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Bioconversion of sulfide to elemental sulfur in trickling filter

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Bioconversion of sulfide to elemental sulfur in trickling filter

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

BIOCONVERSION OF SULFIDE TO ELEMENTAL SULFUR IN TRICKLING FILTER

CHAPTER 1. GENERAL INTRODUCTION

1.1 Background

High strength sulfate-rich or sulfide-rich waste streams are generated by the industries such as petrochemical, photographic processing, textile mills, pulp and paper mills, tannery, molasses fermentation and pharmaceuticals. Such wastewaters are mainly treated by the anaerobic processes due to their inherent benefits, e. g. high loading capacity, saving in aeration energy cost, low sludge production, and methane generation (Fox and Venkatasubbiah, 1996; Janssen *et al.*, 1995; Lens et al., 1998; Ranade and Bhirangi, 2001). The anaerobic treatment of sulfate-rich wastewater however produces sulfide as results of biological sulfate reduction (Rinzema and Lettinga, 1988), which is highly nuisance for several reasons: its toxicity effect on methanogens (Khanal and Huang, 2002), malodorous, corrosivity effect on materials (Hao *et al.*, 1996), and sludge bulking problems in post aerobic treatment (Buisman and Lettinga, 1990). Not to mention, the sulfide so produced significantly lowers the energy value of biogas as fuel (Ranade and Bhirangi, 2001). Because of these ill effects, the emission of sulfide must be stringently controlled.

Today the commonly used methods for sulfide removal are physical-chemical processes that include direct air stripping, chemical oxidation or precipitation. However the physical-chemical methods are often very costly in

daily operation due to high chemical cost and high energy demand (Cork *et al.*, 1986). These shortcomings have led to the development of biological method for sulfide removal, in which sulfide is mainly oxidized to elemental sulfur by the aid of microorganisms. The biological sulfur removal process is highly efficient and does not require a catalyst or oxidant (except for air) and produces little if any biological or chemical sludge for disposal. Sulfate and thiosulfate discharge from the process is minor.

1.2 Objective

Biological sulfide removal process has been well understood and the engineering aspects of biological sulfur removal have been explored as well. However, process simplicity and improvement in the technological and economic feasibility will make this process more attractive and practical. The objective of this study was to explore the potential application of a fixed-film biofilter to remove sulfide from the biogas that contains high hydrogen sulfide level with reclamation of elemental sulfur. The effects of different sulfide loading rates, oxygen contents in gas flow, and gas flow rates were also investigated. A series of batch tests were conducted to quantify the relative contribution of biotic and abiotic components in total sulfide oxidation and to assess the possibility of heterotrophic sulfur reducing activity.

1.3 Thesis Organization

The thesis is organized in four chapters. Chapter 1 is a general introduction providing a brief background of the research. Chapter 2 is the literature review, which includes the previous work in the field and the need for this study. Chapter 3 is the manuscript entitled "Bioconversion of Sulfide to Elemental Sulfur in Trickling Filter", which presents the important findings from the study conducted to evaluate the performance of a trickling biofilter for sulfide removal and also evaluates the relative contribution of biotic and abiotic components in total sulfide oxidation and the possibility of heterotrophic sulfur reducing activity. The general conclusions and some recommendations for future study are presented in Chapter 4. The references for the manuscript are listed at the end of the manuscript.

CHAPTER 2. LITERATURE REVIEW

2.1 Overview

In recent years, anaerobic treatments are becoming popular for the treatment of organic-rich wastewater due to: (a) less energy requirement; (b) energy generation in the form of methane gas; (c) less sludge generation; (d) smaller treatment plant foot print; and (e) less nutrients (nitrogen and phosphorus) requirement. However, anaerobic treatment is not a panacea for the treatment of all types of high strength wastewater, especially from petrochemical industry, photograph processing industry, textile mills, pulp and paper mills, edible oil refinery, molasses fermentation, tannery and pharmaceuticals due to the presence of high concentration of sulfate and or sulfide (Fox and Venkatasubbiah, 1996; Janssen *et al.*, 1995; Lens et al., 1998; Ranade and Bhirangi, 2001). The anaerobic microorganisms utilize sulfate as an electron acceptor thereby producing sulfide as an end product, which is reported to have many ill effects including, toxicity to methanogens, corrosive effect, health effect, and high oxygen demand. Most importantly, the emanation of unpleasant odor of sulfide has been a major source of public complains. From these perspectives, control of sulfide becomes increasingly important before its final disposal.

2.2 Methods for Sulfide Removal

Different methods for sulfide removal can be divided into two groups: physical-chemical processes that include direct air oxidation, chemical oxidation, and precipitation and biotechnological process.

2.2.1 Physical-chemical processes

Direct air oxidation may eliminate the sulfide through abiotic pathway. However, it may result a significant amount of sulfide in the gas phase due to stripping, which also requires further treatment. Uncatalyzed oxidation of sulfide by air/oxygen is a complex and slow process proceeding through a series of chain reactions (Chen and Morris, 1972). Sulfide can be converted to sulfur, thiosulfate, or sulfate in the oxidation process depending on the oxygen level and availability of catalyst. The chemical oxidation includes chlorination, ozonation, potassium permanganate treatment and hydrogen peroxide treatment (Buisman *et al.*, 1991). The rate and efficiency of such oxidation reactions are appreciably high. But the cost of chemicals is often a retarding factor for daily operation.

In chemical precipitation sulfide can be precipitated as insoluble metal sulfides through its reaction with divalent metals such as iron, zinc, copper, etc. (Khanal, 2002). The accumulation of insoluble metal sulfide in direct precipitation reduces the effective volume of treatment unit, causes clogging of pipings and requires further treatment of chemical sludge (Khanal 2002; Cork *et al.*, 1986).

By using biotechnological method, sulfide can be converted to mainly to elemental sulfur and the drawbacks of physical/chemical methods can be alleviated.

2.2.2 Biological oxidation

In biological sulfide oxidation, sulfide is oxidized mainly to elemental sulfur with the aid of microorganisms. The process has several merits, which makes it more attractive than the physico-chemical processes (Buisman *et al.*, 1991) for the following reasons:

a) Low energy requirement.

b) Low chemical and disposal costs, because it does not require a catalyst or oxidant (except for air), and produces little if any biological or chemical sludge for disposal.

c) Reduction of the discharge of sulfate and or thiosulfate.

d) Potential for sulfur recovery and reclamation.

It is preferable that sulfide be oxidized to elemental sulfur instead of sulfate. This is because elemental sulfur is nontoxic and non-corrosive and requires less oxygen in molar basis. From the economic point of view, elemental sulfur is 3 to 8 times more valuable than commercial sulfuring acid (Cork, 1978; Kim and Chang, 1991). Moreover, elemental sulfur is a valuable byproduct, which

can be reused in autotrophic denitrification and bioleaching processes as electron donor (Torres *et al.*, 1995; Tichy *et al.*, 1994).

2.3 Process Microbiology of Biological Sulfide Oxidation

The microbial group involved in sulfide oxidation belongs to a group of colorless sulfur bacteria, of which *Thiobacillus* is the best known.

2.3.1 *Thiobacilli*

The genus *Thiobacillus* consists of a number of species, which are closely related to each other. They are gram negative, facultative autotrophs, non-spore forming, rod shaped, in size of 0.3 × 1 to 3 μm, polarly flagellated and thus motile except for the *Thiobacillus novellas*. All the members of this genus utilize reduced sulfur compounds such as thiosulfate as electron donor and carbon dioxide as carbon source. They are able to oxidize sulfide, elemental sulfur, thiosulfate and polythionite as energy source. They are obligate autotrophs and are not able to utilize organic carbon as an electron and carbon source, with the exception of *T. novellas.* The best growth is reported at 25-35° C and neutral pH, but some species are able to live in highly acidic environments, such as *T. thiooxidans*, which grows best below pH 5 (Vishniac and Santer, 1957).

The five most described members of the *Thiobacillus* species are: *T. thioparus*, *T. denitrificans*, *T. thiooxidans*, *T. intermedius*, and *T. ferrooxidans*.

T. thioparus grows rapidly in a mineral medium containing thiosulfate and is able to precipitate abundant sulfur particles. Its optimal growth can be reached near pH 7 at 30° C.

T. denitrificans differs in that it can utilize NO_3 instead of O_2 as a terminal electron acceptor at anoxic conditions and carry out denitrification. This denitrification is shown in the following bio-chemical reaction:

$$2NO_3^- + S^\circ + H_2O + CaCO_3 = CaSO_4 + N_2 \quad (1)$$

T. thiooxidans grows at much more acidic range. The best growth is in pH range of 2 to 5. Apart from its acid habitat, this species is also distinguished by its higher oxidation rate of elemental sulfur compared to *T. thioparus* and *T. denitrifacans*, which oxidize sulfur rather slowly.

T. intermedius is a facultative chemolithotroph with a pH range of 3 to 7. Its growth is powered by thiosulfate, which acts as an electron donor, and organic matter stimulates its growth.

T. ferrooxidans is a unique organism when taken in the paradigm of *Thiobacillus.* It is strictly aerobic, obligate autotroph, and has a pH growth range of 1.5 to 5. It can also use thiosulfate as an electron donor, but it differs from all the other *Thiobacilli* species in its ability, from which its name is derived, to utilize iron as an energy source instead of thiosulfate.

2.3.2 Other sulfide removal bacteria

The other species of bacteria have also been capable of sulfide removal. Photoautotrophs have been reported to produce a high percentage of elemental sulfur from sulfide (Cork, 1985; Henshaw *et al.*, 1998a, 1998b; Kim *et al.*, 1991, 1992). For photoautotrophs, the light penetrability is a significant factor for the bioreactor design, which is seldom considered in the common reactor design. Hansen *et al.* (1975) found a purple bacterium, which oxidizes sulfide to elemental sulfur and sulfate depending on the influent sulfide concentration. When sulfide concentration exceeds 2 mg/L, elemental sulfur is the major end-product, whereas lower sulfide concentration favors the production of sulfate.

2.4 Biological Sulfur Cycle and Biological Sulfide Oxidation

Microbial conversion processes of different sulfur species are shown in the biological sulfur cycle in Figure 1 (Janssen *et al.*, 1998).

