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Biological Removal of Hydrogen Sulfide from Refinery
Wastewater and Conversion to Elemental Sulfur

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A Thesis Submitted to the
Faculty of Graduate Studies and Research
Through the Department of Civil & Environmental Engineering
in Partial Fulfillment of the Requirements for the
Degree of Master of Applied Science
at the University of Windsor

by

Paul F. Henshaw

Windsor, Ontario, Canada
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Canada

ABSTRACT

The use of the green sulfur bacterium *Chlorobium limnicola* forma specialis *thiosulfatophilum* in a bioreactor is proposed as a means of removing hydrogen sulfide from process water and producing elemental sulfur. For petroleum refineries, this is an alternative to sour water stripping followed by the Claus process.

The analytical methods that were found to work without interference from other sulfur species were: methylene blue for sulfide, cyanide for elemental sulfur and turbidimetric for sulfate.

Elemental Sulfur was successfully produced from sodium sulfide in a batch reactor by *C. thiosulfatophilum*. From 1 to 90% of the sulfide consumed was recovered as elemental sulfur. There was a mild correlation between the initial pH and the percent recovery of sulfur.

The specific growth rate of *C. thiosulfatophilum* was found to be higher than that found in previous work. The Haldane equation for substrate inhibition was used to calculate the maximum specific growth rate as $0.45 \ h^{-1}$. The maximum tolerable level of sulfide was found to be 300 mg/L. The highest rate of substrate utilization was found to be 17.3 mg/L·h.

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CHAPTER ONE

INTRODUCTION

1.1 General

It has been stated that, for Canadians, pollution is more often the cause of water problems than absolute scarcity of supply (SCC,1988). Inorganic compounds of sulfur are receiving increasing attention as water pollutants.

Chemically, sulfide is the most reduced species of sulfur. In water, the sulfide ion (S^{2-}) remains in equilibrium with hydrosulfide ion (HS^-) and hydrogen sulfide (H_2S). H_2S gas is highly toxic and malodorous (Cadena et al.,1988). For humans, its odour threshold is 0.13 ppm (MSDS,1988) and it is fatal at concentrations higher than 13 ppm (Cadena et al.,1988). Sulfide has a high oxygen demand of 2 mol O_2/mol S^{2-} and thus may cause significant depletion of oxygen in receiving waters (Kobayashi et al.,1983).

Sources of sulfide in the environment include domestic wastewater, anaerobic digestion of organic matter containing sulfur, heavy water plants (Gulens et al.,1982) and petroleum refinery processes. Anaerobic decomposition of wastewater releases $H_2S(g)$ which is oxidized to sulfuric acid at the crown of sanitary sewers, leading to the corrosion of concrete pipes and appurtenances (Gaudy et al.,1980). Anaerobic digestion of pulp mill effluent containing sulfite (Salkinoja et al.,1985) and liquified water hyacinth waste (Kobayashi et al.,1983) are also sulfide sources.

Crude oil containing malodorous compounds (H_2S, mercaptans) is

termed as sour crude (MOE,1987). In petroleum refineries, sour gas contains H_2S whereas acid gas contains H_2S ond CO_2. Fuel gases must be treated for H_2S removal in order to reduce the air pollution due to sulfur dioxide formed while burning the H_2S (Sitting,1978). Additional sources of sulfide within the refinery include hydrocrackers, thermal crackers, gas recovery units, hydro desulphurization units and steam (ethylene) crackers (MOE,1987).

Sulfide discharge is regulated by the Federal Refinery Effluent Regulations and Guidelines. Currently its upper limit is 0.3 kg/1000 m^3 of oil refined/day for refineries that commenced operations on or after Nov.1,1973. Refineries that were operating before that date are subject to the guidelines of 0.6 kg/1000 m^3 of oil refined/day (Geadah,1987). The Ontario refineries are further subject to effluent quality objectives. Sulfide is not covered by these guidelines, although the new MISA regulation promises to look at a wider range of contaminants (MOE,1988a). Indeed, the Effluent Monitoring Regulations for the Petroleum Refining Sector require sulfide testing of process effluent thrice weekly (MOE,1988b).

Sulfate is the most oxidized form of sulfur. Although non-toxic, sulfates discharged in large quantities to surface waters can lead to excess mineralization (Maree et al.,1985). The U.S. Environmental Protection Agency (USEPA,1990) set 250 mg/L as a non-enforcable goal for sulfate in drinking water to limit hardness, corrosiveness to metals, and taste owing to salinity. Sources of sulfates include gold, uranium and copper mines (Maree et al.,1985; Cork,1978).

Sulfur species with oxidation states intermediate between sulfide and

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sulfate (SO_4^{2-}) include (in order of increasing valence) : elemental sulfur (S^o), thiosulfate ($S_2O_3^{2-}$) and sulfite (SO_3^{2-}).

1.2 Sulfide Control

Sulfide can be chemically oxidized to sulfate by hypochlorites, chlorine, potassium permanganate, hydrogen peroxide and oxygen. Hydrogen peroxide and oxygen react slowly with sulfide but produce no chemical residue. Hypochlorite and chlorine react to form chloride ions. Permanganate leaves behind manganese oxide. At pH values greater than 7, the product of the oxidation is always sulfate (Cadena et al.,1988). The chemical oxidizers are expensive and energy intensive (Kobayashi et al.,1983).

The partial oxidation of H_2S to S^o instead of sulfate has several advantages. Elemental sulfur is an easily handled and transported noncorrosive solid containing more sulfur per weight than any other form. Also, the agriculture use of elemental sulfur as a nutrient and fungicide is increasing. Finally, even if sulfate could be refined into a commercial product such as $CaSO_4$ (gypsum wallboard) or H_2SO_4 (sulfuric acid) it was worth $36.00/ton in 1978. Elemental sulfur at that time sold for $110.00/ton (Cork, 1978).

For these reasons, petroleum refineries convert H_2S liberated in their processes to elemental sulfur. This is most commonly done by the Claus process (Cork et al.,1986). In the first step of the Claus process, H_2S is partially burned to SO_2 with air. The H_2S/SO_2 mixture is then reacted over a bauxite catalyst to yield S^o and water. Normally 90-95% of the H_2S is converted to S^o. The remaining H_2S is either

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incinerated to SO_2 or converted to sulfur in a tail gas treating unit (Sitting,1978). Alternatively, the Holmes-Stretford process oxidizes H_2S to S^o using a vanadium catalyst in water. Over 99% of the H_2S is removed in this process (Vasan,1978).

Cork *et al.*(1986) have listed several reasons for the high cost of sulfur recovery processes:

(1) The accumulation of soluble sulfates and thiosulfates in the reaction liquid (Holmes-Stretford process) leads to catalyst poisoning, corrosion and lower elemental sulfur production.

(2) The cost of the chemicals, especially the amine solution (Claus process) and the ADA solution (Holmes-Stretford Process).

(3) The expense of tailgas cleanup which is necessary in most Claus plants and the disposal of the slag waste generated during cleanup.

(4) Catalyst lifetime is limited for both the bauxite and the vanadium.

(5) The CO_2 gas saturation in the amine strippers in the Claus process requires greater amounts of lean amine solution.

Petroleum refinery operations produce sour water whenever steam is condensed in the presence of gases containing H_2S (Sitting,1978). Condensates from gas separators may contain up to 5000 mg/L sulfide (Nemerow,1978). The H_2S can be removed by sour water stripping (Figure 1.1). Steam is contacted with heated sour water in the sour water stripper. Sour gas containing some steam leaves the top of the stripper and is partly condensed. Condensate and sour gas are separated in the surge tank. The sour gas is then sent to a sulfur recovery plant (Claus process). The water from the bottom of the stripping column is directed to the wastewater treatment plant (Sitting,1978) where H_2S may escape from

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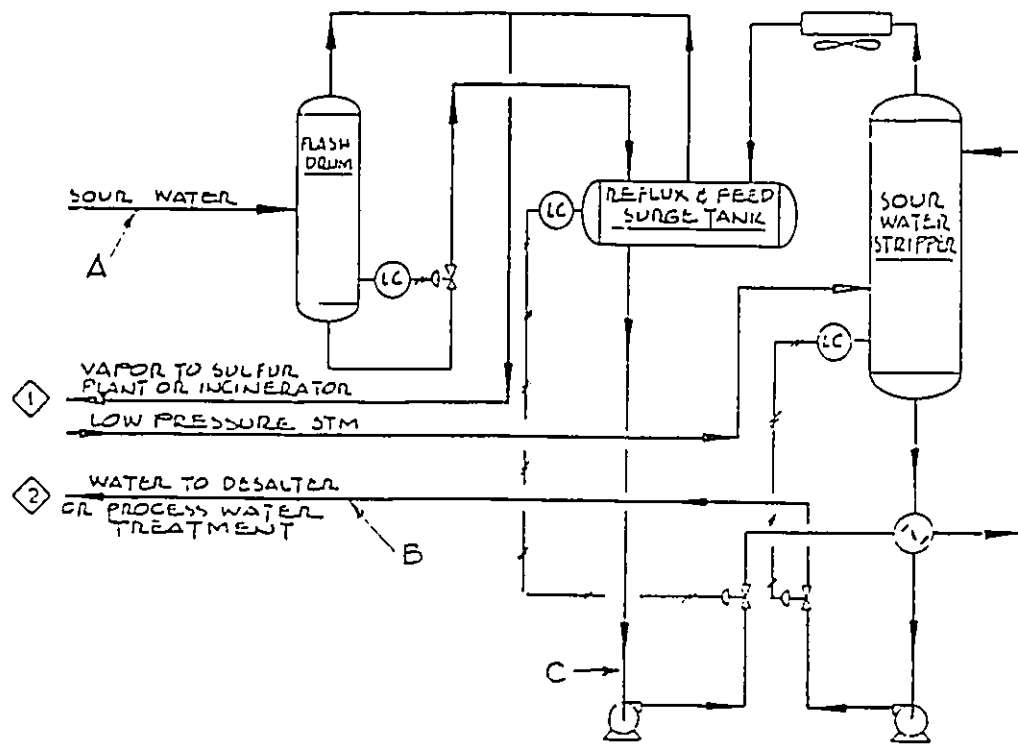
FIGURE 1.1   The Sour Water Stripping Process (Sitting, 1978)

open channels or be oxidized to SO_4^{2-}.

A process which converts the H_2S in the feed to the wastewater treatment plant to S^o can offset its operating costs by the production and sale of elemental sulfur. Such a process can be modified to replace both the sour water stripper and the sulfur recovery processes.

1.3 Objective

The objective of this research was to develop a bioprocess to remove H_2S from the feed to the sour water stripper and convert it to elemental sulfur.

1.4 Scope

The scope of this study was to:

- select and calibrate appropriate analytical methods for different sulfur species and confirm that other sulfur species do not interfere in the analysis;

- demonstrate and quantify the production of elemental sulfur from sulfide in a biological batch reactor;

- quantify the growth rate of bacteria and the maximum tolerable level of sulfide for the selected microorganism in the batch biological reactor; and

- determine the rate of sulfide utilization in the batch biological reactor.

CHAPTER TWO

LITERATURE REVIEW

2.1 Sulfur Cycle

The turnover of sulfur compounds in the biosphere is referred to as the sulfur cycle. Sulfur enters the biosphere as H_2S from volcanic activity, eg. sulfur springs, (Stanier et al.,1957) or through weathering of minerals containing sulfate (Anderson,1978).

The cyclic transformations from H_2S to S^o to SO_4^{2-} and back to H_2S are accomplished entirely by bacteria in a sulfuretum (Figure 2.1). Several ecological niches are formed by the combination of anaerobosis, light, sulfur, carbon dioxide and organic material.

An anaerobic zone is formed in meromictic lakes (lakes which undergo incomplete circulation) or during stratification in homomictic lakes (lakes that undergo a complete circulation) or at the bottom of any body of water due to the pressure of mud and sand which act as a mechanical barrier to mixing and oxidation (Maka,1986). Sulfates and organic matter from surface run-off and domestic and industrial wastes enter the anaerobic zone where they are converted to H_2S by sulfate reducing bacteria such as members of the genus Desulfovibrio. These chemoheterotrophs derive their energy from breaking down and oxidizing organic molecules. Organic matter is also the source of carbon for their new cell material. The H_2S gas produced by these bacteria rises through the water column.

Closer to the surface, but still in the anaerobic zone, the anoxygenic photosynthetic bacteria which include the green sulfur

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Additions of organic matter, sulphates and other salts initiates anaerobic conditions

Water surface. Oxygen available, low sulphide concentration

$H_2S + CO_2 + O_2 \longrightarrow [CH_2O] + SO_4^{2-}$

$S^0 + CO_2 + O_2 \longrightarrow [CH_2O] + SO_4^{2-}$

Chemoautotrophs

Anaerobic, light penetrates

$H_2S + CO_2 \longrightarrow S^0 + [CH_2O]$

Photoautotrophs

Mixing by diffusion and turbulence

Anaerobic, high sulphide concentration

$H_2S + CO_2 \longleftarrow [CH_2O] + SO_4^{2-}$

Dissimilatory sulphate reduction

$H_2S + CO_2 \longleftarrow$ Organic sulphur

Desulphurylation

Organic matter accumulates removing sulphur

FIGURE 2.1 Bacterial Interactions in a Sulfuretum
(Madigan *et al.*, 1988)

bacteria, purple sulfur bacteria and purple non-sulfur bacteria are present. The purple and green sulfur bacteria are photoautotrophs, using the infra-red light available at anaerobic depths as their energy source and carbon dioxide as their carbon source. Green plants assimilate carbon according to the equation:

$$2nH_2O + nCO_2 \xrightarrow{\text{light}} (CH_2O)_n + nO_2 + nH_2O \qquad [2.1]$$
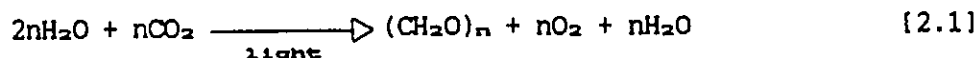
On the other hand, anoxygenic photosynthetic bacteria fix carbon but produce no molecular oxygen (Maka, 1986):

$$2nH_2S + nCO_2 \xrightarrow{\text{light}} (CH_2O)_n + 2nS^o + nH_2O \qquad [2.2]$$
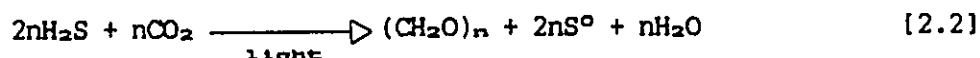
Sulfide in H_2S replaces oxygen in water as the source of four electrons that are transferred to carbon. Some cyanobacteria (Garlick *et al.*,1977) and algae (Knoblock,1966) perform photosynthesis by both paths simultaneously. Most purple and green bacteria oxidize elemental sulfur to sulfate (Stanier *et al.*,1957) and eventually make it available to the sulfate reducing bacteria.

Hydrogen sulfide which reaches the aerobic zone of the lake is oxidized spontaneously by O_2 (Stanier *et al.*,1957) but this reaction is slow (Buisman *et al.*,1989). Chemolithotrophs use the energy of this oxidation to drive their metabolism and also fix carbon from inorganic sources such as carbon dioxide. These colourless sulfur bacteria ultimately produce sulfate as the end-product of sulfide metabolism (Gaudy *et al.*,1980). This sulfate along with decayed biomass from the photo- and chemolithotrophs feed the sulfate reducing bacteria to complete the sulfur cycle.

2.2 Sulfide Oxidizing Bacteria

The desirable bacterium for the bioprocess under investigation should produce S° from sulfide in such a manner that the elemental sulfur is easily extractable from the biomass.

2.2.1 Aerobic Chemolithotrophs

The colourless sulfur bacteria include the genera *Beggiatoa*, *Thiothrix* and *Thiobacillus*.

Thiothrix and *Beggiatoa* are genera of the family Beggiatoacea within the "non-photosynthetic, non-fruiting gliding bacteria" (Tortora,1989). Intracellular granules of elemental sulfur were formed in the presence of H_2S. When the sulfide supply is depleted, the S° dissappears (Stanier *et al.*,1957). In this way, S° acts as an energy-storage medium in the oxidation of sulfide to sulfate. Extracting sulfur, whether by chemical or physical methods, would require rupturing the cell membrane, rendering the cell useless for further sulfur production.

There is some debate whether the *Thiobacilli* produce S° or oxidize H_2S completely to sulfate. Gaudy *et al.*(1980) states," all *thiobacillus* species are small rods, and the sulfur is deposited outside the cell. The final product of oxidation is sulfate." Whereas Roy *et al.*(1970) note, "accumulation of elemental sulfur however, does not invariably accompany sulfide oxidation by *thiobacilli*."

Thiobacillus denitrificans (abrv. *T. denitrificans*) can use the reduction of nitrate to molecular nitrogen as an energy source while oxidizing S° or thiosulfate (Gaudy *et al.*,1980). Sublette *et al.* (1987a,b) demonstrated the use of *T. denitrificans* to oxidize 1 mole % H_2S in a nitrogen gas feed stream. No elemental sulfur was detected in the

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reactor. This process was later demonstrated to be suited to desulfurize natural gas (Sublette *et al.*,1987c). London (1964) concluded that S^o is not a normal intermediate product of sulfide or thiosulfate oxidation for *T. thioparus, T. thiooxidans* and *T. intermedius*. Karavaiko (1977) photographed inclusions in *T. neapolitanus* which were analyzed by x-ray diffraction and found to contain elemental sulfur.

Thiobacillus has been used successfully to treat sulfide-rich wastewaters from Soviet spas. Over 95% of the influent sulfide was converted to sulfate by *T. thioparus* and *T. thiooxidans* in an upflow reactor. Influent H_2S concentrations of 20-60 mg/L were oxidized to less than 1 mg/L in the effluent (Ass *et al.*,1983).

Rozek (1978) patented a process for oxidizing sulfide in sulfur mine wasterwater. Inorganic nutrients and *T. thioparus* were added to the wastewater. After oxidation, the water surface was covered with a layer of elemental sulfur and bacterial cells.

Recently, *Thiobacillus* has been demonstrated to convert sulfide to S^o. *Thiobacillus* growth was promoted from an initial innoculum of ditch mud in an upflow aerobic completely mixed and continuously fed reactor. Sodium sulfide and inorganic nutrients were added to tap water and pH stabilized before being fed into the reactor. Pure oxygen was bubbled into the reactor. On average, 81% of the influent sulfide was converted to S^o, whereas 13% became sulfate (Buisman *et al.*,1989).

Subsequently, the following observations were made using a 20 L reactor with an aeration stone to produce finer bubbles (Buisman *et al.*,1990):

(1) At a sulfide loading rate of 832 mg/L·d, 86% of the influent sulfide

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was converted to S° and approximately 1% of the influent sulfide became sulfate. The sulfide concentration in the reactor effluent was 9-22 mg/L.

(2) At a sulfide loading rate of 1040 mg/L·d, 69% of the influent sulfide was converted to S° but no sulfate was formed. The effluent sulfide concentration was 43 mg/L.

(3) At a sulfide loading rate of 520 mg/L·d, 88% of the sulfide was converted to elemental sulfur but 7% was converted to sulfate resulting in the removal of 95% of the influent sulfide. The reactor effluent contained 2 mg/L sulfide.

There was a compromise between effluent sulfide concentration and sulfate formation. A 100% conversion of all of the removed sulfide to S° was achieved only when sulfide appeared in the effluent. On the other hand, a low sulfide effluent concentration was realised only when sulfate appeared in the effluent. Results were similiar for the biorotor reactor tested. Although these bacteria deposit sulfur extracellularly, 100% removal of H_2S and conversion to S° had not been achieved.

2.2.2 Anaerobic Photolithotrophs

The purple and green bacteria contain bacteriochlorophylls (bchl) which are responsible for light harvesting and transferring energy to the photochemical reaction centre (Stanier *et al.*,1976). It is the type and abundance of these photosynthetic pigments which give the bacteria their characteristic colour.

2.2.2.1 Purple Non-Sulfur Bacteria

Purple bacteria contain either bchl a or bchl b. This group of bacteria are termed "non-sulfur" because its members were historically

found not to metabolize sulfide, that is, they did not produce sulfur granules in the presence of sulfide (Hansen *et al.*,1972). These bacteria are present in sulfureta because they can metabolize sulfur-containing organic compounds, using the reduced sulfur as their electron source and the bound carbon as their carbon source. Therefore, they are called photoheterotrophs (Gaudy *et al.*,1980). This explains their dominance over purple and green sulfur bacteria in sewage sludge (Siefert *et al.*,1978).

Some species of *Rhodospirillum* and *Rhodopseudomonas* have been found to oxidize sulfide and produce extracellular sulfur without production of sulfate. *Rhs. capsulata* tolerated 2 mM (64 mg/L) sulfide in an inorganic medium with growth factors (biotin, niacin, thiamin, potassium-*p*-aminobenzoate) and 3.2 mM (102 mg/L) sulfide when yeast extract was added. Growth in a completely inorganic medium was not reported (Hansen *et al.*,1972). It is doubtful that these bacteria can continuously metabolize sulfide without the addition of expensive and possibly sensitive growth factors.

2.2.2.2 Purple Sulfur Bacteria

Sulfur granules accumulate inside the cells of these motile anaerobes (Roy *et al.*,1970). The genera *Thiocapsa* and *Chromatium* are included in this family.

Slaughter-house effluent was found to turn pink owing to *Thiocapsa roseopersicina*, the major sulfide oxidizing bacteria present (Cooper *et al.*,1975). Sulfide oxidation by *Chromatium okenii* was found to be dependent on the presence of carbon dioxide, and the conversion was reversible (Truper *et al.*,1964). As with *Beggiatoa* and *Thiothrix*, these bacteria store sulfur intracellularly and consequently make it

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inaccessible by simple separation methods.

2.2.2.3 Green Sulfur Bacteria

The green sulfur bacteria (GSB) contain a major light harvesting pigment, bchl c, d or e, and a minor chlorophyll at the photochemical reaction centre which is always bchl a (Stanier *et al.*,1976). These strictly anaerobic non-motile bacteria oxidize sulfide and deposit S^o extracellularly (Roy *et al.*,1970). Several species of the genus *Chlorobium* have been studied.

C. phaebacteriodes had tolerated sulfide levels up to 5 mM (160 mg/L) in the laboratory but was not found in its natural habitat at sulfide levels less than 0.03 to 0.06 mM (1 to 2 mg/L) sulfide (Bergstein *et al.*,1983). *Chlorobium limnicola* forma specialis *thiosulfatophilum* (also known as *Chlorobium thiosulfatophilum*) can utilize thiosulfate as well as sulfide as an electron source (Roy *et al.*,1970).

The mechanism of subsequent conversion of S^o to sulfate is not fully known. Cork (1978) has postulated the reaction :

$$S^o + 3H_2O \longrightarrow SO_3^{2-} + 6H^+ + 4e- \qquad [2.3]$$
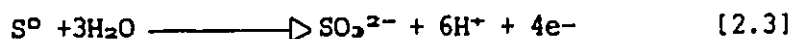
Evidence for this reaction is weak. Truper (1982) has suggested the disproportionation of elemental sulfur:

$$4S^o + 3H_2O \longrightarrow 2H_2S + H_2S_2O_3 \qquad [2.4]$$
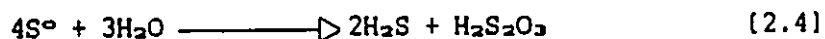
C. thiosulfatophilum possesses a thiosulfate-splitting enzyme which can convert thiosulfate to sulfite:

$$S_2O_3^{2-} + 2e- \longrightarrow S^{2-} + SO_3^{2-} \qquad [2.5]$$
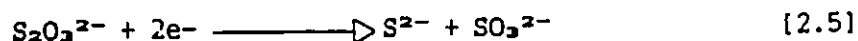
The mechanism of oxidation of sulfite to sulfate is identical to the pathway employed by *Thiobacillus*:

$$SO_3^{2-} + AMP \longrightarrow APS + 2e- \qquad [2.6]$$

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$$APS + PO_4{}^{3-} \longrightarrow ADP + SO_4{}^{2-} \qquad [2.7]$$
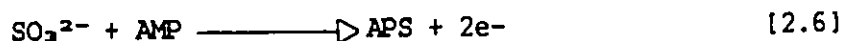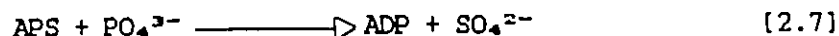
AMP, ADP and ATP are adenosine mono, di, and triphosphate respectively. APS is adenylylsulfate. AMP is regenerated:

$$2\ ADP \longrightarrow ATP + AMP \qquad [2.8]$$

Phosphate is regenerated by cleaving ATP, a hydrolytic process. Thus, the source of the oxygen for conversion of S^o to sulfate is water.

Brune (1982) traced the oxidation of sulfide using an ion-selective electrode. Van Gemerden (1986) found that extracellular sulfur secreted by *C. thiosulfatophilum* remained attached to the cells and was not easily available to other microorganisms. *C. thiosulfatophilum* was inhibited to one half its maximum specific growth rate at a sulfide concentration of 3-4 mM (96-128 mg/L) (Van Gemerden,1984). Mathers *et al.*,(1985) noted that low growth rates of *C.thiosulfatophilum* occurred in sulfide concentrations above 6 mM (192 mg/L).

Maree *et al.*(1985,1986,1987) used an upflow anaerobic packed-bed reactor in series with a photosynthetic reactor to remove sulfate from mine wastewater. Sulfate was reduced to H_2S in the first reactor, and purple and green sulfur bacteria precipitated sulfur in the second reactor. Sulfur yield was not quantified. Kobayashi *et al.* (1983) tested a fixed film upflow photosynthetic reactor and a phototube (plug-flow reactor) for removal of H_2S from anaerobic filters. *Chlorobium* was identified as the common organism in the phototube. Sulfide removal ranged from 70-95% in the column and 99.9% in the phototube (showing excess capacity). Sulfate was the product of oxidation in both reactors.

Cork (1987) patented a process for 95-98% removal of H_2S from natural gas streams containing 0.1 to 65% H_2S. The natural gas was to be bubbled

through a nutrient medium containing *C. thiosulfatophilum*. Cork(1978) also tested *Chromatium vinosum* and *C. thiosulfatophilum* in the photosynthetic reactor of a two-stage process that converted 90% of the influent sulfate to S^o. Sulfate was reduced to H_2S by *Desulfovibrio desulfuricans* in the first stage. The H_2S gas was carried by an inert gas into the photosynthetic reactor for production of S^o. *C. thiosulfatophilum* was found to be superior to *Chromatium* in the following categories :

* production of S^o per unit time

* ratio of S^o produced to other oxidized forms of sulfur produced

* ratio of S^o produced to sulfate input

* tolerance to high sulfide concentration (>4 mM (128 mg/L))

* extracellular production of S^o

Cork claimed that the extracellular S^o could be easily isolated by differential centrifugation (Cork,1978) or rotary filtration (Cork,1987). The optimum pH and temperature were found to be 7.0 and 30°C respectively for *C. thiosulfatophilum*. This process was later patented (Cork,1984) as a means of removing sulfate from copper mining wastewater.

2.2.3 Summary of Previous Work

Cork's (1978,1984,1987) work demonstrated the utility of the green sulfur bacterium *C. thiosulfatophilum* in producing S^o from H_2S. However, the sulfide fed into the photosynthetic reactor was in the gas phase.

Maree *et al.*(1985,1986,1987) and Kobayashi *et al.*(1983) demonstrated the removal of sulfide in the liquid phase by photosynthetic bioprocesses, but sulfur was either not quantified or not produced.

In sour water the H_2S is present in the liquid phase. While

dissolved in water, H_2S is less likely to be an environmental or safety hazard than H_2S gas. Thus, it is advantageous to convert sulfide directly to S^o while in the liquid phase. This has not been attempted within a bioreactor.

2.3 Quantitative Description of Growth

Bacterial growth typically conforms to several phases (Figure 2.2). In the logarithmic growth phase, the increase in microbial population conforms to the equation:

$$\frac{dX}{dt} = \mu X$$

[2.9]

where X = dry weight of the biomass in the population (mg/L)
         t = time (h)
         μ = specific growth rate (h^{-1})

Upon integration between times t_1 and t_2, the specific growth rate becomes (Gaudy et al., 1980):

$$\mu = \frac{\ln X_{t_2} - \ln X_{t_1}}{t_2 - t_1}$$

[2.10]

The specific growth rate depends on the substrate present at the beginning of the growth phase. This dependancy conforms to the Monad equation (Gaudy et al.,1980):

$$\mu = \frac{\mu_{max} S}{K_s + S}$$

[2.11]

where μ_{max} = maximum specific growth rate (h^{-1})
         S = substrate concentration (mg/L)
         K_s = substrate concentration at ½ μ_{max} (mg/L)

When the substrate at higher concentrations also becomes a growth

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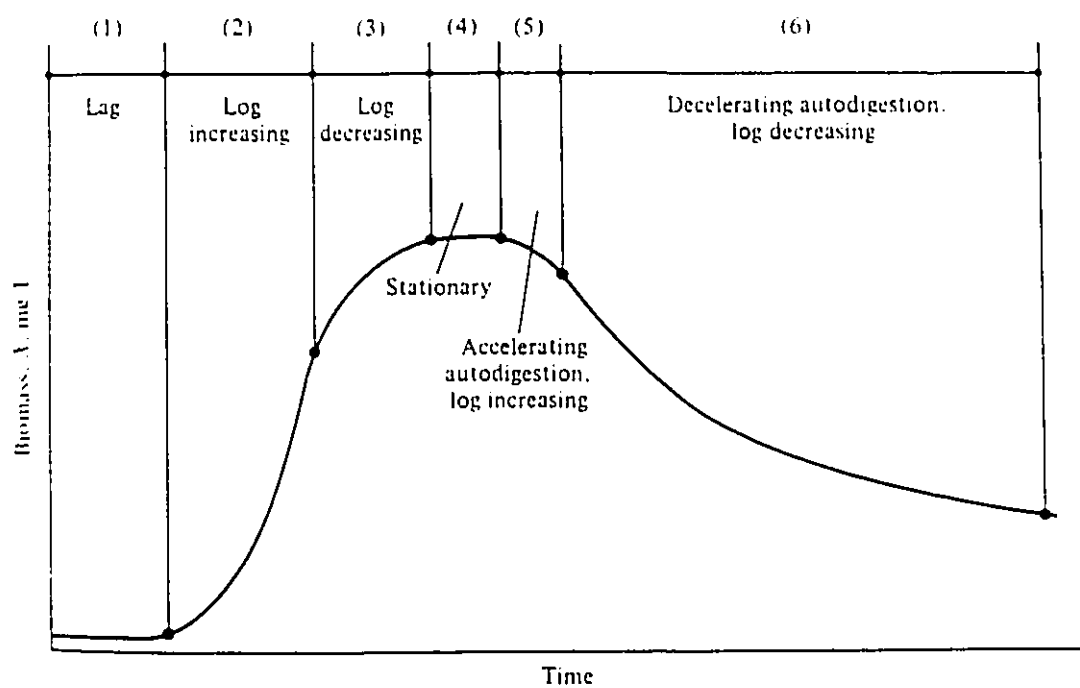
FIGURE 2.2   The Biomass Growth and Decay Curve
(Gaudy *et al.*, 1980)

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inhibitor, the Haldane equation applies (Gaudy *et al.*,1980):

$$\mu = \frac{\mu_{max}S}{(K_s + S)(1 + \frac{S}{K_i})}$$ [2.12]

where K_i = inhibition constant (mg/L)

The cell yield (Y) is the mass of cells produced per unit of substrate utilized (Gaudy *et al.*,1980):

$$Y = \frac{X_{t_2} - X_{t_1}}{S_{t_2} - S_{t_1}}$$ [2.13]

The value of the yield for heterogeneous populations of bacteria of sewage sludge origin growing on various carbon sources is generally 0.4 to 0.6.   This value decreases as the bacteria leave the substrate utilization (log growth) phase. A measure of cell yield taken during or at the end of the substrate removal period is called the true cell yield, Y_t (Gaudy *et al.*,1980).

2.4 Substrate Utilization

The rate of substrate utilization per unit concentration of biomass is termed as the specific substrate utilization rate (Gaudy *et al.*,1980):

$$U = \frac{dS}{dt}\frac{1}{X}$$ [2.14]

where U = specific substrate utilization rate (h^{-1})
         dS = decrease in substrate concentration (mg/L)
         dt = change in time (h)
         X = biomass concentration (mg/L)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Methods of Analysis

Throughout this section, diH_2O refers to distilled water of conductivity 5 to 7 μmho/cm, and deH_2O refers to deionized or distilled, deionized water with a conductivity of less than 1.3 μmho/cm.

All absorbance measurements were made with a Pye Unicam SP500 Series 2 Spectrophotometer in the direct readout mode using a 10mm quartz cuvette. All absorbance measurements are reported in optical density units (O.D.). An International Centrifuge Centra-4 centrifuge with a #215 rotor was used in the sulfate and bacteriochlorophyll assays.

3.1.1 Sulfide

The method of Truper *et al.* (1964) was modified slightly (Section A.2.3 in Appendix A) and used as follows:

3.1.1.1 Reagents

A.   Zn Solution

Roughly 19.1 g zinc acetate (Zn $(acetate)_2 \cdot 2H_2O$) crystals were added to an 800 mL glass bottle. The bottle was capped and shaken after 0.8 L of deH_2O was added. The solution was shaken immediately before use.

B.   DPD Solution

Exactly 2.00 g of dimethyl-*p*-phenylenediamine sulfate (DPD) were added to 1.00 L of 20% H_2SO_4 (200 mL conc. H_2SO_4 + 800 mL deH_2O, cooled) in a beaker and stirred. The mixture was poured into a dark glass bottle for storage.

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C.   FAS Solution

Approximately 18.1 g of ferric ammonium sulfate ($FeNH_4(SO_4)_2 \cdot 12H_2O$) were dissolved in 200 mL deH_2O, then 20 mL of conc. H_2SO_4 and 0.78 L of deH_2O were added to the solution.

3.1.1.2 Procedure

A graduated cylinder was used to measure 20 mL of the Zn solution into a 100.00 mL volumetric flask. A 1 cc plastic syringe graduated to 0.01 mL (Becton Dickinson) with a 7 cm metal needle was rinsed thrice with the sample and filled. The syringe was inverted into a 100.00 mL volumetric flask so that the tip of the needle was less than 1 cm from the zinc solution. Between 0.10 and 0.95 mL of sample (as much as possible but within the range of the method) was squeezed into the Zn solution. The volumetric flask was swirled, and 10.00 mL of DPD solution followed by 0.50 mL of FAS solution were pipetted into the volumetric flask. The flask was made up to the mark with diH_2O, capped and inverted several times. After a waiting period of at least 30 minutes (sometimes overnight) the absorbance was measured at 670 nm against diH_2O.

3.1.1.3 Calculation

The $O.D._{670}$ value was converted to S^{2-} concentration using a calibration equation obtained in the laboratory (see Section A.2.3 in Appendix A):

$$mgS^{2-}/L \; in \; sample = \frac{100.00}{mL \; sample} \left[\frac{O.D._{670} - 0.001}{1.161} \pm 0.022 \right] \quad [3.1]$$

range= 0 to 750 mgS^{2-}/L in sample
$O.D._{670} < 0.86$ in cuvette

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3.1.2 Elemental Sulfur

The colorimetric method of Bartlet *et al.* (1954) was modified (Section A.3.2 in Appendix A) to be used on aqueous samples.

3.1.2.1 Reagents

A. $HgCl_2$ solution

Approximately 25 g $HgCl_2$ and 25 g KCl were added to a 1.5 L erlenmeyer flask. One (1.00) L deH_2O was added, and the mixture was covered with parafilm and stirred to dissolve.

B. Acetone Solution

Exactly 25.00 mL of deH_2O was pipetted into a 500.0 mL volumetric flask. ACS grade acetone was added to the mark and the solution was capped and inverted. Some additional acetone may have been added to compensate for the reduction in volume on mixing.

C. NaCN Solution

Roughly 5.0 g of sodium cyanide (NaCN) were added to 500.0 mL of acetone solution in a 1 L erlenmeyer flask. The mixture was covered with parafilm and stirred to dissolve.

D. $FeCl_3$ Solution

Approximately 0.80 g ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) were added to 200.00 mL of acetone solution in a 250 mL erlenmeyer flask. The mixture was corked and allowed to stand overnight to settle an orange precipitate. The supernatant was decanted into a 100 mL screw-cap bottle for storage.

3.1.2.2 Procedure

Exactly 40.0 mL of $HgCl_2$ solution and a magnetic stir bar were added to a 250 mL erlenmeyer flask with a 24/40 ground glass joint. A 10.00 mL

sample (or a 5.00 mL sample and 5.00 mL deH₂O) was pipetted into the HgCl₂ solution. A teflon or glass stopper was inserted after 20.00 mL of 35-60 petroleum ether were added. The stopper was sealed with parafilm and the mixture was stirred for 45 to 60 minutes. The petroleum ether layer was decanted into a test tube or 25 mL graduated cylinder. A 5.00 mL pipette was rinsed with the organic layer and filled. This solution was transferred to a 25.00 mL volumetric flask and 15.00 mL of NaCN solution were added. The solution was made up to the mark with acetone solution, covered and inverted twice. A 5.00 mL pipette was thrice rinsed with this solution and 5.00 mL were transferred to a test tube. Exactly 5.00 mL of FeCl₃ solution were added to the test tube and the absorbance was measured at 465 nm against a blank consisting of 5.00 mL of FeCl₃ solution and 5.00 mL of acetone solution. There were three samples taken per draw. Only the average of the three is reported in Section 4.1.

3.1.2.3 Calculation

The $O.D._{465}$ value was converted to S^o concentration using a calibration equation developed in the laboratory (see Section A.3.2 in Appendix A):

$$mgS^o/L \text{ in sample} = \frac{200}{mL \text{ sample}} \left[\frac{O.D._{465} - 0.019}{0.2258} \pm 0.24 \right] \quad [3.2]$$

range= 0 to 140 mgS°/L in sample
$O.D._{465} < 1.3$ in cuvette

3.1.3 Sulfate

The APHA (1989) turbidimetric method was modified slightly (section A.4) and limited in use by concentrations of sulfide and elemental sulfur.

