การขจัดไฮโดรเจนซัลไฟด์โดยไนเทรตรีดิวซ์ซิงและซัลไฟด์ออกซิไดซิงแบคทีเรียในถังปฏิกรณ์ชีวภาพไร้ออกซิเจน

นางสาววรรณพร วัฒน์สุนธร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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HYDROGEN SULFIDE REMOVAL BY NITRATE REDUCING AND SULFIDE OXIDIZING BACTERIA IN ANOXIC BIOREACTORS

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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Biotechnology
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Thesis Title	HYDROGEN SULFIDE REMOVAL BY NITRATE REDUCING AND SULFIDE OXIDIZING BACTERIA IN ANOXIC BIOREACTORS
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Field of Study	Biotechnology
Thesis Advisor	Associate Professor Warawut Chulalaksananukul, Ph.D.
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วรรณพร วัฒน์สุนธร : การขจัดไฮโดรเจนซัลไฟด์โดยไนเทรตรีดิวซ์ซิงและซัลไฟด์ออกซิไดซิงแบคทีเรียในถังปฏิกรณ์ชีวภาพไร้ออกซิเจน (HYDROGEN SULFIDE REMOVAL BY NITRATE REDUCING AND SULFIDE OXIDIZING BACTERIA IN ANOXIC BIOREACTORS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. วรวุฒิ จุฬาลักษณานุกูล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร.เอลดอน รีน, 124 หน้า.

ไนเตรทรีดิวซ์ซิงและซัลไฟด์ออกซิไดซิงแบคทีเรีย สายพันธุ์ MAL 1HM19 เป็นแบคทีเรียที่ได้รับการคัดแยกจากบ่อน้ำพุร้อน แม่อุมลองหลวง จังหวัดแม่ฮ่องสอน ประเทศไทย ซึ่งสามารถใช้ก๊าซไฮโดรเจนซัลไฟด์ เป็นตัวให้อิเล็กตรอน และใช้ไนเตรทเป็นตัวรับอิเล็กตรอน พบว่าแบคทีเรียสายพันธุ์ MAL 1HM19 มีประสิทธิภาพในการขจัดก๊าซไฮโดรเจนซัลไฟด์ได้ถึงร้อยละ 100 โดยสามารถขจัดก๊าซไฮโดรเจนซัลไฟด์ที่ความเข้มข้นเริ่มต้น 650 ppm_v หมดภายใน 5.5 ชั่วโมง ภายใต้ภาวะไร้ออกซิเจน ซึ่งเป็นระยะเวลาที่สั้นที่สุดเมื่อเปรียบเทียบกับไอโซเลตอื่นๆ ผลของการจำแนกชนิดของแบคทีเรียด้วยการเปรียบเทียบลำดับเบสของ 16S rDNA กับฐานข้อมูล NCBI พบว่ามีความใกล้เคียงกับแบคทีเรีย *Paracoccus versutus* ถึงร้อยละ 99.93 โดยแบคทีเรีย *P. versutus* สายพันธุ์ MAL 1HM19 สามารถเจริญเติบโตได้ในโซเดียมคลอไรด์ความเข้มข้นร้อยละ 0.03 ถึง 7, อุณหภูมิ 20 ถึง 50 องศาเซลเซียส และพีเอช 7.0 ถึง 9.0 และจากการทดลองแบบกะ พบว่าแบคทีเรียสายพันธุ์นี้สามารถขจัดก๊าซไฮโดรเจนซัลไฟด์ได้หมดภายใต้ภาวะความเข้มข้นที่แตกต่างกันของไนเตรท (60, 120 และ 240 มิลลิกรัมไนเตรท-ไนโตรเจนต่อลิตร) ที่อุณหภูมิ 35 องศาเซลเซียส ภายใน 10 ชั่วโมง โดยมีซัลเฟตหรือซัลเฟอร์เป็นผลิตภัณฑ์ที่ได้จากกระบวนการขจัดก๊าซไฮโดรเจนซัลไฟด์ ซึ่งขึ้นอยู่กับความเข้มข้นของไนเตรทเริ่มต้นที่ใช้ นอกจากนี้เมื่อใช้แบคทีเรียสายพันธุ์ MAL 1HM19 เป็นหัวเชื้อ และตรึงในวัสดุกรองประเภทโพลียูรีเทนโฟม ในถังปฏิกรณ์ชีวภาพไร้ออกซิเจนชนิดไบโอทริคคลิ่งฟิลเตอร์ เป็นเวลา 188 วัน ในระบบกะป้อนและระบบต่อเนื่อง โดยพบว่าความเข้มข้นเริ่มต้นของก๊าซไฮโดรเจนซัลไฟด์ที่ใช้อยู่ระหว่าง 100 ถึง 500 ppm_v ในภาวะคงตัว และ 1000, 2000, 3000 และ 4000 ppm_v ในภาวะ shock loads มีค่าประสิทธิภาพในการลดก๊าซไฮโดรเจนซัลไฟด์อยู่ระหว่างร้อยละ 17 ถึง 100 ซึ่งขึ้นอยู่กับแหล่งคาร์บอน และระบบที่ใช้ในการทดลอง นอกจากนี้ค่าความสามารถสูงสุดในการบำบัดก๊าซไฮโดรเจนซัลไฟด์ที่พบในระบบนี้คือ 121.83 ± 0.1 กรัม ซัลไฟด์ต่อชั่วโมงต่อลูกบาศก์เมตร ร้อยละ 96.5 ของประสิทธิภาพในการลดก๊าซไฮโดรเจนซัลไฟด์) ที่ความเข้มข้นเริ่มต้นของก๊าซไฮโดรเจนซัลไฟด์ 4000 ppm_v จากงานยังพบอีกว่าไนเตรทรีดิวซ์ซิงและซัลไฟด์ออกซิไดซิงแบคทีเรีย *P. versutus* สายพันธุ์ MAL 1HM19 มีประสิทธิภาพในการลดก๊าซไฮโดรเจนซัลไฟด์ทั้งในรูปเชื้ออิสระและเซลล์ตรึงรูป และสามารถนำไปประยุกต์ใช้ในอุตสาหกรรมที่มีการปนเปื้อนของก๊าซไฮโดรเจนซัลไฟด์และไนเตรทได้ในหลากหลายภาวะ

สาขาวิชา เทคโนโลยีชีวภาพ ลายมือชื่อนิสิต .....

ปีการศึกษา 2560 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5672872723 : MAJOR BIOTECHNOLOGY
KEYWORDS: NR-SOB / HYDROGEN SULFIDE REMOVAL / PARACOCCUS VERSUTUS STRAIN MAL 1HM19 / BIOTRICKLING FILTER / ANOXIC BIOREACTORS / HOT SPRINGS

WANNAPAWN WATSUNTORN: HYDROGEN SULFIDE REMOVAL BY NITRATE REDUCING AND SULFIDE OXIDIZING BACTERIA IN ANOXIC BIOREACTORS. ADVISOR: ASSOC. PROF. WARAWUT CHULALAKSANANUKUL, Ph.D., CO-ADVISOR: ELDON RENE, Ph.D., 124 pp.

The strain MAL 1HM19, a nitrate reducing and sulfur oxidizing bacteria (NR-SOB) was successfully isolated from Mae Um Long Luang hot spring from Mae Hong Son province (Thailand) using hydrogen sulfide (H_2S) and nitrate (NO_3^-) as an electron donor and acceptor, respectively. Among the isolates of NR-SOB from different sources, the strain MAL 1HM19 was the most promising novel strain of NR-SOB because of its ability to yield ~100% H_2S removal, under anoxic conditions, within 5.5 h at an initial H_2S concentration of 650 ppm_v. The identification of strain MAL 1HM19 based on the 16S rDNA nucleotide sequence suggests that this strain is closely related to *Paracoccus versutus* and is the member of *Alphaproteobateria* with 99.93% sequence similarity. The *P. versutus* strain MAL 1HM19 was also able to grow at various NaCl concentrations (0.03-7%), in a pH range of 7.0-9.0 and at temperatures of 20-50°C. The ability of H_2S removal by the *P. versutus* strain MAL 1HM19 under the influence of different initial NO_3^--N concentrations (60, 120 and 240 mg NO_3^--N/L), at 35°C, was investigated for 96 h. The results showed that 100% of H_2S oxidation was attained within 10 h, irrespective of the different initial NO_3^--N concentrations. The final end product [sulfate (SO_4^{2-}) or elemental sulfur (S°)] depended on the concentrations of NO_3^--N. In the long-term experiments, i.e. in a biotrickling filter (BTF), biological H_2S removal was investigated by inoculating the pure cultures of *P. versutus* strain MAL 1HM19 in anoxic BTF for 188 d. The BTF was packed with polyurethane foams (PUFs) cubes and the reactor was operated under anoxic conditions in both fed-batch and continuous modes. The H_2S inlet concentrations were varied between 100 and 500 ppm_v during steady-state experiments, while during H_2S shock load tests, the concentrations were increased to 1000, 2000, 3000 and 4000 ppm_v, respectively. The removal efficiency (RE) of H_2S varied between 17 and 100% depending on operational mode of the BTF and the addition of the C source. The maximum elimination capacity (EC_{max}) was achieved at 121.83 ± 0.1 g S/m^3 h (RE - 96.5 %) during H_2S shock load experiments at 4000 ppm_v. The results from this study demonstrated that both free and immobilized cells of *P. versutus* strain MAL 1HM19 can be efficiently used in industrial situations to remove H_2S and NO_3^- under a wide range of operating conditions.

Field of Study: Biotechnology
Academic Year: 2017

Student's Signature .....
Advisor's Signature .....
Co-Advisor's Signature .....

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LIST OF ABRREVIATIONS

AD	Anaerobic digestion
BF	Biofilter
BTF	Biotrickling filter
BS	Bioscrubber
DNA	Deoxyribonucleic acid
EBRT	Empty bed residence times
EDS	Energy dispersive X-ray spectroscopy
EC	Elimination capacity
IC	Ion chromatography
ILR	Inlet loading rate
NR-SOB	Nitrate-reducing and sulfide-oxidizing bacteria
ppm_v	Parts per million by volume
PUF	Polyurethane foam
RE	Removal efficiency
Rpm	Revolution per minute
SEM	Scanning electron microscope
SOB	Sulfur oxidizing bacteria
SQR	Sulfide quinone reductase
SRB	Sulfate-reducing bacteria
TLV	Trickling liquid volume
VFA	Volatile fatty acid

LIST OF SYMBOLS

mCSB	Modified Coleville synthetic brine medium
He	Helium
H_2S	Hydrogen sulfide
N_2	Nitrogen
NO_3^--N	Nitrate-nitrogen
NO_2^--N	Nitrite-nitrogen
^oC	Degree Celsius
SO_4^{2-}	Sulfate
$S_2O_3^{2-}$	Thiosulfate

CHAPTER I
INTRODUTION

1.1 Background

Hydrogen sulfide (H_2S) is a major problem in sewer systems, wastewaters from the pulp and paper industry and during biogas production from anaerobic digestion (AD) due to its unpleasant odor, corrosive nature, and toxicity. It is produced by sulfate-reducing bacteria (SRB) that can use sulfate as a terminal electron acceptor for the degradation of sulfur-containing and other proteinaceous compounds. Biological process has been widely used for H_2S removal because this process is cost effective and environmental friendly. The anaerobic removal of H_2S has proven to be a good strategy to eliminate H_2S because it helps to avoid explosion risk and does not affect the calorific value of the biogas (Pokorna and Zabranska, 2015)In this regard, nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB) play an important role during the biodegradation process because these microorganisms can remove H_2S using nitrate (NO_3^-) as the electron acceptor and oxidizes sulfide to sulfate (SO_4^{2-}) or elemental sulfur (S^0). Literature reports have shown that the stoichiometry of this microbial mediated reaction depends on the ratio of sulfide (S^{2-}) to NO_3^- present in the wastewater (Li *et al*., 2011).

This research focused on the screening, isolation and characterization of NR-SOB from several natural hot springs located in Thailand. The kinetics of H_2S oxidation and its removal from synthetic biogas was tested under batch incubation conditions. Moreover, concerning continuous removal of H_2S, among the different bioreactor configurations tested and reported so far in the literature, anaerobic biotrickling filters (BTF) have proven to be advantageous because it offers low

pressure drop, it does not alter the composition of biogas and reduce the methane concentrations, simple media regeneration procedure, system durability and reliability during shock loads.

1.2 Problem statement

H_2S is a harmful and smelly gas that causes significant effect on the environment and human health. H_2S is produced from various sources including volcanoes and geothermal areas as well as due to human activities (Somma *et al*., 2017). For example, typical H_2S content in raw biogas ranges from 0.1 to 2%, i.e., 1000 to 20,000 ppm_v, resulting in corrosive acids that corrodes metallic components, pipes and other accessories involved in biogas technology (Tóth *et al*., 2015; Staicu *et al*., 2017). Moreover, H_2S is one of the reduced sulfur compounds (RSC) generated from the Kraft's pulping process during the pulp and paper production (Wani *et al*., 1999).

Over the last decades, many methods and technologies have been developed and patented for H_2S removal including physical, chemical and biological processes. The most commonly used methods are the physico-chemical processes. For example, the H_2S contaminants in biogas production are treated by simple adsorption of H_2S on the solid particles (Ryckebosch *et al*., 2011). However, there are many drawbacks including the production of secondary pollutants (Gómez and Cantero, 2007), the high initial investment costs and chemical consumption.

To overcome the problem, biological processes to remove H_2S have been recommended as an appropriate treatment strategy because it has proven to be cost effective, energy efficient and yield high removal of H_2S under steady and transient operating conditions (Fernández *et al*., 2014). Using pure isolates of bacteria for H_2S

biodegradation can be considered as an alternative method because pure cultures have a short lag-phase and require short culture times to deplete the sulfide species (H_2S and S^{2-}) present in wastewater (Watsuntorn *et al*., 2017). Moreover, the use of pure cultures can also improve the removal capacities and efficiencies of bioreactor systems by its incorporation in biofilms (Wang *et al*., 2005; Aroca *et al*., 2007). To the best of our knowledge, there are only few works that have reported the application of pure culture of microorganisms isolated from natural sources for the biodegradation of H_2S in batch and continuous systems under anoxic conditions.

1.3 Objectives

1.3.1 To isolate and characterize new strains of nitrate reducing, sulfide oxidation bacteria (NR-SOB) from natural sources

1.3.2 To apply the screened and selected NR-SOB for biological H_2S removal

1.3.3 To ascertain H_2S removal in batch experiments under different environmental conditions.

1.3.4 To envisage the maximum elimination capacity of a BTF for H_2S removal and determine the degradation kinetics in the presence of NO_3^--N

1.4 Scope of study

1.4.1 This work was carried out at the laboratory scale and under controlled conditions temperature and pressure.

1.4.2 This research aims to isolate and characterized the novel strain of NR-SOB from the hot springs in Thailand.

1.4.3 The novel isolate that showed the best performance for H_2S removal was selected and applied to remove the H_2S in both batch and continuous bioreactor.

1.5 Dissertation structure

This thesis is composed of five chapters. An overview of the structure of this thesis is shown in Figure 1.1.

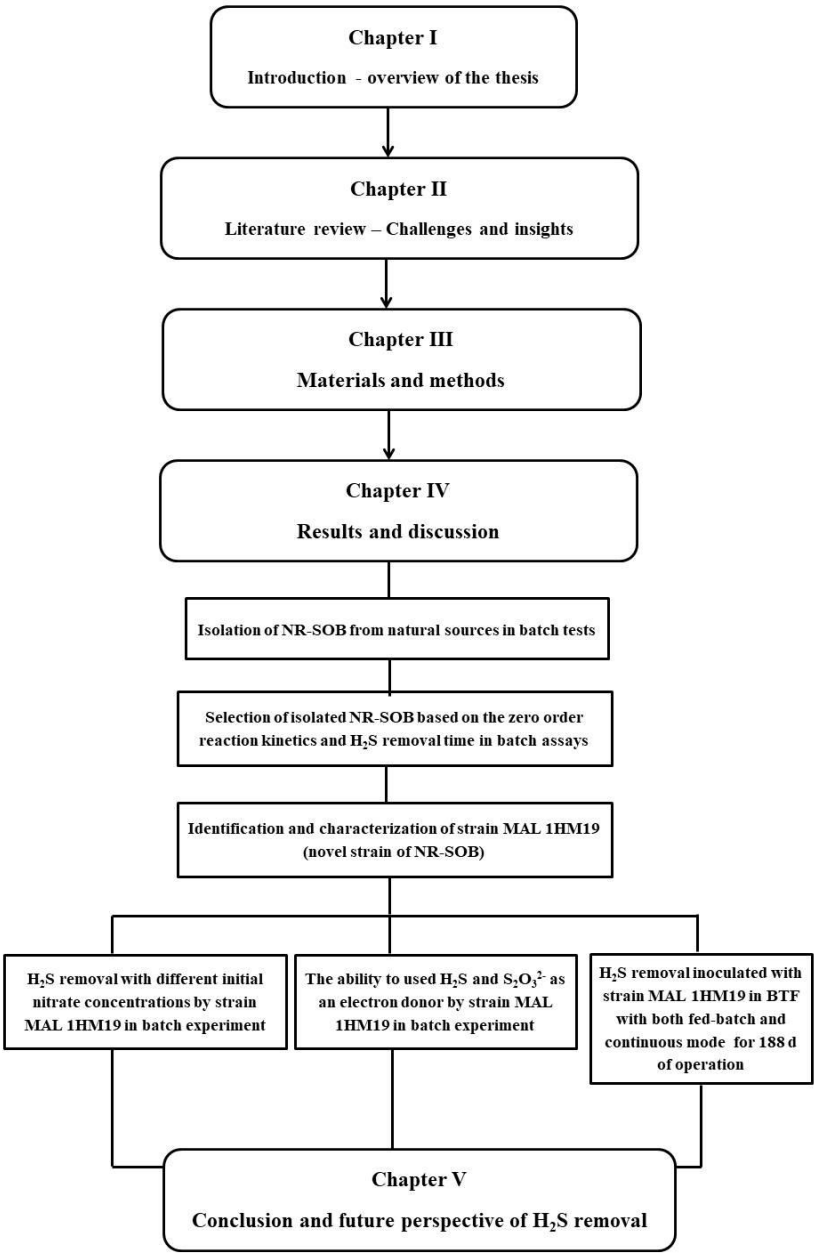


Figure 1.1 Overview of chapters in this Ph.D. dissertation.

CHAPTER II
LITERATURE REVIEW

2.1 Hydrogen sulfide

Hydrogen sulfide (H_2S) is one of the sulfur compounds in the sulfur cycle (Figure 2.1). This compound is highly toxic to living organisms, flammable and it has an unpleasant odor (Chou and WHO, 2003). H_2S is a weak acid which can be dissociated into bisulfide (HS^-) and sulfide (S^{2-}). The pK_a values are 6.90 and 12.92 (at 25°C), respectively (Weast *et al*., 1989). This gas is produced by sulfate reducing bacteria (SRB) *via* the degradation of sulfur-containing compounds and other proteinaceous substances present in water under anaerobic conditions. H_2S is produced naturally from several sources such as hot spring, sulfide spring, crude petroleum, and volcanic gases (Beauchamp *et al.*, 1984). Moreover, various concentrations of H_2S are also generated largely as a by-product from plants. In the pulp and paper industry, this waste gas was generated from the Kraft process. H_2S is also produced from agricultural wastes, anaerobic digestion (AD) and from landfills (Tang *et al*., 2009).

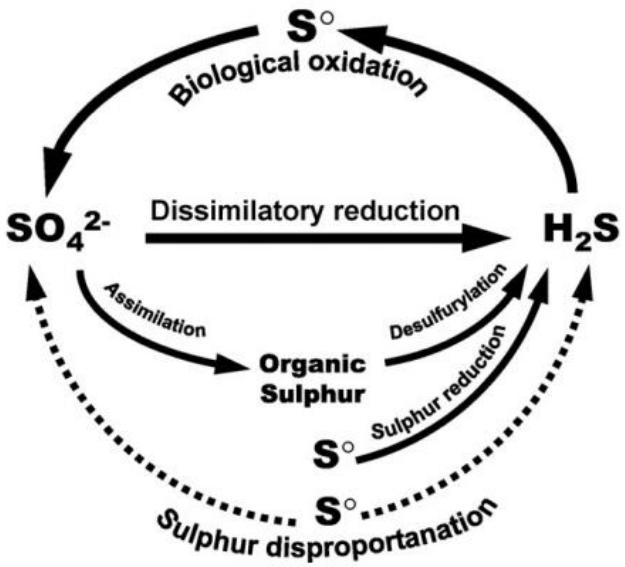


Figure 2.1 Sulfur cycle (Tang *et al*., 2009).

H_2S is highly toxic to the microorganisms. H_2S adversely affects the human health (Beauchamp *et al*., 1984). The toxic effects of H_2S on human health is summarized in Table 2.1 (Beauchamp *et al*., 1984). The maximum allowable concentration (MAC) of H_2S in a workplace is 1.6 ppm_v in the Netherlands and 10 ppm_v for time weighted average (TWA) in USA (van den Bosch, 2008). In brief, inhalation of H_2S can react with the enzymes product in the blood circulation system, that causes pulmonary paralysis and resulting in the death (Syed *et al*., 2006). Inhalation of low concentrations, as low as 10 mg/L, cause the inhibition of the respiration because of the reactions between H_2S and the iron from cytochromes in the human body. There is also evidence showing that, concentrations above 0.002 ppm of H_2S, can have health effects in some insects, suckers, amphipods, minnows and aquatic organisms (Beauchamp *et al*., 1984). Moreover, H_2S can be oxidized to sulfur oxide (SO_2) during combustion process that also causes the acid rain.

Table 2.1 Effect of H_2S on human health at various concentrations of H_2S (Beauchamp *et al*., 1984; Chou and WHO, 2003).

Exposure (mg/m^3)	**Exposure (ppm_v)**	**Effects and observation**
0.011	0.008	Odor threshold
2.8	2	Bronchial constriction in asthmatic individuals
5.0	3.57	Increased eye complaints
7 or 14	5 or 10	Increased blood lactate concentration, decreased skeletal muscle citrate synthase activity, decreased oxygen uptake and sore eye
5-29	3.57-20.71	
28	20	Eye irritation
140	100	Fatigue, loss of appetite, headache, irritability, poor memory and dizziness
> 140	> 100	Loss of smell in 3 to 15 min, may sting eyes and throat
210	150	Olfactory paralysis
350	250	Olfactory nerve paralysis
> 560	> 400	Prolonged exposure may cause pulmonary edema
700	500	Respiratory distress
980	700	Dizziness, breathing ceases in a few minutes, need prompt artificial respiration
1,400	1,000	Unconscious almost instantly and death can occur
7,000	5000	Rapid collapse, nervous system paralysis, unconscious at once and death within minutes
		Imminent death

2.2 H_2S production by sulfate reducing bacteria

Sulfide species (H_2S, HS^- and S^{2-}) are produced by the sulfate reducing bacteria (SRB) which play an important role in the sulfur cycle. Sulfide is generated *via* dissimilatory sulfate reduction pathway under anaerobic condition. The members of this group use sulfate (SO_4^{2-}) as the electron acceptor as well as use various substrates as electron donor such as hydrogen (H_2), ethanol (C_2H_6O), fumalate ($C_4H_4O_4$), lactate ($C_3H_6O_3$), organic acids and other alcohols (Chen *et al*., 2008; Muyzer and Stams, 2008). Despite the utilization of diverse electron donors, H_2 is the most suitable electron donor for SRB as shown by the rank of the affinity of electron donors of SRB in the order of: H_2 > propionate > other organic electron donors (Chen *et al*., 2008; Pokorna and Zabranska, 2015), as shown in equations (2.1), (2.2) and (2.3):

$$4H_2 + SO_4^{2-} + H^- \rightarrow HS^- + 4H_2O,\ \Delta G° = -151.9\ \text{kJ/reaction} \quad (2.1)$$

$$C_3H_5O_2^- + 0.75SO_4^{2-} \rightarrow CH_3COO^- + HCO_3^- + 0.75HS^- + 0.25H^+,\ \Delta G° = -37.7\ \text{kJ/reaction} \quad (2.2)$$

$$C_4H_7O_2^- + 0.5SO_4^{2-} \rightarrow 2CH_3COO^- + 0.5HS^- + 0.5H^+,\ \Delta G° = -27.8\ \text{kJ/reaction} \quad (2.3)$$

2.3 Methods of H_2S removal

Due to the toxic effects of H_2S, the removal of H_2S is of almost importance. The removal of H_2S from the gas phase is the first suggestion in order to prevent the emission of sulfur dioxide (SO_2) during the combustion and control corrosion problems. However, the removal of sulfide from the aqueous phase which is also product in many wastewaters is still important. The chemical, thermal, scrubbing and adsorption are the common methods used for H_2S removal (Shareefdeen *et al*., 2002; Syed *et al*., 2006). There are many physico-chemical methods that have been

commonly used for H_2S removal from the natural gas, landfills gas such as the Lo-Cat and the Claus process (Klok *et al*., 2012).

However, the chemical methods require high energy and require chemical costs as well as leads to generation of secondary waste streams. H_2S removal by biological processes is desirable because these processes can compensate the drawbacks of chemical and physical methods. For example, biological methods are environmental friendly and it can be performed at low cost compared to the chemical and physical processes (De Gusseme *et al*., 2009).

2.4 Pathway of H_2S removal

The H_2S removal is done via the H_2S oxidation pathway. There are two enzymatic pathways, the sulfur-oxidizing (Sox) system pathway and the sulfide quinone oxidoreductase (SQR) pathway to oxidize H_2S to SO_4^{2-} (Figure 2.2). In the Sox system pathway, H_2S is converted to SO_4^{2-} by binding with the complex of SoxY-cysteine-sulfur (Poser *et al*., 2014). In addition, The Sox system can oxidize sulfide and other sulfur compounds including thiosulfate ($S_2O_3^{2-}$), sulfite (SO_3^{2-}) and elemental sulfur (S°) to SO_4^{2-} (Friedrich *et al*., 2005). While in the SQR system, H_2S is first converted to sulfite (SO_3^{2-}). The six electrons from sulfide are transferred to the system of cell electron transport and pass to the terminal electron acceptor. In this case, the terminal electron acceptor is the nitrate (NO_3^-) or nitrite (NO_2^-). After that, adenosine monophosphate (AMP) is produced by the activity of adenosine phosphosulfate reductase and converted to adenosine diphosphate (ADP), respectively. During the step of ADP production, high energy phosphate bond is formed. Finally, the SO_4^{2-} is the final product from the dissimilatory sulfide oxidation pathway (Tang *et al*., 2009).

