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**Biological Removal of Sulfide by the Intermittent
Contact Oxidation Process**

間欠接触酸化法による硫化物の生物学的除去

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LIST OF ABBREVIATIONS

ICOP	Intermittent contact oxidation process
SRB	Sulfate-reducing bacteria
SOB	Sulfur-oxidizing bacteria
BOM	Biodegradable organic matter
SOM	Sulfide-oxidizing bacteria
NR-SOB	Nitrate-reducing, sulfide-oxidizing bacteria
DO	Dissolved oxygen
COD	Chemical oxygen demand
MFCs	Microbial fuel cells
PADMA	P-Aminodimethylaniline solution
NTU's	Nephelometric Turbidity Units

LIST OF UNIT MEASUREMENTS

°C	degree Celsius
d	day
h	hour
L	liter
mL	milliliter
min	minute
g	gram
mg	milligram
m	meter

ABSTRACT

The aim of this study is to evaluate the sulfide removal performance during the intermittent contact oxidation process (ICOP). The discussion is organized in the following way: introduction, literature review, followed by methodology, after that, results and discussion, and finally, conclusions.

The literature review gives a brief summary of sulfur related topics including sulfur compounds, sulfur cycles, sulfide species, the existence forms of dissolved sulfide, and problems caused by hydrogen sulfide gas and sulfide species. Then specifically focuses on the following issues: sulfide sources in sewage, sulfide potential effects on sewers and sewage treatment plant and existing sulfide treatment technologies. Finally, the ICOP is proposed as an alternative method for sulfide-containing wastewater treatment.

During the ICOP, microbial growth and retention are promoted by installing porous media like sponge inside a sewer pipe as habitat. And by utilizing the intermittency of wastewater, sulfide is exposed to headspace air in the sewer pipe and then be aerobically degraded. To explore the sulfide removal performance by ICOP, synthetic feed containing 40 –120mg L⁻¹ of sulfide-sulfur was supplied to a laboratory-scale reactor incorporating the ICOP. First, the sulfide removal rate with different sulfide supply was evaluated. Then, the sulfide transformation during the ICOP was investigated.

The results showed that the maximum sulfide removal rate can up to 29.7gS m⁻² d⁻¹ by ICOP with initial sulfide concentration of 120mgS L⁻¹. And the conversion of removed sulfide consists of intermediate sulfur products and sulfate.

Chapter 1 INTRODUCTION

1.1 Research background

Sulfide can be found in sewage, anaerobic digesters, and some industries[1]. While sulfide exists in water or in solids, it reacts with protons and forms hydrogen sulfide gas. Sulfide and hydrogen sulfide are problematic because of 1) its malodor, 2) corrosion related with the oxidation of hydrogen sulfide precipitated on surfaces of structures, and 3) toxicity to human, and 4) toxicity to microorganisms treating wastewater. For malodor, hydrogen sulfide gas has rotten-egg smell even at 1ppm (1.4mg m^{-3})[2]–[4]. Moreover, hydrogen sulfide gas can cause corrosion of concrete and steel, in detail, it precipitates on their surfaces, oxidized by sulfur oxidizing microorganisms to form sulfuric acid, and this strong acid corrodes concrete and steel[5], [6]. The corrosion related with hydrogen sulfide is one of the main causes of the aging damage of transportation pipelines and processing facilities for sewerage works. Further, exposure to hydrogen sulfide gas can cause nausea, headaches, irritation, and unconsciousness for workers who work in environments with potential existence of hydrogen sulfide[4]. And finally, sulfide species in water especially in the form of hydrogen sulfide can inhibit oxygen utilization and negatively affect removal of organic pollutants.

Conventional chemical and biological technologies to treat sulfide from wastewater streams can be divided into two categories: one is preventive method to inhibit hydrogen sulfide formation, another is removal measure to eliminate produced hydrogen sulfide. Preventive method contains inhibition of SRB activity and increasing redox potential. Removal measures widely used are air stripping, heavy metal addition (Fe or Mn), addition of oxidizing chemicals and addition of nitrate.

Preventive methods are as follows. Inhibition of SRB activity can be achieved by pH elevation, but continuous addition of chemicals like sodium hydroxide (NaOH) to maintain an elevated pH (more than 9) is expensive and may disrupt downstream treatment processes[7]. Increasing redox conditions involves addition of nitrate and oxygen (or air) injection. Addition of nitrate forms anoxic condition and diminish H_2S formation. However, the high load of nitrate reduces COD load in wastewater. Regarding oxygen injection, it can not only inhibit dissolved sulfide occurrence by increasing dissolved oxygen concentration to prevent anaerobic conditions but also oxidize the produced sulfide in the wastewater. However, the restrictive factors are limited oxygen transfer, high energy requirements and H_2S gas emission.

For removal measure, air stripping is a technique in which wastewater and air are intensively brought in contact with each other. However, in this way, hydrogen sulfide gas emits from water phase to air phase, which cause worse corrosion. Further,

the air containing hydrogen sulfide must be treated in an air treatment system. Regarding heavy metal addition, the chemicals are expensive and precipitated sulfide with added heavy metals needs to be handled as sludge. Addition of oxidizing chemicals such as chlorine, hydrogen peroxide, and potassium permanganate can oxidize sulfide to sulfate or to elemental sulfur. However, chlorine's efficiency is limited due to its nature to react not only with sulfide but also with various organic and inorganic compounds in sewage. Moreover, the lifespan of hydrogen peroxide is too short to achieve the desired sulfide removal efficiency. For potassium permanganate, the application is costly. Nitrate injection was also found to stimulate growth of nitrate-reducing and sulfide-oxidizing bacteria (NR-SOB) which can easily oxidize hydrogen sulfide produced by sulfate-reducing bacteria (SRB) by encouraging anoxic activity rather than anaerobic[7]. However, limitations exist including possible negative effect on wastewater treatment plant because of nitrate load, high price and greater chemical consumption for the use of nitrate in large systems. In addition to these conventional ways, technologies such as slow-release solid-phase oxygen ($\text{MgO}_2/\text{CaO}_2$) and microbial fuel cells (MFCs) were also investigated.

In brief, for the conventional chemical sulfide removal methods, there are limitations such as high energy and maintenance requirements, large investment for chemicals and possible harm to sewage treatment plant. Biological technologies have several advantages: 1) no or less chemical compound that is harmful for the environment is used, 2) the generated sludge is less harmful when compared with solids generated from chemical methods, and 3) a wide range of microorganisms is capable of removing hydrogen sulfide. However, for sulfide removal, oxygen supply to achieve aerobic condition in sewage is the key point. As a result, microbiological methods with more feasible and sustainable oxygen supply are necessary to treat sulfide-containing wastewater.

Intermittent contact oxidation process (ICOP) maybe a promising biological technology to remove sulfide from wastewater by installing sponge inside a sewer pipe for the following three merits:

1. Reduced chemical species including sulfide would be entrapped in sponge, then oxidized when the sponge is exposed to air.
2. As water is not bubbled to supply oxygen, emission of H_2S gas is significantly less.
3. With no external energy demand.

Specifically, intermittent contact oxidation process (ICOP) is an enhanced self-purification method in sewer pipes[8]–[10]. In-sewer purification is a natural process where organic pollutants are degraded by microbial activity as it flows through the sewer pipe[11], [12]. As a result, in the ICOP, biomass growth and retention are enhanced by installing porous media like sponge inside a sewer pipe as habitat. And by

utilizing the intermittency of wastewater, when there is high flow, organic pollutants are supplied to microorganisms as feed, and when the flow is low or stopped, organic pollutants, and most probably sulfide also, are aerobically degraded through media exposure to oxygen in sewer atmosphere[12], [13].

Therefore, in this study, the ICOP was investigated as an alternative method to treat sulfide-containing wastewater in sewer pipe transportation process and sewage treatment plants.

1.2 Research Objectives

To date, performance of ICOP for sulfide removal is still unknown. Here, this study set out to evaluate sulfide removal performance during the ICOP. The specific objectives of this study are broken into three parts,

The first specific objective is to evaluate the sulfide removal rate by ICOP. This can be achieved by monitoring the change of sulfide concentration with different sulfide input in synthetic sewage. The range of sulfide concentration was selected based on the typical concentration found in domestic sewage and industrial wastewater.

The second objective is to determine the sulfide transformation process. This can be done by monitoring the changes in concentration of intermediate sulfur species and the end product and making a sulfur mass balance.

1.3 Thesis Structure

The overall structure of this thesis takes the form of five chapters as below:

Chapter 1 briefly introduce this study, including the research background, objectives, and thesis structure.

Chapter 2 gives a related literature review about sulfur, sulfide species, sulfide in sewer and in wastewater treatment plant and some existing technologies to remove sulfide. Moreover, sulfide removal by ICOP and its possible merits are also explained.

Chapter 3 describes the methodology of the experiment. In more detail, this chapter is subdivided into 4 sections including reactor setup, reactor operation, control experiment without sponge and analytical methods.

Chapter 4 explains the results and relevant discussions found in this study.

Chapter 5 summarizes the main findings and limitations of this study.

Chapter 2 LITERATURE REVIEW

The aim of the chapter is to present a review of sulfur cycle and sewer process. The structure of this chapter is as follows. Firstly, sulfur related topics including sulfur compounds and sulfur cycles are described (Section 2.1). Then, in Section 2.2, sulfide species including the existence forms of dissolved sulfide and problems caused by hydrogen sulfide and other sulfide species are explained. Following that, sulfide in sewer and sewage treatment plant are extensively reviewed in detail (Section 2.3). After that, Section 2.4 summarizes the existing sulfide removal technologies. At last, in Section 2.5 and 2.6, ICOP and its merits for sulfide removal are explained, respectively.

2.1 Sulfur

The element sulfur, S, as the sixth most abundant element in biomass, accounts for about 1% of the dry weight of microorganisms[14]. It is crucially significant for life and many essential biological and chemical reactions. Sewage and wastewater environments, which are the focus of this study, also contain sulfur in relatively high concentrations. In this section, inorganic sulfur species are focused, because biological removal of sulfide, the main topic of this study, includes inorganic sulfur species mainly.

2.1.1 Sulfur compounds

Although sulfur exists in an extraordinarily large number of oxidation states (**Table 2.1**), ranging from -2 (most reduced form) to +6 (most oxidized form), only three are abundant in nature and involved in most related reactions, which are -2 in the form of sulfhydryl (R-SH) and sulfide (S^{2-} , HS^- and $H_2S(aq)$), 0 in the form of elemental sulfur (S^0), and +6 in the form of sulfate (SO_4^{2-}) [15].

