermediate thiosulfate metabolic product, polythionate, proved difficult to oxidize. Due to the high oxygen demand of thiosulfate and the high concentration of volatile solids in the reactor, aeration with high purity oxygen was required to maintain an aerobic environment. Continuous operation of the fluidized bed also verified the preference of Thiobacillus bacteria to attach to a solid surface.

Ammonia nitrogen and phosphorus requirements of the fluidized bed Thiobacillus culture were evaluated in a series of batch and oxygen uptake experiments. Unlike nitrogen, phosphorus was demonstrated to

be an influential nutrient in the oxidation of thiosulfate. Trace nutrients which included zinc, iron, molybdenum, copper, and cobalt were also shown to influence the metabolic activity of the Thiobacilli.

Acid decomposition of thiosulfate was briefly evaluated. Acidification below a pH of 3.0 resulted in rapid thiosulfate destruction and suggested a second mechanism for the removal of thiosulfate in biological waste treatment systems.