3.1.3.1 Reagents

A.   Buffer A

Approximately 30 g of magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), 5 g of sodium acetate trihydrate ($Na(CH_3COO) \cdot 3H_2O$), 1 g of potassium nitrate (KNO_3) and 20 mL of glacial acetic acid (CH_3COOH) were added to a 1 L plastic bottle. One (1.0) L of deH_2O was added and the bottle was capped and shaken.

3.1.3.2 Procedure

A portion of the draw from the reactor was centrifuged at 3000 rpm for 30 minutes. A 10.00 (or 5.00) mL sample was pipetted into a 250 mL erlenmeyer flask and 90.0 (or 95.0) mL of diH_2O and a magnetic stir bar were added. The timer was started and 20.0 mL of Buffer A were added. The mixture was stirred mildly for ½ minute then used to rinse and fill a turbidimetric cuvette. The turbidity (NTU_i) of this solution was measured at 360 ± 30 seconds. At a clock time of 120 seconds, ½ mL of barium chloride ($BaCl_2$) crystals were added to the erlenmeyer flask and the solution was stirred vigorously for 60 ± 2 seconds. The solution was allowed to stand, used to rinse the cuvette and the turbidity was measured (NTU_f) at 480 ± 30 seconds clock time. Turbidity measurements were made in nephelometric turbidity units with a Hach model 43900 Ratio/XR Turbidimeter.

3.1.3.3 Calculation

The ΔNTU value was converted to SO_4^{2-}-S concentration using a calibration equation developed in the laboratory (see Section A.4 in Appendix A):

$$\Delta NTU = NTU_f - NTU_i$$

$$mgSO_4^{2-}-S/L \ in \ sample = \frac{120.0}{mL \ sample} \left[\frac{\Delta NTU + 2.0}{13.71} \pm 0.10 \right] \quad [3.3]$$

range= 0 to 240 $mgSO_4^{2-}-S/L$ in sample
$\Delta NTU < 148$ in cuvette

The concentration expression is read "mg sulfur per L in the form of sulfate." The results of this test are valid for a limited range of S^{2-}/S^0 values (see Figure A.11 in Appendix A).

3.1.4 Bacteriochlorophyll

The method of Maka (1986) was used without modification.

3.1.4.1 Procedure

A pipette was used to dispense 10.00 mL of methanol into a centrifuge tube. A 1.00 mL sample was transferred by pipette into the methanol. The tube was covered with parafilm, swirled for one minute and centrifuged at 3000 rpm for 2 minutes. The absorbance of the supernatant was measured at 670 nm against a methanol blank.

3.1.4.2 Calculation

The O.D.670 value was converted to bchl concentration using the conversion given by Maka (1986):

$$mgbchl/L \ in \ sample = 127.9 \ [\ O.D._{670} \ ] \pm 0.66 \quad [3.4]$$

range= $O.D._{670} < 0.5$ has been reported (Uphaus et al.,1983)

3.1.5 pH

A Fisher Accumet model 210 pH meter and Fischer Universal Glass pH Electrode were used. The electrode/meter was calibrated at pH 7.00 and

4.00 using Fischer certified buffer solutions. A 15 mL sample was poured into a 25 mL plastic graduated cylinder, the electrode was inserted and the pH value was read from the meter.

3.2 Apparatus

3.2.1 Bacteria

Chlorobium thiosulfatophilum was ordered from the America Type Culture Collection and subcultured for six months prior to use.

3.2.2 Reactor

A New Brunswick Scientific Co. model F-14 fermentor was used as a batch reactor. Valves were added to the air inlet, sparger inlet and bottom sampling tube. All other openings were sealed. The stir paddles were removed from a second reactor to clamp a Philips IR 175 Watt R-PAR bulb inside the reactor. A photograph of the reactor is shown in Figure 3.1.

Both reactors were mounted in a New Brunswick Scientific Co. model FS-314 fermentor drive assembly. The light source was mounted in the right position with the light shining toward the centre position. This resulted in an average illuminance of 2400 lux (see Appendix B). The waterbath was filled with tapwater and bleach was added to inhibit fouling. The thermostat was set to 30°C. The waterbath temperature was recorded whenever a draw was made from the reactor. The variable speed drive was connected to the medium filled reactor in the centre position with the stirring speed set at 80 rpm during each experiment.

3.2.3 Medium

Initially, a *Chromatium* medium (ATCC, 1985) was used to subculture the ATCC culture of *C. thiosulfatophilum.* Later, the medium of Madigan
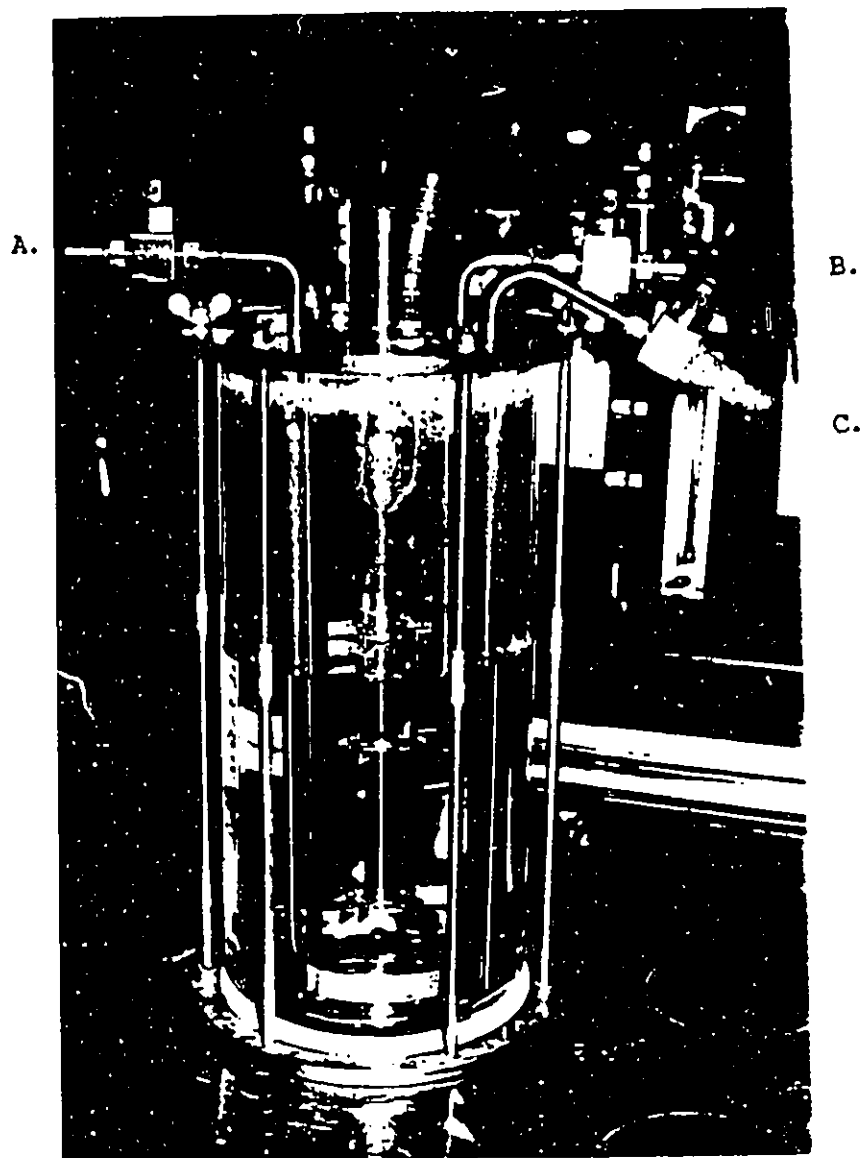
FIGURE 3.1 Photograph of the Bioreactor
Valves were added to the original
A. Sparger inlet,
B. Air inlet,
C. Bottom sampling tube

(1988) was used for culture tubes and the experimental reactors because of its simpler composition.

The recipe for 8.5 L of medium was as follows:

Mineral Salts

$Na_2EDTA \cdot 2H_2O$	0.11 g
$MgSO_4 \cdot 7H_2O$	1.80 g
$CaCl_2 \cdot 2H_2O$	0.45 g
NaCl	3.60 g
NH_4Cl	3.60 g
KH_2PO_4	4.5 g
Trace Elements	9.0 mL
diH_2O	4.5 L

Bicarbonate

$NaHCO_3$	18.0 g
diH_2O	3.6 L
vitamin B_{12} solution (24 µg/mL)	7.4 mL

Sulfide

NaOH	varies
$Na_2S \cdot 9H_2O$	varies
$dadiH_2O$	0.9 L

The mineral salt solution was autoclaved and cooled in a cold water bath. Carbon dioxide was bubbled through the bicarbonate solution for at least ½ hour, after which the vitamin B_{12} solution was added. Deaerated, distilled water was added to an erlenmeyer flask containing sodium sulfide and sodium hydroxide and the flask was gently swirled. The bicarbonate and sulfide solutions were combined in a 5 L dispensing pressure vessel (Millipore Filter Corp.) and pressurized under CO_2. The outlet liquid was forced through a 0.45 µm pore, 47 mm diameter cellulose nitrate filter (Sartorius GmbH) held in a Gelman model 4280 filter holder directly into the sterile culture tube or reactor.

Carbon dioxide was not a limiting nutrient for bacterial growth (see

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Appendix C).

3.2.3.1 Culture Tubes

Culture tubes (70 mL) were half-filled with the mineral salts
solution and autoclaved with the caps on loosely. The tubes were then
taken into an Enviroco EV424 laminar flow hood (Biodynamics Inc.) and
filled with the filter-sterilized bicarbonate/sulfide solution. The pH
and sulfide content of the first and last tubes were checked. The pH was
found to be in the prescribed range of 6.8 to 7.3. The tubes were stored
in a refrigerator until used.

3.2.3.2 One Litre Reactor

A 1 L round bottom flask was fitted with a stopper through which
four glass tubes were inserted. The first tube was covered with a rubber
septum. The second tube extended to 1 cm from the bottom of the flask and
a silicon rubber tube was attached. The third tube was the filling port
for the round bottom flask and the other tube was stuffed with glass wool
and used as a gas outlet while filling.

A magnetic stir bar was added to the flask, and all tubes were
covered before autoclaving. The reactor was purged with CO_2 for 5 minutes
while cooling. The medium recipe was proportioned to 1.5 L volume. The
mineral salts, bicarbonate and sulfide solutions were combined and filter
-sterilized directly into the flask through the filling port. The flask
was kept in a refrigerator for one day prior to innoculation.

3.2.3.3 Fifteen Litre Reactor

The reactor was autoclaved and allowed to cool. Roughly 4.3 L of
the sterile mineral salts solution was vacuumed into the reactor through
the sparger inlet. The reactor was purged through the sparger inlet with

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CO_2 for 5 minutes after which the combined bicarbonate/sulfide solution was filter-sterilized into the reactor. The pre-innoculation fill volume was 8.5 L for each reactor run where 1 L of bacteria medium was to be added. When the reactor was to be monitored as a control, 4.5 L of mineral salts and 4.5 L of bicarbonate/sulfide were added.

The reactor was kept covered at room temperature for 24 hours then placed in the fermentor drive assembly waterbath.

3.3 Experimental Protocol

3.3.1 Innoculation and Incubation

C. thiosulfatophilum was subcultured into fresh culture medium on a weekly basis. About 2 mL of the sediments in the tube were pipetted into a sterile 70 mL culture tube kept under nitrogen while open. A fresh culture tube was innoculated six to thirteen days before commencing the batch reactor test.

Four days (five in Run 7) before the start of the batch reactor test, the 1 L reactor were innoculated. The contents of two 70 mL culture tubes was transferred into four autoclaved 30 mL centrifuge tubes in a laminar flow hood. The tubes were covered with sterile aluminum foil and centrifuged at 3000 rpm for 30 minutes. The tubes were returned to the laminar flow hood and the supernatant was pipetted and discarded. The remaining ½ mL of the supernatant in each tube was used to resuspend the pellet by swirling. The resuspended pellets were drawn into a sterile 5 mL glass syringe (Becton Dickinson) to a total volume of 2 mL. The 1 L flask was placed in a water bath (model 3067, Labline Inc.) at 30°C while stirred and illuminated by a General Electric 250 Watt reflector infrared bulb 150 mm from the flask. The contents of the syringe were injected

into the 1 L flask through the septum while the gas outlet was open. Then all tubes were clamped shut.

The pH and sulfide content of the 15 L reactor were monitored prior to innoculation. The pH was adjusted to the range 6.9 to 7.2 by the injection of sterile 1 M NaOH solution through the septum. The tube leading to the bottom of the 1 L flask was connected to the bottom sampling tube. Nitrogen gas pressure was introduced into the gas outlet port of the 1 L flask forcing the four-day-old bacteria culture into the reactor. The liquid volume in the 15 L reactor was 9.0 to 9.7 L. No innoculation was made to the reactor run as a control.

3.3.2 Batch Reactor Tests

After innoculation, no effort was made to alter the pH and sulfide values. The initial values of pH and concentration of sulfide are shown in Table 3.1 for the seven runs.

Draws were made immediately after innoculation (time = 0 h) and periodically thereafter. The typical interval between draws was 3 hours. A draw consisted of pressurizing the reactor with nitrogen gas to 20 to 30 kPa (3 to 4 psig), then opening the bottom sampling tube valve. The first 25 to 35 mL of the draw were discarded so as not to sample from the stagnant volume inside the bottom sampling tube. From the rest of the draw, a sample was taken immediately for sulfide analysis. Other samples for sulfur, sulfate, bchl and pH were taken thereafter. The time when the sample was added to reagents was recorded and considered to be the time when the reaction had stopped. The measurement of the parameter was considered to be taken at this time.

When the reactor was used for control Run 3, the pH was adjusted by

TABLE 3.1 Initial Values of pH and Sulfide Concentration

	Run	pH_o	$[S^{2-}]_o$ (mg/L)
Batch	1	6.90	72.2± 4.4
Reactor	1 control	6.50	65.8± 4.4
	2	6.96	88.1± 5.5
	3	7.27	120. ± 5.5
	3 control	7.36	123. ± 5.5
	4	7.01	453. ±22
	5	7.04	271. ±22
	6	7.32	23.7± 2.9
	7	7.46	73.2± 3.1

Injection

	Run	pH_o	$[S^{2-}]_o$ (mg/L)
Semi-	initial	6.88	94.3± 5.5
Batch	1	7.35	156. ± 5.5
Reactor	2	7.48	133. ± 5.5
	3	6.45	112. ± 5.5
	4	7.22	120. ± 5.5

injecting sterile 1 M NaOH solution. The sulfide content was lowered by pressurizing the reactor headspace with nitrogen to 20 to 30 kPa (3 to 4 psig) and subsequently venting the headspace through the gas inlet port. Ten repetitions of this venting procedure, while stirring at 3000 rpm reduced the sulfide concentration by 5 to 20 mg/L. The control for Run 3 was performed after Run 3 and thus the timing and volume of the draws were made to mimic those of Run 3. The control for Run 1 was performed before Run 1.

3.3.3 Semi-Batch Reactor Test

In this experiment, the initial consumption of sulfide was monitored as explained in section 3.3.2. After the sulfide was depleted, 1 L of sulfide solution was filter-sterilized and pressurized into the reactor. The sulfide solution was made from washed, dried $Na_2S \cdot 9H_2O$ crystals dissolved in $dadiH_2O$. After monitoring depletion of the sulfide, another 1 L of sulfide solution was injected. Altogether there were four injections of sulfide solution. Prior to the third injection, the pH was adjusted with 1 M NaOH. The values of initial pH and sulfide concentration are given in Table 3.1.

3.3.4 Calculations

For each experimental run or injection, the values for bchl concentration were plotted against time on semi-log paper. The exponential growth phase was determined from this plot and the specific growth rate was calculated by a modified form of Equation 2.10:

$$\mu = 2.303 \left[\frac{\log[bchl]_{t_2} - \log[bchl]_{t_1}}{t_2 - t_1} \right] \qquad [3.5]$$

The values of μ for all runs and injections were plotted against the

substrate (sulfide) concentration at the begining of the log-growth phase. The Haldane Equation 2.12 was fitted to this data using the curve fitting function of the computer program Sigma-Plot Version 4.0 (Jandel Scientific, Sausalito,CA).

True cell yield was calculated also during the log-growth phase. Since bchl was used as an indicator of biomass and sulfide was the substrate, the cell yield (Equation 2.13) was modified to:

$$Y_t^* = \frac{[bchl]_{t_2} - [bchl]_{t_1}}{[S^{2-}]_{t_2} - [S^{2-}]_{t_1}} \qquad [3.6]$$

where Y_t^* = true cell yield based on bchl
$[S^{2-}]$ = sulfide concentration at time t (mg/L)

A sulfur balance was performed by plotting the values of concentrations of sulfur species in a stacked bar graph. Where necessary, the data were interpolated linearly to produce even time intervals. The cumulative uncertainty of all sulfur species concentrations are plotted as an error bar on the graph.

Sulfur recovery was calculated for each run as follows:

$$\% \text{ Recovery} = 100 \frac{[S^0]_{max} - [S^0]_i}{[S^{2-}]_i - [S^{2-}]_{s_m}} \qquad [3.7]$$

where $[S^0]_{max}$ = maximum elemental sulfur concentration for the
run (mg/L)
$[S^{2-}]_i$ = initial measured sulfide concentration
$[S^0]_i$ = elemental sulfur concentration at the time of
the initial sulfide measurement (mg/L)
$[S^{2-}]_{sm}$ = sulfide reading coincident with $[S^0]_{max}$ (mg/L)

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The specific sulfide utilization rate was obtained by calculating the slope of the $[S^{2-}]$ versus time curve between measured data points and dividing by the concentration of bchl interpolated at the midpoint of the time interval:

$$U = \frac{[S^{2-}]_{t_1} - [S^{2-}]_{t_2}}{(t_2 - t_1)[bchl]_{t_{1.5}}}$$ [3.8]

where $[S^{2-}]_t$ = sulfide concentration at time=t (mg/L)
       $[bchl]_{t_{1.5}}$ = bchl concentration at a time midway between t_1 and t_2 (mg/L)

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RESULTS AND DISCUSSION

4.1 Batch Reactor Tests

4.1.1 Data

Figures 4.1 to 4.14 display the data collected in batch reactor runs
1 through 7.   Odd numbered figures show the concentrations of sulfur
species in the reactor and the concentration of bacteriochlorophyll (bchl)
as functions of time.   Where error bars are not shown, the data point
symbol is larger than the error bars.   Even numbered figures display the
reactor environmental conditions (pH, temperature, reactor liquid volume,
reactor headspace) as functions of time.

Data points are joined by straight lines with the exceptions of bchl
in Run 7 and reactor volumes. The reading of bchl at 6 hour in Run 7
indicates a decrease in bchl concentration between 6 and 8 hours even
though sulfide concentration is non-limiting at that time. Therefore, the
6 hour data point was ignored and a second order regression was employed
to join the remaining points.   In the plots of reactor environmental
conditions, liquid volume and headspace volume data points were joined
with a step function corresponding to each withdrawl of liquid.

In general, it can be seen that sulfide depletion is coincident with
an increase in elemental sulfur concentration.   Sulfate concentration
typically   does   not   increase   until   sulfide   is   depleted.
Bacteriochlorophyll increases the fastest when sulfide is being consumed
and continues until elemental sulfur is depleted.

In the control for Runs 1 and 3, it was observed that sulfide

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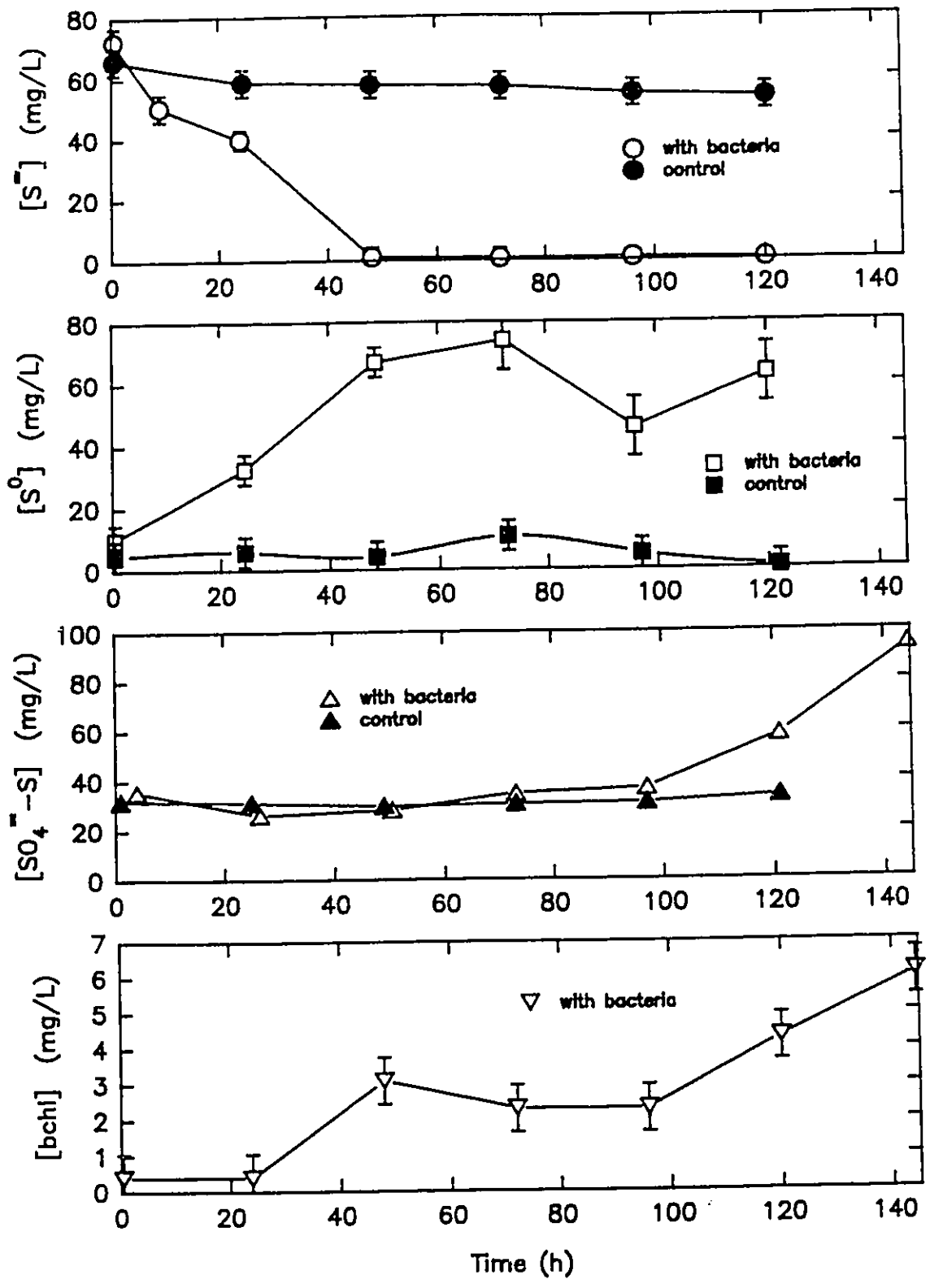
FIGURE 4.1   Concentrations in Batch Reactor Run 1