There are two main reactions in a biological sulfide oxidation:

$$2\,HS^- + O_2 = 2\,S^0 + 2\,OH^-$$
$$\Delta G^0 = -169.35\ \text{kJ/mol}\ HS^- \qquad (2)$$

$$2\,HS^- + 4\,O_2 = 2\,SO_4^{2-} + 2\,H^+$$
$$\Delta G^0 = -732.58\ \text{kJ/mol}\ HS^- \qquad (3)$$

Under oxygen limiting conditions, that is oxygen concentration is below 0.1 mg/L, elemental sulfur is the major end-product, while sulfate is mainly formed under sulfide limited circumstances. (Janssen *et al.*, 1995)

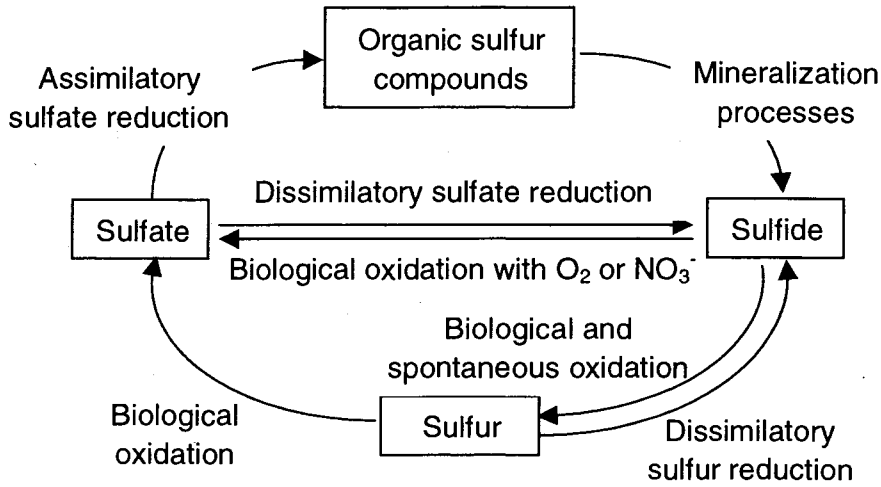

Figure1. Biological sulfur cycle

In addition to the bioconversion of sulfide, chemical oxidation also needs to be taken into account, especially under certain conditions at which biological activity is limited. Under such circumstance, chemical auto-oxidation of sulfide becomes relatively more important. Furthermore, the presence of trace metals and nutrients can also enhance the abiotic sulfide rate and efficiency (Khanal, 2002). Under slightly alkaline conditions, thiosulfate is formed as the major product based on the following equation (Chen and Morris, 1972):

$$2\,HS^- + 2O_2 = S_2O_3^{2-} + H_2O$$
$$\Delta G^0 = -387.35\ kJ/mol \qquad (4)$$

Previous research has shown that in a sulfide-oxidizing bioreactor, the optimal oxygen/sulfide ratio is about 0.7 (Janssen *et al.*, 1995). A maximum sulfur production of around 73 ± 10% occurs at an oxygen/sulfide ratio from 0.6 to 1.0; but not at the stoichiometrical ratio of 0.5 because of the formation of thiosulfate. It also suggest that the sulfide in the influent can not be converted to elemental sulfur completely due to the formation of sulfate, either directly produced by the excess oxygen or indirectly converted from the thiosulfate which is formed under oxygen limited environments.

Elemental sulfur and sulfate are the main oxidation products of the biological sulfide removal process. The concentrations of other sulfur compounds found in the effluent other than elemental sulfur, sulfide and sulfate could be negligible especially at higher pH of 8.0. With a high sulfide concentration, the effluent solution presents dark green color because of the formation of polysulfide as intermediates.

Some researchers found that the biological sulfide oxidation rate was 75 times faster than the chemical non-catalyzed oxidation rate at a sulfide concentration of around 10 mg/L, but with the increase of sulfide concentration, the rate of biological reaction started to decline. The biological rate was only 7 times faster than the chemical oxidation rate at a sulfide concentration of 100 mg/L. (Buisman *et al.*, 1990a). In chemical oxidation of sulfide, it was found that a high sulfide/oxygen ratio favors the production of sulfur (O'Brien and Birkner,

1977; Chen and Morris, 1972). Nevertheless sulfite, thiosulfate and sulfate are formed under a low ratio.

2.5 Factors Affecting Biological Sulfide Oxidation

Important operating parameters of biological sulfide removal process are wastewater flow rate (sulfide-loading rate), oxygen concentration in the reactor, sulfide/oxygen ratio, type of support material, and H_2S concentration in the effluent air (Buisman *et al.*, 1990b; Buisman *et al.*1991).

The optimal pH for biological sulfide oxidation in the range of 8.0-8.5, and the optimal temperature exists in the range of 25-35ºC (Buisman *et al.*, 1989). Buisman *et al.* (1990b) also reported that maximal sulfur production occurs at an oxygen concentration of 0.1 mg/L. The optimum sulfide/oxygen ratio is about 0.7 (Janssen *et al.*, 1995)

Previous research has shown that there is a linear relationship between the logarithm of the sulfide concentration and the redox-potential, and the redox potential is determined by the sulfide concentration kinetically rather than thermodynamically (Bockris and Reddy, 1970; Berner, 1963; Eckert, 1993). Therefore by controlling redox state, an oxygen-limited environment can be achieved to minimize the formation of sulfate will be minimized. The optimal redox value for sulfur formation is between -147 and -137mV (with reference to standard H_2 electrode at 30ºC and pH 8) [Janssen *et al.*, 1998]. However, the direct

placement of ORP electrode in the sulfide oxidizing bioreactor is often poisoned by the fouling due to the attachment of sulfur particle.

2.6 Support Media Used in Biological Sulfide Removal Reactor

To date, different biomass support media have been used in the biological sulfide oxidizing experiments such as reticulated polyurethane (PUR) foam, reticulated polyurethane foam coated with polyvinyl chloride (PVC), PVC Rasschig ring, polypropene hiflow pall rings and polyethene bio-net. It was however found that reticulated polyurethane was not suitable as carrier material for the biological sulfide removal process due to the growth of biomass in the inner part of foam where oxygen level is often below zero (Buisman *et al.*, 1991). Different transparent plastic tube materials (PTMs), including Bev-a-Line (polyethylene liner with ethyl vinyl acetate shell), FEP (fluorinated ethylene propylene), Kynar (polyvinylidene fluoride), PFA (perfluorioalkoxy), polypropylene and Tygon (vinyl chloride-vinylidene chloride co-polymer), were also tested to determine the best supporting material in a fixed-film biological reactor (Henshaw *et al.*, 1999). It was found that the total growth of bacteria was not significantly affected by the presence of PTMs, but the fraction of total growth on the tubing was significantly higher for Tygon and Bev-a-ling tubing than the other PTMs.

2.7 Reactor Configurations for Biological Sulfide Removal System

Rotational Biological Contact (RBC) Reactors, Completely Mixed Tank Reactors (CSTRs), Upflow Reactors, and Batch Reactors (Buisman, *et al.*, 1990c; Janssen, *et al.*, 1995) have been employed for biological sulfide removal. The findings of these researches pointed out the importance of reactor configurations in the conversion of sulfide to sulfur. The upflow reactor and RBC reactor were tested for their suitability for sulfide removal from anaerobic paper mill wastewater, respectively (Buisman and Lettinga, 1990). It was concluded that upflow reactor was not suitable for this kind of wastewater due to frequent clogging problems even though different types of supporting media were tried out. In biorotor reactor a sulfide removal rate of 620 mg/L·h was found at HRT of 13 min with a sulfide removal efficiency of 95%.

Fox and Venkatasubbiah (1996) integrated an attached film biological sulfide-oxidizing reactor with an anaerobic baffled reactor through effluent recycling. This coupled anaerobic/aerobic system was effective in alleviating sulfide inhibition of both methanogenesis and sulfate reduction. The thin film biological reactor could convert sulfide to elemental sulfur without adding excess oxygen, and the product sulfur could be removed from the wastewater stream.

CHAPTER 3. BIOCONVERSION OF SULFIDE TO ELEMENTAL SULFUR IN TRICKLING FILTER

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Qiyong Cao and Shihwu Sung

Abstract

In this study a lab-scale (1L) trickling biofilter was employed to investigate the feasibility of biological sulfide oxidation with the reclamation of elemental sulfur. The effect of different oxygen contents (ranging from 3% to 10 %) in the gas flow was evaluated under two different sulfide loading rates of 120 mg-S/L-hr and 180 mg-S/L-hr. The results showed that at sulfide loading rate of 120 mg-S/L-hr, 93.6% of influent sulfide was removed at an oxygen content of 5% (by volume). However, at a higher sulfide loading rate of 180 mg-S/L-hr, the sulfide removal efficiency dropped to 90.8% even with the increase of the oxygen content to 10%. The gas flow rate was found to have a significant impact on the sulfide removal efficiency. Upon increasing the gas flow to 0.4 L/min from 0.2 L/min, sulfide removal efficiency dropped by 17%.

A series of batch tests were also conducted to quantify the relative contribution of biotic and abiotic components in total sulfide oxidation and to evaluate the possibility of heterotrophic sulfur reducing activity. The batch test results suggest that as high as 88% of the influent sulfide could be oxidized

biologically whereas abiotic oxidation could contribute up to 12% of the total oxidation. The corresponding sulfide removal rates were 811.8 mg-S/L-hr and 112.2 mg-S/L-hr, respectively. A significant sulfur reducing activity was also evident in the presence of organic matter under anaerobic condition.

Key words

sulfide removal, sulfur production, trickling biofilter, oxygen content, biotic/abiotic sulfide oxidation, sulfur-reducing activity

Introduction

Sulfide-laden waste streams are produced during anaerobic treatment of high strength sulfate-rich wastewaters such as molasses fermentation, edible oil refinery, pharmaceutical, sea food processing, distillery etc. (Lens *et al.*, 1998; Fox and Venkatasubbiah, 1996). Sulfide is also directly contributed to the waste streams by other industrial processes such as tanneries, coal gasification, petrochemical plants etc. (Genschow *et al.*, 1996; Janssen *et al.*, 1997). The removal of sulfide is essential for several reasons including: its inhibitory effect on methane producing bacteria (Khanal and Huang, 2002), corrosive effect on materials (Hao *et al.*, 1996), unpleasant odor, chemical oxygen demand (COD) contribution, and sludge bulking problems in post aerobic treatment (Buisman and Lettinga, 1990).