Table 2.1 Oxidation states of key sulfur compounds (modified from Canfield et al. 2012[16])

Compound	Oxidation state of S atom
Sulfide (S^{2-} , HS^- and H_2S)	-2
Organic S(R-SH)	-2
Elemental sulfur (S^0)	0
Thiosulfate ($S_2O_3^{2-}$)	+2

Dithionite	+3
Sulfur dioxide	+4
Sulfite (SO_3^{2-})	+4
Dithionate	+5
Sulfate (SO_4^{2-})	+6

2.1.2 Sulfur cycle

In sewer pipes and sewage treatment plants, complex microbial sulfur processes occur. Under anaerobic condition, sulfide is generated by sulfate reduction or organic sulfur compounds decomposition[16]. It's then oxidized to intermediate sulfur species, including elemental sulfur, thiosulfate, and sulfite, which are all substrates for further microbial oxidation, reduction or disproportionation[17]–[19]. The combination of these processes results in sulfur cycle as shown in **Figure 2.1**. The major reactions related to sulfur cycle are as below:

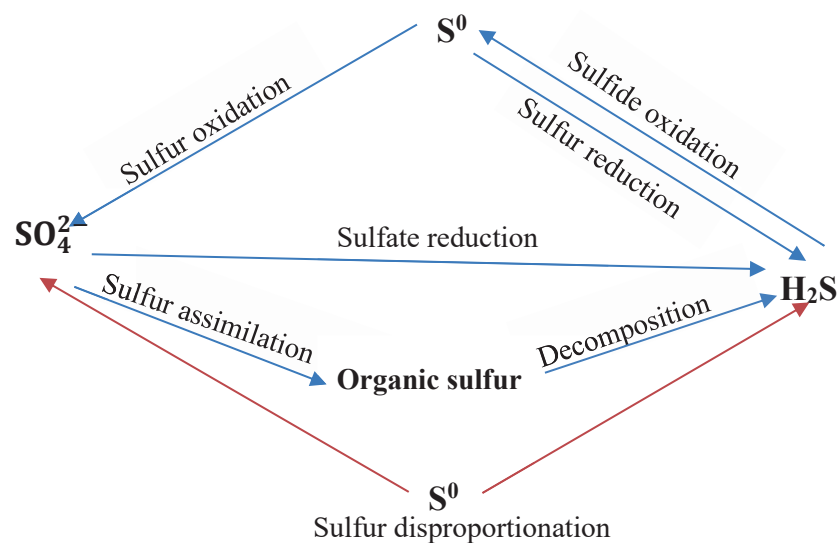
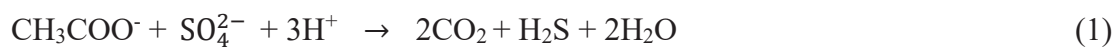


Figure 2.1 Redox cycle of sulfur (modified from Canfield et al. 2012[16])

Sulfate reduction

Under anaerobic conditions, sulfate-reducing bacteria (SRB) use sulfate as the terminal electron acceptor in the degradation of organic compounds, which results in hydrogen sulfide (H_2S) generation[3]. Sulfate-reducing bacteria are widely distributed in anaerobic environment and initiate the sulfur cycle as a primary H_2S producer[20].

Sulfate reduction is always accompanied by the reduction of chemicals like organic matters[21]. For example, when acetate is utilized in sulfate reduction, the reaction is expressed as Eqs.(1):



Sulfide oxidation to elemental sulfur

Sulfide can be oxidized both aerobically and anaerobically to elemental sulfur (S^0). Under aerobic conditions, sulfide oxidation can be rapidly catalyzed by sulfur-oxidizing bacteria (for example, *Thiobacillus* or *Beggiatoa* spp.) as is shown in Eqs.(2)[22]. In addition, anaerobic oxidation of sulfide can occur by phototrophic purple and green sulfur bacteria (for example, *Chlorobium* spp.) using hydrogen sulfide as the reducing agent (electron donor) and produce granules of elemental sulfur (Eqs.(3) and (4)) [23].



Sulfur oxidation and reduction

Elemental sulfur, a kind of sulfur intermediate species with stable chemical properties and insolubility, can be oxidized both under oxic and anoxic conditions to sulfate. Under aerobic condition, it is oxidized by sulfur oxidizing bacteria such as *Thiobacillus* and *Acidithiobacillus* and forms sulfuric acid (Eqs.5), lowering the pH in the environment [24]. Under anoxic condition, sulfur oxidation can be achieved by microorganisms like *Thiomargarita namibiensis* using nitrate (NO_3^-) as the terminal electron acceptors, yielding energy (Eqs.6) [25].



Elemental sulfur can be reduced as well as oxidized. Sulfur-reducing bacteria like *Desulfuromonas acetoxidans*, are able to reduce elemental sulfur (S^0) to hydrogen sulfide (H_2S) by using hydrogen as electron donor to sustain several activities such as respiration for energy generation, in the absence of oxygen[17], [26]. This metabolism is largely found in extreme environments. For example, when acetate is utilized in sulfur reduction, the reaction is expressed as Eqs.(7):



Sulfur disproportionation

In this process, under anoxic environment, inorganic sulfur intermediates like elemental sulfur (S^0), thiosulfate ($S_2O_3^{2-}$), and sulfite (SO_3^{2-}) serve as both electron donor and acceptor to generate hydrogen sulfide and sulfate, as is shown in Eqs.(8), (9) and (10) [15]. These conversions release energy which microorganisms can use for their growth, and sometimes are called "inorganic fermentation" [27]–[29].



Decomposition of sulfur-containing organic material

In addition to inorganic sulfur, various organic sulfur compounds are also metabolized by microorganisms, which enters sulfur cycle as well. A variety of sulfur-containing organic molecules are found in the cell, including amino acids (cysteine and methionine), vitamins, lipids, carbohydrates, and coenzymes [30]. For most of microorganisms, sulfur assimilation can occur by biosynthesizing cysteine and methionine from environmental inorganic sulfur sources such as sulfate (SO_4^{2-}), thiosulfate ($S_2O_3^{2-}$), sulfite (SO_3^{2-}). Further, organic sulfur compounds can be biologically degraded to sulfide (S^{2-}), which provides an important input of inorganic sulfur[31].

2.2 Sulfide species

2.2.1 The existence form of dissolved sulfide

Sulfide species consist of hydrogen sulfide gas (H_2S), aqueous hydrogen sulfide($H_2S_{(aq)}$), bisulfide (HS^-), and sulfide (S^{2-}). This study mainly focusses on dissolved sulfide.

In aqueous solution, hydrogen sulfide is a weak acid and does not dissociate completely, thus, unionized ($H_2S_{(aq)}$) and ionized forms (S^{2-} , HS^-) can coexist. In liquid phase, their relative concentrations are controlled by the equilibrium (Eqs.(11)-(14)) with chemical activity (or molar concentration) of proton [2], [14]:



$$\frac{[H^+][HS^-]}{[H_2S]} = K_{a1} = 1.02 \times 10^{-7} \quad (13)$$

$$\frac{[H^+][S^{2-}]}{[HS^-]} = K_{a2} = 1.21 \times 10^{-13} \quad (14)$$

where $pK_{a,1}$ and $pK_{a,2}$ (equilibrium constant) is 6.99 and 12.92, respectively. For S^{2-} , it practically exists only when pH above 12, so that, in general, the bisulfide form (HS^-) is the dominant species in H_2S -containing water[32]. According to (12), equation can be obtained as below (15):

$$pH = pK_{a,1} + \log \frac{[HS^-]}{[H_2S]} \quad (15)$$

As a result, the concentration of sulfide species is a function of pH (Eqs.(15)), as shown by the equilibrium among $H_2S/HS^-/S^{2-}$ in **Figure 2.2**. The form of dissolved sulfide present in an environment is pH dependent and the dominant species are S^{2-} at $pH > 13$, HS^- at pH range higher than 7 and lower than 13, and H_2S (aq) at acidic pH.

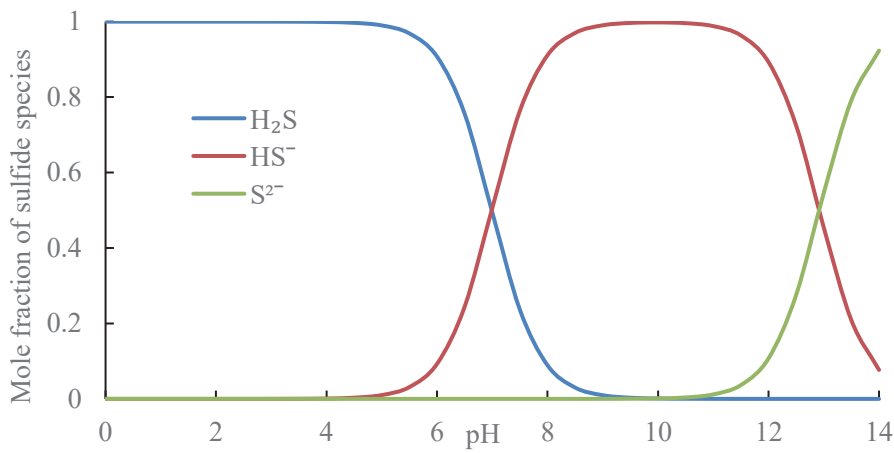


Figure 2.2 pH dependence of sulfide speciation

Hydrogen sulfide ($H_2S(aq)$) can be further released to atmosphere through air-water interface [19]. The concentration of $H_2S(g)$ in the air phase at equilibrium can be estimated from the $H_2S(aq)$ concentration in water phase using Henry's law (16)[15]:

$$p_A = y_A P = H_A x_A \quad (16)$$

where:

p_A : the partial pressure of H_2S in the gas phase(atm)

P : the total pressure in the gas phase(atm)

H_A : Henry's law constant for H_2S (atm)

y_A : mole fraction of H_2S in the gas phase ($mol\ mol^{-1}$)

x_A : mole fraction of H_2S in the liquid phase ($mol\ mol^{-1}$)

As a result, the tendency of hydrogen sulfide to escape from the liquid phase can be quantified by Henry's law.

2.2.2 Problems caused by hydrogen sulfide and other sulfide species

1. Malodor

Hydrogen sulfide is a colorless and malodor gas known for its pungent "rotten egg" smell at low concentrations[14]. Once it emits from the sewage at channels, manholes, vents, and pump stations into the atmosphere, resulting in odor problems. Moreover, hydrogen sulfide in water causes a disagreeable taste and odor to the water[3].

2. Hydrogen sulfide health effects for human

Hydrogen sulfide gas can seriously harm human health. Because it is slightly denser than air, hydrogen sulfide can exist in low-lying and enclosed spaces, such as manholes, sewers, well water and treatment plants[3], [4]. Its presence makes workers in confined spaces potentially very dangerous. The detailed effects of different sulfide concentration are shown in this **Table 2.2** [22]. However, when its concentration is small enough, it's not toxic like in hot springs.

Table 2.2 Symptoms of exposure to hydrogen sulfide gas (Adapted from [32])

Concentration (ppm)	Short Term Symptoms/Effects
0.01-1.5	Odor threshold
2-5	Prolonged exposure may cause nausea, tearing of eyes, headaches, or loss of sleep.
20	Possible fatigue, loss of appetite, headache, irritability, poor memory, dizziness.
100-150	Loss of smell (olfactory fatigue or paralysis)
200-300	Marked conjunctivitis and respiratory tract irritation after 1 hour.
500-700	Staggering, collapse in 5 minutes. Serious damage to the eyes in 30 minutes. Death after 30-60 minutes.
700-1000	Rapid unconsciousness, or immediate collapse within 1 to 2 breaths.
1000-2000	Nearly instant death

3. Toxicity to microorganisms

In aqueous solution, unionized hydrogen sulfide ($\text{H}_2\text{S}_{(\text{aq})}$) is toxic to microorganisms even at nanomolar concentrations [2], [34]. When hydrogen sulfide diffuses into cell membranes, it directly inhibits cellular oxidative metabolism. It was observed that the inhibitory effect is proportional to the pH [35], [36]. Moreover, sulfide toxicity is related to its concentration [37]. According to the study, inhibition of SRB and methanogens was associated with the total sulfide concentration ($100\text{--}800\text{mg L}^{-1}$) and the free hydrogen sulfide concentration ($50\text{--}400\text{mg L}^{-1}$), respectively [38], [39].