37
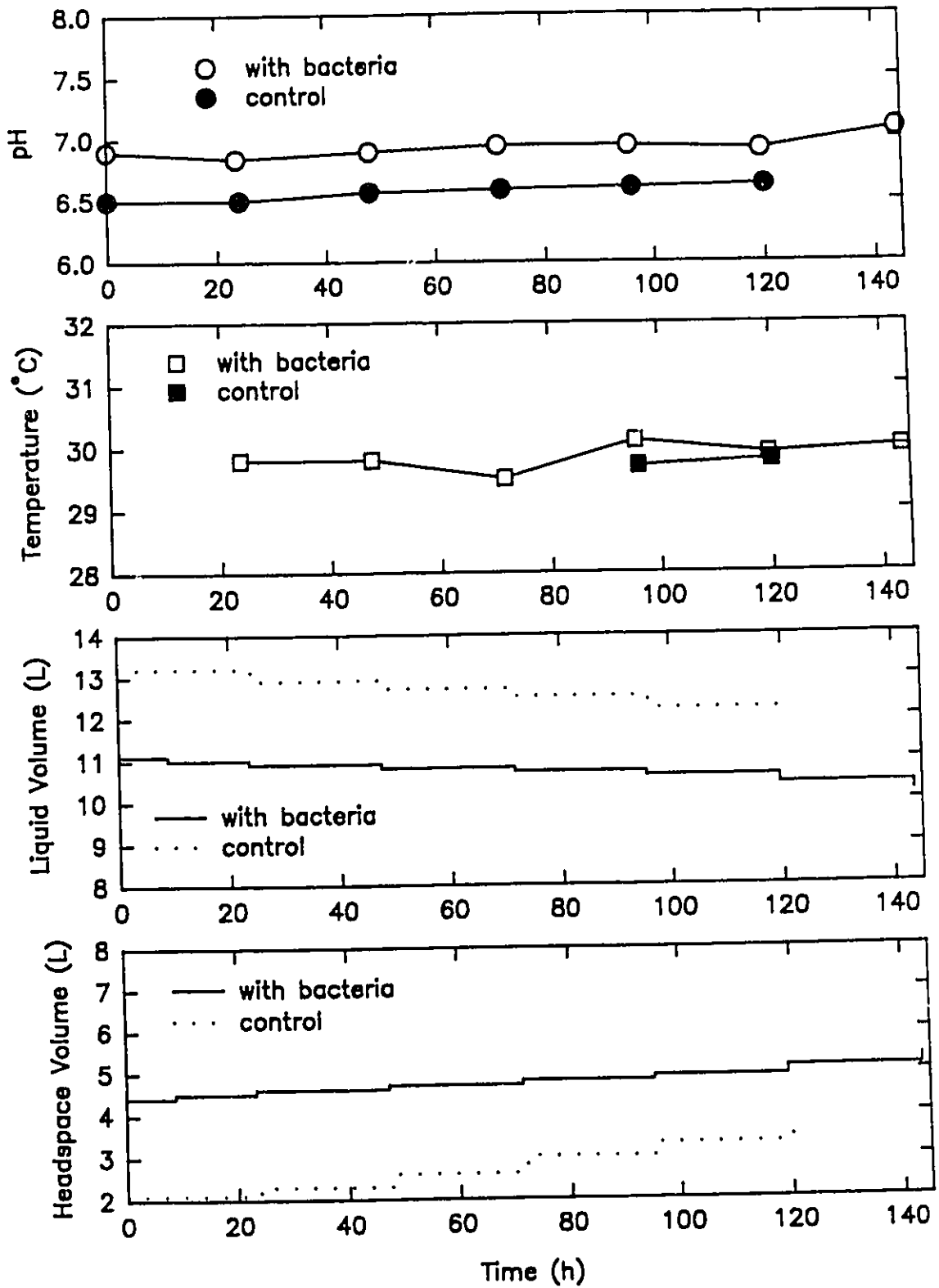
FIGURE 4.2 Reactor Environment for Batch Reactor Run 1
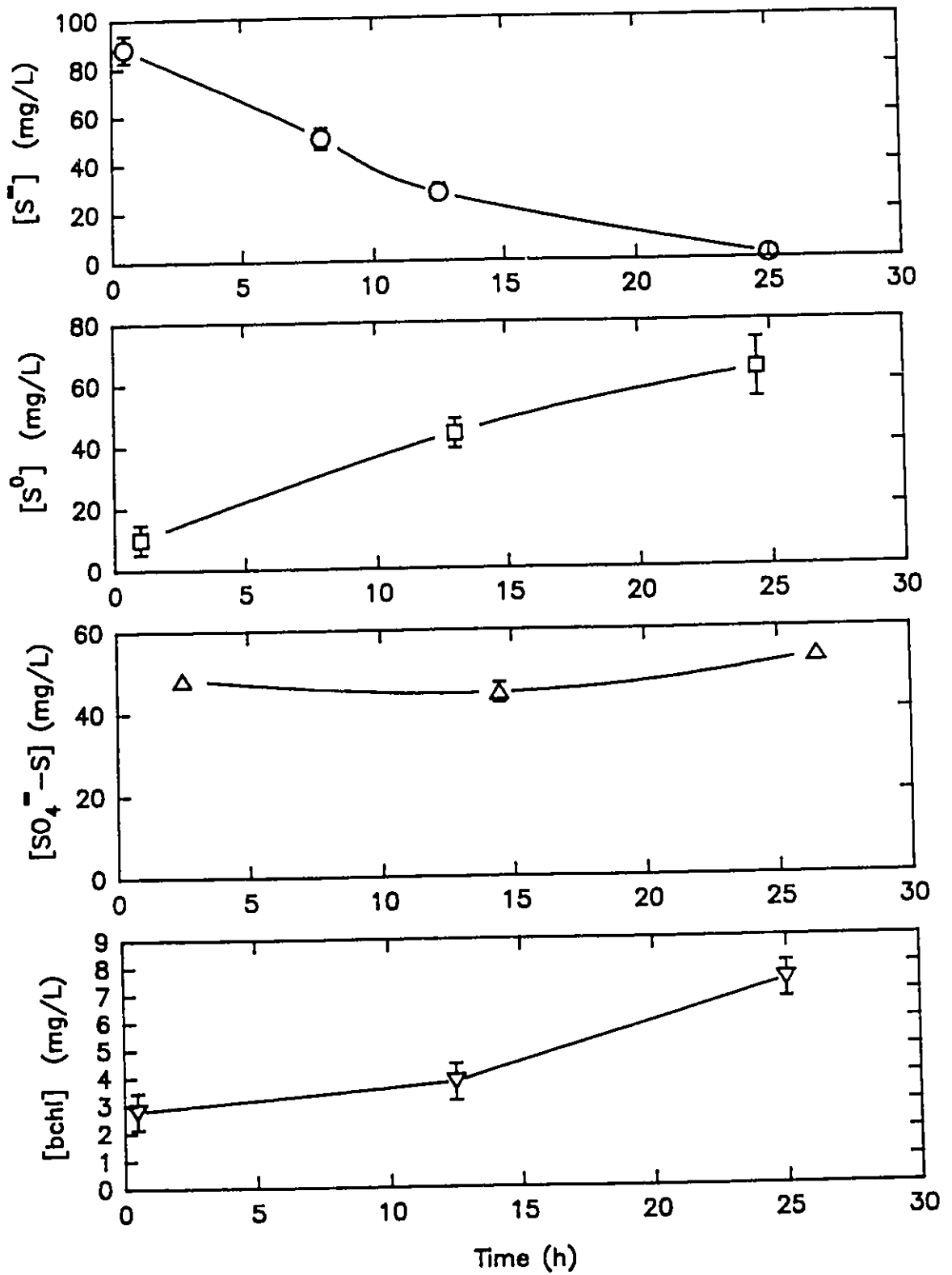
FIGURE 4.3 Concentrations in Batch Reactor Run 2
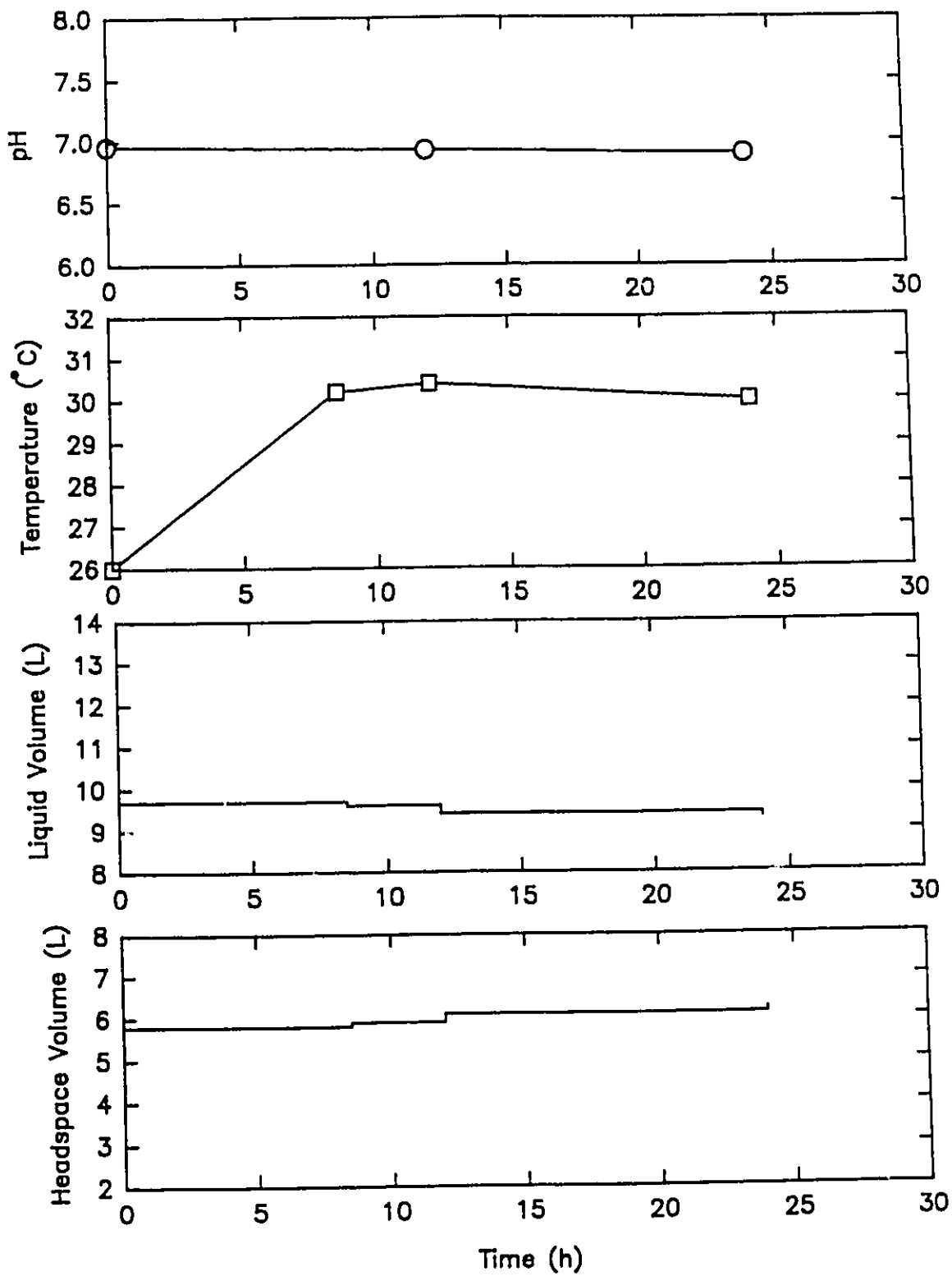
FIGURE 4.4 Reactor Environment for Batch Reactor Run 2
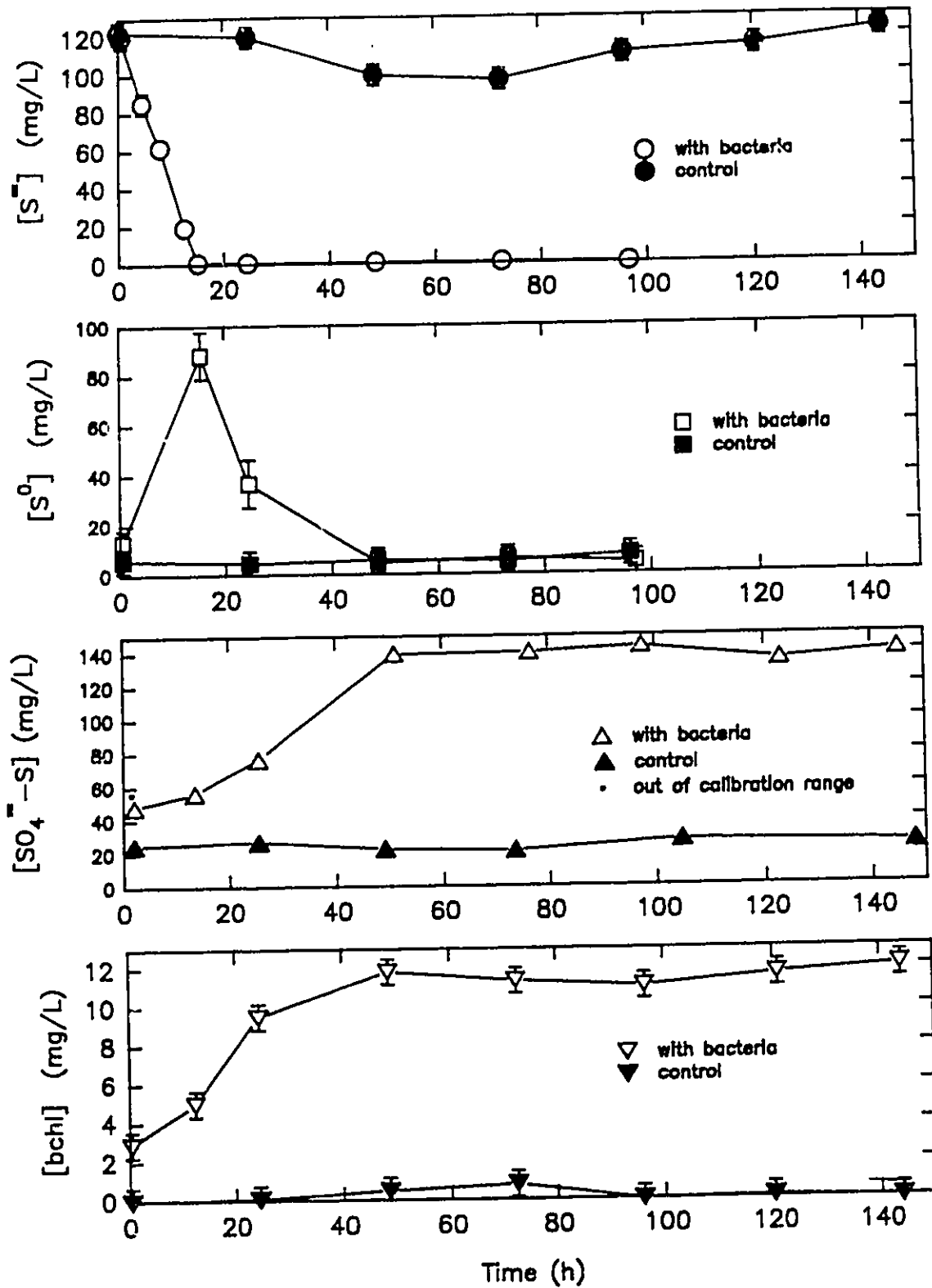
FIGURE 4.5 Concentrations in Batch Reactor Run 3
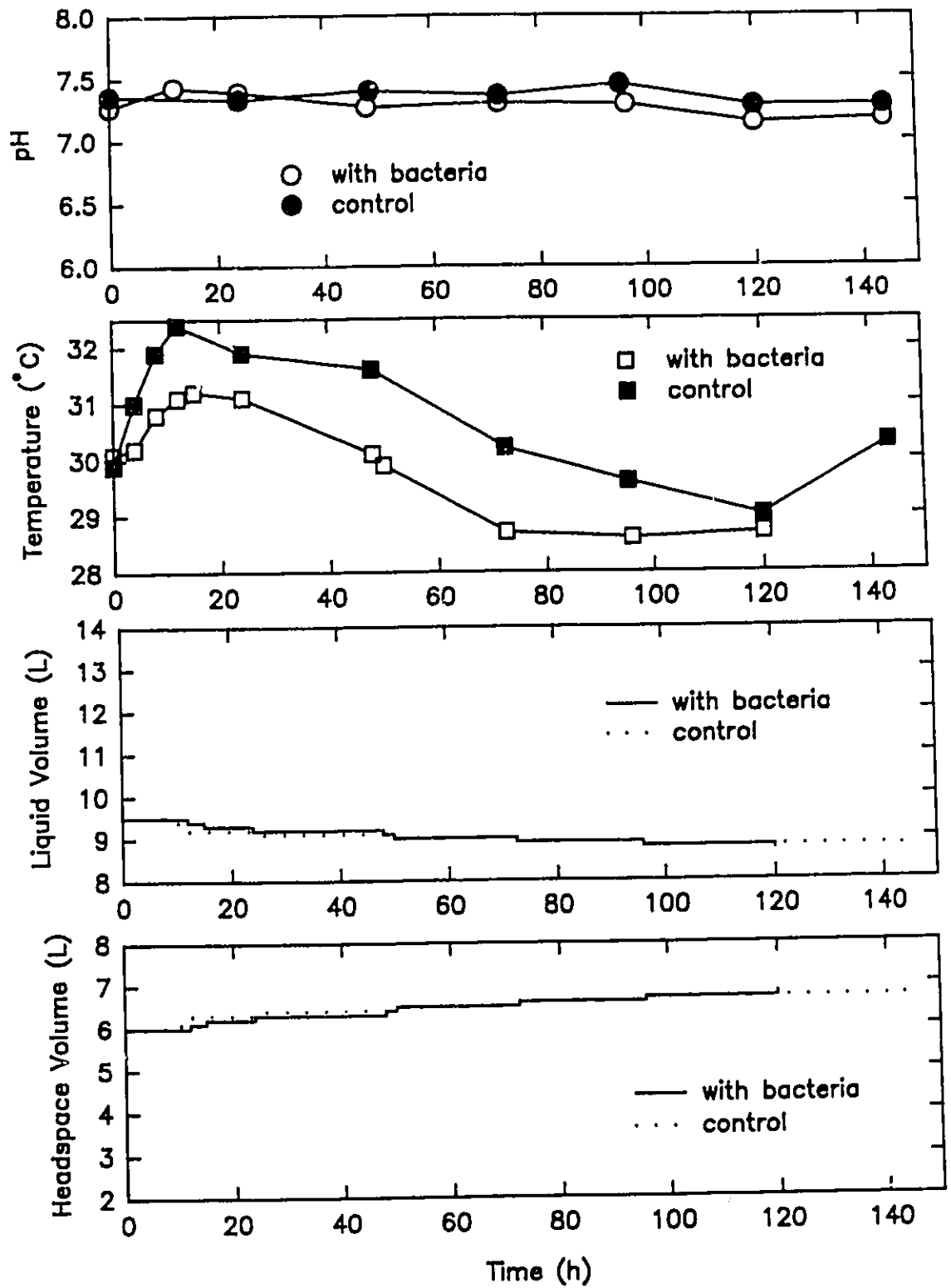
FIGURE 4.6 Reactor Environment for Batch Reactor Run 3
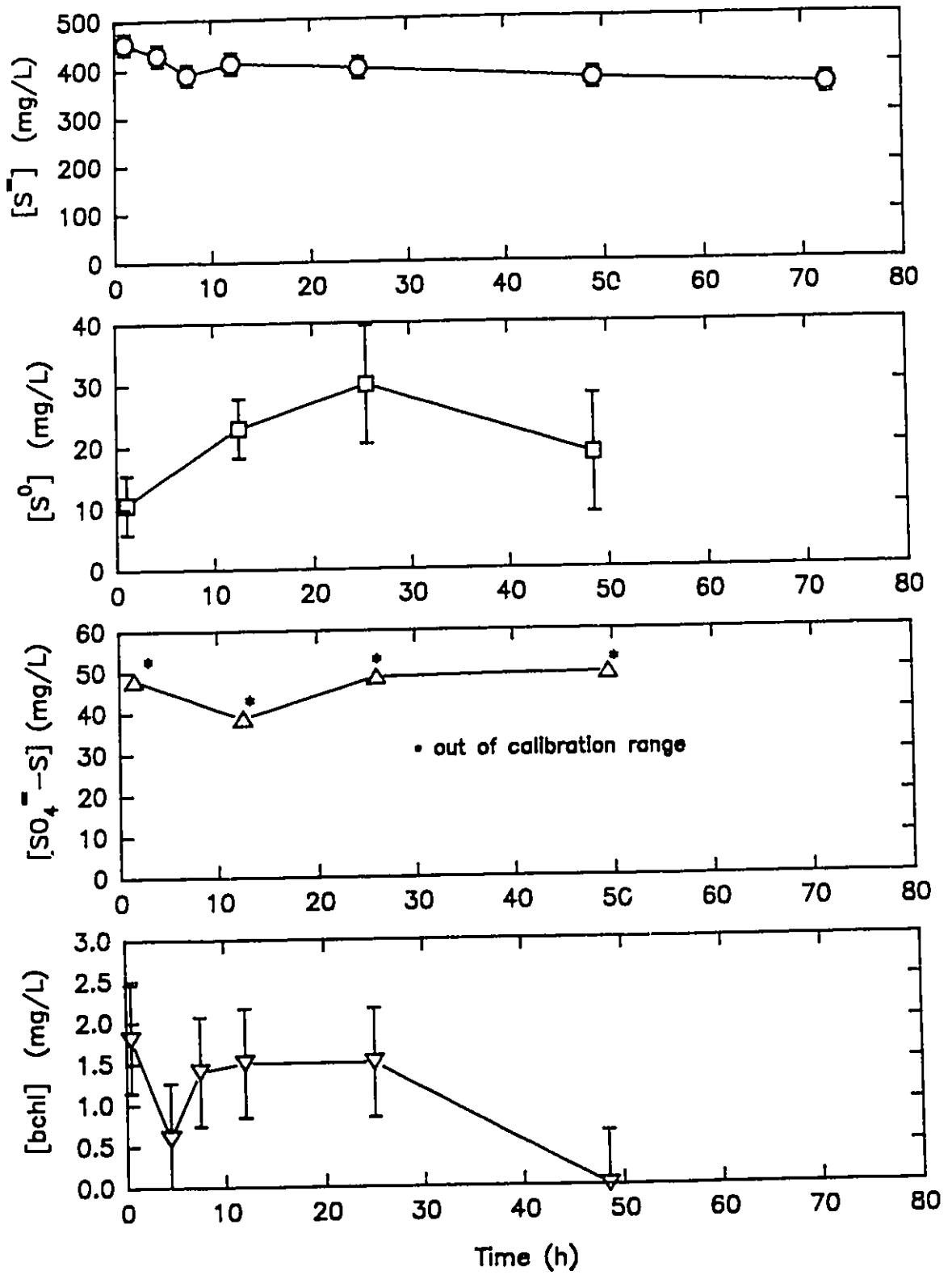
FIGURE 4.7   Concentrations in Batch Reactor Run 4
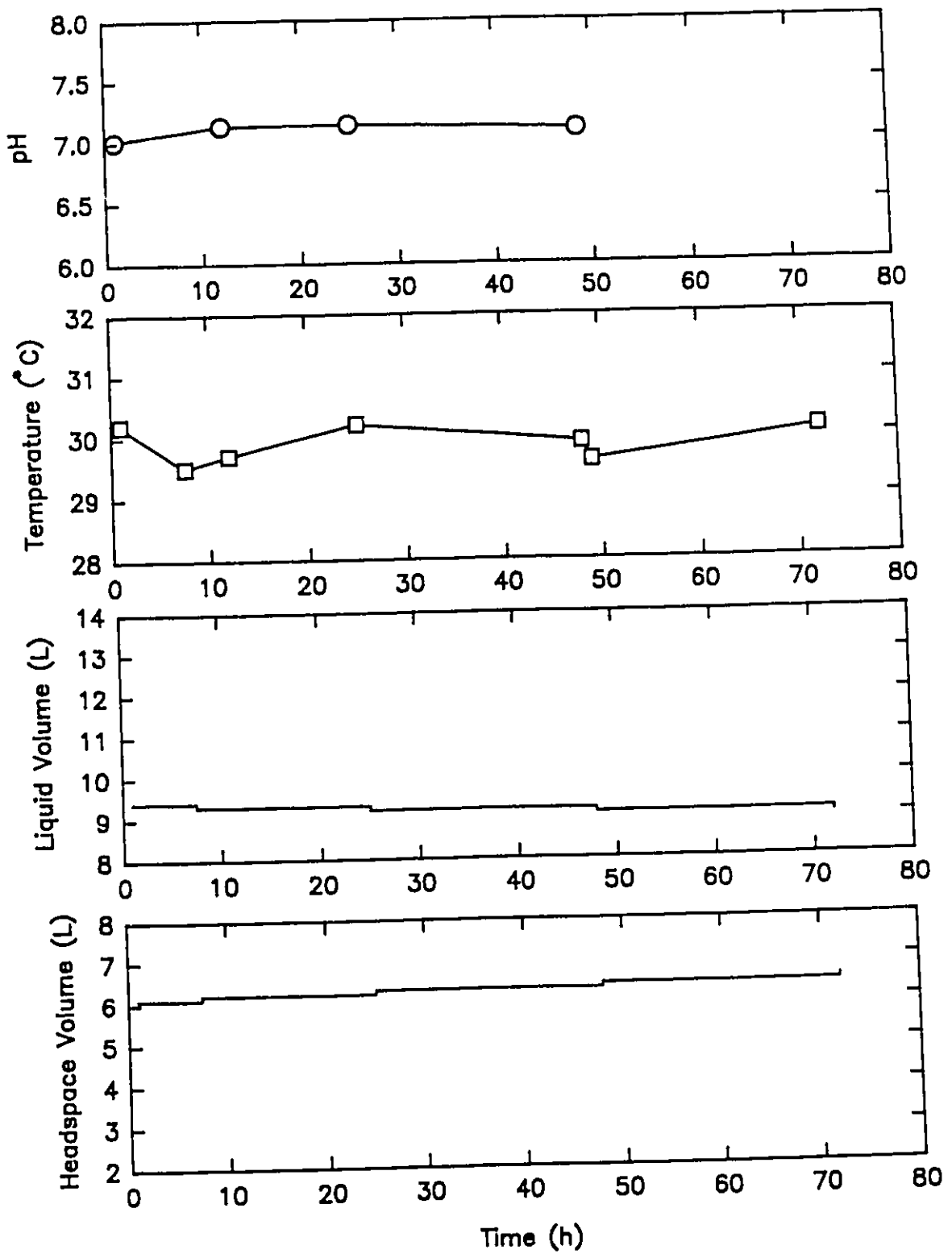
FIGURE 4.8 Reactor Environment for Batch Reactor Run 4
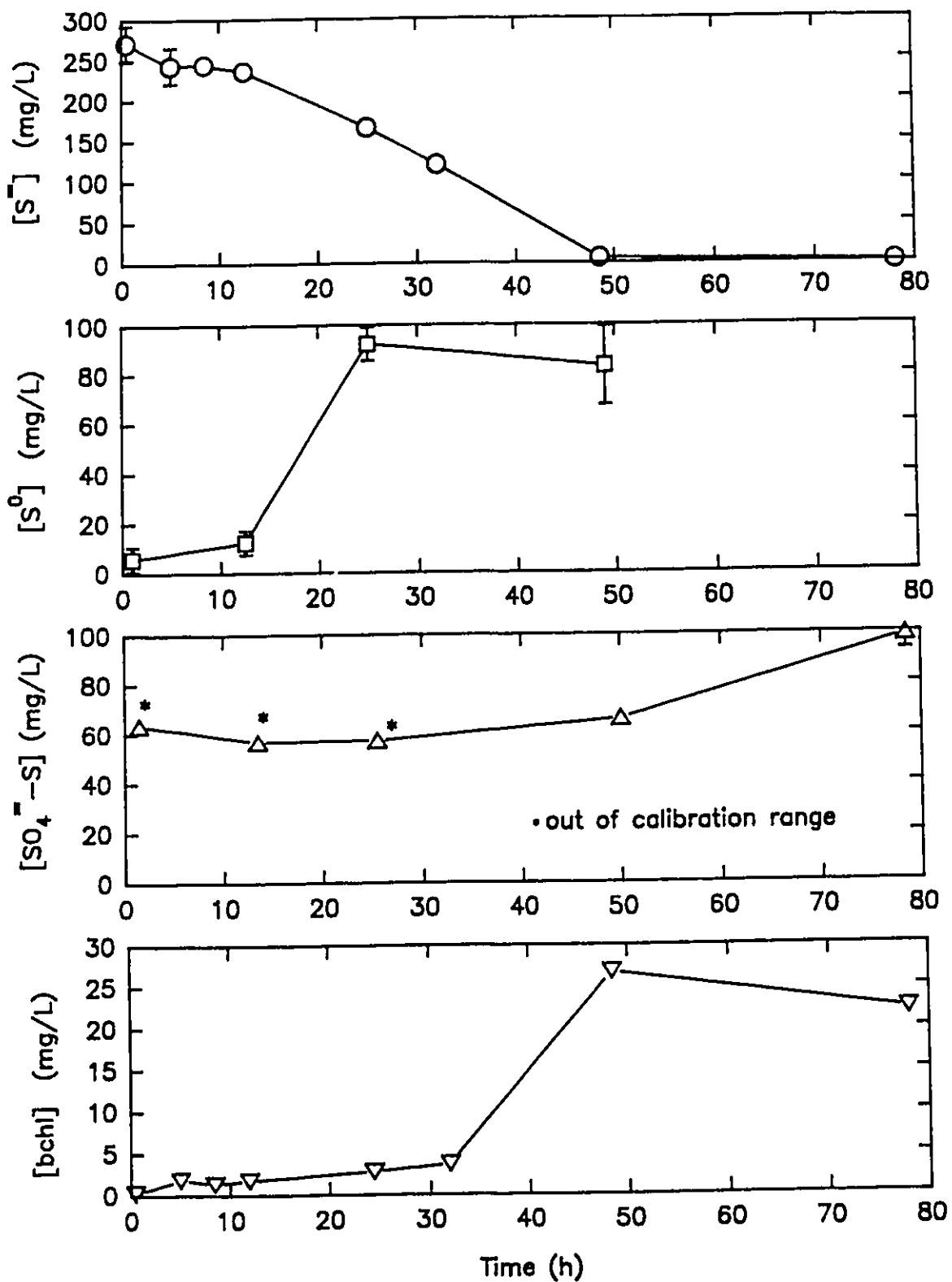
FIGURE 4.9   Concentrations in Batch Reactor Run 5

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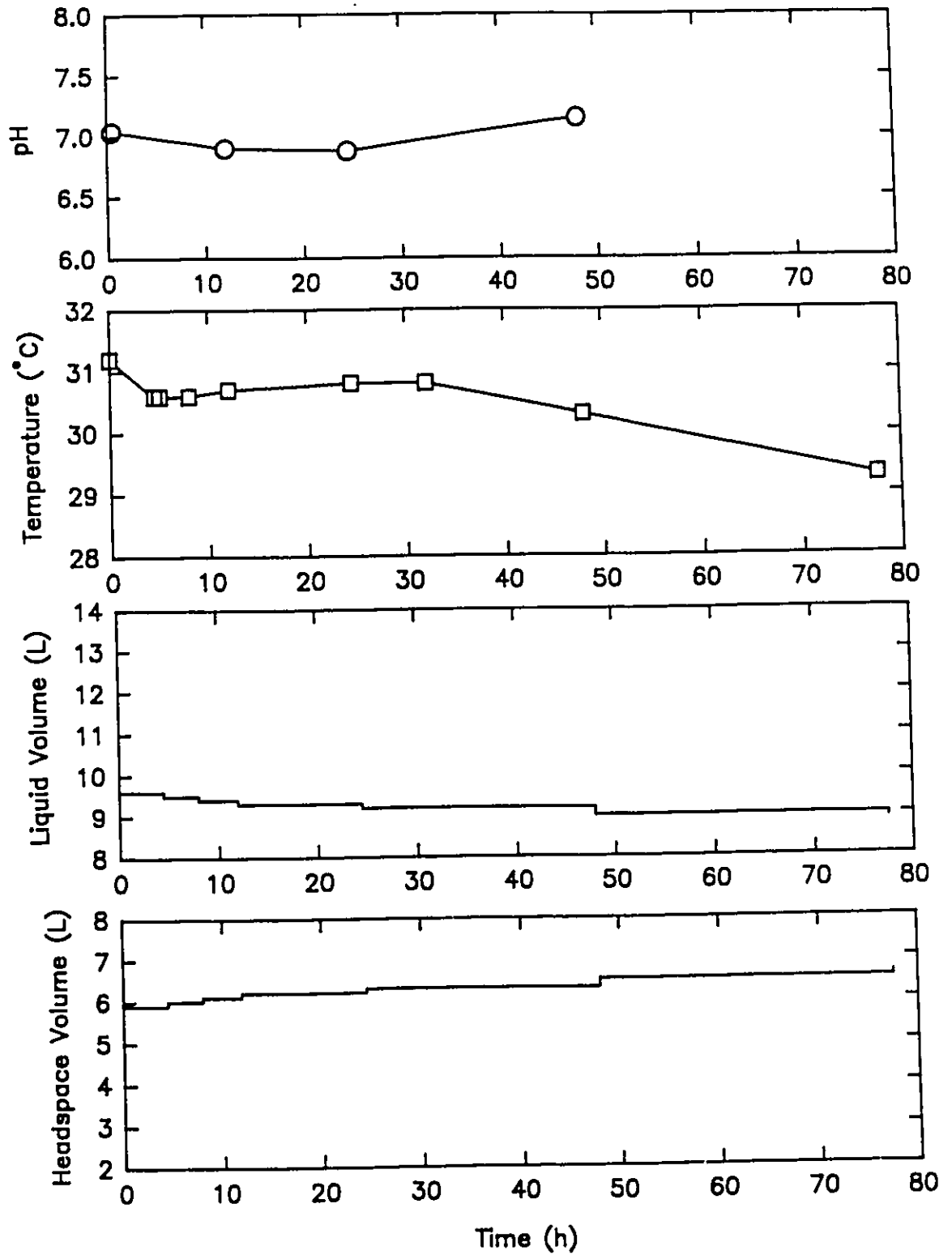
FIGURE 4.10 Reactor Environment for Batch Reactor Run 5
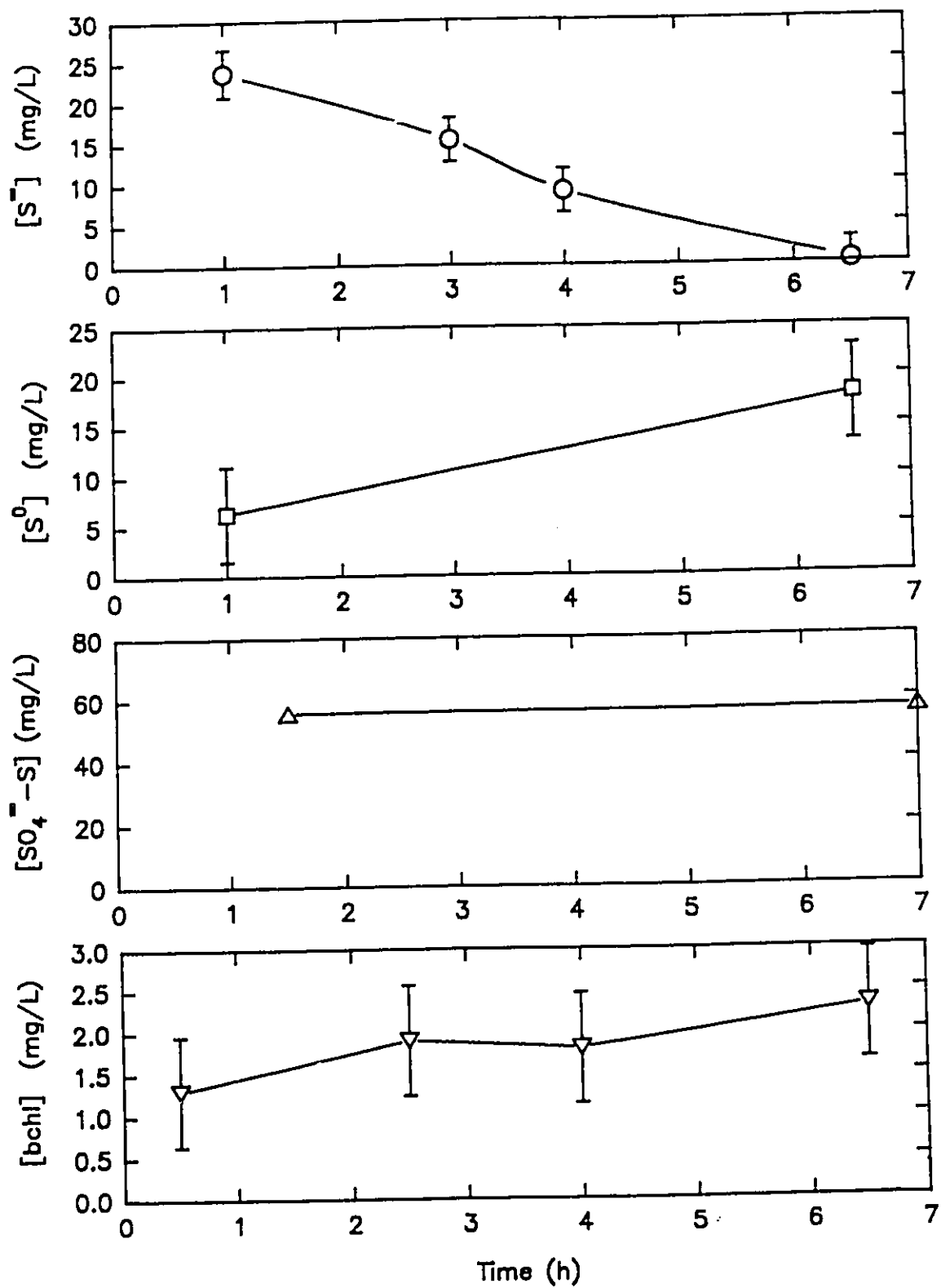
FIGURE 4.11   Concentrations in Batch Reactor Run 6
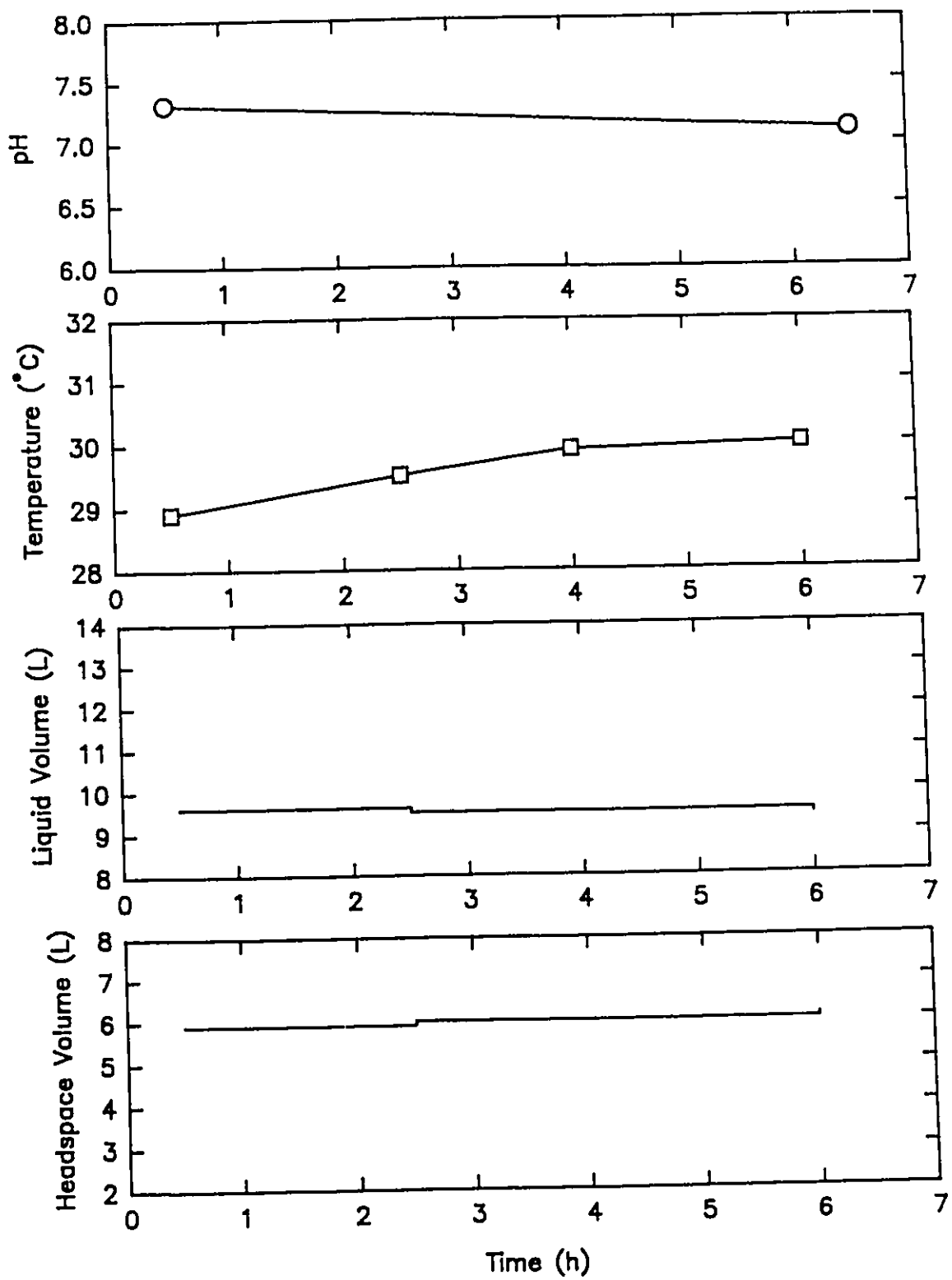
FIGURE 4.12 Reactor Environment for Batch Reactor Run 6
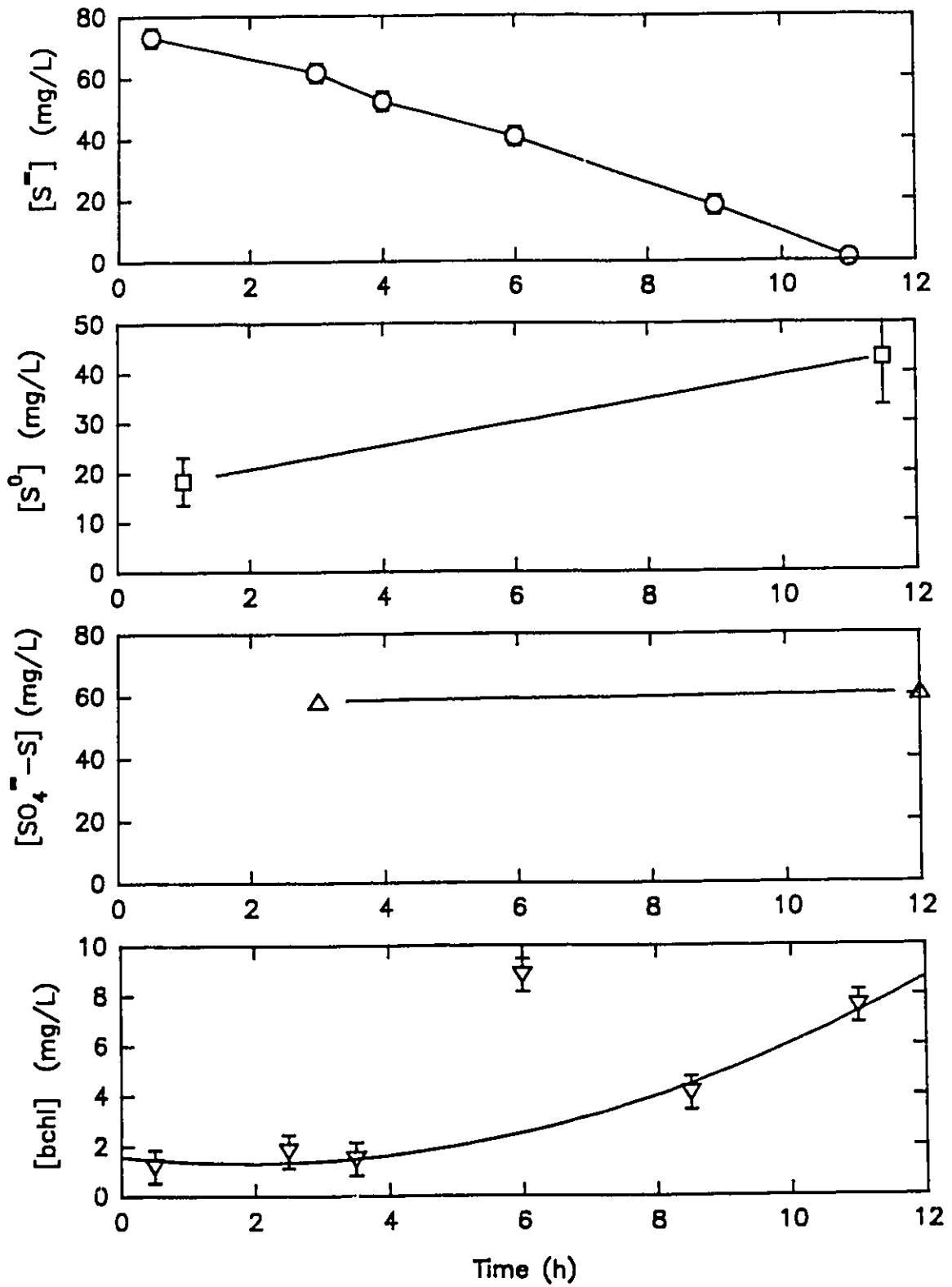
FIGURE 4.13   Concentrations in Batch Reactor Run 7
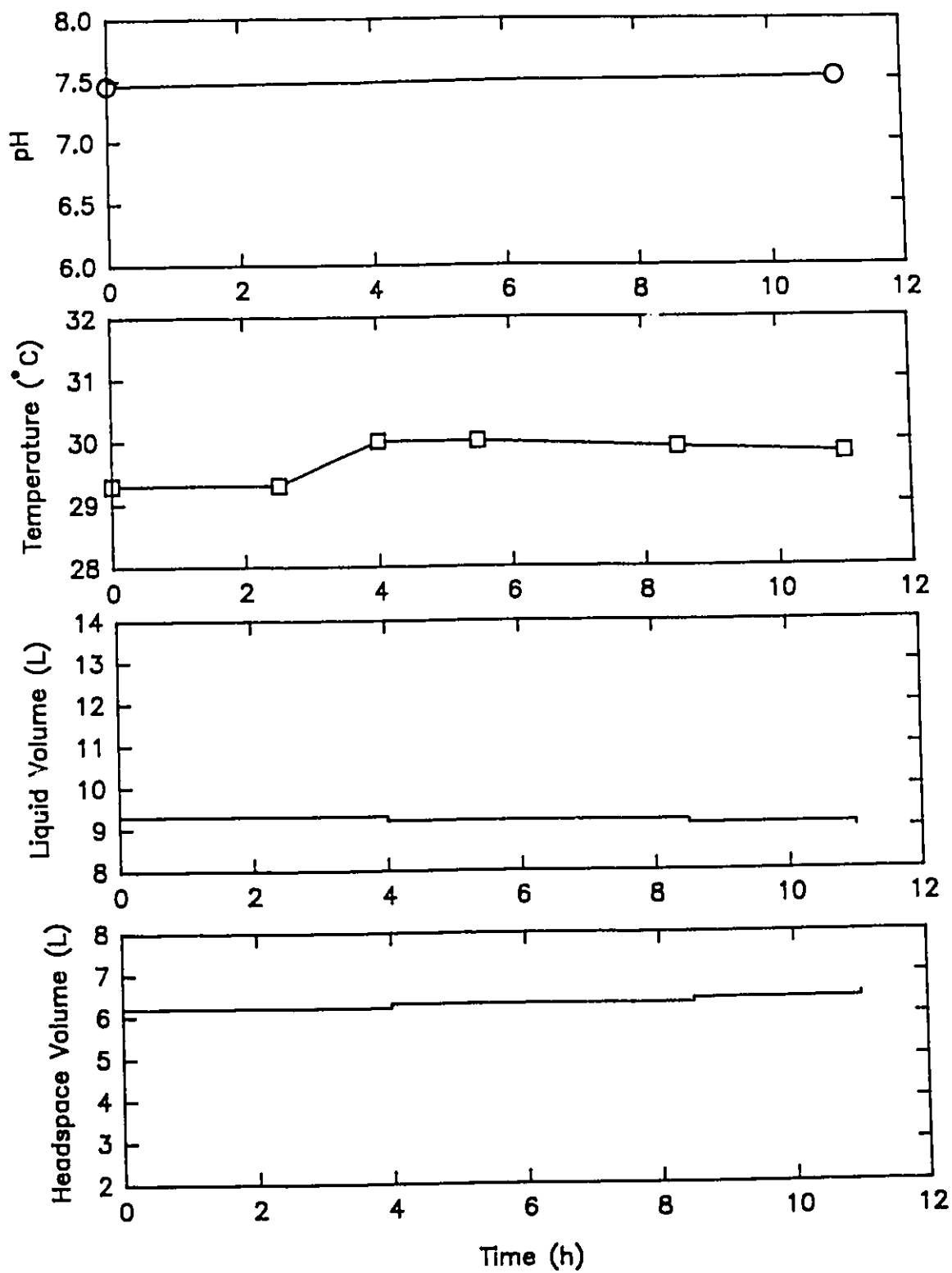
FIGURE 4.14 Reactor Environment for Batch Reactor Run 7

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concentration decreases moderately, either by reacting with oxygen remaining in the reactor or by leakage. Sulfate, elemental sulfur and bchl concentrations do not increase. Sulfate is initially present in the reactor from the dissolved mineral salts.

4.1.2 Growth of Bacteria

The calculations for the specific growth rate (μ) are summarized in Table 4.1. For Run 4, there was a decay of bacteria from the initial concentration to zero because of the inhibitory effect of high sulfide concentration (457 mg/L). For this reason, Run 4 data are not used in the calculation of percent recovery of sulfur, sulfur balance, cell yield or sulfide utilization. Due to the sparsity of data, the steepest section of the [bchl] vs. time plot was considered to be the log growth phase even though as few as two points may have been used to calculate μ. The slope of the log[bchl] versus time plot was determined by linear regression where more than two points were deemed to be in the log growth phase. When the measurement of bchl and sulfide were not coincident, the sulfide value was interpolated or extrapolated to the time of the bchl measurement. The possible errors in the μ values were calculated by adding or subtracting the uncertainty in the bchl analysis to the measured bchl values before taking logarthms. The average deviations of these "worst case" slopes from the stated μ values were used as the uncertainty in the specific growth rate.

The values of specific growth rate are plotted against the sulfide concentration at the begining of the log-growth phase later in Figure 4.26.

TABLE 4.1   Determination of Specific Growth Rate in Batch Reactor Runs

Beginning of Log-Growth Phase

Run #	Time (h)	[S=] at t1 (mg/L)	+	[bchl] at t1 (mg/L)	+
1	24.0	40	3.1	0.4	0.66
2	12.5	28	3.7	3.8	0.66
3	0.5	120	5.5	2.9	0.66
4	0.5	457	22	1.8	0.66
5	32.0	121	7.3	3.7	0.66
6	0.5	26	2.9	1.3	0.66
7	3.5	57	3.1	1.5	0.66

End of Log-Growth Phase

Time (h)	[S=] at t2 (mg/L)	+	[bchl] at t2 (mg/L)	+	# pts in phase	specific growth rate(/h)	+
48.0	1.2	3.1	3.1	0.66	2	0.085	0.05
25.0	1.7	3.1	7.4	0.66	2	0.053	0.021
24.5	0.7	2.9	9.5	0.66	3	0.049	0.013
48.5	373	22	0	0.66	6	-0.036	0.025
48.5	6	7.3	26.7	0.66	2	0.12	0.013
6.5	0.3	2.7	2.3	0.66	4	0.086	0.14
11.0	0.9	2.7	7.5	0.66	3	0.21	0.075

4.1.3 Sulfur Balance and Recovery

Stacked bar charts showing the concentrations of different sulfur species with time are shown in Figures 4.15 to 4.22. The error bars represent the cumulative uncertainties from the analyses of sulfide, elemental sulfur and sulfate. In non-control runs, the increase in S^o did not fully compensate for the net decrease in sulfide. However, as elemental sulfur was depleted, sulfate concentration had increased. The rate of decrease in S^o was greater than the rate of increase in SO_4^{2-} so that there was a depression in the cumulative measured sulfur concentrations (see Figures 4.15, 4.18). Cork (1978) observed that the concentrations of S^o and sulfate did not equal the total concentration of sulfide oxidized by the bacteria. Further studies indicated that thiosulfate was the intermediate form in which sulfur was being accumulated. Thus, in Runs 1 and 3, the "rebound" of total sulfur may have been due to the accumulation and eventual oxidation of thiosulfate, which was not measured in these experiments.