Sulfide removal could be achieved by physico-chemical methods, e.g. direct air stripping, chemical oxidation, or chemical precipitation and biotechnological process. The physico-chemical methods are often very costly in daily operation due

to high chemical cost and high energy demand (Cork *et al.*, 1986). The biotechnological process overcomes those demerits, in which sulfide is mainly converted to elemental sulfur by the aid of microorganisms. The process has several advantages in comparison to the physico-chemical processes (Buisman *et al.*, 1991): a) low energy requirements; b) no chemical and residual disposal costs, (except for air/O_2), and c) effluent with low sulfate and thiosulfate. Elemental sulfur is a desired end product of sulfide oxidation because of its non-toxic, and settleability nature, less oxygen requirement and possibility of reclamation and reuse of sulfur as a valuable byproduct in autotrophic denitrification and metal bioleaching processes (Torres *et al.*, 1995; Tichy *et al.*, 1994)

The bacteria involved in sulfide oxidation belong to a group of colorless sulfur bacteria, of which *Thiobacillus* is the best known. *Thiobacillus* is mostly facultative autotrophic, utilizing reduced inorganic sulfur compounds, e.g. sulfide, elemental sulfur, thiosulfate and polythionite as electron donors and carbon dioxide as a carbon source (Şengül and Müezzìnoġlu, 1991; Janssen *et al.*, 1997). The best growth is reported at 25-35° C and pH of 8.0 - 8.5 (Buisman *et al.*, 1989). The two major bio-chemical reactions of sulfide oxidation are given by:

$$2\,HS^- + O_2 = 2\,S^0 + 2\,OH^-$$
$$\Delta G^0 = -169.35\ \text{kJ/mol}\ HS^- \qquad (1)$$

$$2\,HS^- + 4\,O_2 = 2\,SO_4^{2-} + 2\,H^+$$
$$\Delta G^0 = -732.58\ \text{kJ/mol}\ HS^- \qquad (2)$$

In a suspended growth bioreactor, under oxygen limiting conditions that is oxygen concentration below 0.1 mg/L sulfur is the major end product, whereas sulfate is mainly formed under sulfide limiting circumstances where oxygen/sulfide ratio is greater than 1.0 (Janssen *et al.*, 1995). The researchers also reported that the oxygen/sulfide ratio of about 0.7 is the optimum for maximum sulfur production.

Studied showed that as the sulfide level increased, the chemical oxidation started to predominate over biological one. For example, the biological sulfide oxidation rate was 75 times faster than the chemical non-catalyzed oxidation rate at a sulfide concentration of 10 mg/L, but with the increase of sulfide concentration to 100mg/L, the biological oxidation rate was only 7 times faster than the chemical one (Buisman *et al.*, 1990a). This apparently indicates that when the bioreactor is overloaded or biological activity is limited, auto-oxidation of sulfide predominates. Under such circumstances, significant amount of thiosulfate instead of sulfur formation takes place (Janssen *et al.*, 1997).

The use of fixed-film reactor could eliminate the biomass limiting condition thereby facilitating the conversion of sulfide to predominantly elemental sulfur. Such strategy thus helps to improve the performance of sulfide oxidizing bioreactor. Different biomass support media have been used in sulfide oxidizing bioreactor including reticulated polyurethane (PUR) foam, reticulated polyurethane foam coated with polyvinyl chloride (PVC), PVC Rasschig ring, polypropene hiflow pall rings and polyethene bio-net (Buisman *et al.*, 1991). However, most if not all of these studies were conducted in a submerged media bioreactor such as Rotational Biological

Contact (RBC) Reactors, Completely Mixed Tank Reactors (CSTRs), Upflow biofilters, and Batch Reactors (Buisman *et al.*, 1990b; Janssen *et al.*, 1995), and the use of trickling filter bioreactor for sulfide oxidation has not been well investigated.

This study was therefore conducted to evaluate feasibility of biological sulfide oxidation in a trickling filter. The effects of different oxygen content in gas flow, gas direction and gas flow rate were also investigated. Batch tests were also conducted to quantify the relative contribution of biotic and abiotic components in total sulfide oxidation and to assess the possibility of heterotrophic sulfur reducing activity.

Materials and Methods

Reactor set-up

A bench-scale cylindrical Plexiglas™ reactor was fabricated in the Chemistry Machine Shop at Iowa State University. The reactor with 660 mm internal diameter and 3175 mm height (active volume = 1.0 L), was provided with a series of ports for mixer, influent inlet, effluent outlet, gas release and sample withdrawal. Figure 1 shows the schematic of the experimental set-up. The experimental set-up included a bench-scale trickling filter, an automated pH controlled feed tank, two sulfide scrubbers, and influent and effluent tanks. The bioreactor was operated at room temperature of 22 ± 2ºC throughout the study.

Support media

The media used for supporting biomass growth was 16 mm. Flexirings (Koch Engineering Company, Witchita, KS). The support media provided a porosity of approximately 90% when filled in the reactor and a specific surface area of 98 m^2/m^3.

Seed inoculum and start-up

Activated sludge from the city of Marshalltown wastewater treatment plants was used as seed inocula, which had a volatile suspended solid concentration of 4,350 mg/L. The media was soaked into the seed sludge in which 10 mL of nutrient solution was added and the content was mixed intermittently for 3 days at 37ºC. Thereafter, the whole content was transferred into the biofilter reactor.

Initially the reactor was started with an influent sulfide concentration of 80 mg-S/L at a flow rate of 1.5L/hr. The gas flow rate through the biofilter was maintained at 0.2 L/min with 5% oxygen content in the gas phase. The sulfate, dissolved sulfide, pH in both influent and effluent and oxygen content in gas flow were measured on daily basis. Within five days of the operation, the system reached a quasi-steady state with sulfide removal efficiency of approximately 75%.

Substrate

The feed solution consisted of growth nutrients and synthetic sulfide solution as a source of sulfide. Growth nutrient solution contained 8 g/L of NH_4Cl, 2g/L of $MgSO_4 \cdot 7H_2O$, 5 g/L of KH_2PO_4, and 100 mL trace element solution, which was adopted from Vishniac and Santer (1957) (Table 1). In all the experiments, sulfide feed solution was prepared by dissolving $Na_2S \cdot 9H_2O$ in nanopure water to achieve a desired concentration of aqueous sulfide. For pH control, 0.5 M HCl was used. All the chemicals used for the preparation of synthetic wastewater were of analytical grade.

Bioreactor operation

The reactor was initially started with an influent sulfide concentration of 80 mg-S/L at a flow rate of 1.5L/hr. A gas flow of 0.2L/min containing 3% oxygen was maintained through the reactor. 5 mL nutrient solution was fed into the reactor everyday. The sulfide concentration was then increased to 120 mg-S/L. The oxygen content in the gas flow was varied from 3% to 10%. The desired oxygen content was achieved through online dilution of pure oxygen using nitrogen gas. The feed pH was maintained in the range of 8.0 to 8.4 by using a pH probe and a controller (TBI–Bailey Advantage Series TB84PH Analyzer). The sulfate, dissolved sulfide, thiosulfate, pH in both influent and effluent and oxygen content in gas flow were measured and monitored daily.

Batch tests on biotic/abiotic sulfide oxidation

Biotic/abiotic sulfide oxidation test was conducted in a 0.5-L, air-tight vessel shown in Figure 2 (Khanal, 2002). The vessel was filled with support media to mimic the actual bioreactor scenario. A magnetic stirrer was used for mixing. In batch test, both biotic (biological) and abiotic (chemical) sulfide oxidation rates were evaluated using support media with biofilm and without biofilm, respectively.

Experiments were conducted in three runs. Run 1 was designed to test the total (biological and chemical) sulfide oxidation rate. 300 mL of aqueous sulfide solution and 1 mL of nutrient solution were added into the vessel, and 34 supporting media transferred from the continuous biofilter reactor with biofilm placed in test vessel. In runs 2 and 3, the same amount of Na_2S solution and sterile plastic media were used. The difference between runs 2 and 3 was that there was no nutrient in the aqueous sulfide solution during run 3, but in run 2, 1 mL nutrient solution was added, so that the effect of nutrient supplementation on chemical sulfide oxidation rate and efficiency could be determined. All the Na_2S stock solutions were prepared by dissolving reagent grade $Na_2S{\cdot}9H_2O$ in nano pure water. Nutrient solution was same that of continuous experiment.

The initial pH was adjusted and maintained at 8.0 by using 0.5 M HCl and phosphate buffered system (0.08M Na_2HPO_4 + NaH_2PO_4) (Chen and Morris, 1972). The influent sulfide concentration was varied from 40 mg-S/L to 160 mg-S/L in all the three runs. The oxygen content (3%, 5%, or 8% balanced by N_2 gas) was monitored by flow meters and gas monitor, and gas flow rate was maintained at 0.2 L/min.

During the batch tests, samples were taken at certain time interval until no significant change in residual sulfide was observed. The dissolved sulfide, sulfate, thiosulfate, and pH of sample solution were measured.

Batch tests on heterotrophic sulfur reducing activity

In this batch experiment the sulfur reducing activity under heterotrophic conditions was investigated. In the test, serum bottles (250 mL) were used. Seed sludge was taken from the continuous experiment. The composition of the substrate used in the test is shown in Table 2 (Khanal, 2002). $NaHCO_3$ was used as buffer. 150 mL substrate was added into each bottle allowing 100 mL headspace. The sulfur particle taken from the biofilter was air dried and then was placed into the bottles with the concentrations of 0, 50, 100, 150, 200 and 400 mg-S/L. The pH was adjusted to 7.5 by addition of HCl solution. The bottles were purged with N_2 and capped tightly before being put on an incubator shaker running at 200 rpm at 37ºC. After 5 days, the dissolved sulfide of liquid samples was determined.

Analysis

Dissolved sulfide was determined by the Iodometric Method specified in *Standard Methods* (APHA *et al.*, 1998). Sulfate was analyzed by ion chromatograph [Dionex, model DX 500; Column: Metachem Technologies Inc. AN 300 (150 x 5.5 mm)], and the eluent was 3.5 mM sodium carbonate/1.0 mM sodium bicarbonate at a flow rate of 2 mL/min. Detection was made by a conductivity detector (Dionex

model CD 20). Thiosulfate was detected by this method, but with the eluent of 7.0 mM sodium carbonate/2.0 mM sodium bicarbonate at a flow rate of 1.5 mL/min.

Oxygen content was measured and controlled with a multiple gas detector (BW Defender model D4-2000). The pH of both influent and effluent were measured daily with an pH meter (Cole-Parmer model 05669-20), calibrated at room temperature with standard pH buffers of 7.0 and 10.0 routinely.