4. Corrosion

Hydrogen sulfide gas emitted from sulfide-containing wastewater can precipitate on solid materials then cause corrosion. That is, under aerobic conditions, hydrogen sulfide is oxidized by sulfur-oxidizing bacteria to produce corrosive sulfuric acid (H_2SO_4), which then interacts with concrete or metals such as iron, copper, cadmium and lead, causing corrosion [40]. According to the literature, minor concrete corrosion occurs when the concentration of total sulfide in the wastewater is within the range of $0.1\text{--}0.5\text{mgS L}^{-1}$, while severe concrete corrosion may occur at sulfide concentrations from 2.0mgS L^{-1} [41], [42]. As concrete and metals especially steel are widely used in infrastructure such as buildings, pipes, and machines, corrosion can cause serious damage on infrastructure.

5. Consumption of oxygen in water

Reduced sulfide species in wastewater can react with oxygen, as a result, when sulfide-containing wastewater is directly discharged to water environment without treatment for sulfide removal. It will cause oxygen depletion in water body, leading to fish kills and death to other aquatic organisms.

2.3 Sulfide in sewer and sewage treatment plant

2.3.1 Source of sulfide in sewer and sewage treatment plant

For domestic wastewater, the primary source of sulfur is sulfate (SO_4^{2-}) in a concentration range of $40\text{--}200\text{mg L}^{-1}$, which is equivalent to $13\text{--}70\text{mgS L}^{-1}$, and sulfur-containing organic matters like proteins [3], [43]. Besides, sulfide-containing waste streams are generated by a number of industries such as petrochemical plants, tanneries, viscose rayon manufactures, the gasification of coal for electricity production, or by the anaerobic treatment of sulfate-containing wastewaters [14]. As sulfide-containing wastewater flow through sewer pipes, sulfur cycle occurs in five phases of sewer pipes including sewer atmosphere, sewer wall, biofilm, bulk water phase, and sewer sediment (**Figure 2.3**). Moreover, with the increasing flow rate and turbulence, hydrogen sulfide gas emits from gas-liquid interface. As a result, treatment

of sulfide rich waste streams is highly desirable due to its toxic effects upon environment and human health.

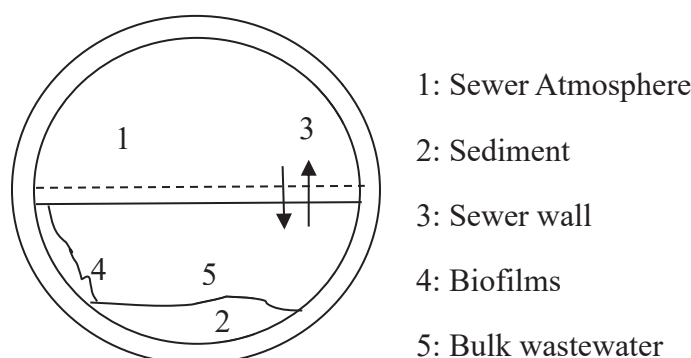


Figure 2.3 Cross-section representation of a concrete sewer pipe (modified from Zhang, et al., 2008[44])

2.3.2 Sewer and metal corrosion

Hydrogen sulfide (H_2S) gas emission in sewer collection systems and wastewater treatment processes will indirectly cause corrosion problem. Corrosion in sewers and sewage treatment plant potentially costs millions of dollars to maintain and repair the affected sewer pipes and equipment[45]. In sewer pipes and sewage treatment plants, complex microbial sulfur processes occur under the dynamic anaerobic-anoxic-aerobic environment.

In sewer system, hydrogen sulfide corrosion is usually divided into four steps according to the different environments that exists in the sewer [46]:

- ♦ Firstly, sulfate ions, the main sulfur source in sewage, is biologically reduced into dissolved sulfide by sulfate-reducing bacteria (SRB) in the anerobic areas like the submerged sediments.
- ♦ Secondly, with the aid of sewage turbulence (turbulence increases the gas release), H_2S (aqueous) is released into the sewer atmosphere and exists as H_2S gas.
- ♦ Thirdly, hydrogen sulfide gas dissolves in the moist sewer walls or crown in accordance with its partial pressure in the sewer atmosphere, further, under aerobic conditions, a variety of sulfur-oxidizing bacteria (SOB) present on the moist sewer wall surface oxidize H_2S to some intermediate sulfur products such as elemental sulfur and then eventually form highly corrosive sulfuric acid through both chemical and biological pathways[47].
- ♦ Finally, sulfuric acid reacts with the compounds of concrete thereby resulting in concrete softening, expansive cracking and deterioration.

The corrosion mechanism was discussed in detail as follows [30]:

For concrete corrosion, sulfuric acid that is generated on the moist sewer surface initiates sulfate attack by penetrating into concrete pore and react with the alkaline

substances of concrete[48]. Two processes are generally responsible for sulfate attack in concrete. First is the formation of calcium sulfate (gypsum) by the combination of sulfate ions and calcium hydroxide ($\text{Ca}(\text{OH})_2$) in the concrete (17), causing softening and loss of concrete mass and strength[49]. Further, this formed calcium sulfate may then react with the calcium aluminate hydrate to form ettringite ($3\text{CaO} \cdot \text{Al}_2\text{O}_3 \cdot 3\text{CaSO}_4 \cdot 32\text{H}_2\text{O}$) (18), resulting in an increase in solid volume, expansion and cracking[49], thus more chance for sulfuric acid penetration.



As a result, sulfuric acid production, plaster formation, and production of ettringite, all contribute to the weakening of the sewer pipes, which results in the ultimate collapse and structure failure within a few years[48].

Moreover, hydrogen sulfide (H_2S) gas can corrode metal such as steel, iron, copper, and lead as well and then cause serious damage to metal compounds in pumping stations and wastewater treatment plants as well as the sewer structures with electronic equipment [33]. Hydrogen sulfide is a weak acid that reacts with most heavy metals and produces a corresponding metal sulfide with a low solubility in water [50], [51]. Take the reaction with iron as an example (Eqs.(19)):



2.3.3 Factors affecting hydrogen sulfide generation and emission

The generation and emission of hydrogen sulfide is affected by several factors, as following[52]:

1. Biodegradable organic matter (BOM)

Biodegradable organic matter is required as an electron donor for sulfate-reducing bacteria and as a substrate for biomass growth (another influential factor in dissolved sulfide generation). According to the study, in the presence of organic matter such as ethanol, lactate and glucose, sulfate reduction in sewer systems can be enhanced[20], [53]. Therefore, compared with the sulfate reduction rate in typical domestic wastewater, industries wastewater with relatively high concentration of BOM is higher.

2. Temperature

High temperature facilitates the microbial activity. It was found that the sulfate reduction rate (or hydrogen sulfide production rate) is highly temperature dependent[54]. Furthermore, high temperature lowers oxygen solubility and leads to anaerobic sewer conditions which in turn encourages the conversion of sulfate to sulfide.

3. Turbulence

With high turbulence in a sewer, chances for re-aeration and chemical oxidation increase, resulting in growth of sulfide-oxidizing bacteria (SOM) and lowered potential for sulfide generation. Sources of turbulence include manholes with flows dropping in from the side, manholes with flows colliding, sections with steep slopes and force main discharges[55]. Moreover, sewage flow with high velocity (more than 1.5 ms^{-1}) could potentially release the dissolved sulfide (in the form of H_2S) into the sewer atmosphere[56].

4. DO (dissolved oxygen)

Low dissolved oxygen in wastewater not only encourages the activity of SRB but also promote the conversion of sulfate to sulfide.

2.4 Sulfide removal technology

The presence of hydrogen sulfide gas (H_2S) in sewer collection networks and sewage treatment plant causes nuisance odor, detrimental working environment and corrosion. Moreover, oxygen depletion occurs when sulfide-containing sewage is discharged without removal. To solve these problems, several technologies have been explored, for example, (1) increasing ventilation and optimizing the hydraulic design of sewers to minimize sulfide generation, (2) application of protective coatings like plasticized PVC sheets for existing systems and corrosion-resistant pipe materials for new construction, (3) removing sulfate from the source such as urine separation or pretreatment, (4) inhibiting hydrogen sulfide emission from water phase [57], [58].

This section focuses on the inhibition of hydrogen sulfide gas formation and the elimination of produced sulfide.

Firstly, the inhibition of hydrogen sulfide gas formation can be achieved by increasing redox potential and preventing SRB activity. Increasing redox conditions includes addition of electron acceptor such as oxygen, nitrate. SRB activity can be inhibited by pH elevation or inhibitors such as molybdat [59].

Further, the elimination of produced sulfide consists of chemical and biological technologies. Chemical sulfide removal involves addition of iron salts and oxidizing chemicals (e.g., chlorine, hydrogen peroxide, and potassium permanganate), which can be regarded as ultimate treatments in sewage plants to remove sulfide. For biological technologies, addition of nitrate may also favor biological oxidation of sulfide by means of nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB) [60], [61].

A variety of technologies have been developed and utilized to treat sulfide-containing wastewater. This section summarizes the current state and explores emerging possibilities.

2.4.1 Inhibition of hydrogen sulfide formation

2.4.1.1 Increasing redox potential

1. Addition of air or oxygen

Air or pure oxygen injection can prevent dissolved sulfide occurrence in wastewater by increasing dissolved oxygen concentration to prevent anaerobic conditions[62]. Moreover, when there is dissolved oxygen (DO) around biofilm layer in the sewers, sulfide can be both chemically and biologically oxidized to higher oxidation state[63]. It is reported that, a dissolved oxygen level of 0.5mg L^{-1} can achieve dissolved sulfide inhibition in the wastewater and produced dissolved sulfide oxidation[62]. For air injection in wastewater, the typical DO range is 3 to 5mg L^{-1} . It has merits including simultaneous organic matter degradation during sulfide removal and cost effectiveness[64]. And the disadvantage is the limited oxygen transfer into water phase, which can be overcome by pure oxygen injection to achieve higher DO level ranging from 5 to 7mg L^{-1} due to its five times higher solubility than air [58]. However, since the pure oxygen is flammable, which represents fire and explosion risks, more attention should be paid when applying this method[65].

Overall, in this way, although hydrogen sulfide can be prevented or oxidized, there is a serious disadvantage of hydrogen sulfide gas emission together with exhaust oxygen, thus causing worse corrosion.

2. Nitrate addition

Nitrate (NO_3^-) addition to an anaerobic wastewater system encourages anoxic activity due to the increased redox potential by using nitrate as an electron acceptor. It was observed that the nitrate inhibition to hydrogen sulfide production can be achieved both for short and long term by microbial preferential reduction of nitrate over sulfate. According to report, hydrogen sulfide production can be prevented for 29 days by adding 1mg L^{-1} nitrate[66], [67]. Since nitrate is more soluble in water than oxygen, there is less restriction for dosage rate and location. However, due to high nitrate load in wastewater, this could affect the performance of the wastewater treatment plant by reducing COD load and by increasing nitrogen load to it, further, considering the high price and greater chemical consumption for the use of nitrate in large sewer systems, it is cost prohibitive for real application[57], [68].

The addition of nitrate as a biological oxidation technology by means of NR-SOB will be described in Section 2.4.2.2.

