ประสิทธิภาพและกลไกการบำบัดน้ำเสียปนเปื้อนซัลเฟตด้วยเซลล์เชื้อเพลิงชีวภาพที่อัตราส่วนซีโอดีต่อซัลเฟตต่างๆ

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Performances and mechanisms of MFC treating organic wastewater at various COD: sulfate ratio

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วิชยุตม์ นิยม : ประสิทธิภาพและกลไกการบำบัดน้ำเสียปนเปื้อนซัลเฟตด้วยเซลล์เชื้อเพลิงชีวภาพที่อัตราส่วนซีโอดีต่อซัลเฟตต่างๆ (Performances and mechanisms of MFC treating organic wastewater at various COD: sulfate ratio) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.เบญจพร สุวรรณศิลป์, 269 หน้า.

ในงานวิจัยนี้ศึกษาการประยุกต์ใช้เซลล์เชื้อเพลิงชีวภาพแบบสองห้องย่อยชนิดห้องเดี่ยวใช้อากาศเพื่อบำบัดน้ำเสียที่ปนเปื้อนสารอินทรีย์และซัลเฟตสูงพร้อมทั้งการผลิตกระแสไฟฟ้าจากเซลล์เชื้อเพลิงชีวภาพที่อัตราส่วนซีโอดีต่อซัลเฟตเป็น 1 3 และ 6 โดยศึกษาทั้งอัตราการบำบัดสารอินทรีย์ ซัลเฟต ซัลไฟด์ การผลิตกระแสไฟฟ้า รวมถึงกลไกที่เกิดขึ้นภายในเซลล์เชื้อเพลิงชีวภาพที่อัตราส่วนซีโอดีต่อซัลเฟตต่างๆ ทำการเดินระบบอย่างต่อเนื่องด้วยระยะเวลากักเก็บน้ำ 24 ชั่วโมง ในห้องย่อยที่ 1 ของเซลล์เชื้อเพลิงชีวภาพด้วยน้ำเสียสังเคราะห์โดยใช้กลูโคสความเข้มข้น 3,000 มิลลิกรัมซีโอดีต่อลิตร และความเข้มข้นซัลเฟตเป็น 3,000 1,000 และ 500 มิลลิกรัมซัลเฟตต่อลิตรในเซลล์เชื้อเพลิงชีวภาพ1 3 และ 6 ซึ่งมีอัตราส่วนซีโอดีต่อซัลเฟตเป็น 1 3 และ 6 ตามลำดับ จากผลการทดลองพบว่าประสิทธิภาพการบำบัดซีโอดีในห้องย่อยที่ 1 ของเซลล์เชื้อเพลิงชีวภาพเป็น 56.06 ± 10.67 62.49 ± 11.21 และ 63.22 ± 11.57% ในเซลล์เชื้อเพลิงชีวภาพ1 3 และ 6 ตามลำดับ การบำบัดซัลเฟตมีค่าเป็น 1,209 ± 455 964 ± 93 และ 492 ± 44 มิลลิกรัมซัลเฟตต่อลิตร ในเซลล์เชื้อเพลิงชีวภาพ 1 3 และ 6 ตามลำดับ และมีปริมาณซัลไฟด์ละลายน้ำเป็น 400 ± 69 265 ± 59 และ 119 ± 32 มิลลิกรัมซัลไฟด์ต่อลิตร ในเซลล์เชื้อเพลิงชีวภาพ1 3 และ 6 ตามลำดับ จากการวิเคราะห์กลุ่มประชากรจุลินทรีย์ด้วยเทคนิค 16S rRNA gene amplicon sequencing (MiSeq, Illumina) ตรวจพบ *Tolumunas* spp. เป็นแบคทีเรียหลักในห้องย่อยที่ 1 ซึ่งเป็นแบคทีเรียกลุ่มหมัก โดยจะทำหน้าที่หมักกลูโคสให้กลายเป็นสารอินทรีย์ระเหยง่ายและอะซิเตทเพื่อให้แบคทีเรียกลุ่มรีดิวซ์ซัลเฟต (*Desulfovibrio* spp.) และจุลินทรีย์กลุ่มสร้างมีเทน (Methanoregulaceae และ Methanosaetaceae) ใช้ต่อไป ในส่วนของการบำบัดน้ำเสียในห้องย่อยที่ 2 พบการบำบัดซัลไฟด์เป็น 49.51 ± 57.74 24.08 ± 13.74 และ 15.69 ± 21.30 มิลลิกรัมซัลไฟด์ต่อลิตร ในเซลล์เชื้อเพลิงชีวภาพ1 3 และ 6 ตามลำดับ ซึ่งการบำบัดเกิดขึ้นผ่านกระบวนการ abiotic sulfide oxidation ซึ่งเป็นกลไกหลักที่ก่อให้เกิดกระแสไฟฟ้าให้แก่ระบบ โดยพบกำลังไฟฟ้าสูงสุดมีค่า 15.3 มิลลิวัตต์ต่อตารางเมตร ในเซลล์เชื้อเพลิงชีวภาพที่ 1 ภายหลังหลังจากการเปลี่ยนขั้วแอโนด การสะสมของซัลไฟด์บริเวณขั้วซึ่งตรวจสอบได้จากการวิเคราะห์พื้นผิวบนขั้วด้วย Scanning electron microscopy ต่อด้วย Energy-dispersive X-ray (SEM/EDX) และการก่อตัวของกลุ่มจุลินทรีย์ *Klebsiella Tolumonas* และ *Methanoseata* ซึ่งเป็นกลุ่มจุลินทรีย์หลักบนขั้วแอโนดที่ไม่สามารถผลิตกระแสไฟฟ้าได้ คาดว่าเป็นสาเหตุทำให้ค่าความต่างศักย์และกำลังไฟฟ้าที่เกิดขึ้นมีค่าลดลงตามเวลา

ภาควิชา วิศวกรรมสิ่งแวดล้อม ลายมือชื่อนิสิต .....

สาขาวิชา วิศวกรรมสิ่งแวดล้อม ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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WITCHAYUT NIYOM: Performances and mechanisms of MFC treating organic wastewater at various COD: sulfate ratio. ADVISOR: ASST. PROF. BENJAPORN SUWANNASILP, Ph.D., 269 pp.

In this study, three identical two compartment single-chamber air-breathing microbial fuel cells (MFC) were used to treat sulfate-rich wastewater simultaneously with electricity generation at the COD:SO_4^{2-} ratio of 1, 3, and 6 in MFC1, MFC3, and MFC6, respectively. COD, sulfate, and sulfide removal, electricity generation, and mechanisms in MFCs were investigated. The MFCs were continuously operated at a hydraulic retention time of 24 hr in the first compartment. Glucose equivalent to 3,000 mgCOD/L and sulfate concentrations of 3,000, 1,000, and 500 mgSO_4^{2-}/L were fed into MFC1, MFC3, and MFC6, corresponding to the COD:SO_4^{2-} ratio of 1, 3, and 6, respectively. For the first compartments, COD removal efficiencies were 56.06 ± 10.67, 62.49 ± 11.21, and 63.22 ± 11.57% in MFC1, MFC3, and MFC6, respectively. Sulfate removal was 1,209 ± 455, 964 ± 93, and 492 ± 44 mgSO_4^{2-}/L in MFC1, MFC3, and MFC6, respectively, whereas dissolved sulfide concentrations of 400 ± 69, 265 ± 59, and 119 ± 32 mgS^{2-}/L were observed in MFC1, MFC3, and MFC6, respectively. From the microbial community analysis with 16S rRNA gene amplicon sequencing (MiSeq, Illumina), *Tolumunas* spp. were predominant species in the first compartments of all of the MFCs. These microorganisms were the fermenters that can ferment glucose into VFAs and acetate, which can be further consumed by sulfate-reducing bacteria (SRB, *Desulfovibrio* spp.) and methanogens (Methanoregulaceae and Methanosaetaceae). For the second compartments of MFCs, sulfide concentrations of 49.51 ± 57.74, 24.08 ± 13.74, and 15.69 ± 21.30 mgS^{2-}/L were removed in MFC1, MFC3, and MFC6, respectively, whereas electricity was generated via abiotic sulfide oxidation process on the anode electrodes. The maximum power generation of 15.3 mW/m^2 was achieved in MFC1 on the first day after replacing the anode electrode with a new one. Sulfur accumulation on the anode electrodes suggested by the results from scanning electron microscopy equipped with energy-dispersive X-ray (SEM/EDX) and non-exoelectrogenic microorganisms such as *Klebsiella, Tolumonas,* and *Methanoseata* on the anode electrodes might increase the voltage losses in the systems and decrease the power density over time.

Department:	Environmental Engineering	Student's Signature	----------
Field of Study:	Environmental Engineering	Advisor's Signature	----------
Academic Year:	2015		

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Chapter 1
Introduction

1.1 Introduction

Nowadays, various types of industries have been developed to serve human needs and population growth. Several types of industries have generated organic wastewater containing high-sulfate, for examples, paper mill industry, rubber industry, pharmaceutical industry, mining industry, and tannery industry (Lens et al., 2000). Despite large amount of sulfate-rich wastewater released to the environment, sulfate is not categorized into the water quality standard due to its low adverse effects, compared with other substances. Nevertheless, high concentration of sulfate in drinking water can cause diarrhea in human. Moreover, sulfate can be converted to hydrogen sulfide by sulfate-reducing bacteria (SRB) under anaerobic conditions, resulting in many adverse effects, such as sulfide toxicity, bad odor, corrosion in concrete pipes, and decreases in quantity and quality of biogas produced from anaerobic processes.

High-strength organic wastewater is typically treated using combined biological treatment processes of anaerobic treatment, followed by aerobic treatment. In the first stage, anaerobic treatment is more suitable, since it can receive high organic loading rate while recovering energy from wastewater in a form of biogas. In the second stage, aerobic treatment is added to ensure the effluent quality. However, in cases of organic wastewater contaminated with sulfate, sulfate reduction and sulfide production by SRB can occur in anaerobic process, thereby lowering the quantity and quality of biogas. Microbial fuel cells (MFC) capable of treating organic wastewater containing sulfate should, therefore, be developed as an alternative for the treatment and energy recovery from this type of wastewater.

Several studies have investigated the treatment of sulfide using microbial fuel cells (Rabaey et al., 2005; Rabaey et al., 2006; Sun et al., 2010; Zhao et al., 2008). The results suggest that sulfide can be oxidized at the anode electrodes by abiotic sulfide oxidation and/or microbial mediated sulfide oxidation. In addition, MFCs have been used to treat organic wastewater containing sulfate with simultaneous electricity generation (Rabaey et al., 2005; Rabaey et al., 2006). The results show that the MFCs can remove sulfide by oxidation process in the anodic chamber.

However, research on the effects of COD:SO_4^{2-} ratio on MFC performances is still limited, especially under continuous operation (Ghangrekar et al., 2010; Zhang et al., 2012). Since the COD:SO_4^{2-} ratio can have an influence on microbial activities in the MFCs (Ghangrekar et al., 2010), the treatment and electricity generation mechanisms in the MFCs tend to depend on COD:SO_4^{2-} ratio. Therefore, in this study, two-compartment single-chamber MFCs will be used to investigate the treatment of organic wastewater with different COD:SO_4^{2-} ratio, including the treatment and electricity generation mechanisms and microbial communities in the systems. The results from this study will provide us a better understanding on the treatment of organic wastewater containing sulfate in MFC systems.

1.2 Objectives

1. To investigate the removal efficiencies of COD and sulfate and electricity generation in two- compartment single-chamber air-breathing microbial fuel cells at different COD: SO_4^{2-} ratio
2. To investigate the mechanisms of treatment and electricity generation in the two-compartment single-chamber air-breathing microbial fuel cells at different COD: SO_4^{2-} ratio
3. To identify microbial communities in the two-compartment single-chamber air-breathing microbial fuel cells at different COD: SO_4^{2-} ratio

1.3 Scope of study

This study was conducted as laboratory experiments at the Department of Environmental Engineering, Faculty of Engineering, Chulalongkorn University. The scopes of this study include:

1. Three identical two-compartment single-chamber air-breathing microbial fuel cells were used in this study. Each MFC consisted of two compartments for wastewater treatment and electricity generation in the first and second compartment, respectively.
2. Three MFCs were operated at room temperature continuously at different COD:SO_4^{2-} ratio, which were 1, 3, and 6. The hydraulic retention time (HRT) was controlled at 24 hr and 7.5 hr in the first and second compartment, respectively.
3. Synthetic wastewater containing glucose as an organic substrate at COD of 3,000 mg/L was used in this study.
4. Seed sludge from an anaerobic bioreactor from Thai Quality Starch Co.,Ltd., Karnjanaburi, Thailand, was used a seed sludge for the MFCs.

5. Microbial communities were analyzed using 16S rRNA amplicon sequencing by MiSeq system (Illumina) using universal primers for bacteria and archaea (515F/806R).

1.4 Expected outcomes

1. This study can build up the knowledge for the treatment of organic wastewater containing sulfate, which could be applied to many industries for example thermomechanical pulping, acid mine drainage, and tannery industry.

2. The study provides the guidelines for energy recovery by producing electricity from organic wastewater at different ratio of COD:SO_4^{2-}.