Results and Discussion

Effect of oxygen content and sulfide loading rate

Figure 3 summarizes the results of experiments conducted at different oxygen contents and sulfide loading rates. From the figure, it is apparent that at O_2 content of 3%, the sulfide removal efficiency was 85% at sulfide loading rate of 120 mg-S/L-hr. When oxygen content was increased to 5%, the sulfide removal efficiency increased to as high as 94%. With further increasing the oxygen content to 8%, the removal efficiency didn't improve significantly. However at a higher sulfide loading rate of 180 mg-S/L-hr, the sulfide removal efficiency reduced to as low as 50% at 5% of oxygen content. With further increase oxygen content to 8% and 10%, the remove efficiency was improved significantly. The respective removal efficiencies were 85% and 91%.

The poor sulfide removal efficiency at lower O_2 content was attributed to oxygen limiting condition. This is because at higher sulfide level, the available

oxygen was not enough to oxidize the sulfide. Thus, the optimal oxygen content was found to depend on sulfide loading rate. In this study, 5% and 10% oxygen contents were optimum for 120 and 180 mg-S/L-hr, respectively.

Figure 4 presents the effluent sulfide and sulfate concentrations for the biofilter operating at sulfide loading rate of 120 mg-S/L-hr (i.e., influent sulfide concentration of 80 mg-S/L and flow rate of 1.5 L/hr). The oxygen percentage was varied from 3% to 5% then to 8%, and the gas flow rate was maintained at 0.2 L/min. It was evident from the figure that the mean sulfide removal efficiencies were 86%, 94%, and 87%, respectively at O_2 content of 3%, 5%, and 8%. Thus, 5% oxygen content was found to be optimum for sulfide oxidation at influent sulfide concentration of 80 mg-S/L. The results also showed a concomitant increase in effluent sulfate levels. The increased level of sulfate in the effluent was at higher oxygen content was most likely contributed by biological conversion of sulfide to sulfate. Several researchers reported an optimum oxygen/sulfide (molar) ratio of 0.7 in a suspended growth system to prevent the formation of excess sulfate and at higher ratio, sulfate formation would become dominant (Buisman *et al.*, 1990; Janseen *et al.*, 1997). In this study the oxygen:sulfide (molar) ratio of varied from 4.3 to 11.4. However, the optimum ratio for biooxidation of sulfide in a trickling filter has so far been not reported. Abiotic oxidation of sulfide was also reported to contribute to effluent sulfate level (Chen and Morris, 1972). However, a series of abitioc batch oxidation study showed that such contribution was not significant (will be discussed later).

Effect of gas flow rate

The effect of gas flow rate on sulfide removal efficiency is shown in Figure 5. When gas flow rate was increased from 0.2 L/min to 0.4 L/min at constant oxygen content of 5%, sulfide removal efficiency dropped appreciably from 93.6% to 76.6%. The low sulfide removal efficiency at higher gas flow rate was possibly due to the low gas residence time (GRT). This is because at higher flow rate, the contact time between oxygen and attached biomass was insufficient for through sulfide oxidation. This finding was consistent with Ranade and Bhirangi (2001) who also observed improved H_2S removal efficiency at longer GRT. The effluent sulfate was found to decrease with increase in gas flow rate. This finding further substantiate that the bioreactor was operating under oxygen limiting condition. The operation of sulfide oxidizing bioreactor under oxygen limiting condition was found to favor sulfur formation instead of sulfate (Janssen *et al.*, 1997).

Effect of gas flow direction

During the continuous operation, the system was run at influent sulfide concentration of 80 mg/L, gas flow rate of 0.2 L/min with a direction from top to down (co-current with liquid flow), and oxygen content of 5%. After the system reached steady state (the steady study was believed to reach when effluent sulfide and sulfate levels did not vary more than 5% for 10 consecutive days of operation), the gas direction was then reversed to counter current to the liquid flow. Figure 6 shows

the performance of the biofilter before and after the change of gas flow direction. It was observed that after the gas direction was reversed, sulfide removal efficiency was improved from 94% to 96%, and the effluent sulfate concentration dropped slightly. Thus, the counter current direction of gas and liquid flows was most appropriate for effective sulfide oxidation. When the gas and liquid flows run in opposite directions, the contact time for both phases was longer. Most importantly, the liquid film resistance is greatly reduced and better oxygen transfer to the biofilm could be achieved. As a result, efficient sulfide oxidation was achieved compared to co-current direction. Therefore, in the following experiments, the biofilter was operated under counter current mode.

Sulfur mass balance

A sulfur mass balance was carried out during the continuous sulfide biooxidation experiment to quantify the distribution of sulfur species. The influent sulfide was considered to end up into the following components: a) effluent sulfate; b) effluent thiosulfate; c) residual sulfide in the effluent; and d) elemental sulfur. The sulfide consumed in biomass synthesis and/or metal precipitation was regarded as unaccounted fraction. All the species of sulfur were expressed as the sulfur equivalent. The elemental sulfur was not measured; instead it was estimated by subtracting the sum of (a), (b) and (c) from the influent sulfide concentration. This is because at pH of 8.0, the formation of polysulfides and thionates was insignificant (Janssen *et al.,* 1997). The sulfur balance result is summarized in Table 3 and the plot is shown in Figure 7. It was obvious that with the increase of oxygen content,

the effluent sulfide was decreased progressively with concomitant improvement in sulfide removal efficiencies from 50.7% to 85.1% then to 90.8% at oxygen percentage of 5%, 8%, and 10%, respectively. The major part of the influent sulfide recovered was elemental sulfur and thiosulfate, accounting respectively 49% and 33% of the total influent sulfide of 120 mg/L at the end of the experiment. The effluent sulfate also increased with the increase of the oxygen percentage, which was in close agreement with earlier studies on sulfide bio-oxidation (Buisman *et al.*, 1989, 1990b). Detection of high concentration of thiosulfate in the effluent apparently suggests a significant auto-oxidation of sulfide during oxygenation. The auto-oxidation may have taken place in the reactor within the void spaces where the sulfide could have come in contact with oxygen in absence of sufficient biomass.

Biological/chemical sulfide oxidation rate

A series of batch tests were conducted to evaluate the quantitative contribution of biological and chemical components on overall sulfide oxidation rate. The results of the batch tests are shown in Figure 8. The results apparently showed that biological sulfide oxidation rate was significantly higher than the chemical oxidation rate. The maximum biological oxidation rate was 811.8 mg-S/L-hr whereas chemical oxidation rates were just 112.2 mg-S/L-hr (with trace metals/nutrients) and 27.6 mg-S/L-hr (without trace metals/nutrients). The biological reaction rate was about 7 times faster than the chemical one at a sulfide concentration of 80 mg/L, which was close to the other researcher's results (Buisman *et al.*, 1990). The batch studies further suggest that biological sulfide oxidation could have contributed as

high as 88% of the total oxidation where as chemical oxidation could have contributed up to 12% of the total oxidation in the bioreactor. Moreover, the presence of trace metals and nutrients was found to have significant catalytic effect on chemical sulfide oxidation rate. This finding was in close agreement with that of O'Brian and Birkner (1977).

Figure 9 reflects the quantitative distribution of different sulfur components at various influent sulfide levels and different operation conditions. The elemental sulfur was estimated indirectly with the same method used in continuous experiment. It is observed that a considerable amount of thiosulfate was formed in the batch tests, which proves that abiotic sulfide oxidation played an important role in sulfide removal.

Heterotrophic sulfur reducing activity

In the real wastewater treatment system, sulfide oxidation becomes more complicated due to the presence of organic compounds, such as acetate and propionate. Because oxygen can only penetrate 150 to 200 μm into a biofilm (Hooijmans *et al.*, 1990; Wijffels et al., 1995), it is likely that an anaerobic circumstance prevails within the sulfur sludge and sulfur reducing bacteria may grow inside sulfur sludge particles in the presence of organic matter so as to reduce sulfur back to sulfide.

Widdel and Bak (1991) reported that the genus Desulfuromonas are able to use acetate as electron donor to reduce elemental sulfur to sulfide according to the following reaction:

$$CH_3COO^- + 4S + 4\ H_2O = 2\ HCO_3^- + 4H_2S + H^+ \quad (3)$$

In this batch test, black precipitate could be visually observed due to the formation of FeS (Figure 10). Figure 11 reflects the existence of sulfur reducing activity under different sulfur concentration. It is most likely due to sulfur accepts electron from organic compounds and was reduced to sulfide according to equation (3). It is shown that with the increase of sulfur concentration, the reduction activity was changed from first-order reaction to zero-order reaction.

Figure 12 shows that the sulfide removal efficiency of the biofilter decreased once acetate of 200mg/l was dosed into the system. The system was operated at influent sulfide concentration of 120 mg/L, gas flow rate of 0.2 L/min, and oxygen content of 10%. Only in 5 days, the removal efficiency dropped seriously from 91.7% to 78.1%. At the same time, abundant thiosulfate was produced as a major end-product. The sulfate level in effluent didn't change obviously. Buisman and Lettinga (1990) also found that the sulfide removal efficiency markedly decreased with the removal of acetate while treating anaerobic wastewater of a papermill. They also conducted an anaerobic batch test in which sulfide was detected while acetate and propionate were removed.

Conclusions

In this study, a trickling biofilter was developed to remove sulfide and convert it to elemental sulfur. It was observed that sulfide removal efficiencies of 93.6% and 90.8% were obtained at sulfide loading rates of 120 mg-S/L-hr and 180 mg-S/L-hr

respectively with O_2 contents of 5% and 10%. The results indicate that gas flow rate is an important parameter for the system performance. It was found that a flow rate of 0.2 L/min was favorable to the sulfide oxidation with a removal efficiency of 93.6%, whereas only 76.6% of sulfide was removed under a higher gas flow rate of 0.4 L/min.

A sulfide removal rate of 811.8 mg-S/L-hr was found in biological batch study at a sulfide concentration of 80 mg/l, whereas under the same conditions the rates of chemical oxidation were only 112.2 (with trace metal/nutrients) and 27.6 (without trace metal/nutrients) mg-S/L-hr. The biological rate was about 7.2 times faster than the chemical oxidation rate. It was suggest that as high as 88% of the influent sulfide could be oxidized biologically whereas abiotic oxidation could only contribute up to 12% of the total oxidation.

With the presence of organic compounds, the removal efficiency of the biofilter deteriorated most likely due to the existence of sulfur reduction, which was approved in the batch test on heterotrophic sulfur reducing activity. Except for the recovery of elemental sulfur, sulfate and thiosulfate were detected as end products most likely due to the chemical sulfide oxidation.