2.4.1.2 Inhibition of SRB activity

The most common and effective method for inhibiting the microbial activity is pH elevation. However, continuous addition of chemicals like sodium hydroxide (NaOH) to maintain an elevated pH (more than 9 to minimize the existence of $\text{H}_2\text{S}(\text{aq})$) is costly, moreover, wastewater with high pH needed to be isolated or diluted to prevent the effects on subsequent treatment processes[62].

According to the study, a pH shock treatment for sodium hydroxide can increase the pH in wastewater to the range of 12.5–13.0 for 20–30min. And with this high pH, SRB activity can be inhibited up to 2 weeks[69].

Other methods like utilizing molybdates has also been considered[70]. Molybdate is an essential trace element required by biological systems including the anaerobic sulfate-reducing bacteria (SRB); however, detrimental consequences may occur if molybdate is present in high concentrations in the environment. It was reported that 0.08mM (12.8mg L^{-1}) molybdate, a molar ratio molybdate/sulfate of 0.004, is sufficient to inhibit the activity of sulfate-reducing bacteria for 168h [42].

2.4.2 Elimination of produced sulfide

2.4.2.1 Chemical removal of sulfide

Precipitation by iron salts

Iron is widely distributed in municipal wastewater in the typical concentration of 0.4–1.5mg L^{-1} [71]. Addition of iron salts is an effective way to control the dissolved sulfide concentration, and thus was universally applied to control sulfide production in sewer networks[42].

Iron salts were used to precipitate the produced dissolved hydrogen sulfide in the wastewater. As a result, the emission of hydrogen sulfide become significantly less. For example, ferrous iron (Fe^{2+}) is utilized to remove sulfide by precipitation as insoluble ferrous sulfide (FeS) in anaerobic wastewater as shown in reaction (20)[71]. Ferric iron (Fe^{3+}) can remove sulfide by chemical oxidation to form elemental sulfur while being reduced to ferrous iron (Fe^{2+}), which can subsequently produce FeS (21) [41]. The proper ratio of iron to sulfide is 1-2.5 portions of iron to 1 portion of sulfide, which achieves the sulfide removal efficiency of 88-100%[42], [50].

In general, it is a common practice for large sewer systems for its simplicity and cost effectiveness compared with other chemicals, but the precipitated sulfide with added heavy metals need to be handled as sludge.





Addition of oxidizing chemicals

Addition of oxidizing agents can chemically oxidize sulfide to sulfate or to elemental sulfur, thereby decreasing the amount of dissolved sulfide.

1. Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is added to oxidize dissolved sulfide and is decomposed into water and oxygen, thus creating aerobic environments[72]. The proper ratio of H_2O_2 is 1.3–4.0 $\text{mgH}_2\text{O}_2 \text{ L}^{-1}$ to 1 mgS L^{-1} , which achieve an average sulfide elimination efficiency of 85–100%[73]. And it is estimated that the application cost of hydrogen peroxide is at US\$2.38-5.26 per kg of sulfide. However, the lifespan of hydrogen peroxide (less than 90min) is too short to achieve the desired sulfide removal efficiency[73]. Therefore, several dosing locations of hydrogen peroxide in sewer networks is important in terms of effectiveness.

2. Chlorine

Chlorine oxidizes sulfide to sulfate or to elemental sulfur, depending on the pH. However, chlorine's efficiency is limited due to its capacity to react with various organic and inorganic compounds in sewage[74]. It can be injected to wastewater as an aqueous solution (e.g., NaClO or $\text{Ca}(\text{ClO})_2$) with the ratio of chemicals to sulfide of 1.8–2.0:1 (w/w) or directly as a gas with the ration of 9.0–15.0:1 (w/w). Moreover, the chemical oxidation rate of chlorine is slow when aqueous sulfide concentration is lower than 1 mg L^{-1} [72], It is estimated that the application cost of chloride is at US\$26.50-38.20 per kg of sulfide[76]. Moreover, as chlorine gas is very toxic and dangerous, human exposure to it should be limited when it is applied.

3. Potassium permanganate

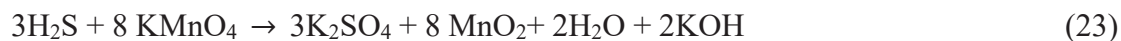
Potassium permanganate (KMnO_4) is a strong oxidant that oxidizes sulfide very quickly (on the order of seconds), making it a good choice for application in short duration hydrogen sulfide control. Since potassium permanganate is a granular dry product, it is usually applied as aqueous solution at 6% concentration[77].

The drawback of potassium permanganate application is its high cost, which estimated to US\$54.27-66.23/kg sulfur in wastewater[78]. Potassium permanganate oxidizes hydrogen sulfide according to the following equations (22), (23), and the dose ratios of KMnO_4 to sulfide are dependent on the pH of the wastewater being treated [77].

Neutral-Acid conditions:



Alkaline conditions:



2.4.2.2 Biological oxidation of sulfide

In addition to the physicochemical processes to remove dissolved sulfide, biological oxidation of H_2S through microbial activity was also investigated for its cost-effectiveness and high reaction rate.

Injection of nitrate into oil reservoirs was investigated to reduce the sulfide produced by SRB through the activity of nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB)[81]. *Thiomicrospira denitrificans* and *Thiobacillus denitrificans* are two species found to be able to oxidize sulfide with nitrate as the electron acceptor [66], [79]. In this process, HS^- is converted into elemental sulfur in the presence of nitrate, which ultimately leads to pH increase of the environment (24), (25). On the other hand, elemental sulfur can be further oxidized into SO_4^{2-} (26), leading to the decrease of pH [82]. According to the report, in plant-scale tests, sulfide removal efficiencies up to 94% can be achieved by *Thiomicrospira denitrificans* after nitrate injection to the wastewater [83].



However, limitations exist including possible negative effect on wastewater treatment plant because of nitrate load, high price and greater chemical consumption for the use of nitrate in large systems [61], [63].

2.4.3 Future possibilities

Recently, several technologies were explored for promising future application, such as slow-release solid-phase oxygen ($\text{MgO}_2/\text{CaO}_2$) and Microbial fuel cells (MFCs).

Slow-release solid-phase oxygen ($\text{MgO}_2/\text{CaO}_2$) was developed for oxidation of hydrogen sulfide to sulfur in oil wells[85]. When $\text{MgO}_2/\text{CaO}_2$ in contact with water, oxygen is slowly released and then magnesium hydroxide and calcium hydroxide ($\text{Mg}(\text{OH})_2$ and $\text{Ca}(\text{OH})_2$) are formed[86]. As a result, both chemical oxidation of sulfide and biotic inhibition of SRB function on sulfide removal in water. After a series of lab-scale batch tests, 0.4% MgO_2 was found to be able to inhibit the formation of H_2S for more than 40 days in an SRB-enriched environment [85]. By providing long-term inhibition of the SRB activity, slow-release solid-phase oxygen provides a good alternative means of controlling the generation of H_2S in water. However, it faces the issue of being washed off when applied in solid phase in sewer pipes[85].

Microbial fuel cells (MFCs) is an emerging biological methods, which is capable of converting chemical energy to electrical energy through electrochemical reactions

associated with biological processes[44]. As a result, it can convert dissolved sulfide (fuel) to elemental sulfur with electricity generation in the presence of oxidants, but further investigation is needed for the stability of this process on the longer term and the influence of the precipitated sulfur on the electrode [87].

2.5 Intermittent contact oxidation process (ICOP)

Intermittent contact oxidation process (ICOP) maybe a promising technology for sulfide removal through enhanced self-purification in sewer pipes.

In-sewer purification is a natural process in the sewer pipe where organic pollutants and sulfide are degraded by microbial activity as it flows through the sewer pipe [91]. In this way, sewer system is regarded as a biological reactor rather than the primary tool to physically collect and transport sewage [92] A variety of physical, chemical and biological reactions and transformations occur in this dynamic and complicated sewer environment. However, the capacity of natural in-sewer purification is limited compared to sewage volume [13].

Intermittent contact oxidation process (ICOP) is an enhanced self-purification method with no external energy demand[8]. In the ICOP, microbial growth and retention are promoted by installing porous media like sponge inside a sewer pipe as habitat[9]. And by utilizing the intermittency of wastewater, when there is high flow, organic pollutants are supplied to microorganisms as substrate, and when the flow is low or stopped, organic pollutants which are entrapped in the sponge are subsequently exposed to headspace air in the sewer pipe and then be aerobically degraded.

Two kinds of sewer structure utilizing ICOP was studied. One is a novel purifying pipe system with double-layer structure as shown in **Figure 2.4**[93]. The sewer pipe was divided into the upper deck and the lower deck by the installation of a separator. The upper deck was designed to achieve smooth transportation of wastewater, while for the lower deck, it was used for promoting self-purification by installing a sponge media as biomass habitat and retention. The holes in the separator can achieve high wastewater turbulence when it drops to lower deck, increasing the chance for re-aeration and chemical oxidation.

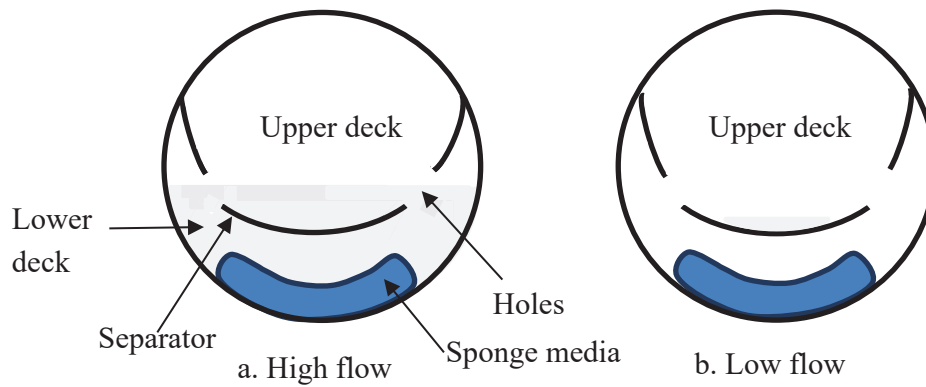


Figure 2.4 The schematic of double-layer structure

The other is single-layer structure incorporating ICOP [7]–[9], [11], [12]. For both sewer structures utilizing ICOP, sponge exposure to air during the low flow condition achieves organic pollutants and sulfide oxidation. The single-layer ICOP structure is as shown in **Figure 2.5**.

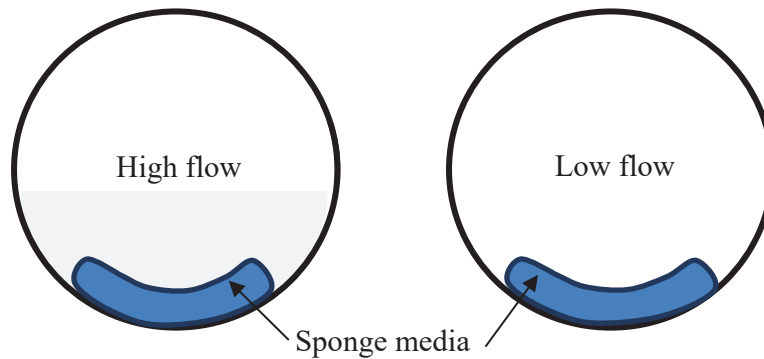


Figure 2.5 The schematic of single-layer structure

2.6 Potential merits of ICOP for sulfide removal

When ICOP is applied to treat sulfide-containing wastewater. It may has following possible advantages:

1. Reduced chemical species including sulfide would be entrapped in sponge, then oxidized when the sponge is exposed to air.
2. As water is not bubbled to supply oxygen, emission of H_2S gas is significantly less.
3. With no external energy demand.