Chapter 2

Background and literature review

2.1 Organic wastewater containing high sulfate.

In general, water can be contaminated with sulfate by two ways including natural process and anthropogenic process. In natural process, sulfur form in the earth is washed down into groundwater rainwater percolation resulting in high-sulfate water. An example of this sulfate contamination is acid mine drainage (AMD). In some cases, the organic compounds can also be contaminated in this type of water. For anthropogenic process, many industries can generate wastewater containing high COD and sulfate, for examples, paper mill industry, rubber industry, pharmaceutical industry, mining industry, and tannery industry (Lens et al., 2000; Lens et al., 1998). The contaminated concentrations are different in each process of the industry. The data of organic wastewater containing high sulfate are shown in Table 2.1

Figure 2.1 Sources of organic wastewater containing high sulfate

Table 2.1 Types and concentration of organic wastewater containing high sulfate

Source	COD (g/l)	Sulfate (g/l)	Reference
Natural Emission			
1. Acid mine drainage	0 – 4.6	0.35 - 0.55 (moderate) 1.5 – 7.2 (high)	(Farmer et al., 1995; Maree and Du Plessis, 1994)
Anthropogenic Emission			
1. Pulp and Paper Industry			
1.1 Thermomechanical pulping	2 – 5	0.2 – 0.7	(Habets and De Vegt, 1991; Rintala et al., 1991)
1.2 chemo-thermomechanical pulping	7.5 – 10.4	1.2 – 1.5	(Habets and De Vegt, 1991)
2. Food processing industry			
2.1 Wine	17	1 – 4	(Ehlinger et al., 1992)
2.2 Molass	50.6	2.9	(Carrondo et al., 1983)
2.3 Seafood-processing industry	12.4 – 16.9 (mussel, tuna, octopus)	2.1 – 2.7	(Mendez et al., 1995)
	55.4 (fish meal)	0.6	(Mendez et al., 1995)
3. Tannery industry	4.8 – 8.0	1.2 – 2.0	(Shin et al., 1995)
4. Photoprocessing	67.8	8	(Rooden et al., 1995)
5. Trinitrotoluene manufacturing process	68.5	51.4	(Hao et al., 1993)

2.2 Sulfur cycle

Sulfur cycle (Figure 2.2) is the process that converts elemental sulfur to other forms by chemical reactions and biological reactions. Sulfur compounds can be found in three phases with different oxidation numbers (2^+ - 6^-) including solid phase (elemental sulfur, S^0), liquid phase (sulfate, SO_4^{2-}), and gas phase (hydrogen sulfide, H_2S). There are four main processes in sulfur cycle as following:

1. Mineralization of organic sulfur to inorganic form such as sulfate, hydrogen sulfide, and elemental sulfur.
2. Oxidation of elemental sulfur, sulfide, sulfite (high oxidation number) to sulfate (SO_4^{2-}, oxidation number = -6)
3. Reduction of sulfate to sulfide
4. Incorporation of sulfide to organic sulfur

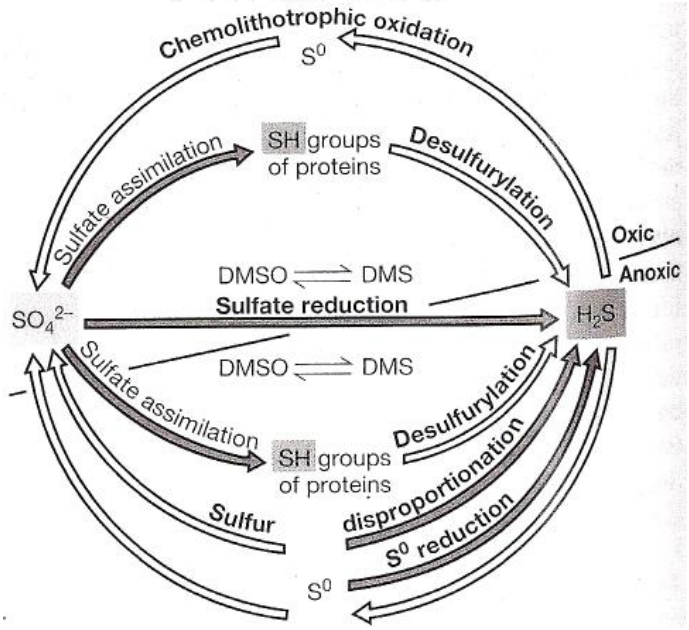


Figure 2. 2 Sulfur cycle (Madigan et al., 2003)

Bacteria are the most important factor in sulfur cycle. They can change one of sulfur form to others by biological processes. Table 2.2 shows reactions in sulfur cycle and the examples of bacteria which can catalyze the reactions.

Table 2.2 the reaction in sulfur cycle (Madigan et al., 2003).

Reaction	Bacteria
Sulfide/Sulfur oxidation ($H_2S \longrightarrow S^0 \longrightarrow SO_4^{2-}$) Aerobic process Anaerobic process	Sulfur chemolithotrophs (*Thiobacillus*, *Beggiatoa*) Purple and green phototrophic bacteria and some chemolithotroph
Sulfate-reduction (Anaerobic) ($SO_4^{2-} \longrightarrow H_2S$)	*Desulfovibrio, Desulfobacter*
Sulfur-reduction (Anaerobic) ($S^0 \longrightarrow H_2S$)	*Desulfuromonas and* hyperthermophilic *Archaea*
Sulfur disproportionation ($S_2O_3^{2-} \longrightarrow H_2S+SO_4^{2-}$)	*Desulfovibrio*
Organic sulfur compound oxidation or reduction ($CH_3SH \longrightarrow CO_2 + H_2S$) (DMSO $\longrightarrow$ DMS)	Many microorganisms
Desulfurylation (organic-S $\longrightarrow H_2S$)	Many microorganisms

2.3 Sulfate removal technique

Regarding sulfate-rich water such as sea water, cooling water, and acid mine drainage, there are several processes used to treat wastewater, which include chemical and physicochemical processes. Physicochemical techniques, namely electrodialysis, nanofiltration, and reverse osmosis (Bilstad, 1992), are highly effective for removing sulfate from water; however, the materials for these techniques are very expensive, and the process requires post-treatment to dispose of the brine in sulfate-rich water. Therefore, chemical treatments by precipitation of $BaSO_4$ (Equation 2.1) and $CaSO_4$ (Equation 2.2) have been developed to replace physicochemical process to reduce cost of treatment system. Nevertheless, Ba^{2+} is toxic for human lives when human acquires Ba^{2+} at high concentration, so precipitation of $CaSO_4$ is widely used for sulfate removal in sulfate-rich water.

$$SO_4^{2-} + BaCO_3 + Ca^{2+} \rightarrow BaSO_4 + CaCO_3 \quad (2.1)$$

$$SO_4^{2-} + Ca^{2+} \rightarrow CaCO_3 \quad (2.2)$$

For high-organic wastewater containing high sulfate, the most treatment process used to treat wastewater is anaerobic biological treatment process. This is because it is a cost-effective and easy to operate when compares to others physicochemical and chemical process. Sulfate content will be removed from wastewater by sulfate reduction process of sulfate reducing bacteria in anaerobic treatment process. The applications of sulfate removal by using biological treatment process are shown in Table 2.3

Table 2.3 Overview of reactor types, influent, and SO_4^{2-} removal in anaerobic digestion of sulfate-rich wastewater (Lens et al., 1998).

Reactor type	Influent			SO_4^{2-} removal	
	Type	COD (g/l)	SO_4^{2-} (g/l)	Efficiency (%)	% COD flow by SRB
CSTR	Molasses	40.9	4.2-5.1	38-71	3-8
CSTR	Edible oil	2.2-3.9	3.6-6.3	30-59	47-75
UASB	Sucrose	5.6	1.4-2.0	60-70	13-16
UASB	White water TMP	1.9-2.2	1.0-1.5	24-64	NR
UASB	Acetate	1.5-2.1	0.7-3.4	70	50-90
UASB	Ac/Prop/But	3.3-4.0	1.6-8.3	NR	50-95
EGSB	Ac/Prop/But	0.5-2.5	1.2-4.6	27-68	59-97
MUSB	Acetate	0.2-0.4	0.1-0.2	40-80	100
FSB	Cane juice stillage	26.0	1.5	95	4
AF	Acetate	5.0	0.6-15.0	3-38	3-7
AF	EtOH	5.0	0.6	99	22
AF	Molasses	40.9	4.5-5.1	47-100	4-11
AF	Molasses	49.8	6.0-11.4	100	13-25
AF	Edible oil	2.1-3.3	3.5-4.5	43-54	38-83
AF	Citric acid	25.8	3.4	93	18
HYBRID	Prop/But/EtOH	5.0	4.0	NR	50
HYBRID	Landfill leachate	19.6-42.0	5.9	>90	NR
CAD	Sea food	10-60	0.6-2.7	96.0	3-12

Note: **CSTR**=completely stirred tank reactor, **UASB**=upflow granular sludge bed reactor, **EGSB**=expended granular sludge bed reactor, **MUSB**=microaerophilic granular sludge bed reactor, **FSB**=flocculant sludge bed reactor, **AF**=anaerobic filter, **HYBRID**=hybrid reactor, **CAD**=central activity digester, **NR**=not reported.

Sulfate-reduction process

In anaerobic biological treatment process, sulfate-reducing bacteria (SRB) can be present when sulfate or sulfite appears in wastewater, which consequently results in sulfate-reduction process. Sulfate-reduction is the process which converts sulfate to sulfide by SRB in the absence oxygen. SRBs can use hydrogen, acetate, or other volatile fatty acids as electron donors and use sulfate as an electron acceptor to produce sulfide as a final product.

$$H_2, \text{Acetate, VFA} + SO_4^{2-} \xrightarrow{Sulfate-reducing\ bacteria} \text{Product} + H_2S \qquad (2.3)$$

Sulfide, produced by SRB, can cause many adverse effects which are (Tanthulawes, 2003):

1. Decrease COD removal efficiency because 1 g of sulfide equivalent to 2 g of COD
2. Give off bad odor
3. Decrease quality and quantity of methane production
4. Corrosion in pipe systems
5. Inhibit anaerobic bacteria at high concentrations of sulfide

Theoretical stoichiometry of COD:SO_4^{2-} ratio is 0.67. It means that 1 g SO_4^{2-} is equivalent to 0.67 g COD. This is the minimum COD requirement for sulfate wastewater treatment using bacteria. Figure 2.3 shows the calculation of electron equivalents of COD and sulfate.

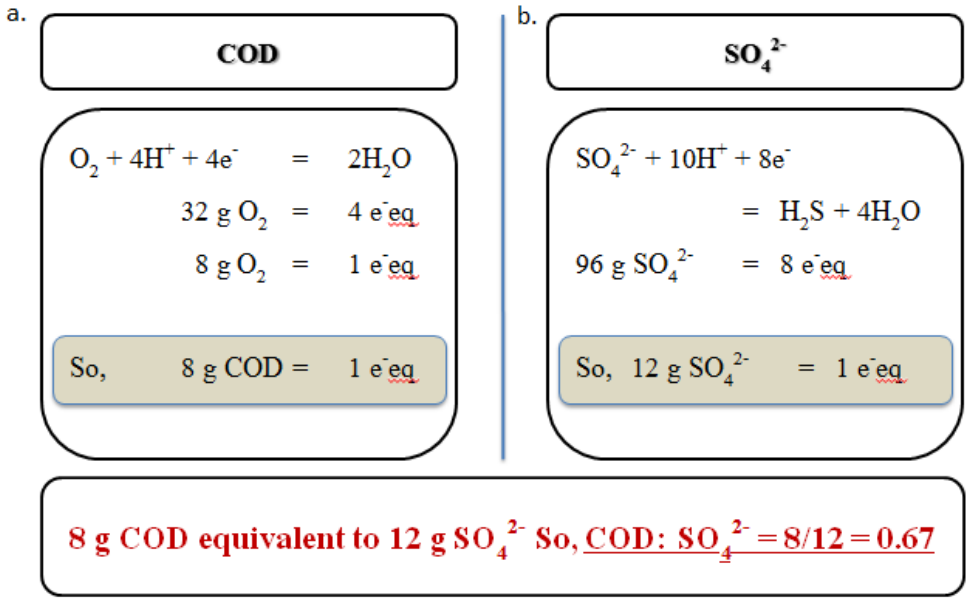


Figure 2.3 Theoretical stoichiometry of COD:SO_4^{2-} ratio. a) electron equivalent of COD and b) electron equivalent of sulfate.

Sulfide, the final product of sulfate-reduction process, can be removed from wastewater by several methods such as aeration process, precipitation by oxidizing agent (Zhang et al., 2008), adsorption (Kante and Bandosz, 2007), chemical, and biological oxidation (Ateya et al., 2003). Nevertheless, energy consumption for aeration process, oxidizing agent, and chemical used require high cost for operate; therefore, microbial fuel cells will be developed to remove sulfide from wastewater simultaneously generate electricity from oxidation process.

2.4 Microbial fuel cell

Firstly discovered by Potter (Potter, 1911), Microbial fuel cell (MFC) is currently a new interesting highly effective technology for wastewater treatment. Apart from having high efficiency of treating wastewater containing high COD, MFC also plays an important role on recovering energy and converting it into electricity by using an electrochemical reaction between two electrodes including anode and cathode electrodes. Chemical energy from organic substrates, such as lactate, acetate, and

glucose, is transformed into electrical energy by groups of microorganisms under anaerobic conditions. Meanwhile, organic complex is decomposed by microorganisms into carbon dioxide (CO_2), protons (H+), and electrons (e-) as shown in Equation (2.4). Then, protons move through proton exchange membrane to the cathode, resulting in a potential difference between cathode and anode. Therefore, electrons are transferred from the anode to the cathode by a conductive wire in order to combine with protons and oxygen (O_2) at the cathode (electron acceptor) as shown in Equation (2.5), causing an electrical current.

Anodic reaction:

$$C_6H_{12}O_6 + 6H_2O \xrightarrow{microorganism} 6CO_2 + 24H^+ + 24e^- \quad (2.4)$$

Cathodic reaction: $$6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O \quad (2.5)$$

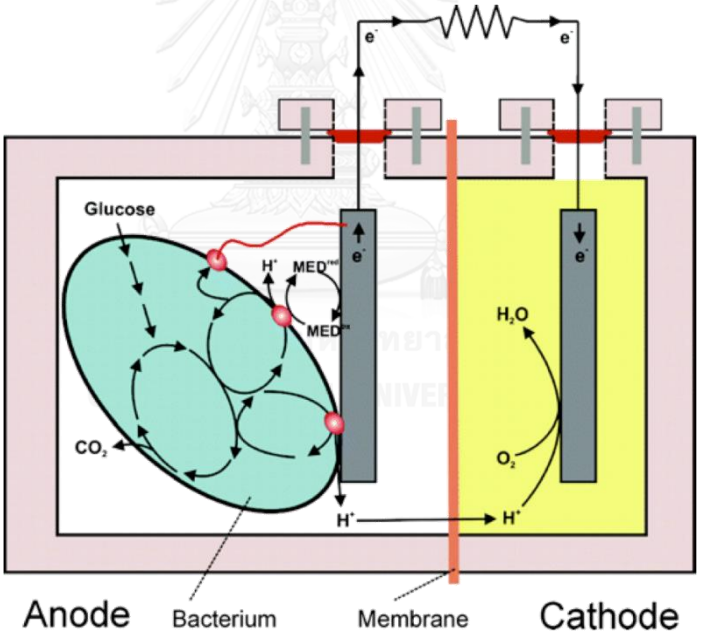


Figure 2.4 principle of microbial fuel cell (Rabaey et al., 2005)

Microbial fuel cell consists of following components (Du et al., 2007):

1. Anode: Anode is the electrode that is used for accepting electron from the oxidation of organic compounds by microorganisms. Anode can be made from many materials including graphite, graphite felt, carbon paper, carbon-cloth, Pt, Pt black, and reticulated vitreous carbon (RVC).
2. Cathode: Cathode is the electrode where electron acceptors, oxygen in this case, can accept electrons and protons for completing a cycle of electrochemical process. Graphite, graphite felt, carbon paper, carbon-cloth, Pt, Pt black and RVC are mostly selected as materials for cathode. Cathode is usually coated by catalyst, such as platinum (Pt) to improve the reaction rates.
3. Proton exchange membrane (PEM) is a membrane that allows only protons to pass through. The example of PEM is Nafion, polyethylene. In other words, PEM serves as electrolyte for MFC.
4. Cathode and anode chamber: Cathode and anode chambers are the compartments in MFC in which biological and chemical processes occur. They can be made from glass, polycarbonate, or Plexiglas.
5. Electrical devices: conductive wire and external resistance.
6. Microorganisms: Microorganisms in anodic chamber oxidize organic substance and then transferred the electrons to the anode.

One of the important key factors in microbial fuel cell is microorganisms in the anodic chamber. However, there are only certain groups of microorganisms which can transfer electrons to anode. This is because common anaerobic microorganisms typically have cytochromes in the inner cell wall, thereby preventing the delivery of electrons to the anode. Nevertheless, certain groups of microorganisms contain outer membrane cytochromes, such as *Geobacter sulfurreducens* (Magnuson et al., 2001). The electrons can be transferred to the anode directly to produce electricity. This

group of microorganisms is known as exoelectrogenic microorganisms (EEM). Transfer of electrons to the anode can occur in three ways:

1. Microorganisms transfer electrons directly: Microorganisms adhering to an anode area, oxidizing organic compounds, and transferring electrons to the anode.
2. Transferring electrons by redox mediators: Redox mediators, such as humic acid, Fe^{3+}, Mn^{4+}, and threonine perform as an electron carrier, sending the electrons to anode.
3. Transferring electrons by nanowire: Some bacteria can produce conductive nanowire. When these groups of bacteria adhere to the anode area and create such nanowire, it allows the electrons to move to the anode and produce electricity.