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Tables and Figures

Table 1. Trace element solution compositions

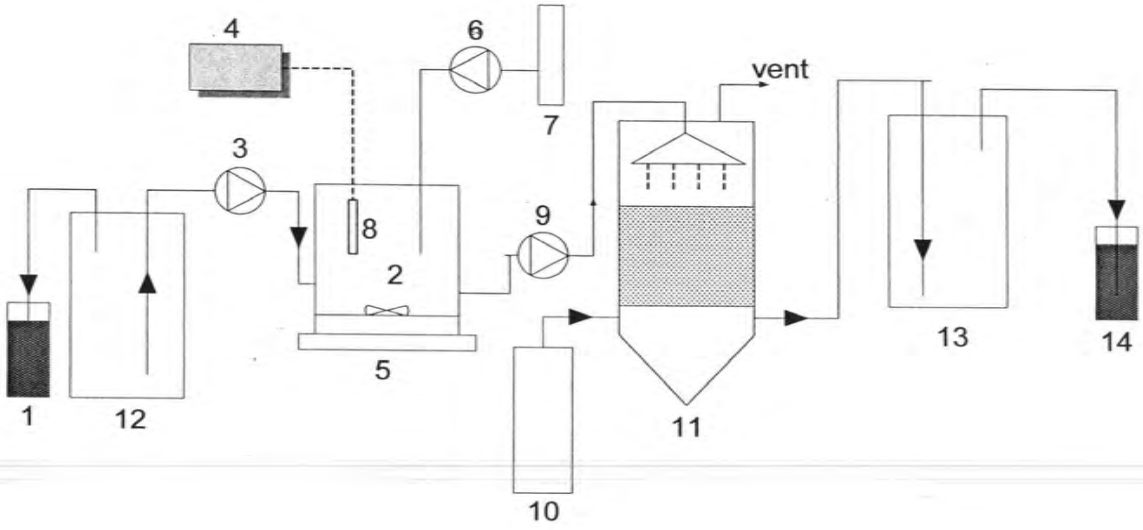
Constituents	Concentrations (g/L)
Ethylenediamine tetra-acetic acid	50.0
$ZnSO_4 \cdot 7H_2O$	22.0
$CaCl_2$	5.54
$MnCl_2 \cdot 4H_2O$	5.06
$FeSO_4 \cdot 7H_2O$	4.99
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	1.10
$CuSO4 \cdot 5H_2O$	1.57
$CoCl_2 \cdot 6H_2O$	1.61

Table 2. Substrate compositions (for 500 mg/l COD) for batch tests on heterotrophic sulfur reducing activity

Constituents	Concentrations (mg/L)
$C_6H_{12}O_6$	467.29
NH_4Cl	44.64
KH_2PO_4	4
K_2HPO_4	8.01
$MgCl_2 \cdot 6H_2O$	15
$CaCl_2$	20
$CoCl_2 \cdot 6H_2O$	0.28
$FeCl_3$	3.55
$NiSO_4 \cdot 6H_2O$	4
$CuSO_4 \cdot 5H_2O$	1.57
$NaHCO_3$	187.5

Table 3. Sulfur mass balance

Date	Influent Sulfide (mg-S/L)	$S_2O_3^{2-}$ (mg-S/L)	SO_4^{2-} (mg-S/L)	S^{2-} (mg-S/L)	S^0 (mg-S/L)	S^{2-} Removal Efficiency (%)
1	120	25.76	5.22	87.99	1.03	26.68
2	120	44.92	7.28	62.68	5.12	47.77
3	120	32.36	7.73	75.21	4.70	37.33
4	120	34.61	6.70	74.00	4.69	38.33
5	120	37.67	7.39	68.57	6.37	42.86
6	120	45.74	5.33	63.63	5.30	46.98
7	120	43.49	2.48	59.16	14.87	50.70
8	120	41.92	3.30	52.87	21.91	55.94
9	120	40.06	4.96	45.70	29.28	61.92
10	120	40.30	9.70	34.97	35.03	70.86
11	120	46.54	12.31	29.41	31.74	75.49
12	120	56.07	11.24	27.34	25.35	77.22
13	120	46.91	10.73	25.11	37.25	79.08
14	120	45.43	11.03	23.58	39.96	80.35
15	120	60.08	10.75	21.77	27.40	81.86
16	120	63.66	11.94	20.69	23.71	82.76
17	120	64.38	10.79	19.20	25.63	84.00
18	120	47.64	13.22	17.87	41.27	85.11
19	120	64.34	14.19	15.36	26.11	87.20
20	120	51.17	16.25	14.20	38.38	88.17
21	120	49.36	13.85	14.77	42.02	87.69
22	120	49.17	14.15	14.06	42.62	88.28
23	120	40.93	16.59	15.83	46.65	86.81
24	120	47.55	16.10	13.97	42.38	88.36
25	120	39.79	16.42	12.75	51.04	89.38
26	120	38.82	15.11	11.82	54.25	90.15
27	120	38.15	13.46	11.46	56.93	90.45
28	120	39.70	12.03	11.80	56.47	90.17
29	120	39.61	10.77	11.02	58.60	90.82



1,14. Sulfide Scrubbers 2. pH Controlled Feeding Tank 3, 9. Feeding pump
4. pH Controller 5. Magnetic Stirrer 6. HCl dosage pump 7. HCl tank
8. pH probe 10. Gas feed tanks 11. Reactor 12. Influent Tank 13. Effluent tank

Figure 1. Schematic of continuous biofilter reactor

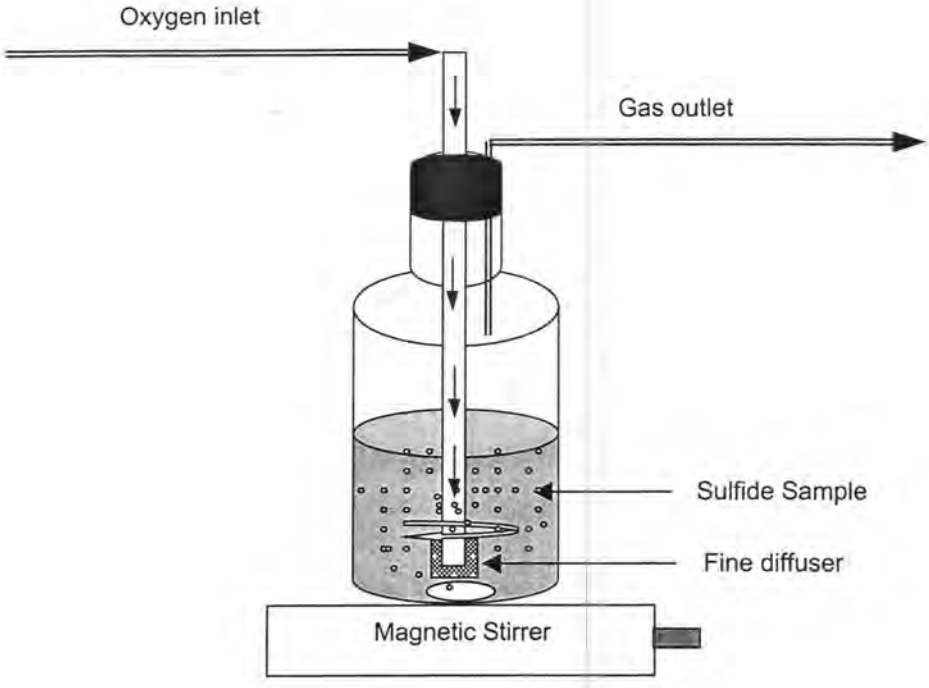


Figure 2. Completed mixed reactor for batch studies on biotic/abiotic sulfide oxidation

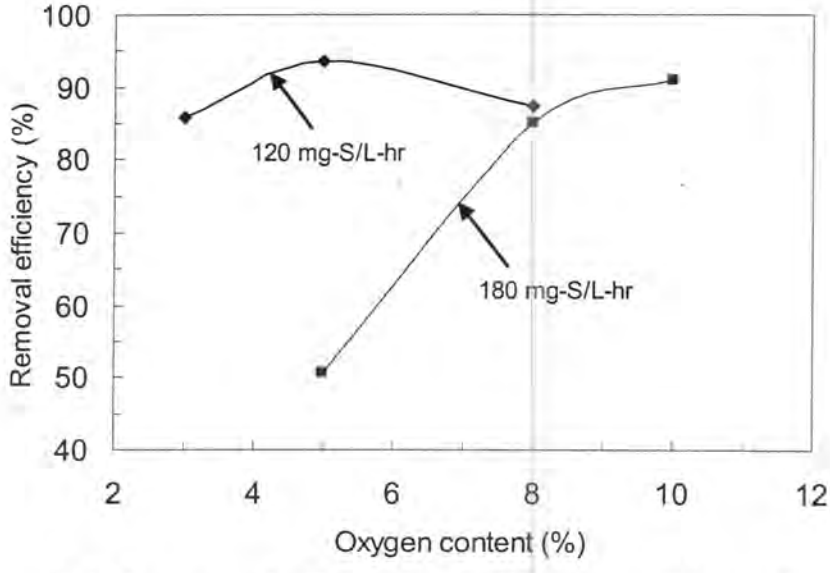


Figure 3. Sulfide removal efficiencies at different sulfide loading rates and oxygen contents