As a result, in this process, sulfide-containing wastewater is pre-treated, and sewage quality is improved. ICOP technology has the potential to be regarded as an alternative way to treat sulfide containing wastewater.

2.7 Chapter Summary

In this chapter, sulfur and sulfide related topics were explained, as well as the potential of ICOP as a biological sulfide removal technology. The summaries are described as follows:

1. Sulfur is significant for many essential biochemical reactions. And the transformation between sulfur compounds forms sulfur cycles.
2. Dissolved sulfide can occur as bisulfide (HS^-) at neutral pH, sulfide ions (S^{2-}) at alkaline pH, and H_2S at acidic pH. Only hydrogen sulfide (H_2S) can be further released to sewer atmosphere.
3. Hydrogen sulfide gas is a serious health hazard for human and a malodorous compound that can create extreme corrosion problems in the sewer networks and in sewage treatment plants. Moreover, it can inhibit cellular redox processes, causing toxicity to microorganisms.
4. In sewage, the source of sulfur comes from domestic wastewater and various industries. The main source of sulfur in domestic wastewater is sulfate in a concentration range of $40\text{--}200\text{mg L}^{-1}$, which is equivalent to $13\text{--}70\text{mgS L}^{-1}$, and sulfur-containing organic matters.
5. A variety of chemical and biological technologies has been investigated to inhibit hydrogen sulfide formation or decrease produced hydrogen sulfide in sewer systems and sewage treatment plants. However, technologies utilizing biological sulfide removal are relatively more cost-effective.
6. Installing sponge media in the sewer pipes could enhance in-sewer purification and it may have potential to remove sulfide in sewage without energy and aeration input.

Chapter 3 METHODOLOGY

The objective of this chapter is to evaluate the performance of sulfide removal during the ICOP. To do so, a lab-scale ICOP reactor was fabricated and then operated with different sulfide concentrations in the synthetic feed. This chapter is organized as follows: first, reactor setup, then, reactor operation, followed by control experiment without sponge, and finally, the analytical methods for sulfide concentration, sulfate concentration, intermediate sulfur products concentration and turbidity are described.

3.1 Reactor setup

A lab-scale reactor incorporating the ICOP was fabricated. The schematic is as shown in **Figure 3.1**. This reactor consisted of a rectangular channel made of 0.5 cm thick transparent polyvinylchloride plastic plates with a dimension of 48.5 cm (length) \times 7 cm (width) \times 6 cm (depth), a piece of sponge (Model BCD-2, pore cell density 6 cell/cm, Achilles Corporation Tokyo, Japan) made of polyurethane with a dimension of 43.5 cm (length) \times 7 cm (width) \times 1 cm (depth) for microorganism habitat and carrier, a recirculation tank (working volume of 1 L) for synthetic feed supply. The slope of this reactor was around 2%. The ambient temperature was maintained at 25 °C for this whole reactor during the study.

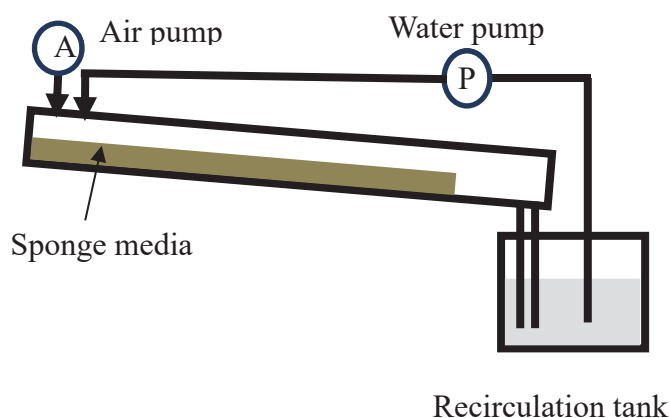


Figure 3.1. The schematic of reactor setup

A recirculation pump (Masterflex Model 77201-60, USA) recycled synthetic feed in the recirculation tank through the channel. An air pump (Masterflex Model 77201-60, USA) was operated to refresh the oxygen inside the reactor. These pumps were connected to an Arduino UNO microcontroller board which was used for controlling pump operation and collecting data.

3.2 Reactor operation

The reactor was operated as follows. One liter of synthetic feed containing different concentrations of sulfide was basically daily prepared with ultrapure water (MilliporeSigma™ Milli-Q™ Advantage A10, Fisher Scientific, New Hampshire, USA) and placed in the recirculation tank. The composition of the synthetic feed is described later in this section. Then the synthetic feed was recirculated intermittently by the recirculation pump through the channel for 5min every 20 minutes at a flowrate of 200mL m⁻¹ repeatedly. The air pump was operated for 5 minutes every 1 hour to refresh the air inside the channel. The timeline of the reactor's operation is tabulated in **Table 3.1**.

Table 3.1 Reactor operation history

Conditions	Sulfide concentration (as Na ₂ S·5H ₂ O) (mgS L ⁻¹)	Operation period	Monitoring date
Inoculation and biomass development	40	March 2021	-
Monitoring and operation improvement		From March to the end of July (with organic matters in the feed)	-
		From August to September (with no organic maters in the feed)	-
Run 1		4-10 Oct	7,9,10 Oct
Run 2	80	11-25 Oct*	16,17,21 Oct
Run 3	120	29 Oct-27 Dec	18,22,27 Dec
Without sponge	120	28 Dec	28 Dec

*: During run 1, on 14 and 18 Oct, feed was omitted.

The initial seven months of operation was devoted mainly for acquiring operating and analytical skill and for acclimatization of microorganisms. During this period until the end of July, the feed contained 40mgS L⁻¹ (as initial concentration) together with readily biodegradable organic matter. The feed composition was basically the same as the one for Run 1 except that it was supplemented with readily biodegradable organic matter. The feed composition for Run 1 is described later in this section. During the very initial three days, around 10mL of activated sludge (around 5,000mg L⁻¹) from a municipal wastewater treatment plant was added to the daily prepared synthetic feed as the inoculant. After August, organic matter in the feed was omitted and the same feed composition for Run 1 matter was used. till September.

Then experiment from Run 1 to Run 3 as well as without sponge were operated in subsequent three months from October to December. In detail, different sulfide concentration in the synthetic feed were supplied for Runs 1 to 3, respectively in the order of time sequence, and for each run, the detailed monitoring was conducted for 3 days. Experiment without sponge was operated for 1 day after Run 3 was done. The synthetic feed volume was all 1L.

The feed for Runs 1 to 3 and the control experiment without sponge were prepared as follows. Stock solutions A and B with their composition shown in **Table 3.2** were prepared. Here, the composition of the trace metal solution described by Smolder et al[95] for stock solution A is shown in **Table 3.3**. Then, sodium sulfide pentahydrate (Na₂S·5H₂O), stock solution A, stock solution B, and sodium bicarbonate were mixed in a composition as shown in **Table 3.4**. Sodium bicarbonate was added with the aim to adjust pH. Feed sulfide concentrations in Runs 1, 2, and 3 were 40, 80, and 120mgS L⁻¹, respectively.

Table 3.2 Preparation for stockfeed solution A and B

	Chemicals	Concentration (g L⁻¹)	Manufacture
Stock solution A	MgSO ₄ ·7H ₂ O	3	FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan
	NH ₄ Cl	115	
	Trace metal solution	5 (ml L ⁻¹)	
Stock solution B	K ₂ HPO ₄	25	Kishida Chemical Co. Ltd, Osaka, Japan
	Na ₂ HPO ₄ ·7H ₂ O	50	

Table 3.3 Preparation for trace metal solution

Chemicals	Concentration (g L ⁻¹)	Manufacture
CuSO ₄ ·5H ₂ O	0.075	Kanto Chemical Co., Inc., Tokyo, Japan
Na ₂ MoO ₄ ·2H ₂ O	0.15	
ZnSO ₄ ·7H ₂ O	0.3	
FeCl ₂ ·6H ₂ O	3.75	Wako Pure Chemical Corporation, Osaka, Japan
CoCl ₂ ·6H ₂ O	0.375	
MnCl ₂ ·4H ₂ O	0.3	Kishida Chemical Co. Ltd, Osaka, Japan
EDTA	25	
KI	0.45	FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan
H ₃ BO ₃	0.375	SIGMA-ALDRICH CHEMIE GmbH, Steinheim, Germany

Table 3.4 The whole synthetic feed preparation per liter

Conditions	Run 1	Run 2	Run 3	Without sponge
Target sulfide concentration (mgS)	40	80	120	120
Target sulfide concentration as Na ₂ S·5H ₂ O (mg)	210	420	630	630
Target sulfide-sulfur concentration (mgS)	40	80	120	120
Stock solution A (mL)	10			
Stock solution B (mL)	10			
NaHCO ₃ (mg)	460			

During the experiment, samples were all collected from recirculation tank. And the sample collection pattern for each run was different.

For Run 1, 15 samples were collected from recirculation tank every 20 minutes from 0 – 200min, and every one hour from 260 to 440min, and 1440 minutes. For Run 2, 22 samples were collected every 20 minutes from 0 – 200min, and every one hour from 260 to 680min, and 1440 minutes. For Run 3, 32 samples were collected every 20 minutes from 0 – 200min, and every one hour from 260 to 1440 minutes. The time 0- and 1440-minutes sample refer to the freshly prepared synthetic feed, and the one after one day's operation, respectively.

3.3 Control experiment without sponge

To figure out the chemical oxidation contribution and the extent of sponge media to sulfide removal rate during the ICOP, a control experiment was undertaken without sponge media after all the old piping and pipe connectors were replaced and reactor was thoroughly cleaned. This experiment was conducted after Run 3 was finished.

In this experiment, synthetic feed with 120mgS L^{-1} , as described in the previous section, was recirculated intermittently through the channel without sponge for 5min every 20 minutes repeatedly for one day to explore the sulfide removal rate, sulfide transformation process and turbidity change during this condition. And the sampling schedule was the same as Run 3, which was 32 samples every 20 minutes from 0 – 200min, and every one hour from 260 to 1440 minutes.

3.4 Analytical methods

For each sample collected in each run and in control experiment, the following parameters were measured: sulfide concentration, intermediate sulfur products concentration, sulfate concentration and turbidity. Protocols for each analysis are described in this section.

3.4.1 Sulfide concentration

A typical method for hydrogen sulfide determination is the methylene blue spectrophotometric method. While there are different protocols proposed, I employed the protocol by Yano et al. [96], which include stabilization of sulfide by adding glycerol and EDTA under alkaline condition. The reagents used and related preparation methods in this study are:

1. Fixation solution (for sulfide fixation)
10g NaOH was dissolved in 50mL ultrapure water, after vortexed well, 125mL glycerol and 1.0g EDTA-2Na were added into the mixture and then was made up to 250mL.
2. Dilution liquid
Solution [1] was diluted by 10 times with ultrapure water.
3. p-Aminodimethylaniline (PADMA) solution
27mL concentrated H_2SO_4 was dissolved in ultrapure water, after cooling, the mixture was made up to 100mL (10N H_2SO_4). After that, 0.2g PADMA-HCl was dissolved in it.
4. FeCl_3 solution
0.54mL H_2SO_4 was added into ultrapure water and was made up to 100mL (1% (w/w) H_2SO_4). After that, 0.55g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was dissolved in the mixture.
5. Sulfide standard solution

263mg $\text{Na}_2\text{S}\cdot 5\text{H}_2\text{O}$ was added in ultrapure water and then the solution was filled up to 50mL (1000mgS L^{-1}).