In Runs 2, 5, 6 and 7, the sulfate concentration stayed constant as sulfide was converted to elemental sulfur. These experiments were ended when sulfide was depleted. A sulfur balance was not performed on Run 4 data as there was no elemental sulfur produced in this run.

Table 4.2 shows that 35 to 90% of the sulfide oxidized was converted to elemental sulfur. When the elemental sulfur concentration measurement was not coincident with the initial sulfide measurement, the S^o data were extrapolated. Again, when sulfide was not determined at the same time as the highest measured S^o concentration, the sulfide data were interpolated or extrapolated. In this way, the interpolation or extrapolation was
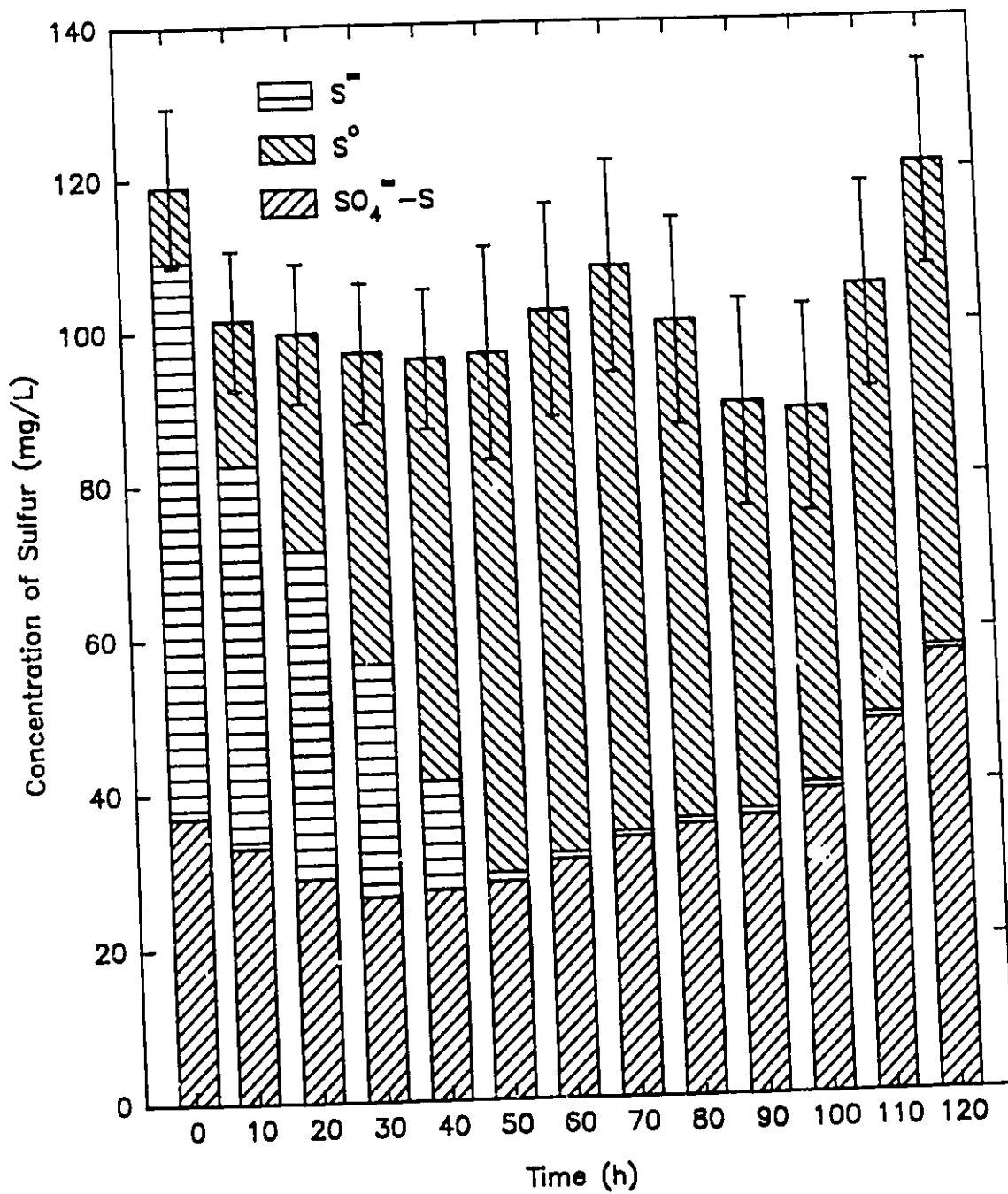
FIGURE 4.15 Sulfur Balance for Batch Reactor Run 1
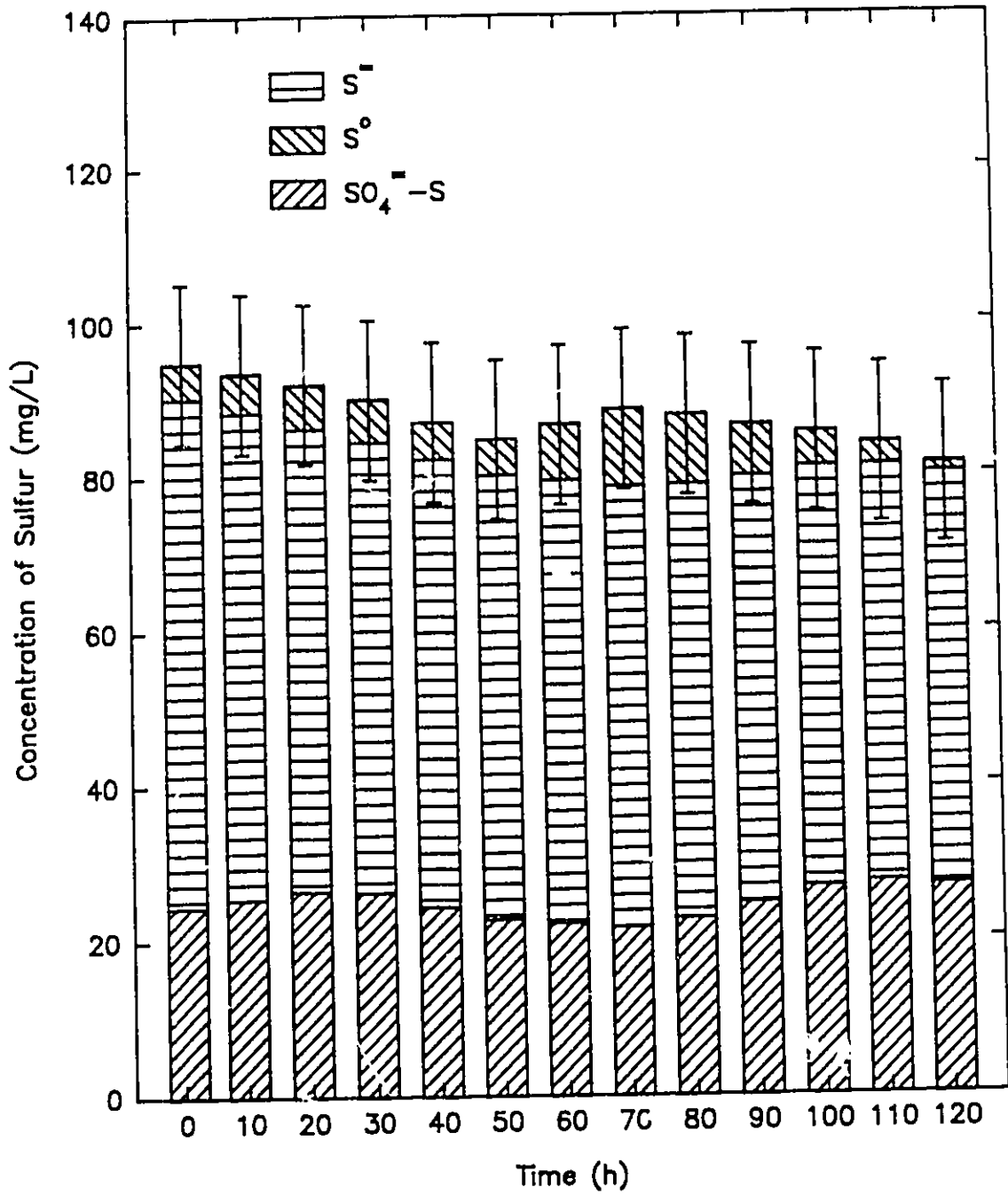
FIGURE 4.16 Sulfur Balance for Batch Reactor
Control Run 1
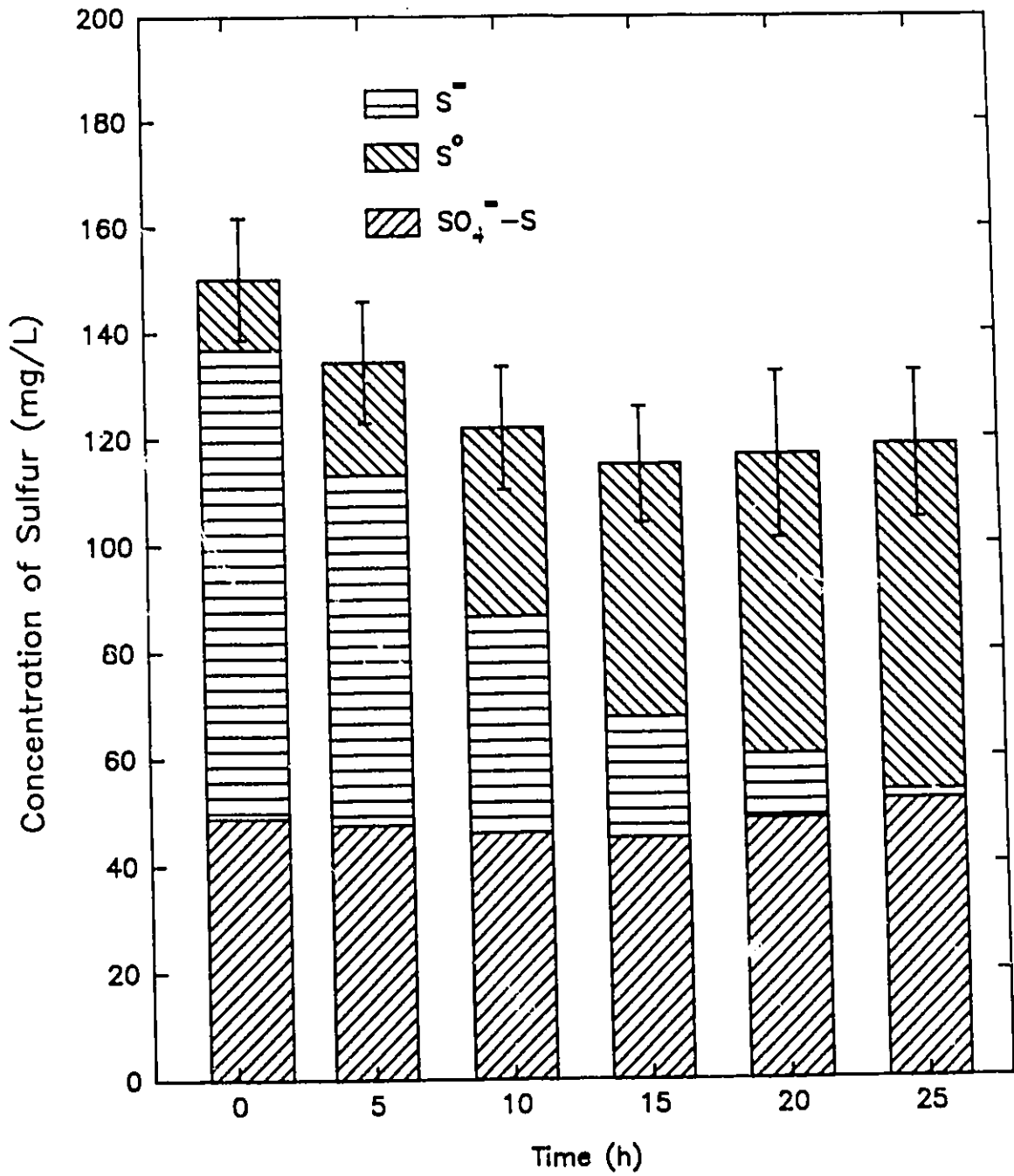
FIGURE 4.17   Sulfur Balance for Batch Reactor Run 2
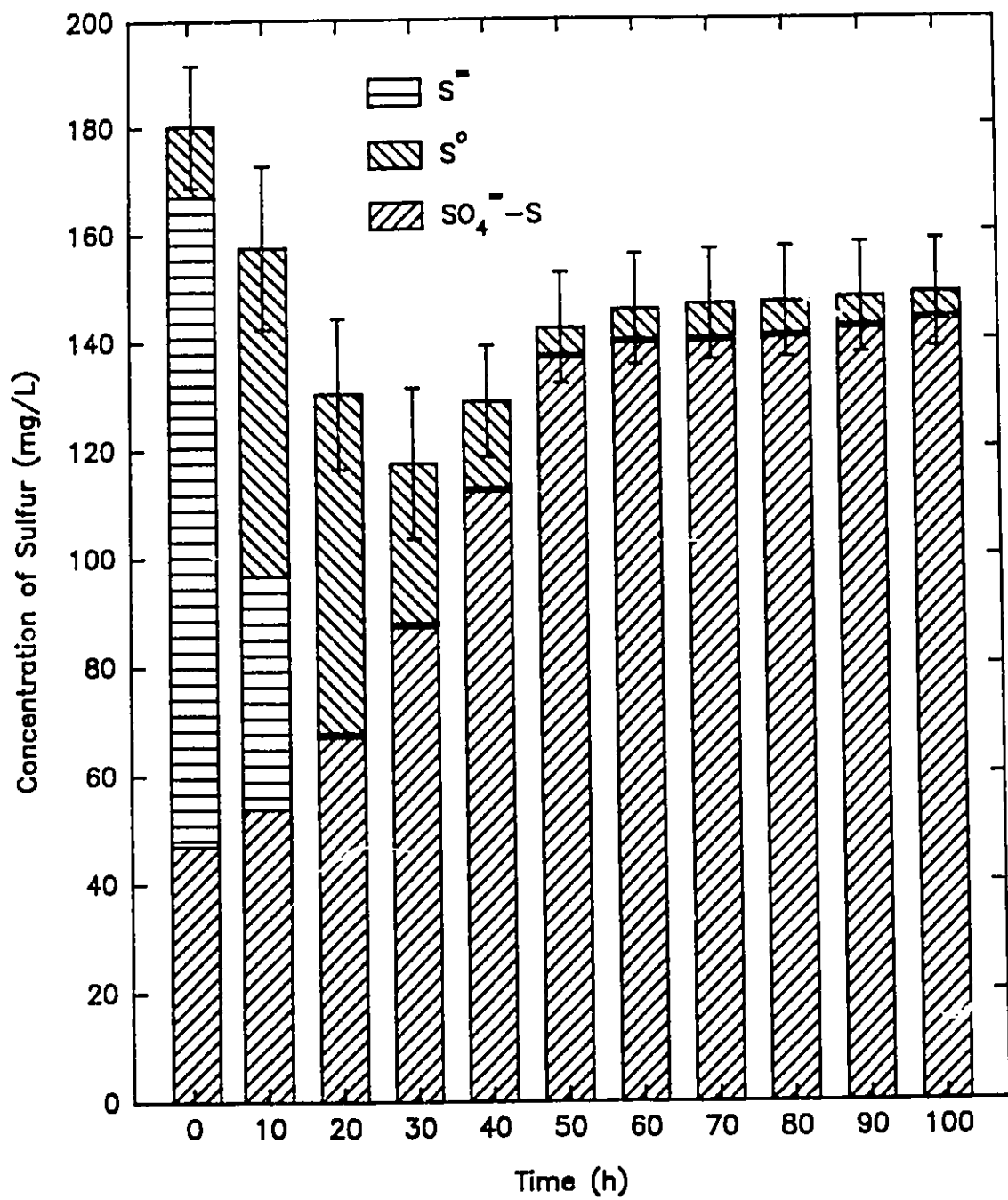
FIGURE 4.18   Sulfur Balance for Batch Reactor Run 3
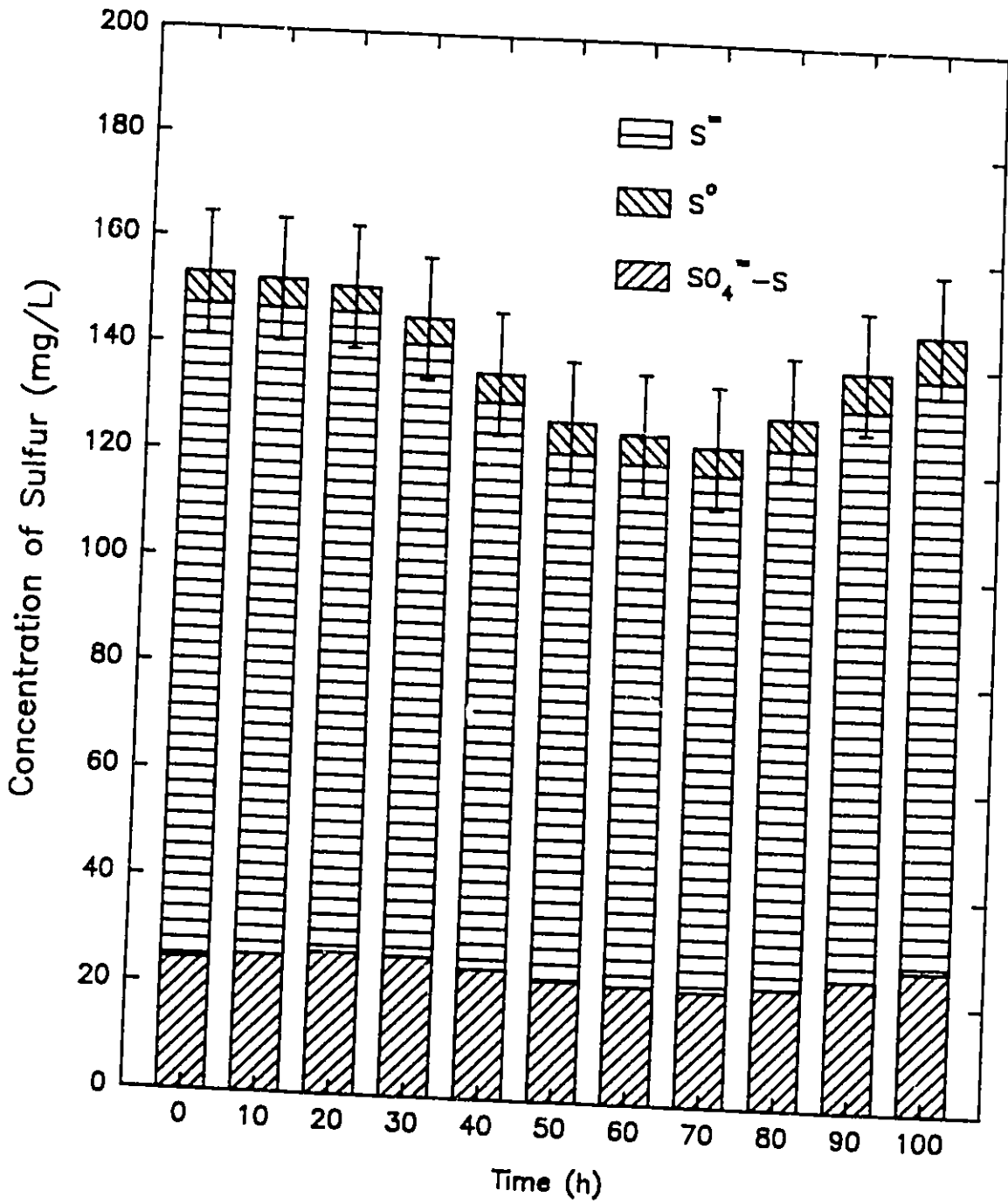
FIGURE 4.19   Sulfur Balance for Batch Reactor
Control Run 3

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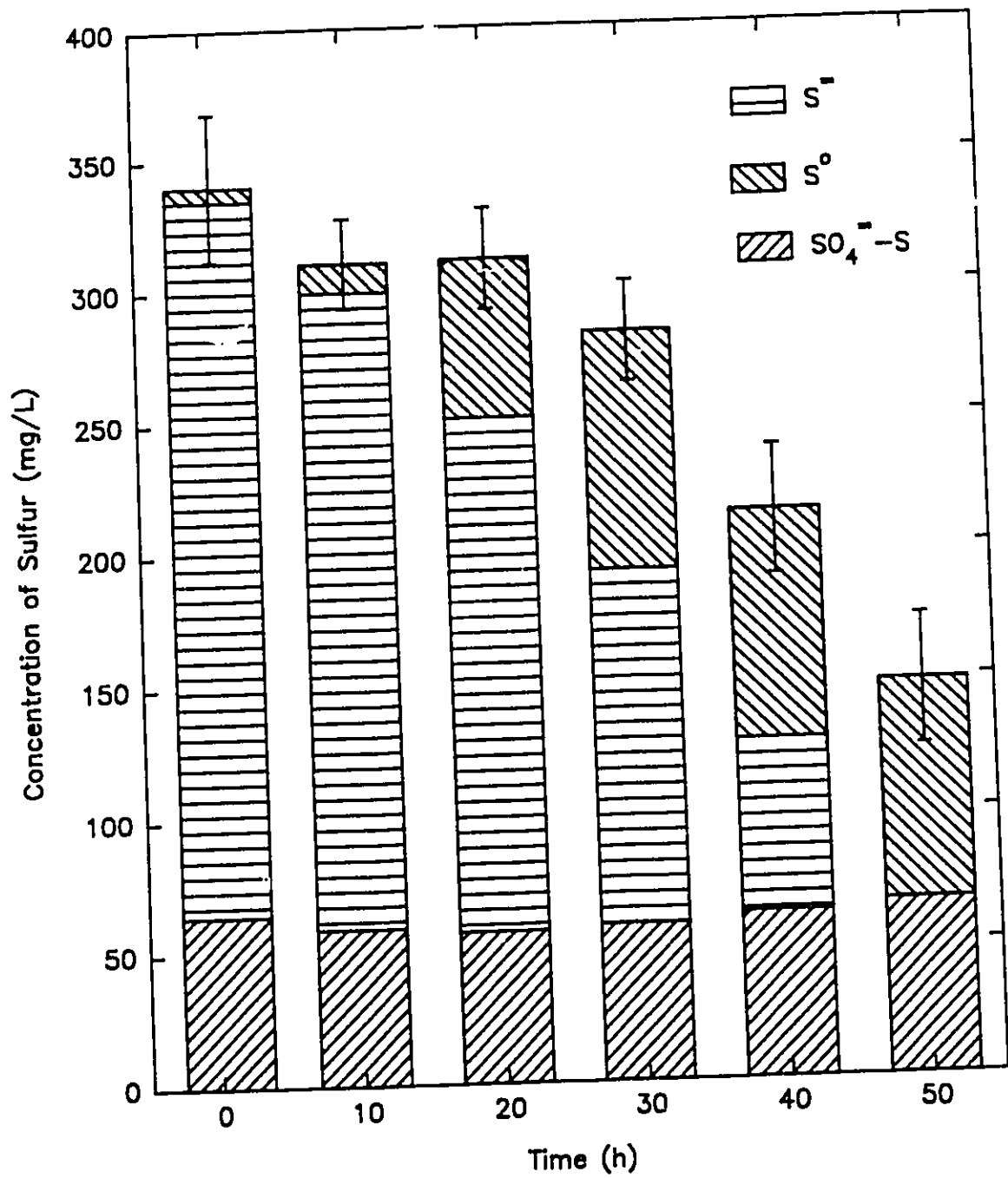
FIGURE 4.20 Sulfur Balance for Batch Reactor Run 5
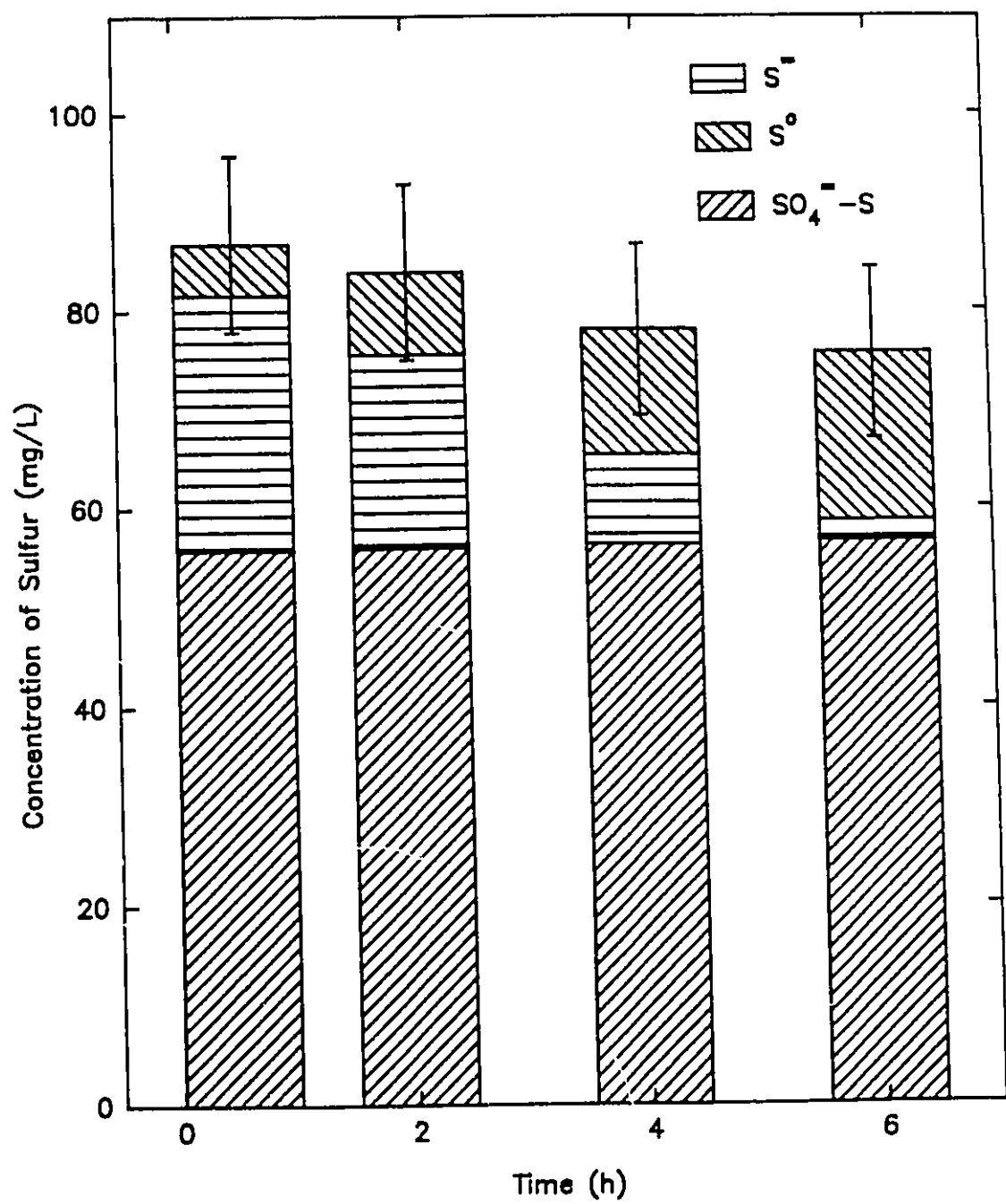
FIGURE 4.21   Sulfur Balance for Batch Reactor Run 6
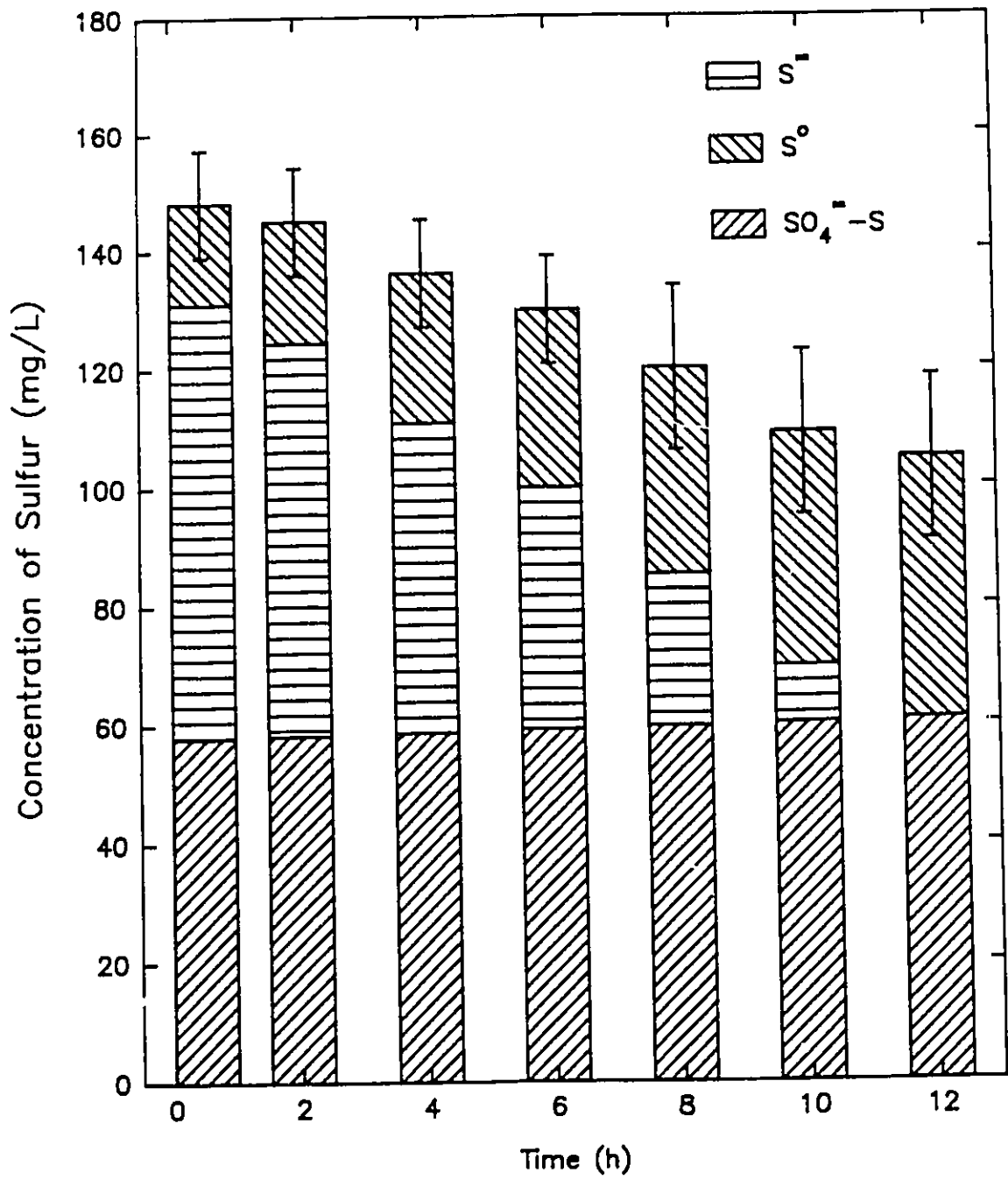
FIGURE 4.22 Sulfur Balance for Batch Reactor Run 7

TABLE 4.2   Sulfur Recovery for Batch Reactor Runs

Run #	[S=]i (mg/L)	+	[S=] at [So]m (mg/L)	+	Δ[S=] (mg/L)	+	[So]i (mg/L)	+	[So]m (mg/L)	+	Δ[So] (mg/L)	+	Δ[So]/Δ[S=] (%)	+
1	72	4.4	1	3.1	72	7.5	10	4.8	74	9.6	64	14	90	29
2	88	5.5	3	3.1	85	8.6	9	4.8	64	9.6	55	14	65	23
3	120	5.5	1	3.1	119	8.6	13	4.8	88	9.6	75	14	63	56
5	271	22	166	11	104	33	6	4.8	92	9.6	87	12	83	38
6	24	2.9	0	2.7	23	6.6	6	4.8	18	9.6	12	9.6	50	54
7	73	3.1	0	2.7	73	5.8	17	4.8	43	9.6	26	14	35	22

performed at the lowest concentration of each sulfur species. The highest S° concentration occurs typically at the end of sulfide depletion, but in Run 5 it occurred mid-way through the sulfide utilization phase. As shown in Figure 4.30 later, the uncertainty of this recovery value was high owing to the subtraction of S° measurements of moderate uncertainty. There was no correlation between percent recovery and the initial sulfide concentration.

4.2 Semi-Batch Reactor Tests

4.2.1 Data

Concentrations of sulfur species and bchl versus time are shown in Figure 4.23. Data points are joined by straight lines. Where error bars are not shown, the data point symbol is larger than the error bar. The corresponding reactor environmental conditions are plotted in Figure 4.24. The sudden increase in sulfide concentration at each injection was accentuated by drawing the line through a low point immediately before the time of the injection. The sulfide value at this point was considered to be equal to the value of the preceding point. As in the batch reactor tests, the decrease in sulfide was coincident with an increase in elemental sulfur. Sulfate, as the end-product of oxidation of sulfur accumulated in the reactor, reaching a concentration of 420 mgS/L. Bchl readings were zero within the method uncertainty for the initial depletion of sulfide. The methanol used for the absorbance blanks in the bchl assay was found to be contaminated at 168 hours, so the results between 25 hours and 168 hours were discarded. Bchl measurements for Injections 2 to 4 ranged from 7.5 to 37.9 mg/L and were generally higher than in the batch reactor runs.

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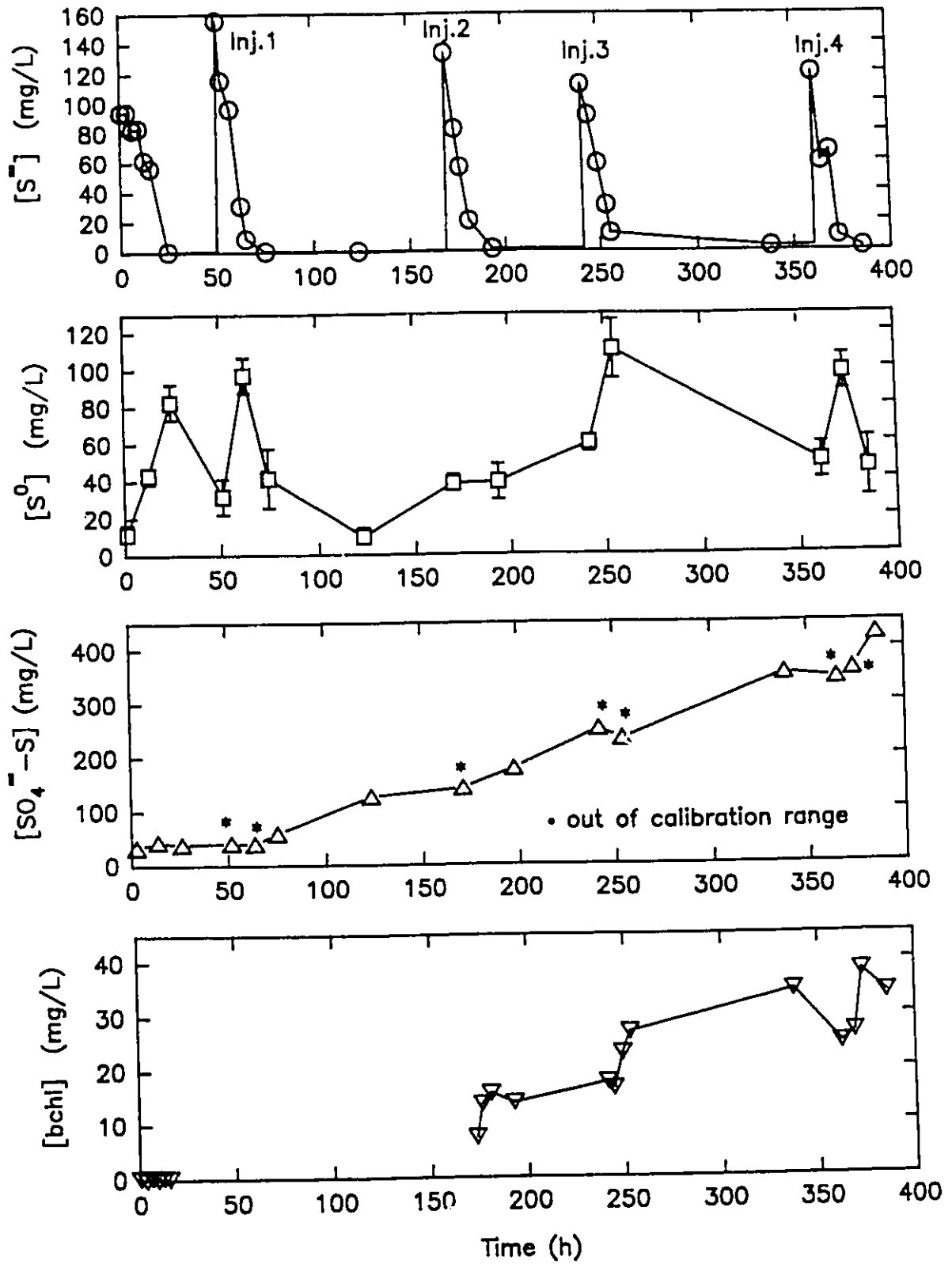
FIGURE 4.23   Concentrations in Semi–Batch Reactor

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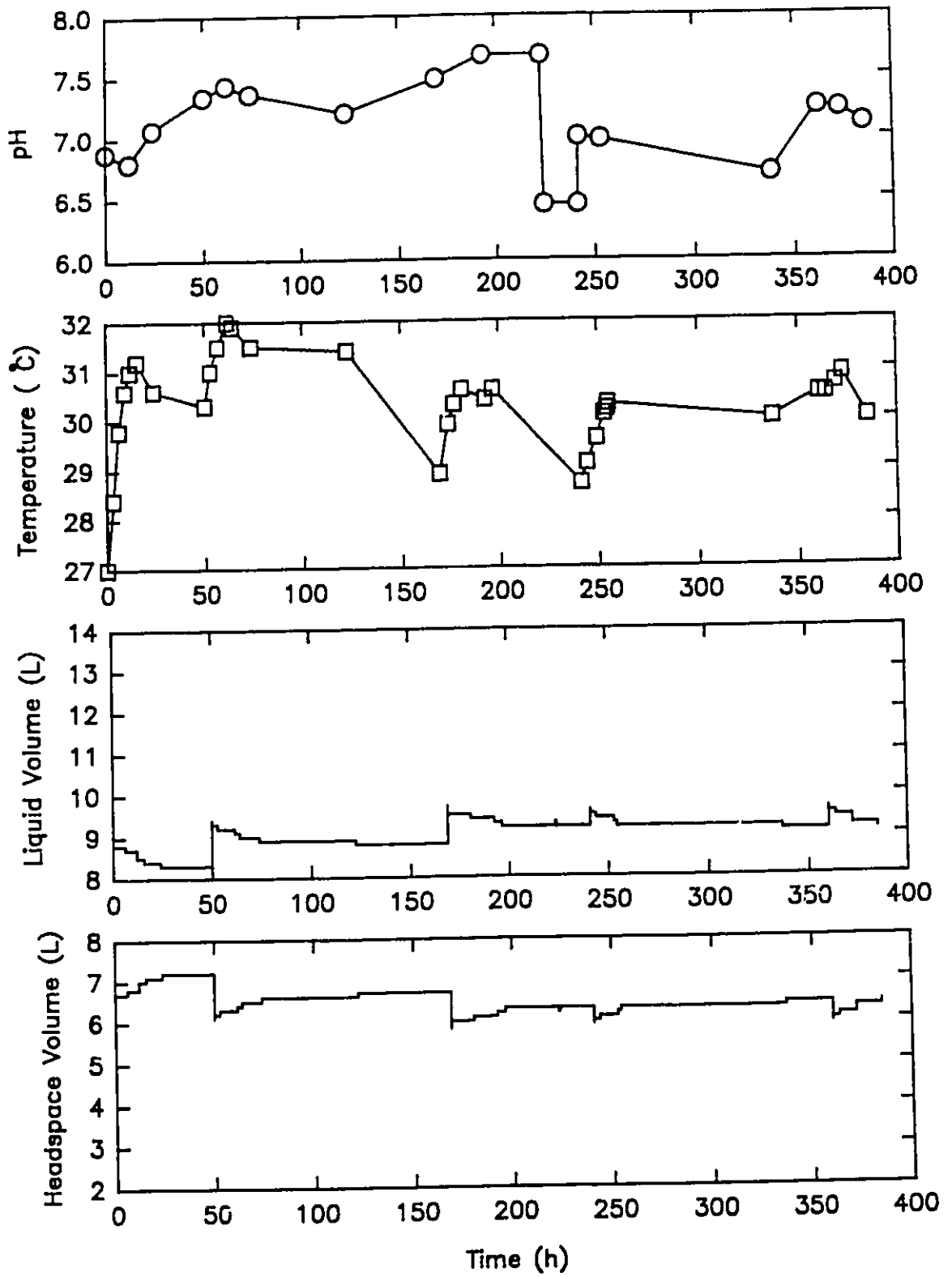
FIGURE 4.24 Reactor Environment for Semi—Batch
Reactor

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At each injection, approximately 1 L of sulfide solution was added to the reactor thereby causing the dilution of elemental sulfur, sulfate and bchl concentrations. This is most evident in Injection 4 where sulfate and bchl measurements were taken 24 hours before the injection.