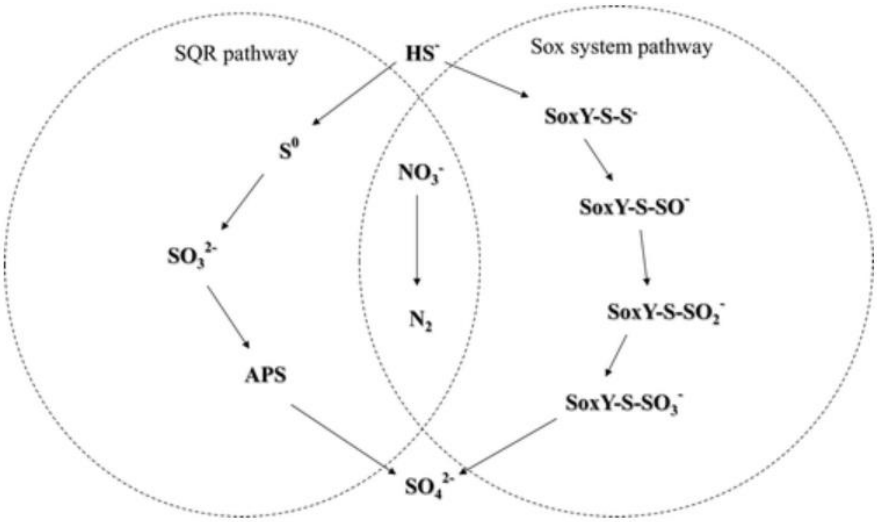


Figure 2.2 Sulfide oxidation pathway (Poser *et al*., 2014).

2.5 Nitrate reducing and sulfide oxidizing bacteria

Biological H_2S removal methods using nitrate-reducing and sulfide-oxidizing bacteria (NR-SOB) have been developed during the past two decades (Watsuntorn *et al*., 2017). NR-SOB are the group of bacteria that can use H_2S as an electron donor and nitrate (NO_3^-) as an electron acceptor. NR-SOB play the major roles for removal both H_2S and nitrate by linking between sulfur and nitrogen cycles. The H_2S in both gas and liquid is converted *via* the sulfide oxidation pathway and $N\text{-}NO_3^-$ via the nitrate reduction pathway following equation (2.4) and (2.5):

$$S^{2-} + 1.6NO_3^- + 1.6H^+ \rightarrow SO_4^{2-} + 0.8N_2 + 0.8H_2O,\ \Delta G° = -743.9\ \text{kJ/reaction} \quad (2.4)$$

$$S^{2-} + 4NO_3^- \rightarrow SO_4^{2-} + 4NO_2^-,\ \Delta G° = -501.4\ \text{kJ/reaction} \quad (2.5)$$

NR-SOB consume H_2S, producing sulfur oxidized compounds such as sulfides, elemental sulfur, sulfites, and thiosulfates. These bacteria are chemoautotrophs and belong to Proteobacteria (classes α-, β-, γ-, ε-), Chlorobia, and Chloroflexi (Friedrich *et al*., 2005; Ghosh and Dam, 2009). The NR-SOB can be classified into three types by their carbon source: (i) the autotrophic bacteria, this

group of NR-SOB use the inorganic compounds as a carbon source such as carbon dioxide (CO_2), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) for production of new cell materials and their growth. Another group is the heterotrophic bacteria, a large variety of organic compounds such as acetate, glucose can be used as the carbon source (Syed *et al*., 2006). (iii) Facultative bacteria than can possibly use both type of carbon including organic and inorganic (Pokorna and Zabranska, 2015). There are many genera of NR-SOB such as *Thiobacillus, Thiomicropsira and Paracoccus* that have been reported for their ability of H_2S removal under anaerobic conditions (Table 2.2).

According to the previous work by Tóth *et al*. (2015), the well-known of genus *Thiobacillus* has been widely studied for H_2S oxidation. *Thiobacillus* sp. is the *betaproteobacteria*. Same as the other NR-SOB, this genus can oxidized the sulfur compounds as electron donor including H_2S, SO_4^{2-}, $S_2O_3^{2-}$ and S° for production of their growth and energy. Several species of the members of this genus are rod shaped and gram negative (Robertson *et al*., 2006). Some of them are acidophilic that can grow in acidic conditions (Tang *et al*., 2009). The sources of *Thiobcillus* sp. can be found in the sewage, wastewater under anoxic environment. The optimal pH and temperature are 6.9 and 29.5 °C, respectively (Kelly and Wood, 2000). Not only the ability of *Thiobacillus* sp. for sulfide removal but also this genus has been widely used for the NO_3^- removal *via* the denitrification pathway. In the denitrification step, some of the microorganisms can be use inorganic or organic compounds as carbon sources (Alzate, 2015).

Sulfurimona denitrificans (previous name: *Thiomicrospira denitrificans*) is also one of NR-SOB which is characterized as obligate chemolithotrophic bacteria and belong to *epsillonproteobacteria*. This microorganism can oxidize H_2S as an

electron donor to SO_4^{2-}. In addition, *Sufurimona* sp. can use $S_2O_3^{2-}$ and S° as an energy source. Moreover, there are many compounds for serving as an electron acceptor including NO_3^-, Iron (III) (Fe^{3+}) and oxygen (O_2). Many researches were reported about the capability of H_2S removal by *Sulfurimona denitrificans (Thiomicrospira denitrificans*) strain CVO under anoxic conditions (Gevertz *et al*., 2000; Gadekar *et al*., 2006; An *et al*., 2010).

Another genus of NR-SOB is *Paracoccus* sp., which belongs to *betaproteobacteria*. This genus was first isolated by Beijerinck and Minkman (Kelly *et al*., 2006). This genus is well-known as the denitrifying bacteria. Most of them have potential to remove NO_3^- in aquatic environment and wastewater. Watsuntorn *et al*., 2017). About the sulfide removal, like other NR-SOB the members of this genus capable use the H_2S and other elemental sulfur such as $S_2O_3^{2-}$. The sources of isolation of several strains of genus *Paracoccus* are polluted environment. For example, *Paracoccus* sp. strain TRP was isolated from the activated sludge from the pesticide plant in China (Li *et al*., 2011).

Table 2.2 H_2S removal rates reported for different NR-SOB cultures (Watsuntorn *et al*., 2017).

Microbial culture	**Source of biomass**	**[H_2S/sulfide]**	**H_2S/sulfide removal rate**	**References**
Paracoccus sp. strain MAL 1HM19	Sediments and water from Mae Um Long Luang hot spring, Thailand.	700-800 ppm_v	139.11 ppm_v/h 126.89 ppm_v/h 71.96 ppm_v/h	Wannapawn *et al*., 2017
Paracoccus versutus DSM 582	DSMZ		71.32 ppm_v/h	
Thiobacillus denitrificans strain D_4	Anaerobic activated sludge	200 mg/L	- 0.88-0.95	Wang *et al*., 2005
NR-SOB Consortium dominated by *Thiomicrospira* sp. stain CVO	Produced water of the Coleville oil field, Saskatchewan, Canada	6-6.5 mM	mM/h < 0.76 mM/h	An *et al*., 2010

2.6 Isolation of NR-SOB from hot spring

Isolation is the process in which the individual cells are physically divided from each other or from the matrix substances such as soil sediments, eukaryotic tissues, water and air samples. The isolation techniques are the most important step during the process of acquiring pure cultures. “Culture” and “cultivation” are used to recount the microorganism that can be grown and propagated in the laboratory which not only refers to pure culture but also include the stable mixed cultures in the laboratory (Zengler, 2009). The isolation is a traditional technique which is usually a culture dependent method. The isolation of pure culture microorganisms shows the detailed physiology of microorganisms. Moreover, this method is more important for understanding the properties and characteristics of microorganisms include the testing of Koch’s postulates, identification of organisms that carry specific genetic information (gene or pathway), evaluation of morphology and physiology, as well as determining the role of novel enzymes for industrial and pharmaceutical applications (Hugenholtz *et al*. 1998; Schleifer, 2004).

NR-SOB can be found from various natural and man-made environment sources that are rich in sulfur compounds. These sulfur compounds provide sufficient nutrients for maintaining their cell activity and growth (Sorokin *et al*., 2006; Hidayat *et al*., 2017). Moreover, many NR-SOB are capable to adapt in various conditions including the normal atmospheric conditions and extreme conditions such as high concentration of salt due to their high energy yield resulting from the complete oxidation of sulfide to SO_4^{2-} (Sorokin *et al*., 2006).

Hot spring is one of the natural sources because this place is often rich in high reduced and saturated sulfur compounds owing to the presence of mud and sediment.

Hot spring is a potential source of mesophilic or thermophilic SOB (Otaki *et al*., 2012; Tamazawa *et al*., 2012) and such environments provide the electron donors required for the growth of SOB (Hidayat *et al*., 2017). The activity of microorganisms in hot spring relies on the biogeochemical cycle, e.g. the sulfur cycle, and other limited nutrients (Skirnisdottir *et al*., 2001). Nowadays, there are only few studies that have focused on the isolation of SOB from hot springs.

2.7 Biotrickling filter

Biotrickling filter (BTF) is one type of the biological reactor for waste gas treatment that becomes widely acceptable which has advantages over the conventional techniques because this method is environmental friendly, significantly cost saving. Moreover, using BTF can be completed at ambient temperature resulting in no production of the secondary waste (Cox and Deshusses, 1998). BTF has been investigated increasingly for more than two decades by many applications of industry (Kennes *et al*., 2009). In this system, the nutrient for the microorganisms presented as a liquid phase is constantly trickled over the inert packing materials in any situations. The waste gas passed through the packing material where the microorganisms can grow and develop as the biofilm and the biodegradation is occurred within the biofilm formation (Khoshnevisan *et al*., 2017). The microorganisms consumed the pollutants as an energy source for their growth by absorbing atmospherics contaminants to the area of microbial biofilm (Mudliar *et al*., 2010). BTFs provide the pH control, less pressure drops and volume requirement as well as good process stability (Schiavon *et al*., 2016). The schematic of the BTF is shown on Figure 2.3.

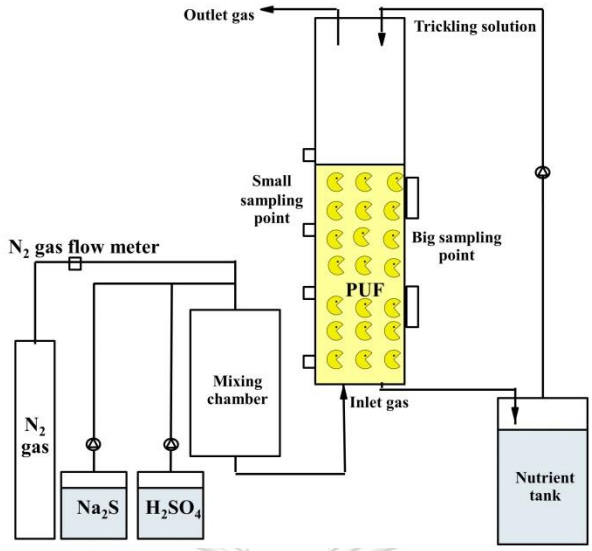


Figure 2.3 Schematic of the anoxic BTF system.

The advantages of the BTF are less operating cost, small footprint, low pressure drop. Moreover, BTF has the ability for volatile organic acid (VOC) (Yang *et al*., 2010). The first BTF was established in 1923 for applying the H_2S from the wastewater. After that BTFs have been used to remove various VOCs, ammonia (NH_3), sulfur dioxide (SO_2), nitrogen oxides (NO_x) (Grove *et al*., 2009; Philip and Deshusses, 2003, du Plessis *et al*., 1998). Nowadays, more than 7,500 systems of biological waste gas treatment have been generally used all over Europe. Half of them were installed at the composting sites and treatment plants (Yang *et al*., 2010). However, there are some of disadvantages of BTF such as the secondary waste formation, the redundant biomass accumulation (Mudliar *et al*., 2010).

2.8 Biofilm formation on the BTF

The biofilms are the aggregation of microorganisms which is called the biological community and implant in polymer matrix. When microorganisms attach on the support media due to the attractive forces and physical movement, grow on the media by consuming the waste gas as their nutrient. After that the microbial

aggregations are developed because of the forces of microorganisms and polymer matrix structure of biofilms is generated because of the hydrodynamic shear forces. The crucial thing of the biofilm formation is the microbial attachment to the surface area of a packing material of BTF for the initial biofilm generation for biodegradation of pollutant gases (Figure 2.4) (Yang *et al*., 2010). Moreover, extracellular polymeric substances (EPSs) are formed as a part of biofilms also importantly contributed to the biofilm production. Previous research reported about the advantage of secretion of EPSs that assists the combination between cell to cell (Costerton *et al*., 1995). In BTF operation, the trickling recirculation from the storage tank provides the greater operation resulting in the high water content in biofilm. The pH and the nutrient can be controlled in BTF and might stimulate the exuviae of the biofilms resulting in the decreasing clogging (Devinny and Ramesh, 2005).

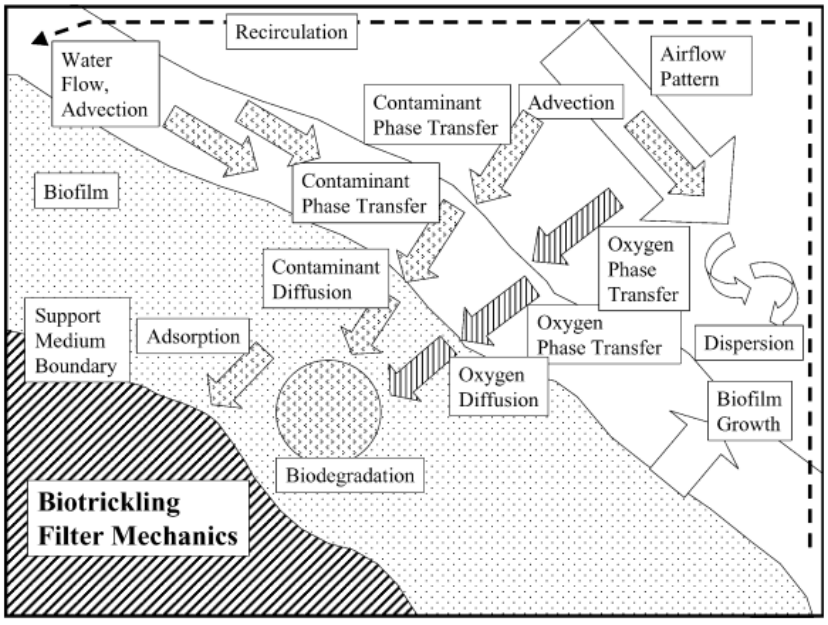


Figure 2.4 Circumstance involved in the BTF operation (Devinny and Ramesh, 2005).

2.9 Effect of important parameters on the performance of BTF

Removal efficiency (RE), Inlet loading rate (ILR) and elimination capacity (EC), and empty bed gas residence time (EBRT) are generally important parameters to specify the performance of the BTF (Iranpour *et al*., 2005; Schiavon *et al*., 2016).

2.9.1 Removal efficiency (RE)

The first parameter is RE that shows the waste gas represents the percentage of the pollutant removed from the contaminated stream.

2.9.2 Inlet loading rate (ILR)

ILR is the rate of the contaminant that is fed to the BTF. ILR has an effect on the performance of BTF. ILR depends on the concentration of the contaminant and the flow rates (Q). The lower pollutant loading rate leads to higher pollutant removal (Matanhike, 2017). Consequently, the rate of contaminant biodegradation is investigated by the mass transfer rate of the contaminants throughout the media.

2.9.3 Elimination capacity (EC)

EC is the amount of pollutant removed per unit volume of the packed filter bed. Normally, the equality between EC and IL (EC = ILR) is normally observed in the well-established BTF with the low inlet loading which demonstrates nearly 100% of RE (Figure 2.5). However, of the pollutant load to the BTF is increased beyond its critical load, the RE will decrease to values lower than 100% (Alzate, 2015).

2.9.4 Empty bed gas residence time (EBRT)

Another important parameter is the empty bed residence time (EBRT) which is the ratio of empty trickling bed volume and the gas flow rate. The higher EBRT leads to higher RE because of the longer contact time between the waste-gas and the microorganisms present in the BTF.

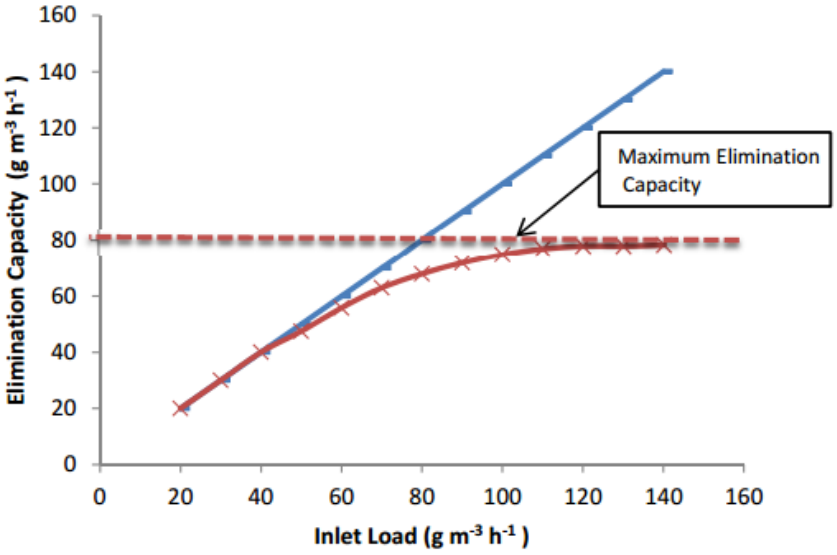


Figure 2.5 Elimination capacity (Alzate, 2015).

2.10 The operation parameter for BTF

The various parameters for the operation of BTF are described as follow:

2.10.1 Trickling liquid volume (TLV)

TLV is the velocity of the recirculated liquid through the media filter which directly affect to the mass transfer between the aqueous and gas phases. For example, the higher removal capacity is caused by a high mass transfer. The optimal TLV of BTF based on the surface area and filter media used (Fernández *et al*., 2014).

2.10.2 Shock loading

The shock loading means the loading of large amount of contaminant gases into the BTF. The pollutant concentrations impact on the ability of contaminant removal by microorganisms. Many researches have focused on evaluating the performance of BTFs under steady-state conditions. However, the objective of investigating shock load or transient-state experiments is to gain better insight of the BTF in terms of its stability and resilience capacity (Jiang *et al*., 2009). Increasing of gas flow rate or high inlet concentration leads to the suppression of microbial activity.

On the other hand, the scarcity of the contaminant loading rate also affects the microbe activity. Moreover, the microorganisms can adapt to the higher inlet concentration. The increasing of the inlet concentration of H_2S from low to very high concentration has the effect on the microbial activity (Duan *et al*., 2005).

Kim *et al*. (2008) carried out H_2S shock load experiments by varying the inlet load from 0.1-10 g H_2S/m^3 h and it was reported that the RE decreased from 100% to 78%. Fortuny *et al*. (2008) studied the effect of high inlet concentration (1500 to 6000 ppm_v of H_2S) as well as the loading of H_2S (42 to 167 g $H_2S/m^3 \cdot h$) resulting in the reduced RE of H_2S from 100% to 90% after 5 d of operation at high inlet concentrations.

2.10.3 Temperature

Maintaining proper temperature during BTF operation is important for the growth of microbial community (Feizi *et al*., 2016). For full-scale H_2S oxidation in a biofilter (BF), temperature ranging between 24 and 32 °C has been recommended (Shao *et al*., 2010). For H_2S oxidation in a BF, the genus *Thiobacillus* such as *Thiobacillus thioparus* and *Thiobacillus denitrificans* are well-known chemolithotrophic sulfide oxidizing bacteria which has been widely used in BF and BTF (Vikrant *et al*., 2018). Previous works have shown good performance of H_2S oxidation in BTF or BF at 30 °C (Massoudinejad *et al*., 2008; Abdehagh *et al*., 2011). However, Fernández *et al*. (2014) reported the inhibition of NR-SOB at temperatures below 30 °C.

2.10.4 pH

Many studies were reported wide range of pH with the BTF operation. Few studies focus on the removal H_2S under acidic condition. The complete simultaneous removal of H_2S and methanol (CH_3OH) (100%) was achieved under the acidic condition at pH 2 (Jin *et al*., 2007). Most of the prior works about BTF were done at the neutral pH. The range of pH between 7.3-7.5 showed the high efficiency for the H_2S removal (99%). Moreover, the slightly alkaline pH improve the conversion NO_3^- to nitrogen gas (N_2) as well as the avoiding of the intermediate formation such as nitrous oxide (N_2O) (Fernández *et al*., 2014). The pH control is necessary because H_2S oxidation generate the S° or SO_4^{2-}or sulfuric acid (H_2SO_4) as a product. The decreasing pH occurs from the accumulation of H_2SO_4. In addition, decreasing of pH might be affect the activity of microbes (Jin *et al*., 2005).

2.10.5 Nutrients

Nutrient is important factor in the biological process for growth of bacteria. In addition, the nutrient is necessary to the biofilm formation. Normally, the trickling nutrient in BTF composed of the inorganic compounds such as potassium, nitrogen and phosphorus (Jin *et al*., 2007). From our best knowledge, there are poor information reported about the particular nutrient requirement for the denitrifying autotrophic bacteria. For NR-SOB, the nitrogen source of NR-SOB can be NO_3^- or nitrite (NO_2^-) and should be limited to evade the metabolism of bacteria.

2.11 H_2S removal using anoxic BTF

H_2S oxidation in the BTF can be performed under aerobic and anoxic conditions depending on the source of the waste gas stream and also the purpose of the treatment step. In the case of biogas upgrading using an anoxic BTF to remove

H_2S, the level of oxygen needs to be controlled because biogas contains oxygen that might pose an explosive risk (Sereanu *et al*., 2009). In such cases, aerobic treatment is not preferred because the mixing between air and biogas would result in the dilution of the methane (CH_4) concentrations (Fortuny *et al*., 2008). Consequently, the removal of H_2S under anoxic conditions provides more advantages over the aerobic processes. During anoxic conditions, NO_3^- can serve as an electron acceptor instead of the oxygen and the presence of sulfide leads to high activities of NR-SOB that plays an important role in the H_2S removal process. Researches pertaining to H_2S degradation using anoxic BTF are of much interest to the scientific community in recent years (Soreanu *et al*., 2008; Soreanu *et al*., 2010; Li *et al*., 2016). In a previous study, the anoxic BTF was operated for 178 d in order to remove H_2S and the NO_3^- containing wastewater was used as the nutrient solution for the microorganisms attached to the porous support matrix within the BTF (Soreanu *et al*., 2008). The authors reported 100% H_2S removal without effects on the CH_4 concentrations and this system was reported to be highly efficient during long-term operation. The performance of the anoxic BTF depends on the H_2S concentrations and the biogas flow rate (Soreanu *et al*., 2010). Li *et al*. (2016) compared the performance of H_2S removal between an anoxic BTF and biobubble column (BBC) reactor and showed that the H_2S elimination capacity was more stable in the BTF than the BBC system.

2.12 Future perspective in biological H_2S removal

The future perspective of H_2S removal should be shifted towards the biological process owing to its advantages over the conventional physico-chemical processes: environmental friendly, less chemical requirements, less operational and maintenance costs and less energy requirements. Several previous works have

reported high performances in biological H_2S removal using laboratory and pilot scale systems. It is however important to also mention that, anoxic H_2S removal using BTF still has some drawbacks. At high concentration of H_2S (> 1000 ppm_v), the removal of H_2S decreases due to the toxicity effects, thereby reducing the activity of the microorganisms (Fortuny *et al*., 2008; Montebello *et al*., 2012). Besides, from a process intensification and control view-point, anoxic BTF should be optimally designed and operated in order to make it cost-effective. The application of artificial intelligence tools such as fuzzy logic and artificial neural networks should be tested in order to monitor and control the operation of BTF using online data acquisition systems. Besides, the nutrient requirements are also high in BTF and the source of N should also be carefully selected. Hence, future anoxic bioreactor systems should also test novel biocatalysts and integrated bioreactor configurations that are able to show stable, yet high H_2S removal performance during normal as well as shock or transient operating conditions.

CHAPTER III
MATERIAL AND METHODS

3.1 Overview of the experiments

The conceptual experimental framework to this research is shown in Figure 3.1

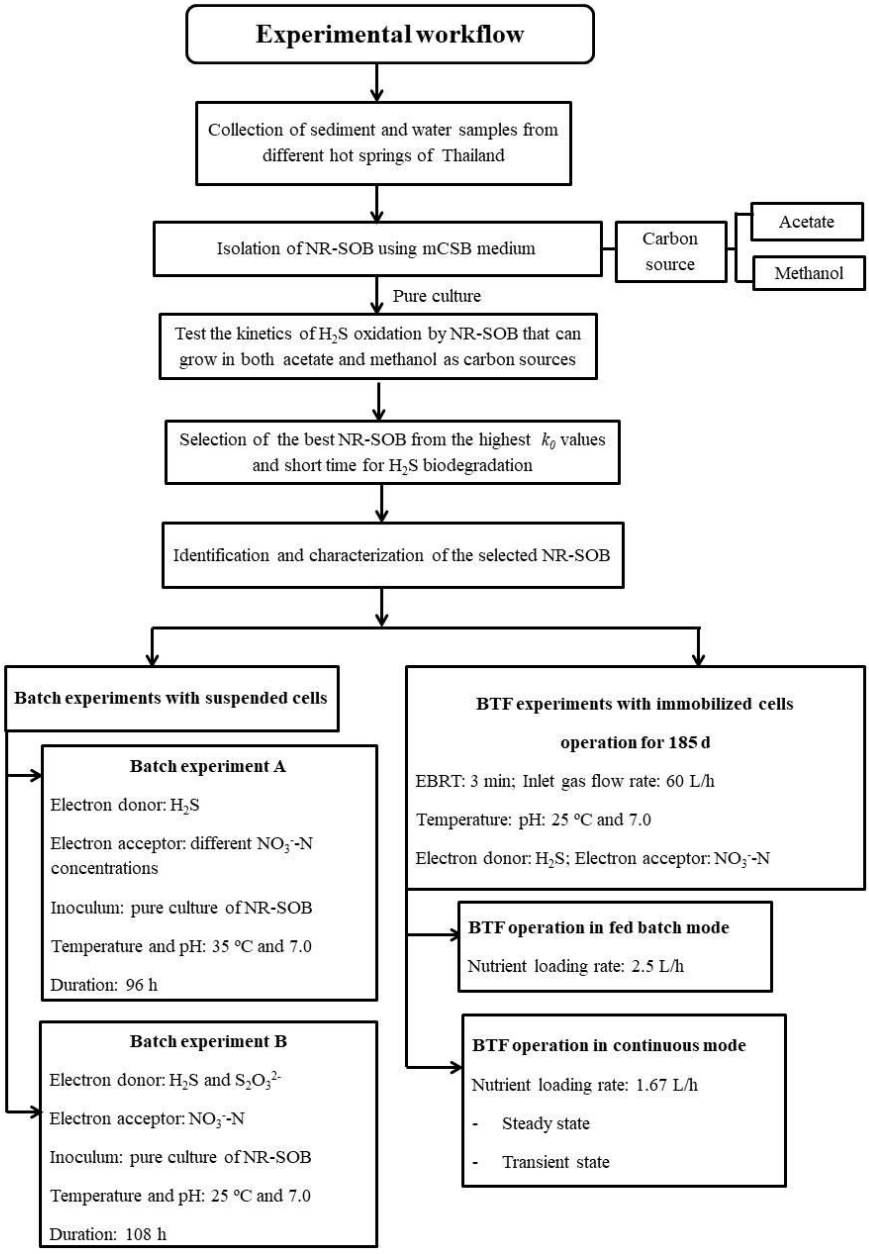


Figure 3.1 Experimental framework of this Ph.D. research.