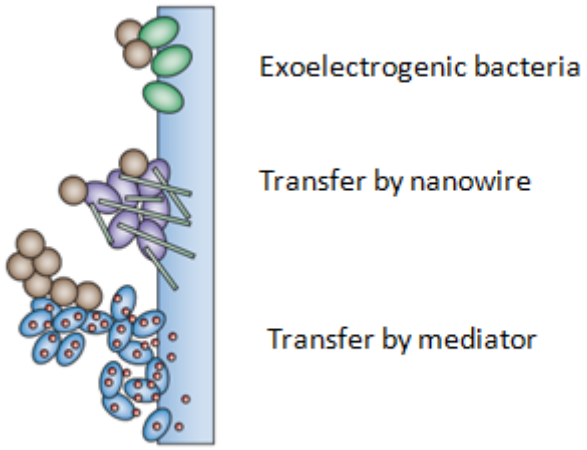


Figure 2.5 Types of electron transfers from microorganisms to the anode (Logan, 2009).

Types of Microbial Fuel Cell

Microbial fuel cells (MFC) can be categorized into two types, double-chamber microbial fuel cells and single-chamber microbial fuel cells.

1. Double-chamber microbial fuel cells consist of two chambers, an anode chamber and a cathode chamber. An anode chamber is a chamber for microbial treatment; the anode was added in this chamber for electrical generation. In this chamber, wastewater serves as a fuel for electricity generation. For a cathode chamber, it contains a cathode and the solution which contains electron acceptor such as oxygen. In some cases, ferricyanide is added into a solution to increase the electron transfer rates to the electron acceptors.
2. Single chamber microbial fuel cells have been developed to replace double-chamber MFC to improve the MFC efficiencies while reducing operational costs and offering smaller footprint. Unlike anode being inside the single chamber, the cathode is attached to the outside of the MFC. The proton exchange membrane is a barrier between cathode and the solution inside the anodic chamber. The other side of cathode is exposed directly to the atmosphere where oxygen in the air accepts electrons from the cathode.

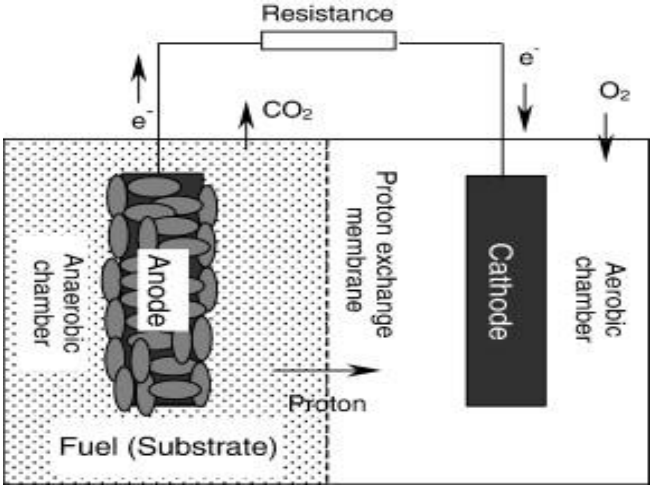


Figure 2.6 Double-chamber MFC (Du et al., 2007).

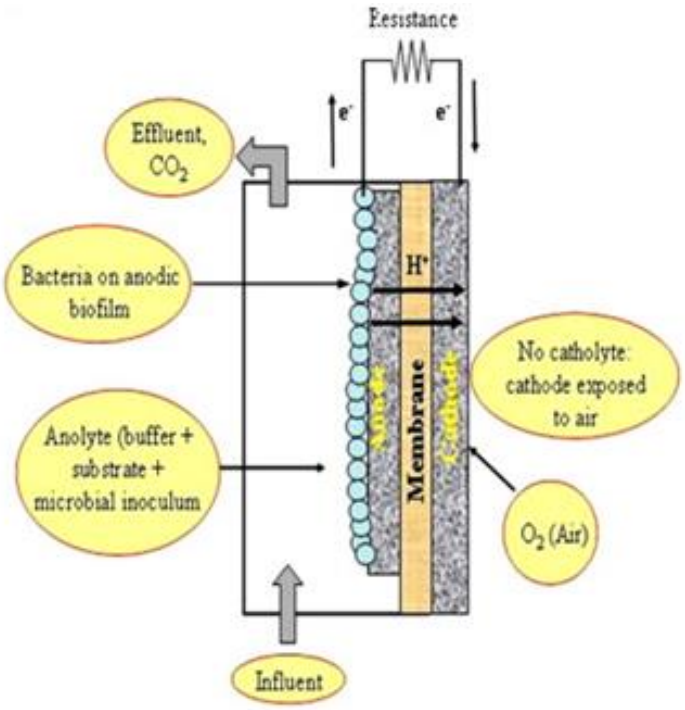


Figure 2.7 Single-chamber MFC (Pant et al., 2010).

2.5 Electrical Measurement

MFC can be considered as a galvanic cell, in which the electricity is generated from the different potentials between anode and cathode. Therefore, the electricity generated from the MFC is a direct current (DC).

In general, the power is calculated per unit area or volume of the smallest area of electrode anode because the power generation is limited by the smallest area of electrode which consists of anode, cathode, and PEM (Logan et al., 2006). Power generation tends to increase when the surface area of electrode increases (Logan et al., 2007). Electrical current generated in MFC is a function of external resistance (R_{ext}) and the electrical potential (V) between the anode and the cathode. Therefore, in order to determine the actual electrical current (I), it is necessary to calculate it by

using a mathematical function (See Equation 2.6). After that, the power density can be continuously measured from Equation 2.7 and 2.8.

$$V = I \times R_{ext} \quad (2.6)$$

Power density per unit area $$P = \frac{I \times V}{Area} \quad (2.7)$$

Power density per unit volume $$P = \frac{I \times V}{volume} \quad (2.8),$$

where P = Power density per unit area or units volume (W/m^2), (W/m^3)

I = Current (Amp)

V = Voltage (Volt)

Area = The smallest area of electrode (m^2)

Volume = Volume of anode chamber (m^3)

R_{ext} = External resistance (Ohm).

Regarding the Equation (3.6) and (3.7, it is clearly shown that the power density depends on the electric current (I) and the electric potential (V) of the MFC, which can vary relying on the external resistances (Equation 3.6). As a result, various power densities (P) of the MFC can be obtained if the external resistances (Rext) are different. Therefore, using a polarization curve (I versus V) and a power density curve (I versus P) can help describe the MFC performance in a wide range of operation (Figure 2.8). In addition, these curves can specify the external resistant that corresponds to the maximum power density, allowing us to know the best condition that the MFC can operate.

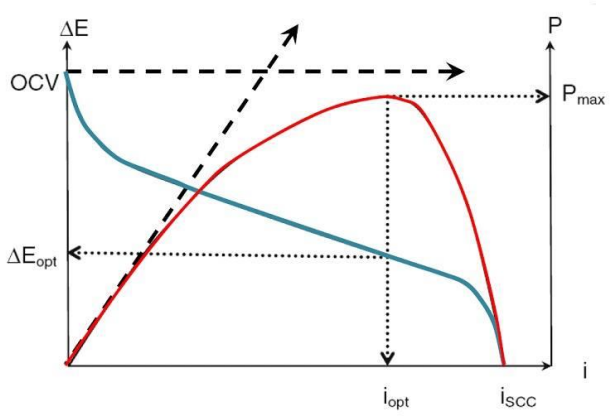


Figure 2.8 Polarization curve (I versus V) and power density curve (I versus P)

In addition, the polarization curve (blue line in Figure 2.8 and Figure 2.9), plotted between electrical current (x-axis) and electrical potential (y-axis), can be used to estimate the voltage loss of MFC system. The polarization curve can be divided into 3 parts representing 3 types of voltage loss, including activation loss, ohmic loss, and concentration loss (O'hayre et al., 2009).

Activation loss: Activation loss can be observed in the first part of the polarization curve (Figure 2.9). Activation loss is primarily caused by chemical reactions in MFC systems. Activation loss can be decreased by using proper electrode catalysts, increasing electrode surface areas, increasing operating temperature, and improving the anode surface to increase the amount of biofilm on the anode. Activation loss can be calculated from Tafel Equation (Equation 3.9).

$$\eta_{act} = [a_A + b_A \ln(j)] + [a_C + b_C \ln(j)] \quad (3.9),$$

where

a_A, a_C = x-intercept of Tafel Equation of anode and cathode, respectively

b_A, b_C = Tafel slope of anode and cathode, respectively

j = current density

In some cases, activation loss can be simplified to Equation (3.10), in which the unknown coefficients are easy to be determined. Equation (3.10) combines a_A, and a_C into a, while combining b_A and b_C into b.

$$\eta_{act} = a + b\ln(j) \quad (3.10)$$

Ohmic loss: Ohmic loss is the voltage loss that corresponds to the linear part of the polarization curve. Ohmic loss can occur due to the loss of electron flow between the electrodes and interconnections. The ohmic loss can be minimized by decreasing the distance between anode and cathode, improving proton transfers through proton exchange membranes, reducing the loss at any interconnections, and increasing solution conductivity. Ohmic loss can be explained by Equation (3.11).

$$\eta_{ohmic} = jR_{ohmic} \quad (3.11),$$

where

j = current density

R_{ohmic} = external resistance

Concentration loss: Concentration loss, the third part of polarization curve, is the voltage loss occurring at high current due to the limit of mass transfers of substrates and products. Concentration loss can be estimated by Equation (3.12).

$$\eta_{conc} = c\ \ln[\frac{j_L}{j_L - j}] \quad (3.12),$$

where

c = an empirical constant

j = current density

j_L = The limiting current density

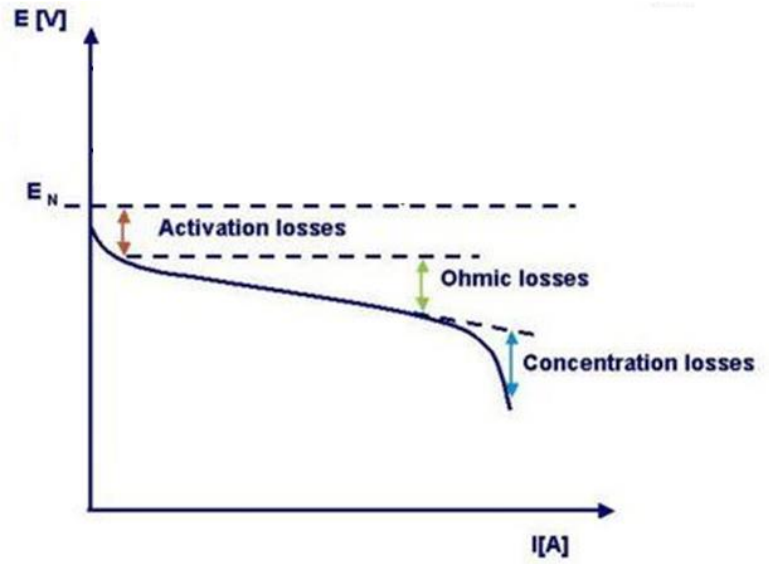


Figure 2.9 Polarization curve

Due to the fact that MFC is a galvanic cell, which can generate electricity from redox reactions by itself, the free energy of the redox process driving the MFC must be thermodynamically favorable. Table 2.4 shows reduction potentials (E^0) of common conjugate redox pairs. The higher the reduction potentials, the more favorable the reduction process is. According to Table 2.4, oxygen is the most favorable electron acceptor compared to other compounds. On the other hand, the reduction of CO_2 → glucose is the least favorable. In other words, glucose is the most favorable electron donor. However, the reduction potentials of system will be decreased when mixing solution are used as fuel.

Table 2.4: Reduction potential of common redox couples (Madigan et al., 2003)

Conjugate redox pair	**Reduction potential, E_0 (V)**
CO_2/glucose (24 e^-)	-0.43
$2H^+/H_2$ (2 e^-)	-0.42
CO_2/methanol (6 e^-)	-0.38
CO_2/acetate (8 e^-)	-0.28
S^0/H_2S (2 e^-)	-0.28
SO_4^{2-}/H_2S (8 e^-)	-0.22
Pyruvate/lactate (2 e^-)	-0.19
$S_4O_6^{2-}/S_2O_3^{2-}$ (2 e^-)	+0.024
Fe^{3+}/Fe^{2+} (1 e^-), pH7	+0.2
NO_3^-/NO_2^- (2 e^-)	+0.42
$NO_3^-/0.5N_2$ (5 e^-)	+0.74
Fe^{3+}/Fe^{2+} (1 e^-), pH2	+0.76
$0.5O_2/H_2O$ (2 e^-)	+0.82

From Figure (3.11), the real voltage of MFC decreases with increasing current density. Three types of voltage loss in MFC include activation loss, ohmic loss, and concentration loss. The real voltage can be found from thermodynamic prediction as shown in Equation (3.13).

$$V = E_{emf} - \eta_{act} - \eta_{ohmic} - \eta_{conc} \quad (3.13),$$

where

V = real output voltage of fuel cell

E_{emf} = thermodynamically predicted voltage

η_{act} = activation losses

η_{ohmic} = ohmic losses

η_{conc} = concentration losses

E_{emf} is a thermodynamically predicted voltage between anode (E_{an}) and cathode (E_{cat}). E_{an} and E_{cat} can calculate by Equation (3.14) and Equation (3.15) respectively and E_{emf} can calculate by Equation (3.16).

$$E_{an} = E^0_{an} - (RT/nF)\ln(\prod) \quad (3.14)$$

$$E_{cat} = E^0_{cat} - (RT/nF)\ln(\prod) \quad (3.15)$$

$$E_{emf} = E_{cat} - E_{an} \quad (3.16),$$

where

E_{an} = reduction potential of anode

E_{cat} = reduction potential of cathode

R = the gas constant (8.31447 J/mol-K)

T = absolute temperature (K)

n = the number of electron transferred

F = Faraday's constant (96,485 C/mol)

$\prod$ = the reaction quotient ($\frac{[product]^p}{[reactants]^r}$)

Moreover, to evaluate the MFC performance in terms of energy conversion efficiencies, Coulombic efficiency is needed. This parameter indicates the system potentials with respect to the conversion of electrons from organic substrates to electricity generated from MFC. Equation (3.17) describes the calculation of Coulombic efficiency.

$$\mathrm{CE} = \frac{\mathrm{Cp}}{\mathrm{Ct}} \times 100 \quad (3.17),$$

where

CE = Coulombic efficiency

C_p = Accumulated current (Coulomb)

C_t = Number of electrical charge of decreased Substances potential regarding the theory (Coulomb),

where

$$C_t = \frac{F \times b_i \times S_i \times V}{M},$$

where

F = Faraday's constant (96,458 Coulomb/mole of electrons)

b_i = Number of mole of produced electrons per mole of organic substances

S_i = Concentration of organic substances (g/l)

V = Volume (l)

M = Molecular weight of organic substances

2.6 Potential groups of microorganisms in MFC treating wastewater containing high COD and sulfate

In MFC treating wastewater containing high COD and sulfate, microbial communities and relationship among microorganisms in the systems can be complex. There could be many types of relationship, such as mutualism, commensalism, protocooperation, and competition, because each group of microorganism provides different functions and requires different substrates in the MFC. Potential groups of microorganisms that can play important roles in the MFC system used in this study include:

1. **Fermentative bacteria**

In general, fermentative bacteria can be found in anaerobic treatment systems. Fermentative bacteria use specific enzymes to convert complex organic substances, such as protein, carbohydrate, and lipid into simple organic substances. This process is so-called hydrolysis. Hydrolysis could be the slowest reaction and become the rate-

limiting step in anaerobic treatment process. The rate of a hydrolysis reaction also depends on concentrations of the organic substances, enzymes, temperature, and pH.