The pH was adjusted at 224 hours as seen in Figure 4.24 by the addition of sterile 1M HCl solution.

4.2.2 Growth of Bacteria

Specific growth rates were calculated as in the batch reactor. These data are presented in Table 4.3 and plotted later in Figure 4.26.

4.2.3 Sulfur Balance and Recovery

Figure 4.25 shows the sulfur balance for the semi-batch reactor. An attempt was made to depict the sulfur species profile immediately after the injection of sulfide, at the elemental sulfur maximum and after sulfide depletion. The error bars represent the cumulative uncertainties from the analyses of sulfide, elemental sulfur and sulfate. As in the case of the batch reactor, conversion of sulfide to S^o is not 100%. The "loss" of sulfur to other species is evident after Injections 1 and 2. Sulfur recovery ranged from 1 to 78% as shown in Table 4.4. Interpolations were performed as outlined in Section 4.1.3. In Injection 3, the draw at 193 hours probably missed the elemental sulfur peak resulting in a reported sulfur recovery of 1%.

4.3 Discussion

4.3.1 Growth of Bacteria

The values of specific growth rate obtained from batch and semi-batch reactor experiments are plotted against the initial concentration of sulfide (at the begining of the log-growth phase) in Figure 4.26. The

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TABLE 4.3   Determination of Specific Growth Rate in Semi-Batch Reactor Injections

Injection #	Begining of Log-Growth Phase					End of Log-Growth Phase					# pts in phase	specific growth rate (/h)	+
	Time (h)	$[S=]$ at t1 (mg/L)	+	[bchl] at t1 (mg/L)	+	Time (h)	$[S=]$ at t2 (mg/L)	+	[bchl] at t2 (mg/L)	+			
2	173.5	88	4.4	7.5	0.66	176.5	56	4.4	13.7	0.66	2	0.20	0.046
3	244.5	91	5.5	16.5	0.66	253.0	30	3.7	26.9	0.66	3	0.058	0.008
4	369.0	67	4.4	26.6	0.66	373.0	9.5	3.1	37.9	0.66	2	0.089	0.011
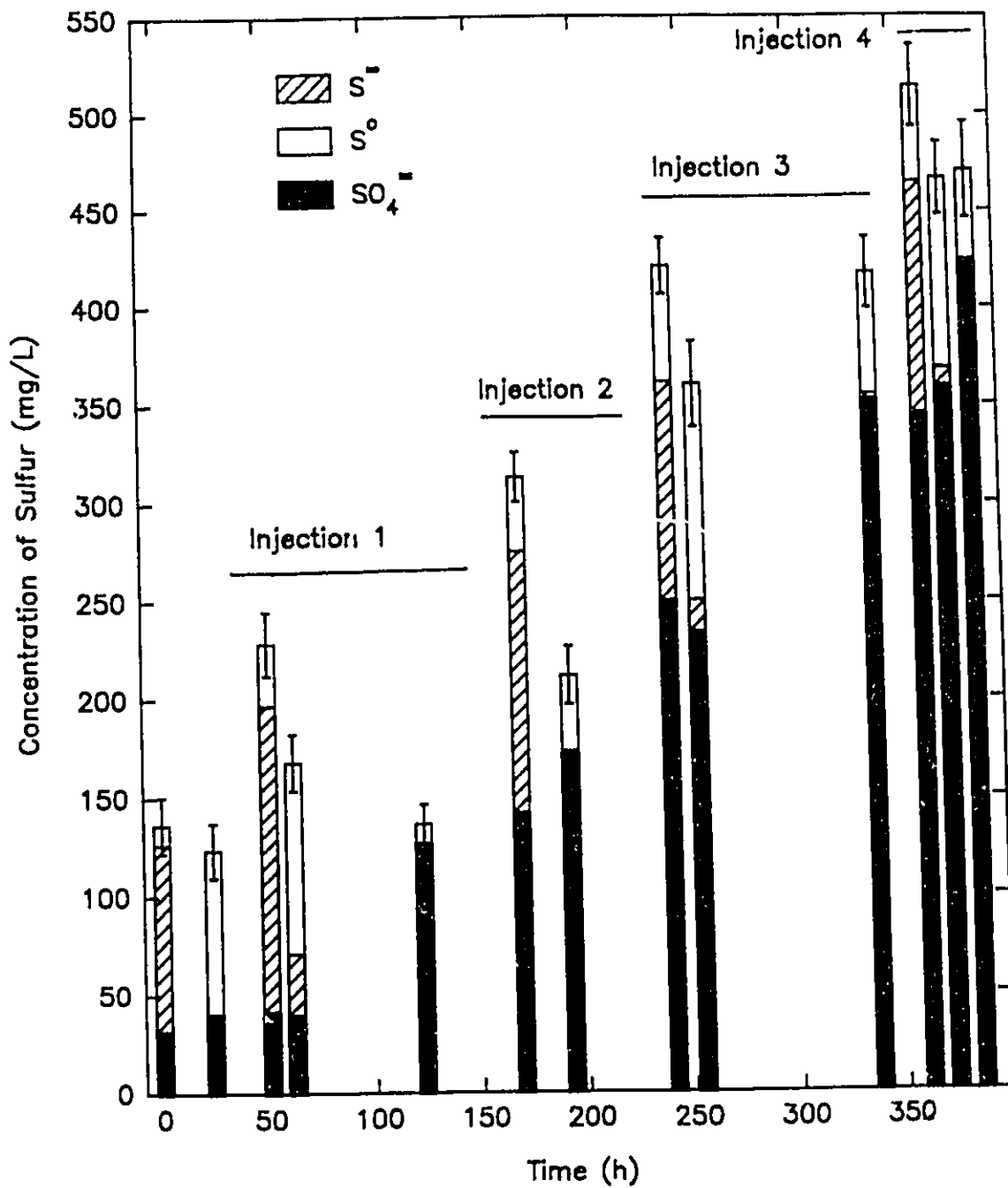
FIGURE 4.25   Sulfur Balance for Semi—Batch Reactor

TABLE 4.4   Sulfur Recovery for Semi-Batch Reactor

Injection #	[S=]i (mg/L)	+	[S=] at [So]m (mg/L)	+	Δ[S=] (mg/L)	+	[So]i (mg/L)	+	[So]m (mg/L)	+	Δ[So] (mg/L)	+	Δ[So]/Δ[S=] (%)	+
initial	94	5.5	1	3.1	94	8.6	10	4.8	83	9.6	73	14	78	22
1	156	5.5	31	3.7	124	9.2	32	9.6	97	9.6	66	19	53	19
2	133	5.5	1	2.7	132	8.2	38	4.8	39	9.6	1	14	1	11
3	112	5.5	16	3.1	96	8.6	60	4.6	110	16	51	21	53	26
4	120	5.5	10	3.1	110	8.6	49	9.6	98	9.6	48	19	44	21
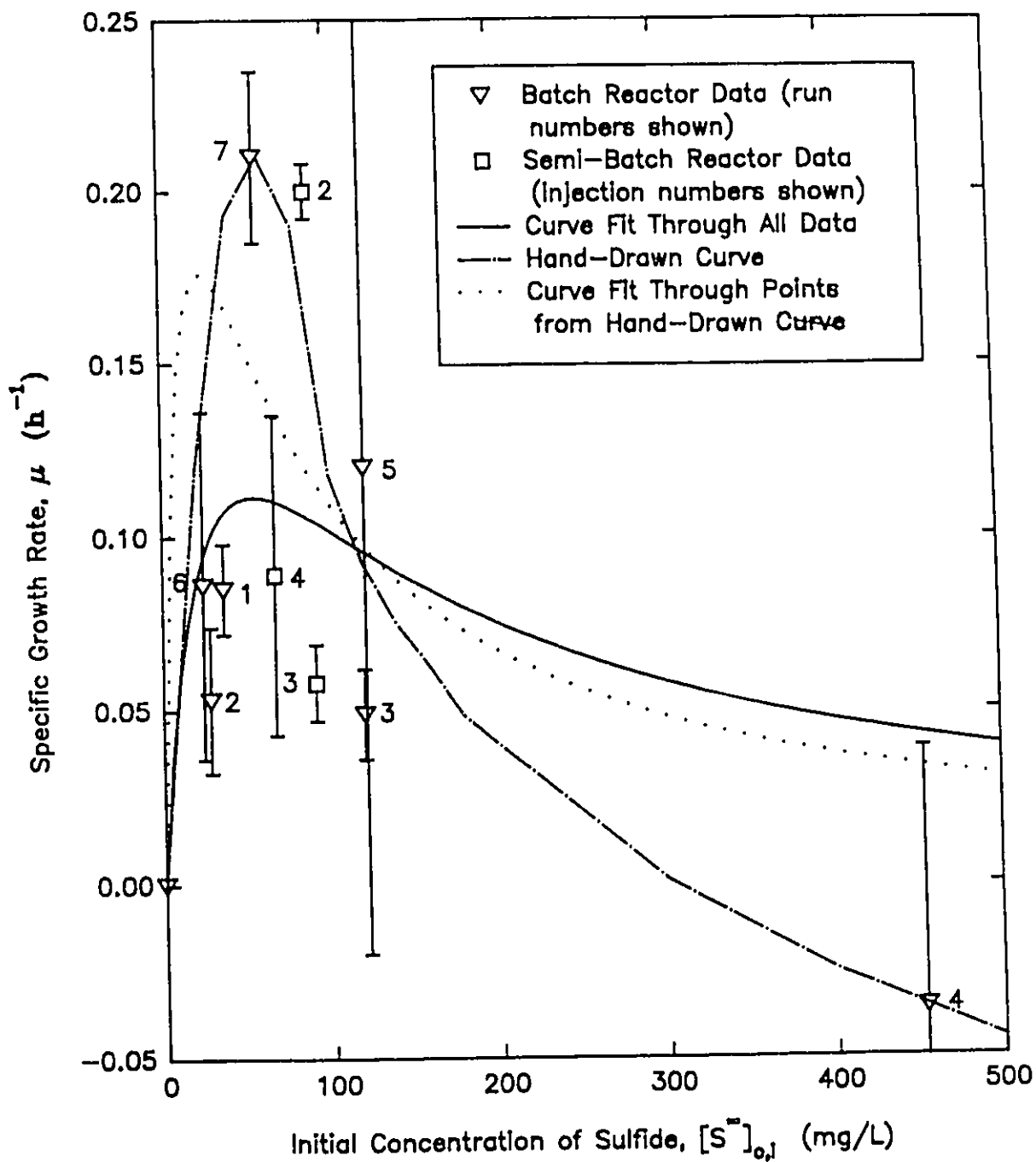
FIGURE 4.26 Specific Growth Rate of Bacteria versus Sulfide Concentration at Begining of Log-Growth Phase of Bacteria

data were fitted to the Haldane Equation 2.12 with and without the point at (0,0) with no difference in the result. The calculated values for parameters μ_{max}, Ks and Ki, based on the entire data, were used to generate the solid line in Figure 4.26. Similarily, the data points from the hand-drawn curve (dot-dash line) were entered into the curve fitting program and the resultant parameters were used to draw the dotted line. The form of the equation does not allow a duplication of the hand-drawn line. For comparison, the parameters value are:

Source of Data	μ_{max} (h^{-1})	Ks (mg/L)	Ki (mg/L)
experiment	0.446	54.7	54.6
hand-drawn	0.705	23.8	23.8

Maka (1986) obtained μ values of 0.01 to 0.15 h^{-1} in gas-fed static cultures of *C. thiosulfatophilum* where the total light energy ranged from 0.3 to 4.9 W. Cork *et al.* (1985) reported μ values of 0.033 to 0.050 h^{-1} in a similar reactor receiving a total of 2.1 to 28.8 W of light energy. The majority of μ values in Figure 4.26 are in the 0.045 to 0.090 h^{-1} range, obtained with an incident light level of 7.1 W. From these cummulative data, it would seem that there is not a direct correlation between the specific growth rate and the incident light energy.

In batch studies with toxic substrates, the observed μ values do not approach μ_{max} with an increase in the substrate concentration, as they do for non-toxic substrates. This is due to the fact that the inhibitory effect increases as the substrate (food) concentration increases. In addition, the μ_{max} values in batch culture are usually lower than in continuous culture due to the dilution effect on the high substrate feed concentration (Gaudy *et al.*,1980).

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The maximum level of sulfide tolerable by *C. thiosulfatophilum* can be read from Figure 4.26. The lines of best fit do not cross the $\mu = 0$ line, bound by the form of the Haldane equation. However, the observations of bacterial death at 457 mgS^{2-}/L and survival at 270 mgS^{2-}/L set the practical level for no bacteria growth at 300 mgS^{2-}/L. At this sulfide concentration, S^{2-} would be metabolized by the available *C. thiosulfatophilum* until its concentration is about 120 mgS^{2-}/L, at which point the bacteria population would grow logarithmically.

The specific growth rate is affected mildly by the initial pH of the run or injection in addition to the sulfide concentration (Figure 4.27). The correlation coefficient indicates that the null hypothesis that μ and pH_o are not related can be rejected with 97.5% confidence. However, in sets of runs which have similar pH_o values but different sulfide concentrations at the begining of the log-growth phase, the Haldane relation applies. An example is the set of batch reactor Runs 6, 7 and 3. Although the pH_o values are between 7.27 and 7.47, the specific growth rates are much different and conform to the peak of Figure 4.26.

Figure 4.28 shows plots for calculating the true cell yield. The slope of the lines in this figure are equal to the true cell yield. The increase in bchl from the beginning of the log-growth phase was calculated by subtracting the concentration of bchl at the beginning of the log-growth phase from all subsequent bchl measurements. The decrease in concentration of sulfide was calculated by subtracting all sulfide values in the log-growth phase from the initial sulfide concentration at the beginning of the log-growth phase.

Values of Y_c^* range between 0.021 and 0.20. In all semi-batch
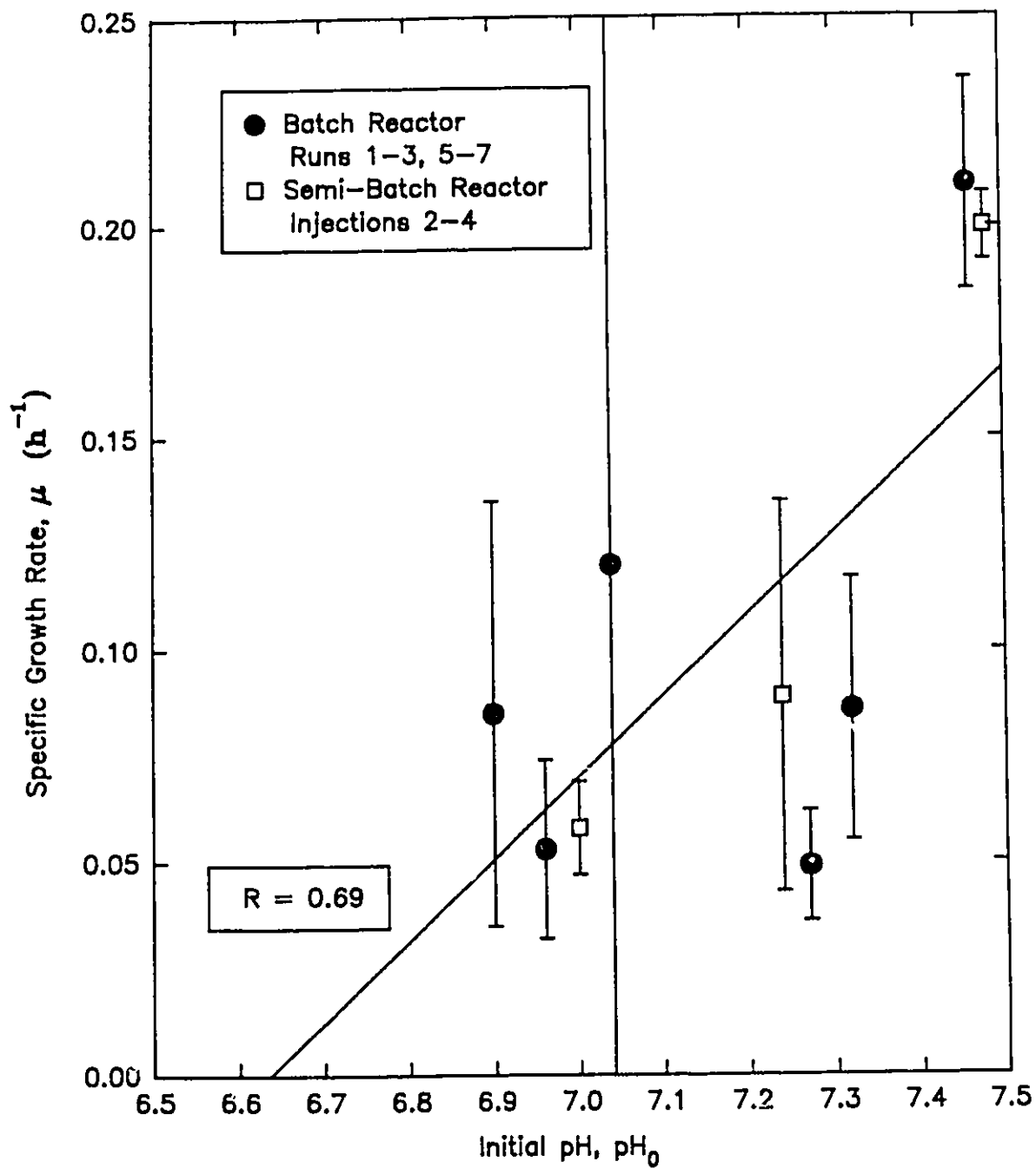
FIGURE 4.27   Specific Growth Rate versus Initial pH
in Batch and Semi−Batch Reactors

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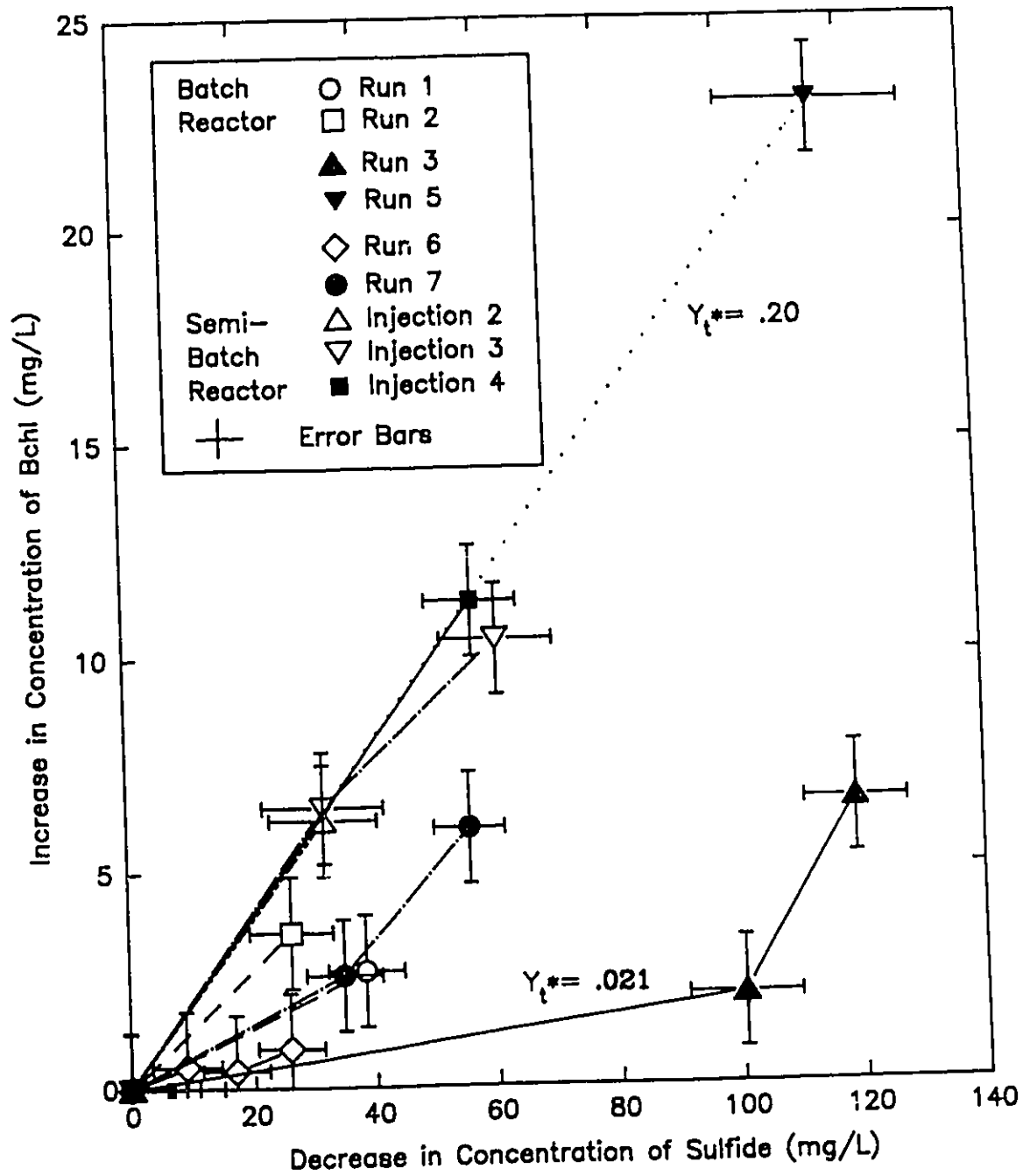
FIGURE 4.28 Plot for Calculation of True Cell Yield

Injections,this value was consistently around 0.20. Kakidas (1982) has reported that 1 mg/L of bchl corresponds to 36 mg dry weight/L of biomass. Converting these yield values to (mg dry weight biomass)/L per (mg sulfide utilized)/L gives a yield range of 0.76 to 7.2. This is up to eighteen times the typical values reported for wastewater (Section 2.3). Kakidas (1982) does not report the source of the ratio of bchl concentration to dry weight.

The theoretical maximum yield is calculated from Equation 2.2 as follows:

$$Y_t = \frac{2 \text{ mmole } CO_2 \text{ fixed}}{\text{mmole } S^* \text{ consumed}} \times \frac{\dfrac{1 \text{ mmole } CH_2O \text{ formed}}{\text{mmole } CO_2 \text{ fixed}} \times \dfrac{30 \text{ mg } CH_2O}{\text{mmole } CH_2O}}{\dfrac{32.06 \text{ mg } S^*}{\text{mmole}}}$$

$$= \frac{1.8 \text{ mg biomass formed}}{\text{mg } S^* \text{ consumed}}$$

Thus, if all of the metabolism of the bacterium was directed at growth, 1.8 mg of cell dry mass as CH_2O would be formed for every mg of sulfide utilised. The actual cell mass created was different for two reasons. Bacteria need electron donors to carry out their metabolism. These anaoxygenic photosynthetic bacteria obtain electrons both from the oxidation of sulfide and the activation of photosynthetic pigments. Thus sulfide is not the only source of electrons. Secondly, not all of the compounds needed for cell growth can be derived from CO_2. Some of the cell mass is created by assimilating nitrogen and sulfur compounds.

The μ_{max} and Y_t^* constants can be used in developing a preliminary model for a continuous flow reactor without recycle. At steady state, the specific growth rate in the reactor is equal to the dilution rate (D) or flow rate per unit volume. Rewriting the Monad Equation 2.11 and substituting D for μ, yields:

$$S = [S^{2-}] = [S^{2-}]_{eff} = K_s \left(\frac{D}{\mu_{max} - D} \right) \qquad [4.1]$$

The bchl concentration can be calculated from the change in substrate concentration by:

$$[bchl] = Y_t^* \left([S^{2-}]_{infl} - [S^{2-}]_{eff} \right)$$

$$[4.2]$$

Both $[S^{2-}]_{eff}$ and [bchl] are plotted as functions of the dilution rate in Figure 4.29. This is referred to as the dilute-out curve because it shows that without recycle there is a dilution rate at which the biomass concentration will be flushed out of the reactor.

4.4.2 Sulfur Recovery

There is a correlation between the recovery of sulfur and the initial pH in the reactor at the beginning of a run/injection (Figure 4.30). The corelation coefficient of 0.82 allows us to reject the null hypothesis that percent recovery of sulfur and initial pH are not related with 99% confidence. The relationship may be due to the fact that sulfate is the more stable form of sulfur at higher pH (Chen,1974) and thus would be formed in preference to sulfur.

4.3.3 Sulfide Utilization

The rate of sulfide utilization, $d[S^{2-}]/dt$ was found to be
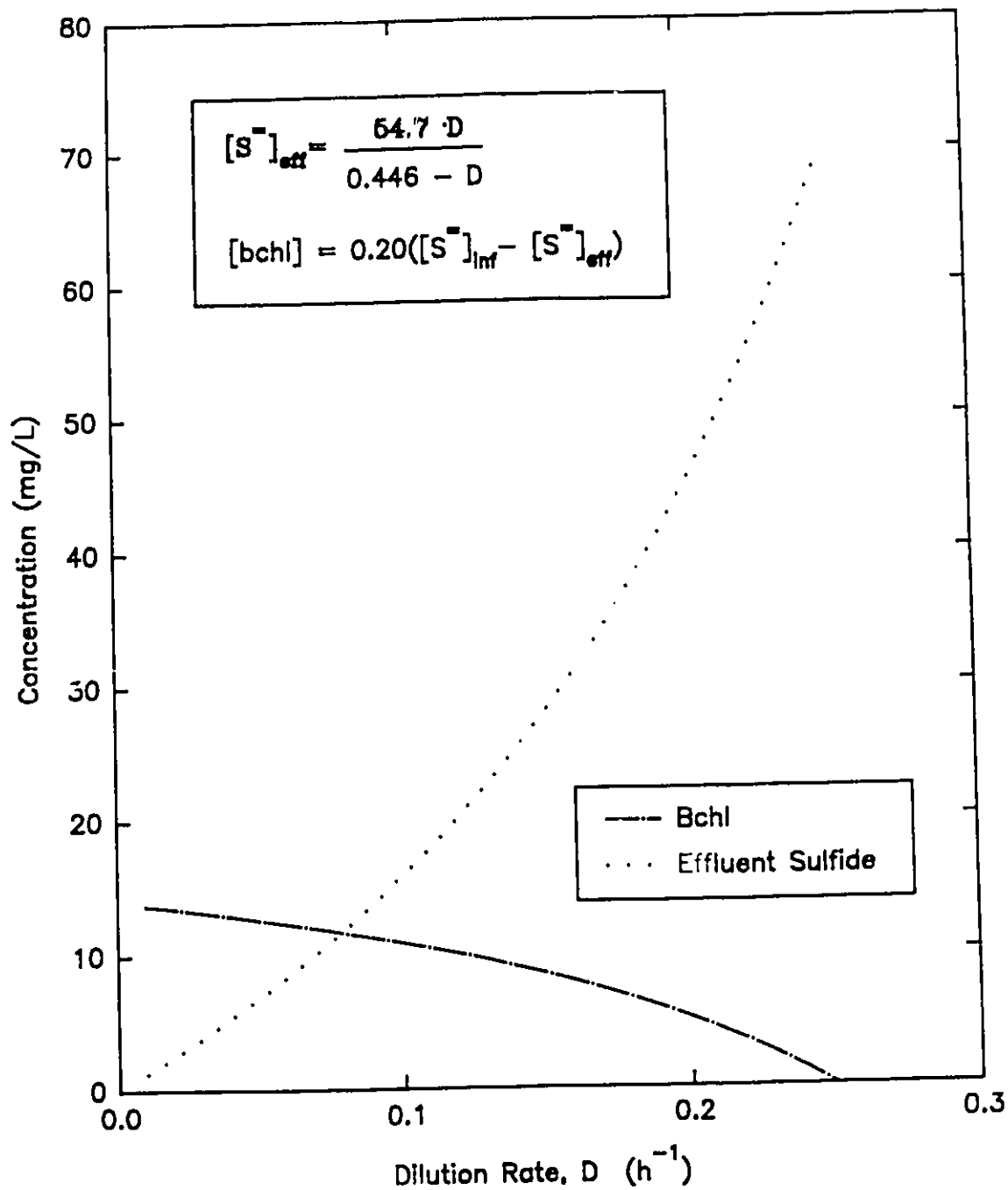
FIGURE 4.29   Dilute—out Curves in a Continuous—Flow
Reactor Without Recycle
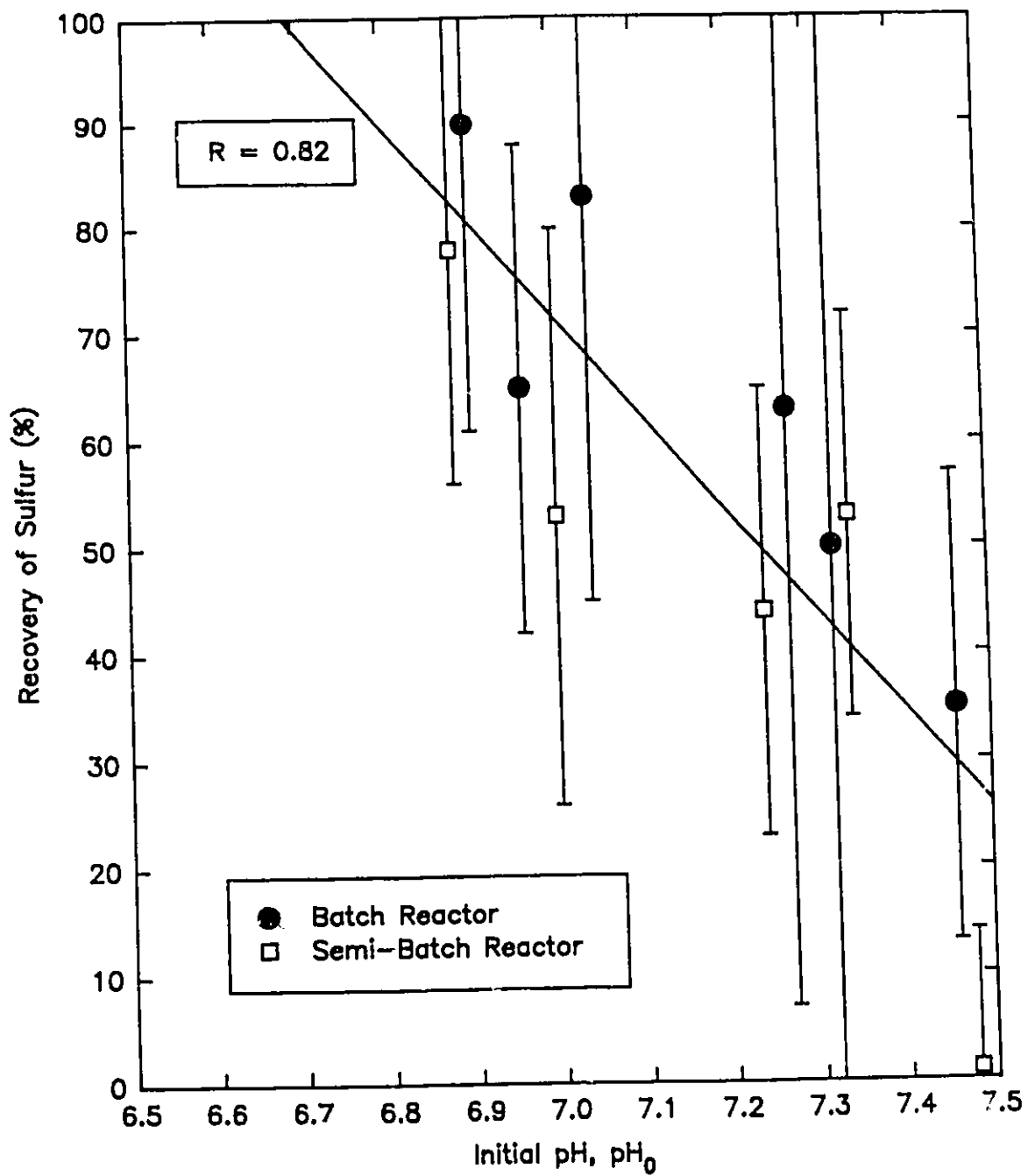
FIGURE 4.30 Recovery of Sulfur versus Initial pH in Batch and Semi-Batch Reactors

uncorrelated to the concentration of sulfide in the reactor (Figure 4.31) or the concentration of bci.l in the reactor (Figure 4.32). Similarily, there was no correlation (R = 0.15) between the specific utilization rate and sulfide concentration (Figure 3.33). U is considered to be a constant in continuous flow design equations.