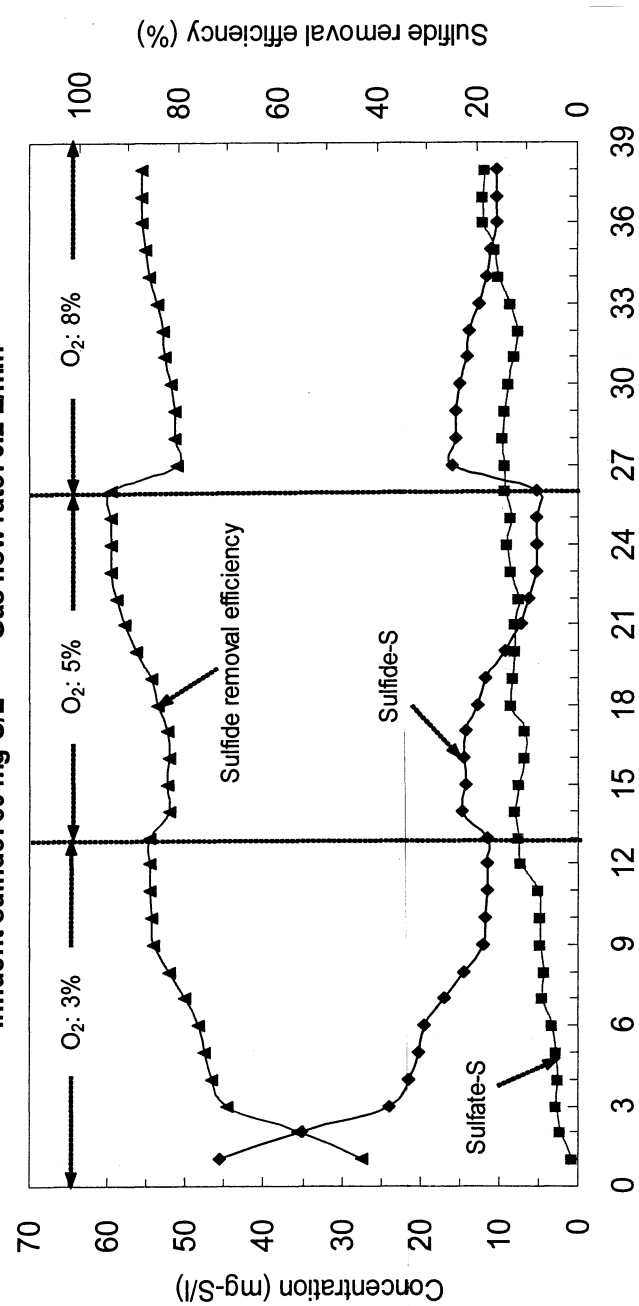


Figure 4. Effluent sulfides and sulfates at different oxygen contents

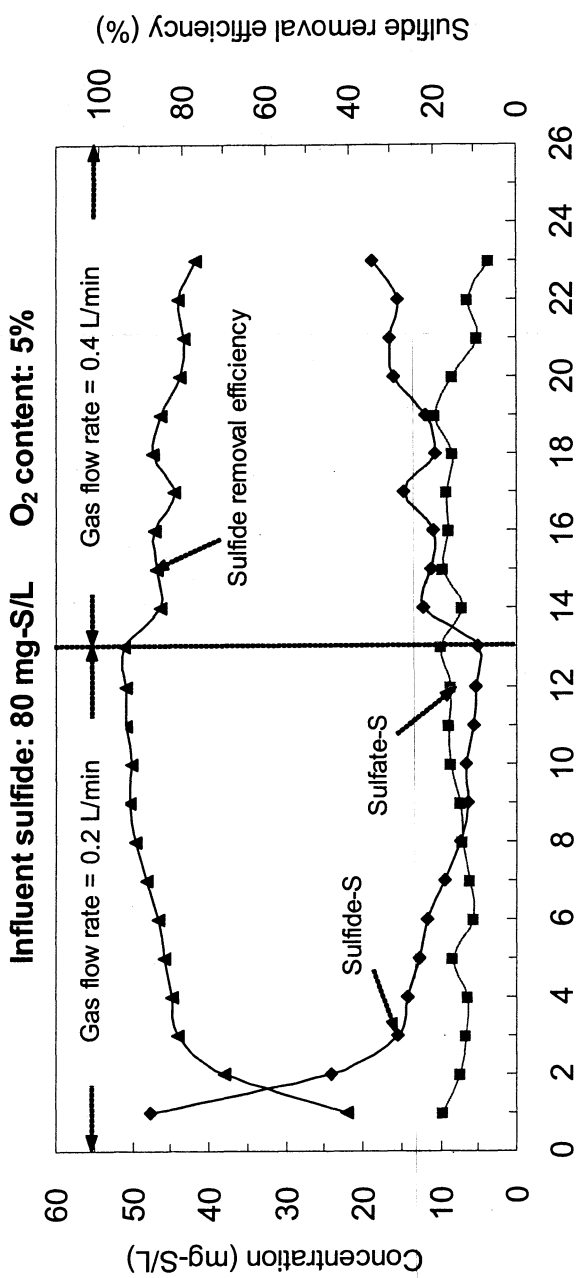


Figure 5. Effluent sulfides and sulfates at different gas flow rates

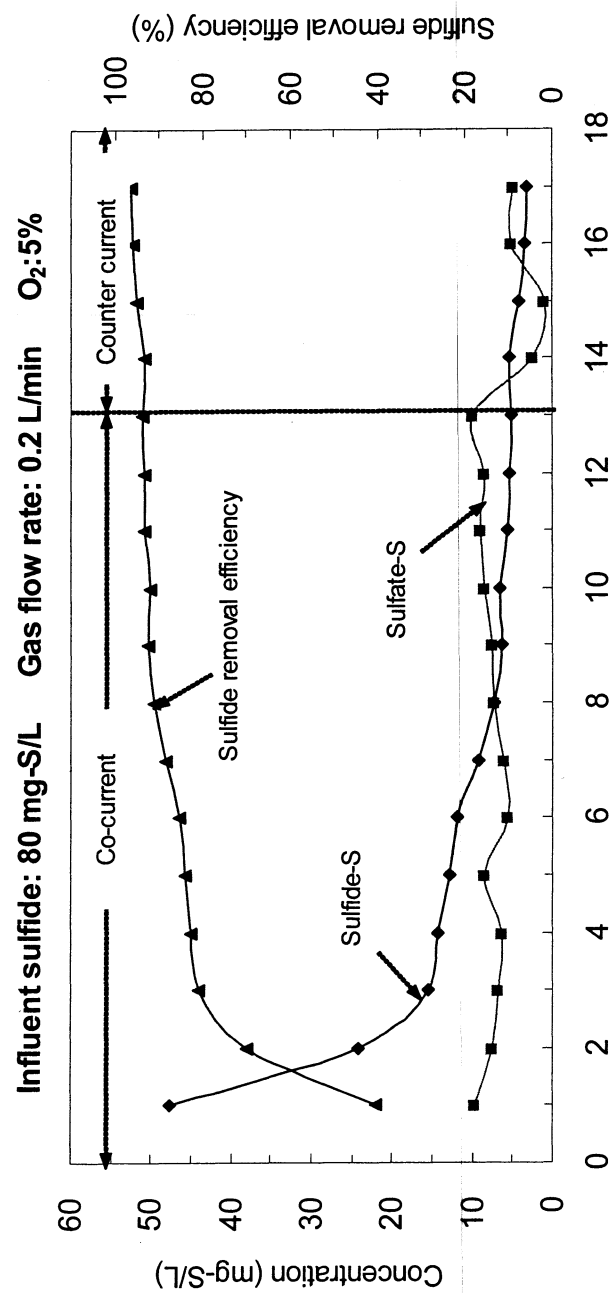


Figure 6. Effluent sulfides and sulfates at different gas flow direction.