After the hydrolysis process, a simple organic substance is converted to volatile fatty acid (VFA), such as acetic acid, propionic acid, and butyric acid by fermentative bacteria. Therefore, this group of microorganisms can also be called as acidogenic bacteria. Examples of acidogenic bacteria are Pseudomonas, Flavobacterium, Alcaligenes, Escherichia, and Aerobactor. An example of the oxidation process of glucose to pyruvic acid is shown in Equation (3.18)

$$C_6H_{12}O_6 + 2NAD^+ + 2ADP + 2P \rightarrow 2CH_3COCOOH + 2NADH^+ + ATP \quad (3.18)$$

2. Sulfate-reducing bacteria (SRB)

Sulfate-reducing bacteria are anaerobic chemoheterotroph that can obtain energy from a redox reaction of organic substances in the system. They generally have two shapes including bacillus and spiral. SRB can reduce sulfate (SO_4^{2-}) to hydrogen sulfide (H_2S) using organic compounds as electron donors and sulfate as an electron acceptor. SRB can also use hydrogen as an electron donor instead of organic substances. The redox reactions of SRB for different electron donors are following in Equation (3.19), (3.20), and (3.21):

Hydrogen as electron donor: $4H_2 + SO_4^{2-} \rightarrow S^{2-} + 4H_2O$ (3.19)

Acetate as electron donor:

$$CH_3COOH + SO_4^{2-} \rightarrow S^{2-} + 2CO_2 + 2H_2O \quad (3.20)$$

Large organic compound as electron donor:

$$CH_3CH_2COOH + 1.75SO_4^{2-} \rightarrow 1.75S^{2-} + 3CO_2 + 3H_2O \quad (3.21)$$

SRB can be classified into two groups according to types of organic oxidation, including (Tanthulawes, 2003):

2.1 Incompletely oxidizing sulfate-reducing bacteria (I-SRB). The end-product of organic oxidation by this group of SRB is acetate, since I-SRB lack of enzymes degrading acetate. However, in some cases, I-SRB can use acetate as a carbon source when it uses hydrogen or formate as electron donors.

2.2 Completely oxidizing sulfate-reducing bacteria (C-SRB). This group of SRB can oxidize organic compounds completely to CO_2.

Table 2.5 Examples of organic decomposition of I-SRB and C-SRB (Tanthulawes, 2003).

No.	Electron donor	Type of bacteria	Reduction reaction
1.	Hydrogen	I-SRB C-SRB	$4H_2 + SO_4^{2-} + H^+ \rightarrow 4H_2O + HS^-$
2.	Acetate	C-SRB	$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$
3.	Propionic	C-SRB I-SRB	$4CH_3CH_2COO^- + 7SO_4^{2-} \rightarrow 12HCO_3^- + 7HS^- + H^+$ $4CH_3CH_2COO^- + 3SO_4^{2-} \rightarrow 4CH_3COO^- + 4HCO_3^- + 3HS^- + H^+$
4.	Butyrate	C-SRB I-SRB	$2CH_3(CH_2)_2COO^- + 5SO_4^{2-} \rightarrow 8HCO_3^- + 5HS^- + H^+$ $2CH_3(CH_2)_2COO^- + SO_4^{2-} \rightarrow 4CH_3COO^- + HS^- + H^+$
5.	Lactate	C-SRB I-SRB	$2CH_3CHOHCOO^- + 3SO_4^{2-} \rightarrow 6HCO_3^- + 3HS^- + H^+$ $2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2HCO_3^- + 3HS^- + H^+$
6.	Benzoate	C-SRB I-SRB	$4C_6H_5COO^- + 15SO_4^{2-} + 16H_2O \rightarrow 28HCO_3^- + 15HS^- + 9H^+$ $4C_6H_5COO^- + 3SO_4^{2-} + 16H_2O \rightarrow 12CH_3COO^- + 4HCO_3^- + 3HS^- + 9H^+$

Note: I-SRB is incompletely oxidizing sulfate-reducing bacteria
C-SRB is completely oxidizing sulfate-reducing bacteria

Factors affecting sulfate-reducing bacterial activities

- Temperature – In general, pure cultures of sulfate-reducing bacteria have optimum temperatures in the range of 30 - 40°C (Tanthulawes, 2003). Changes in

temperature can have great impacts on sulfate-reducing bacterial activities. At temperature below optimum temperature, increases in temperature typically result in higher growth rates and higher activities.

- Iron concentration – Since sulfate-reducing bacteria require iron for their living, it is essential that sufficient iron is available to sulfate-reducing bacteria in the systems. However, the presence of iron can lead to ferrous sulfide precipitation (FeS), which decrease the amount of available iron in the aqueous phase, and thereby reducing sulfate-bacterial activities (Tanthulawes, 2003).

- Salt demand and salt tolerant - sulfate-reducing bacteria can be classified regarding the salt concentrations in their habitats including 1) sea water, containing proper salt concentrations of ~20 g/l, 2) brackish water, containing proper salt concentrations of ~0.5 g/l, and 3) fresh water. Sulfate-reducing bacteria originated from freshwater might be inhibited at salt concentrations over 27 g/l (Tanthulawes, 2003).

- COD: sulfate ratio - COD:SO_4^{2-} ratio is one of the factors controlling groups of microorganisms in the systems. Sulfate-reducing bacteria usually can outcompete methanogens when COD:SO_4^{2-} ratio is less than 1.1 (Chou et. al, 2008).

- pH - Optimum pH for sulfate-reducing bacteria is usually between pH 6-9. The pH values higher or lower than this range can suppress sulfate-reducing bacterial activities (Tanthulawes, 2003).

- Sulfide toxicity – Sulfide toxicity could occur to microorganisms living in the treatment systems in which sulfate-reducing bacteria coexist. Sulfide is generated from the reduction of sulfate by sulfate-reducing bacteria. However, sulfate-reducing bacteria usually have higher thresholds for sulfide than other groups of microorganisms.

3. Sulfur-oxidizing bacteria (SOB)

Sulfur-oxidizing bacteria, such as *Thiobacillus* and *Thiomicrospora* have many types of shapes including bacillus, coccus, and spiral. SOB live under aerobic conditions using carbon dioxide as a carbon source and obtaining energy from a redox process with sulfur as an electron donor and oxygen as an electron acceptor. The end product of reactions is sulfate as shown in Equation (3.22), (3.23), and (3.24).

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+ \quad (3.22)$$

$$S + H_2O + 1.5O_2 \rightarrow SO_4^{2-} + 2H^+ \quad (3.23)$$

$$S_2O_3^{2-} + H_2O + O_2 \rightarrow SO_4^{2-} + 2H^+ \quad (3.24)$$

From previous research, in MFC treating wastewater containing high COD and sulfate or sulfide, SOB has been found in the anode chamber of the MFC (Sun et al., 2009; Sun et al., 2010). SOB can also co-active with other groups of microorganisms such as sulfate-reducing bacteria and exoelectrogenic microorganisms to produce electricity in the MFC (Pant et al., 2012). Therefore, it is likely to find SOB in MFC when it is used to treat wastewater containing high COD and sulfate.

4. Methanogens

Methanogens are anaerobic chemoheterotrophic microorganisms. Methanogens cannot use VFA which have carbon more than 2 atoms; therefore, only 10 types of substrates can be used for the growth of methanogens as shown in Table 2.6.

Table 2.6 Substrates that can be used by methanogens.

Type of substance	**Type**	**Chemical reaction**	$\Delta G^{0\prime}$ **(kJ/ reaction)**
1. Carbon dioxide type	- Carbon dioxide - Formate - Carbon monoxide	$CO_2+4H_2 \rightarrow CH_4+H_2O$ $4HCOO^-+4H^+ \rightarrow CH_4+3CO_2+H_2O$ $4CO+2H_2O \rightarrow CH_4+3CO_2$	-131 -145 -210
2. Methyl type	- Methanol - Methylamine - Dimethylamine - Trimethylamine - Methyl-mercaptan - Dimethylsulfide	$4CH_3OH \rightarrow 3CH_4+CO_2+2H_2O$ $4CH_3NH_3^+ +2H_2O \rightarrow 3CH_4+CO_2+4NH^+$ $(CH_3)_2NH_2^+ +2H_2O \rightarrow 3CH_4+CO_2+2NH_4^+$ $4(CH_3)_3NH_2^+ +6H_2O \rightarrow 9CH_4+3CO_2+4NH_4^+$	-319 -230 -230 -666
3.Acetate	- Acetate	$CH_3COO^-+H^+ \rightarrow CH_4+CO_2$	-31

Factors affecting methanogen activities (Tanthulawes, 2003).

- Temperature – There are two ranges of temperature that are suitable for methanogens which are 8 – 45 ℃ and 40 – 70 ℃ for mesophilic and thermophilic methanogens, respectively.

- pH – pH 5 – 10 is suitable for the growth of methanogens.

- VFA and alkalinity – In anaerobic reactors, alkalinity should be in the range of approximately 1,500 – 2,000 mg/l as CaCO3 to prevent pH drop in the system from VFA. VFA concentrations for effective reactors should be kept at 20 – 200 mg/l as acetate. VFA: alkalinity ratio should be maintained to be less than 0.4.

- Nutrient – Macronutrients for cell synthesis of methanogen are 100:10:1:1 for C:N:P:S. Iron, cobalt, nickel, and sulfur are also important elements for methanogens.

- Toxicity – There are several inorganic compounds than can inhibit the methanogen activities if they are contaminated at high levels, such as cations (Na^+, K^+, Mg^{2+}, Ca^{2+}), heavy metals, ammonia, sulfide, and oxygen.

5. Exoelectrogenic microorganisms

Shewanella and *Geobactor* are examples of exoelectrogenic microorganisms which have cytochromes outside cells, resulting in electron transfer outside the cell that can generate electricity. Electron transfer of bacteria to the anode can occur via three mechanisms including:

1. Electron transfer by direct contact to the anode. An example of microorganisms that use this mechanism is *Geobactor sulfurreducens.*
2. Electron transfer to the anode via redox mediator, such as humic acid and ferric. An example of microorganisms that use this mechanism is *Shewanella.*
3. Electron transfer to the anode via nanowire in biofilm. Examples of microorganisms that use this mechanism are *Geobactor sulfurreducens, Pelotomaculum thermopropionicum.*

2.7 Toxicity effect from sulfur species and cations

2.7.1 Inhibition by cations

High concentrations of cations (Na^+ and Ca^{2+}) in high sulfate wastewater can inhibit anaerobic bacteria such as methanogens. The range of concentrations of Na^+ that can cause 50% inhibition of methanogen is 6 – 40 g/l (De Baere et al., 1984; Omil et al., 1995). However, in the case of sulfate-reducing bacteria, although the presence of high sodium concentration can increase growth rate of marine SRB, it can also inhibit freshwater SRB . The levels of sulfate suitable for freshwater SRB and marine SRB are 2 and 5.5 – 6 g/l, respectively. Nevertheless, they will become an inhibitor, if the sodium concentrations increase up to more than 11 g/l (Visser, 1995).

Calcium has not been reported to have direct toxicity on anerobic microorganisms; however, the precipitation of calcium carbonate and calcium phosphate can cause down performances of reactors by scaling. Clogging problems in pipe system also occur at high levels of calcium.

2.7.2 Inhibition by sulfite

Anaerobic microorganisms are inhibited by sulfite. Maaskant and Hobma (1981) reported that 50% of methanogens was inhibited when 150 – 200 mg/l SO_3^{2-} was dosed in the first cycle of batch test; however, after they added sulfite in the reactor in the second cycle, the inhibition rate decreased because SRB in sludge oxidized sulfite to sulfide. Therefore, sulfite inhibition would probably be inconsiderable in continuous reactors.

2.7.3 Inhibition by sulfide

Lens et al. (1998) stated that such inhibition of the purification process or even a total process failure can occur if there is an existence of toxic sulfide in the process. Types of sludge in anaerobic treatment process will be suppressed by sulfide at different concentrations. The 50% inhibition occurs at sulfide concentration of 50 – 130 mg/l, 50 mg/l, and 250 mg/l for suspended sludge, fix bed, and sludge granule, respectively. For a pure culture of SRB (*Desulfovibrio desulfuricans*), 50% inhibition was observed when operated at 250 mg/l of sulfide.

2.8 Microbial community analysis by 16S rRNA gene amplicon sequencing

Normally, 16S rRNA gene is often used as the marker to categorize microorganisms since all microorganisms contain 16S rRNA gene with different sequences among the groups and species. There are various advanced instruments and techniques used for DNA sequencing, including Roche 454 system, Illumina MiSeq system, AB SOLiD system, Sanger system. Each technique has different advantages and disadvantages. The selection of appropriate genome sequencing techniques, therefore, depends on the expected outputs and results. The differences of each genome sequencing instrument and technique are summarized in Table 2.7.

Table 2.7 Comparison of mechanism, cost, advantages and disadvantages of different sequencers. (Liu et al., 2012)

Sequencer	**454 GS FLX**	**MiSeq**	**SOLiDv4**	**Sanger 3730xl**
Sequencing mechanism	Pyrosequencing	Sequencing by synthesis	Ligation and two-base coding	Dideoxy chain Termination
Read length	700 bp	Up to 2x300 bp	50 + 35 bp or 50 + 50 bp	400～900 bp
Accuracy	99.9%*	98%, (100PE)	99.94%	99.999%
Reads	1M	3G	1200～1400M	—
Output data/run	0.7Gb	0.3 –51 Gb	120 Gb	1.9～84 Kb
Time/run	24 Hours	4- 56 hours	7 Days for SE 14 Days for PE	20Mins～3Hours
Advantage	Read length, fast	High throughput,	Accuracy	High quality, long read length
Disadvantage	Error rate with polybase more than 6, high cost, low throughput	Short read assembly	Short read assembly	High cost low Throughput
Cost	$7000 (per run)	$6000 / 30x	$15,000/100Gb	$4 per 800 bp
Cost/ million base	$10	$0.07	$0.13	$2400
Bacterial Sequencing	Yes	Yes	Yes	No

16S rRNA gene amplicon sequencing has many steps including 1) DNA extraction, 2) polymerase chain reaction (PCR), 3) clean-up, and 4) genome sequencing.

DNA extraction. There are 3 main steps to extract DNA from the cells, including 1) breaking the cell by a lysis solution consisting of detergent and proteinase K, 2) separating DNA from protein and debris using a salt solution causing clump proteins and debris, and 3) isolating concentrated DNA which can be done by many techniques. Examples of DNA purification are ethanol precipitation, phenol-chloroform extraction, and minicolumn purification.