3.2 Part I: Isolation of NR-SOB from natural sources

3.2.1 Sample collection

Mixed water and sediment samples were collected from Mae Um Long Luang hot spring, Mae Hong Son province and Thep Pha Nom hot springs, Chiang Mai province (Figure 3.2) according to the method suggested by Gevertz *et al*. (2000). Samples were collected from 3 different sites of each hot spring with 3 replicates. All samples were collected in 100 mL sterile glasses bottles containing N_2 for providing the required anaerobic conditions and stored in an ice box and transported to the laboratory within 2 d.

Figure 3.2 Sources of NR-SOB isolation: (A) Thep Pha Nom hot spring, (B) sampling site of Thep Pha Nom hot spring, (C) Mae Um Long Luang hot spring, and (D) sampling site of Mae Um Long Luang hot spring.

3.2.2 Sample preparation

The steps involved in sample preparation were done according to the adopting the methodology used by Luo *et al.* (2013). One hundred mL of water samples was suspended in sterile phosphate buffer solution (PBS, pH 8.0) and mixed with sterile glass beads by agitating for 30 min, at 35 °C. The suspension was transferred to conical centrifuge tubes and centrifuged at 6000 × g for 5 min (Hanil Scientific Inc., Korea). The pellets were re-suspended twice in 100 mL of PBS and were maintained at 4°C.

3.2.3 Enrichment medium

The modified Coleville synthetic brine medium (mCSB) was used for the isolation of NR-SOB. The medium composition consisted of (in g/L) NaCl 0.3; KNO_3 1.0; NH_4Cl 0.02; KH_2PO_4 0.027; $NaHCO_3$ 1.9; $MgSO_4.H_2O$ 0.68; $CaCl_2.H_2O$ 0.24; resazurin 0.0001; and trace elements 1.0 mL. Two carbon sources including acetate (CH_3COO^-) in form of sodium acetate ($NaC_2H_3O_2.3H_2O$) (0.68 g/L) and methanol (CH_3OH) (2 mM) were added separately in order to test the ability of acetate and methanol utilization by the NR-SOB. Trace elements solution had the following composition (in g/L): 50.0 g/L of H_2SO_4, 2.28 g/L of $MnSO_4·H_2O$, 0.5 g/L of $ZnSO_4·7H_2O$, 0.5 g/L H_3BO_3, 0.025 g/L of $CuSO_4·5H_2O$, 0.025 g/L of $Na_2MoO_4·2H_2O$, 0.045 g/L of $CoCl_2·6H_2O$ and 0.58 g/L of $FeCl_3$, respectively (Gevertz *et al.*, 2000). The pH of the mCSB medium was adjusted to 7.0 ± 0.2 and flushed with N_2 gas for 5 min to provide the required anaerobic conditions. Prior to use, all experimental serum bottles were autoclaved for 15 min at 121°C. 0.5 mL of sterile $Na_2S.xH_2O$ from 1 M of stock solution was added to each serum bottle in order

to get the desired final concentration of ~5 mM. Thereafter, 0.5 mL of sterile HCl from a 2 M stock solution was added to re-adjust the pH to ~7.0 ± 0.2.

3.2.4 Isolation of pure cultures of NR-SOB

Five mL of prepared samples were inoculated into serum bottles containing 50 mL of modified CSB medium and the bottles were incubated in a rotary shaker (Daihan scientific, Korea) (100 rpm) at 35°C for 3 d. The growth of each isolated NR-SOB was detected by measuring the increase in turbidity of the modified CSB in terms of the optical density at 600 nm (OD_{600}). After that, the culture samples were spread on mCSB agar plates and incubated at 35°C, pH 7.0. One colony was taken from the plate and sub-cultured several times to obtain the pure culture.

3.3 Part II: Selection of the isolated NR-SOB

3.3.1 Selecting the appropriate NR-SOB by estimating the kinetics of H_2S oxidation

The appropriate NR-SOB was selected based on the highest rate of H_2S removal by determining the kinetics of H_2S oxidation according to the methodology suggested by Chung *et al.* (1996). The kinetics of H_2S oxidation by different strains of NR-SOB were performed at 35 ºC and pH 7.0 using mCSB medium with acetate as the carbon source. The batch serum bottles (1000 mL) comprised of 180 mL sterile mCSB medium and 20 mL (10% *v/v*) of 3 d old of each NR-SOB strain as an inoculum. The pH was adjusted to 7.0 ± 0.2 by the addition of sterile HCl and then purged with nitrogen (N_2) gas for 10 min to provide the desired anaerobic conditions. All batch experiments were performed in duplicates. Prior to use, all experimental serum bottles containing mCSB medium were autoclaved for 15 min at 121°C. 0.6 mL of $Na_2S.xH_2O$ from 1 M of stock solution was added to each serum bottle to

generate H_2S concentrations of 650-1000 $ppm_{v.}$ After that, 0.6 mL of sterile HCl from 2 M of stock solution was added to re-adjust the pH to~7.0 ± 0.2. Control bottles without the addition of NR-SOB were also performed.

Initial and final H_2S concentrations were diluted and measured by gas detector tubes (RAE, USA).The time series experimental data was used to estimate the kinetic parameters by fitting the data to zero-order reaction kinetics. Zero-order reaction rate was calculated using equation (3.1). (Chung *et al*., 1996):

$$C_0 - C_e = k_o t \tag{3.1}$$

Where C_0 is the inlet concentrations of H_2S (ppm_v), C_e is the outlet concentrations of H_2S (ppm_v), t is the reaction time (h), and k_0 is the zero-order constant

The NR-SOB that showed the highest removal rate of H_2S and remove H_2S from the gas phase within 10 h was selected for further study.

3.3.2 Analytical procedures

13 mL of gas samples were collected from the headspace from the different batch incubations and diluted ten times with N_2 before the H_2S measurements using a H_2S detector tube (range 0 to 2000 ppm_v) (RAE, USA). Gas samples were collected once every 1 or 2 h and were continued until the H_2S was totally removed (0 ppm_v). Sulfide ion (S^{2-}), SO_4^{2-}, NO_3^--N, and NO_2^--N concentration were examined once every 24 h. Sulfide concentrations were analyzed once every 24 h following the method described by Cord Ruwisch (1985), SO_4^{2-} were determined by the turbidimetric method described in APHA (2005), while the NO_3^--N and nitrite (N-NO_2^-) concentrations were determined using the standard 4500-NO_3^--B ultraviolet spectrophotometric screening and 4500-NO_2^--B colorimetric method, respectively

(APHA, 2005). pH was measured using a pH meter (PL700PCS, Pacific Sensor Technologies, Australia).

3.3.3 Statistical analysis

The experimental data are presented in the form of mean ± SD values. One-way analysis of variance (ANOVA) was done using the SPSS software (SPSS Inc., USA) followed by the Tukey's post-hoc test. The level of statistical significance was set at $P < 0.05$.

3.4 Part II: Identification and characterization of appropriate NR-SOB

The selected NR-SOB was identified using morphology, 16S rDNA gene sequencing and phylogenetic analysis.

3.4.1 Morphological characteristics of the strain MAL 1HM19

Cell morphology was examined using a light microscope (Olympus CH30, Olympus, Japan). The cellular morphology of the selected NR-SOB was examined using scanning electron microscopy (SEM) (JSM 7610F microscope, JEOL, Japan) by outsourcing the sample to Scientific and Technological Research Equipment Centre (Chulalongkorn University, Thailand). Gram staining was done according to the Hucker method (Doetsch, 1981).

3.4.2 16S rDNA, *soxB* gene sequence and phylogenetic analysis

Genomic DNA was extracted from the pure isolates using the bacterial DNA kit (OMEGA Bio-Tek, USA). A PCR product for sequencing 16s rDNA regions was prepared using the following two primers, viz., 27F (5'-AGAGTTTGGATCCTGGCTCAG'-3) and 1492R (5'-CGGTTACCTTGTTACGACTT'-3), respectively (Gillan *et al*.,1998). PCR amplification was programed to carry out an initial denaturation step at 98 °C for 30 s;

30 amplification cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s and extension at 72 °C for 10 s; followed by a final extension at 72 ^{o}C for 10 min with the *Taq* polymerase protocol (Biolabs, UK). While *soxB* primers 704F/1199R and the *soxB* gene fragments protocol were followed, the PCR amplification was programed to carry out an initial denaturation step at 94 °C for 3 min, followed by two cycles of 94 °C for 30 s, annealing at 65 °C for 45 s and extension at 72 °C for 60 s. After that, the annealing temperature was decreased by 1 °C for each cycle until it reached 55 °C, followed by a final extension at 72 ^{o}C for 7 min (Luo *et al*., 2011). The PCR products were purified and sequenced by Pacific Science Co., Ltd (Bangkok, Thailand). All PCR products were evaluated by electrophoresis on a 1% (*w*/*v*) agarose gel in 1× Tris-acetate EDTA (TAE) buffer with UV emission. PCR amplified 16S rDNA and *soxB* gene fragments were commercially purified and sequenced by Pacific Science Co., Ltd. (Bangkok, Thailand) and Gibthai Co., Ltd. (Bangkok, Thailand), respectively.

Both nucleotide sequences were compared by sequence similarity to those in the GenBank database using the NCBI Basic Local Alignment Search Tool (BLAST; BLASTx) database (Altschul *et al*., 1997). Phylogenetic trees of 16S rDNA and *soxB* were generated by the neighbor-joining (NJ) distance method using the MEGA 5 software (Tamura *et al*., 2011). The evolutionary distances were computed using the Kimura 2-parameter model, while the bootstrap values were obtained based on 500 replicates. The nucleotide sequence of the 16S rDNA and *soxB* gene from strain MAL 1HM19 have been deposited in the GenBank database under the accession numbers KY427435 and KY434634, respectively.

3.4.3 Characterization of appropriate NR-SOB

The characterizations of the NR-SOB strain MAL 1HM19 were conducted by following the modified methodologies of Gevertz *et al*. (2000) and Kantachote *et al*. (2008). The effect of temperature (30, 35, 40, 50, 60 °C) sulfide concentrations (5, 10 and 15mM) and pH (4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) on the growth of NR-SOB was investigated. Moreover, the mCSB medium was supplemented with each of the following substrates (2 mM): acetate, glucose, lactate, pyruvate and methanol, at 35°C for testing the effect of substrate on the growth of NR-SOB under anaerobic conditions. For anaerobic sulfur utilization, the ability of strain MAL 1HM19 to use tetrathionate ($S_4O_6^{2-}$) and thiosulfate ($S_2O_3^{2-}$) at 5 mM was tested. Three replicates were conducted for each experimental condition and the MAL 1HM19 growth was determined by measuring the optical density at 600 nm (OD_{600}) using a spectrophotometer (Thermo Scientific, USA). The OD_{600} values measured at different time intervals during the experiments were reported as the percent increase in the growth of strain MAL 1HM19 with respect to those measured at the start of the experiment (at time t = 0 h).

3.5 Part IV: H_2S removal in batch experiments

3.5.1 Batch experiment at different NO_3^--N concentrations (60, 120 and 240 mg NO_3^--N/L) and different sulfur sources including sulfide and thiosulfate ($S_2O_3^{2-}$)

For the experiment including different NO_3^--N concentrations, the mCSB medium described in Section 3.2.3 was used in this study. Potassium nitrate (KNO_3) was added to the different incubations to give the desired initial NO_3^--N concentrations of 60, 120 and 240 mg NO_3^--N/L, respectively. All batch experiments

were performed in triplicates. The zero-order kinetics of H_2S oxidation under the influence of different NO_3^--N concentrations were performed at 35°C and pH 7.0. The batch experiments were inoculated with the strain MAL 1HM19 (10% *v/v*), with three replicates. Control cultures were investigated under the same conditions without the addition of strain MAL 1HM19.

For the experiment with different sulfur sources, the mCSB medium (Section 3.2.3) was used in this study at 25 ºC and at pH 7.0. In this study, the sulfur sources including sodium sulfide ($Na_2S.9H_2O$) (96 mg S^{2-}S/L) and sodium thiosulfate ($Na_2S_2O_3$) (96 mg $S_2O_3^{2-}$-S/L) were added separately for each experiment. All batch experiments were performed in duplicates.

3.5.2 Analytical procedures

For batch experiment at different NO_3^--N concentrations (60, 120 and 240 mg NO_3^--N/L), the measurements of H_2S in gas phase, sulfide, SO_4^{2-}, NO_3^--N, NO_2^--N and pH were carried out as described in Section 3.3.2. For batch experiment with different sulfur sources, (sulfide and $S_2O_3^{2-}$), the sulfide concentrations in aqueous phase was measured by the acid volatile sulfide (AVS) method (Kruis, 2014). Briefly, the samples were mixed with 0.5 M sodium hydroxide (NaOH) in order to trap the sulfide. After that, diamine reagent (MDR) reagent was added resulting in the development of a methylene blue color. A UV/Vis spectrophotometer (Lambda 365, PerkinElmer, USA) was used to measure the absorbance at 670 nm after 30 min. $S_2O_3^{2-}$ and SO_4^{2-} concentrations in the liquid phase were determined using ion chromatography (IC) (Dionex ICS-100, ThermoFisher Scientific, USA). NO_3^--N and NO_2^--N concentrations were determined using the standard 4500-NO_3^--B ultraviolet spectrophotometric screening and 4500-NO_2^--B colorimetric method, respectively

(APHA, 2005). CH_3COO^- concentrations were measured using a gas chromatography (Varian 430-GC, Varian Inc., USA), equipped with a flame ionization detector (FID), a CP WAX-58 CB column and using Helium (He) as the carrier gas. pH was measured using a pH meter (Präzisions E 510, Metrohm, Switzerland).

3.5.3 Statistical analysis

The significance of data from batch experiments at different NO_3^--N concentrations were analyzed by performing one-way analysis of variance (ANOVA) using the SPSS Software (SPSS, USA), Version 19.0, while the averages were compared by the Duncan's test. The differences with *P*-value less than 0.05 were considered to be statistically significant.

3.6 Part V: Anoxic biotrickling filter

3.6.1 Inoculum preparation and nutrient solution

The pure culture of strain MAL 1HM19 was used as the inoculum. The mCSB nutrient solution described in Section 3.2.3 was used.

3.6.2 Anoxic biotrickling filter (BTF) set up and operation

The laboratory scale BTF column used in this study was made from glass, having the total height of 60 cm. The BTF was packed to a height of 30 cm using inner diameter of 12 cm and a total volume of filter bed 3 L (Figure 3.3). The temperature of the BTF was maintained between 22 and 25 °C, and the pH was 7.0 ±1.0. Different diameters of Tygon tubes were used supply the nutrient and the gas-phase H_2S to the BTF. The H_2S gas was generated by mixing 1M H_2SO_4 and 0.1 and 0.3 M $Na_2S{\cdot}9H_2O$ in different preparation to obtain the desired H_2S concentrations (100-4000 ppm_v). The mCSB medium was trickled from the top of the BTF using a peristratic pump (Masterflex, USA). The mCSB medium provided the nutrient

requirement for the microbes and also helped to maintain the moisture content of the filter bed. The inlet gas including H_2S and N_2 were passed to the BTF in an up flow mode. The BTF installation is shown in the Figure 3.3. Operational parameters and conditions of anoxic BTF are shown in Table 3.1-Table 3.3.

Polyurethane foams (PUFs) cubes of size 8 cm^3 were used as the packing material. The porosity and density of the PUFs were 98% and 28 kg/m^3, respectively (Eregowda *et al*., 2018). PUFs were autoclaved for 15 min, at 121 °C in order to provide the sterile conditions. 1.5 L of PUFs were inundated in the mCSB medium containing 10% (*v/v*) of *P. versutus* strain MAL 1HM19. After 3 d of incubation, 1.5 L of the PUFs containing the microbes were added to the BTF as the packing material and mixed with sterile PUFs, there by maintaining a final filter bed volume of 3 L.

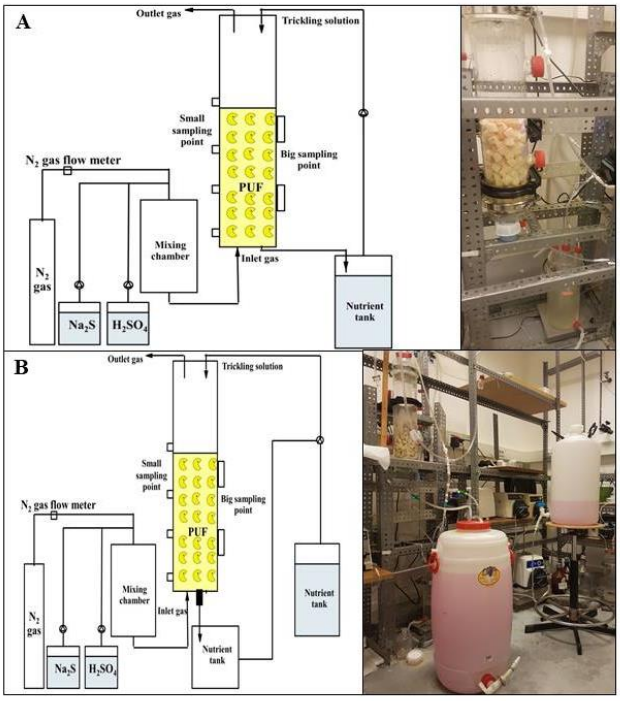


Figure 3.3 Schematic of the experimental BTF: (A) fed batch mode, and (B) continuous mode of operation.

Table 3.1 Operational parameters of the anoxic BTF.

Operation parameters	**Conditions/values**
Temperature	22-25 °C
pH	7.0-8.0
Electron donor	H_2S
Electron acceptor	NO_3^--N
Trickling nutrient solution	mCSB medium
Nutrient trickling rate (L/h)	60
Inlet gas flow rate (L/h)	60
EBRT (min)	3
Volume of the filter bed (L)	3
Packing material	PUFs

Note: EBRT- Empty bed residence time; PUFs-Polyurethane foams; mCSB- modified Coleville synthetic brine medium

Table 3.2 Conditions during phase I of anoxic BTF operation.

Period	Conditions	Duration (days)	Inlet H_2S concentration (ppm_v)	Inlet H_2S concentration (g/m^3)
I	Presence of CH_3CHOO^-	0-27	100	0.14
II	No CH_3CHOO^-	28-54	100	0.14
III	Presence of CH_3CHOO^-	55-69	300	0.44
IV	Presence of CH_3CHOO^-	70-90	300	0.44
V	Presence of CH_3CHOO^-	91-107	500	0.70

Table 3.3 Conditions during phase II and phase III of anoxic BTF operation.

Phase	Period	Conditions	Duration (days)	Inlet H_2S concentration (ppm_v)	Inlet H_2S concentration (g/m^3)
II	I	Presence of	133-162	500	0.7
II	II	CH_3CHOO^-	162-174	500	0.7
III	I	No CH_3CHOO^-	175-178	500-4000	0.7-5.7
III	II	No CH_3CHOO^-	184-189	500-4000	0.7-5.7
		No CH_3CHOO^-			

3.6.3 Performance parameters of the BTF

The performance parameters of the BTF including removal efficiency (RE), inlet loading (ILR), trickling liquid velocity (TLV), elimination capacity (EC) and global nitrate demand (GN) were estimated using equations (3.2) to (3.6):

$$RE\ (\%) = \frac{C_{in} - C_{out}}{C_{in}} \cdot 100 \quad (3.2)$$

$$TLV\ (\text{m/h}) = \frac{Q}{A} \quad (3.3)$$

$$ILR\ (\text{g S/m}^3\ \text{h}) = C_{in}\frac{Q}{V} \quad (3.4)$$

$$EC\ (\text{g S/m}^3\ \text{h}) = ILR \cdot \frac{RE(\%)}{100} \quad (3.5)$$

$$GN\ (\text{mg } NO_3^-\text{-N/mg } H_2S \text{ removed-day}) = \frac{M_0 - M_f}{t \times \left(Q \times (C_{in} - C_{out}\right) \times 24} \quad (3.6)$$

Where C_{in} and C_{out} are the inlet and outlet H_2S concentrations (ppm_v or g S/m^3), Q is the gas flow rate (m^3/h), A is the cross sectional area of the BTF (m^2), V is the volume of the packing media (m^3); M_o is the initial nitrate present in the nutrient solution (mg NO_3^--N), M_f is the final nitrate present in the nutrient solution (mg NO_3^--N), and t is time of processing the nutrient solution or the time of operating the BTF with that medium before being replaced (d).

3.6.4 Analytical techniques

3.6.4.1 H_2S and sulfide measurements

H_2S concentrations (0-500) were measured using two H_2S sensors including, (i) Dräger X-am 7000 (Dräger, Lübeck, Germany) (H_2S measurement range: 0-500 ppm_v), and (ii) Biogas 5000 (Geotech, UK) (H_2S measurement range: 0-5,000 ppm_v). H_2S concentration was shown as ppm_v units in both the sensors. Sulfide ion (S^{2-}) was measured by the modified methylene blue color method as described by Kruis (2014).

The samples measured using a UV/Vis spectrophotometer (PerkinElmer, USA) at an absorbance of 670 nm.

3.6.4.2 Sulfide, $S_2O_3^{2-}$, SO_4^{2-}, NO_2^-, NO_3^- and CH_3COO^- measurements

The protocol used for measuring sulfide, $S_2O_3^{2-}$, SO_4^{2-}, NO_2^-, NO_3^- and CH_3COO^- concentrations has been described in Section 3.5.3.

3.6.4.3 SEM images and energy dispersive X-ray spectroscopy (EDS)

The surfaces of the PUFs were monitored using SEM (JSM-6010LA, JEOL, Japan). In brief, the PUF samples were fixed with 0.5% formaldehyde and 0.5% glutaraldehyde, in phosphate buffer (pH: 7.4) for 24 h and then dried in a graded series of ethanol (30, 50, 70, 80, 90 and 100% *v*/*v*). In addition, EDS was used to investigate the elemental composition (%) of the PUF samples.

3.6.5 Kinetics of H_2S removal in the BTF

The kinetics of H_2S removal in the BTF was determined using the modified Michaelis-Menten equation in order to obtain the maximum EC (EC_{max}), according to the procedure reported by Kim *et al*. (2008) and Rene *et al*. (2010) shown in equation (3.7). The analysis of kinetic data can be used to compare the performance and characteristics of BTF as well as used for the application in reactor design which has been reported by Tsang *et al*. (2015).

$$\frac{1}{EC} = \frac{K_s + C_{ln}}{EC_{max} C_{ln}} \tag{3.7}$$

Where $C_{ln} = \frac{C_{in} - C_{out}}{ln(\frac{C_{in}}{C_{out}})}$, C_{in} is the inlet H_2S concentration (g/m^3), C_{out} is the outlet concentrations of H_2S (ppm_v or g S/m^3), K_s is the half saturation constant (g/m^3), EC is the elimination capacity (g/m^3) and EC_{max} is the maximum elimination capacity (g/m^3).

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Part I: Isolation of NR-SOB from the hot springs

The NR-SOB strains were isolated by the streak plate technique. 18 and 20 isolates comprising of different bacterial colonies were isolated from the Mae Um Long Luang and Thep Pha Nom hot springs, respectively. The growth of NR-SOB was ascertained by visual observation of turbidity development in the mCSB medium (Appendix B, Figures 4.1 and 4.2). From Tables B.1 and B.2, during the screening step, 11 strains of NR-SOB showed the development of turbidity in different types of carbon sources including CH_3COO^- and CH_3OH in the mCSB medium, confirming the growth of bacteria. However, among the 38 isolates, three isolates of NR-SOB strain namely MAL 1HM19, TPN 1HM1 and TPN 3HM1 were able to grow on both CH_3COO^- and CH_3OH. Moreover, all the three NR-SOB strains were also able to remove H_2S in the gas-phase within 10 h. Their colonies were observed after 3 d of incubation. The morphology of their colonies is described in Table 4.1.

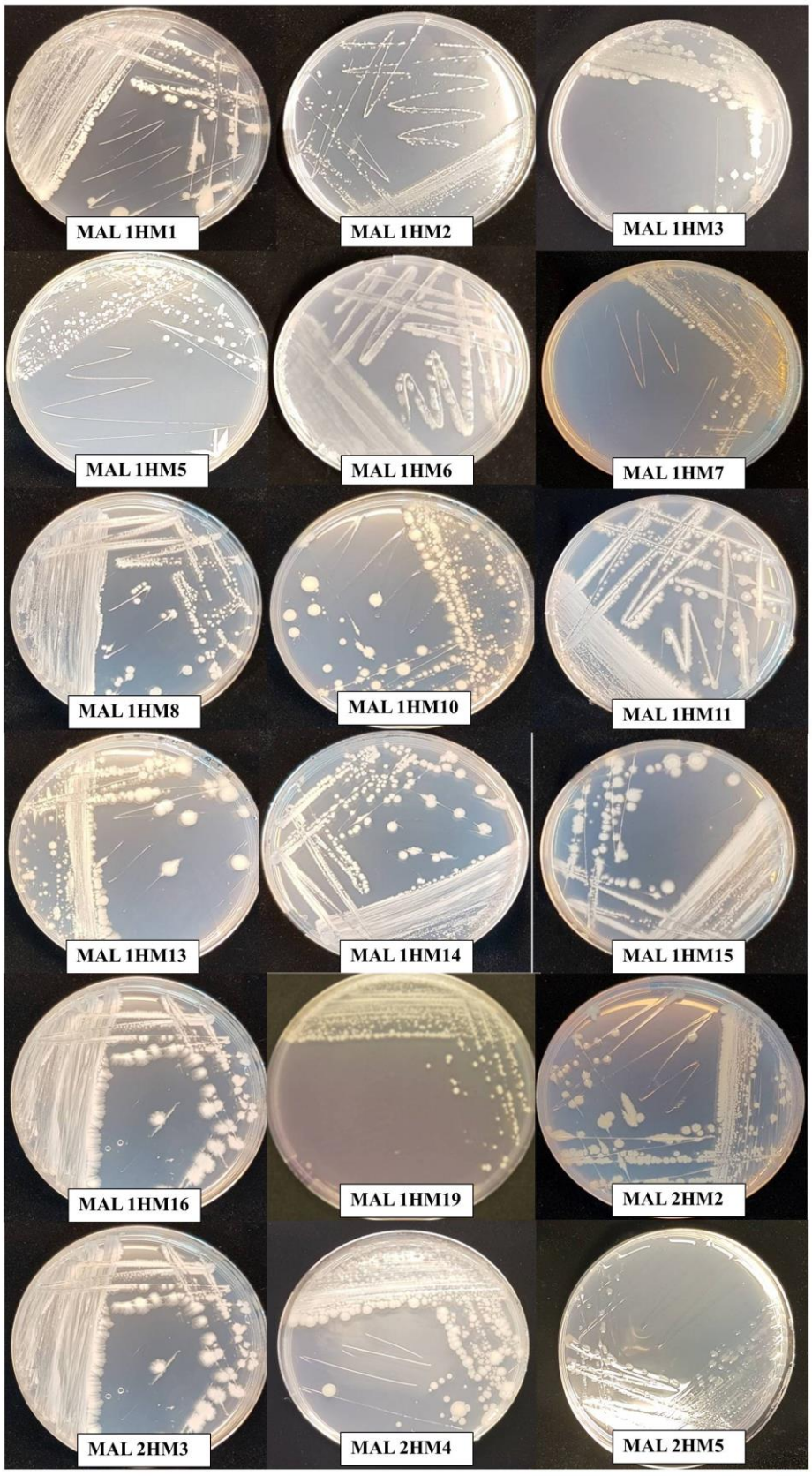


Figure 4.1 The different NR-SOB isolated from the Mae Um Long Luang hot spring.