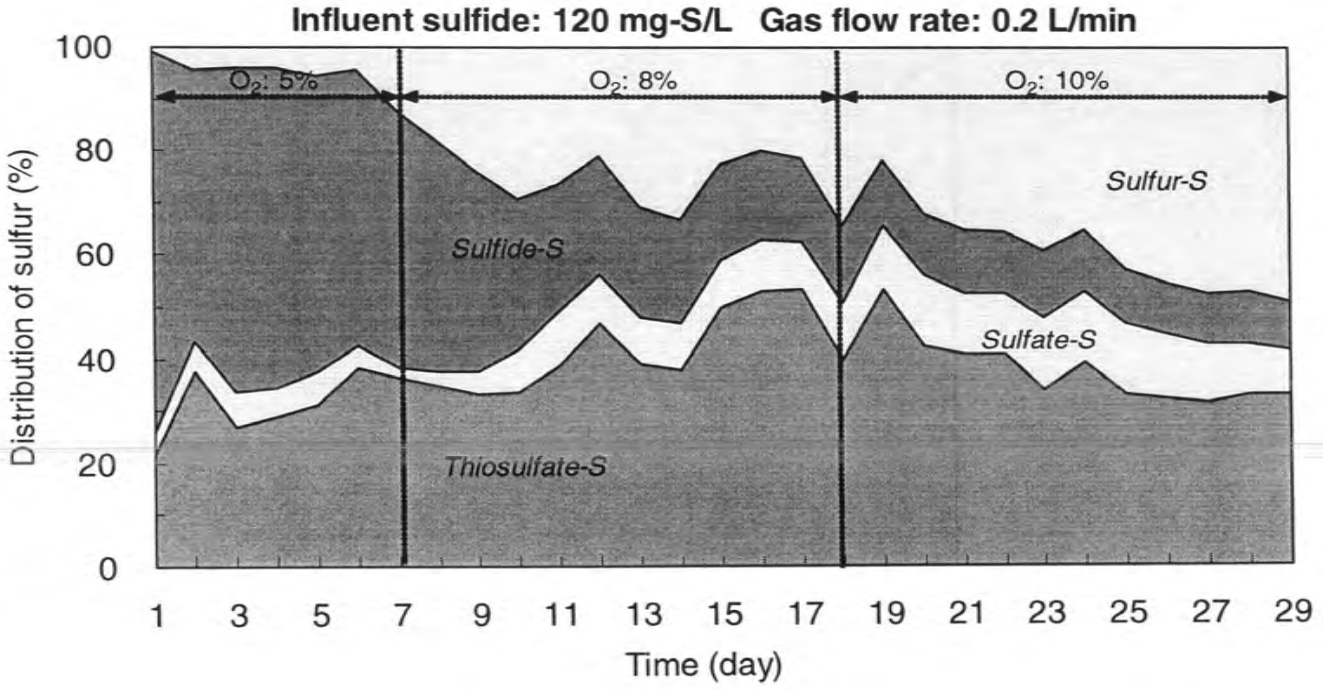


Figure 7. Sulfur mass balance at different oxygen contents

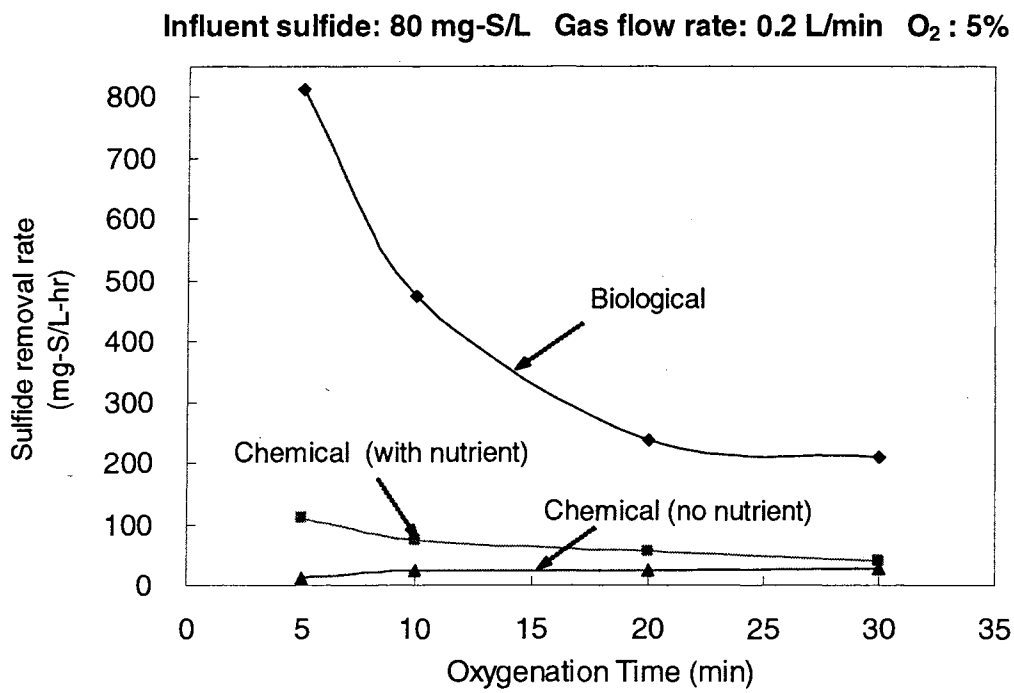


Figure 8. Biotic/abiotic sulfide oxidation in batch studies

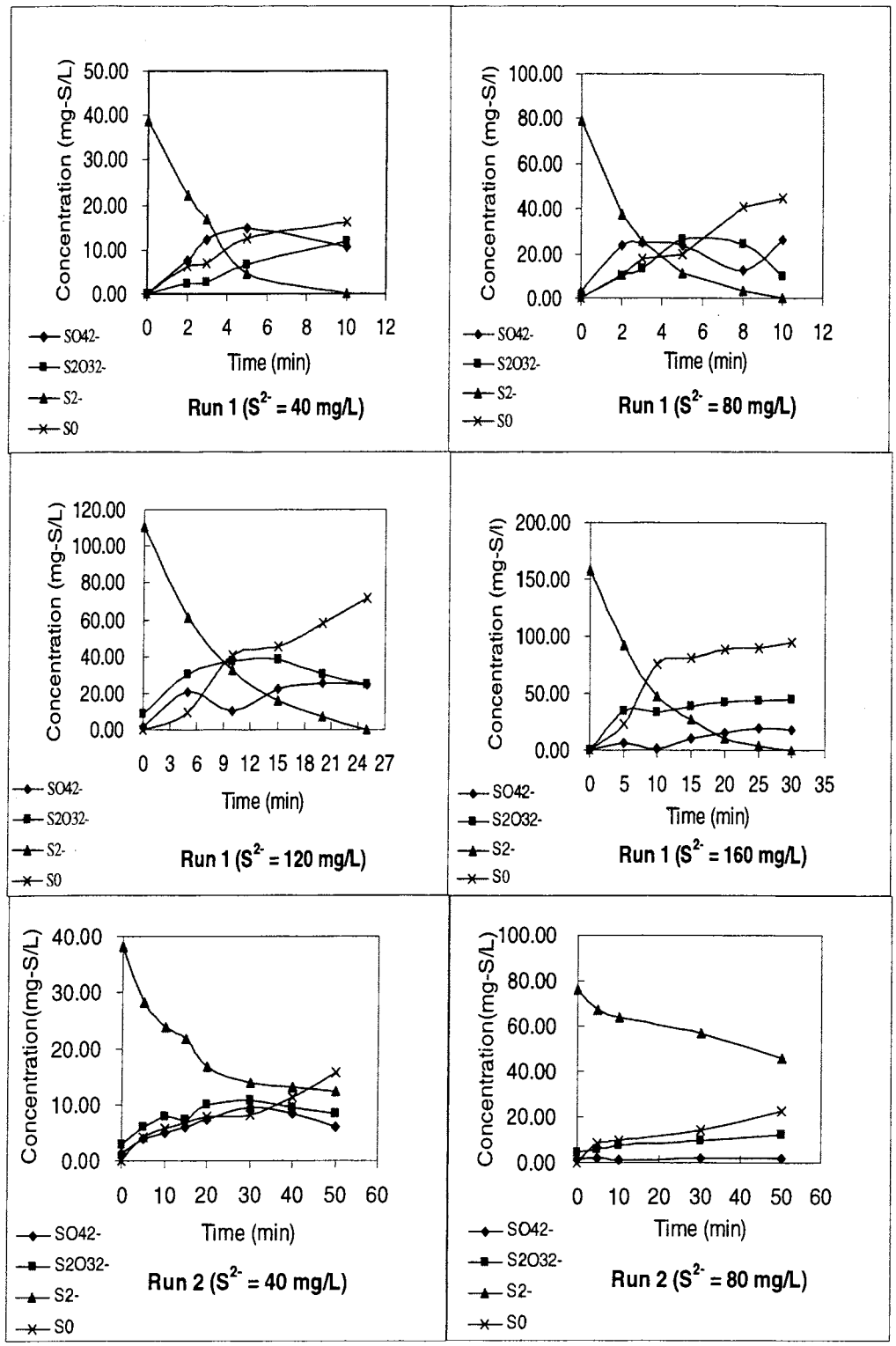


Figure 9. Quantitative distribution of sulfur components in biotic/abiotic sulfide oxidation batch studies

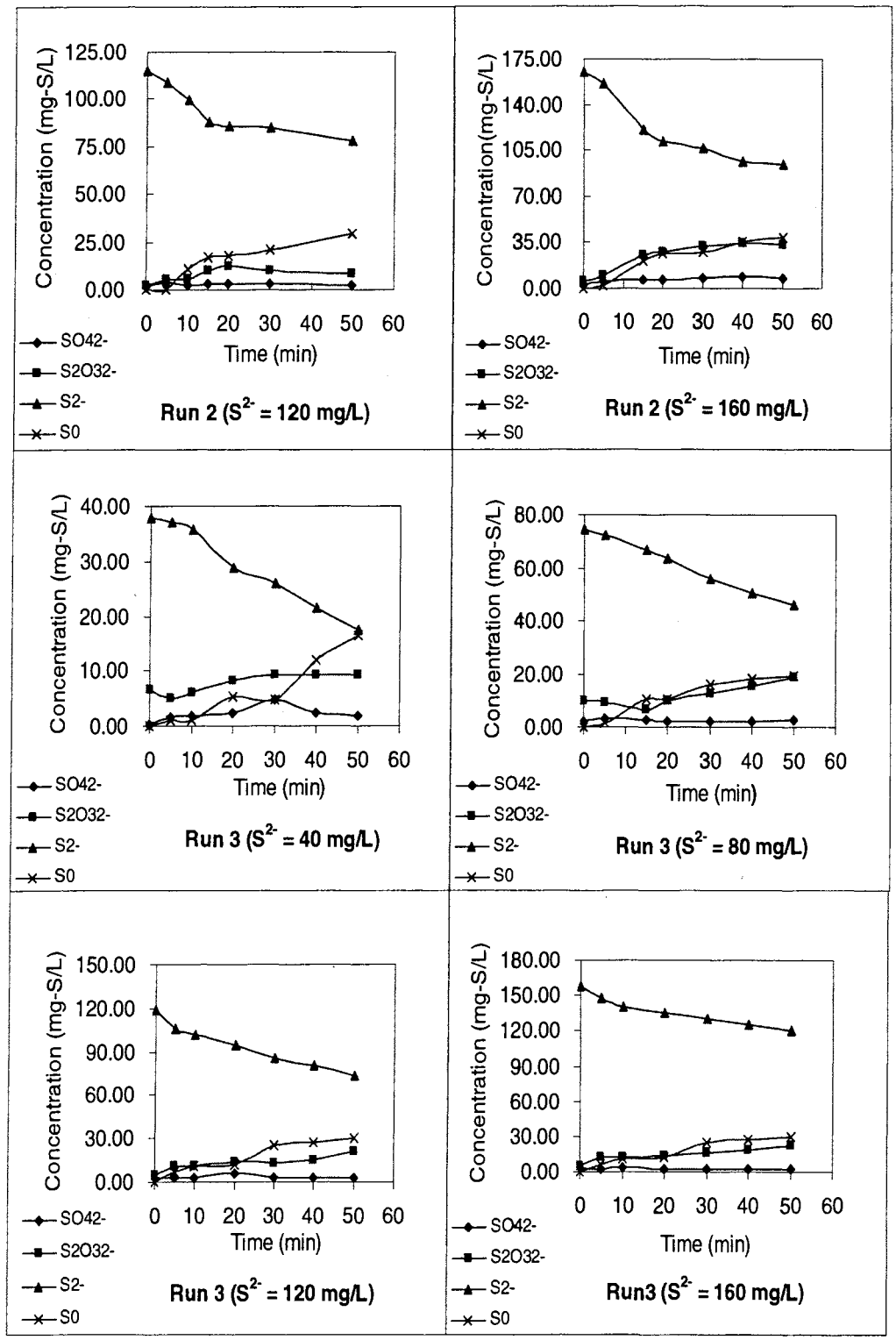


Figure 9. Quantitative distribution of sulfur components in biotic/abiotic sulfide oxidation batch studies (Contd.)

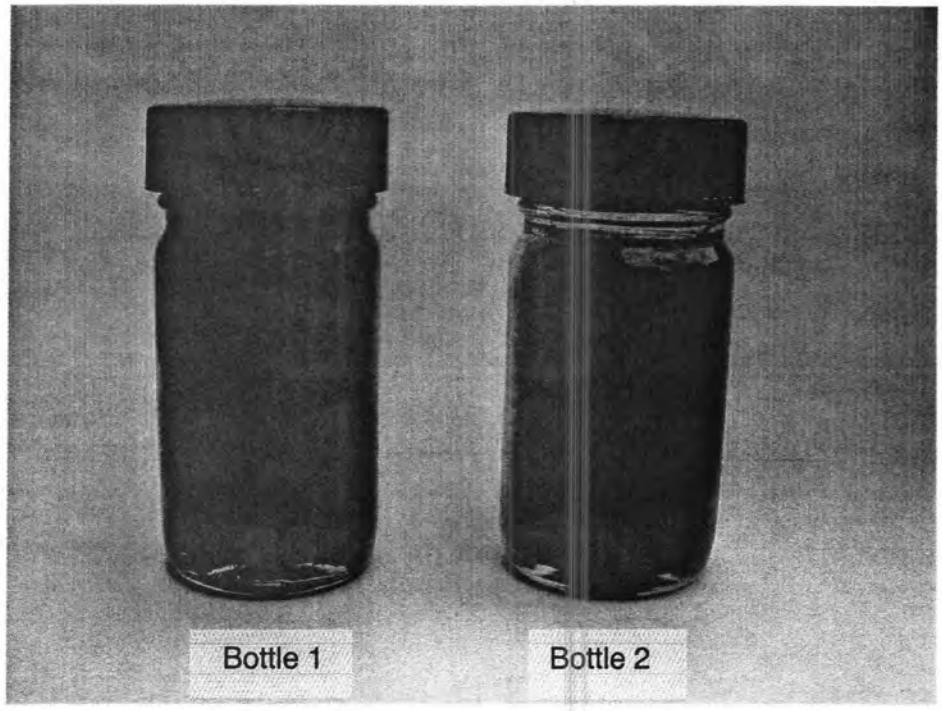


Figure 10. Heterotrophic sulfur reducing activity. Bottle 1 contained the sample of 200 mg-S/L. Bottle 2 was the blank sample.