Polymerase chain reaction (PCR). PCR is a method of amplifying DNA of microorganisms in the samples. PCR is designed to simulate DNA replication in the cells. PCR consists of seven components, including DNA template, primers, thermostable DNA polymerases, PCR buffer, $MgCl_2$, nNTPs, and PCR machine. In 16S rRNA gene amplicon sequencing, the amplified region will be at v3 or v3-v4 of 16S rRNA gene using universal primers. There are three steps for PCR operation, including denaturation, annealing, and extension which is shown in Figure (3.10).

Denaturation: Denaturation is a process of denaturing or separating double-stranded DNA into single stranded DNA in the PCR machine at a high temperature (94 – 98 °C for 20 – 40 second) to disrupt hydrogen bonds in DNA.

Annealing: In this process, a primer will be matched with single stranded DNA at a low temperature (40 - 62 °C for 20 - 40 second).

Extension: Extension is a step to synthesize new DNA by adding dNTPs. The temperature for extension is at approximately 72 – 74 °C, depending on DNA polymerase used.

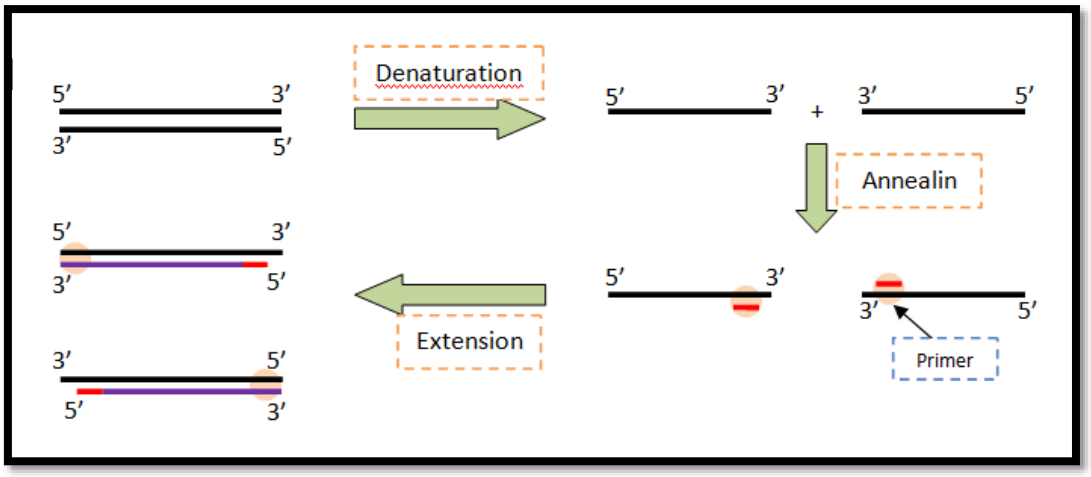


Figure 2.10 Polymerase chain reactions.

Clean-up is a process to remove free primers and primer dimers from the PCR products.

DNA sequencing. There are several approaches for DNA sequencing as mentioned earlier. In this study, the Illumina Miseq technique will be used to sequence nucleotide because it has many advantages such as exceptional data quality, simple and intuitive instrument workflow, rapid sequencing, cost-effective, and adjustable read length. The data outcomes of analyzing microbial community by using Illumina MiSeq technique are shown in bacterial and archaeal categories. Illumina MiSeq can group microbial community in the series of kingdom to species; therefore, it is easy to interpret the results to find the predominant species in the MFCs. There are two main steps in DNA sequencing by using Illumina MiSeq which consists of bridge amplification and genome sequencing.

Bridge amplification: in this step, a single stranded DNA will be amplified in flow cell channels which are coated by adapters and complementary adapters. Adapters will function similarly to a primer in PCR process (Figure 2.11a.). After that, DNA will be amplified to double-stranded bridge (Figure 2.11b.) by nucleotide and enzyme. Then, amplified DNA will become the DNA template for other amplification

process. At the end of this step, approximately 1,000 DNA will be obtained (Figure 2.11c.).

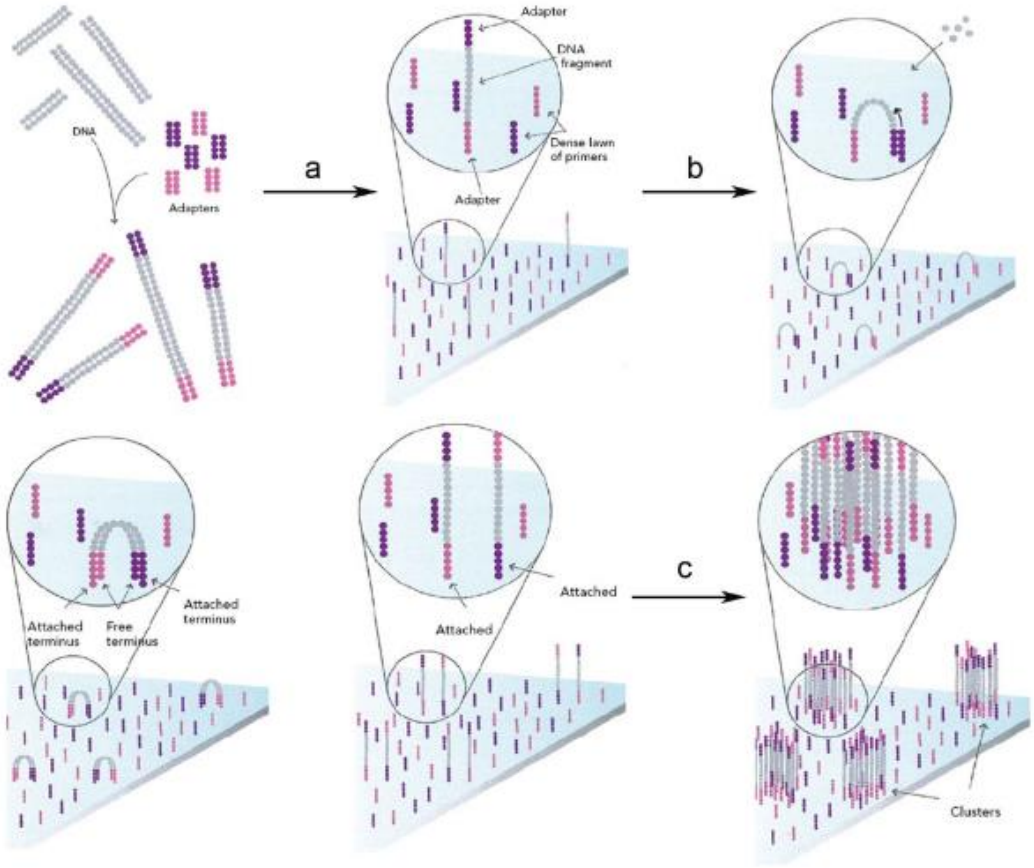


Figure 2.11 Bridge amplification. (a) single stranded DNA randomly attached to adaptor on a flow cell channel (b) bridge amplification to double stranded DNA (c) double stranded DNA denatures to single stranded DNA (Wilantho et al., 2012).

Sequencing DNA: After the bridge amplification process, a lot of single stranded DNA is attached on a flow cell. Each nucleotide base on single stranded DNA will be analyzed one by one from top to bottom of the flow cell by adding DNA polymerase enzyme and fluorescent-nucleotide in the cell. In each sequencing base, fluorescent attached on DNA will show specific color for each base by stimulating with laser light. The result will be recorded on computer (Figure 2.12b.). After that, fluorescent dye and terminator will be washed out of the flow cell. New DNA

polymerase enzyme and fluorescent-nucleotide will be added again to sequence the next base of DNA. The process of sequencing DNA can be seen in Figure 2.12a.

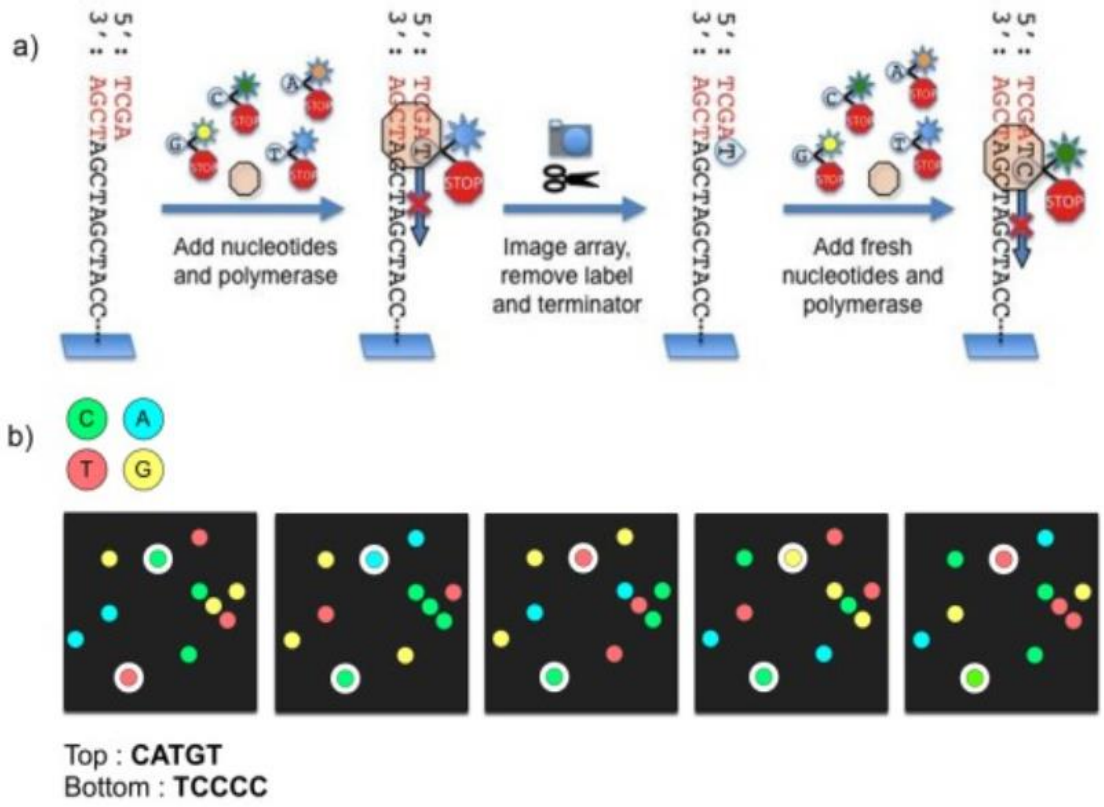


Figure 2. 12 Sequencing of DNA in single stranded DNA (a) illumina sequencing (b) sequencing cycle for analysis (Wilantho et al., 2012).

2.9 Literature review

Nowadays, the studies of various topics and technologies regarding renewable energy are increasingly conducted. Microbial fuel cell is one of the promising technologies for energy recovery from wastewater. Many researchers tend to apply MFC to treat many types of wastewater such as wastewater containing sulfate (Rabaey et al., 2005), wastewater contaminated with sulfide (Eaktasang et al., 2013; Rabaey et al., 2006; Sun et al., 2010), sulfide and nitrate wastewater (Cai and Zheng, 2013; Cai et al., 2013), and organic wastewater (Ahn and Logan, 2010; Chae et al., 2010). Treatment of sulfate and sulfide contaminated wastewater using MFC is now attentively interesting, as it is an alternative to recover energy from this type of wastewater, which

could substitute low quality biogas due to sulfide contamination if conventional anaerobic treatment is used.

Rabaey et al. (2006) investigated the electricity production from the sulfide oxidation process in a double-chamber MFC using hexacyanoferrate as an electron acceptor in a cathodic chamber. Wastewater containing only sulfide (0.1 g/l) was used as an electron donor in a batch mode. The results showed that double-chamber MFC could remove all sulfide from wastewater after 1 day of operation and generate maximum current of 11 mA, which corresponded to 37 mW/L NAC (the anode internal volume of 320 ml). This study also investigated simultaneous COD and sulfide removal with electricity generation in a tubular microbial fuel cell. The results showed that MFC had COD and sulfide removal efficiencies of 46% and 98%, respectively. Power generation in tubular MFC of 101 mW/l NAC (210 ml) was observed. This study has initiated the uses of MFC for the treatment of sulfide wastewater.

Sulfide oxidation in MFCs can spontaneously occur via abiotic sulfide oxidation and/or microbially mediated sulfide oxidation at the anode (Dutta et al., 2008; Sun et al., 2009; Sun et al., 2010; Zhao et al., 2008). Sun et al. (2010) set an experiment to compare power generation between an abiotic reactor and a biotic reactor. They found that sulfide oxidation through microbial catalysis in an MFC could produce a higher and more persistent current density than an abiotic reactor, in which only abiotic sulfide oxidation occurred at the anode.

Zhao et al. (2008) have explored suitable materials for an anode electrode in a single chamber microbial fuel cell in a batch mode with substrate recirculation. Three different electrodes were used in the experiment including activated carbon cloth, graphite foil, and carbon fiber veil. The results showed that the activated carbon cloth was the most effective material for sulfide oxidation in MFC, compared with graphite

foil (GF) and carbon fiber veil (CFV). The current density of activated carbon cloth was higher than the CFV with a factor of 7.6 and higher than GF with a factor of 20. Sulfide could be removed from wastewater by the oxidation process to elemental sulfur and polysulfide species (S_n^{2-}, n=2-5) at the anode. In addition, sulfide and polysulfide could also be converted back to sulfate under the low level of anode potential (Zhao et al., 2009). Therefore, the anode potential was one of the important factors which control such products in MFCs. Then, another experiment was carried out by adding sulfide to the MFC using the same electrode. Regarding the results of the study, the current density of MFC was lower than the previous experiment probably because sulfur content on the anode electrode could block the electrons transferring to anode.

Eaktasang et al. (2013) indicated that the double chamber MFC could also remove hydrogen sulfide in domestic wastewater in the batch mode of operation. The electricity production pattern had two increasing steps which had maximum current density of 118.6 ± 7.2 mA/m^2 and 176.8 ± 9.4 mA/m^2 for the first and the second maximum current density, respectively. The redox potential and sulfate levels in anode were observed for an explicit understanding of the results. In the first 80 hours, the redox potential near the anode was in the high range (-30 to -50) with a slightly decrease of sulfate level. Hence, the study concluded that electrical generation in this step did not occur from sulfide oxidation. However, after that both redox potential and sulfate level decreased rapidly, suggesting that SRB plays a major role in this step. Another experiment was set up to prove that the sulfide in wastewater could be removed in MFC by comparing between open-circuit and close-circuit of MFC. The results showed that sulfide was accumulated in an open-circuit MFC over time. On the other hand, the sulfide in a close-circuit MFC was rather constant. The results confirmed that the MFC can remove sulfide in wastewater by the oxidation process.

Microbial community is also the important factor in MFC operation. Sun et al. (2010) reported that in the treatment of organic wastewater containing high sulfide using MFCs, two communities of bacteria, anode-attached and planktonic communities, were found. The 16S rRNA clone library analysis was used to analyze microbial communities in the MFC. Sulfate-reducing bacteria, sulfur-oxidizing bacteria, and exoelectrogenic microorganisms were observed in this study. Sulfate-reducing bacteria were found in solution as suspended solids. Most of sulfur-oxidizing bacteria lived on the anode surface. Exoelectrogenic microorganisms existed on both anode surface and solution. According to their study, *Pseudomonas spp.* predominated on the anode surface while *Comamonus spp.* predominated in the solution in a sulfide-fed MFC. Sulfur-oxidizing bacteria, sulfate-reducing bacteria, and exoelectrogenic bacteria appeared to work together to treat wastewater and generate electricity in MFCs (Pant et al., 2012).