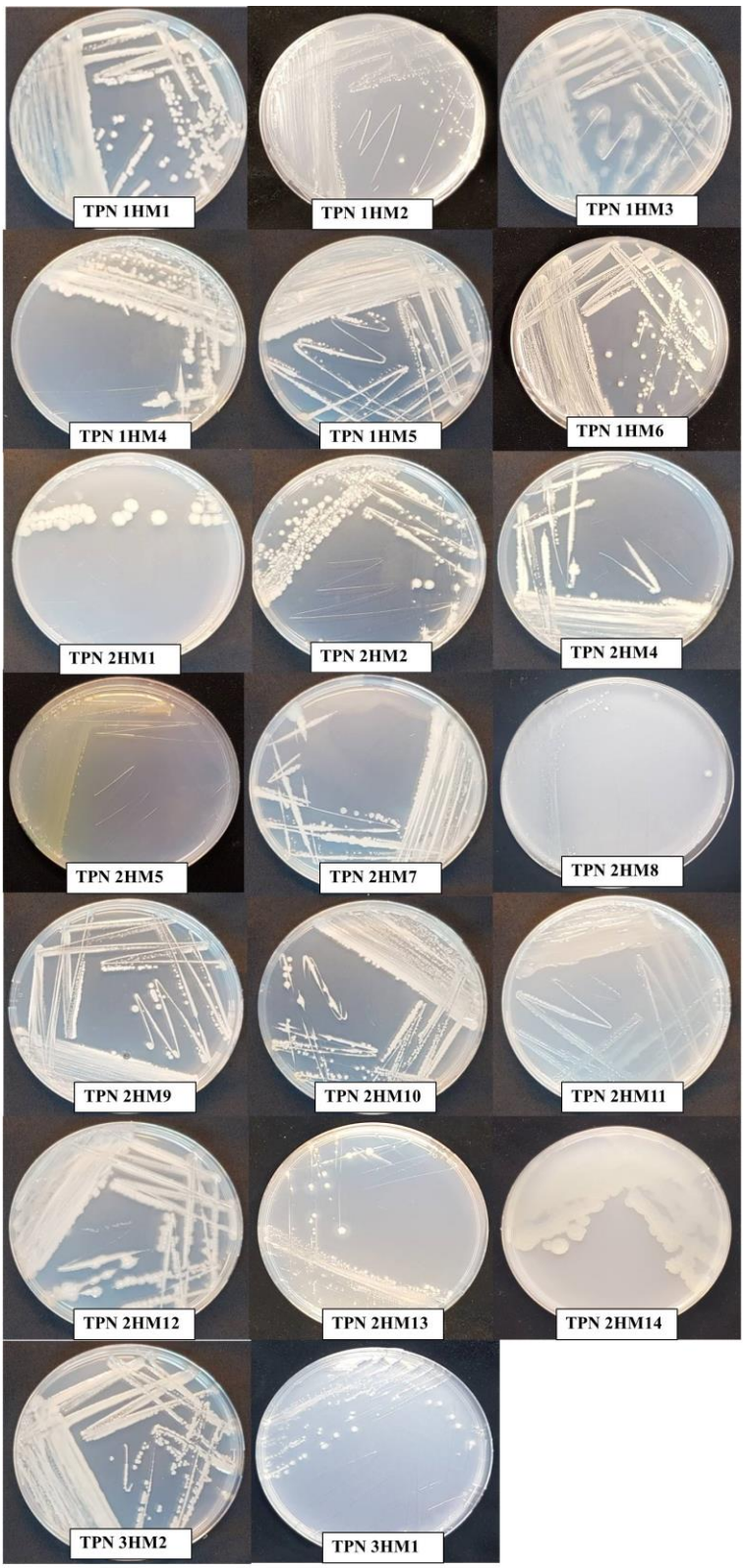


Figure 4.2 The different NR-SOB isolated from the Thep Pha Nom hot spring.

Table 4.1 Morphological characteristics of the NR-SOB strains MAL 1HM19, TPN 1HM1 and TPN 3HM1.

Strains of NR-SOB	Colony morphology
MAL 1HM19	Color: Creamy-white Form: Circular Elevation: Raised Margin: Entire
TPN 1HM1	Color: Creamy Form: Circular Elevation: Convex Margin: Entire
TPN 3HM1	Color: Creamy Form: Circular Elevation: Convex Margin: Entire

4.2 Part II: Selecting the appropriate NR-SOB by estimating the kinetics of H_2S oxidation

4.2.1 Estimation of the zero-order constant for H_2S oxidation in headspace samples

As mentioned previously, among the 38 isolates, four isolates of NR-SOB strain namely MAL 1HM19, TPN 1HM1 and TPN 3HM1 was able to grow on CH_3COO^- and CH_3OH. Moreover, three of them were able to remove H_2S in gas phase within 10 h. The k_0 values of MAL 1HM19, TPN 1HM1 and TPN 3HM1 were 117.0, 113.0, 75.0 ppm_v/h, respectively. From these results, it is evident that strain

MAL 1HM19 showed the highest k_0 values when compared to other strains of NR-SOB. This good result suggested that strain MAL 1HM19 can degrade the H_2S faster than the others. Moreover, in this study, the strain *Paracoccus pantotrophus* DSM 2944 which is one of the well-known NR-SOB was also used as one of the inoculum to examine its ability to remove gas phase H_2S. No H_2S removal was observed during the 24 h period of incubation in the presence of *P. pantotrophus* DSM 2944 (Figure 4.3).

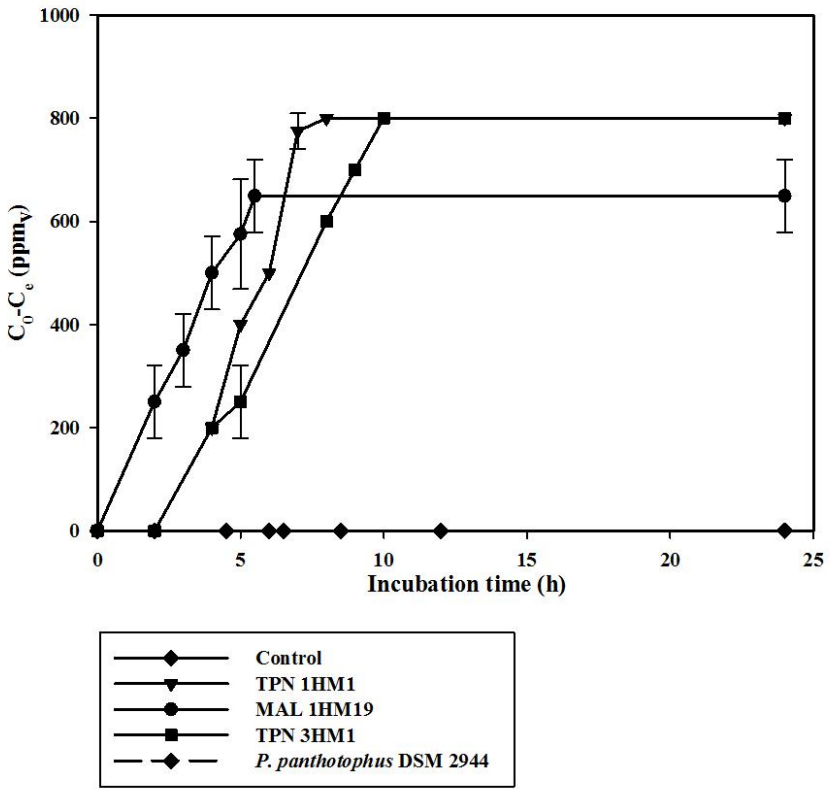


Figure 4.3 Zero-order reaction of H_2S oxidation by strains TPN 1HM1, MAL 1HM19, TPN 3HM1 and *P. panthotophus* DSM 2944. All data are shown as the mean ± SD, derived from duplicate repeats, except for the data of using *P. pantotrophus* DSM 2944 as the inoculum.

Although the optimal temperature for the growth of *P. pantotrophus* DSM 2944 is 28 °C, there are previous studies that have reported good activity at 30 °C (Friedrich *et al*., 2000). When compared to the temperature used in this study (35 °C), this temperature is still within the range of the optimal temperature for this microorganism. Consequently, there might be other environmental factors/conditions that might have affected the ability of *P. pantotrophus* DSM 2944 to remove H_2S.

4.2.2 Hydrogen sulphide (H_2S) removal by pure cultures of NR-SOB

Comparing the time taken by the different NR-SOB strains to remove H_2S, the strain MAL 1HM19 was able remove an initial H_2S concentration of 650 ppm_v within 5.5 h. The strains TPN 1HM1 and TPN 3HM1 consumed an initial concentration of 800 ppm_v within 8 and 10 h, respectively. No degradation of H_2S in the control sets was observed which clearly shows that H_2S removal was solely due to the NR-SOB activity

4.2.3 Sulfide (S^{2-}) and sulfate (SO_4^{2-}) profiles in the liquid phase by pure strains of NR-SOB

The end product of H_2S oxidation by the NR-SOB strains was also reflected in the sulfide (S^{2-}) and sulfate (SO_4^{2-}) production profiles. Meanwhile 100% of sulfide removal from the aqueous phase was observed (Figure 4.4A). The sulfide oxidation was almost instantaneous and it was achieved within 8 h. In a recent study, the sulfide degradation by the group of haloalkaliphilic bacteria composing of *Thioalkalimicrobium aerophilum* and *Thioalkalivibrio versutus* were reported to occur at a slower rate (Ang *et al*., 2017).

SO_4^{2-} concentrations were noticed as the product *via* the sulfide oxidation (Figure 4.4B). The percentage of SO_4^{2-} formation in this study varied from 7 to 34%

for the different inocula of NR-SOB. The N/S molar ratio of this study is 3.3 and it can be correlated to the ratio of stoichiometry shown in equation (4.1) (Huang *et al.*, 2016):

$$S^{2-} + 1.25CH_3COOH + 3.6NO_3^- + 1.6H^+ \rightarrow SO_4^{2-} + 2.5CO_2 + 1.8N_2 + 2.3H_2O + 2OH^-$$

$$\Delta G° = -2100.1 \text{ kJ/reaction} \quad (4.1)$$

The final product of sulfide oxidation is usually linked to the NO_3^--N concentration (Tan *et al.*, 2016). As previously reported, the presence of excess NO_3^- concentration causes excess SO_4^{2-} production (Cardoso *et al*., 2006; Gadekar *et al*., 2006).

4.2.4 Nitrate (NO_3^--N) removal profiles by pure cultures of NR-SOB

As seen from Figure 4.4C, strain MAL 1HM19 showed the highest percentage of NO_3^--N removal (76.2%), while strains TPN 1HM1 and TPN 3HM1 demonstrated 67.5 % 67.3% NO_3^--N removal, respectively. Concerning the NO_3^--N removal rate, strains MAL 1HM19 and TPN 1HM1 showed the same highest NO_3^--N removal rate of 4.0 mg NO_3^--N/L·h and the NO_3^--N removal rate of strain TPN 3HM1 was 3.8 mg NO_3^--N/L·h. Comparing the removal rate of NO_3^--N with previous reports, the rate of NO_3^--N by strain MAL 1HM19 and TPN 1HM1 was higher than the NO_3^--N removal rate by *Rhodococcus* sp. CPZ24 under aerobic denitrification conditions (0.93 mg NO_3^--N/L·h) as well as the NO_3^--N removal rate of 1 mg NO_3^--N/L·h by *Chryseobacterium* sp. R31 isolated from abattoir wastewater (Kundu *et al*., 2014).

4.2.5 Growth of NR-SOB strains and pH profiles

The growth of pure cultures of strains NR-SOB including MAL 1HM19, TPN 1HM1 and TPN 3HM1, as represented by their OD_{600} values, are shown in Figure 4.4D. The growth patterns of strains NR-SOB were similar in all the cultures. The

highest OD_{600} values were recorded at the same time that the H_2S in the gas phase was also completely removed. The OD_{600} values for strain MAL 1HM19 were lower than the other strains; however, this strain still showed good H_2S and NO_3^--N removal. For practical use in anoxic biofilters, the use of strain MAL 1HM19 can limit biomass growth and prevent clogging problems during long term operation.

The initial pH of this study was ~ 7.0 and it increased to 7.8 by the end of the 96-h incubation time. One reason to support the stable pH values might be due to the presence of CH_3CHOO^- which acts as the carbon source in mCSB medium. According to Qambrani *et al.* (2013), the constant pH profiles can be maintained in the sulfur denitrification process by the addition of acetate. The authors also mentioned that the pH can be easily maintained by the proton balance created using the simultaneous sulfur autotrophic and heterotrophic denitrification process. Furthermore, the culture media pH values are mostly stable within the optimal pH range from 6.0 to 9.0 which also supported the activity of both autotrophic and heterotrophic denitrifying bacteria (Oh *et al*., 2001).

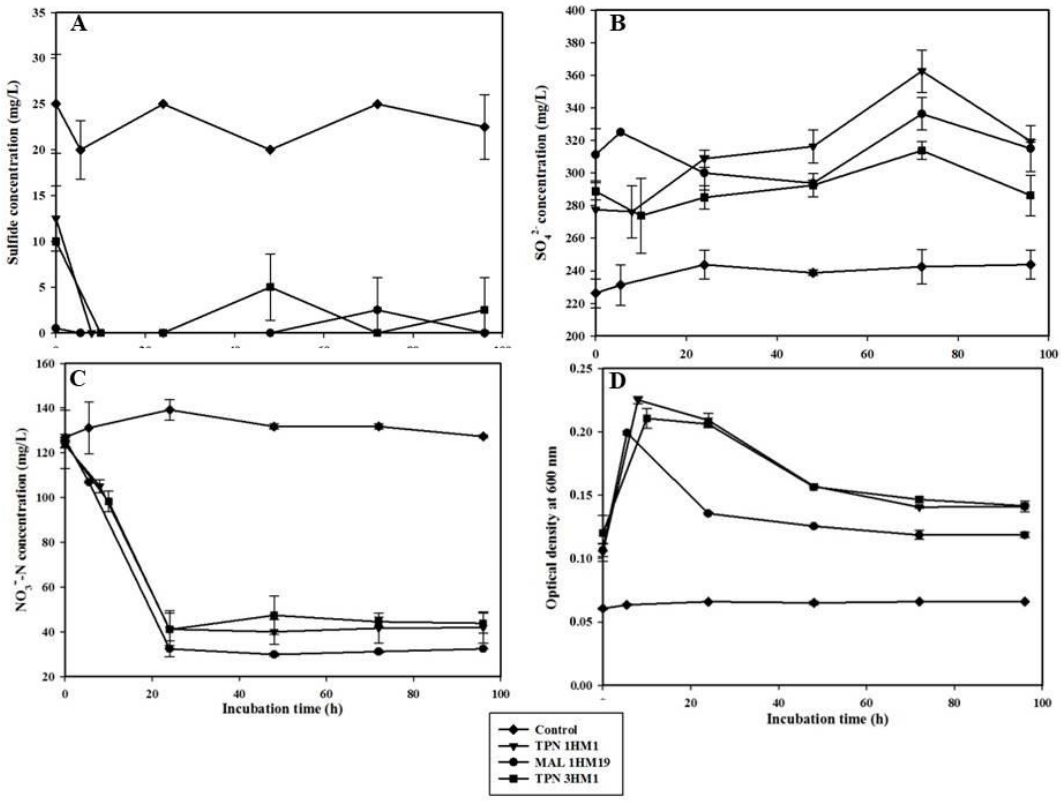


Figure 4.4 Concentrations of (A) sulfide, (B) SO_4^{2-}, (C) NO_3^--N, and (D) optical density values at 600 nm for the pure cultures of MAL 1HM19, TPN 1HM1 and TPN 3HM1 at 35 °C and an initial pH of 7.0 (N/S molar ratio = 3.3). Data are shown as the mean ± SD, derived from duplicate repeats.

From the above results, it was clearly evident that MAL 1HM19 was the best performing strain in terms of its ability to remove H_2S at faster rates, and it was able to perform simultaneous removal of gas/liquid phase sulfide and NO_3^--N. Therefore, the strain MAL 1HM19 was selected for further batch and continuous bioreactor experiments.

4.3 Part III: Identification and characterization of appropriate NR-SOB

(The results of Sections 4.3 and 4.4 have been published in modified form:

Watsuntorn, W., Ruangchainikom, C., Rene, E. R., Lens, P. N., Chulalaksananukul, W., 2017. Hydrogen sulfide oxidation under anoxic conditions by a nitrate-reducing, sulfide-oxidizing bacterium isolated from the Mae Um Long Luang hot spring, Thailand. *International Biodeterioration and Biodegradation*, 124: 196-205.)

4.3.1 The effect of carbon and sulfur sources on the growth of strain MAL 1HM19

About the carbon sources characterization, MAL 1HM19 was able grow on the media containing galactose ($C_6H_{12}O_6$), fructose ($C_6H_{12}O_6$), acetate ($C_2H_3O_2^-$), glucose ($C_6H_{12}O_6$), pyruvate ($C_3H_3O_3^-$) and succinate ($C_4H_4O_4^{2-}$) (Table 4.2). However, the strain MAL 1HM19 did not utilize lactose as the carbon source in these tests. Meanwhile, the growth of strain MAL 1HM19 in the medium containing sulfide and $S_2O_3^{2-}$ was observed and no growth was noticed in the presence of tetrathionate ($S_4O_6^{2-}$).

4.3.2 Morphological characteristics, pH and temperature range of strain MAL 1HM19

The colonies of strain MAL 1HM19 grown on mCSB medium were entire, opaque, circular, smooth, creamy and raised after 2 d of culturing. Strain MAL 1HM19 is rod-shaped, gram-negative, non-spore former with a length and width of 0.8 to 1.6 µm, respectively (Table 4.2, Figure 4.5). Strain MAL 1HM19 is also able to grown in the pH range of 7.0 to 9.0. The growth of this strain was observed in a temperature range of 20 to 50 °C. The strain MAL 1HM19 is mesophilic bacteria although this bacteria was isolated from the hot spring where the temperature is ~ 60-70 °C. However, the place where the water and the sediment samples were collected had lower temperatures.

Table 4.2 Major characterization of strain MAL 1HM19 (Watsuntorn *et al*., 2017).

Parameter	**Value**
Morphology	
Shape	Rod-shaped
Length (µm)	0.8-1.6
Width (µm)	0.4-0.8
Length (µm)	0.8-1.6
Spore formation	-
Gram stain	-
Anaerobic growth	
Sulfide (5-15 mM)	+
Thiosulfate	+
Tetrathionate	-
Carbon source	
Acetate	+
Glucose	+
Galactose	+
Fructose	+
Lactose	-
Pyruvate	+
Succinate	+
NaCl concentration (w/v)	
0.03%	+
0.7%	+
3%	+
7%	+
Growth pH	7.0-9.0
Growth temperature	20-50 ^{o}C

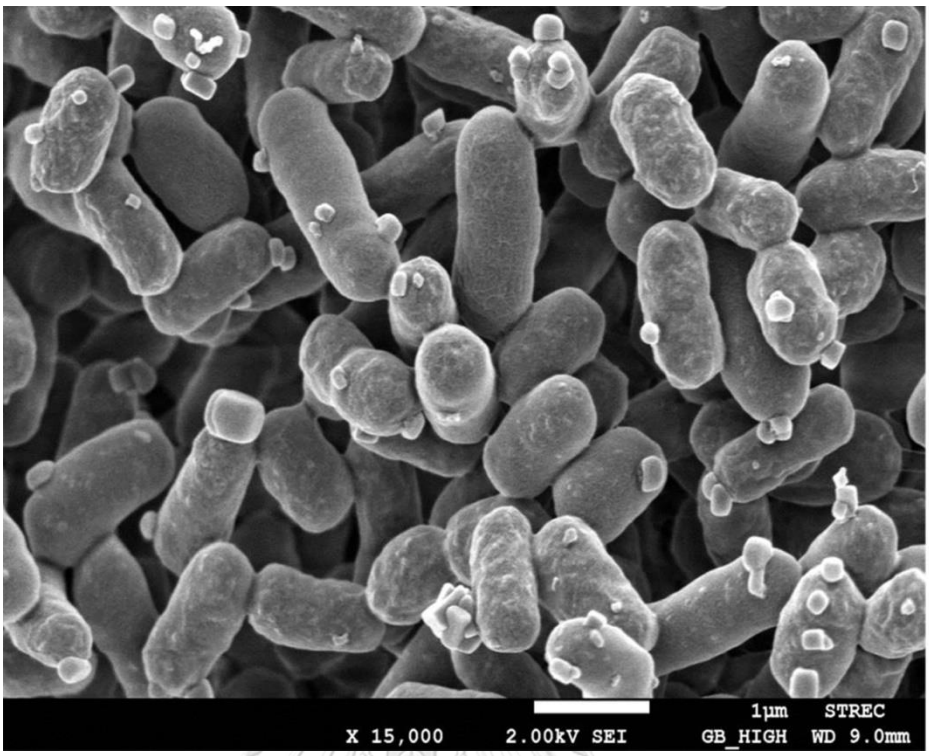


Figure 4.5 SEM micrographs of strains MAL 1HM19 grown on 96 mg/L sulfide and 120 mg/L NO_3^--N (bar = 1 µm).

4.3.3 Effect of NaCl concentration on the growth tolerance of strain MAL 1HM1

The effect of different NaCl concentrations in the mCSB medium such as 0.03%, 0.7%, 3% and 7% (*w/v*) that represents the fresh, brackish, saline and brine conditions, respectively, on the growth of NR-SOB were tested. The highest growth of strain MAL can be detected with 0.7% (*w/v*) NaCl under the brackish conditions. 150% increase in biomass growth (OD_{600}) compared to the control sets (Figure 4.6) was observed. Although the original source of this strain is fresh water from Mae Um Long Luang hot spring, the strain MAL 1HM19 was able to grow under mild saline conditions. At high concentrations of NaCl, the strain MAL 1HM19 might have experienced considerable physiological stress leading to significant decrease in its cell-specific activity and growth.

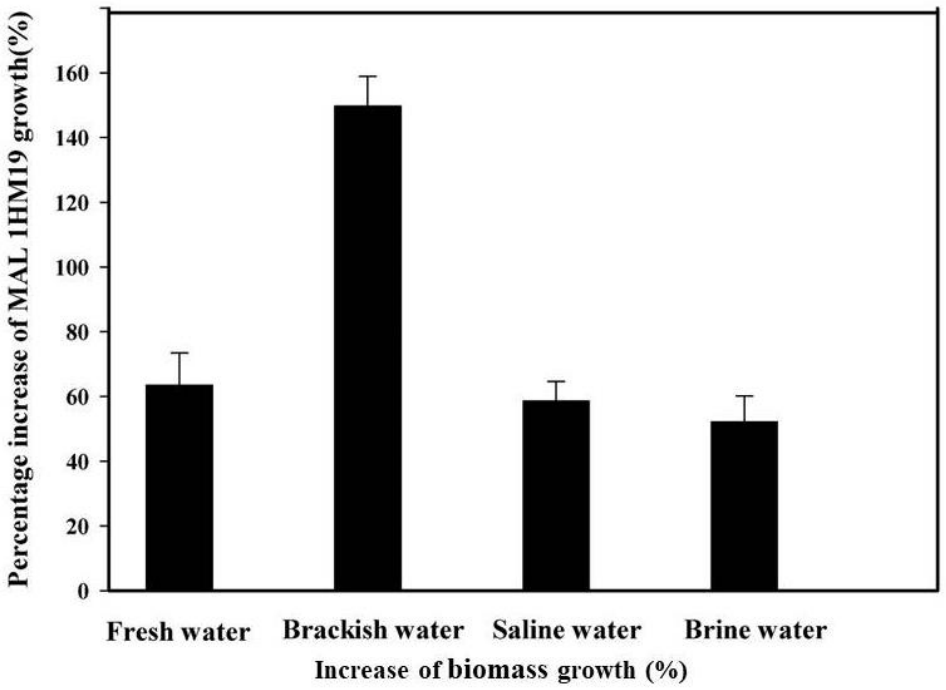


Figure 4.6 The increase of biomass growth of strain MAL 1HM19 at different NaCl concentrations**.**

4.3.4 *soxB* gene and 16S rDNA sequence and phylogenetic analysis of strain MAL 1HM19

The PCR product of *soxB* gene (~550 bp) from strain MAL 1HM19 was exposed (Figure 4.7). The *soxB* sequences demonstrated 98% identity to the genus *Paracoccus versutus* (formerly known as *Thiobacillus versutus*). *soxB* is one gene from the sulfur oxidation system which has been widely studied for its role in the sulfide oxidation pathway (Friedrich *et al*., 2001; Luo *et al*., 2011; Tourova *et al*., 2013). From previous literature, it was show that the *sox* gene clusters is the major pathway of chemotrophic *Alphaproteobacteria* such as the *Paracoccus* sp. (Ghosh and Dam, 2009).

The 16S rDNA nucleotide sequences of the strain MAL 1HM19 showed 99.93% sequence similarity to the 16S rDNA sequences of *P. versutus* over the 1600

bp sequence which corresponded to the *soxB* gene results. From the results of 16S rDNA and *soxB* partial sequences, it can be confirmed that the strain MAL 1HM19 is attributed to the genus *P. versutus* belonging to *Alphaproteobacteria* (Figure 4.8). Furthermore, the Neighbor Joining (NJ) phylogenetic tree, using *E. coli* as the out-group, placed strain MAL 1HM19 within the sub-class *Alphaproteobacteria* and the genus *Paracoccus*. The 16S rDNA sequence similarity above 97 to 98% based on molecular operational taxonomic unit (MOTU) for the same MOTU/species (Janda and Abbott, 2002; Mahmood *et al*., 2009). Consequently, strain MAL 1HM19 can be considered as a *P. versutus*.