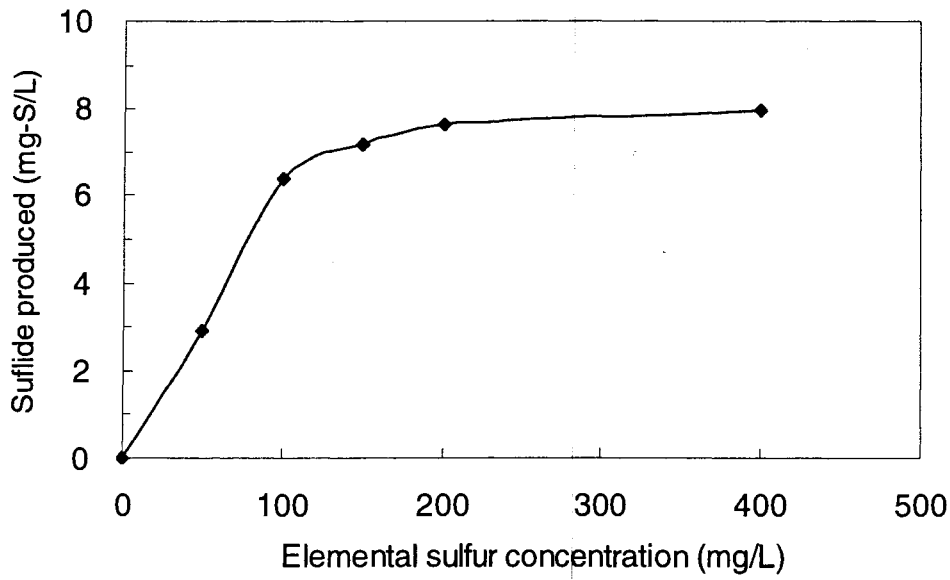


Figure 11. Sulfur reducing activity

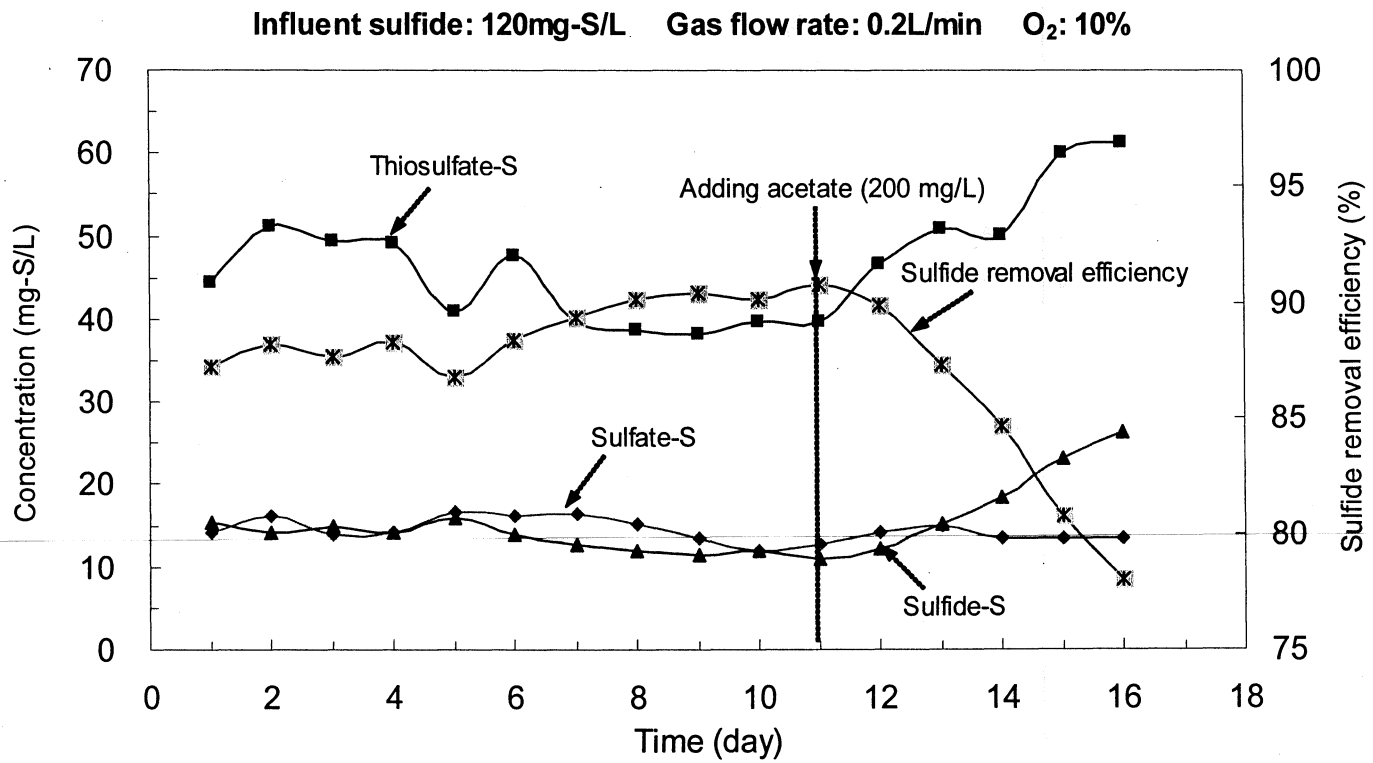


Figure 12. Effluent sulfides, sulfates & thiosulfates before and after adding acetate

CHAPTER 4. GENERAL CONCLUSIONS

4.1 Engineering Significance

In this study, a fixed-film trickling filter was developed and its suitability for biological sulfide removal was investigated. Different operation parameters were examined to optimize the bioconversion of sulfide to elemental sulfur.

The City of Cedar Rapids Water Pollution Control has constructed a new anaerobic pretreatment facility treating high strength industrial wastes. This facility employs a chemical-scrubbing unit followed by a Continuous Stirred Tank Reactor (CSTR) for biological sulfide removal. The capital investments required for these proprietary systems are exorbitant.

The biofilter reactor, as proposed in this study, may have several advantages over the CSTR system. The use of fixed-film reactor eliminates the biomass limiting condition thereby facilitating the rapid conversion of sulfide to predominantly elemental sulfur and thus improves the performance of sulfide oxidizing bioreactor. Capital savings in the form of smaller reactor volumes, elimination of aeration blowers, and fewer solids settling tanks can make the biofilter design an economically attractive option. The primary end product sulfur can be used as fertilizer, feed substrate for recuperating heavy metal contaminated sludge or electron donor for autotrophic denitrification. With these

potential economic and operative advantages, biological sulfide removal may find full-scale applications in future.

4.2 Summary and Conclusions

A trickling biofilter was developed and investigated to oxidize sulfide to elemental sulfur. Different sulfide loading rates, oxygen contents in gas flow, and gas flow rates were evaluated in this study. The relative contribution of biotic and abiotic components in total sulfide oxidation and the possibility of heterotrophic sulfur reducing activity were also evaluated. Based on this study, the following conclusions were drawn:

1. Sulfide removal efficiencies of 93.6% and 90.8% were obtained at sulfide loading rates of 120 mg-S/L-hr and 180 mg-S/L-hr, respectively, with an O_2 content of 5% and 10%.

2. Gas flow rate was found to be an important parameter for the system performance. It was found that sulfide was removed 93.6% at a flow rate of 0.2 L/min, however with the increase of gas flow to 0.4 L/min, sulfide removal efficiency dropped significantly to 76.60%.

3. The batch test results suggest that as high as 88% of the influent sulfide could be oxidized biologically whereas abiotic oxidation could only contribute up to 12% of the total oxidation. The corresponding

sulfide removal rates were 811.8 mg-S/L-hr and 112.2 mg-S/L-hr respectively at sulfide concentration of 80 mg/L. The biological oxidation rate was about 7.2 times faster than the chemical rate.

4. With the presence of organic compounds, the sulfide removal efficiency of the biofilter dropped from 91.7% to 78.1% only in five days. It was most likely due to the existence of sulfur reduction, which was approved in the batch test on heterotrophic sulfur reducing activity.

5. Except for the recovery of elemental sulfur, sulfate and thiosulfate were detected as end products most likely due to the chemical sulfide auto-oxidation.

4.3 Recommendations for Future Study

In this study, most of the parameters affecting biological sulfide oxidation have been investigated and effort has been made to clarify as many hypotheses as possible within the limited time of the study. However, some hypotheses require further study. Recommendations for future study are listed as follows:

1. Other than synthetic wastewater, feasibility of biological sulfide oxidation in the trickling filter from real wastewaters should be tested.

2. At the terminal stage of continuous experiments, acetate as organic substrate was added into the feeding water to evaluate the impact of organic compound to the system. It was found that sulfide removal efficiency dropped obviously, and the situation became more complicated. The effect of organic compound to the sulfide oxidation should be further investigated.

3. A great amount of elemental sulfur was formed during the sulfide removal process, thus the potential for recovery and reuse of the sulfur should be further studied.

4. Batch test results show the potential of heterotrophic sulfur reducing activity. It can be concluded that part of the formed sulfur attached to the wall of the biofilter reactor and support media could be reduced to sulfide again under anaerobic circumstance. Further study is needed to quantify the heterotrophic sulfur reducing activity in the biofilter reactor.

5. This study mainly focused on aqueous sulfide oxidation, further investigation is needed to study the feasibility of gaseous sulfide oxidation by integrating the sulfide oxidizing bio-reactor with scrubbing unit.

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