The maximum rate of sulfide utilization was found to be 9.4 mg/L·h in the batch reactor experiments. This value was obtained in Runs 3 and 7. The highest rate of sulfide utilization achieved in the semi-batch reactor experiments was 17.3 mg/L·h in Injection 4.
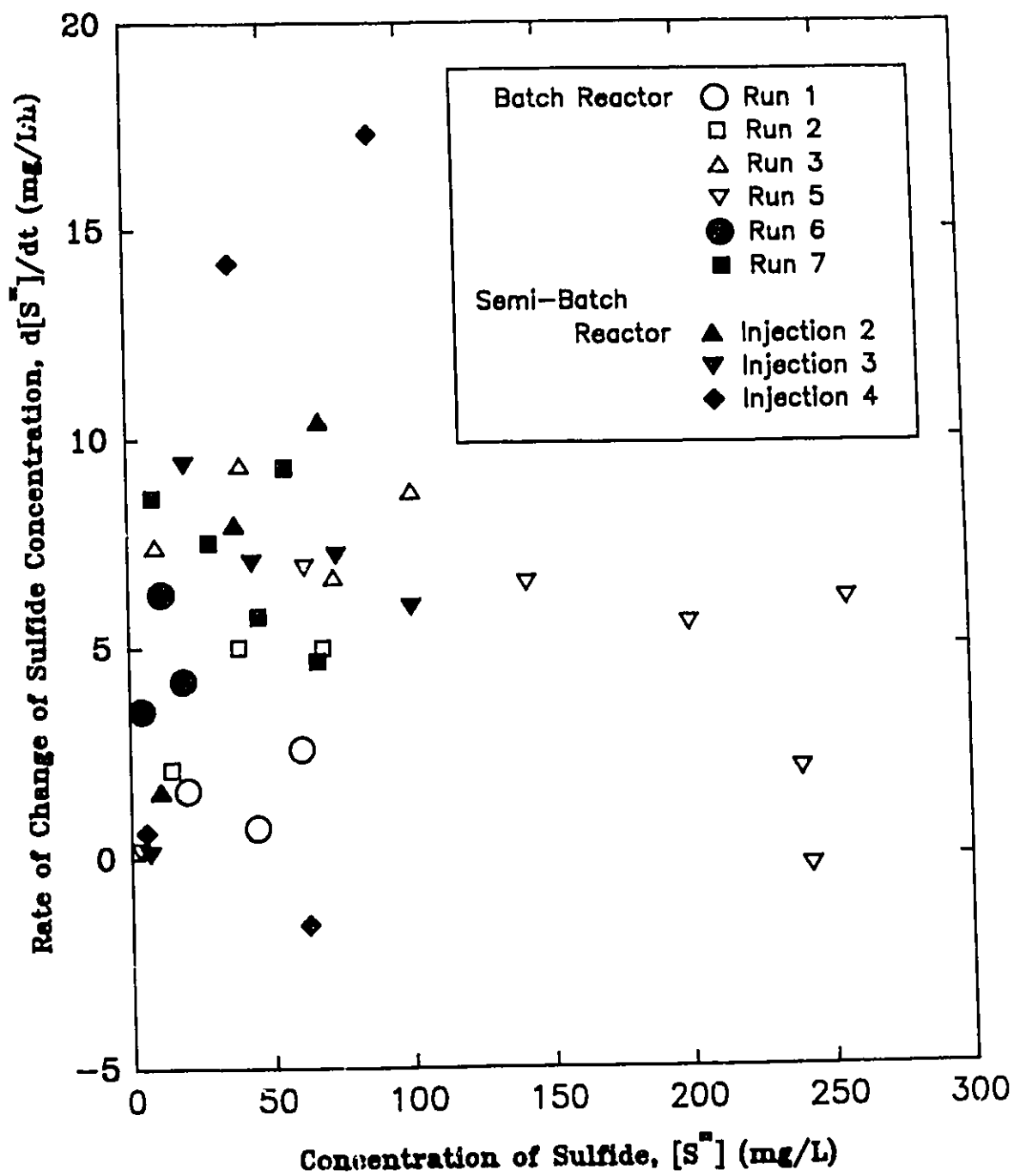
FIGURE 4.31   Rate of Change in Sulfide Concentration
versus Sulfide Concentration in Batch and
Semi-Batch Reactors
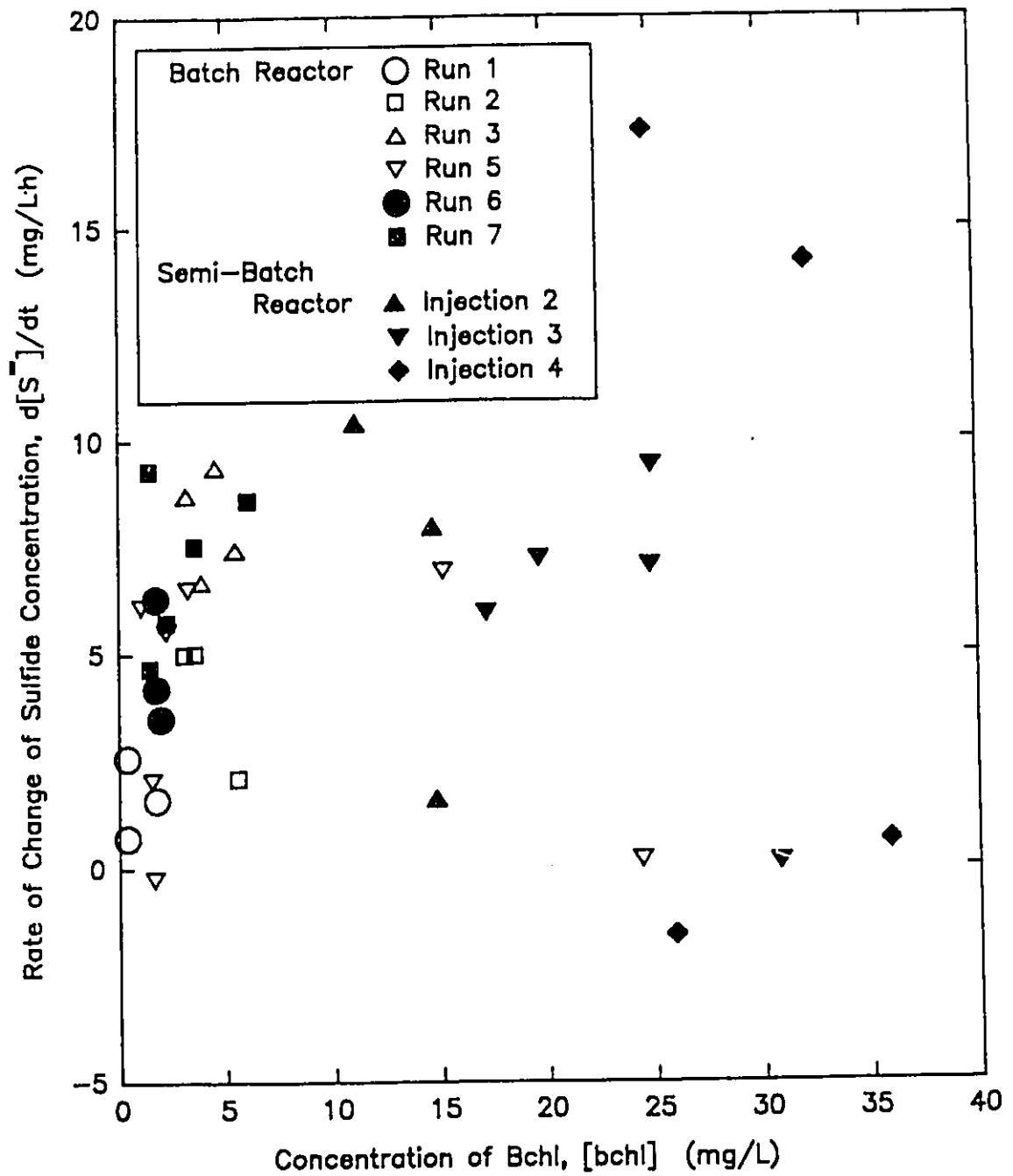
FIGURE 4.32 Rate of Change of Sulfide Concentration
versus Bchl Concentration in Batch and
Semi—Batch Reactors
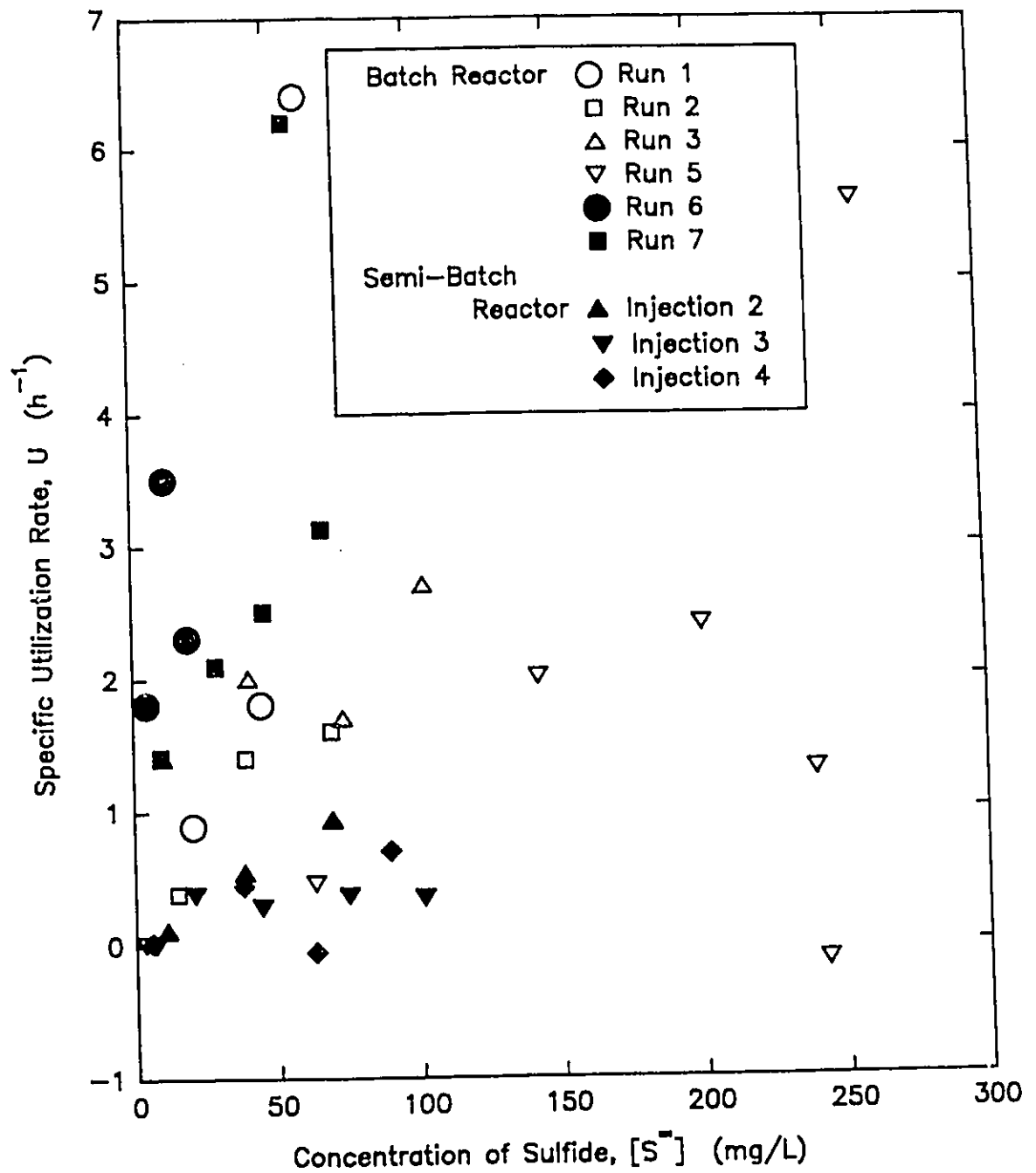
FIGURE 4.33   Specific Utilization Rate of Sulfide versus
Sulfide Concentration in Batch and Semi–
Batch Reactors

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Based on batch and semi-batch experiments conducted in the laboratory, it is concluded that:

•Elemental sulfur was produced successfully from sulfide by *C. thiosulfatophilum* in a batch reactor.

•From 1 to 90% of the initial sulfide was converted to elemental sulfur. By increasing the frequency of elemental sulfur sampling, the peak of sulfur concentration in a particular batch reactor run can be found more accurately, resulting in more realistic measured recovery values. There was no correlation between the % recovery of sulfur and the initial sulfide concentration. However, the initial pH affected the sulfur recovery. This effect should be quantified further by using a more precise method of measuring elemental sulfur in an aqueous matrix. In addition, the operating conditions should be modified to minimize the amount of sulfide conversion to thiosulfate.

•The values for specific growth rate of *C. thiosulfatophilum* ranged from 0.049 to 0.21 h^{-1} and were found to be similar to those obtained by others using a gas-fed semi-batch reactor. The maximum specific growth rate values have not been reported in these other studies.

•The maximum tolerable level of sulfide for *C. thiosulfatophilum* was found to be 300 mg/L.

•The rate of sulfide utilization was determined to be independant of sulfide or bacteriochlorophyll concentration. The maximun rate of

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substrate utilization was found to be 17.3 mg/L·h.

5.2 Recommendations

It is recommended that:

•A more precise and rapid method for measuring different sulfur species be developed.

•The batch reactor be converted to a continuous flow reactor and operated in once-through mode. Using this tool, the parameters obtained in batch flow experiments can be confirmed and other parameters unique to continuous flow reactors can be determined.

•Once these parameters are established, a method of separating elemental sulfur from the bacteria, and the bacteria from water must be developed so that the continuos flow reactor can be operated by recycling the biomass.

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DEVELOPMENT OF METHODS OF ANALYSIS

A.1 General

A.1.1 Reagents

diH$_2$O is distilled water (conductivity = 5 to 7 μmho/cm)

deH$_2$O is deionized water (conductivity = 1.3 μmho/cm)

dadeH$_2$O is deaerated, deionized water. Deionized water was boiled in a covered beaker or erlenmeyer flask for at least five minutes. The beaker or flask was placed in a cold water bath and cooled until tepid.

Sulfide stock solution was made by rinsing sodium sulfide (Na$_2$S·9H$_2$O) crystals in deH$_2$O, patting them dry with a paper towel, weighing them and transferring them to a volumetric flask. The bulk of the flask was filled with dadeH$_2$O and deH$_2$O was used to adjust the liquid level to the mark. In Sections A.3 and A.4, the methylene blue method of Section 3.1.1 was used to determine the sulfide content of this stock on the day of use.

Sulfur stock colloidal solution was made by adding a measured amount of recrystallized (in benzene), dried roll formed sulfur to an erlenmeyer flask with a ground glass joint. 95% ethanol was added (0.8 to 1.0 mL/mgS°) and the mixture was cooled until boiling stopped and poured into a volumetric flask half full of deH$_2$O. The "milk of sulfur" formed was stable for one to two days. On the second day, the precipitated sulfur could be re-suspended by stirring. This solution was used as a sulfur interference for testing the sulfide and sulfate methods of analysis.

Sulfate stock solution was made by dissolving a measured quantity of

dried sodium sulfate (Na$_2$SO$_4$) in deH$_2$O in a volumetric flask. The solution was made up to the mark with deH$_2$O.

A.1.2 Apparatus

All absorbance measurements were made with a Pye Unicam SP500 Series 2 Spectrophotometer in the direct readout mode using a 10 mm quartz cuvette. All absorbance measurements are reported in Optical Density units (O.D.).

An International Centrifuge Centra-4 with a #215 rotor was used to centrifuge samples.

A.1.3 Statistical Analysis

$\overline{x}$ is the mean of a set of values.

U_{95} is the uncertainty of a mean at the 95% confidence level. The value is calculated by (APHA,1989):

$$U_{95} = t_{.025,n-1}(s/\sqrt{n}) \qquad \text{[A.1]}$$

where   s   = sample standard deviation
        n   = number of values
        $t_{.025,n-1}$ = critical value at the 95% confidence level

CV is the coefficient of variation.   It is calculated by (APHA,1989):

$$CV = 100(s/\overline{x}) \qquad \text{[A.2]}$$

Linear regressions were performed by the computer program Sigmaplot Version 4.0 (Jandel Scientific, Sausalito,CA). The 95% confidence limits and the sample correlation coefficient, R of the linear regression line were also computed by this program. The correlation coefficient is a measure of how accurately the line of best fit accomodates the data.

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A.2 Sulfide

A.2.1 Iodometric Method

Standard Methods 4500-S^{2-} E. (APHA,1989) describes the iodometric method wherein an iodine (I_2) solution is added in excess to a sulfide sample. The iodine reacts with the sulfide, after which the excess iodine is back-titrated with a thiocyanate solution. The sodium thiocyanate ($Na_2S_2O_3$) solution had been standardized against a potassium bi-iodate ($KH(IO_3)_2$) solution. The iodine solution had been standardized against the thiosulfate solution.

This method suffers interference from substances that react with iodine (thiosulfate, sulfite) so a sample pretreatment is suggested (APHA, 1989). The pretreatment consists of adding zinc acetate to the sulfide sample to precipitate zinc sulfide. The precipitate is filtered and re-suspended in water for use in the iodometric test. An investigation into the precision and accuracy of the iodometric method with and without sample pretreatment was undertaken. The quantities of reagents used are given in Table A.1. The procedures used were as follows:

A. Blank

Deionized water (deH_2O) was added to a 250 mL erlenmeyer flask. The standardized iodine solution was added from a pipette or burette to the flask and HCl was added. The solution was titrated with standardized thiosulfate solution from a 50 mL burette until the brown/red iodine colour faded to a straw colour. Then, a starch indicator was added, turning blue in the presence of iodine so that the endpoint could be detected to within a drop.

TABLE A.1 Quantities of Reagents Used to Investigate Iodometric Method for Sulfide Analysis

	Blank	Non-Pretreated	Pre-Treated
August 9	20.00 mL deH2O 20.0 mL 24.9 mN I2 2.0 mL 6N HCl titrate w/ 25.0 mN S2O3=	5.00 mL sample 26.2 mL 24.9 mN I2 2.0 mL 6N HCl titrate w/ 25.0 mN S2O3=	5.00 mL sample 10 drops 2.0N Zn (CH3COO)2 2 drops 0.62N NaOH filter 20.0 mL deH2O 20.0 mL 24.9 mN I2 2.0 mL 6N HCl titrate w/ 25.0 mN S2O3=
August 10	50.0 mL deH2O 20.0 mL 24.9 mN I2 2.0 mL 6N HCl titrate w/ 25.0 mN S2O3=	50.0 mL deH2O 5.00 mL sample 20 mL 24.9 mN I2 2.0 mL 6N HCl titrate w/ 25.0 mN S2O3=	10.0 mL deH2O 10 drops 2.0N Zn (CH3COO)2 5.00 mL sample 2 drops 0.62N NaOH filter 100 mL deH2O 20 mL 24.9 mN I2 2.0 mL 6N HCl titrate w/ 25.0 mN S2O3
August 11	10.0 mL deH2O 10 drops 2.0N Zn (CH3COO)2 2 drops 0.62N NaOH let stand 30 min. filter rinse w/ 0.6N HCl 100 mL deH2O 20.0 mL 24.9 mN I2		10.0 mL deH2O 10 drops 2.0N Zn (CH3COO)2 2 drops 0.62N NaOH 5.00 mL sample let stand 30 min. filter rinse w/ 0.6N HCl 100 mL deH2O

TABLE A.1 (cont'd)

	Blank	Non-Pretreated	Pre-Treated
August 11 (cont'd)	2.0 mL 6N HCl titrate w/ 25.0 mN S_2O_3=		20.0 mL 24.9 mN I_2 2.0 mL 6N HCl titrate w/ 25.0 mN S_2O_3=
August 15 Step 2	15.0 mL deH_2O let stand 30 min. 10.00 mL 24.9 mN I_2 50 mL deH_2O 2 mL 6N HCl stir 30 min. titrate w/ 25.0 mN S_2O_3=	10.00 mL sample 10.0 mL deH_2O let stand 30 min. 10.00 mL 24.9 mN I_2 2.0 mL 6N HCl titrate w/ 25.0 mN S_2O_3=	10 drops 2.0N Zn $(CH_2COO)_2$ 10.00 mL sample 2 drops 0.62N NaOH let stand 30 min. filter 20.0 mL deH_2O 10.00 mL 24.9 mN I_2 50.0 mL deH_2O 2.0 mL 6N HCl stir 30 min. titrate w/ 25.0 mN S_2O_3=
Step 6	15.0 mL deH_2O let stand 30 min. 5.00 mL 24.9 mN I_2 50.0 mL deH_2O 2.0 mL 6N HCl stir 30 min. titrate w/ 25.0 mN S_2O_3=	5.00 mL sample 10.0 mL deH_2O let stand 30 min. 5.00 mL 24.9 mL I_2 2.0 mL 6N HCl titrate w/ 25.0 mN S_2O:=	10 drops 2.0N Zn $(CH_3COO)_2$ 5.00 mL sample 10.00 mL deH_2O 2 drops 0.62 N NaOH let stand 30 min. filter 20.0 mL deH_2O 5.00 mL 24.9 mN I_2 50.0 mL deH_2O 2.0 mL 6N HCl stir 30 min.

TABLE A.1 (cont'd)

Step 6 cont'd

	Blank	Non-Pretreated	Pre-Treated
August 29	50.0 mL deH_2O 10.0 mL 25.1 mN I_2 2.0 mL 6N HCl titrate w/ 25.2 mN $S_2O_3^=$	50.0 mL deH_2O 10.00 mL sample 12.0 mL 25.1 mN I_2 2.0 mL 6N HCl titrate w/ 25.2 mN $S_2O_3^=$	titrate w/ 25.0 mN $S_2O_3^=$ 10.0 mL deH_2O 10 drops 2.0N Zn $(CH_3COO)_2$ 2 drops 0.62N NaOH 10.00 mL sample let stand 30 min. filter 50.0 mL deH_2O 12.0 mL 25.1 mN I_2 2.0 mL 6N HCl stir 30 min. titrate w/ 25.2 mN $S_2O_3^=$

B. Non-Pretreated

The sulfide sample and deH₂O were added to a 250 mL erlenmeyer flask and either titrated without delay or allowed to stand 30 minutes. A quantity of the standard iodine solution and HCl were added and the mixture was titrated as in section A.2.1.A Blank.

C. Pretreated

Typically, the sulfide sample was added to a 250 mL erlenmeyer flask to which zinc solution and hydroxide solution had been added. After allowing 30 minutes for precipitation, the solution was filtered through a 42.5 mm diameter Whatman's GF/C glass fibre filter in a Buchner funnel. The flask and filter cake were rinsed with 10-20 mL of deH₂O or HCl as indicated in Table A.1. The filter paper and filter cake were carefully folded and inserted back into the erlenmeyer flask. Deionized water, standard iodine solution, HCl and a magnetic stir bar were added to the flask. The mixture was titrated either immediately or stirred for 30 minutes as indicated in Table A.1. Titration was performed with standardized thiosulfate solution as indicated in Section A.2.1.A. Blank.

The results of five trials of the iodometric method without pretreatment and six trials with pretreatment are shown in Table A.2. On three occasions, the iodometric method without pretreatment gave readings of sulfide greater than the amount added. The coefficient of variation for these trials averaged 0.7%. With pretreatment, the iodometric method gave more accurate readings but precision suffered. Sulfide readings were equal to or less than the quantity of sulfide added to the sample within the experimental limits of uncertainty. Sulfide readings indicating less sulfide than the amount added may have been due to spontaneous sulfide

TABLE A.2 Results of Trials of Iodometric Method for Sulfide Analysis

Date	[S=] in Sample	Sample Matrix	Non-Pretreated Measured (mgS=/L)	$\bar{x} \pm$ U95 (mgS=/L)	CV	Pretreated Measured (mgS=/L)	$\bar{x} \pm$ U95 (mgS=/L)	CV
August 9	<1.4g/L*	de water +10% SAOB**	2.0 ± .1 2.0 ± .1 2.0 ± .1	2.1 ± .03	0.6%	0.99±.1 0.80±.1 0.93 ± .1	.91 ±.24	10%
August 10	<1.4g/L*	date water	1.2 ± .07 1.2 ± .07 1.2 ± .07	1.2 ± 0.0	0%	0.714 ± .07 0.714 ± .07 0.752 ± .07	.73 ± .05	3%
August 1i	<1.4g/L*	date water				0.590 ± .01 0.430 ± .01 0.510 ± .01	.51 ± .08	20%
August 15	<0.35g/L*	dade water	0.278 ± .02 0.278 ± .02 0.278 ± .02	0.279 ± .006	0.8%	0.18 ± .04 0.16 ± .04 0.18 ± .04	.17 ± .03	8%
August 15	<0.10g/L*	g/L: 0.19 SO4=-S 0.34 S° 0.12 (SO3=+S2O3=) as S dade H2O	0.14 ± .03 0.14 ± .03 0.14 ± .03	0.14 ± 0.0	0%	0.06 ± .02 0.06 ± .02 0.06 ± .02	.06 ± 0.0	0%
August 29	0.35g/L	not recorded	.411 ± .025 .411 ± .025 .411 ± .025	.422 ± .023	2%	0.338 ± .025 0.364 ± .025 0.378 ± .025	.360 ± .050	6%

* potassium sulfide was used instead of sodium sulfide in sulfide stock solution
** sulfide anti-oxidant buffer

oxidation or degassing of $H_2S(g)$. The coefficient of variation averaged 8% with pretreatment indicating lower precision than the iodometric method without pretreatment.

A.2.2 Potentiometric Method

In this method aqueous sulfide was complexed with lead to form lead sulfide, a black precipitate. The activity of sulfide in the solution was monitored electrochemically by a silver/sulfide electrode against a double junction reference electrode. The equivalence point was reached when there was a sudden rise in the potential between the electrodes (Orion,1980). Lead perchlorate $(Pb(ClO_4)_2)$ is recommended (Orion,1980; Baumann,1974) for the standard lead solution but is unacceptable as a primary standard because it is unstable (extremely hygroscopic). Disodium ethylenediaminetetraacetic acid $(Na_2H_2EDTA \cdot 2H_2O)$ was dried at 80°C to remove superficial moisture and was stable enough to be used as a primary standard (Skoog et al.,1982). A measured volume of the EDTA solution was titrated with lead perchlorate solution using the electrode combination mentioned above. The volume of lead solution added was plotted against the mV reading. The point of inflection of this curve was taken as the equivalence point and used to calculate the concentration of the lead solution.

Sulfide anti-oxidant buffer (SAOB) stock was made up as follows van Gemerden,1984):

> 80 g NaOH
> 67 g $Na_2H_2EDTA \cdot 2H_2O$
> 35 g ascorbic acid
> make up to 1 L with deH_2O

After the first trial, a SAOB stock was made up from the above ingredients

less the ascorbic acid. Roughly 1.8 g ascorbic acid and 50.0 mL SAOB stock were added to the titration beaker immediately before the sulfide sample was pipetted (Table A.3). The function of the ascorbic acid was to remove oxygen from the solution so that the sulfide would not be oxidized. After standing three to four hours, the titration mixture formed a copper-brown layer on the surface indicating oxidation of the ascorbate. The mixture was continuously stirred while being titrated with lead solution. After each addition, about ¼ minute was needed for the drift in the potential to stabilize to less than one mV/min. Then, the reading was taken. The endpoint was determined by two methods:

A. Calculation

The mV reading after each addition of titrant was subtracted from the reading before the addition. This difference (ΔmV) was divided by the volume of the addition (ΔmL). The quotient ΔmV/ΔmL is the slope of the titration curve. The slope is highest at the point of inflection of the titration curve (equivalence point). Thus, the highest ΔmV/ΔmL value occurs at the end point.

B. Graph

In this method, mV readings were plotted against volume of titrant added. The sigmoidal curve that resulted was approximated by drawing three straight lines. The line through the initial (lead) part of the titration curve and the line through the tail were approximately parallel. The line in the middle (vertical) part of the graph intersected the two previously drawn lines. The equivalence point was taken as the mid-point of the vertical line between the upper and lower intersection points.

Table A.4 is a summary of the results of eleven trials of the

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TABLE A.3 Reagents and Procedures Used to Investigate Potentiometric Method for Sulfide Analysis

Date	[EDTA]	[Pb]	Ascorbic Acid Added to SAOB*	Electrode Polished	Samples Sequential	Volume Ratio Sample: SAOB*
Sept. 2, 1988	10.13 mM±.03%	10.0 mM±1%	to SAOB stock, 200 mL made for day	no	drawn from stock as needed	1:2
May 11, 1989	20.04 mM±.03%	8.83 mM±.2%	to beaker	no	drawn from chemostat feed as needed	1:5
May 24, 1989	20.04 mM±.03%	8.83 mM±.2%	to beaker	no	drawn from chemostat feed as needed	1:2
May 25, 1989	20.04 mM±.03%	8.83 mM±.2%	to beaker	no	drawn from chemostat feed as needed	1:2
June 21, 1989	20.04 mM±.03%	8.83 mM±.2%	to beaker	no	drawn from chemostat feed as needed	1:2
June 23, 1989	20.04 mM±.03%	8.83 mM±.2%	to beaker	no	drawn from chemostat feed as needed	1:2
Aug. 30, 1989	20.04 mM±.03%	8.83 mM±.2%	to beaker	no	drawn from r.b.f.** as needed	1:2

*sulfide anti-oxidant buffer   ** round bottom flask

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TABLE A.3 (cont'd)

Date	[EDTA]	[Pb]	Ascorbic Acid Added to SAOB*	Electrode Polished	Samples Sequential	Volume Ratio Sample: SAOB*
Aug. 31, 1989	20.04 mM±.03%	8.83 mM±.2%	to beaker	thoroughly between titrations	drawn from r.b.f.** as needed	1:2
Sept. 1, 1989	20.04 mM±.03%	8.83 mM±.2%	to beaker	thoroughly between titrations	drawn at same time from r.b.f.**	1:2
Sept. 13, 1989	20.04 mM±.03%	8.83 mM±.2%	to beaker	lightly between titrations	drawn at same time from r.b.f.**	1:2
Sept. 22, 1989	20.04 mM±.03%	8.83 mM±.2%	to beaker	thoroughly between titrations	drawn at same time from vol. flask	1:2

* sulfide anti-oxidant buffer
** round bottom flask

TABLE A.4 Results of Trials of Potentiometric Method for Sulfide Analysis

Date	[S=] in Sample	Sample Matrix	Calculation				Graph			
			Measured (gS=/L)	$\bar{\chi}$ (gS=/L)	U95 (gS=/L)	CV	Measured (mgS=/L)	$\bar{\chi}$ (mgS=/L)	U95 (mgS=/L)	CV
Sept. 2/88	<0.129g/L	dadeH2O	.085±.001							
			.086±.0009	.085	±.001	.07%				
			.085±.01							
May 11/89		diH2O, mineral salts, NaHCO3, PO4^3- buffer	1.01±.007							
			1.02±.007	1.01	±.01	.6%				
			1.01±.007							
May 24/89		diH2O, mineral salts, NaHCO3, PO4^3- buffer	.10±.001							
			.099±.001	.099	±.004	2%				
			.097±.001							
May 25/89		diH2O, mineral salts, NaHCO3 PO4^3- buffer	.098±.001	.099	±.004	1%	.10±.001	.099	±.004	1%
			.10±.001				.098±.001			
June 21/89		diH2O, mineral salts, NaHCO3	.088±.0009	.084	±.04	6%	.088±.0009	.084	±.04	6%
			.081±.0008				.081±.0008			

TABLE A.4 (cont'd)

Date	[S=] in Sample	Sample Matrix	Calculation				Graph			
			Measured (gS=/L)	x̄ (gS=/L)	U95 (gS=/L)	CV	Measured (mgS=/L)	x̄ (mgS=/L)	U95 (mgS=/L)	CV
June 23/89		diH2O,	.151±.0016				.147±.0016			
		mineral salts, NaHCO3	.146±.0016	.146	±.007	3%	.147±.0016	.145	±.009	4%
			.147±.0016				.149±.0016			
			.140±.0015				.137±.0015			
August 30/89		diH2O,	.099±.001				.10±.001			
		mineral salts, NaHCO3	.095±.001	.092	±.007	6%	.099±.001	.093	±.008	7%
			.089±.0009				.092±.0009			
			.090±.0009				.090±.0009			
			.085±.0009				.085±.0008			
August 31/89		diH2O,	.097±.001				.10±.001			
		mineral salts, NaHCO3	.095±.001	.094	±.009	4%	.097±.001	.097	±.009	4%
			.090±.0009				.093±.0009			
Sept.1/89		diH2O,	.086±.0009				.086±.0009			
		mineral salts, NaHCO3	.086±.0009	.086	±.0008	.6%	.087±.0009	.086	±.0009	7%
			.087±.0009				.086±.0009			
			.086±.0009				.087±.0009			

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TABLE A.4 (cont'd)

Date	[S=] in Sample	Sample Matrix	Calculation				Graph			
			Measured (gS=/L)	x̄ (gS=/L)	U95 (gS=/L)	CV	Measured (mgS=/L)	x̄ (mgS=/L)	U95 (mgS=/L)	CV
Sept. 13/89		diH2O, mineral salts, NaHCO3	.140±.003 .137±.0015 .134±.0015	.137	±.007	2%	.134±.0015 .137±.0015 .134±.0015	.135	±.004	1%
Sept. 22/89	<.3350g/L	dadeH2O	.151±.0016 .149±.0016 .151±.0016 .149±.0016	.150	±.0018	.8%	.151±.0016 .150±.0016 .151±.0016 .148±.0016	.150	±.0022	.9%

potentiometric method. The average coefficients of variation for the calculation and graphical methods were both 3% for those trials where both methods were used. In addition, the calculation method gave a sulfide concentration that was higher twice, the same 4 times and lower twice of the eight times that both methods were used. There is no significant difference in the coefficients of variation or the average sulfide concentrations between the use of the calculation and graphical methods. Therefore, the calculation method is preferred because: it does not require graphing, it can be done during the titration and, there is no need to titrate much beyond the equivalence point. The use of the calculation method results in a savings of ½ hour per titration over the graphical method.

In terms of the accuracy of the potentiometric method, in the first and last trials the sulfide concentration was well under the known sample maximum. In terms of precision, the average coefficient of variation for all trials was 2%. In trials on August 30 and 31, where samples were taken as needed from a single round bottom flask, there was a decrease in the values of successive readings. This may have been due to oxygen introduced to the flask while sampling or equilibration of H_2S between the gas and liquid phases.

This method is superior in precision to the iodometric method with pretreatment and as such was used to calibrate stock sulfide solutions for the methylene blue method.

A.2.3 Methylene Blue Method

Table A.5 compares the reagents used in the Standard Methods' Methylene Blue Method 4500-S^{2-} D. (APHA, 1989) and that reported by Truper

TABLE A.5 Comparison of Reagents in Methylene Blue Methods of Sulfide Analysis

	Truper et al. (1964)	APHA (1985)
max. S= (mmole)	.0039	.058
Zn (CH3COO)2 (mmole)	2.2	-
DPD (mmole)	.085	.11
Fe^{3+} (mmole)	19	17
H2SO4 (mL)	2.0	3.1

et al.,(1964). In both methods, sulfide reacts quantitatively with excess dimethyl-*p*-phenylenediamine (DPD) to make leuco-methylene blue. This compound reacts with ferric ion to form methylene blue and ferrous ion. In the latter method, the sulfide sample is added to a zinc acetate solution to precipitate zinc sulfide. This step preserves the sulfide content of the sample until DPD can be added. Truper's method requires less than 1 mL of sample whereas the APHA method requires 7.5 mL.

In Truper's method 20 mL of 2% $Zn(CH_3COO)_2$ solution was measured into a 100.00 mL volumetric flask. A 1 mL syringe with graduations of 0.01 mL and a 70 mm needle was used to draw sulfide samples. Then, the syringe was inverted into the volumetric flask so that the distance between the end of the needle and the zinc solution was less than ten mm. The sample (0.1 to 0.95 mL) was then forced out from the syringe so that it immediately contacted the zinc solution and was stabilized. A pipette was used to add 10.00 mL of 0.2% dimethyl-*p*-phenylenediamine sulfate in 20% H_2SO_4 and 0.50 mL of 10% ferric ammonium sulfate in 2% H_2SO_4. The flask was filled to the mark with distilled water, capped and inverted twice. A minimum of 30 minutes were required for the colour to develop (Figure A.1). After this time the absorbance was measured spectrophotometrically at 670 nm against a diH_2O blank. Sulfide samples of 0, 0.1, 0.2, 0.3, 0.4 and 0.5 mL were analyzed by the methylene blue method on a day on which the sulfide stock solution was analyzed potentiometrically. Sulfide concentration in the cuvette was calculated (Table A.6) and a linear regression was performed (Figure A.2). This correlation was used to determine the sulfide stock concentration in further tests in Section A.2.3.