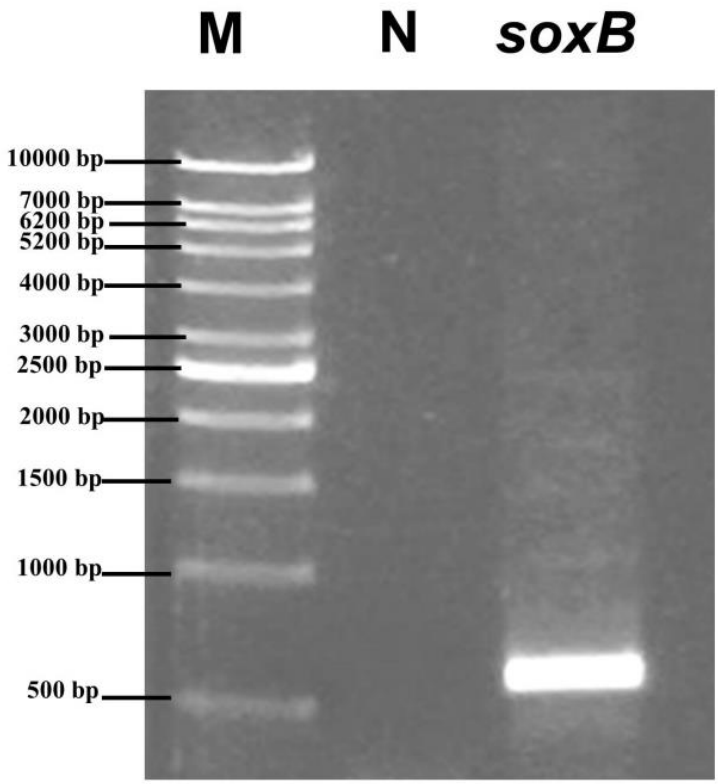
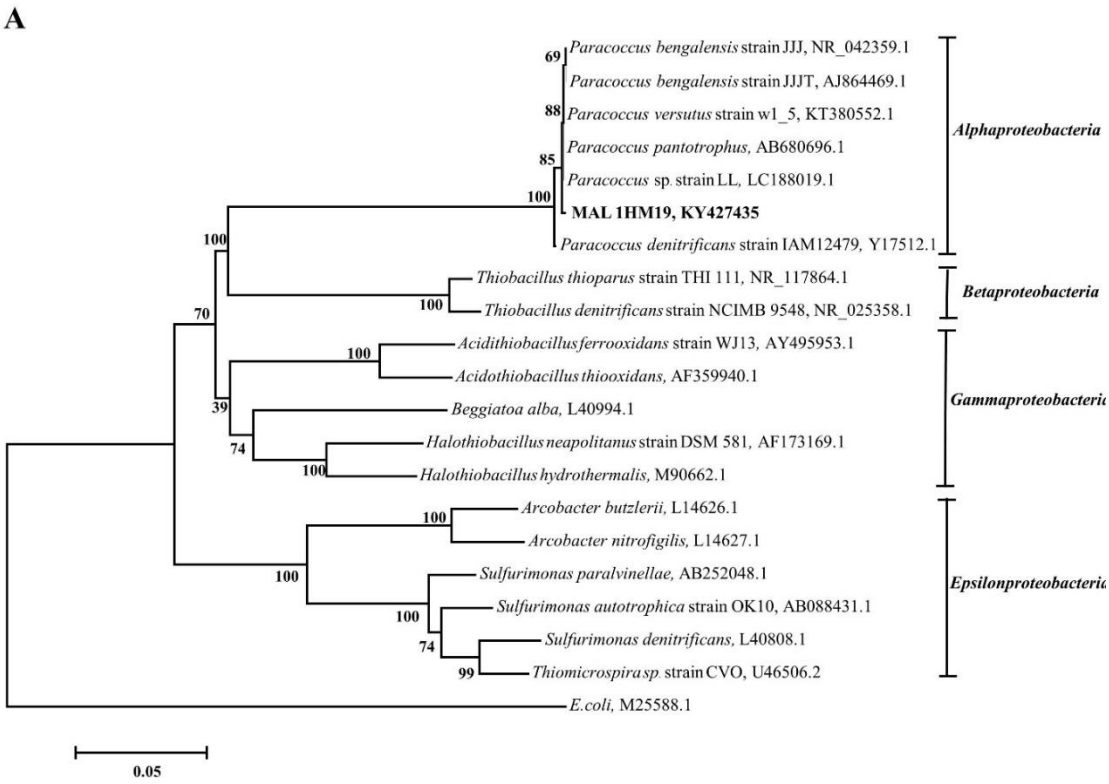


Figure 4.7 The PCR products of *soxB*. M was the DNA marker and N was the negative**.**



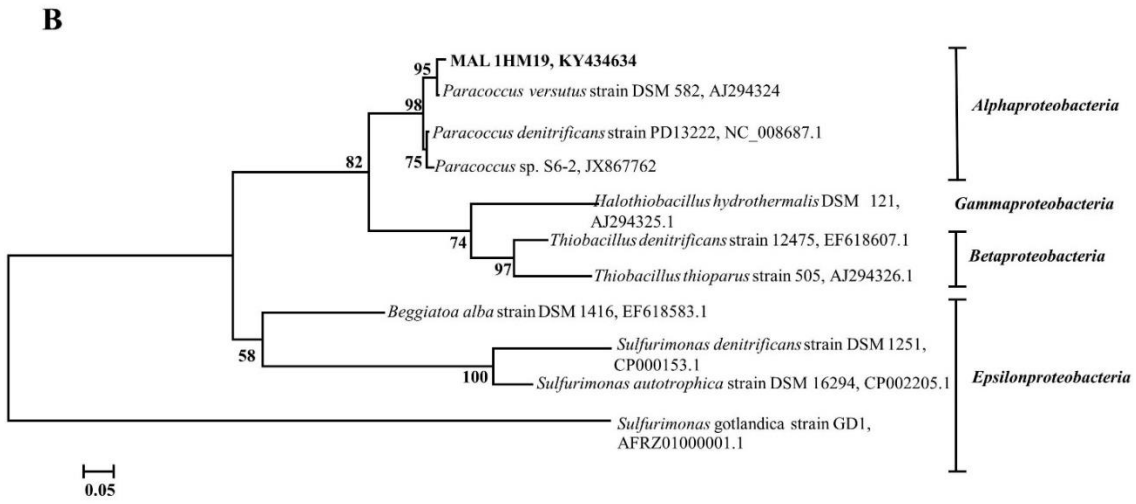


Figure 4.8 Phylogenetic trees using the Neighbor-Joining (NJ) distance method and GenBank accession numbers are shown after the species name. A) based on 1,600 bp of the 16S rDNA gene sequence for strain MAL 1HM19, and B) based on 450 bp of the soxB gene sequence for strain MAL 1HM19. Scale bar substitutions represents a nucleotide divergence of 5%.

4.4 Part IV: H_2S removal in batch experiments under anoxic conditions

4.4.1 The effect of different NO_3^--N concentrations on H_2S oxidation

The zero-order equation, as described in equation (3.1), was used in this study to determine the performance of strain MAL 1HM19. The H_2S in gas phase was removed completely (100%) in 5, 6 and 10 h at initial NO_3^--N concentrations of 60, 120 and 240 mg NO_3^--N/L, respectively. From the zero-order equation, k_0 values of this study were 60.2, 129.7 and 159.7 ppm_v/h at initial NO_3^--N concentrations of 240, 120 and 60 mg NO_3^--N/L, respectively (Figure 4.9). From these results, it was observed that *P. versutus* strain MAL 1HM19 demonstrated the highest k_0 value at the lowest concentration of NO_3^--N. Consequently, the MAL 1HM19 growth at 60 mg

NO_3^--N/L was the most suitable NO_3^--N concentration for H_2S oxidation in the gas-phase which can be seen from the highest k_0 values (159.7 ppm_v/h) for completely removing 700 ppm_v of H_2S within 5 h. The high concentration of NO_3^--N (240 mg NO_3^--N/L) in this study might affect the H_2S removal. The decrease of H_2S removal at high NO_3^--N concentrations might be due to the suppression by NO_3^-. Literature reports have shown than high NO_3^--N concentrations of 47 mM of can inhibit the activity of NR-SOB (Oh *et al*., 2000; Cardoso *et al*., 2006).

Furthermore, k_0 from *P. versutus* DSM 582 was 77.0 ppm_v/h at 120 mg NO_3^--N/L. The k_0 values by strain MAL 1HM19 was almost 2 folds higher than the k_0 value obtained from *P. versutus* DSM 582 which implies that MAL 1HM19 had the faster H_2S oxidized reaction than *P. versutus* DSM 582. From these results, it was affirmed that strain MAL 1HM19 had a potential for H_2S removal, at high concentrations and within a short time.

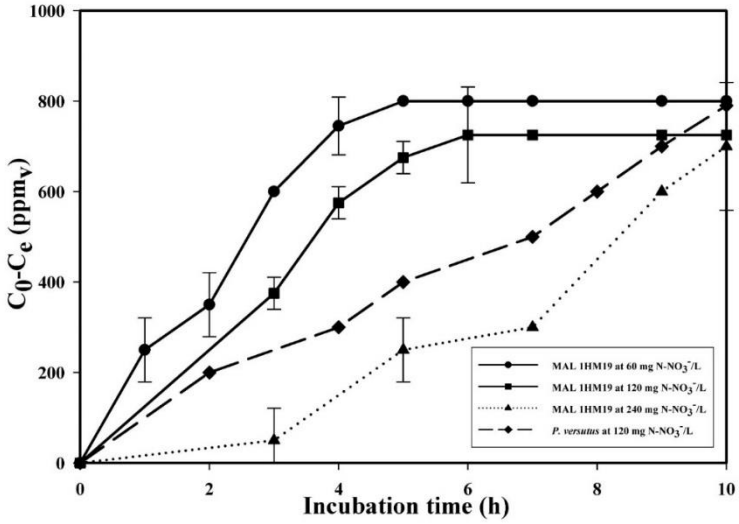


Figure 4.9 Zero-order reaction of H_2S oxidation by strain MAL 1HM19 at different initial N-NO_3^- concentrations of (●) 60, (■) 120 and (▲) 240 mg N-NO_3^-/L, and (♦) compared to *P. versutus* DSM 582. All data are shown as the mean ± SD, derived from three independent replicates, except for the data of using *P. versutus* DSM 582 as inoculum which was derived from one independent replicate.

4.4.2 Sulfide (S^{2-}) and sulfate (SO_4^{2-}) removal in aqueous phase

The sulfide profiles at different initial concentrations of NO_3^--N are shown in Figure 4.10. Sulfide removal in aqueous phase was 28% and 25% during incubation with an initial NO_3^--N concentration of 120 and 60 mg NO_3^--N/L, respectively. The control sets without any biomass also showed H_2S removal, which was presumably due to spontaneous chemical sulfide oxidation. The H_2S concentrations in the media at initial NO_3^--N concentration of 240 mg NO_3^--N/L was constant in both strain MAL 1HM19 and the cell-free control. Even the novel strain MAL 1HM19 demonstrated its good ability to remove H_2S in gas phase. In order to understand the dynamics of H_2S removal, further tests should be performed using living and dead cells (sterilized controls), and the initial concentrations of both H_2S and NO_3^--N should be varied from low to high levels to understand the synergistic or antagonistic effects.

According to Bentzen (1995), the addition of NO_3^--N can increase the microbial competition by altering the degradation pathway of H_2S in sewage. On the other hand, the presence of NO_3^--N is beneficial because it alters the redox potential and supports the growth of autotrophic nitrate-reducing, sulfide-oxidizing bacteria (Garcia-de-Lomas *et al*., 2006; Widdel, 1989).

The major product of the sulfide oxidation reaction of this study was SO_4^{2-}. 15% and 18% of SO_4^{2-} were detected at initial concentrations of 120 and 240 mg NO_3^--N/L (Figure 4.10). The SO_4^{2-} concentrations in all the incubations did not increase exponentially because SO_4^{2-} was also present initially in the medium at concentrations of ~ 225 mg/L (Equation (2.1)). The main end product during incubations with low concentration of NO_3^- is S° which supported by previous reports (Krishnakumar and Manilal, 1999; Cardoso *et al*., 2006). Nonetheless, there are some discrepancies in the literatures because another study has shown the formation of S° at high concentrations of NO_3^- (Reyes-Avila *et al*., 2004).

4.4.3 NO_3^--N, NO_2^--N profiles and pH in the liquid-phase

~97% of NO_3^--N was reduced at initial NO_3^--N concentration of 60 mg/L. 87% and 44% of NO_3^--N were removed at an initial NO_3^--N concentration of 120 and 240 mg/L, respectively. The same patterns of NO_3^--N removal were noticed in all the tested experimental conditions. NO_3^--N was removed instantly within the first 24 h and after that, its concentration remained constant in the bottles (Figure 4.10). From these results, it can be assumed that this strain of NR-SOB can quickly use the NO_3^--N as an electron acceptor. In a previous study by An *et al*. (2010), the consortium dominated by *Thiomicrospira* sp. strain CVO showed slow NO_3^- removal rate throughout the phase of SO_4^{2-} production at initial concentrations of 2.1 mM sulfide

and 10 mM NO_3^-. In this study, the NO_2^--N concentrations were 0.57, 0.31 and 0.57 mg N-NO_2^- at 60, 120 and 240 mg NO_3^--N/L, respectively. After complete H_2S removal from the gas phase, the NO_2^--N was not detected and it can be presumed that the production of N-NO_2^- was only temporary and it was converted to other form of nitrogen according to the denitrification pathway: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow NO_2 \rightarrow N_2$ (Prescott *et al*., 2003; Soreanu *et al*., 2008). The pH values throughout the experiment were in the range of 7.0 to 8.0 which is the optimum pH for the denitrification (Cai *et al*., 2008). The increase of pH might induce the accumulation of NO_2^- resulting in the toxicity to microorganisms and may speed up the instability of the system. Based on the results of this study, the initial concentration of 60 mg NO_3^--N/L was found to be the most appropriate for the H_2S removal ($P < 0.05$) because this condition obtained the highest sulfide and NO_3^--N removal without SO_4^{2-} accumulation.

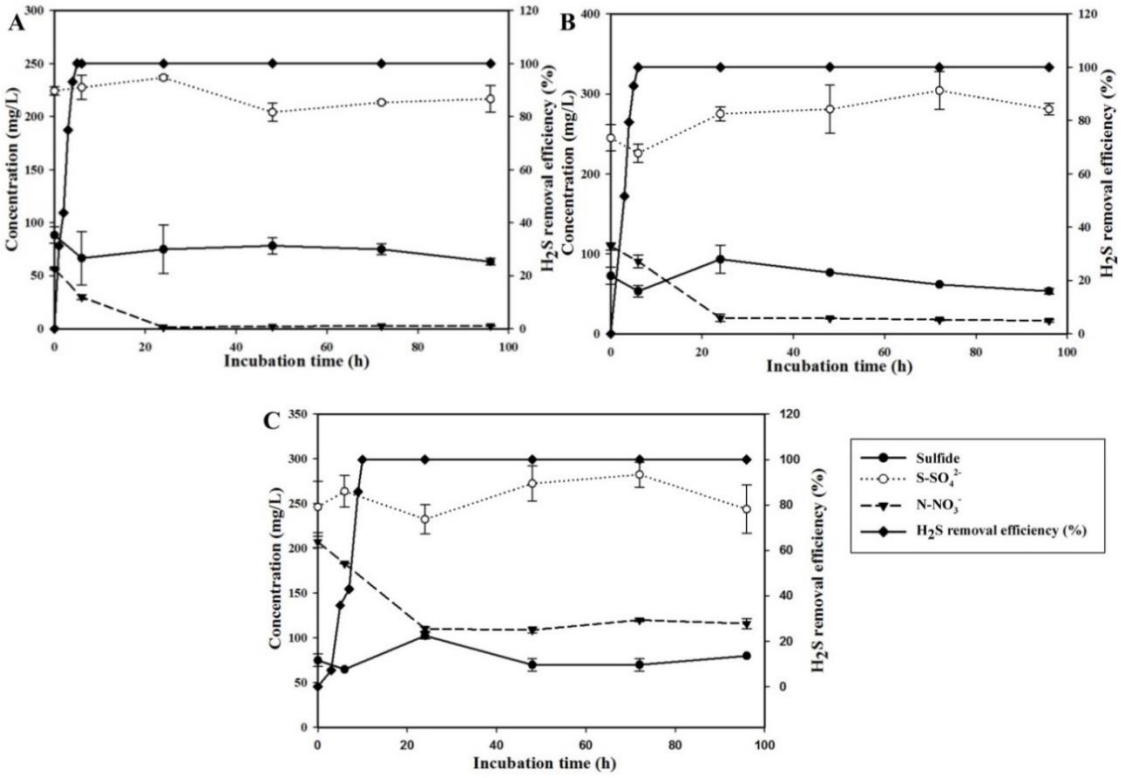


Figure 4.10 H_2S removal efficiency and sulfide (S^{2-}), sulfate (SO_4^{2-}), and nitrate (NO_3^--N) concentration profiles at initial NO_3^--N concentrations of (A) 60, (B) 120 and (C) 240 mg/L. Data are shown as the mean ± SD, derived from three independent replicates.

The highest OD_{600} values in the different incubations were observed at the time when H_2S in the gas phase was completely removed. After that, the turbidity as well as the OD_{600} values slightly decreased (Figure 4.11). These results can be correlated to the previous findings described in Section 4.2.5.

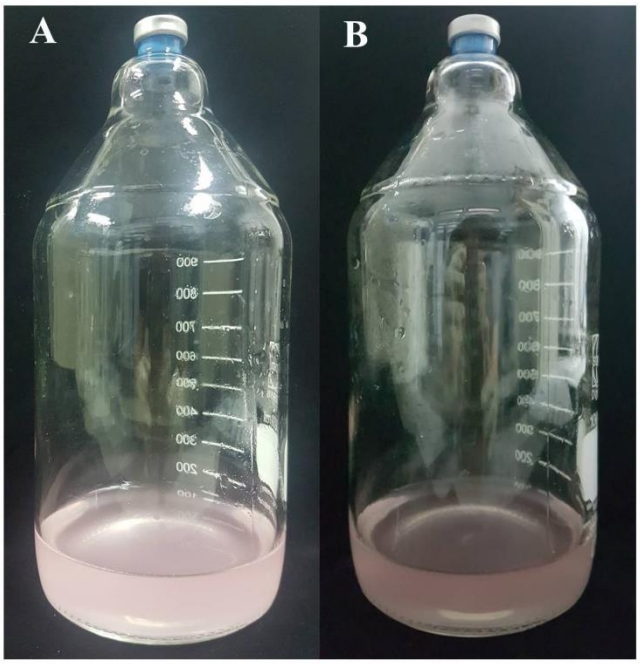


Figure 4.11 The turbidity of mCSB medium (A) at 6 h of incubation with an initial NO_3^--N concentration of 120 mg NO_3^--N/L, and (B) after 6 h of incubation.

4.4.4 Comparison between sulfide (S^{2-}) and thiosulfate ($S_2O_3^{2-}$) as sulfur sources for the growth of MAL 1HM19

4.4.4.1 Sulfide and $S_2O_3^{2-}$ profiles in batch experiments

In order to test the ability of the microorganisms to use $S_2O_3^{2-}$ instead of sulfide, different batch experiments were carried out. The results showed that the sulfide concentrations in the aqueous phase were reduced completely within 48 h (Figure 4.12A). These results correlated to the previous finding as shown in this thesis, as well as the previous study by Watsuntorn *et al.* (2017). Moreover, this results strongly supported the fact that the strain MAL 1HM19 can use sulfide as an energy source. In the case of $S_2O_3^{2-}$, its concentrations in the aqueous phase were almost constant and it only slightly increased from 72 h until 108 h (Figure 4.12B). The metabolic pathway of $S_2O_3^{2-}$ utilization by the *P. versutus* strain MAL 1HM19

was not clearly understood in this study. However, it can be assumed that the strain MAL 1HM19 cannot use $S_2O_3^{2-}$ as a sole energy source. $S_2O_3^{2-}$ is the key intermediate of the sulfur cycle (Matanhike, 2017). Tang *et al*. (2009) reported $S_2O_3^{2-}$ oxidation by *Thiobacillus denitrificans* and *Thiomicrospira denitrificans* which are normally known as sulfur bacteria and belong to the NR-SOB that can also simultaneously perform anaerobic denitrification by reducing NO_3^- to N_2 as shown in equation (4.2):

$$S_2O_3^{2-} + 1.6NO_3^- + 0.2H_2O + 2SO_4^{2-} + 0.8N_2 + 0.4H^+, \Delta G^O = 765.7 \text{ kJ} \quad (4.2)$$

This observation and results are different from previous researches which have reported that the metabolic activity of NR-SOB was considerably higher with $S_2O_3^{2-}$ compared to other sulfur compound such as S^o or sulfide. In addition, there are many literatures that have reported that $S_2O_3^{2-}$ is an intermediate compound of sulfate (SO_4^{2-}) oxidation and it can also be used as an electron donor than sulfide. Due to the non-toxic and readily bioavailable properties of $S_2O_3^{2-}$, the use of $S_2O_3^{2-}$ has shown to facilitate high denitrification and sulfur oxidation rates (Cardoso *et al.,* 2006). $S_2O_3^{2-}$ oxidation to SO_4^{2-} is a detoxification method by heterotrophic bacteria. $S_2O_3^{2-}$ can improve the carbon assimilation rate resulting in the higher biomass concentration of bacteria (Nicola *et al*., 2010). Cardoso *et al*. (2006) reported that $S_2O_3^{2-}$ was the most used electron donors than sulfide and S° and the rates of NO_3^- removal in the presence of $S_2O_3^{2-}$ were 4.6 times higher than the other sulfur sources.

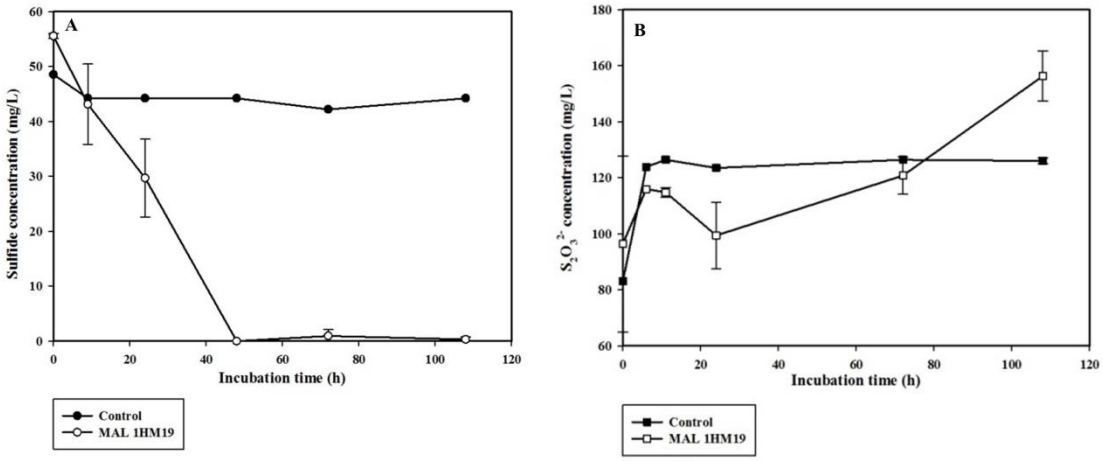


Figure 4.12 (A) H_2S and (B) $S_2O_3^{2-}$ profiles. Data are shown as the mean ± SD, derived from two independent replicates.

4.4.4.2 SO_4^{2-} profiles in batch experiments

SO_4^{2-} was detected in both experiments (Figure 4.13). The SO_4^{2-} formation using sulfide as an electron donor was generated from the biological activity by strain MAL 1HM19 *via* the complete sulfide oxidation pathway. These results fit well with the previous study by Watsuntorn *et al*. (2017) as well as the batch experiment reported in Section 4.4.2. However, SO_4^{2-} formation was also detected in the experiment when $S_2O_3^{2-}$ was used as the electron donor despite no removal of $S_2O_3^{2-}$ from the aqueous solution. The formation of SO_4^{2-} as a product using $S_2O_3^{2-}$ might also be from $S_2O_3^{2-}$ disproportionation reaction or the spontaneous chemical reaction that does not involve the biological activity of strain MAL 1HM19 ($S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + H_2S$). According to these chemical mechanisms, the disproportionation of $S_2O_3^{2-}$ yields only a small amount of free energy and it is an important process for the transformation of $S_2O_3^{2-}$ in aquatic ecosystems. Nevertheless, further studies should be carried out to understand the real biochemical mechanisms using adequate control experiments at varying pH conditions.

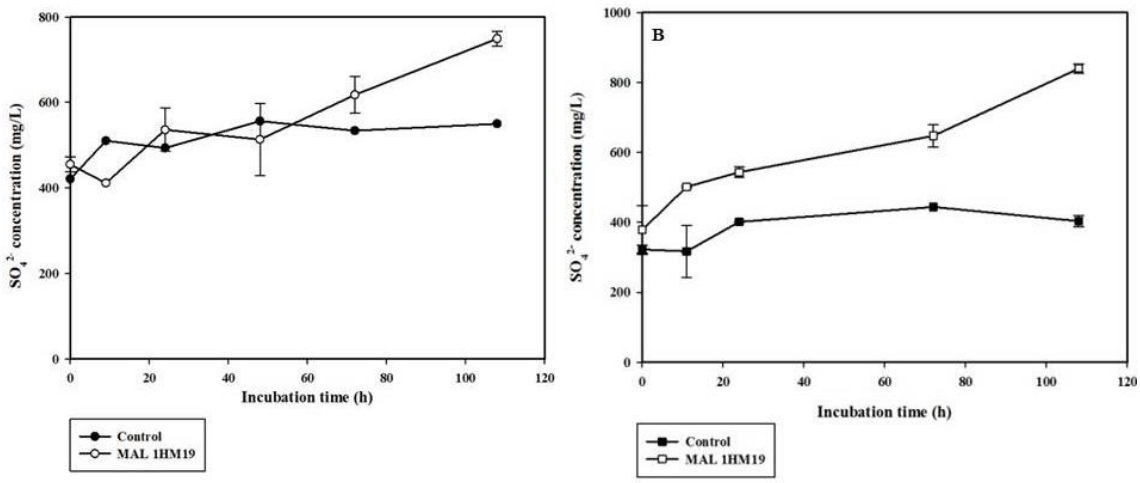


Figure 4.13 SO_4^{2-} profiles during (A) sulfide as the sulfur source and (B) $S_2O_3^{2-}$ as the sulfur source. Data are shown as the mean ± SD, derived from two independent replicates.

4.4.4.3 NO_3^--N profiles in batch experiments

44 % of NO_3^--N concentrations was removed when sulfide was used as the electron donor and > 91% NO_3^--N removal was achieved when $S_2O_3^{2-}$ was used as the electron donor (Figure 4.14). The NO_3^--N removal in the presence of $S_2O_3^{2-}$ was higher than the removals observed using sulfide in all the batch experiments. One of the probable reason might be that the strain MAL 1HM19 can use NO_3^--N and CH_3COO^- without using the sulfur oxidation pathway. *P. versutus* is one of the denitrifying bacteria that can consume only NO_3^--N as an electron acceptor.

The genus of *Paracoccus* is widely present in aquatic and deep sub-surface as well as waste treatment plants (Baker *et al*., 1998; Kumar and Spiro, 2017). *Paracoccus* sp. have also demonstrated high efficiency of denitrification reactors (Wang *et al*., 2015) and several studies have applied this genus of bacteria for NO_3^--N removal from wastewater under both anaerobic and aerobic conditions (Liu *et al*.,

2012; Shi *et al*., 2013). Previously, Liu *et al*. (2012) reported the efficiency of *Paracoccus* sp. strain YF1 isolated from activated sludge for the removal of NO_3^--N contamination in the wastewater and showed a NO_3^--N removal rate of 4.57 mg NO_3^- /L h at an initial concentration of 100 NO_3^- mg/L (23 mg NO_3^--N/L). Furthermore, *P. versutus* LYM which was isolated from seabed sludge served as an efficient candidate to remove high NO_3^--N concentrations from inorganic medium under aerobic conditions (Shi *et al*., 2013).