The procedure of this method is as follows:

1. A calibration curve was established by a series of sulfide standard solutions ($0\text{--}1.0\text{mgS L}^{-1}$) using the dilution liquid.
2. 0.9mL sample taken from the feed tank and 0.1mL fixation solution was added in a 10mL screw cap glass tube.
3. Sample was diluted with dilution liquid immediately after sampling so that the sulfide concentration falls within the range of 0 to 1mgS L^{-1} . In detail, an aliquot of sample (0.1mL for Runs 1 and 3, and 0.05mL for Run 3 and the control experiment) was added to a volume of dilution liquid (4.9mL for Runs 1 and 2, and 4.95mL for Run 3 and the control experiment) right after sampling and mixed immediately.
4. 0.5mL PADAMA solution and 0.25mL FeCl_3 solution were added into the diluted sample, after mixing well, left for coloring for 15 minutes.
5. Absorbance was measured in a spectrophotometer (HACH Company, Mod: DR 3900, Germany) at the absorbance wavelength of 668 nm.
6. The concentration of sulfide for each sample was calculated from the calibration curve established with sulfide standard series solution.

3.4.2 Sulfate and intermediate sulfur products

For sulfide oxidation process, sulfide is expected to be finally converted to sulfate. During the conversion process, it's expected to involve accumulation of intermediate sulfur products such as elemental sulfur and thiosulfate. The determination methods for sulfate and intermediate products concentration are explained respectively here.

1) Sulfate concentration measurement

Sulfate was determined by ion chromatography. An ion chromatograph IC-3000 (Dionex, Sunnyvale, USA) with an anion exchange column AS-12A (4 mm i.d., 20 cm length, Dionex) was employed with bicarbonate buffer ($2.7\text{mmol L}^{-1} \text{Na}_2\text{CO}_3$ and $0.3\text{mmol L}^{-1} \text{NaHCO}_3$) at a flow rate of 1.5 mLmin^{-1} as the eluent. Samples were filtered through $0.45\mu\text{m}$ membrane filter 25AS020, Advantec, Tokyo, Japan) to remove particles, and then injected to the ion chromatograph. Standard solutions were prepared using the Anion Mixture Standard Solution 1 (Fujifilm Wako, Osaka, Japan). The sulfate concentration was manually converted to sulfate-sulfur concentration.

2) Intermediate sulfur products concentration

The protocol is based on the one proposed by Yokoyama 2019 [97]. The determination of intermediate sulfur products in the sample was performed as follows. To an aliquot of 1.8mL sample in a 10mL screw cap glass tube, 0.2mL 1M HCl was

added, vortexed, and the mixture was kept open to air so that sulfide in the sample escape as H₂S gas. Then, 1.5mL 0.3N KOH ethanol solution and 0.5mL 30% H₂O₂ were added to the mixture, after mixing well, the tube was closed with a cap and then heated for 2hr to oxidize intermediate sulfur products. The heating temperature for Run 1 and Run 2 was 100°C, for Run 3 and control experiment, it was 80°C. After cooled to room temperature, 0.45mL 1M HCl was added and mixed to neutralize the mixture, and then this mixture was analyzed for sulfate in the same way as described in. 1). Intermediate sulfur product concentration can be obtained by the difference of sulfate concentration with and without oxidation. That is,

$$\text{Intermediate sulfur products concentration} = \left(\frac{4.45}{1.8} * X - C\right) \text{ mgS L}^{-1}.$$

where:

X: Sulfate-sulfur concentration detected after oxidation (mgS L⁻¹)

C: Sulfate-sulfur concentration in original sample (mgS L⁻¹)

3.4.3 Turbidity

During the experiment, elemental sulfur, which is one form of intermediate sulfur products and insoluble, was expected to occur in the recirculation tank, causing the turbidity in the tank. Indeed, in July when feed contained organic matter, significant development and disappearance of turbidity was observed after replacement of synthetic feed, strongly indicating that elemental sulfur colloids were accumulated to cause turbidity in the course of oxidation of sulfide to sulfate. Thus, as the indicator of elemental sulfur formation, turbidity measurement was employed. Turbidity was measured by a turbidity meter (HACH 2100Q01).

Chapter 4 RESULTS AND DISCUSSIONS

This section describes the results and discussions of the experiments.

4.1 Results

4.1.1 The sulfide removal rate

Changes in sulfide concentration with different sulfide supply in Run 1 (40mgS L⁻¹), Run 2 (80mgS L⁻¹), and Run 3 (120mgS L⁻¹) are shown in **Figure 4.1**.

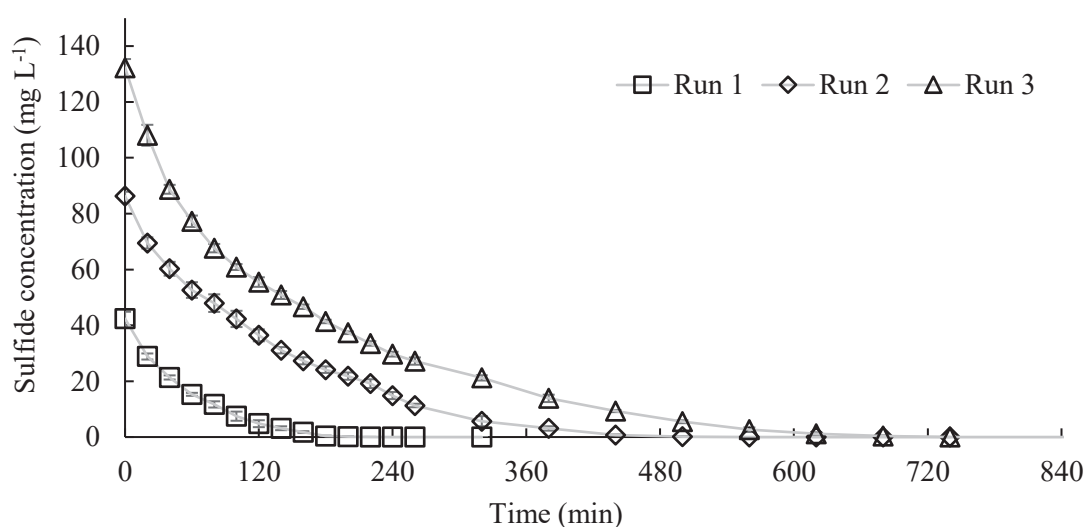


Figure 4.1 Changes in sulfide concentration in recirculation tank in Run 1 to Run 3. Each of the plots is from 3 days' average monitoring results with an error bar representing the standard deviation.

As is shown in **Figure 4.1**, during the ICOP, for Run 1 to Run 3, sulfide concentration was fully reduced in initial 200, 440 and 620 minutes after the synthetic feed was introduced, respectively. Further, all the sulfide removal showed a consistent trend of initially faster and later slower. From the sulfide reduction during the initial 80 minutes, the initial removal rates per area of sponge were calculated to be 16.2, 20.1 and 29.7gS m⁻² d⁻¹, respectively.

4.1.2 Sulfide transformation to sulfate

Sulfate is the final product for both biological and chemical sulfide oxidation process. Changes in sulfate concentration in Run 1 to Run 3 are illustrated in **Figure 4.2**.

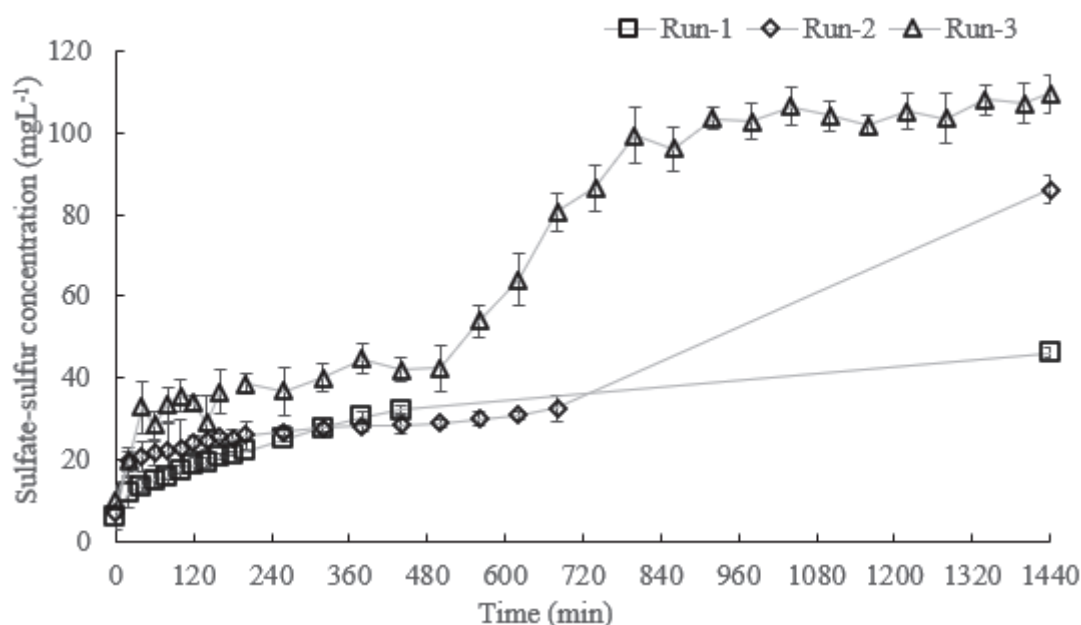


Figure 4.2 Changes in sulfate-sulfur concentration in recirculation tank for Run 1 to Run 3. Error bar: standard deviation of sulfate-sulfur concentration, $n = 3$.

Generally, sulfate concentration showed an increasing trend for all the concentrations supplied throughout the monitoring. Specifically, for Run 1, sulfate concentration was observed to gradually increase during the initial 440 minutes, and after one day's operation, the final sulfate-sulfur concentration was close to the added sulfide-sulfur concentration at 1440 minutes. For Run 2, sulfate concentration increased slowly till 680min, and it was found that the final sulfate-sulfur concentration after the reactor was operated for 1440 minutes came back to the added concentration of sulfide.

However, for Run 2, samples were not taken between 700 min and 1440 min, and thus the exact time needed for total sulfur recovery in the form of sulfate could not be known. There was possibility that sulfur concentration already reached around the added concentration before the last sample. As a result, more samples were taken and analyzed for Run 3 to determine the exact sulfate-sulfur recovery pattern. And as is shown, for Run 3 (120mgS L^{-1}), sulfate concentration increased rapidly twice in the initial 40 min and between 500min to 800min, after that, it slowly increased to around 110mgS L^{-1} at 1440 minutes, which was less than the added sulfide-sulfur concentration (120mgS L^{-1}). It indicates that more time was needed for total sulfur recovery.

4.1.3 Sulfide transformation to intermediate sulfur products

For sulfide oxidation process, in between sulfide and sulfate, there will be some intermediate products such as elemental sulfur and thiosulfate produced. **Figure 4.3** shows the variation of intermediate products concentration in Run 1 to Run 3.