Moreover, the predominant group of microorganisms in MFCs could depend on COD:SO_4^{2-} ratio. At COD:SO_4^{2-} ratio less than 1.3, SRB are likely to outcompete methanogens. However, at COD:SO_4^{2-} ratio greater than 2.0, methanogens are likely to outcompete SRB (Chou et al., 2008). This competition was found in anaerobic filters which had organic loading of 2.5 kg COD/m^3.d. SRB have specific substrate utilization rate of 0.19-0.24 mg acetate/mg.VSS.d, which is lower than methanogens (0.31–0.59 mg acetate/ mg.VSS.d). Similarly, Zhang et al. (2013b), reported that the more COD in wastewater the less sulfate reduction in MFC.

However, Hu et al. (2015) studied about the effect of influent COD:SO_4^{2-} ratio on UASB treatment of a synthetic sulfate-containing wastewater by using acetate and ethanol as electron donors at the average concentration of 3,000 mgCOD/L. They indicated that methanogens could still active at COD:SO_4^{2-} of 0.5 (sulfate 6,000 mgSO_4^{2-}/L), which was inconsistent with previous studies (Choi and Rim, 1991; Chou et al.,

2008). The results showed that methanogens including acetate-utilizing and hydrogen-utilizing methanogens were still dominant species under this condition. Since, only 1,000 mgSO_4^{2-} was removed in this study, sulfide inhibition was not observed. Therefore, the competition between SRB and methanogens should not depend on only COD:SO_4^{2-} ratio. It should have several factors.

Sulfate concentration in the wastewater might be one factor affecting on microbial activities and microbial communities. Wu et al. (2015) reported that both COD and sulfate removal efficiencies of expended granular sludge bed (EGSB) decreased from 65% and 95% for COD and sulfate at sulfate concentration of 2,000 mgSO_4^{2-}/L, respectively, to 59% and 65% for COD and sulfate at sulfate concentration of 3,000 mgSO_4^{2-}/L, respectively. They also found that the increase in sulfate concentration from 2,000 mgSO_4^{2-}/L to 3,000 mgSO_4^{2-}/L could decrease the percentage of SRB in the system from 15.3 to 12.7%.

The MFC removal efficiency and MFC columbic efficiency depend on many factors, such as hydraulic retention time (HRT), temperature, materials of MFC, and COD:SO_4^{2-}. Ghangrekar et al. (2010) reported that at COD:SO_4^{2-} of 0.8 the highest removal efficiency and the highest power generation were observed in the batch mode of double chamber MFC using sucrose (445 mg/l) as organic substrate and sulfate varying from 0 to 4,280 mg/l. The ratio of COD:SO_4^{2-} in this experiment consisted of six ratio including 500, 20, 1, 0.8, 0.5, and 0.3. In this experiment, elemental sulfur was converted back to sulfide probably by sulfur-reducing bacteria and methanogens. Then, sulfide could also be oxidized to sulfate under MFC operation.

In addition, Zhang et al. (2013b) found various effects of COD:S^{2-} ratio on sulfide oxidation in microbial fuel cells in a range of 4.85 to 18.53. The results showed that COD removal increased when COD increased. However, the power generation did not

differ within the range of COD:S^{2-} between 4.85/1 and 18.53/1. The maximum power density observed in this experiment was 14.5 W/m^3 V_{anode}, and the internal resistance of 36.5 ohm was observed.

Most previous research focused on sulfide wastewater treatment using microbial fuel cells in the batch mode of operation (Dutta et al., 2008; Eaktasang et al., 2013; Ghangrekar et al., 2010; Rabaey et al., 2006; Sun et al., 2009; Sun et al., 2010; Zhang and Ni, 2010; Zhang et al., 2009; Zhang et al., 2013b; Zhao et al., 2009). Only few studies focused on sulfate wastewater treatment using MFC under continuous mode of operation (Rabaey et al., 2005; Rabaey et al., 2006; Zhao et al., 2008). In order to apply MFCs in real wastewater treatment systems, which usually have large amount of wastewater, continuous operation of MFCs should be further investigated and developed. Energy obtained from MFC was considered to be clean; however, several factors are still needed to be investigated to enhance MFC efficiencies.

COD:SO_4^{2-} ratio is one of the important factors that could affect treatment efficiencies and electricity generation. Until now, there was only one previous study investigating the effects of COD:SO_4^{2-} on the performance of MFC under continuous operation (Zhang et al., 2012). The study by Zhang et al. (2012) used UASB-MFC integrated system to investigated the effects of COD:SO_4^{2-} ratio and hydraulic retention time on sulfate removal and electricity generation using the response surface methodology. The results showed that the highest total sulfate removal efficiency was observed at the COD:SO_4^{2-} of 3.7 and at the hydraulic retention time of 55.6 hr. Maximum power output was achieved at the COD:SO_4^{2-} of 2.3 and at the hydraulic retention time of 54.3 hr. However, microbial communities in this system were not well characterized. In addition, only short term operation (10 d) was conducted at each sulfate concentration, which may not reflect the differences in microbial communities at different COD:SO_4^{2-} ratio during long term operation.

Understanding the mechanisms of electricity generation and treatment in MFCs at different COD:SO_4^{2-} ratio will assist us in the improvement of the MFC systems. Therefore, this study aims to investigate the performances of two-compartment single-chamber MFCs at different COD:SO_4^{2-} ratio. Treatment and electricity generation mechanisms of the MFCs as well as microbial communities at different COD:SO_4^{2-} ratio will also be investigated.

2.10 Expected mechanisms in two-compartment single-chamber microbial fuel cell treating sulfate-rich wastewater

Sulfate-rich wastewater had been interested in a decade. The microbial communities treating sulfate-rich wastewater have been studied (Hu et al., 2015; Wu et al., 2015). There are three groups of mechanisms growing on this type of wastewater consisting of 1) fermentative bacteria, 2) SRB, and 3) methanogens. Figure 2.13 shows the co-existence of microorganisms in anaerobic bioreactors treating sulfate-rich wastewater using glucose as initial substrate. Firstly, glucose was fermented by fermentative bacteria to volatile fatty acids (VFAs) and acetate in the first compartment of MFC. Then, both VFAs and acetate were oxidized to CO_2 (or acetate when used other VFAs except acetate) via SRB by using sulfate as the electron acceptor. Sulfide is a final product of sulfate reduction process. On the other hand, methanogens can use acetate as an electron donor and use CO_2 as an electron acceptor to produce methane as a final product. There are some groups of methanogens such as Methanobacterium (Hu et al., 2015) that can produce methane from hydrogen.

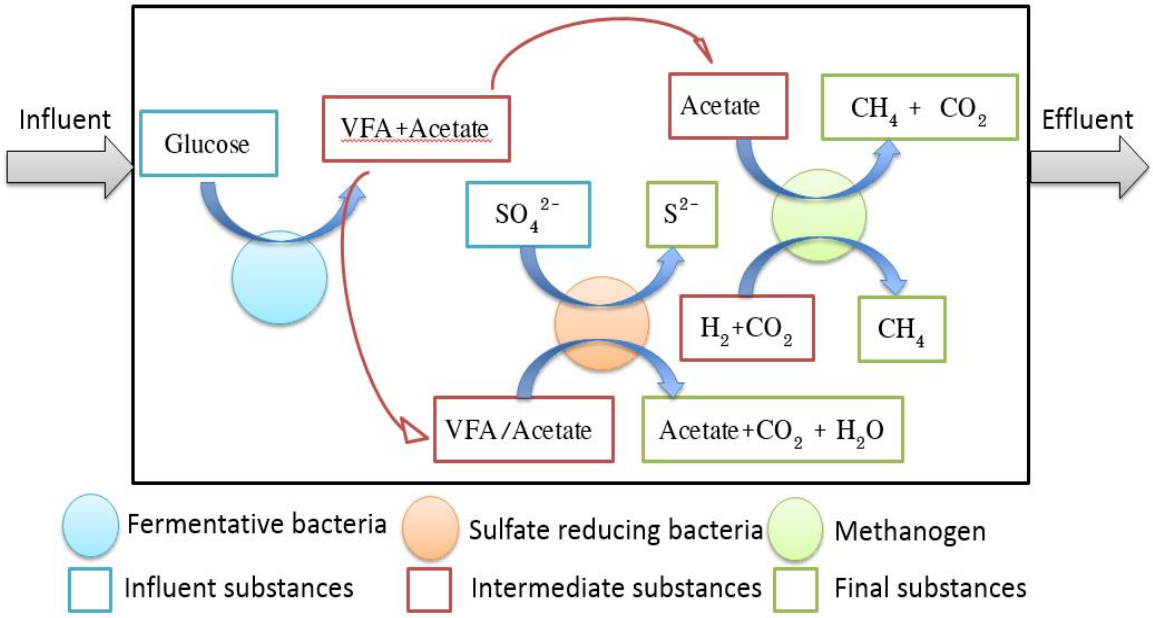


Figure 2.13 Expected mechanisms in the first compartment of MFC

In the second compartment of MFC, the remaining substrates in the first compartment consisting of glucose, VFAs, acetate, sulfate, sulfide, methane, CO_2, and hydrogen were used as the initial substrates in the second compartment. Microorganisms growing in the suspended solids and on anode electrodes as biofilms could remove both COD and sulfate remaining from the first compartment with the same mechanisms as in the first compartment. For electrical generation, there are two main pathways consisting of 1) abiotic electricity generation and 2) biotic electricity generation (Rabaey et al., 2006) to generate electricity in MFCs treating sulfide-rich wastewater. The possible mechanisms are shown in Figure 2.14

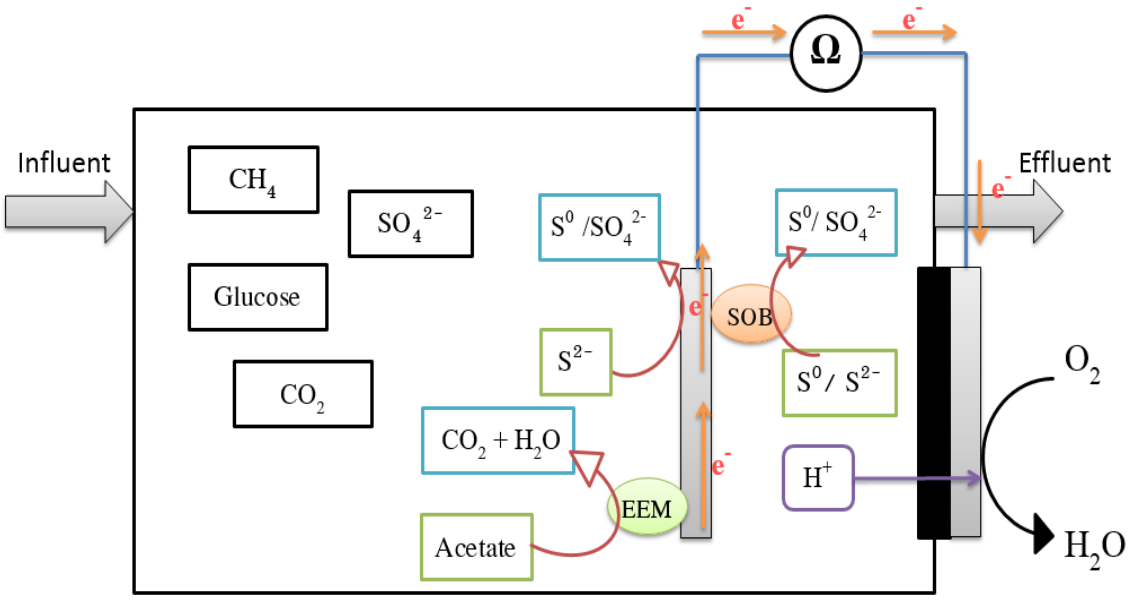


Figure 2.14 Possible mechanisms for electricity generation in the second compartment

There are four possible reactions on electricity generation on the anode electrodes which consist of 1) microbial sulfide oxidation via SOB, 2) microbial sulfur oxidation via SOB, 3) abiotic sulfide oxidation on anode electrodes and 4) microbial oxidation via exoelectrogenic microorganisms. On the other hand, the reaction on the cathode electrode is the reduction process of oxygen which generates H_2O as a final product. Table 2.8 shows the electron transport of mechanisms in MFC.

Table 2.8 Electron transport in MFC

Reaction	e^- donor	e^- acceptor
Fermentation	Organic	Organic
Methanogenesis	Organic	CO_2
Microbial sulfate reduction	Organic	SO_4^{2-}
Microbial sulfide oxidation	S^{2-}/S^0	Anode electrodes
Abiotic sulfide oxidation	S^{2-}	Anode electrodes
Microbial organic oxidation at anode electrodes	Organic	Anode electrodes

Chapter 3
Methodology

3.1 Experimental framework

This research is divided into four experiments, including 1) anaerobic bioreactor start-up, 2) MFC operation, 3) abiotic fuel cell operation, and 4) microbial community analysis. In the first experiment, the objective is to select the groups of microorganisms that are suitable for synthetic wastewater at different COD:SO_4^{2-} ratio. For the second experiment, MFCs were operated in order to generate the electricity by using the products from the first compartment of MFCs. In this part, the voltages and power densities were observed whereas the mechanisms in MFCs were analyzed. In the third experiment, abiotic fuel cells fed with sulfide were operated to investigate the role of abiotic sulfide oxidation in electricity generation in MFCs. In the fourth experiment of this study, microbial communities were analyzed by 16S rRNA gene amplicon sequencing (MiSeq sequencing system, Illumina). For all experiments, MFCs and reactors were operated at room temperature, approximately 30 – 35°C. The experimental framework can be seen in Figure 3.1

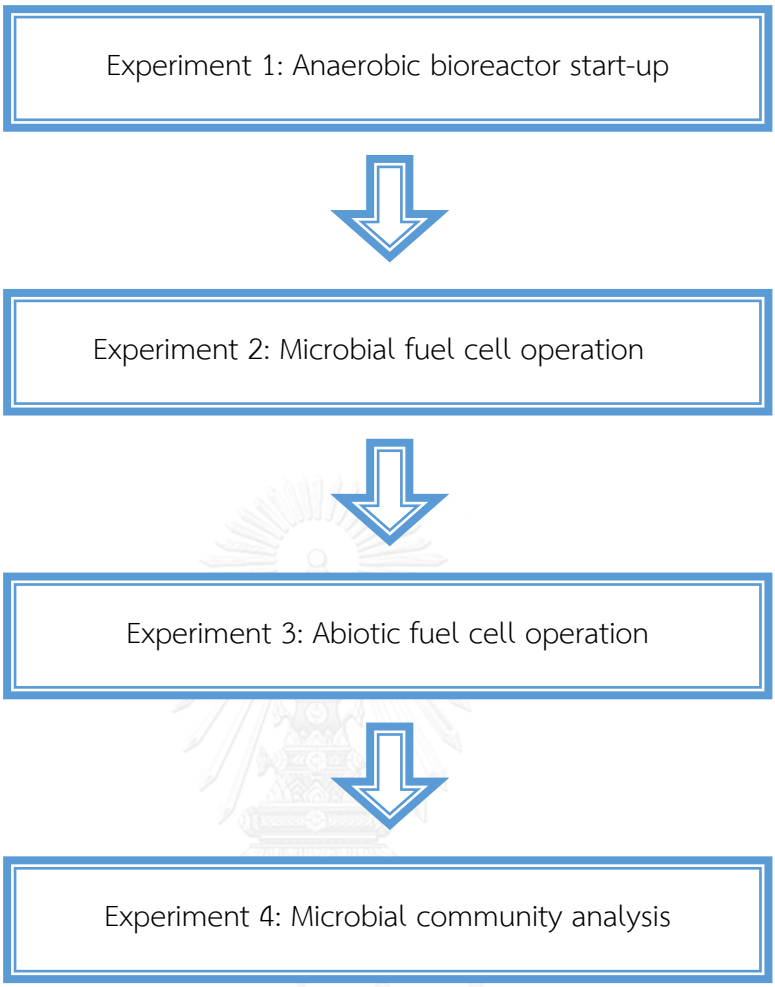


Figure 3.1 Overall experimental framework

3.2 Experiment 1: Anaerobic bioreactor start-up

The objective of this experiment was to enrich microorganisms that were suitable for the treatment with different COD:SO_4^{2-} ratio. This experiment was divided into 2 parts with different types of reactors: 1) 5 liter-plastic bottles and 2) the first compartments of MFCs. In Experiment 1-1, plastic bottles which were called as Reactor1, Reactor2, and Reactor4, were used for the enrichment of microorganisms at the COD:SO_4^{2-} ratio of 1, 2, and 4. Because there was no significant difference in both COD and sulfate removal in both Reactor2 and Reactor4. Therefore, COD:SO_4^{2-} ratio in Reactor2 and Reactor4 had been changed to 3 and 6, respectively, being the same range of predominance groups with the previous value in Experiment 1-2. Then, the sludge from Reactor1, Reactor2, and Reactor4, was transferred to the first compartments of MFC1, MFC3, and MFC6, which were operated at the COD:SO_4^{2-} ratio of 1, 3, and 6, respectively.