In this study, NO_2^--N formation was observed as an intermediate product *via* the denitrification step in both experiments. The highest NO_2^--N concentrations were 7.2 and 11.0 mg/L of NO_2^--N from the experiments with $S_2O_3^{2-}$ and sulfide, respectively. After that, the NO_2^--N was not detected in both the experiments. It was therefore assumed that NO_2^--N was completely removed to N_2 according to the denitrification pathway shown in Section 4.4.3.

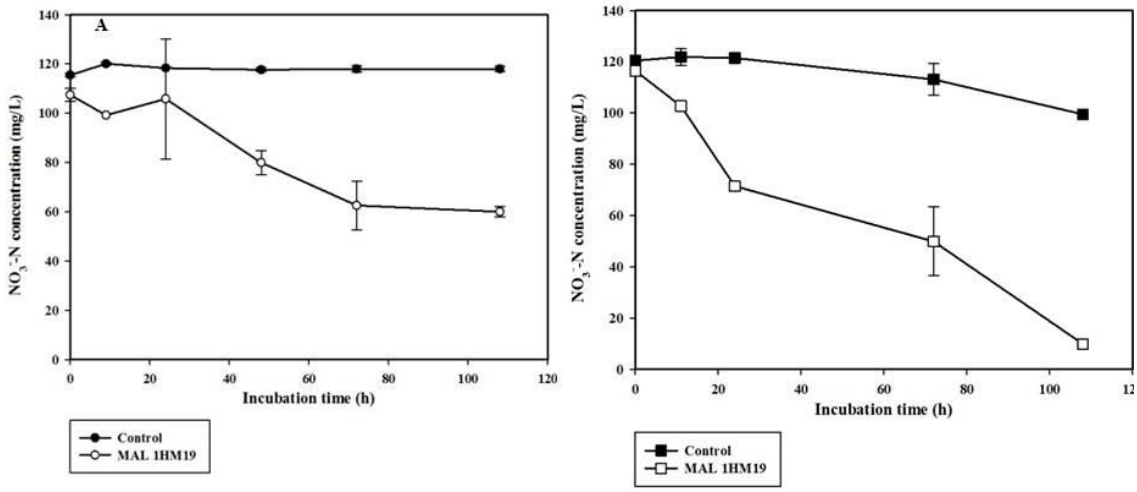


Figure 4.14 NO_3^--N profiles during (A) sulfide as the sulfur source, and (B) $S_2O_3^{2-}$ as the sulfur source. Data are shown as the mean ± SD, derived from two independent replicates.

4.4.4.4 CH_3COO^- profiles in batch experiments

CH_3COO^- consumption was almost 100% (Figure 4.15) in both experiments. This clearly indicates the fact that CH_3COO^- is the main carbon source of this study and it was totally degraded by the activity of *P. versutus* strain MAL 1HM19. The CH_3COO^- oxidation occurred in order to gain the energy source for strain MAL 1HM19 apart from the sulfide oxidation. Moreover, the presence of CH_3COO^- has shown to enhance the total denitrification rate as well as decrease the lag phase of denitrification (Cardoso *et al*., 2006).

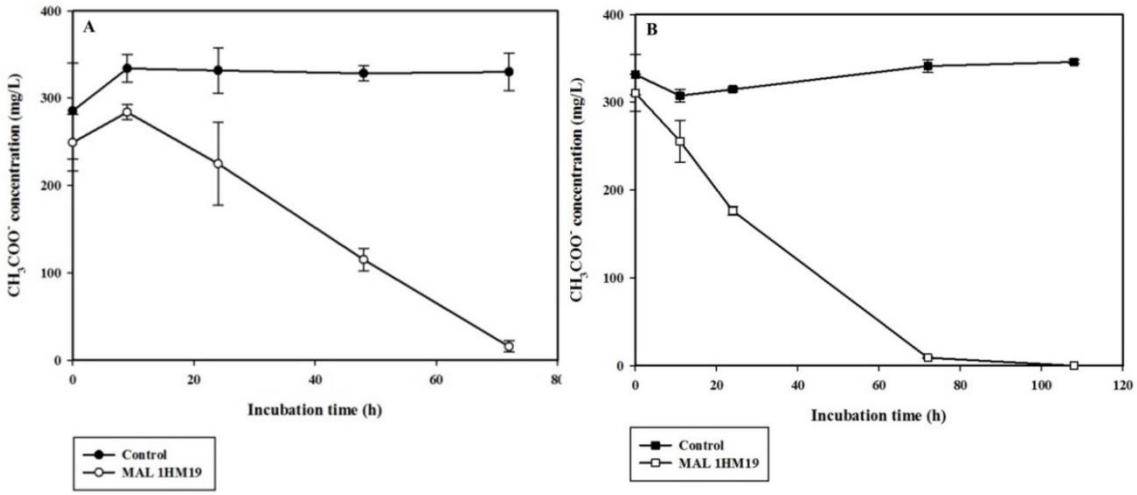


Figure 4.15 CH_3COO^- profiles during (A) sulfide as the sulfur source, and (B) $S_2O_3^{2-}$ as the sulfur source. Data are shown as the mean ± SD, derived from two independent

4.4.4.5 Growth of NR-SOB strains

The growth patterns of strain MAL 1HM19 in the presence of both sulfur sources, as shown by their OD_{600} values, were similar in the batch assays (Section 4.2.5). Although the strain MAL 1HM19 cannot utilize $S_2O_3^{2-}$ as the sulfur source, the OD_{600} value was higher than the OD_{600} value with sulfide (Figure 4.16). The highest OD_{600} value with $S_2O_3^{2-}$ was obtained at 24 h while the highest OD_{600} value with sulfide was obtained at 48 h. This observation clearly indicates that the strain MAL 1HM19 grew faster in the presence of $S_2O_3^{2-}$ despite its inability to use $S_2O_3^{2-}$ as a sulfur source. Hence, sulfide, in the form of H_2S (gas), was selected and used as the electron donor for the strain MAL 1HM19 in further experiments that were performed in an anoxic biotrickling filter (BTF).

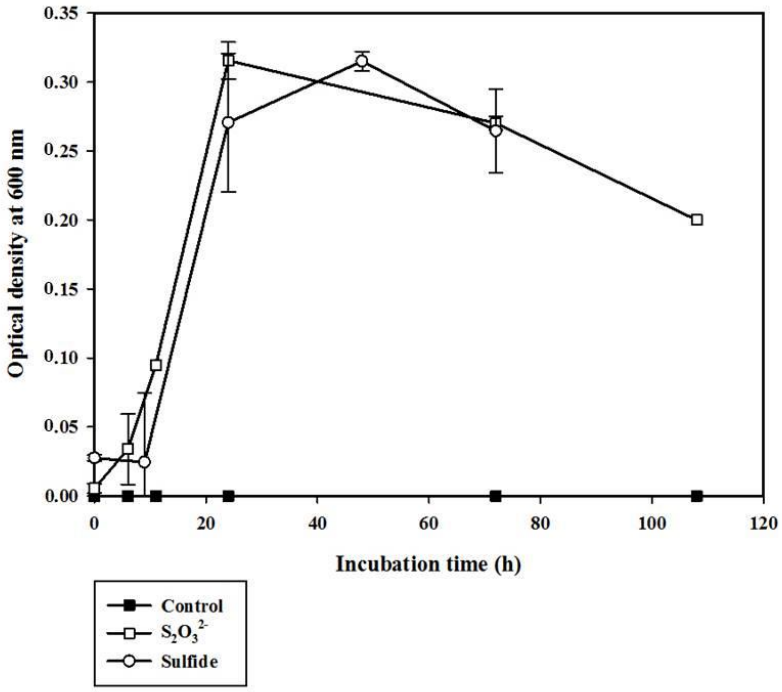


Figure 4.16 Optical density values at 600 nm of the pure cultures of MAL 1HM19 using (A) sulfide as the sulfur source, and (B) $S_2O_3^{2-}$ as the sulfur source. Data are shown as the mean ± SD, derived from two independent replicates.

4.5 Part V: H_2S removal in an anoxic biotrickling filter (BTF)

The BTF, inoculated with *P. versutus* strain MAL 1HM19, was operated for 188 d using NO_3^--N as an electron acceptor (liquid-phase) and continuously passing H_2S (gas-phase) under anoxic conditions. In this study, the BTF operations were divided into three phases including (i) fed-batch mode, (ii) continuous mode and (iii) shock loads with different, yet high concentrations of H_2S. In order to evaluate the ability of continuous H_2S removal under anoxic BTF conditions, N_2 was used as the carrier gas and it was passed at a flow rate of 60 L/h.

4.5.1 H_2S profiles in the anoxic BTF

4.5.1.1 BTF operation with nutrient supply in fed-batch mode

During Phase I, period I of the anoxic BTF operation, 100% H_2S removal was detected instantly after starting the BTF, within almost one day (Figure 4.17A) resulting in the production of SO_4^{2-} which clearly demonstrated the biological activity of the immobilized cells of strain MAL 1HM19 to remove gas-phase H_2S. In this study, the good removal of H_2S was also possibly due to the continuously trickling nutrient solution, i.e. from the top of the filter bed, which might have also absorbed the gas-phase H_2S. The acclimatization stage of this study was nearly similar to a previous work by Fernández *et al*. (2014). In that study, high RE of H_2S was observed since the beginning of anoxic BTF operation because the inoculum was obtained from a previously operated BTF operated under the same conditions. During the acclimation stage, 100% RE of H_2S was achieved. The initial H_2S concentrations used in this study was greater than the previous report by Nisola *et al*. (2010). According to the authors, the inlet concentration of H_2S for the BTF inoculated with *Bordetella* sp. Sulf-8 was 50 ppm_v and it was completely consumed within 3 d. The inoculation of microorganisms in BTF is more arduous than the BF due to difficulty to form the biofilm by microorganisms on the inert material as well as the shear stress created due to the trickling nutrient solution (Jiang *et al*., 2009).

During period II of BTF operation, i.e. in the absence of CH_3COO^-, the RE of H_2S dramatically reduced from 100% to almost 17% in 7 d; however, it reached a RE of 100% after replacing the nutrient medium. Nevertheless, the RE of H_2S was slightly reduced to 31-46% when the NO_3^--N was complete consumed. The RE of H_2S was ~70%; it might be because the microorganisms was able to easily adapt to the new conditions without CH_3COO^- as the carbon source. The RE of H_2S depended on the concentration of NO_3^--N in the liquid-phase. During this period, the dependence of

NO_3^--N was noticed. The RE of H_2S decreased only slightly when the concentration of NO_3^--N in the trickling medium was almost zero and it immediately increased to values as high as 100% when NO_3^--N was introduced to the BTF. This anoxic BTF was fed a mCSB medium that was refreshed once every week following the recommendations of a previous study by Prado *et al*. (2004)**.** The RE of H_2S reached >89% when the new nutrient solution containing CH_3COO^- and NO_3^--N was passed to the reactor. In this study, the NO_3^--N concentrations did not significantly affect the H_2S removal. Even when the NO_3^--N was completely consumed, the RE of H_2S was still > 89%.

During the period IV, the inlet H_2S concentration was increased to 300 ppm_v, correspond to an inlet load of 8.37 g $S/m^3 \cdot h$, resulting in a slight decline in RE to 73%. When the inlet H_2S concentration was increased further to 500 ppm_v (loading rate - 14.14 g $S/m^3 \cdot h$), the RE of H_2S was still > 72%.

4.5.1.2. BTF operation with continuous supply of nutrients

When the operational mode of the BTF was changed from fed-batch mode to continuous mode, at an inlet H_2S concentration of 500 ppm_v, the RE of H_2S increased to values > 92% (Figure 4.17B). These RE values were higher than the RE of H_2S observed during fed-batch mode of operation. This might be due to the continuous supply and availability of nutrients for the microorganism attached to the PUF packing material of the BTF. Such operation might have increased the activity of the biomass, leading to good removal of both H_2S and NO_3^--N in the BTF.

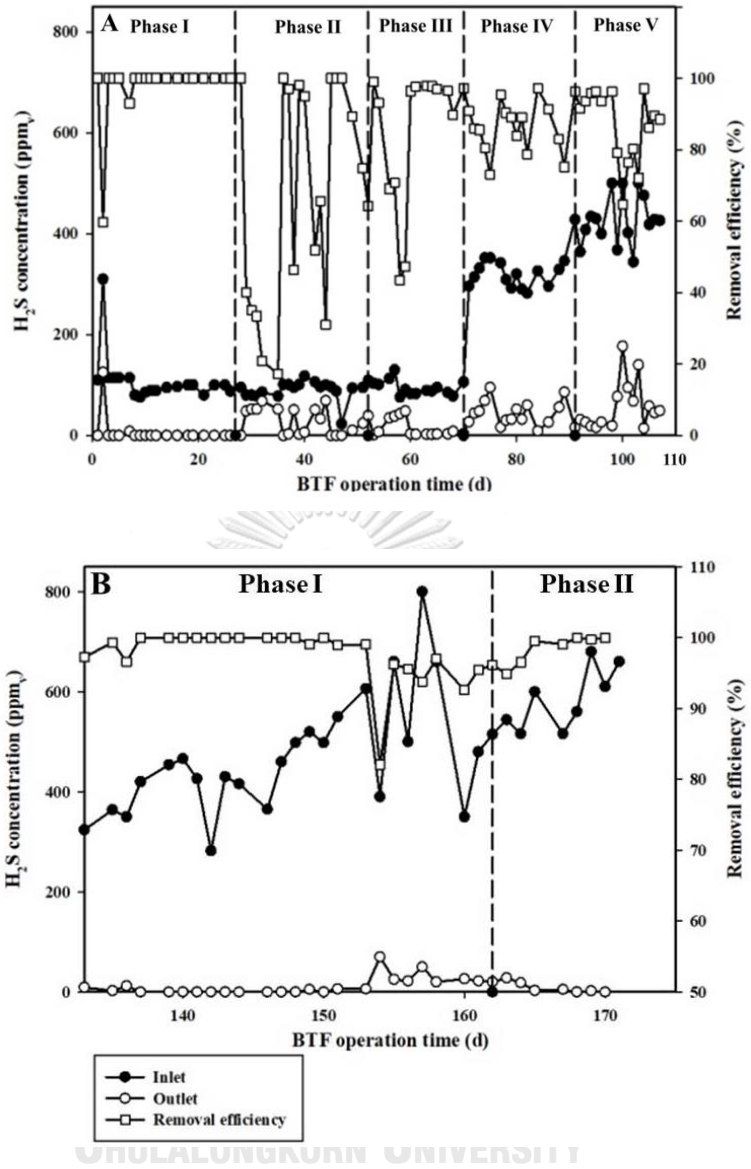


Figure 4.17 H_2S profiles and removal efficiencies in the anoxic BTF: (A) fed-batch, and (B) continuous mode of nutrient supply.

4.5.1.3 Effect of shock loads on H_2S removal

In industrial settings, the concentrations of pollutants in emissions can vary depending on the unit operation, the raw materials used, the products and also the time of operation in a day. Hence, it is important to design bioreactors that can be versatile in handling fluctuating inlet concentrations and/or flow rates. At the lab-scale, it will be useful to test such conditions in order to understand the consequences

of shock loading situations, i.e. a sudden, unexpected increase or decrease in gas flow rate and concentration, and also envisage the time required by the biomass to recover and demonstrate stable/steady-state removal efficiencies (Kasperczyk and Urbaniec, 2015). Several previous studies have tested the effect of shock-loading or feast and famine conditions in BTF. The results from these studies have proven that an active BTF will not fail completely under such situations, instead, it will be able to operate under fluctuating conditions of pollutant loads for a short time and once the loads are restored to values that are less than the critical load of that system, the BTF will yield maximum degradation of the pollutant (Rene *et al*., 2005; Kim *et al*., 2008).

The results from shock loading experiments are shown in Figure IV-18. Two sets of experiments were conducted in order to ascertain the repeatability of the transient tests and understand the activity of the microorganisms. In the first set of experiments (Figure 4.18A), the inlet H_2S concentration was increased from a previous value of 500 ppm_v to 1000 ppm_v during the first shock load. Thereafter the H_2S concentrations were increased to 2000, 3000, and 4000 ppm_v, respectively, corresponding to an inlet load of 28.29, 56.58, 84.87 and 113.161 g $S/m^3 \cdot h$, respectively. During these shock loads, the high H_2S concentration was maintained for ~3. 5 h. The RE of H_2S during these successive shock load tests were > 96%, > 91%, > 83% and 61% at inlet loads of 28.29, 56.58, 84.87 and 113.161 g $S/m^3 \cdot h$ (Figure 4.18A), respectively. During the restoration of the original concentrations, i.e. a H_2S concentration of 500 ppm_v, the RE increased to 96% (0.14 g $S/m^3 \cdot h$).

For the second set of shock loading experiments (Figure 4.18B), the inlet load of H_2S was 56.58, 84.87 and 113.161 g $S/m^3 \cdot h$ (concentrations - 2000, 3000, and 4000 ppm_v of H_2S). During all these increased H_2S loading conditions, the RE was

greater than 96% and the BTF was able to show almost 100% RE when the inlet load was decreased to the 0.14 g $S/m^3 \cdot h$ at an inlet H_2S concentration of 500 ppm_v within 1.5 h (Figure 4.18B). The RE of H_2S during the second set of shock loading experiments were higher than the first successive shock loading experiments and a satisfactory bioreactor performance was demonstrated by the BTF. Usually, in BTF or biofilters (BF), a sudden exposure to very high H_2S concentration might cause toxicity to the microbial community, leading to a inhibition of microbial activity and a decline in the RE by ~40 to 50% (Kim *et al*. 2008). These good results suggests that the anoxic BTF would easily cope with shock loading conditions and still maintain high gas-phase H_2S removal in the presence of liquid-phase NO_3^--N.

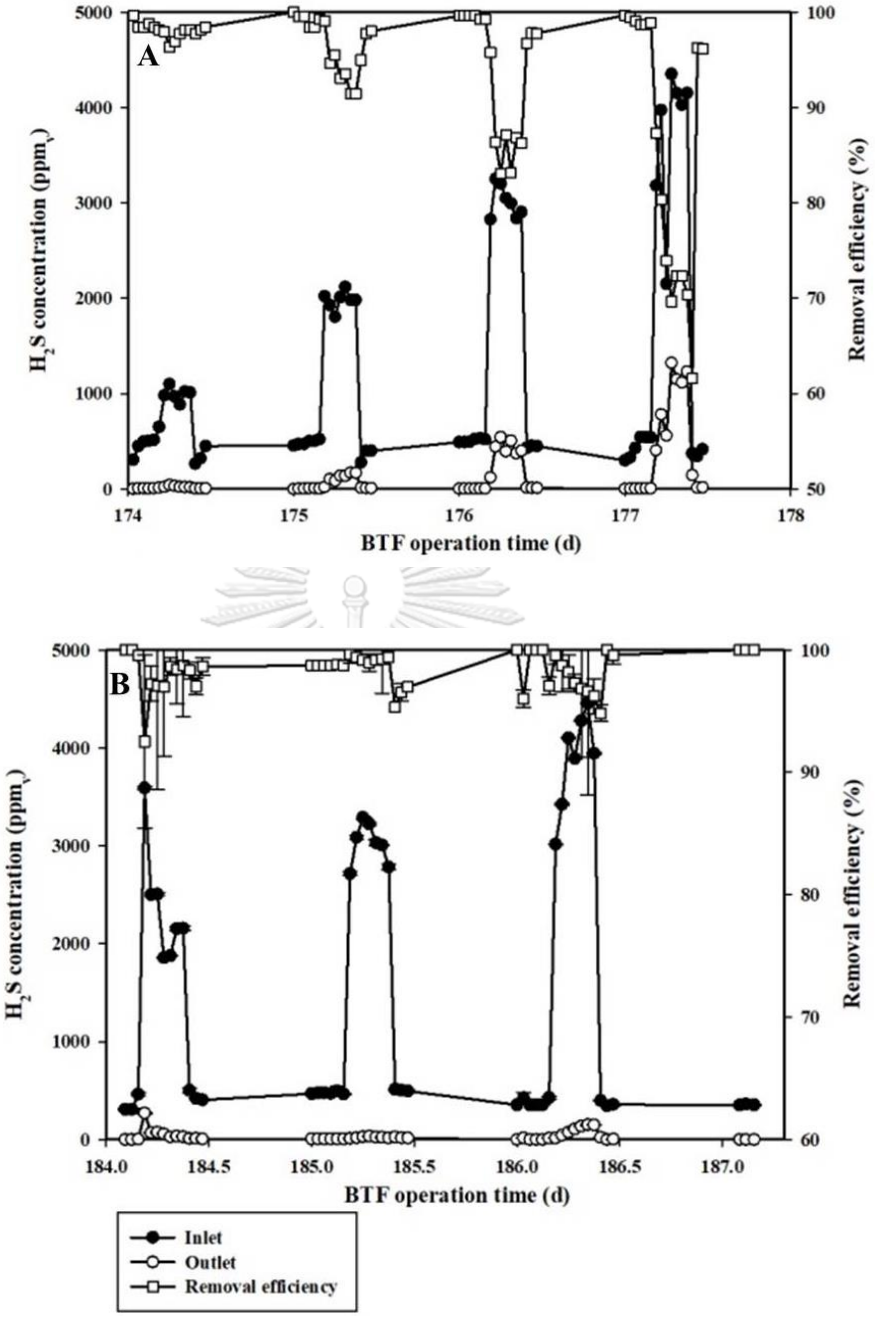


Figure 4.18 Inlet H_2S concentration profiles and removal efficiencies in the BTF: (A) 1st set of H_2S shock loading experiments and (B) 2nd set of H_2S of shock loading experiments.

4.5.2 Sulfide (S^{2-}) and SO_4^{2-} profiles in the BTF

Trace amounts of sulfide concentration in the liquid-phase (0-0.4 mg/L) were detected in the anoxic BTF during fed-batch mode of operation (Figure 4.19A). The maximum sulfide concentration in the liquid-phase during the H_2S shock load experiments was 0.7 mg/L which had no inhibitory effect on the microorganisms present in the anoxic BTF. As reported in previous works, the inhibitory concentration of undissociated and dissolved dissociated sulfide and H_2S forms are in the range of 50 to 400 and 100 to 800 mg/L, respectively (Parkin *et al*., 1990; Pokorna *et al*., 2015).

SO_4^{2-} is the major product of the sulfide biodegradation pathway under anoxic conditions. The concentration of SO_4^{2-} was in the range of 900 to 1600 mg/L even when the H_2S concentration was increased from 100 to 500 ppm_v (Figure 4.19B). Consequently, an increase in the H_2S concentration did not affect the SO_4^{2-} generation profiles. As shown in a previous study by Vikromvarasiri *et al*. (2017), the SO_4^{2-} production rate was constant when the inlet concentration of H_2S was below 570 ppm_v.

The highest SO_4^{2-} concentration was 1,600 mg/L during phase I of BTF operation, at an inlet H_2S concentration of 500 ppm_v (Figure 4.19B). In this study, SO_4^{2-} formation or the presence of SO_4^{2-} in the trickling nutrient medium did not have an inhibitory effect on the microorganism nor an effect on the removal of gas-phase H_2S. This observation is consistent with the results reported in a previous BTF study by Ramírez *et al*. (2009). In that study, the BTF was inoculated with *Thiobacillus thioparus* and inhibition was not reported even when the SO_4^{2-} concentration was as high as 5,000 mg/L. According to another report, inhibition by SO_4^{2-} occurred above

1900-2400 mg/L because high SO_4^{2-} concentrations might increase the ionic strength of the nutrient solution (Ongcharit *et al*., 1991). According to Jin *et al*. (2005), in a BTF inoculated with activated sludge from a wastewater treatment plant, SO_4^{2-} concentrations > 1,900 mg SO_4^{2-}/L was shown to inhibit H_2S biodegradation. As shown in equations (2.4) and (2.5), the end-product of sulfide oxidation is associated with the initial NO_3^--N concentration, which acts as an electron acceptor. Furthermore, complete sulfide oxidation are more thermodynamically favorable and they strongly depend on the variations of standard Gibbs free energy (Moraes *et al*., 2012). The SO_4^{2-} concentrations during the continuous mode of BTF operation was less than the SO_4^{2-} formed during fed-batch mode of operation (247-593 mg/L) because sulfur (S^o) was also produced as one of the other end-products.

During BTF operation in continuous mode, a cream-whitish layer of precipitate covering the PUFs was observed in the BTF (Figure 4.20B). EDX analysis affirmed these precipitates as S^o particles (Figure 4.21). However, during fed batch mode of BTF operation, S^o formation was not evidenced (Figure IV). Thus, when the mode of BTF operation was changed from fed-batch mode to continuous mode, it caused variations in the S/N molar ratio. The S/N molar ratio (0.1) during continuous mode was ten times higher than the S/N molar ratio of fed batch mode (0.01-0.05). This clearly explains the fact that the presence of excess sulfide in the nutrient solution results in partial oxidation and S^o is generated as the intermediate product (Moraes *et al*., 2012 Pokorna *et al*., 2015). S^o formation is thermodynamically favorable in the presence of sulfide. The S^o is the primary product from sulfide oxidation which is faster than the second step to SO_4^{2-} (Reyes Avila *et al*., 2004; Dolejs *et al*., 2015). Another possible reason for the formation of S^o might be due to

the high loads of H_2S that was passed to the BTF. According to Li *et al*. (2011), S^o increased in the nutrient solution of a BTF at inlet loads ranging between 16.3 and 54.5 g $H_2S/m^3 \cdot h$. The end-product of the sulfide oxidation pathway not only depends on the NO_3^- concentration but also on the sulfide or other S compounds present in the bioprocess. However, it is noteworthy to mention that the accumulation of end-products from H_2S oxidation including SO_4^{2-} or S^o did not have any negative effect on the microorganism's activity. However, the presence of S^o might lower the chemolithotrophic denitrification rates because the solid from of S^o would cause unexpected clogging problems and also affect the mass transfer characteristics of the reactor (Beristain *et al*., 2005).