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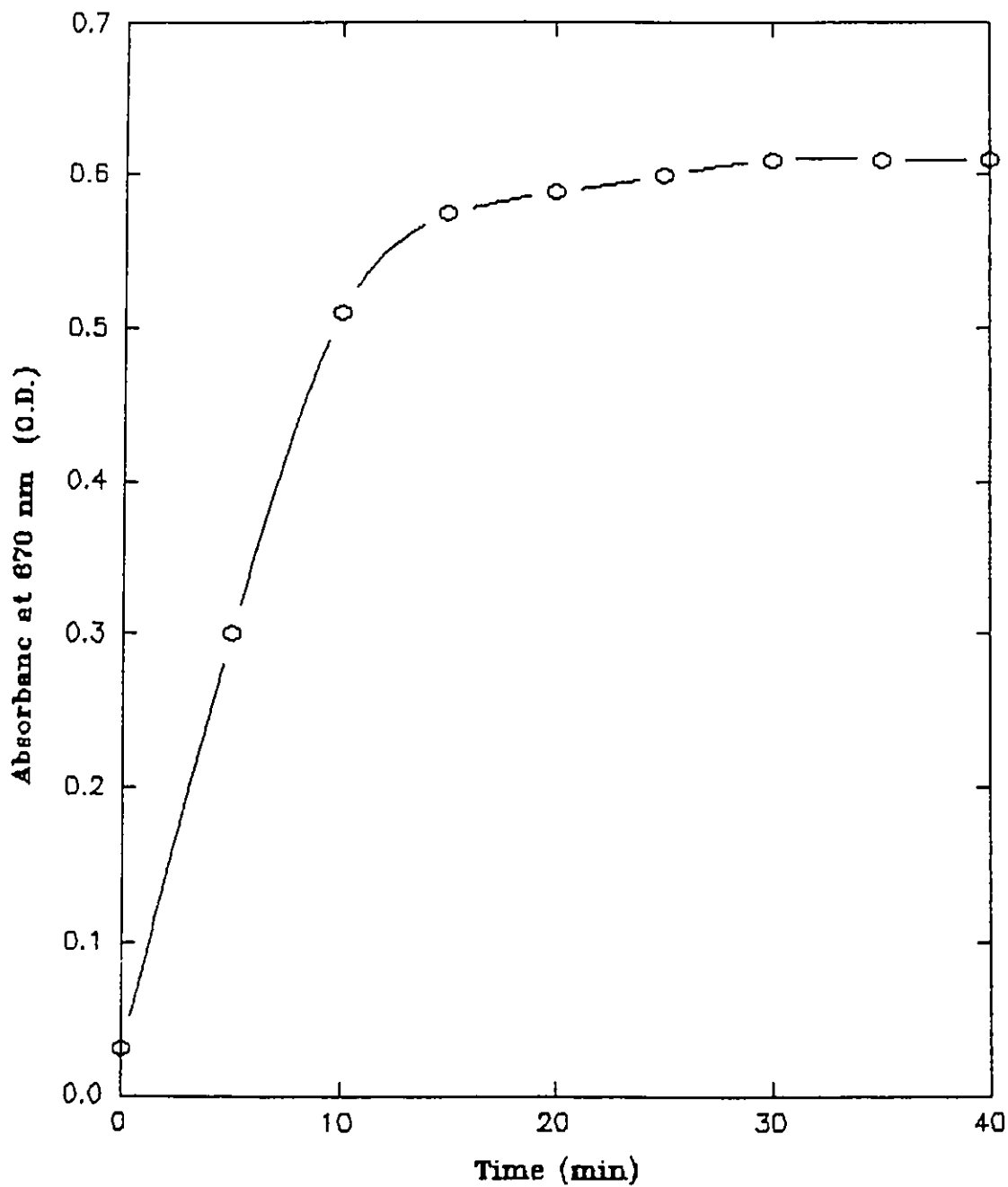
FIGURE A.1 Colour Development in Truper's Methylene
Blue Method of Sulfide Analysis

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TABLE A.6 **Calibration of Truper's Methylene Blue Method of Sulfide Analysis   Without Added Sulfur Compounds**

Flask No.	Volume S= Stock [150±1.8 mg/L] (mL)	[S=] in Cuvette (mg/L)	Absorbance, average of 3 readings (O.D.670)
1	0	0	.003
2	.100	.150	.149
3	.200	.300	.338
4	.300	.450	.517
5	.400	.600	.680
6	.500	.750	.860
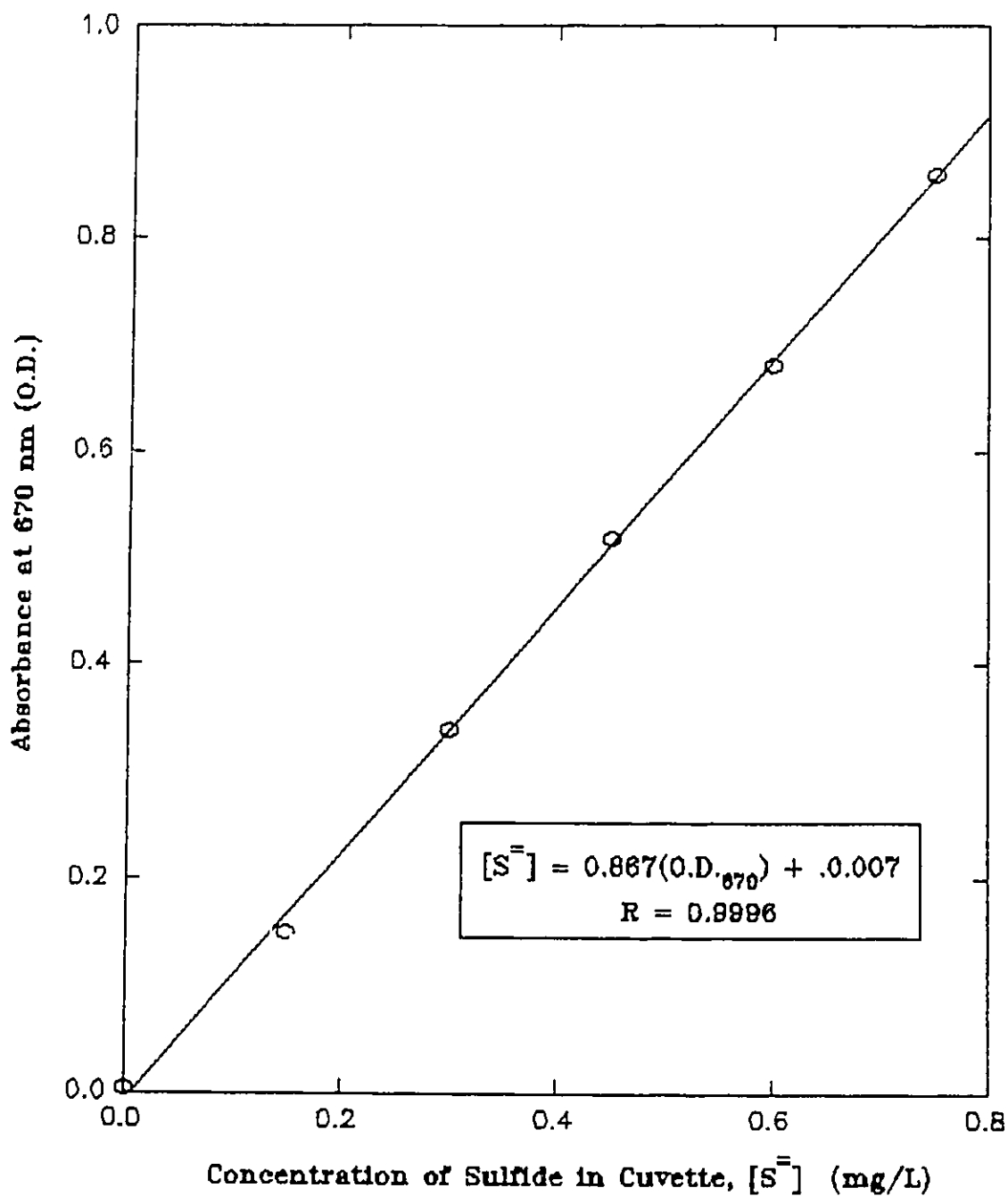
FIGURE A.2 Calibration of Truper's Methylene Blue
Method of Sulfide Analysis Without Added
Sulfur Compounds

To determine the effects of various interferences on the methylene blue method of measuring sulfide, stock solutions of sulfide, sulfur, and sulfate were combined in various proportions and sulfide was measured. In addition, repetitions of sulfide tests at the same concentration were performed to establish the uncertainty of the sulfide test. The results are shown in Table A.7.

The results of all methylene blue sulfide tests shown in Table A.7 were plotted in Figure A.3. The data were considered to be from the same population. The line of best fit and the 95% confidence limit envelope are shown. The widths of this envelope at the lowest and highest in-range values were measured. The greatest width value was divided by two and used as the uncertainty of the concentration in the cuvette.

A.3 Elemental Sulfur

A.3.1 Ethanol Method

Cork (1978) has described the method of Schmidt and Kamen (1970) where an aqueous sample was diluted 1:2 with 95% ethanol. The sample was centrifuged and the absorbance of the supernatant measured at 264 nm, varied linearly with the sulfur concentration. Maka (1986) has referred to the technique of van Gemerden (1968) where the aqueous sample was diluted 1:40 with 95% ethanol and refluxed for two hours. Subsequently, the ethanol mixture was centrifuged at 3000 rpm (clinical centrifuge) for 30 minutes. The absorbance of the supernatant was measured at 264 nm against a 95% ethanol blank. Van Gemerden (1968) measured absorbance at 260 nm.

Initially, a calibration curve was developed by diluting a stock solution of roll formed elemental sulfur in 95% ethanol and measuring the

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TABLE A.7 Calibration of Truper's Methylene Blue Method of Sulfide Analysis With and Without Added Sulfur Compounds

Date	[S=] Stock (mg/L)	Vol. S= Stock (mL)	[S°] Stock (mg/L)	Vol. S° Stock (mL)	[SO4=] Stock (mgS/L)	Vol.SO4= Stock (mL)	Vol. deH2O (mL)	Vol. Sample (mL)	[S=] Cuvette (mg/L)	Absorbance (O.D.670)
Sept. 22	150	-	-	0	-	0	0	0	0	.003
	150	-	-	0	-	0	0	.100	.150	.149
	150	-	-	0	-	0	0	.200	.300	.338
	150	-	-	0	-	0	0	.300	.450	.517
	150	-	-	0	-	0	0	.400	.600	.680
	150	-	-	0	-	0	0	.500	.750	.860
Nov. 17	296	-	-	0	-	0	0	.200	.592	.674
	296	-	-	0	-	0	0	.200	.592	.684
	296	-	-	0	-	0	0	.200	.592	.644
	296	-	-	0	-	0	0	.200	.592	.672
	296	-	-	0	-	0	0	.200	.592	.697
Nov. 17	41.4	-	-	0	-	0	0	.800	.331	.382
	41.4	-	-	0	-	0	0	.800	.331	.331
	41.4	-	-	0	-	0	0	.800	.331	.374
	41.4	-	-	0	-	0	0	.800	.331	.410
Nov. 23	228	5.00	-	0	-	0	5.00	.500	.570	.650
	228	5.00	-	0	-	0	5.00	.500	.570	.636
	228	5.00	-	0	-	0	5.00	.500	.570	.585
	228	5.00	-	0	-	0	5.00	.500	.570	.728
	291	1.00	-	0	-	0	9.00	.500	.146	.170
	291	1.00	-	0	-	0	9.00	.500	.146	.162
	291	1.00	-	0	-	0	9.00	.500	.146	.138
	291	1.00	-	0	-	0	9.00	.500	.146	.170

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TABLE A.7 (cont'd)

Date	[S=] Stock (mg/L)	Vol. S= Stock (mL)	[S°] Stock (mg/L)	Vol. S° Stock (mL)	[SO4=] Stock (mgS/L)	Vol.SO4= Stock (mL)	Vol. deH2O (mL)	Vol. Sample (mL)	[S=] Cuvette (mg/L)	Absorbance (O.D.670)
Nov. 16	320	0	-	0	256	5.00	5.00	.900	0	.003
	320	1.00	-	0	256	5.00	4.00	.900	.288	.323
	320	2.00	-	0	256	5.00	3.00	.700	.448	.579
	320	3.00	-	0	256	5.00	2.00	.500	.480	.590
	320	4.00	-	0	256	5.00	1.00	.400	.512	.634
	320	5.00	-	0	256	5.00	0	.300	.480	.630
	317	0	258	5.00	-	0	5.00	.900	0	.020
	317	1.00	258	5.00	-	0	4.00	.900	.285	.304
	317	2.00	258	5.00	-	0	3.00	.700	.444	.542
	317	3.00	258	5.00	-	0	2.00	.500	.476	.601
	317	4.00	258	5.00	-	0	1.00	.400	.507	.554
	317	5.00	258	5.00	-	0	0	.300	.476	.583
Nov. 23	225	7.50	540	3.75	-	0	3.75	.500	.562	.627
	225	7.50	540	3.75	-	0	3.75	.500	.562	.775
	225	7.50	540	3.75	-	0	3.75	.500	.562	.708
	225	7.50	540	3.75	-	0	3.75	.500	.563	.724
	225	7.50	540	3.75	-	0	3.75	.500	.563	.693
	225	1.50	540	3.75	-	0	9.75	.500	.112	.105
	225	1.50	540	3.75	-	0	9.75	.500	.112	.125
	225	1.50	540	3.75	-	0	9.75	.500	.113	.257
	225	1.50	540	3.75	-	0	9.75	.500	.113	.117
	270	0	542	5.00	513	5.00	10.00	.500	0	.012
	270	2.00	542	5.00	513	5.00	8.00	.500	.135	.127
	270	2.00	542	5.00	513	5.00	8.00	.500	.135	.127
	270	2.00	542	5.00	513	5.00	8.00	.500	.135	.122
	270	2.00	542	5.00	513	5.00	8.00	.500	.135	.137
	270	4.00	542	5.00	513	5.00	6.00	.500	.270	.304
	270	6.00	542	5.00	513	5.00	4.00	.500	.405	.466

TABLE A.7 (cont'd)

Date	[S=] Stock (mg/L)	Vol. S= Stock (mL)	[S°] Stock (mg/L)	Vol. S° Stock (mL)	[SO4=] Stock (mgS/L)	Vol.SO4= Stock (mL)	Vol. dcH2O (mL)	Vol. Sample (mL)	[S=] Cuvette (mg/L)	Absorbance (O.D.670)
Nov. 23 cont'd	270	8.00	542	5.00	513	5.00	2.00	.500	.540	.600
	270	10.00	542	5.00	513	5.00	0	.500	.675	.738
	270	10.00	542	5.00	513	5.00	0	.500	.675	.754
	270	10.00	542	5.00	513	5.00	0	.500	.675	.762
	270	10.00	542	5.00	513	5.00	0	.500	.675	.750
	270	10.00	542	5.00	513	5.00	0	.500	.675	.742

114
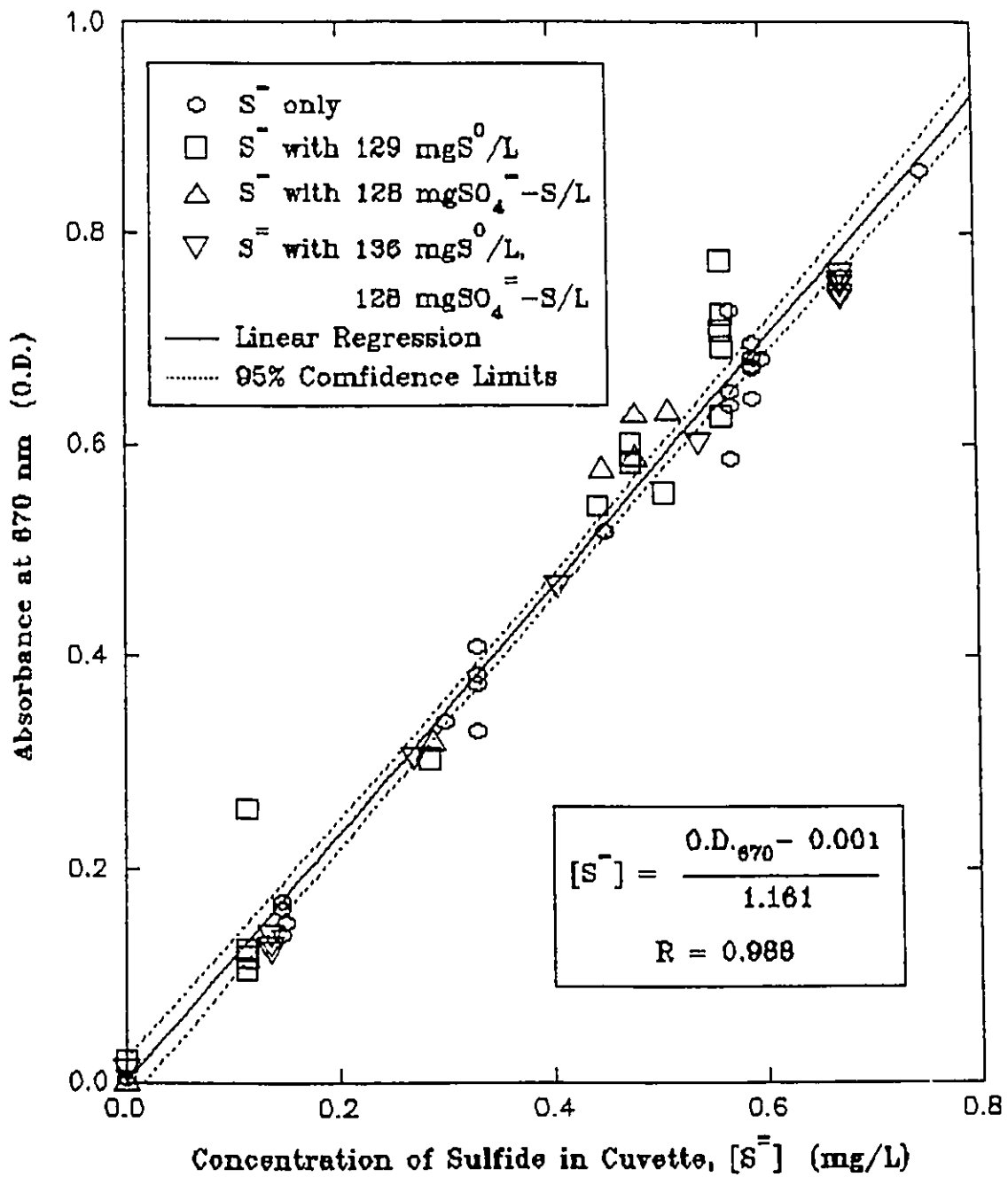
FIGURE A.3   Calibration of Truper's Methylene Blue
Method of Sulfide Analysis With and
Without Added Sulfur Compounds

absorbance at 262 nm. The calibration curve was linear in the range 0 to 33 mgS°/L with a correlation coefficient of 0.9994. A mixture of known amounts of potassium sulfide stock solution, Na_2SO_4, Na_2SO_3, $Na_2S_2O_3$ and elemental sulfur in deH_2O was filtered through Whatman's GF/C filter paper. The filter paper was transferred to an erlenmeyer flask and refluxed in 95% ethanol for 2 hours. The heated ethanol was transferred to a volumetric flask and made up to volume with 95% ethanol. The absorbance of this solution at 262 nm yielded a sulfur content equal to 98% of the amount added. The sulfur added was not finely dispersed in the aqueous mixture but remained in a layer on top. Thus a sample from the bulk liquid would give a false indication of the amount of sulfur present. Also, sulfur produced by bacteria may be more finely dispersed and may not be filterable. Thus, a second test of this sulfur method was performed using a fine suspension of sulfur in a sulfide/sulfate solution.

A second calibration of the ethanol method was performed this time measuring absorbance at 264 nm (Figure A.4).

A second test was performed to determine the effects of other sulfur species. Stock solutions of $Na_2S \cdot 9H_2O$ and Na_2SO_4 were made in deH_2O. The solubility of $S°$ in 95% ethanol is 1.4 g/L at reflux temperature and 0.3 g/L at room temperature. In order to achieve a high sulfur content in the sample but minimize the ethanol content so as to best simulate a reactor sample, a sulfur colloidal solution was made (see Section A.1.1). Twenty-five (25.00) mL aliquots of all stock solutions were combined in test tubes. A 10.00 mL sample was taken from each combination and stock solution. The sample was added to approximately 65 mL of 95% ethanol and refluxed for 2 hours. After cooling, the contents of the reflux vessel

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$$[S^0] = \frac{O.D._{284} - 0.025}{0.02553}$$

$$R = 0.9990$$
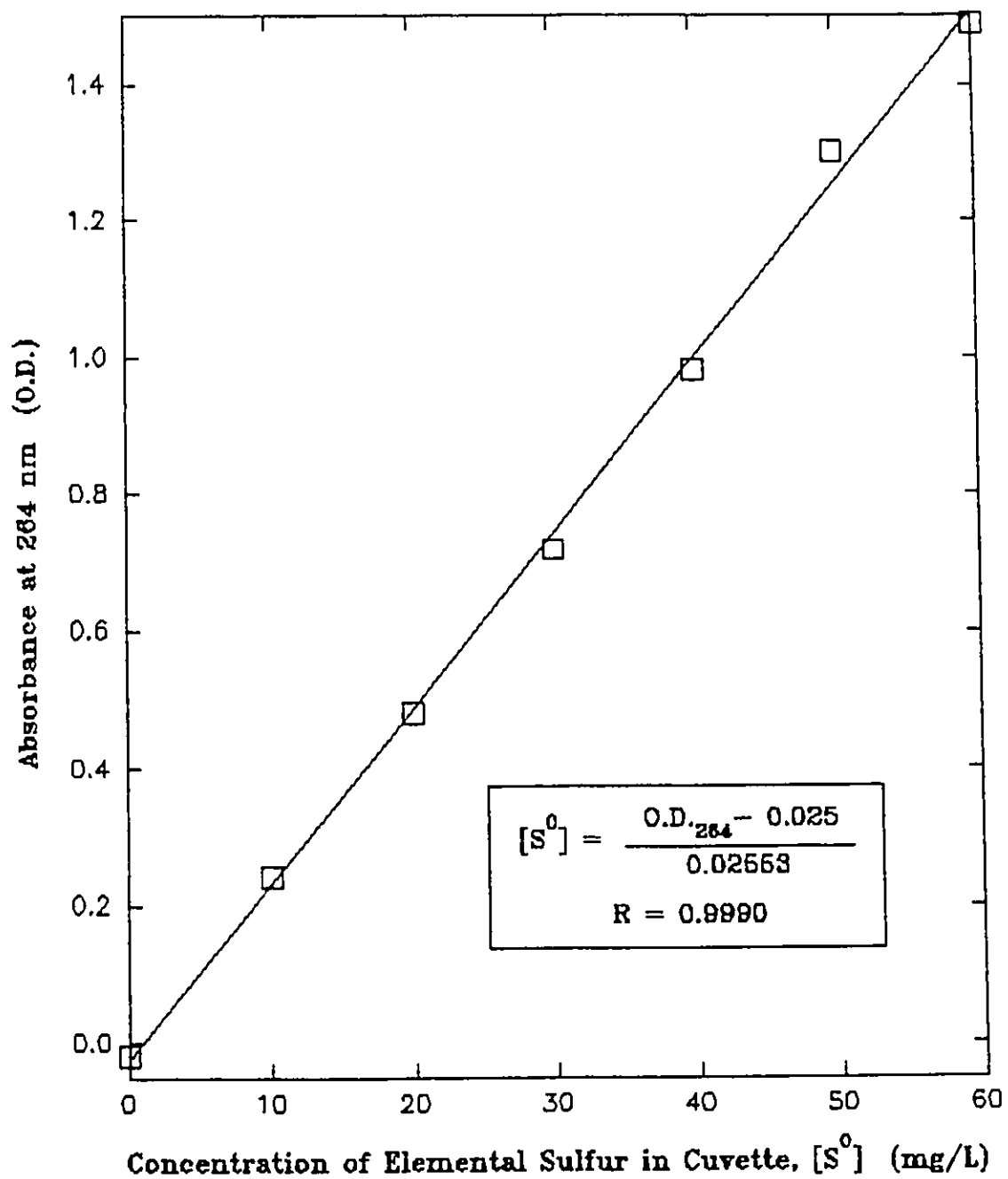
FIGURE A.4 Calibration of Ethanol Method of Elemental Sulfur Analysis

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was poured into a 100.00 mL volumetric flask and made up to the mark with 95% ethanol. The absorbance of this solution was measured at 264 nm and converted to sulfur concentration using the calibration curve developed previously (Figure A.4). The results of this study are shown in Table A.8. Sulfur recovery was 78% without interference and dropped to 14% in the presence of sulfide. The samples containing sulfide and elemental sulfur exhibited a yellow colour owing to the formation of polysulfides (S_2^{2-} to S_5^{2-}). Polysulfides have a light absorption peak at 285 to 290 nm (Chen,1974) in water and presumably this shift occurs in ethanol as well, decreasing the sulfur peak at 264 nm. On acidification, solutions of polysulfides form H_2S, free sulfur (milk of sulfur) in white colloidal form and small amounts of hydrogen polysulfides (Chen,1974).

The acidification of samples by hydrochloric acid was tested as a means of removing $H_2S(g)$. Aliquots of a Na_2S solution and a sulfur colloidal solution were added to an erlenmeyer flask (Table A.9). The effects of HCl on sulfide are shown in Figure A.5. The effect of the addition of sulfide to the sulfur colloidal solution was confirmed. The yellow colour of the sulfide/sulfur mixture confirmed the presence of polysulfides. The addition of HCl turned solutions from cloudy to clear but the colour (white or yellow) was unchanged. The acidified sulfide/sulfur combination gave a higher than normal absorption at 264 nm. At pH < 0 the formation of elemental sulfur is favoured in oxidizing environments (Chen, 1974). This may be the reason for the high absorbance. It was concluded that sulfur cannot be measured in the presence of sulfide by the ethanol method without severe modification of the method.

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TABLE A. 8   Results of Second Test of Ethanol Method of Elemental Sulfur Analysis With Added Sulfur Compounds

Stock Solutions Combined	Matrix	$[S^\circ]$ Added (mg/L)	$[S^\circ]$ Detected (mg/L)
$S^=$	123 mgS$^=$/L	0	11
S°	-	129	100
$SO_4^=$	128 mg $SO_4^=$-S/L	0	5
$S^= + S^\circ$	62 mg $S^=$/L	64	9
$S^= + SO_4^=$	62 mg $S^=$/L	0	10
	64 mg $SO_4^=$-S/L		
$S^\circ + SO_4^=$	64 mg $SO_4^=$-S/L	64	36
$S^= + S^\circ + SO_4^=$	41 mg $S^=$S/L	43	6
	43 mg $SO_4^=$-S/L		

TABLE A.9 Test of Acid Correction of Sulfide Interference on Ethanol Method of Elemental Sulfur Analysis

Volume Added (mL)				Sample Vol. (mL)	[S°] in Cuvette (mg/L)	Absorbance (O.D.$_{264}$)
201 mgS°/L	139 mgS=/L	12N HCl	deH2O			
5.00	0	0	10.00	15.0	20.1	.379
2.50	0	0	12.50	15.0	10.0	.217
5.00	0	5.0	5.00	15.0	20.1	.53
2.50	0	5.0	7.50	15.0	10.0	.265
5.00	5.00	0	5.00	15.0	20.1	.023
2.50	5.00	0	7.50	15.0	10.0	.021
5.00	5.00	5.0	0	15.0	20.1	.64
2.50	5.00	5.0	2.50	15.0	10.0	.328
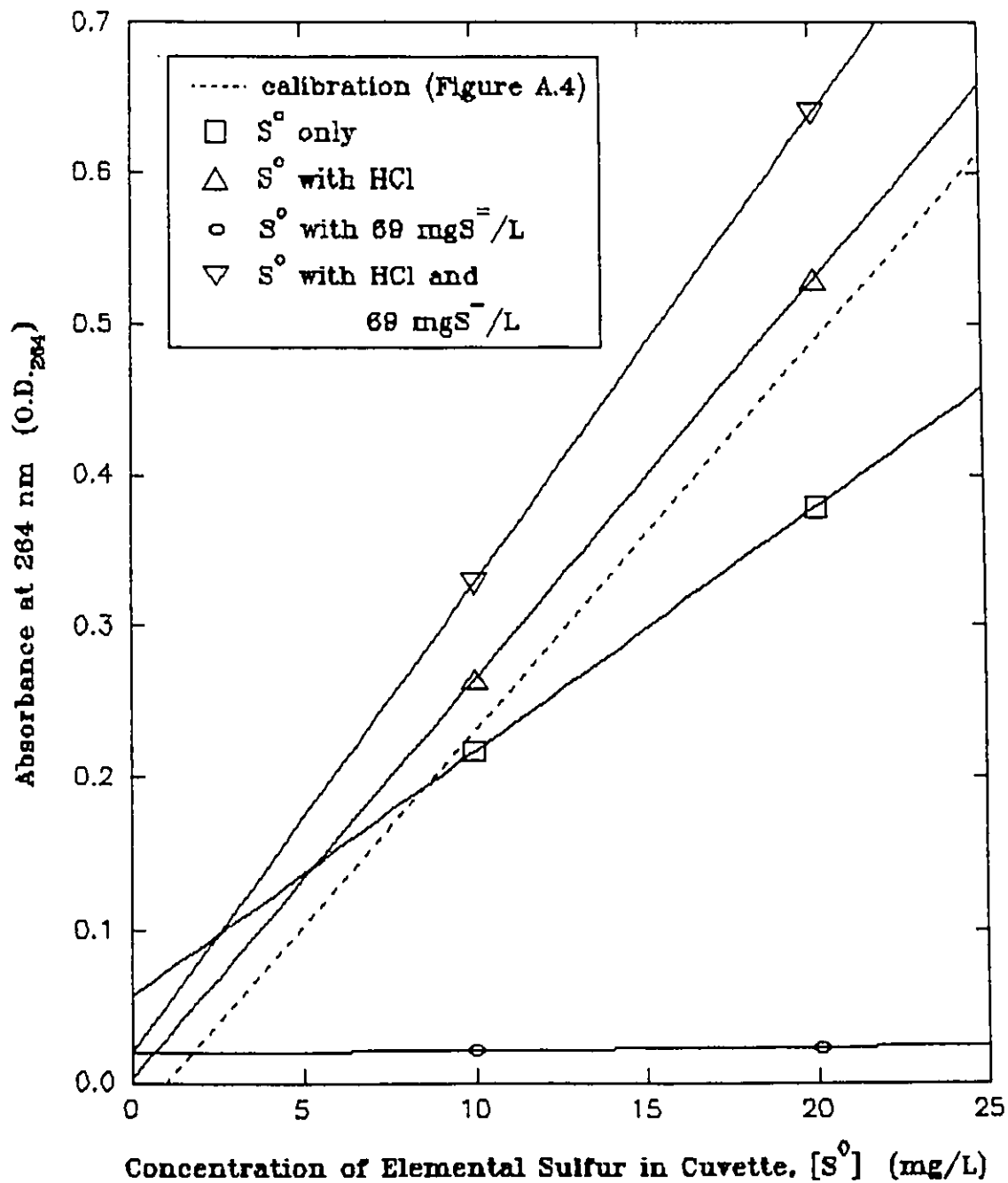
FIGURE A.5   Test of Acid Correction of Sulfide
              Interference on Ethanol Method of
              Elemental Sulfur Analysis

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A.3.2 Cyanide Method

The colorimetric method of Bartlett *et al.*(1954) measures elemental sulfur dissolved in hydrocarbons. In acetone solution, elemental sulfur reacts quantitatively with cyanide ion to yield thiocyanate ion (CSN^-). Thiocyanate can be measured colorimetrically by the addition of ferric chloride. A calibration curve was developed using a stock solution of elemental sulfur in petroleum ether (Figure A.6).

Bartlett's method was modified to extract sulfur from aqueous solutions. A sulfur colloidal suspension was used in the sulfide and sulfate calibrations to confirm or deny sulfur interference. In preliminary calibrations of the cyanide method, erroneous indications of sulfur resulted, presumably because of the inhomogeneity of the colloidal solution. For calibration, a stock solution of elemental sulfur in ethanol was made by refluxing recrystallized (in benzene) elemental sulfur in 95% ethanol for two hours. After some cooling, the solution was poured into a volumetric flask and made up to the mark with 95% ethanol.

Combinations of sulfur, sulfide and sulfate stock were added directly to a 250 mL erlenmeyer flask with a 24/40 ground glass joint. The total volume of stock additions was made up to 10.0 mL with deH$_2$O (Table A.10). Exactly 40.0 mL of HgCl$_2$ solution (25.5–26.0 g HgCl$_2$, 25.3–25.8 g KCl in 1 L deH$_2$O), a teflon-coated magnetic stir bar and 20.0 mL of 35–60 petroleum ether were added to the flask which was capped with a ground glass or teflon plug and sealed with parafilm. The mixture was stirred thoroughly for at least 45 minutes to extract sulfur into the organic phase. The upper layer was decanted into a test tube or 25 mL graduated cylinder so that a pipette could be used to transfer 5.00 mL of

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FIGURE A.6 Calibration of Bartlett's Cyanide Method of
Elemental Sulfur Analysis

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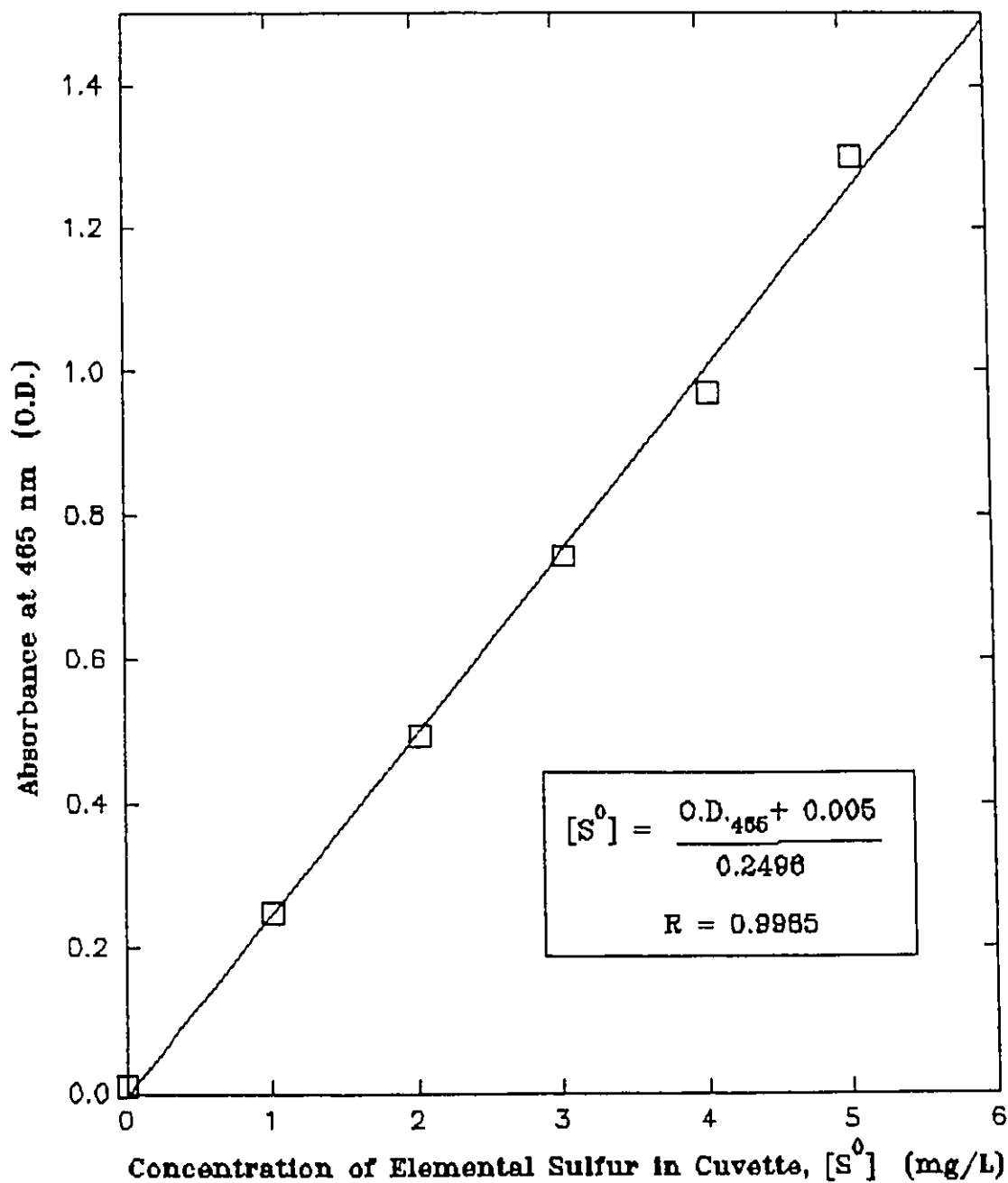**TABLE A.10   Calibration of Cyanide Method of Elemental Sulfur Analysis With and Without Added Sulfur Compounds**

Date	[S=] Stock (mg/L)	Vol. S= Stock (mL)	[S°] Stock (mg/L)	Vol. S° Stock (mL)	[SO4=] Stock (mgS/L)	Vol.SO4= Stock (mL)	Vol. deH2O (mL)	[S°] in Cuvette (mg/L)	Absorbance (O.D.465)
Nov. 30	-	0	257	4.00	-	0	6.00	5.14	1.357
	-	0	257	4.00	-	0	6.00	5.14	1.229
	-	0	257	4.00	-	0	6.00	5.14	1.244
	-	0	257	4.00	-	0	6.00	5.14	1.222
	-	0	257	1.00	-	0	9.00	1.28	.311
	-	0	257	1.00	-	0	9.00	1.28	.326
	-	0	257	1.00	-	0	9.00	1.28	.312
Dec. 7	584	2.50	257	0	513	2.50	5.00	0	.058
	584	2.50	257	0.50	513	2.50	4.50	.642	.208
	584	2.50	257	1.00	513	2.50	4.00	1.28	.356
	584	2.50	257	2.00	513	2.50	3.00	2.57	.631
	584	2.50	257	3.00	513	2.50	2.00	3.86	.870
	584	2.50	257	4.00	513	2.50	1.00	5.14	1.208
Dec. 8	538	2.50	257	1.00	513	2.50	4.00	1.28	.331
	538	2.50	257	1.00	513	2.50	4.00	1.28	.333
	538	2.50	257	1.00	513	2.50	4.00	1.28	.334
Dec. 13	397	3.30	257	1.00	513	2.50	3.20	1.28	.320
	397	3.30	257	1.00	513	2.50	3.20	1.28	.280
	397	3.30	257	1.00	513	2.50	3.20	1.28	.303
Dec. 15	610	2.10	257	2.50	513	2.50	2.90	3.21	.684
	610	2.10	257	2.50	513	2.50	2.90	3.21	.684
	610	2.10	257	2.50	513	2.50	2.90	3.21	.664
	610	2.10	257	2.50	513	2.50	2.90	3.21	.668
	610	2.10	257	3.00	513	2.50	2.40	3.86	.674
	610	2.10	257	3.00	513	2.50	2.40	3.86	.812

the petroleum ether to a 25.00 mL volumetric flask. Then, 15.0 mL of NaCN solution (1.0 g NaCN/L in 5% deH$_2$O in ACS grade acetone) was added to the flask. The solution was made up to the mark with 5% deH$_2$O in acetone and inverted twice. Exactly 5.00 mL of this solution was pipetted into a test tube to which 5.00 mL of FeCl$_3$ solution (4.0 g FeCl$_3 \cdot$6H$_2$O/L in 5% deH$_2$O in acetone) was added. The absorbance at 465 nm was measured immediately against a mixture of 5.00 mL of the FeCl$_3$ solution and 5.00 mL of 5% deH$_2$O in acetone. The absorbance was plotted against concentration of sulfur in the cuvette in Figure A.7. The line of best fit and the 95% confidence limit envelope are shown. The widths of this envelope at the lowest and highest in-range values were measured. The greatest width value was divided by two and used as the uncertainty of the concentration in the cuvette. Due to the large range in uncertainty in this calibration, measures of sulfur were performed in triplicate.

A.4 Sulfate

Standard Methods 4500-SO$_4$$^{2-}$ E. (APHA,1985) describes the turbidimetric method wherein sulfate ion is precipitated in a hydrochloric acid medium with barium chloride (BaCl$_2$) so as to form barium sulfate crystals of uniform size. The turbidity of the precipitated solution was found to be linear with the concentration of the sulfate in the cuvette (Figure A.8). Turbidity mearsurements were made in nephelometric turbidity units with a Hach model 43900 Ratio/XR Turbidimeter.