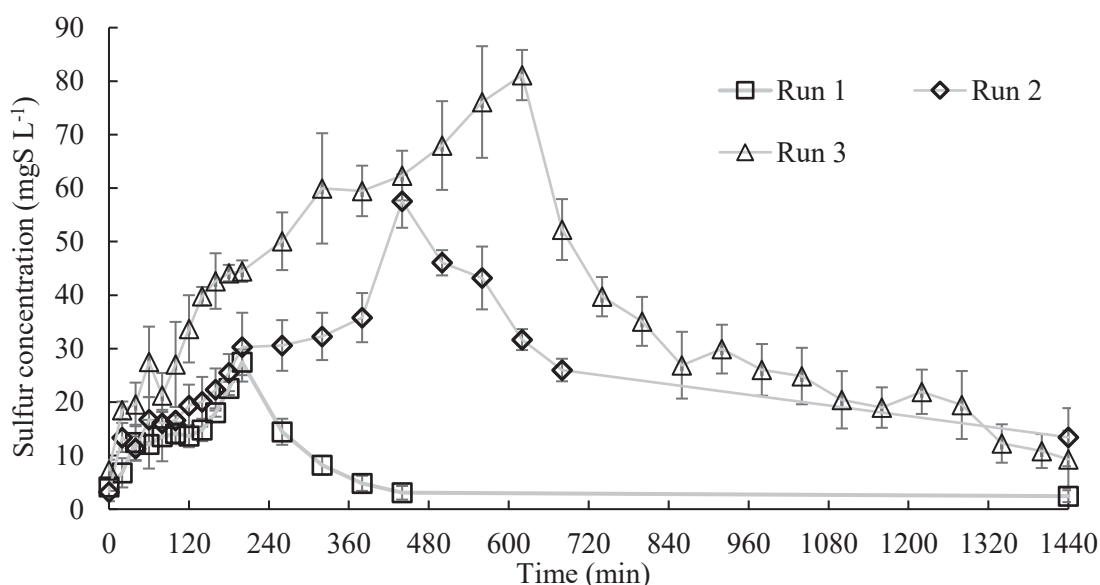


Figure 4.3 Changes in intermediate sulfur product concentration for Run 1 to Run 3. The error bar represents the standard deviation, where $n = 3$.

As is shown in this figure, for both Run 1 to Run 3, the concentration of intermediate sulfur product gradually increased and then reached its maximum at 200, 440 and 620 minutes, respectively. And these time points were exactly the time when sulfide was completely removed as shown in **Figure 4.1**. After that, intermediate sulfur product concentration was all observed to decrease till the end of monitoring at 440 and 680 minutes for Run 1 and Run 2, respectively. Moreover, in Run 3, it was observed that intermediate sulfur product concentration showed a continues downward trend from the maximum concentration at 620 minutes to 1440 minutes.

4.1.4 Turbidity

During the experiment, in order to confirm the presence of elemental sulfur, which is insoluble, turbidity of each sample taken from the recirculation tank for Runs 1 to 3 was measured. The results are shown in **Figures 4.4 to 4.6**.

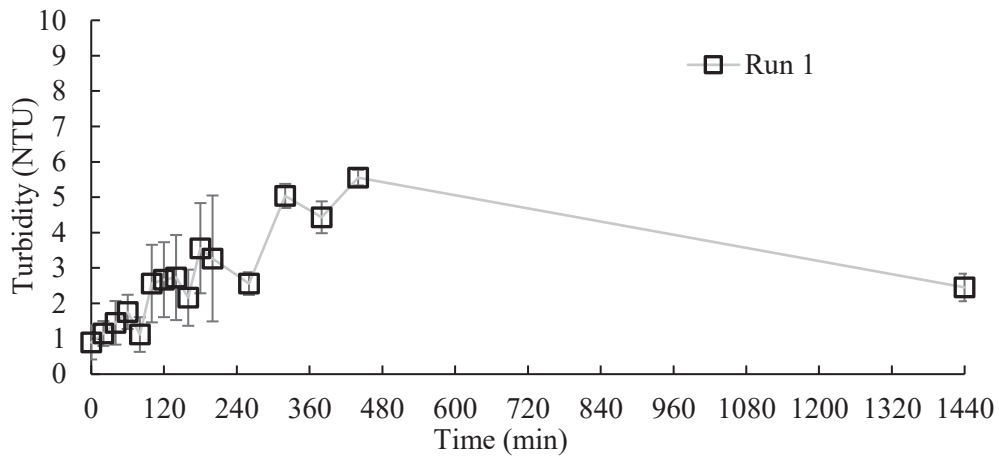


Figure 4.4 Variation of turbidity for Run 1. The error bar represents the standard deviation, $n = 3$.

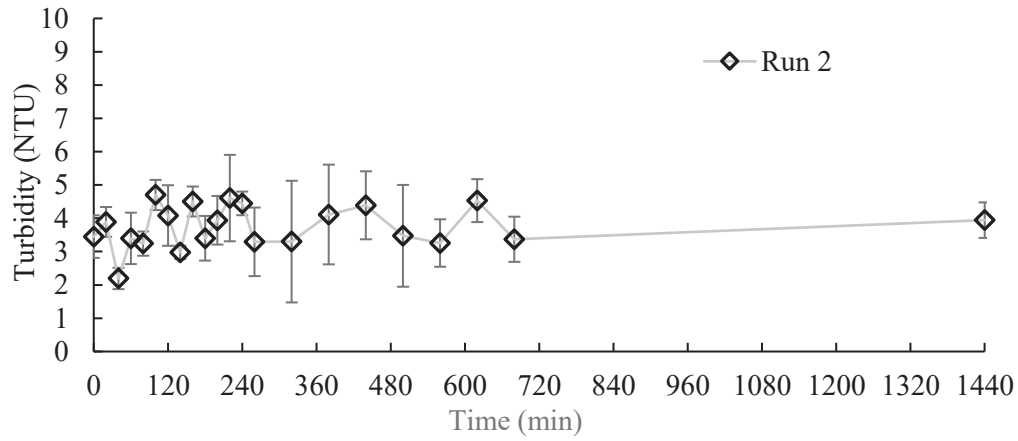


Figure 4.5 Variation of turbidity for Run 2. The error bar represents the standard deviation, $n = 3$.

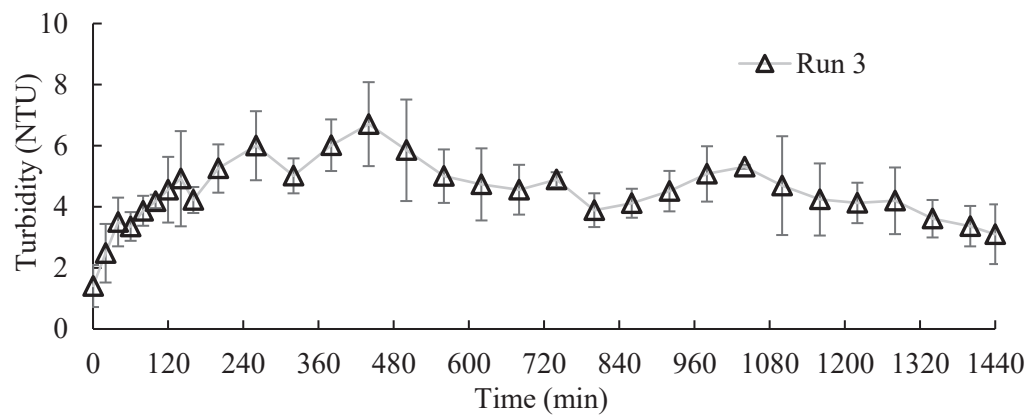


Figure 4.6 Variation of turbidity for Run 3. The error bar represents the standard deviation, $n = 3$.

As is shown in the figures above, for all the runs, during the experiment, the value of turbidity for each sample was very small and no more than 10, and the increase of turbidity value was not significant. It maybe because oxygen supply in the reactor was enough for sulfide oxidation, so there was insignificant elemental sulfur. When compared intermediate products concentration and turbidity, it was observed that there was no obvious connection between them, which indicates that the intermediate products cannot be elemental sulfur, and a large part of it may be other soluble reduced sulfur such as thiosulfate.

4.1.5 Control experiment without sponge

In this experiment, the possibility of chemical sulfide oxidation was considered. In order to clarify the effect of sulfide oxidation by chemical reaction, one liter of freshly prepared synthetic feed with 120mgS L^{-1} was recirculated through the reactor without sponge. Four parameters including sulfide concentration, sulfate concentration, intermediate sulfur concentration and turbidity were observed. The variation of each related parameter was monitored and plotted against time as shown in **Figure 4.7**.

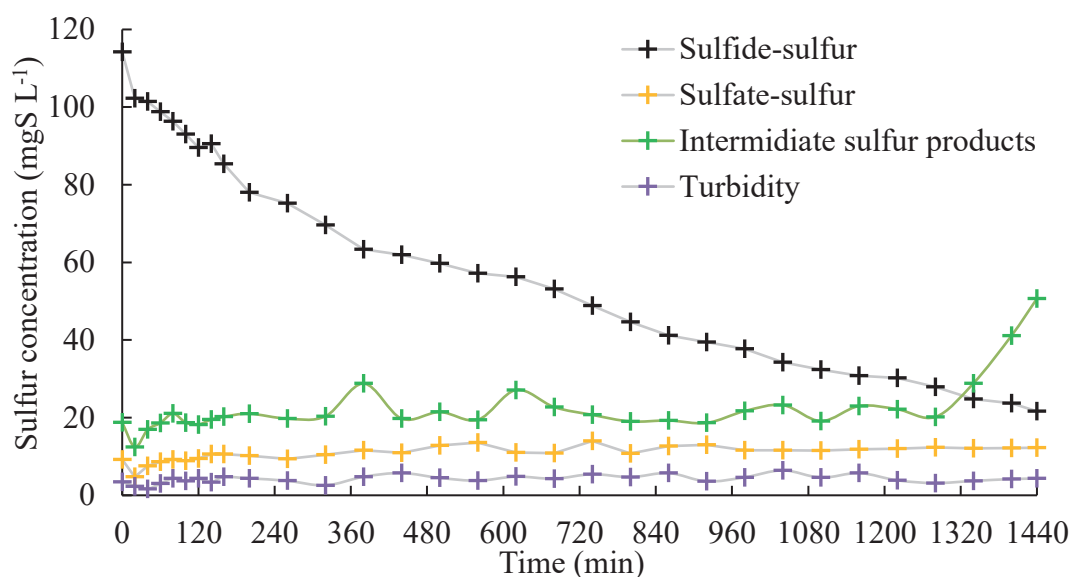


Figure 4.7 Sulfide transformation and turbidity change for control experiment. Each of the plots is from 1 days' monitoring results.

From the figure, it was found that sulfide concentration showed a gradually decreasing trend throughout the monitoring. Initial sulfide removal rate per area of sponge during the initial 80 minutes was $10.72\text{gS m}^{-2} \text{d}^{-1}$, which was about one third of the removal rate in Run 3 (120mgS L^{-1} and with sponge) of $29.7\text{gS m}^{-2} \text{d}^{-1}$.

According to the result, even without biomass and sponge, sulfide was removed to some extent. It reveals that sulfide removal was partly attributed to chemical oxidation. For sulfate concentration, it gradually increased during the initial 160

minutes to around 10mgS L⁻¹ and then maintained a slowly increasing trend till the end of the monitoring to reach around 12mgS L⁻¹. The intermediate sulfur concentration maintained low level and experienced several small fluctuations before, and then increased dramatically after 1320 minutes' operation till 1440 minutes. During the experiment, turbidity was very small and the variation of turbidity value was not significant.