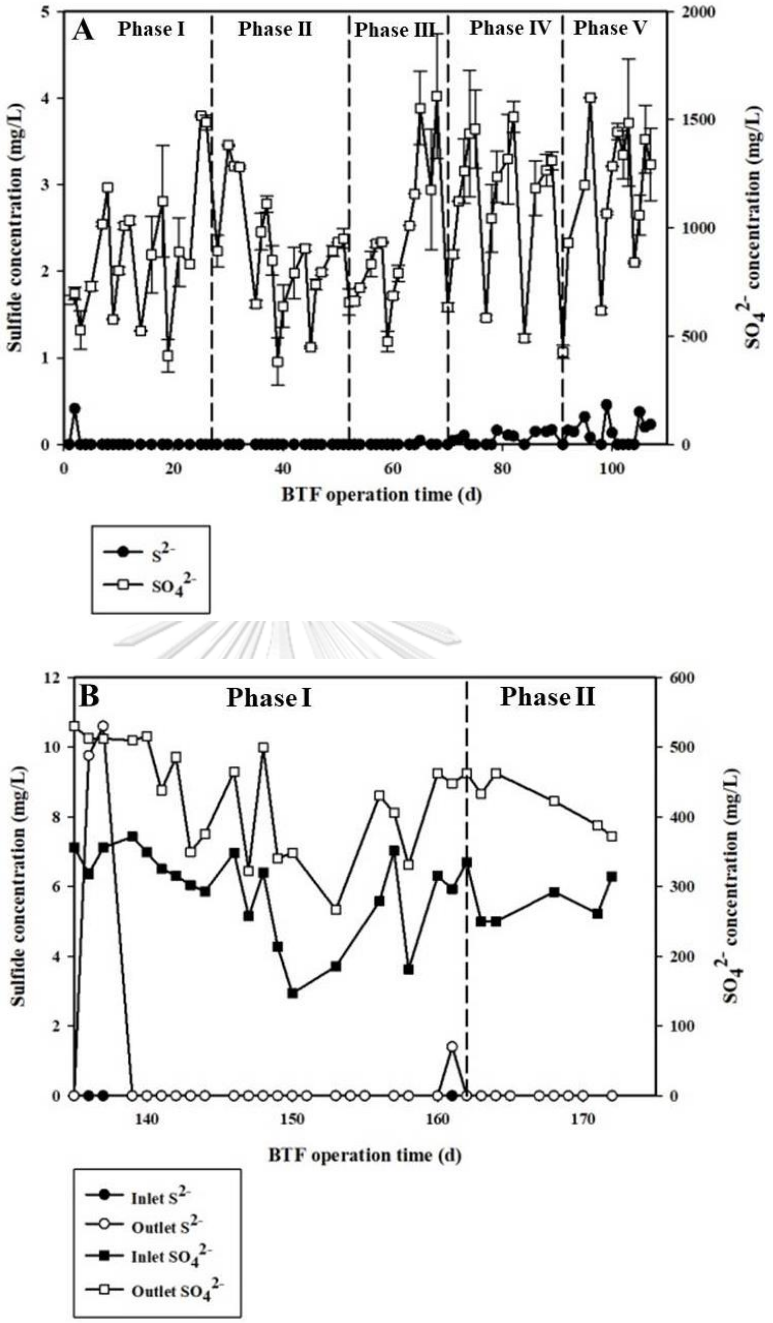


Figure 4.19 Sulfide and SO_4^{2-} profiles in the BTF during (A) fed-batch mode, and (B) continuous mode of operation.

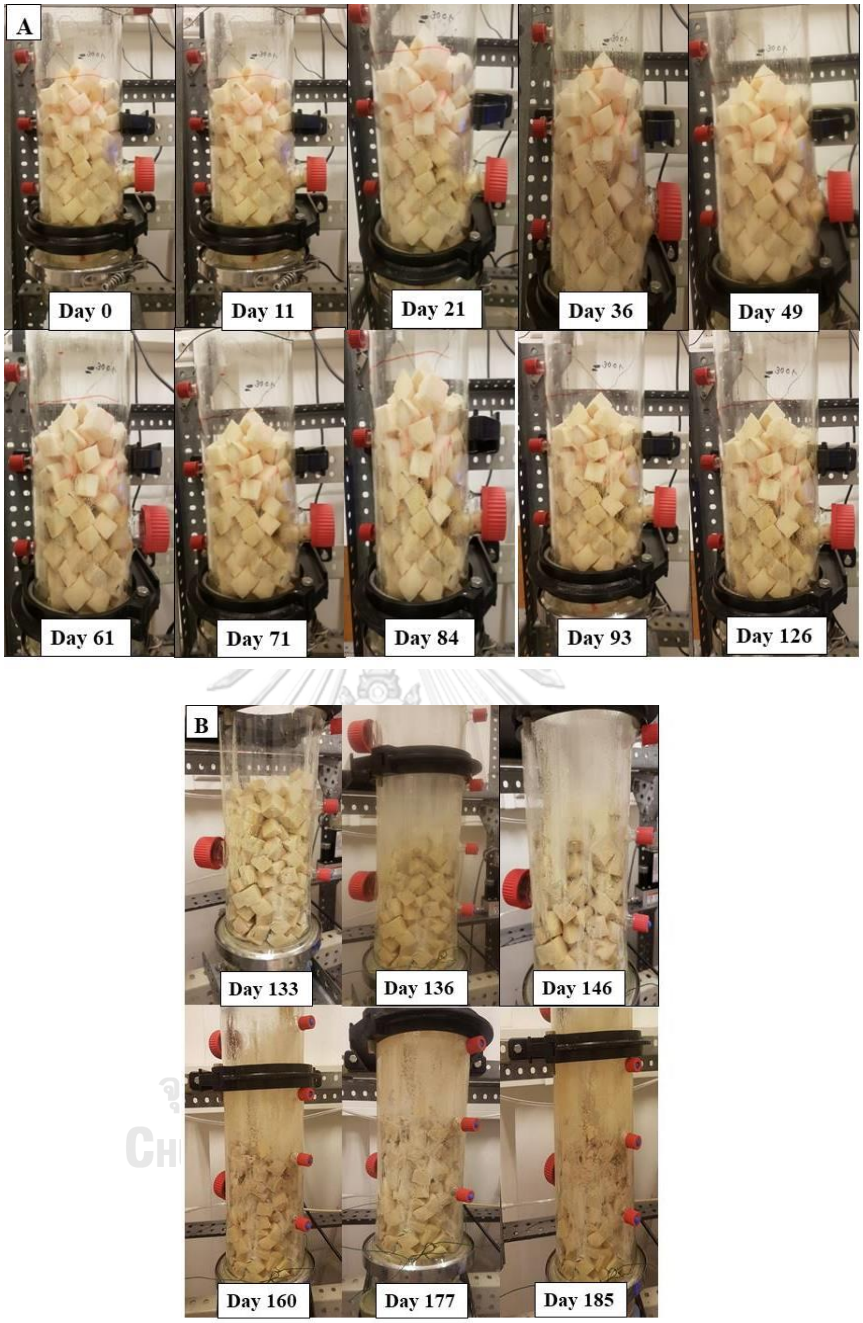


Figure 4.20 Biomass development and S° accumulation on the packing material of the BTF during (A) fed batch mode, and (B) continuous mode of operation.

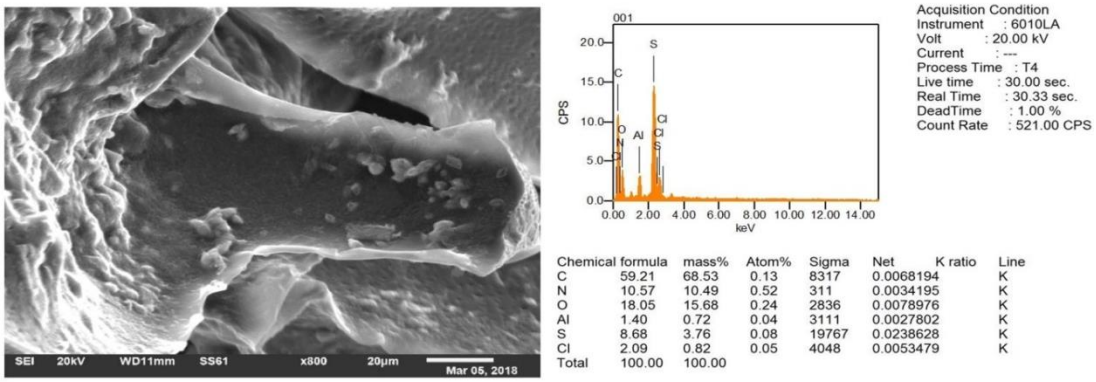


Figure 4.21 SEM image of PUF morphology using SEM-EDS analysis from samples collected during continuous mode of BTF operation.

4.5.3 Nitrate (NO_3^--N) and nitrite (NO_2^--N) profiles in the anoxic BTF

The NO_3^--N removal profiles throughout the anoxic BTF operation were nearly similar and almost instantaneous. During the fed-batch mode, the NO_3^--N concentration was removed completely (~100%) within 2 d for each cycle (Figure 4.22A). Nearly >82% NO_3^--N consumption was also detected during the BTF operation when the nutrient medium was continuously trickled over the filter bed (Figure 4.22B). As discussed before, NO_3^--N is a crucial parameter for gas-phase H_2S oxidation (Soreanu *et al.*, 2008). In a previous study, Reyes-Avila *et al*. (2004) indicated that the presence of CH_3COO^- could enhance the biodegradation of NO_3^--N and sulfide as discussed before in Section 4.5.4.

NO_2^--N was detected as an intermediate product (0-55.0 mg NO_2^--N/L) which was in the range of concentration that was not inhibitory to the microorganisms. The maximum accumulation of NO_2^--N in the system was observed for 3 d. The NO_2^--N profiles during the continuous mode was lower than 14 mg NO_2^--N/L. The accumulation pattern of NO_2^--N was usually followed by its complete conversion to N_2 gas. This observation is different from the report of De Gusseme *et al*. (2009). According to that study, the accumulation of NO_2^--N (43 mg NO_2^--N/L) in a

continuous stirred tank reactor (CSTR) indicated partial denitrification and it was explained that some NR-SOB species do not have the ability to completely reduce nitrate to N_2.

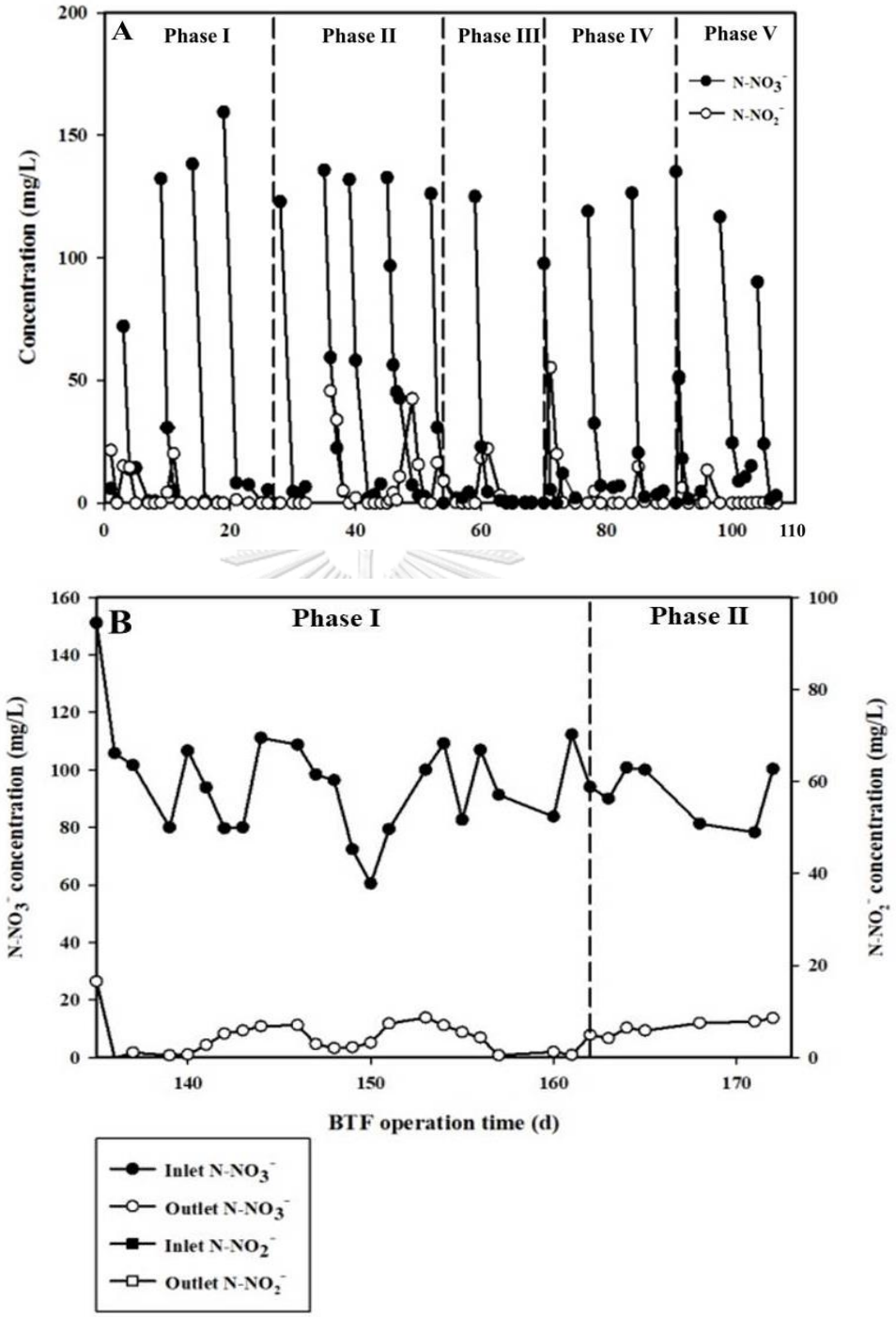


Figure 4.22 Nitrate (NO_3^--N) and nitrite (NO_2^--N) profiles in the BTF during (A) fed-batch mode, and (B) continuous mode of operation.

4.5.4 Acetate (CH_3COO^-) and pH profiles in the anoxic BTF system

100% CH_3COO^- removal was observed during 188 d of anoxic BTF operation (Figure 4.23). In Phase I, period II, it was evident that the RE of H_2S dramatically decreased in the absence of CH_3COO^-. However, after phase II, period II, the BTF was operated in continuous mode and CH_3COO^- was not added to the reactor. Yet, the RE of H_2S was still high which indicates that the microorganisms were able to adapt to this operating condition. CH_3COO^- is presumed to be easily absorbed than other inorganic carbons and consequently it improves the cell yield of bacteria. Furthermore, the lag phase of autotrophic denitrification was shorter when CH_3COO^- was present in the system. In engineered systems, there are numerous NR-SOB which are adaptable to the autotrophic, heterotrophic and mixotrophic conditions. They can use both inorganic and organic carbon as their energy source, for instance *P. versutus*, *Paracoccus denitrificans*, *P. pantotrophus and Beggiatoa* spp. (Hagen and Nelson, 1997; Otte *et al*., 1999). In this study, the anoxic BTF was inoculated with pure culture of *Paracoccus versutus* strain MAL 1HM19 which is one of the NR-SOB that was reported previously to grow in the presence or absence of CH_3COO^-.

In both fed-batch and continuous mode of anoxic BTF operation, the initial pH value was ~6.5-7.0 and the final pH value was ~7.7-8.1 without any adjustment of pH throughout the long term operation of anoxic BTF (Figure 4.23). It can therefore be concluded that the removal of H_2S coupled to NO_3^--N reduction at this pH value was mainly due to biological activity of NR-SOB (Soreanu *et al*., 2008). Oh *et al*. (2001) reported that the optimum pH is in the range of 6.0-9.0 for autotrophic and heterotrophic denitrifying bacteria which is in the same pH range maintained in this study. The pH value has an impact on the concentration of sulfide in the nutrient

solution of BTF (Solcia *et al*., 2014). The accumulation of sulfide in the liquid-phase causes toxicity to the bacteria present in the system such as chemolithoautotrophic denitrifying bacteria (Cardoso *et al*., 2006).

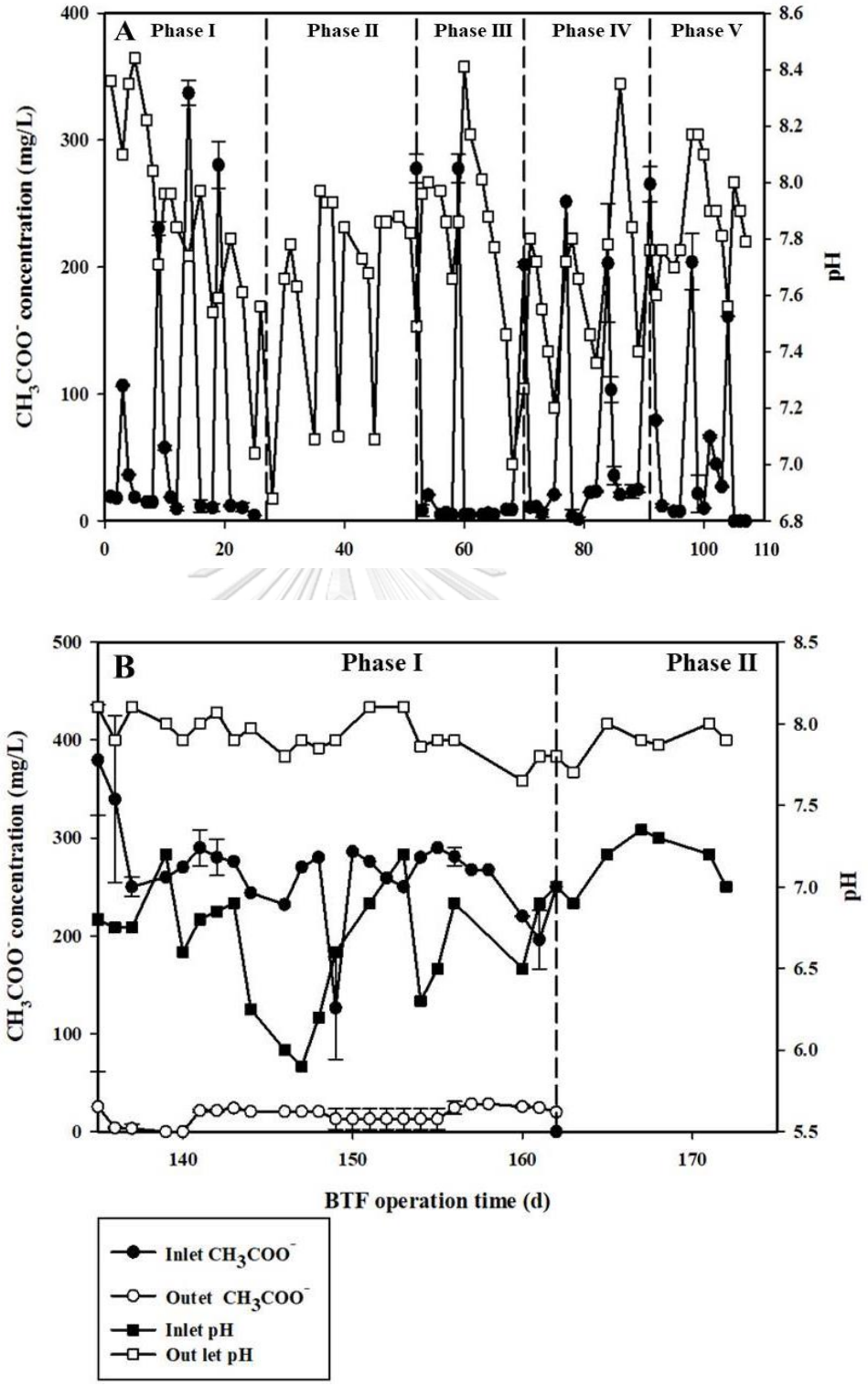


Figure 4.23 Acetate (CH_3COO^-) and pH profiles in the BTF during (A) fed-batch mode, and (B) continuous mode of operation.

4.5.5 Elimination capacity (EC) of the BTF

Figure 4.24 shows the EC *versus* inlet loading rate for all the experimental conditions tested in this study. To the best of our knowledge, information concerning the performance of *P. versutus* to remove H_2S in a BTF has never been reported. The EC values indicate the mass of pollutant that is removed per unit of filter bed volume per time. The EC values is normally correlated to the inlet loading rate and it allows to compare the performances of different bioreactors for waste-gas treatment (Rene *et al*., 2010).

The EC values achieved during the anoxic BTF operation are summarized in Table 4.3 It is evident from this table that the EC of phase I, period I was 2.86 $\pm$ 0.66 g S/m^3.h, at an inlet H_2S concentration of 100 ppm_v. The critical inlet loading rate (ILR_{crit}) of the BTF was 17.26 g S/m^3.h (RE = 100%) during continuous mode of reactor operation (Phase II, period II). The value of ILR_{crit} observed in this study is higher than the previously reported values by Ramírez *et al*. (2009). The authors reported an ILR_{crit} of 14.9 g S/m^3.h (RE = 99.8%) for the biodegradation of H_2S by *Thiobacillus thioparus* which was immobilized on PUFs in an aerobic BTF. However, the value of ILR_{crit} from this study was less than the value of ILR_{crit} observed in an aerobic BTF inoculated with *Acidithiobacillus thiooxidans* (58.7 g S/m^3.h; RE > 98%) (Ramírez *et al*. 2011).

The maximum elimination capacity (EC_{max}) values during fed batch and continuous mode of BTF operation were 10.67 ± 0.0 and 21.22 ± 0.0 g S/m^3.h, respectively. The EC_{max} value during H_2S shock load experiment was 121.83 ± 0.1 g S/m^3.h (RE = 96.5 %) which was achieved at an inlet loading of 126.08 ± 0.14 g S/m^3.h. The performance of the anoxic BTF inoculated with *P. versutus* strain MAL

1HM19 was also compared with the literature reports (Table 4.4). It can be clearly seen that the anoxic BTF showed higher EC_{max} values compared to the EC_{max} observed in other bioreactors inoculated with heterotrophic sulfur oxidizing bacteria (SOB) as well as mixed cultures of autotrophic bacteria. The EC_{max} values of this study is comparatively lower than the EC_{max} values obtained from experiments carried out using autotrophic SOB. The good performance of the BTF in terms of reaching high EC_{max} values during shock loading tests and consistently high H_2S removal clearly indicates the versatility of the strain MAL 1HM19 to handle gas-phase H_2S. Although further studies are required to understand the metabolic engineering aspects of different modes of BTF operation, the results from this study suggest that the anoxic BTF assures specific advantages for removing H_2S and other trace contaminants present in biogas.

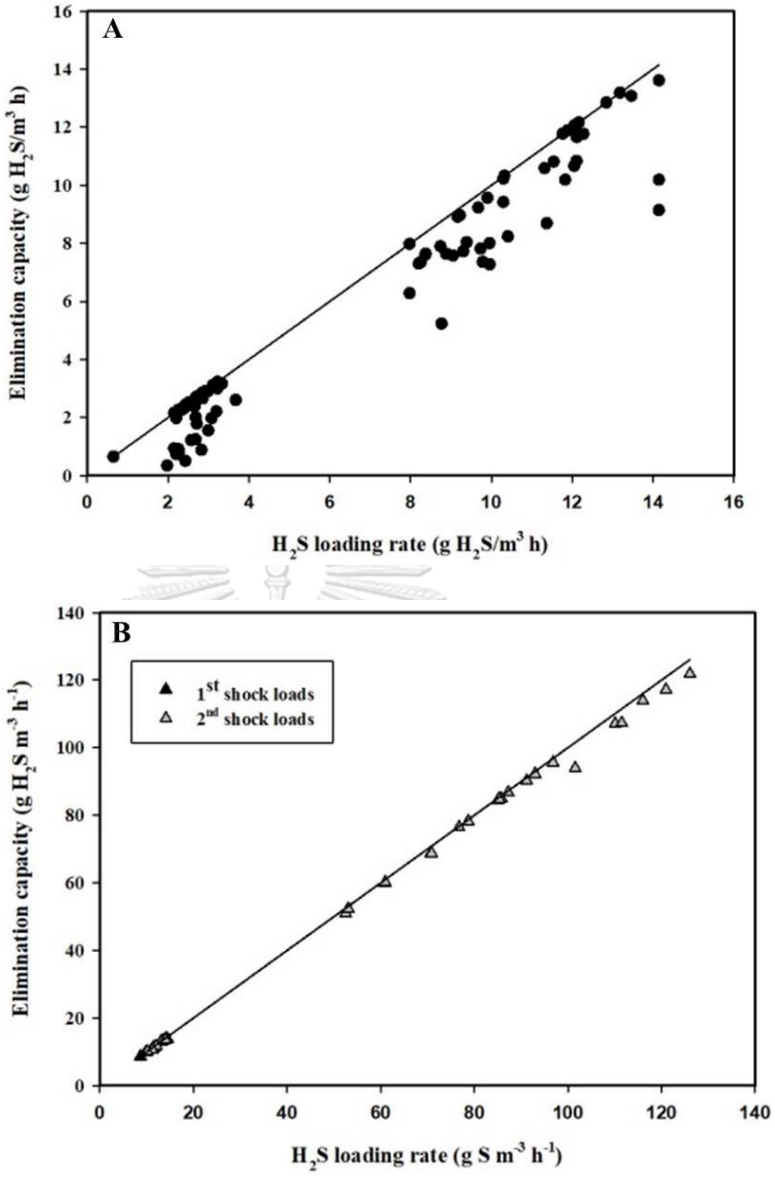


Figure 4.24 Influence of H_2S loading rate on the elimination capacity of the BTF during different modes of operation: (A) fed-batch and continuous, and (B) H_2S shock load tests.

Table 4.3 Inlet loading rate (ILR) and elimination capacity (EC) values obtained in this study during different phases of biotrickling filter (BTF) operation.

Phase	Period	Inlet load rate ($g\ S/m^3.h$)	Elimination capacity ($g\ S/m^3.h$)
I	I	2.86 ± 0.66	2.86 ± 0.66
	II	1.85 ± 0.94	1.85 ± 0.94
	III	2.12 ± 0.55	2.12 ± 0.55
	IV	7.73 ± 0.70	7.73 ± 0.70
	V	10.52 ± 1.63	10.52 ± 1.63
II	I and II	13.67 ± 3.21	13.67 ± 3.21
III	H_2S shock loads	126.08 ± 0.14	121.84 ± 0.10

Table 4.4 EC_{max} values reported in the literature for H_2S removal using different bioreactor configurations (modified from Nisola *et al*., 2010).

Microorganism	**EC_{max} ($g/m^3.h$)**	**Reactor type**	**References**
Thiobacillus thiooxidans AZ11	398 - 428	BF	Cho *et al*., 2000
Acidithiobacillus thiooxidans	83, 370	BTF	Sersu *et al*., 2005; Aroca *et al*., 2007
Bordetella sp. Sulf-8 BTF 94	94	BTF	Nisola *et al*., 2010
Activated sludge	51, 67	BTF	Ruokojärvi *et al*., 2001; Hirai *et al*., 2001
Pseudomonas putida	25	BF	Huang *et al*., 1996
Thiobacillus thioparus	14	BF	Aroca *et al*., 2007
Acinetobacter sp. and	20	BTF	Potivichayanon *et al*.,
Alcaligenes sp.		BS	2006
Alcalicgenes faecalis MU2-03	6		
Acinetobacter sp. MUI-03	3		
Mixed cultures of autotrophic bacteria	8	BF	Kim *et al*., 2008

Note:
BF-biofilter; BTF-biotrickling filter; BS-bioscrubber

4.5.6. Biokinetics of BTF operation

An analysis of the biokinetics was done in order to estimate the maximum elimination capacity (EC_{max}) of the BTF (Rene *et al*., 2010). The kinetic constants of the BTF were evaluated by using a modified Michaelis-Menten equation. The kinetic parameters including half saturation constant (K_s) and EC_{max} were determined from a linear plot which takes the form of the Lineweaver-Burk equation (equation 3.9). The K_s value represents the affinity of microorganism to the contaminants present in the waste gas stream, i.e. H_2S in gas-phase (Rene *et al*., 2010).