3.2.1 Experiment 1-1: Anaerobic bioreactor start-up in Reactor1, Reactor2, Reactor4

Reactor configuration

Three 5 liter-plastic bottles were used as anaerobic bioreactors to enrich microorganisms at different COD:SO_4^{2-} ratio. Figure 3.2 shows the schematic diagram of the reactor used in Experiment 1-1. The influent inlet and effluent outlet were pierced through at the top of the bottle in which the level of influent inlet was higher than effluent outlet. A hole of 0.1 mm diameter was drilled to release the gas produced from the system.

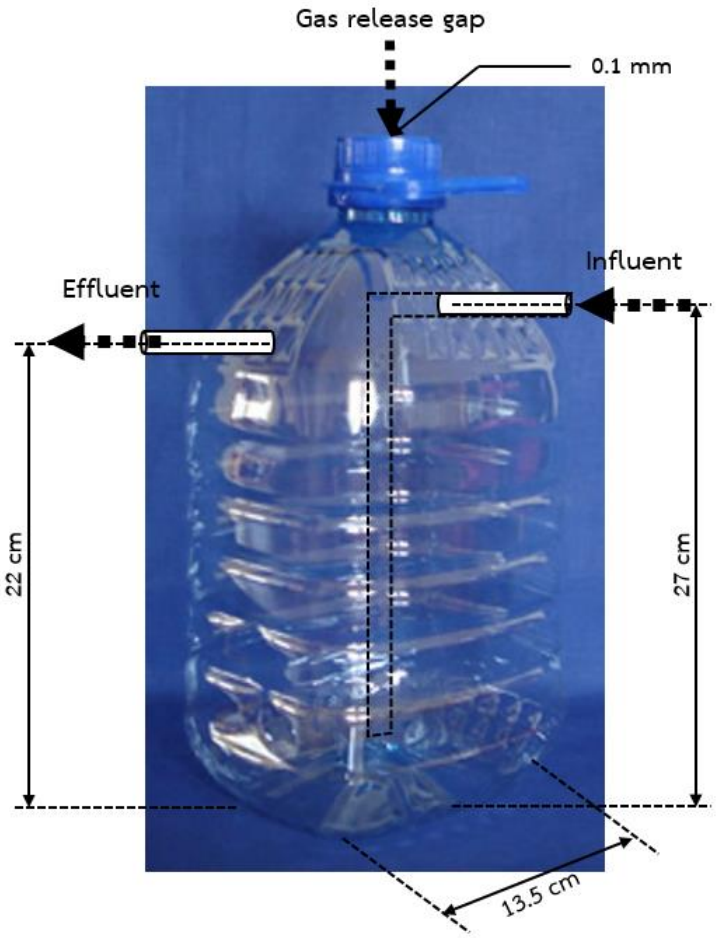


Figure 3.2 Reactor configuration in Experiment 1-1

Reactor operation

Figure 3.3 shows the framework of Experiment 1-1. Sludge from an anaerobic treatment process (Thai Quality Starch Co.,Ltd., Karnjanaburi, Thailand) was used as initial sludge at 30 % v/v of the reactor. Synthetic wastewater with three different values of COD:SO_4^{2-} ratio, consisting of 1, 2, and 4, was fed into Reactor1, Reactor2, and Reactor4, respectively. Each ratio was selected as a representative for each situation of competition between SRB and methanogens as shown in Table 3.1. The COD concentrations of synthetic wastewater continuously fed into all reactors were approximately 3,000 mgCOD/L. Reactor1 was operated for 30 days whereas Reactor2 and Reactor4 were operated for 40 days. Since the operation of Reactor1 began 10

days after the operation of Reactor2 and Reactor4, the operation time of Reactor1 was 10 days shorter than that in the other reactors.

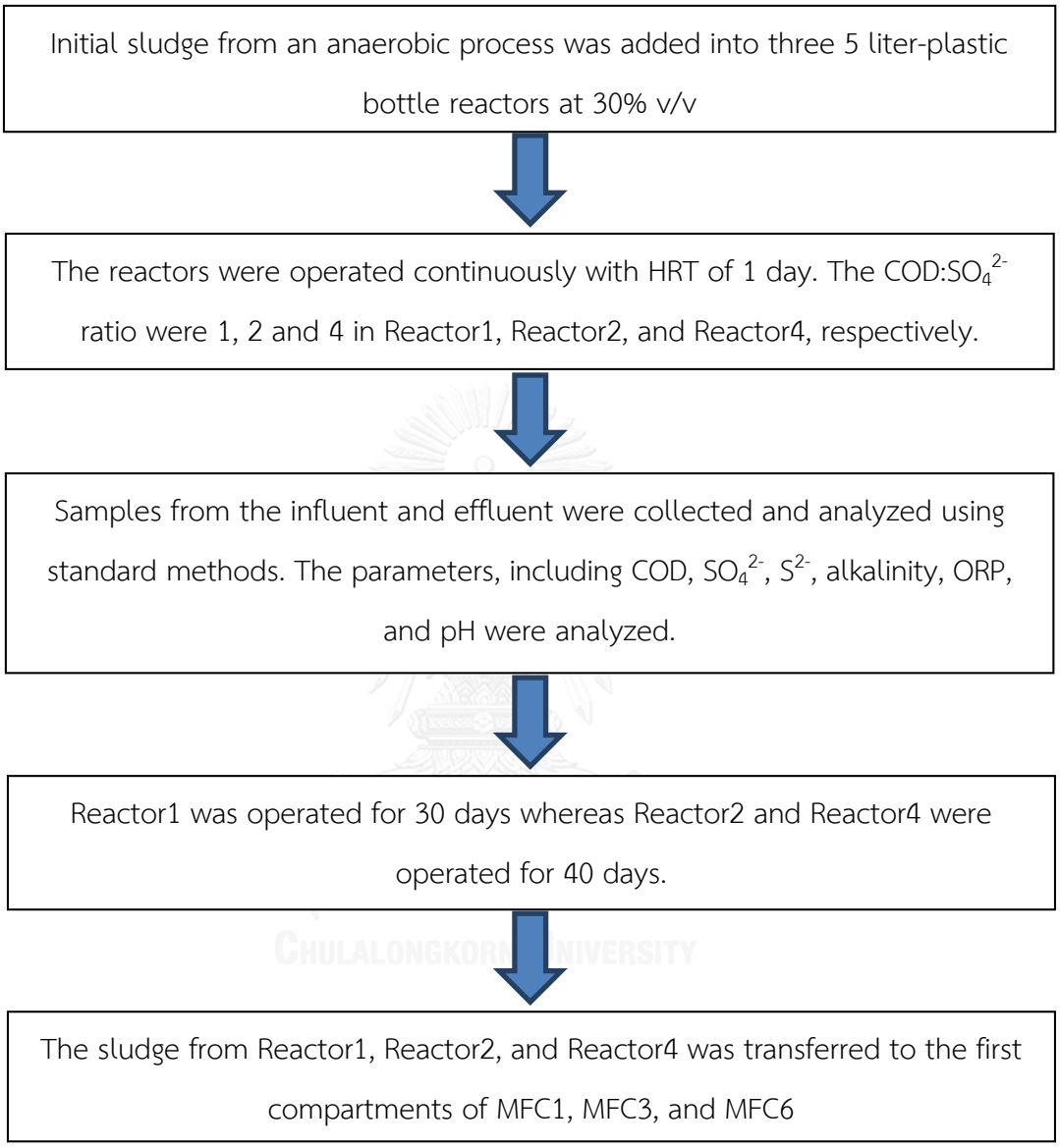


Figure 3.3 Framework of Experiment 1-1

Table 3.1 COD:SO_4^{2-} ratio of each reactor.

Reactor	**COD:SO_4^{2-} ratio**	**Reasons**
Reactor1	1	- Sulfate-reducing bacteria are predominant in the system **(SRB > methanogens)**
Reactor2	2	- Sulfate-reducing bacteria and methanogens can grow together **(SRB VS methanogens)**
Reactor4	4	- Methanogens are predominant in the system **(SRB < methanogens)**

Glucose (1g Glucose = 1.067 gCOD) was used as a substitute for COD in the synthetic wastewater and sodium sulfate (Na_2SO_4) was used as sulfate contaminated in wastewater. The concentrations of COD in the synthetic wastewater were approximately at 3,000 mgCOD/L (2,812 mgGlucose/L). Nevertheless, in each MFC, the concentrations of sulfate were varied by the COD:SO_4^{2-} ratio of 1, 2, and 4, which corresponded to the sulfate concentrations of 3,000 mg/l, 1,500 mg/l, and 750 mg/l, respectively. For the COD:SO_4^{2-} ratio of 1, the COD:SO_4^{2-} of 4 was set as an initial ratio to inoculate SRB growing in the system. Then, the ratio was decreased to 2, 1.67, and 1.428 on day 9, day 17, and day 26 of operation, respectively. On the other hand, sodium bicarbonate (2,000 mg/l) were used as a pH buffer in the system. Macronutrients and micronutrients were added in the synthetic wastewater according to Rittmann and McCarty (2001) as shown in Table (3.2).

The synthetic wastewater was fed into the MFCs using peristaltic pumps (model 505U of ATSAN MARLOW brand) at the influent flow rate of 0.167 L/hr, which corresponded to the HRT of 24 hours (1 day). The synthetic wastewater was prepared every 2 days to avoid the decrease in COD concentration before feeding into the MFCs.

The samples from the influent were collected for COD and sulfate analysis whereas the samples from the effluent outlet were collected for COD, sulfate, sulfide, alkalinity, ORP, and pH measurement. Gas production of each reactor was released from the system via a small hole at the bottle cap. The methods of parameter analysis and the sampling frequencies are shown in Table (3.3). In addition, the sludge was temporarily transferred from Reactor4 to the first compartment of an MFC on day 32 of operation. After that, the sludge was moved back to Reactor4 after day 40 of operation. Moreover, Reactor2 was changed from a continuous mode to a batch mode of operation for 5 days to investigate the effects of increasing HRT on COD and sulfate removal efficiencies. After Reactor1 was operated for 30 days whereas Reactor2 and Reactor4 were operated for 40 days, the sludge from Reactor1, Reactor2, and Reactor4 was transferred to the first compartments of MFC1, MFC3, and MFC6, respectively.

Table 3.2 Composition of synthetic wastewater(Rittmann and McCarty, 2001).

Element	Molecular weight (g)	Concentration (mg/L)	Substance Compound	Molecular weight (g)	Concentration (mg/L)
Electron donor					
Glucose	-	-	$C_6H_{12}O_6$	180	3,000
Electron accepter					
Sulfate in Exp 1-1	-	-	Na_2SO_4	142	3,000, 1,500, 750
Sulfate in other Exp					3,000, 1,000, 500
Macronutrients					
Nitrogen	14	58	NH_4Cl	53.5	221.6
Phosphorus	30	11.32	$NaH_2PO_4.2H_2O$	156	58.9
Sulfur	32	6.6	$MgSO_4.7H_2O$	246.5	50.8
Micronutrients					
Iron	56	10.024	$FeCl_2.4H_2O$	199	35.6
Cobalt	59	0.0224	$CoCl_2.6H_2O$	207	0.1
Nikel	57	0.0232	$NiCl_2.6H_2O$	237.7	0.1
Zinc	65	0.036	$ZnCl_2$	136.3	0.1
Copper	64	0.0232	$CuCl_2.2H2O$	170.5	0.1
Maganese	55	0.0232	$MnCl_2.4H_2O$	198	0.1
Boron	11	0.0232	H_3BO_4	77.8	0.2
Common cations					
Sodium	23	150	NaCl	58.5	381.5
Potassium	39	300	KCl	74.6	573.8

Table 3.2 Composition of synthetic wastewater(Rittmann and McCarty, 2001). (Cont.)

Element	Molecular weight (g)	Concentration (mg/L)	Substance Compound	Molecular weight (g)	Concentration (mg/L)
Calcium	40	150	$CaCl_2$	111	416.3
Magnesium	24	160	$MgCl_2$	95	633.3
pH Buffer					
Sodium Bicarbonate	-	-	$NaHCO_3$	84	2,000

Table 3.3 Methods of parameter analysis and frequencies of wastewater sampling in Experiment 1-1, Experiment 1-2 and Experiment 2.