4.2 Discussions

The discussions focus on two issues. First, in 4.2.1, sulfur removal rate is discussed. Then, in 4.2.2, sulfide transformation process during the ICOP is discussed

4.2.1 Sulfide removal rate

As was mentioned in section 4.1.1 using **Figure 4.3**, sulfide removal was faster initially and later slower. Considering the sulfide oxidation process, it may be attributed to the lower oxygen availability for sulfide in the reactor. Initially, the amount of produced intermediate sulfur products was not significant, as a result, the oxygen in the reactor can be primarily used by sulfide. However, as the reaction progressed, intermediate sulfur products accumulated much more than before in the reactor, thus there will be competition between sulfide and intermediate sulfur products for oxygen. Therefore, the sulfide removal rate became slower.

The sulfide removal rate per unit area of sponge in initial 80 minutes was calculated as follows:

$$\text{Sulfide removal rate (gS m}^{-2} \text{ d}^{-1}) = \frac{(\text{Sulfide}_1 - \text{Sulfide}_2) \times \text{Volume}}{\frac{A}{\left(\frac{80}{1440}\right) \times 1000}}$$

Sulfide₁ = the sulfide concentration of freshly prepared synthetic feed (mg L⁻¹)

Sulfide₂ = the sulfide concentration measured at 80 minutes after treatment (mg L⁻¹)

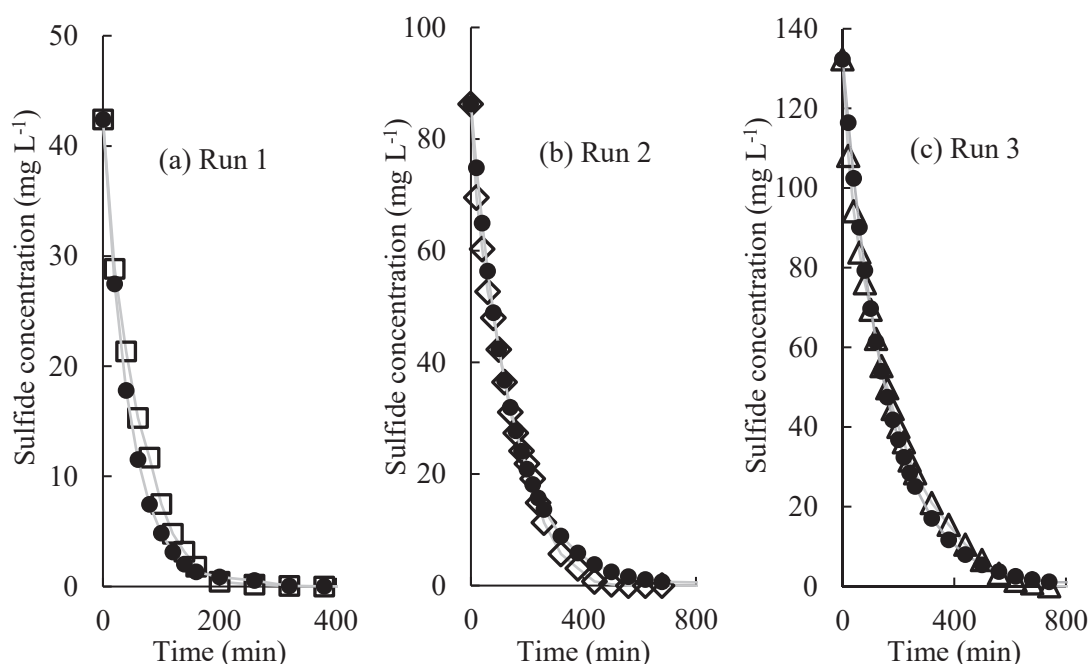
A = surface area of sponge (m²)

Volume = volume of synthetic sewage supplied (L d⁻¹)

The result of sulfide removal rate in initial 80 minutes for each run was different as was described in Section 4.1.1. In order to figure out the sulfide removal kinetics, the first order model for each run was proposed in **Table 4.1**, and then compared the expected removal pattern from first order model with the actual observation. As shown in following **Figure 4.8**:

Table 4.1. First order reaction model for each run

Conditions	Run 1 (40mgS L ⁻¹)	Run 2 (80mgS L ⁻¹)	Run 3 (120mgS L ⁻¹)
First order reaction model	$C=42.39*e^{(-0.0217t)}$	$C=86.28*e^{(-0.0071t)}$	$C=135.34*e^{(-0.0064t)}$

**Figure 4.8** Comparison between sulfide removal from experimental data and model estimation for Run 1 to Run 3.

It was found that there were some discrepancies between the experimental data and expected sulfide removal model, and the rate constant for Run-1 was quite different from that of Run 2 and Run 3. For Run 2 and Run 3, the similar low rate constant may be attributed to the decrease of microorganism activity caused by the inhibition from produced hydrogen sulfide.

The factors that affect the sulfide removal rate during the ICOP may include initial sulfide concentration, microbial activity, and oxygen availability to microorganisms. Moreover, whether the removal rate in initial 80 minutes can be sustained for one day is unknown, thus flow-through experiment is necessary to achieve continuous high sulfide load supply. To generalize this removal pattern for real application, experiments under different conditions such as different temperature and oxygen concentration should be conducted. Note that in my study, the meaning of sulfide

removal only refers to the disappearance of sulfide, and it need not involve the full oxidation to form sulfate. However, if sulfide remaining in water as intermediated products, it can consume oxygen in latter treatment process.

4.2.2 Sulfide transformation process

To grasp the sulfur conversion from sulfide to sulfate from a whole picture during the ICOP, sulfur mass balance for each run and for control experiment was made (**Figure 4.9**). And several implications were obtained from it:

1. During the ICOP, some part of the removed sulfide was converted into intermediate products and then finally to sulfate.
2. From the figures, the total sulfur mass showed a decreasing trend first and then finally came back in Runs 1 to 3. The disappearing sulfur was suspected to be captured in the sponge. However, the results from control experiment without sponge also showed the disappearing sulfide. In control experiment, as sulfide was gradually removed, intermediate sulfur products, sulfate as well as turbidity did not increase significantly. It indicated that the disappearing sulfide was not oxidized to elemental sulfur but was converted to other soluble sulfur species, which has higher oxidation number than elemental sulfur and cannot be detected by the protocols used in this study to measure intermediate products. Further exploration is needed to determine this form.

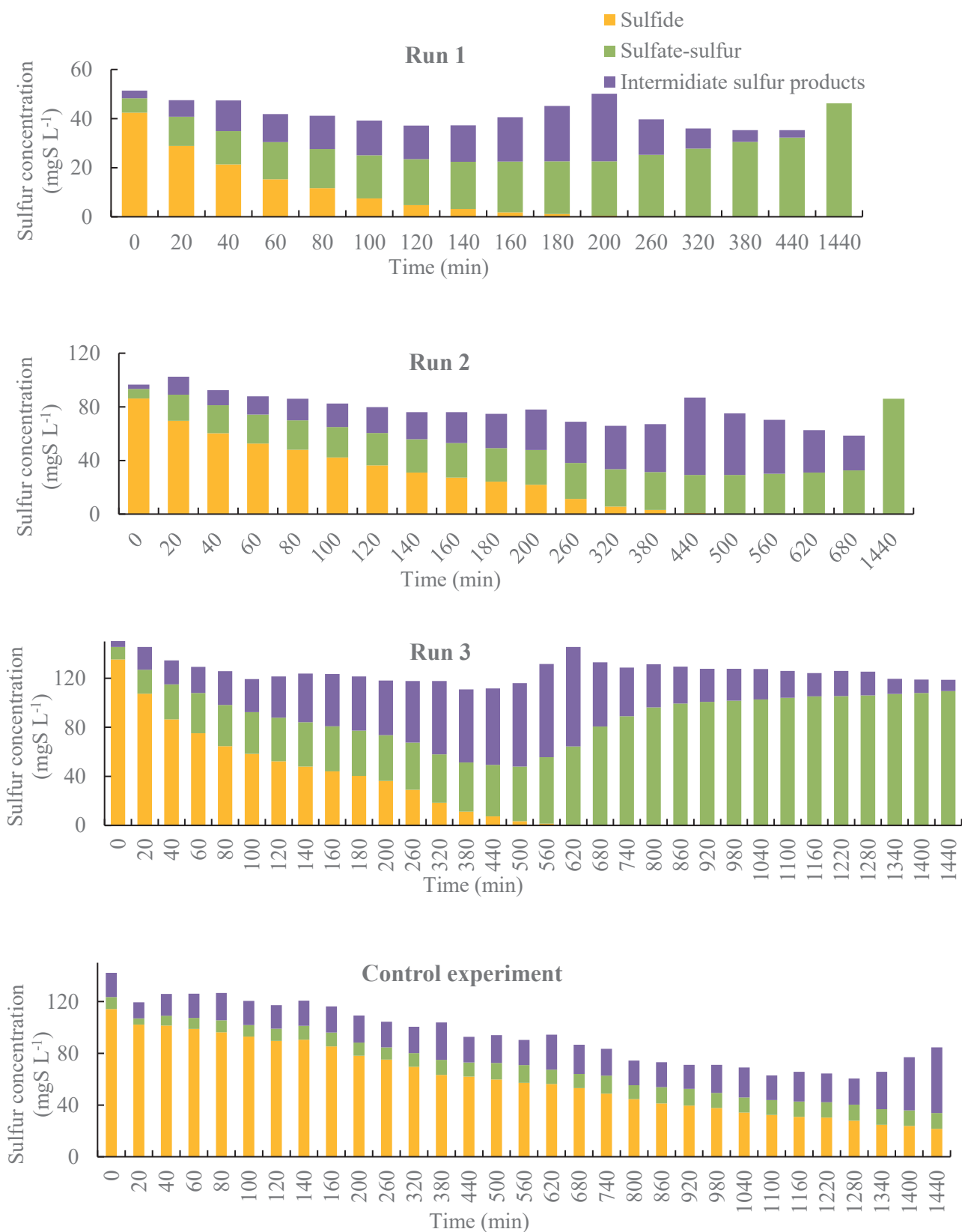


Figure 4.9 Sulfur mass balance for Runs 1 to 3 and for control experiment.

Chapter 5 CONCLUSIONS

5.1 Key Findings

This study was generally aimed to evaluate sulfide removal performance during the ICOP. This aim constitutes of two specific objectives, findings of which are provided below:

The first specific objective is to evaluate the sulfide removal rate by ICOP. From this study, the maximum sulfide removal rate can up to $29.7 \text{ gS m}^{-2} \text{ d}^{-1}$ by ICOP with initial sulfide concentration of 120 mgS L^{-1} .

The second objective is to determine the sulfide transformation process. It was found that removed sulfide was finally converted to sulfate via intermediate products. Further, during this process, while chemical reaction contributed to some extent, microbial activity played significant role in the removal and oxidation of sulfide.

5.2 Limitations and suggestions

Limitations and suggestions of this study are described as follows.

In my study, there will be some limitations in the practical application of the reactor used in this study due to the short length, rectangular shape as well as recycled flow compared with real sewer pipe and one-flow sewage.

ICOP shows promising sulfide removal performance with no energy need for oxygen supply. It has high potential to be applied as an alternative method to treat sulfide-containing wastewater in sewer pipe transportation process and sewage treatment plants. However, to further understand sulfide removal mechanism, sulfide removal in the sponge media and the real composition of intermediate sulfur products should be investigated. More exploration is needed for practical application of ICOP for the removal of sulfide.

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