The experimental data from the fed-batch mode, continuous mode and the H_2S shock load experiments were used separately to estimate the biokinetics of the process under each operational conditions (Figure 4.25). From the mathematical model the values of EC_{max} from fed-batch mode, continuous mode and shock loads experiments were 13.05, 25.84 and 100 $g/m^3.h$, respectively. The K_s values were 0.12, 0.10 and 0.86 g/m^3 during the fed-batch mode, continuous mode and shock loads experiments, respectively. The experimentally determined EC_{max} value is in close agreement with the EC_{max} derived from the kinetic equation.

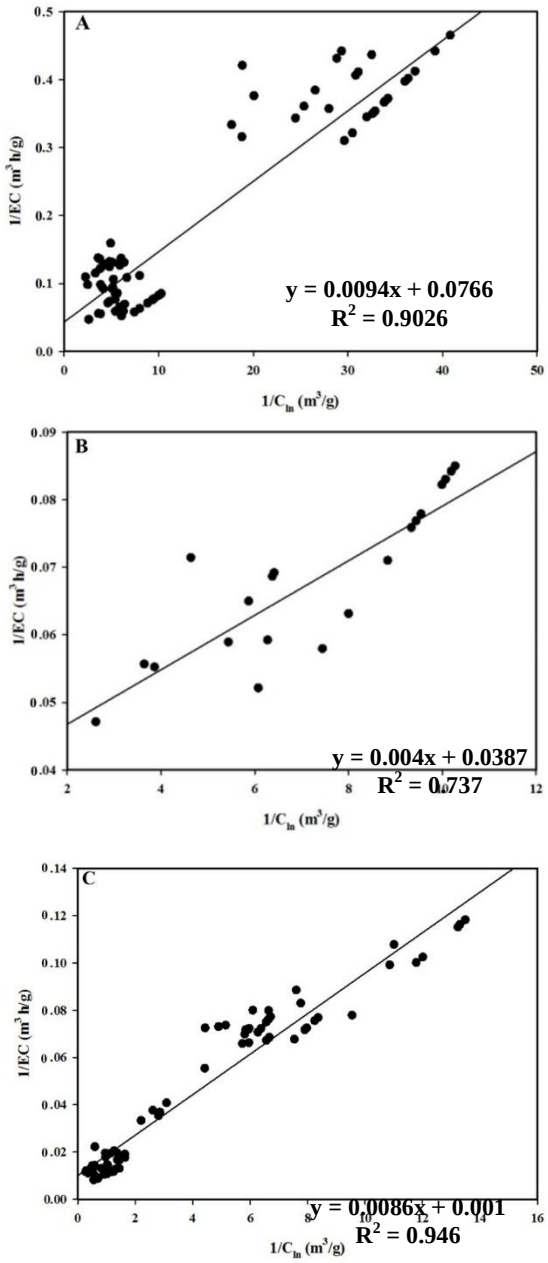


Figure 4.25 Relationship between 1/EC and $1/C_{ln}$ for H_2S removal during BTF operation: (A) fed-batch mode, (B) continuous mode, and (C) shock load tests.

4.5.7 Global nitrate demand

The global nitrate (GN) demand values estimated from different phases of BTF operation are shown in Table 4.5. The values of GN from phase I, periods IV and V as well as phase II was lower than the GN values obtained from Phase I, periods I-III. Moreover, the NO_3^--N removal rates obtained from this study was constant (~80 - 102 mg NO_3^--N /L·d) during the BTF operation (Table 4.5) and the NO_3^--N removal rates of this study were higher than the previously reported values (20.5 mg NO_3^--N/L·d) by Soreanu *et al*. (2008) with simultaneous removal of 100 % H_2S in the BTF. This observation clearly implies the fact that the anoxic BTF is able to demonstrate high performance for the removal of high concentrations of H_2S (gas-phase) and NO_3^--N (liquid-phase). Concerning the practical implication, the BTF can be applied to remove H_2S and NO_3^--N rich wastewater that are commonly generated from various unit operations of the pulp and paper industry (Example - Kraft mill process).

Table 4.5 Global nitrate (GN) demand profiles during steady-state BTF operation.

Phase	Period	N/S ratio	H_2S degradation rate (mg H_2S /L·d)	NO_3^--N degradation rate (mg NO_3^--N /L·d)	GN value (mg NO_3^--N/mg H_2S)
I	I	100	186.50 ± 26.63	101.57 ± 0.00	0.58 ± 0.00
	II	100	147.83 ± 65.10	81.87 ± 11.79	0.35 ± 0.03
	III	100	182.053 ± 22.05	98.76 ± 4.77	0.63 ± 0.00
	IV	30	568.31 ± 45.39	94.93 ± 10.05	0.16 ± 0.01
	V	22	700.31 ± 66.59	86.07 ± 2.17	0.12 ± 0.00
II	I and II	14	675.51 ± 211.43	96.04 ± 26.83	0.15 ± 0.07

CHAPTER V

CONCLUSIONS AND FUTURE PERSPECTIVES

5.1 Conclusions

In this study, *P. versutus* strain MAL1HM19, a novel strain of NR-SOB was successfully isolated from the sediments and water samples of Mae Um Long Luang hot spring (Thailand). The strain MAL 1HM19 was selected as a promising NR-SOB for H_2S removal due to its efficiency to degrade 100 % hydrogen sulfide (H_2S) within 5 h. The identification and characterization of strain MAL 1HM19 was also successfully carried out in this study. The results of 16s rDNA and the whole genome sequence analysis of strain MAL 1HM19 revealed that this strain belongs to the genus *P. versutus*. *P. versutus* strain MAL1HM19 was able to grow under a wide range of environmental conditions such as temperature, pH, sulfur sources, carbon sources and salt concentrations.

Based on the nature of the experimental work carried out in batch and continuous conditions, the ability of *P. versutus* strain MAL 1HM19 for H_2S removal was evidenced. The results from short-term experiments, i.e. in batch tests, showed that *P. versutus* strain MAL 1HM19 was able to remove H_2S in gas phase completely within 10 h, irrespective of the different initial nitrate (NO_3^--N) concentrations. The strain MAL 1HM19 removed 700 to 800 ppm_v gas-phase H_2S within 5, 6 and 10 h, respectively, and the final product *via* the H_2S oxidation pathway depended on the initial NO_3^--N concentration. Anew, batch assay results also confirmed the fact that the *P. versutus* strain MAL 1HM19 cannot utilize thiosulfate ($S_2O_3^{2-}$) as its sulfur source. Although $S_2O_3^{2-}$ has been widely reported as an intermediate product of the

sulfide (S^{2-}) oxidation pathway and also a key intermediate of the sulfur cycle, the novel strain did not show removal of $S_2O_3^{2-}$. However, interestingly, the presence of $S_2O_3^{2-}$ enhanced the removal of NO_3^--N when compared to batch assay results performed in the presence of sulfide.

During long term operation in a biotrickling filter (BTF), the practicality of *P. versutus* strain MAL 1HM19 to remove H_2S gas under anoxic conditions was demonstrated in both fed-batch and continuous mode of reactor operation (188 d). The results showed that the anoxic BTF, inoculated with pure cultures of *P. versutus* strain MAL 1HM19, was able to show high removal efficiency (RE) of H_2S within its first 10 d of operation, which clearly indicates the rapid startup of the reactor. The RE of H_2S during the different phases of operation varied between 17 and 100% depending on the operational mode, as well as the presence of an organic carbon source (CH_3COO^-). Under transient state operations, i.e. in the form of high H_2S shock loads, the anoxic BTF system demonstrated good response and resilience capacity. The RE was restored immediately when the normal load was revered again which clearly shows its capability to handle fluctuating concentrations of H_2S. The presence of NO_3^--N is also an important factor for H_2S oxidation and the RE of NO_3^--N depended on the mode of BTF operation (fed-batch or continuous). Concerning the kinetics of H_2S removal by *P. versutus* strain MAL 1HM19, the strain showed high affinity to H_2S gas and yielded high EC_{max} values. The good results obtained from different batch and continuous experiments showed the suitability of *P. versutus* strain MAL 1HM19 for H_2S and NO_3^--N removal.

5.2 Future perspectives

In this study, the potential of *P. versutus* strain MAL 1HM19 as a potential biocatalyst for H_2S removal was demonstrated. However, further studies should be carried out to address some of the important issues concerning the mechanism of contaminant removal in liquid and gas phases:

(i) Proteomic and metabolomics studies should be performed to understand the metabolism of different microbial communities involved in the sulfur cycle. Molecular biology techniques such as RNAseq and microarray should be used to ascertain which specific genes are active during H_2S oxidation. The gene expression level under each set of operating condition should be determined in order to better empathize the role of metabolic processes in wastewater engineering applications.

(ii) Use artificial intelligence tools such as artificial neural networks (ANNs) and fuzzy logic to predict the performance of bioreactors carrying out simultaneous removal of gas-phase H_2S and NO_3^--N.

(iii) For practical applications, it will also be interesting to perform vigorous bioaugmentation studies and test the effect of adding *P. versutus* strain MAL 1HM19 with other microorganisms. Thus, apart from the removal of volatile inorganic pollutants, volatile organic compounds (VOCs) can also be removed in one bioreactor configuration.

(iv) Studies should also be carried out using different reactor configurations and the hydrodynamics of the reactors should be

evaluated under a wide range of operating conditions. If possible, pilot scale anoxic (bio)reactors should be operated using biogas containing H_2S and other odorous substances (for example - biogas from an anaerobic digester) in order to understand the limitations and performance of the bioreactor in an industrial setting.

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APPENDIX

Appendix A: Calibration curves

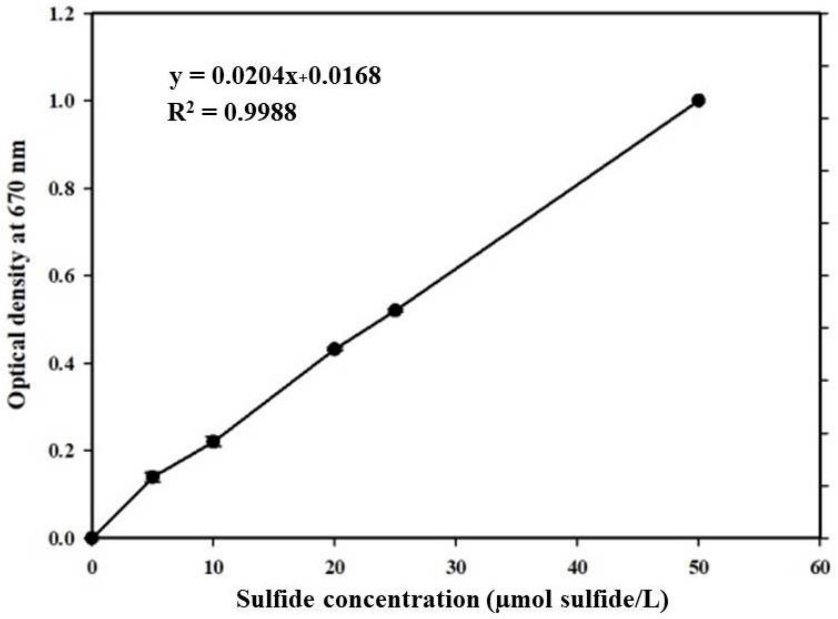

Figure A.1 Calibration curve for sulfide in liquid-phase

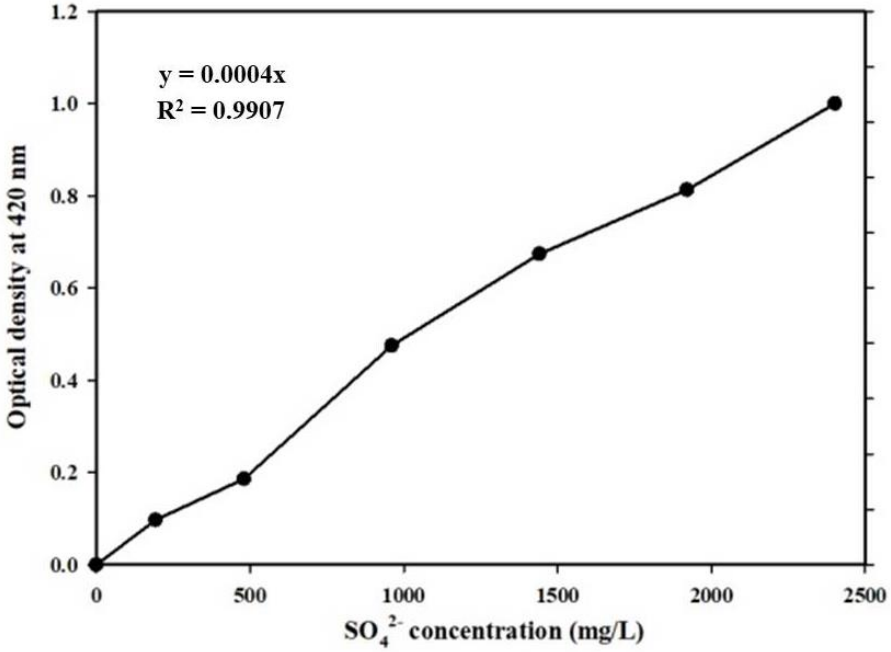

Figure A.2 Calibration curve for SO_4^{2-}

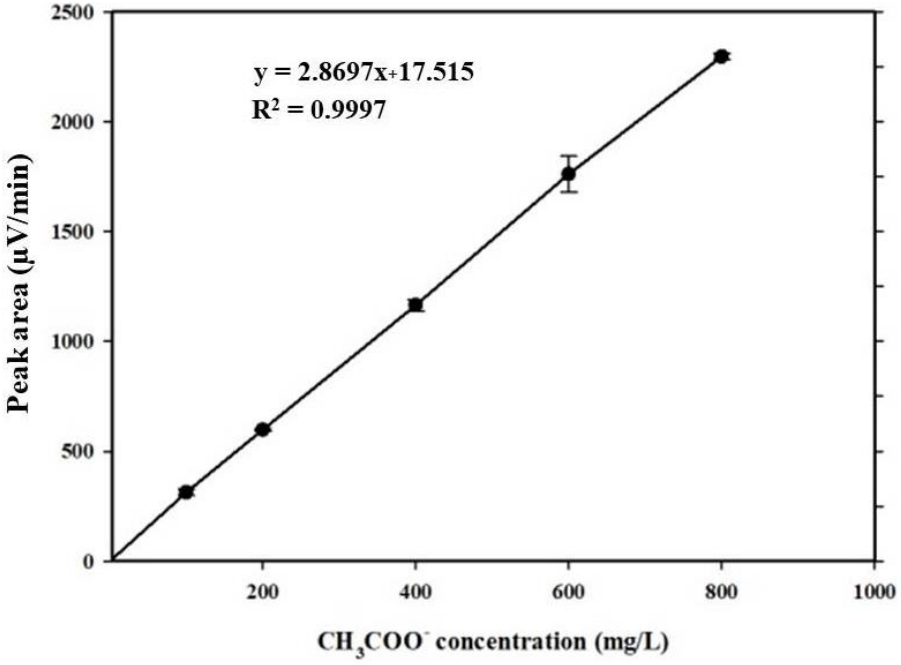

Figure A.3 Calibration curve for CH_3COO^-

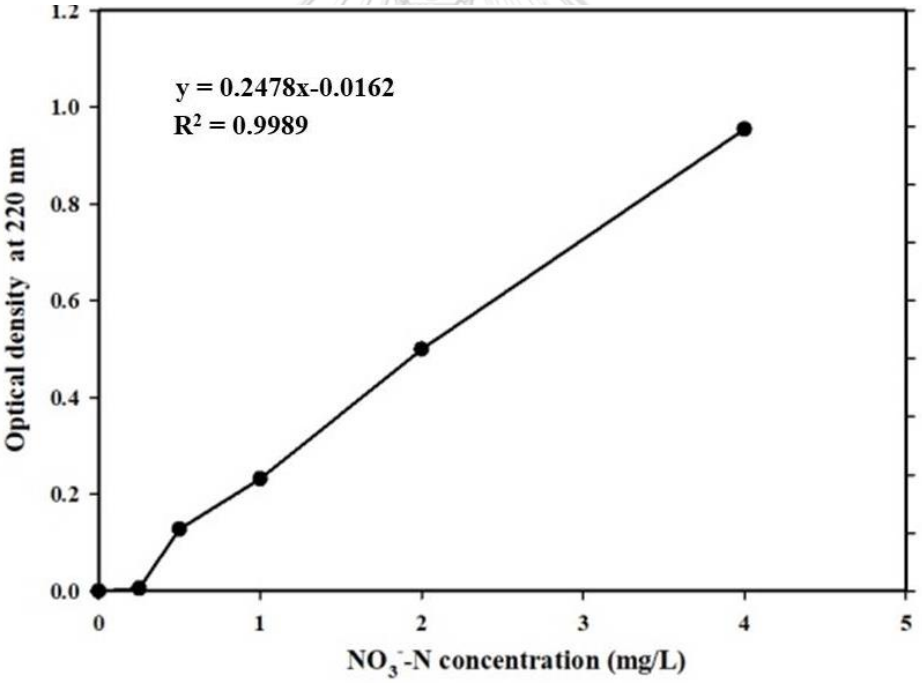

Figure A.4 Calibration curve for NO_3^--N

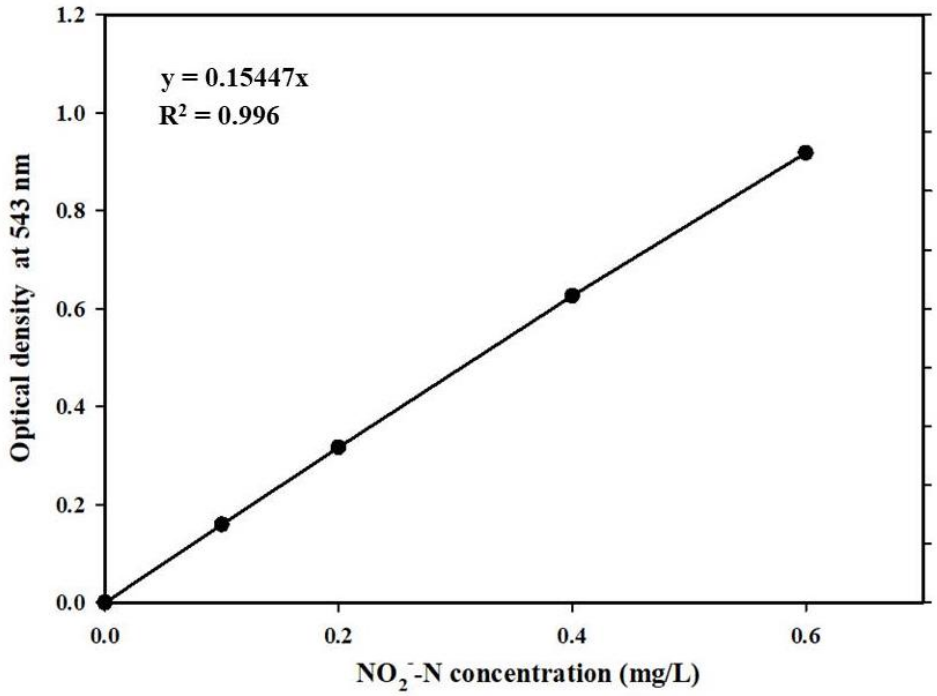

Figure A.5 Calibration curve for NO_2^--N

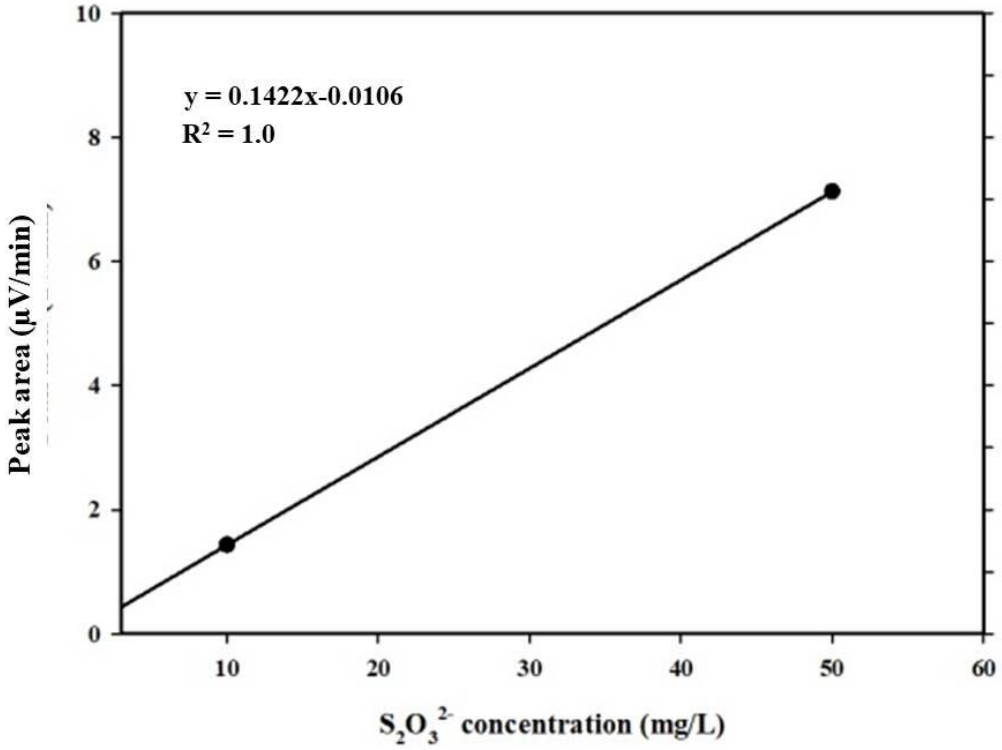

Figure A.6 Calibration curve for $S_2O_3^{2-}$

Appendix B

Table B.1 Various isolates of NR-SOB from the Mae Um Long Luang hot spring.

Strains	Growth with H_2S and acetate	Growth with H_2S and methanol
1. MAL 1HM1	+	-
2. MAL 1HM2	+	-
3. MAL 1HM3	+	-
4. MAL 1HM5	-	+
5. MAL 1HM6	+	+
6. MAL 1HM7	+	-
7. MAL 1HM8	+	+
8. MAL 1HM10	+	+
9. MAL 1HM11	+	+
10. MAL 1HM13	+	-
11. MAL 1HM14	+	-
12. MAL 1HM15	+	+
13. MAL 1HM16	-	-
14. MAL 1HM19	+	+
15. MAL 2HM2	-	-
16. MAL 2HM3	+	-
17. MAL 2HM4	-	-
18. MAL 2HM5	+	+

Note:

‘’+‘’ refers to the detection of turbidity in the mCSB medium

‘’-‘’ refers to the absence of turbidity in the mCSB medium

Table B.2 Various isolates of NR-SOB from the Thep Pha Nom hot spring.

Strains	Growth with H_2S and acetate	Growth with H_2S and methanol
1. TPN 1HM1	+	+
2. TPN 1HM2	-	-
3. TPN 1HM3	+	-
4. TPN 1HM4	+	-
5. TPN 1HM5	+	-
6. TPN 1HM6	+	-
7. TPN 2HM1	+	-
8. TPN 2HM2	+	-
9. TPN 2HM4	+	-
10. TPN 2HM5	+	-
11. TPN 2HM7	+	+
12. TPN 2HM8	+	-
13. TPN 2HM9	+	-
14. TPN 2HM10	+	-
15. TPN 2HM11	+	-
16. TPN 2HM12	+	+
17. TPN 2HM13	+	-
18. TPN 2HM14	-	-
19. TPN 3HM1	+	+
20. TPN 3HM2	+	-

Note:
‘’+’’ refers to the detection of turbidity in the mCSB medium
‘’-‘’ refers to the absence of turbidity in the mCSB medium

VITA

Miss Wannapawn Watsuntorn was born on November 12th, 1990. She completed her Bachelor's degree in Genetics from Faculty of Science, Chulalongkorn University in 2012. After that she studied as a Ph.D. student in the Biotechnology program, Chulalongkorn University (Thailand). Her Ph.D. research focuses on the removal of hydrogen sulfide using nitrate-reducing, sulfide oxidizing bacteria (NR-SOB) that was isolated from hot spring. Her PhD research is supported by the 100th Anniversary Chulalongkorn University doctoral scholarship. During her Ph.D. life, she got the mobility to do a research for eleven months at Laboratory of IHE Delft Institute for Water Education, the Netherlands under Eldon R. Rene, Ph.D. (Co-advisor) and Prof. Piet N.L. Lens. Ph.D.

Outcome of this study

Publications:

1. Watsuntorn, W., Seehone, W., Ruangchainikom, C., Sachakamol, P., Chavanparit, O., Chulalaksananukul, S., Chulalaksananukul, W., 2014. Hydrogen sulfide reduction in gas production system by biological processes. Srinakharinwirot Sci. J. 30, 187-202. (In Thai) (Review article).

2. Watsuntorn, W., Ruangchainikom, C., Rene, E.R., Lens, P. N. L., Chulalaksananukul, W., 2017. Hydrogen sulfide oxidation under anoxic conditions by a nitrate-reducing, sulfide-oxidizing bacterium isolated from the Mae Um Long Luang hot spring, Thailand. Int. Biodeterior. Biodegrad. 124, 196-205.

3. Watsuntorn, W., Ruangchainikom, C., Rene, E.R., Lens, P.N.L., Chulalaksananukul, W., 2018. Comparison of sulfide and nitrate removal from synthetic wastewater by pure and mixed cultures of nitrate-reducing, sulfide-oxidizing bacteria isolated from hot springs in Thailand. (Submitted to Bioresource Technology).

4. Watsuntorn, W., Chulalaksananukul, W., Rene, E.R., Lens, P.N.L., 2018. Anoxic biotrickling filter inoculated with pure culture of Paracoccus versutus strain MAL 1HM19 and artificial neuron network analysis. (In preparation).

Conference presentations

Oral presentation

Watsuntorn, W., Ruangchainikom, C., Rene, E.R., Lens, P.N.L., Chulalaksananukul, W., 2016. Isolation and characterization of nitrate-reducing, sulfide-oxidizing bacteria from Mae Um Long Luang hot spring (Thailand): Anaerobic hydrogen sulfide oxidation under brine conditions. Challenges in Environmental Science and Engineering (CESE), 6-10 November 2016, Kaohsiung, Taiwan.

Posters

Watsuntorn, W., Rene, E.R., Lens, P.N.L., Chulalaksananukul, W. 2017. Simultaneous H2S and NO3- removal using different NR-SOB strains isolated from hot springs in Thailand. 7th International Conference on Biotechniques for Air Pollution Control and Bioenergy (Biotechniques-2017), La Coruña, 9-21 July 2017. Spain. (Best poster award).

Watsuntorn, W., Rene, E.R., Lens, P.N.L., Chulalaksananukul, W. 2017. Simultaneous H2S and NO3- removal using different NR-SOB strains isolated from hot springs in Thailand. PhD symposium, Delft, 2-3 October 2017. The Netherlands.