The presence of sulfide and elemental sulfur in the sample caused a falsely high reading of turbidity even though the sample was centrifuged at 3000 rpm for 5 minutes to settle any suspended solids (Maka,1986). In the presence of 49 mgS^{2-}/L, 338 mgS$^{\circ}$/L and 191 mgSO$_4$$^{2-}$-S/L, the turbidity

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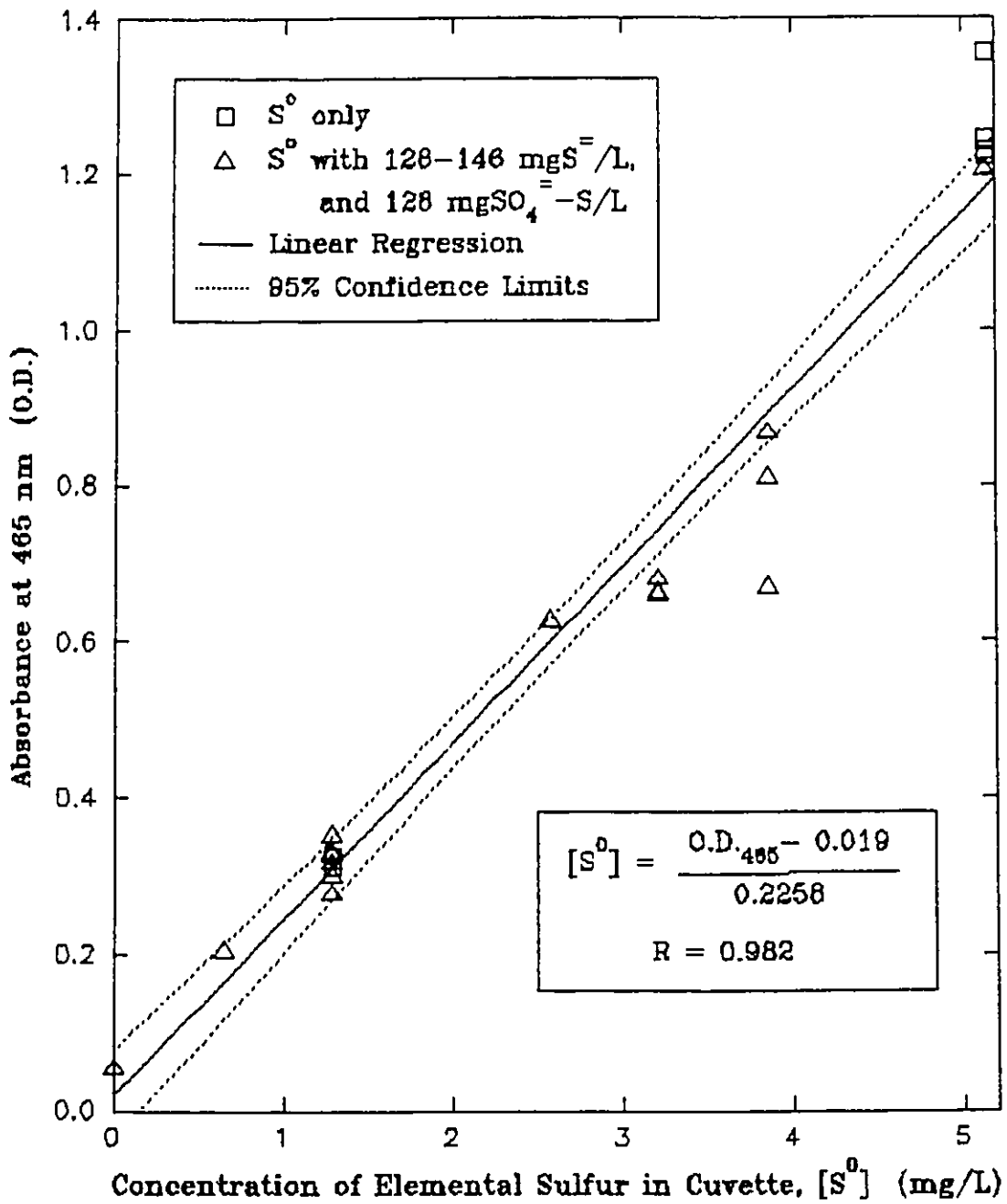
FIGURE A.7   Calibration of Cyanide Method of Elemental
             Sulfur Analysis With and Without Added
             Sulfur Compounds

$$[SO_4^= - S] = \frac{NTU + 13.2}{14.98}$$

$$R = 0.9997$$

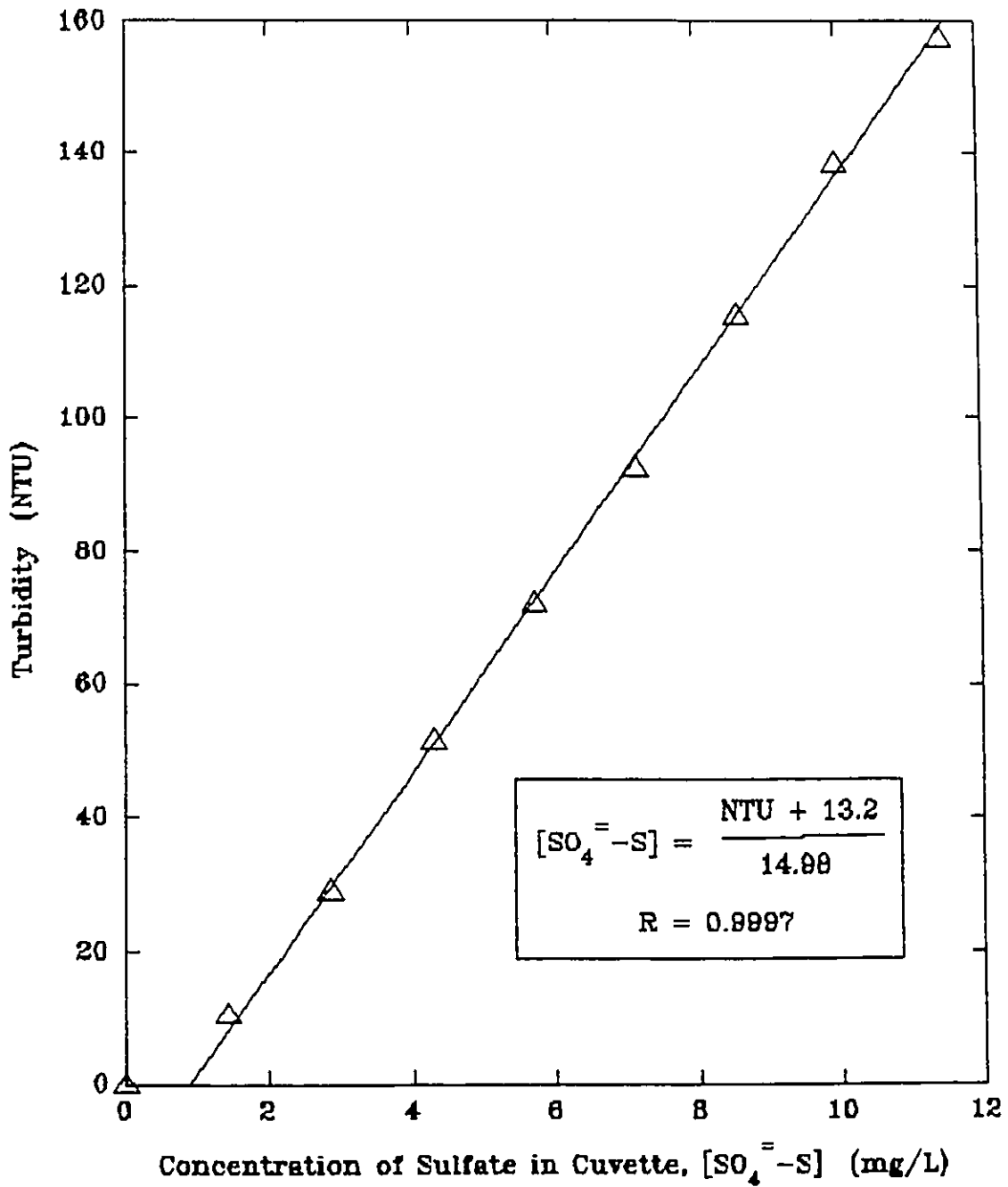FIGURE A.8 Calibration of Turbidimetric Method of
Sulfate Analysis Without Added Sulfur
Compounds

indicated a sulfate concentration of 313 $mgSO_4^{2-}-S/L$. In another experiment where 41 mgS^{2-}/L, 43 mgS^o/L and 43 $mgSO_4^{2-}-S/L$ were present, the turbidimetric method indicated that 60 $mgSO_4^{2-}-S/L$ were present. Further investigation revealed that in the presence of both sulfide and elemental sulfur, the buffered sample solution turned cloudy before barium chloride was added.

An investigation was performed using various combinations of sulfide and sulfur to interfere with the sulfate test. Solutions were added to 30 mL centrifuge tubes as indicated in Table A.11. After centrifuging for 30 minutes at 3000 rpm a 10.00 mL sample was pipetted into a 100.00 mL volumetric flask which was filled to the mark with diH_2O. The contents of the volumetric flask was poured into a 250 mL erlenmeyer flask and a magnetic stir bar was added. 5.0 mL of buffer A (per L diH_2O : 30 g $MgCl_2 \cdot 6H_2O$, 5 g $Na(CH_3COO) \cdot 3H_2O$, 1 g KNO_3, 20 mL glacial acetic acid) was added to the erlenmeyer flask. After mild stirring, the turbidimeter cuvette was rinsed and filled. The timer was started and approximately ½ mL of $BaCl_2$ crystals was added to the erlenmeyer flask. Stirring was started immediately and lasted for 60±2 seconds. When the time read 300 seconds the NTU of the sample without $BaCl_2$ was read. Then the cuvette was rinsed twice and filled with the contents of the erlenmeyer flask. The turbidity was read at 360±30 seconds. The difference between the turbidity without $BaCl_2$ and with $BaCl_2$ was nearly a constant up to a S^o concentration of 130 mg/L in the sample (Table A.11, Figure A.9). Thus, under conditions of limited sulfide and S^o concentrations, this difference in NTU could be used as an indicator of sulfate concentration.

Bergstein *et al.*(1983) monitored sulfide and sulfur in a static

TABLE A.11 Effect of Concentrations of Sulfide and Elemental Sulfur on Turbidimetric Method of Sulfate Analysis

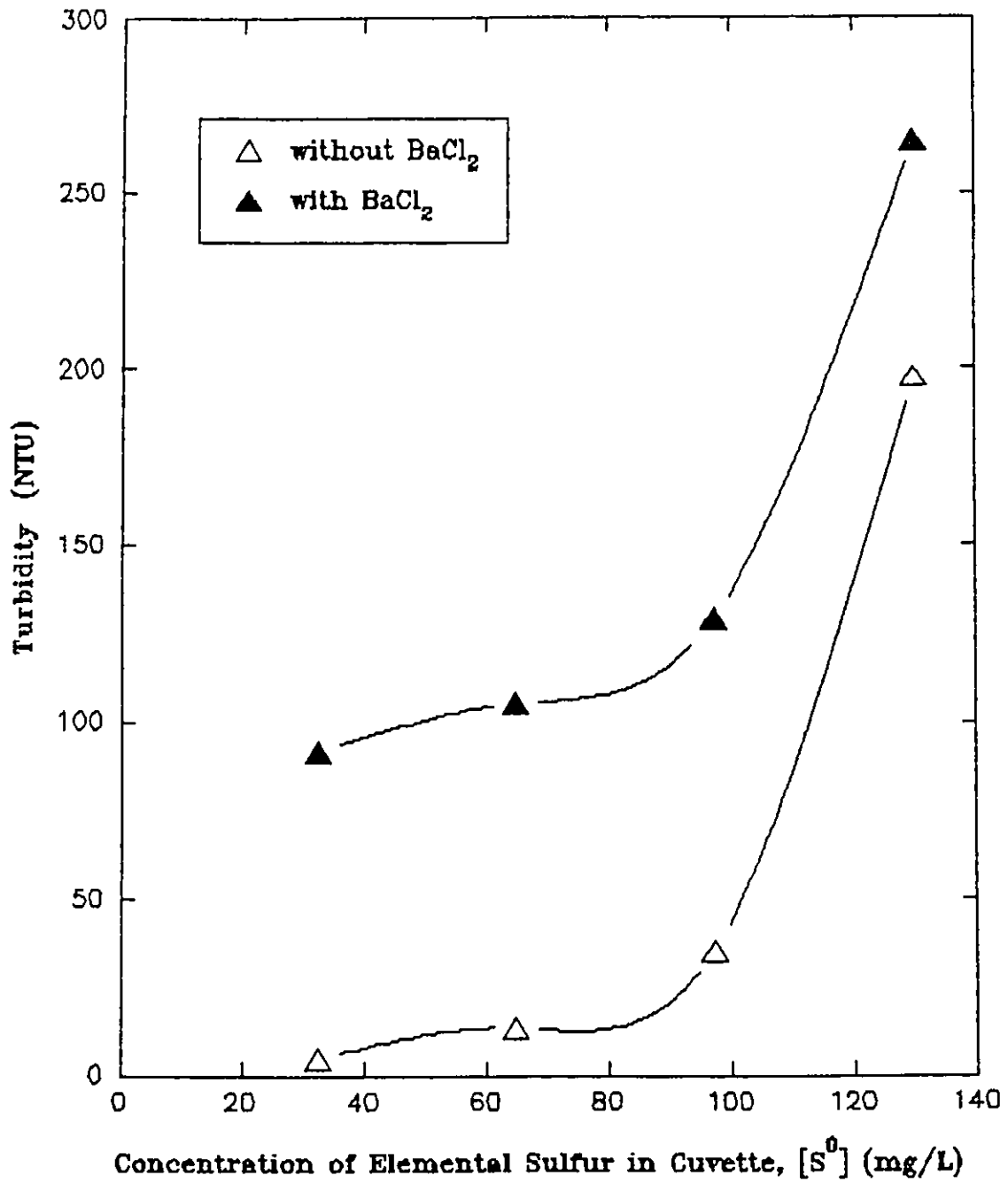| [S=] Stock (mg/L)	Vol. S= Stock (mL)	[S=] Stock (mg/L)	Vol. S° Stock (mL)	[SO4=] Stock (mgS/L)	Vol.SO4= Stock (mL)	Vol. deH2O (mL)	NTU w/o BaCl2	NTU w/ BaCl2	ΔNTU
370	10.00	389	10.00	384	5.00	5.00	197.6	265	67.4
370	7.80	389	2.50	384	5.00	14.70	4.6	91.4	86.8
370	5.20	389	5.00	384	5.00	14.80	13.3	105.1	91.8
370	2.60	389	7.50	384	5.00	14.90	35.1	129.0	94.3

FIGURE A.9   Effect of Concentrations of Sulfide and
Elemental Sulfur on Turbidimetric
Method of Sulfate Analysis

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culture of *Chlorobium phaeobacteriodes* and found that from an initial sulfide concentration of 5.05 mM (162 mg/L), S° peaked at 0.9 mM (29 mg/L) when the sulfide concentration was 2.7 mM (87 mg/L). These values were used in the test of combined S° and sulfate interference in the sulfate calibration.

Sulfate test calibration and interference check were performed as shown in Table A.12. Quantities of sulfide, sulfur and sulfate stock solutions were measured into a 30 mL centrifuge tube which was covered with parafilm and centrifuged at 3000 rpm for 30 minutes. A pipette was used to transfer 10.00 mL of the supernatant into a 250 mL erlenmeyer flask. Then, 90.0 mL of diH$_2$O were added using a graduated cylinder and a magnetic stir bar was added. A graduated cylinder was used to add 20.0 mL of buffer A, the time clock was started and mild stirring proceeded for about 30 seconds. The turbidimeter cuvette was rinsed once with the buffered solution and filled. The turbidity of this solution was measured (NTU$_1$) 360±30 seconds after adding the buffer. To the remaining solution, ¼ mL of BaCl$_2$ crystals was added at a clock time of 120 seconds and stirred until 180 seconds. This solution (with barium) was used to fill the cuvette after rinsing it. The turbidity of the solution with barium was measured at 480±30 seconds.

Figure A.10 shows that those samples with sulfide and S° in their matrices give turbidities not significantly different than for those samples without S^{2-} and S°. The line of best fit and the 95% confidence limit envelope are shown. The widths of this envelope at the lowest and highest in-range values were measured. The greatest width value was divided by two and used as the uncertainty of the concentration in the

TABLE A.12   Calibration of Turbidimetric Method of Sulfate Analysis With and Without Added Sulfur Compounds

Date	[S=] Stock (mg/L)	Vol. S= Stock (mL)	[S°] Stock (mg/L)	Vol. S° Stock (mL)	[SO$_4$=] Stock (mgS/L)	Vol.SO$_4$= Stock (mL)	Vol. deH2O (mL)	[SO$_4$=] in cuvette (mgS/L)	NTU$_i$	NTU$_f$	ΔNTU
Jan. 17	-	0	-	0	384	0	30.00	0	0.14	.45	.31
	-	0	-	0	384	1.20	28.80	1.28	0.12	12.80	12.68
	-	0	-	0	384	2.50	27.50	2.67	0.1	32.7	32.6
	-	0	-	0	384	5.00	25.00	5.33	0.1	68.7	68.6
	-	0	-	0	384	7.5	22.50	8.00	0.1	108.3	108.2
	-	0	-	0	384	10.00	20.00	10.7	0.1	144.4	144.3
	-	0	-	0	384	4.00	26.00	4.27	0.1	58.3	58.2
	-	0	-	0	384	4.00	26.00	4.27	0.1	58.3	58.2
	-	0	-	0	384	4.00	26.00	4.27	0.1	56.7	56.6
	-	0	-	0	384	4.00	26.00	4.27	0.1	56.1	56.0
	-	0	-	0	384	9.00	21.00	9.60	0.1	128.6	128.5
	-	0	-	0	384	9.00	21.00	9.60	0.1	125.2	125.1
Jan. 18	474	8.10	-	0	384	0	21.90	0	0.14	2.1	2.0
	474	8.10	-	0	384	1.00	20.90	1.07	0.17	12.51	12.34
	474	8.10	-	0	384	2.50	19.40	2.67	0.13	37.0	36.9
	474	8.10	-	0	384	5.00	16.90	5.33	0.1	74.3	74.2
	474	8.10	-	0	384	7.50	14.40	8.00	0.1	109.6	109.5
	474	8.10	-	0	384	10.00	11.90	10.7	0.2	147.2	147.0
Jan 19	-	0	385	10.00	385	0	20.00	0	34.0	34.0	0.0
	-	0	385	10.00	385	1.00	19.00	1.07	1.285	10.35	9.06
	-	0	385	10.00	385	2.50	17.50	2.67	2.22	33.9	31.7
	-	0	385	10.00	385	5.00	15.00	5.35	2.06	75.8	73.7
	-	0	385	10.00	385	7.50	12.50	8.02	.423	112.1	111.7
	-	0	385	10.00	385	10.00	10.80	10.4	.190	148.3	148.1
	453	5.70	385	2.25	385	0	22.05	0.00	11.55	11.96	.41
	453	5.70	385	2.25	385	1.00	21.05	1.07	6.47	17.59	11.12
	453	5.70	385	2.25	385	2.50	19.55	2.67	17.52	51.7	34.2

TABLE A.12 (cont'd)

| Date	[S=] Stock (mg/L)	Vol. S= Stock (mL)	[S°] Stock (mg/L)	Vol. S° Stock (mL)	[SO4=] Stock (mgS/L)	Vol.SO4= Stock (mL)	Vol. deH2O (mL)	[SO4=] in cuvette (mgS/L)	NTU$_i$	NTU$_f$	ΔNTU
Jan 19 cont'd	453	5.70	385	2.25	385	5.00	17.05	5.35	15.97	89.5	73.5
	453	5.70	385	2.25	385	7.50	14.55	8.02	18.20	128.6	110.4
	453	5.70	385	2.25	385	10.00	12.05	10.7	21.70	164.6	142.9
	479	5.40	385	2.25	385	4.00	18.35	4.27	4.92	57.8	52.9
	479	5.40	385	2.25	385	4.00	18.35	4.27	4.68	57.5	52.8
	479	5.40	385	2.25	385	4.00	18.35	4.27	4.30	57.4	53.1
	479	5.40	385	2.25	385	9.00	13.35	9.60	3.74	134.5	130.8
	479	5.40	385	2.25	385	9.00	13.35	9.60	18.60	149.7	131.1
	479	5.40	385	2.25	385	9.00	13.35	9.60	21.1	149.1	128.0
	479	5.40	385	2.25	385	9.00	13.35	9.60	17.09	145.2	128.1
	479	5.40	385	2.25	385	9.00	13.35	9.60	25.7	154.2	128.5
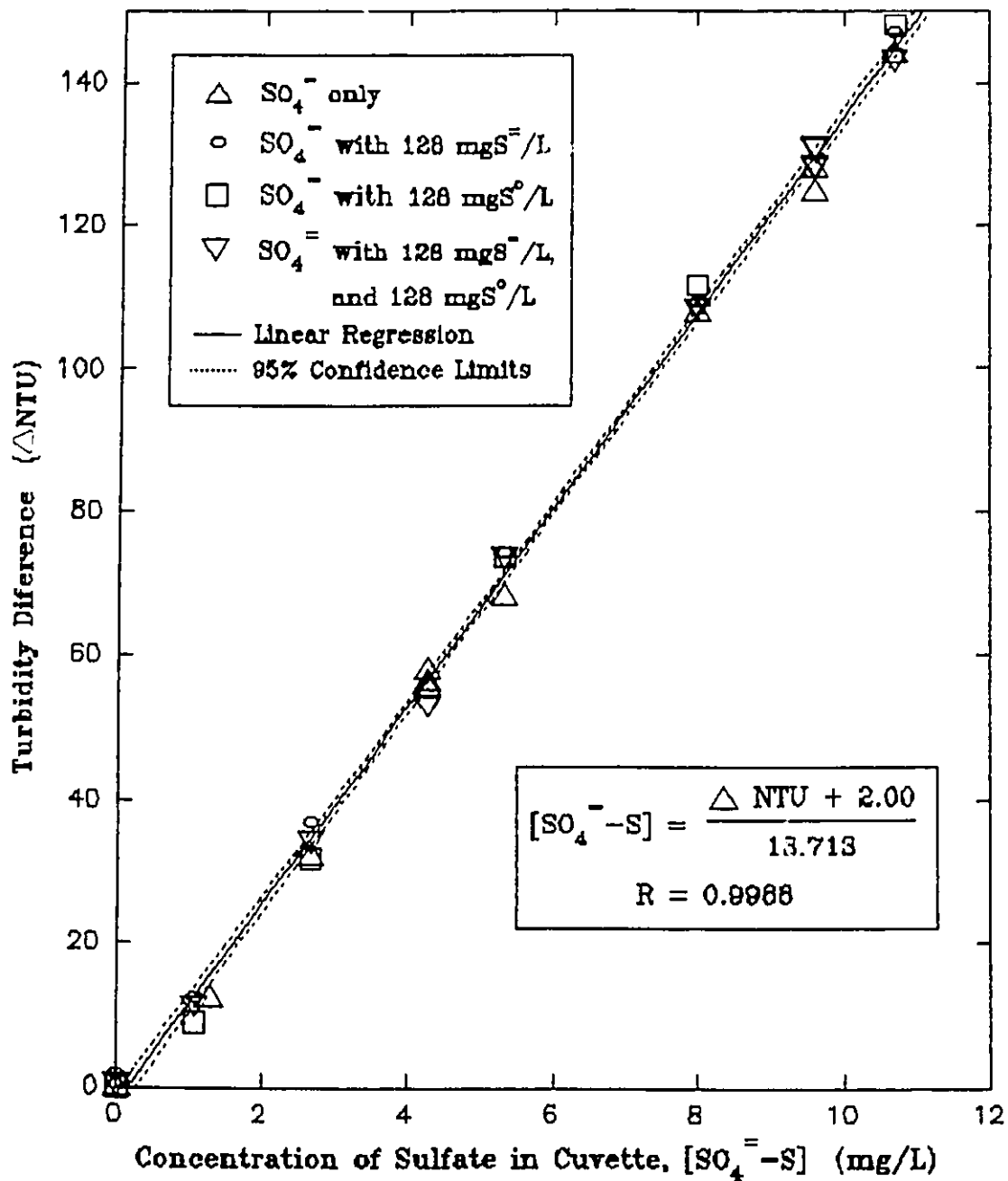
FIGURE A.10   Calibration of Turbidimetric Method of
Sulfate Analysis With and Without Added
Sulfur Compounds

cuvette. Figure A.11 shows the region where this modified sulfate method is valid.

A.5 Bacteriochlorophyll

Kakidas (1982) has shown there is a linear relationship between the number of cells and the mass of bacteriochlorophyll (bchl) for *C. thiosulfatophilum*. She also makes reference to the ratio of 900 mg/L dry cell weight to 25 mg/L bchl. Therefore, the concentration of bchl in a sample of reaction mixture can be used as an indicator of biomass.

The procedure of Maka (1986) was used but the quantities were doubled (see Section 3.1.4). In order to quantify the uncertainty of this measurement, several repetitive measurements of the same reactor solution were made in groups of two (one group of four). The absorbance readings of each group were averaged and the concentration of bchl in the cuvette was calculated using the formula given by Maka (1986) based on that average. The values of absorbance were plotted against the concentration of bchl in the cuvette (Figure A.12). The line of best fit and the 95% confidence limit envelope are shown. The widths of this envelope at the lowest and highest in-range values were measured. The greatest width value was divided by two and used as the uncertainty of the concentration in the cuvette.
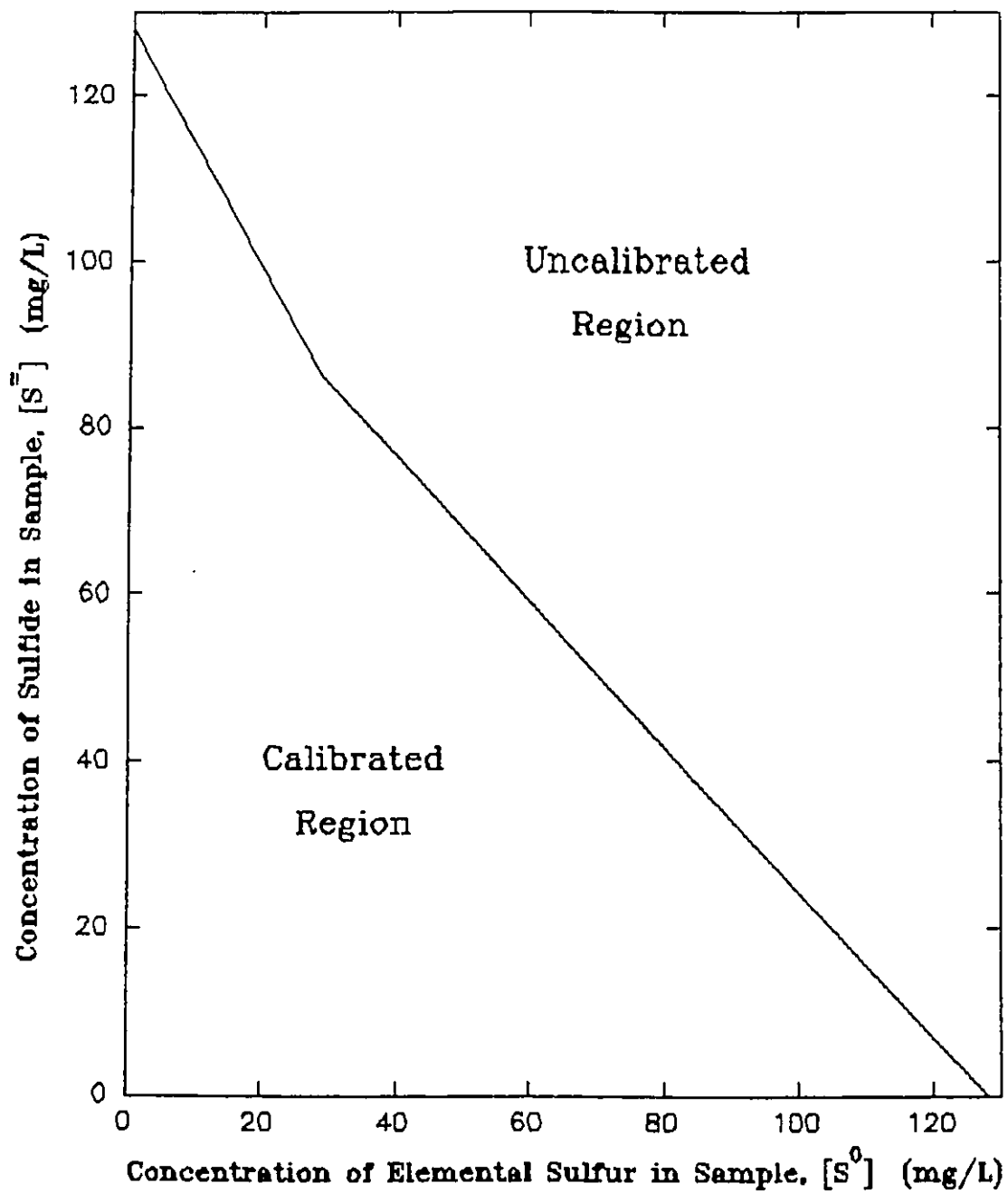
FIGURE A.11   Calibrated Region for Turdidimetric
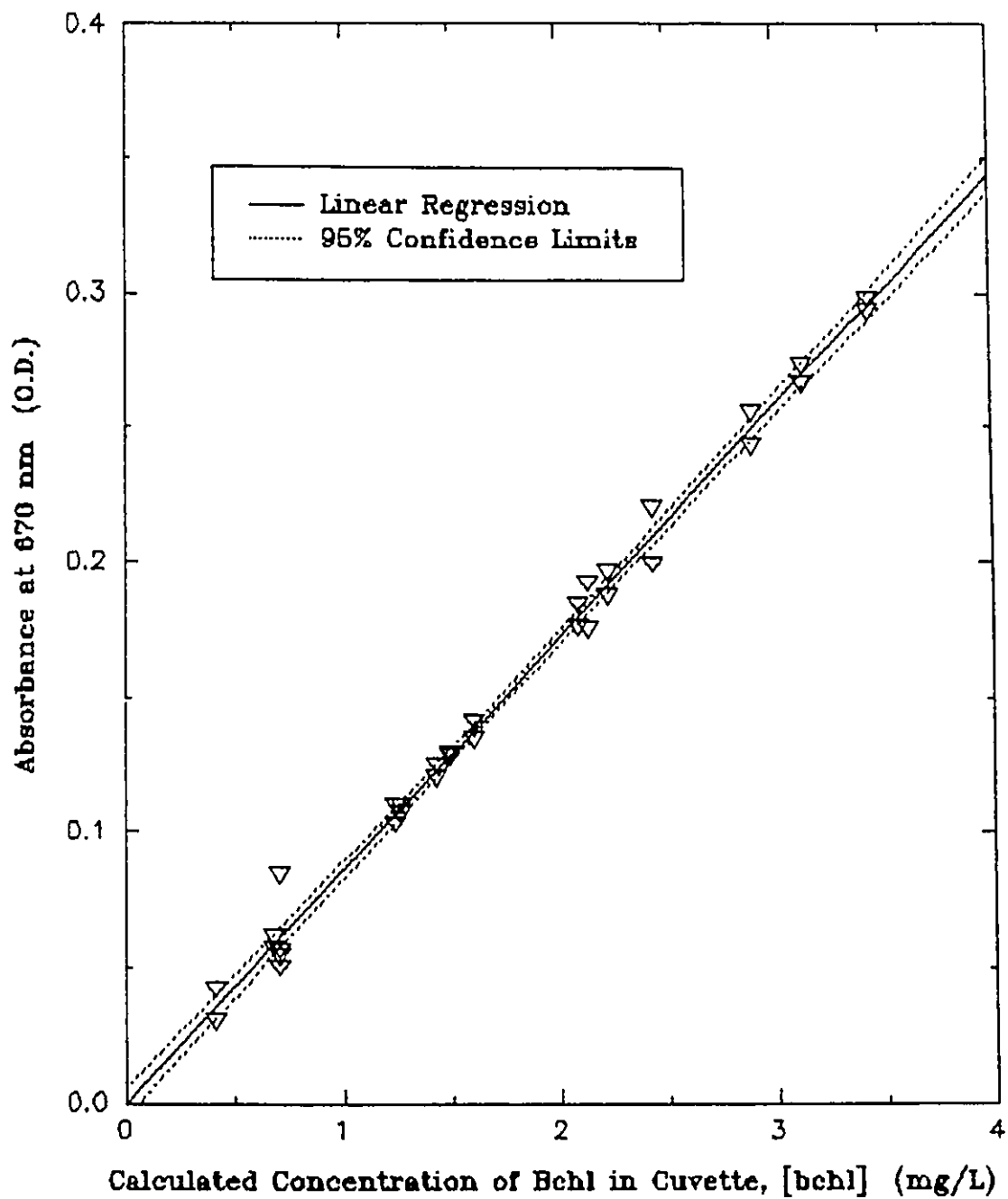Method of Sulfate Analysis

FIGURE A.12   Plot for Calculation of Uncertainty in
Bchl Analysis

APPENDIX B

MEASUREMENT OF REACTOR ILLUMINANCE

B.1 Materials and Methods

The top of the 15 L reactor was removed and the glass reactor was immersed in the fermentor drive assembly water bath as in an experimental run (Section 3.2.2). Weights were added to the bottom of the flask to keep it from floating while the waterbath was filled. The sensor of an International Light IL 1700 Research Radiometer was positioned inside the reactor and normal to the light source at twelve locations as shown in Figure B.1. The light intentsity was measured with SED038/F/W, SED038/Y/W and SED038/TFDR/W filter combinations.

The resultant values for each sensor combination were integrated over the frontal area of the reactor. The locations of the sensor during measurements were considered as nodes. The reactor front projection area was divided into 20 rectangles having nodes at 1 to 4 corners. The average of the light measurements at the four nodes was multiplied by the area of the rectangle. Where a light measurement was not available, a node value was linearly extrapolated from the previous two nodes in-line. When extrapolated node values were negative, zero was used as the node value. The sum of the average values x rectangle areas was divided by the total frontal area of the reactor.

B.2 Results

The following table summarizes the measurements of light illuminance:

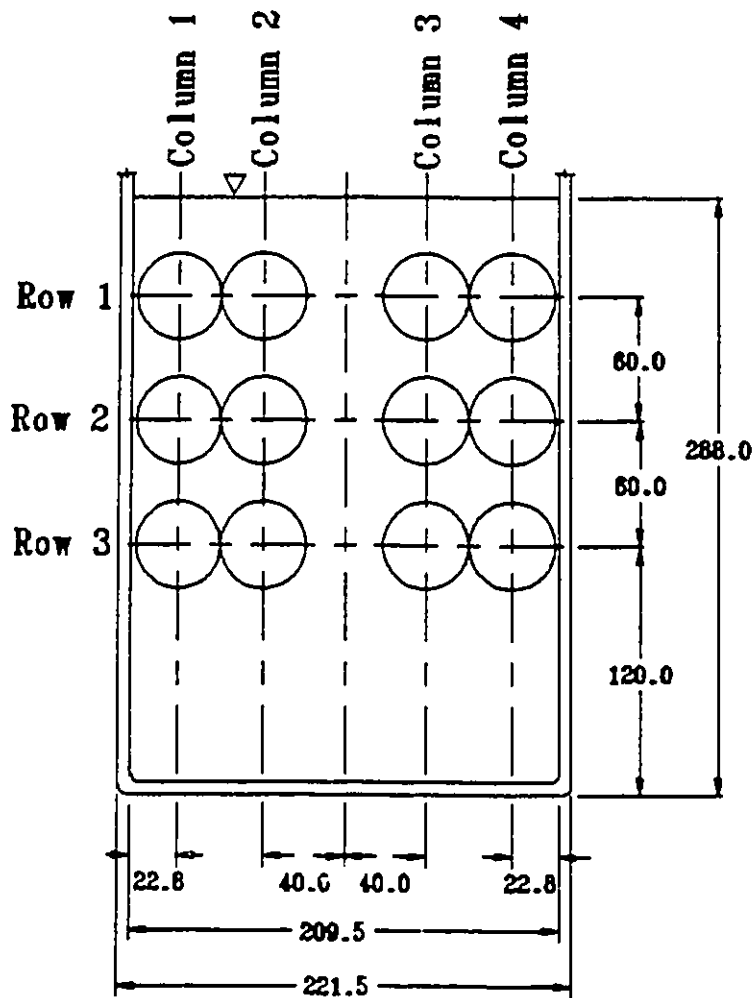| Filter	Range of Light Measured	Incident Radiation	Reactor Illuminance
SED 038/Y/W	Visible		2400 lux
SED 038/F/W	400 - 700 nm	7.09 W	1.2×10^{-2} W/cm^2
SED 038/TFRD/W	Infra-Red	2.78 W	4.7×10^{-3} W/cm^2

FIGURE B.1   Sensor Locations for Measuring
Reactor Illuminance

all dimensions in mm

APPENDIX C

CALCULATION OF THE AMOUNT OF AVAILABLE CARBON DIOXIDE

C.1 Carbon Dioxide Inputs

1. 3.6 L of diH_2O saturated with CO_2

   • solubility of CO_2 in water at 25°C = 0.145 g/100mL (CRC, 1980)

   3.6 L x 1.45 gCO_2/L = 5.2 gCO_2

2. 18 g of $NaHCO_3$

   18 g $NaHCO_3$ x $\dfrac{44.01\ gCO_2}{84.0\ gNaHCO_3}$ = 9.4 gCO_2

3. Total

   5.2 gCO_2 + 9.43 gCO_2 = 14.6 gCO_2

C.2 Sulfide That Can be Utilized

1. Refer to equation [2.2]

   14.6 gCO_2 x $\dfrac{2\ moles\ H_2S}{mole\ CO_2}$ x $\dfrac{32.06\ gS^{2-}/mole\ H_2S}{44.01\ gCO_2\ /mole\ CO_2}$

   = 21.3 gS^{2-}

2. Equivalent Concentration of S^{2-}

   $\dfrac{21.3\ gS^{2-}}{9.5\ L}$ = 2240 mgS^{2-}/L

3. Therefore there is enough CO_2 to allow for an initial concentration of 2240 mgS^{2-}/L in the reactor, or a series of injections where the sum of sulfide concentrations is 2240 mgS^{2-}/L. This is a conservative estimate since neither the CO_2 retained in the headspace nor that dissolved in the mineral salts solution upon purging the reactor were taken into account.

VITA AUCTORIS

1961	Born in Windsor, Ontario, Canada, on June 14.
1978	Awarded Ontario Secondary School Graduation Diploma from Centennial Secondary School, Windsor, Ontario.
1979	Awarded Ontario Secondary School Honour Graduation Diploma from Centennial Secondary School, Windsor, Ontario.
1979	Awarded University of Western Ontario Entrance Scholarship.
1981	Awarded NSERC University Summer Research Scholarship.
1982	Completed the Degree of Bachelor of Science in Chemistry at the University of Western Ontario, London, Ontario, Canada.
1984	Awarded NSERC Industrial Summer Research Scholarship.
1985	Completed the Degree of Bachelor of Engineering Science in Mechanical Engineering at the University of Western Ontario, London, Ontario, Canada.
1985	Began employment as Jr. Mechanical Engineer at Chorley & Bisset Ltd., Consulting Engineers, London, Ontario, Canada.
1987	Began employment as Mechanical Engineer at Wilson, Dario & Associates, Consulting Engineers, Windsor, Ontario, Canada.
1989	Awarded Ontario Graduate Scholarship.
1990	Awarded NSERC Postgraduate Scholarship.