Parameter	Standard method	Frequency	Experiment	Sampling point
COD	Standard method 5220 C. / 1999 Close reflux method	Three times a week	1-1, 1-2, & 2	Influent Effluent 1 Effluent 2
Sulfate	Standard method 4500-SO_4^{2-} E. / 1999 Turbidimetric method	Three times a week	1-1, 1-2, & 2	Influent Effluent 1 Effluent 2
Sulfide	Standard method 4500-S^{2-} G. / 1999 Sulfide ion selective electrode	Three times a week	1-2 & 2	Effluent 1 Effluent 2
Suspended solid	Standard method 2540D. / 1999.	Before and after operation	1-1, 1-2, & 2	Solution in compartment 1 & 2
pH	Standard method 4500-H^+ B. / 1999 pH meter	Every two day	1-1, 1-2, & 2	Solution in compartment 1 & 2
ORP	Standard method 2580 / 1999 ORP meter	Three times a week	1-1	Solution in Effluent
Alkalinity	Standard method 2320 B. / 1999 Titration method	Initial of the Experiment	1-1 & 1-2	Effluent 1
Voltage	Multimeter	Every two day	2	Across the electrodes
Current	Calculate from Equation	Every two day	2	-
External resistance	Multimeter	Two times a week	2	-
VFA	Standard method 5560 B. / 1999 Titration method	Initial of the Experiment	1-1 & 1-2	Effluent 1

3.2.2 Experiment 1-2: Anaerobic bioreactor start-up in the first compartments of MFCs

MFC configuration

A rectangular chamber was used as a microbial fuel cell, which was made from acrylic to prevent any unexpected reactions with the reactor material. The MFC was divided into two compartments by multiple baffles to separate the reactions in the first compartment and the second compartment. The first compartment had the total volume of 2,970 mL with the dimension of 10 cm x 13.5 cm x 22 cm while the working volume of this compartment is 2,025 mL with the dimension of 10 cm x 13.5 cm x 15 cm. On the other hand, the second compartment had the working volume of 630 mL with the dimension of 10 cm x 7 cm x 9 cm. In the second compartment, 5 cm x 5 cm proton exchange membrane and cathode assembly was installed on the same side with the outlet. The MFC had six valves for controlling the inlet, outlet, water sampling points, and sludge sampling points as shown in Figure 3.4. On the top of MFC, there were four opening slots for releasing gas production (3 opening slots) and inserting a titanium wire (1 opening slot closing with a rubber stopper). Figure (3.4) and (3.5) show the configuration of the MFCs.

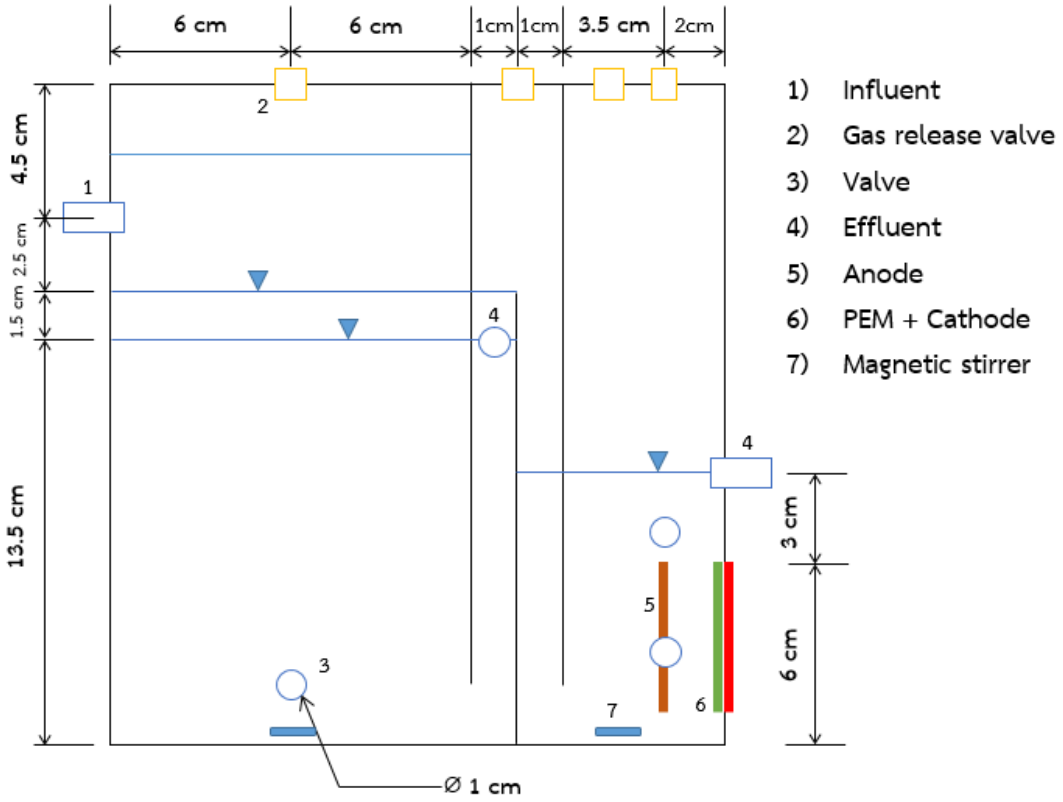


Figure 3.4: Front view of the MFC

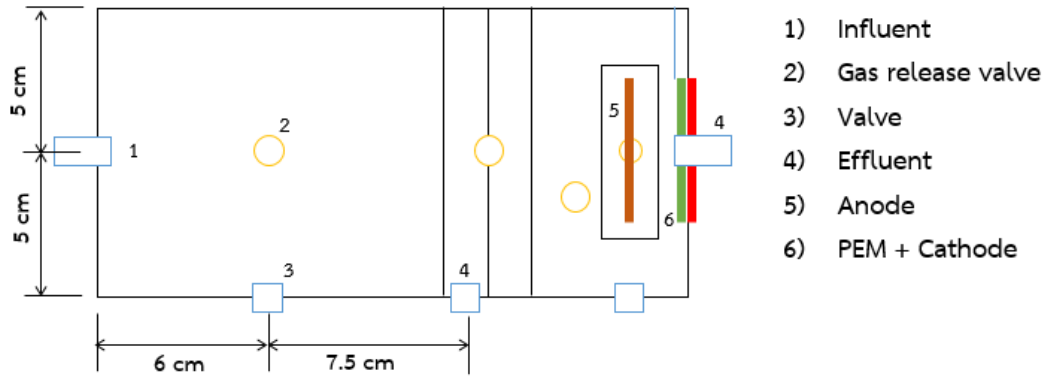


Figure 3.5 Top view of the MFC

A piece of 5 cm x 5 cm rectangular activated carbon cloth (Figure 3.6a), the highest power generation when compared with graphite foil and carbon fiber veil (Zhao et al., 2008), was used as an anode electrode that connected with the cathode

electrode through an external resistor (Figure 3.6d) with 1 mm-diameter of titanium wire (Figure 3.6c). The external resistances were set to 1,000 ohms when the MFC was operating in Experiment 2. A piece of 5 x 5 cm^2 of 30% PTFE wet-proof carbon cloth (Pt loading: 0.5mgPt/cm^2) was used as the cathode electrode to improve power densities of the single chamber air breathing microbial fuel cell (CHENG et al., 2006). The distances between the anode electrode and the cathode electrode were 2 cm. Nafion117 was used as a proton exchange membrane (PEM). The cathode and the PEM were assembled by hot-pressing (Figure 3.6b). Silver mesh was attached to the cathode to help collect the electrical current. The locations of the anode electrode, cathode electrode and PEM in the MFC system are shown in Figure (3.4) and Figure (3.5).

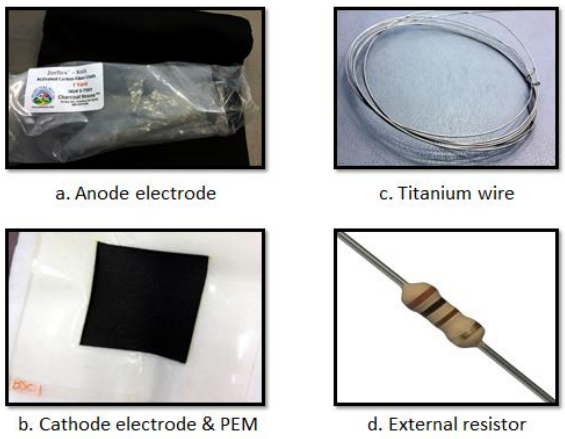


Figure 3.6 Electrical equipment in MFCs a) anode electrode, b) cathode electrode and PEM, c) titanium wire, and d) external resistance

Operation of the first compartments of MFCs

Figure 3.7 shows the framework of Experiment 1-2. MLSS concentration of the sludge in each reactor in Experiment 1-1 was measured in order to equally transfer the sludge into the first compartment of MFC. The sludge from Reactor1, Reactor2, and Reactor4 was transferred into the first compartments of MFC1, MFC3, and MFC6, respectively, to obtain the MLSS concentration of 8,000 mgMLSS/L. The synthetic wastewater was fed into the MFCs using peristaltic pumps at the influent flow rate of 0.084 L/hr, which corresponded to the HRT of 24 hr. Magnetic stirrers were used to mix

wastewater and suspended sludge in the first compartments. However, the mixing was canceled after 8 days because the suspended sludge was lost from the reactors. The points of wastewater sampling chosen to be analyzed in this experiment consisted of the influent inlet and the effluent outlet in the first compartment of the MFC. The methods of parameter analysis and the sampling frequencies were similar to those in Experiment 1-1 as summarized in Table 3.3.

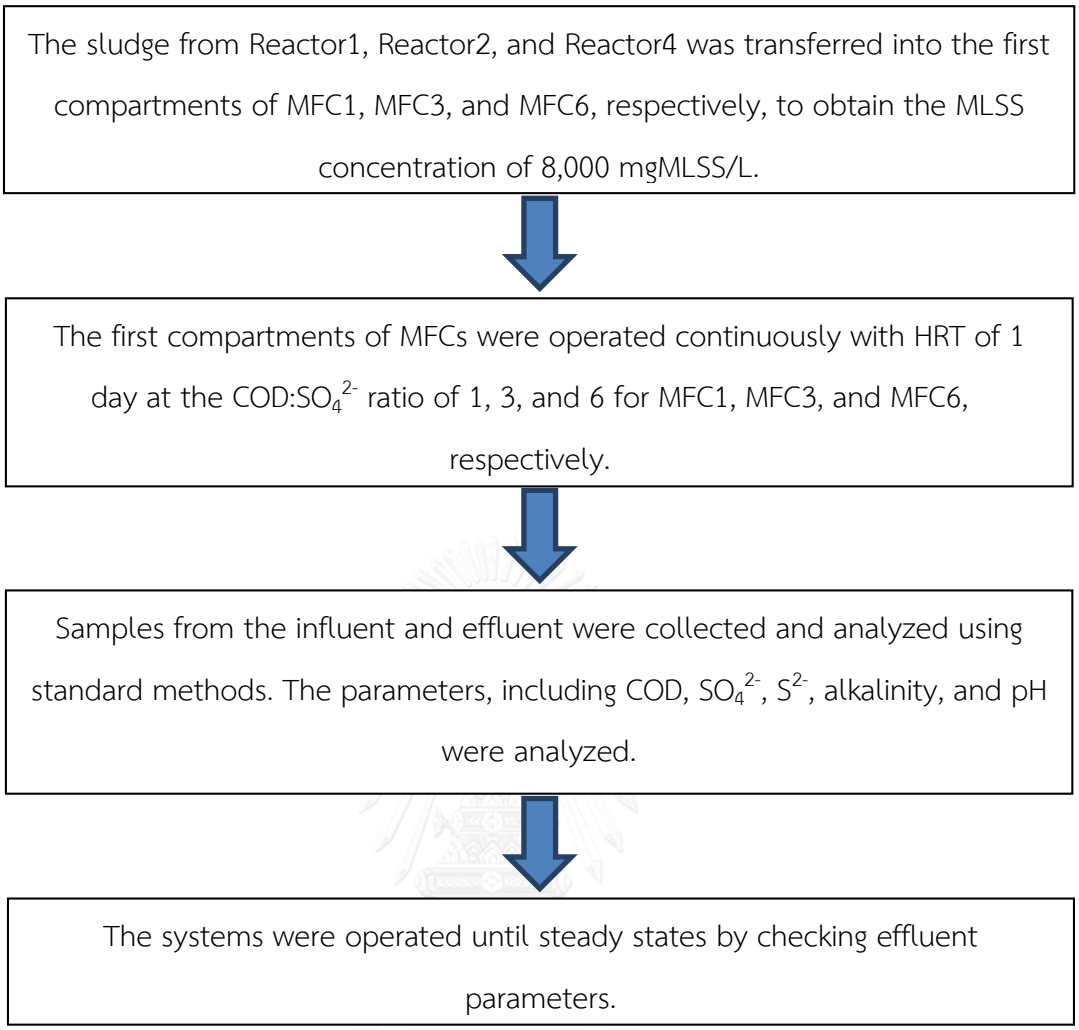


Figure 3.7 Framework of Experiment 1-2

Table 3.4 COD:SO_4^{2-} ratio of each reactor.

Reactor	**COD:SO_4^{2-} ratio**	**Reasons**
MFC1	1	- Sulfate-reducing bacteria are predominant in the system (SRB > methanogens)
MFC3	3	- Sulfate-reducing bacteria and methanogens can growth together (SRB VS methanogens)
MFC6	6	- Methanogens are predominant in the system (SRB < methanogens)

According to the results from Experiment 1-1, the COD:SO_4^{2-} ratio were changed from 1, 2, and 4 to 1, 3, and 6, respectively. These COD:SO_4^{2-} ratio were still the representatives of the same situation of competition between sulfate-reducing bacteria and methanogens as shown in Table 3.4. Therefore, sulfate concentrations in Experiment 1-2 were set to 3,000, 1,000, and 500 mgSO_4^{2-}/L for COD:SO_4^{2-} of 1, 3, and 6, respectively.

The MFCs were operated only in the first compartments by opening the effluent outlets in the first compartments to prevent the wastewater from getting into the second compartments. Figure 3.8 shows MFC set up in Experiment 1-2. The first compartments of the MFCs were operated until reaching steady states, which could be observed by analyzing the parameters in the effluent. Alkalinity was increased from 2,000 mg/L as $CaCO_3$ to 3,000 mg/L as $CaCO_3$ to prevent the pH drop in the systems on day 10 of operation. Both alkalinity and volatile fatty acids were analyzed only on day 1 – day 20 of operation to check the stability of the systems.

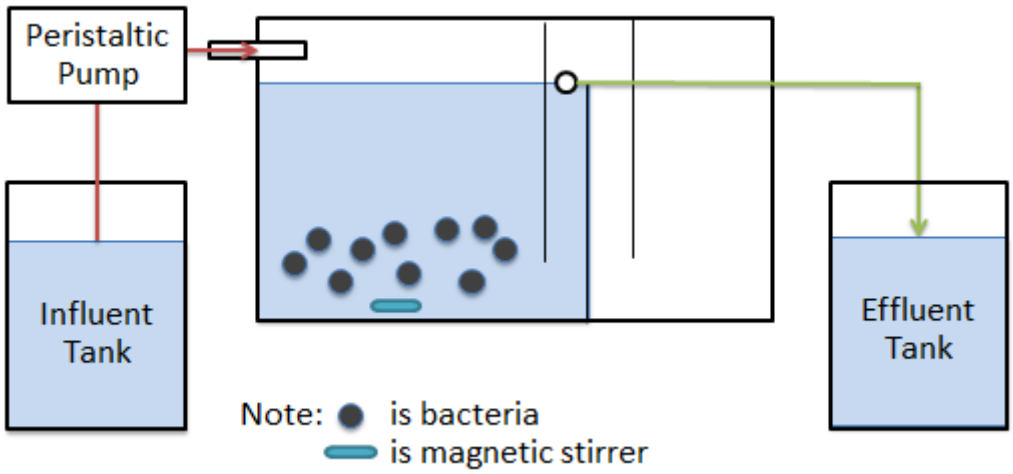


Figure 3.8 MFC set up in Experiment 1-2

3.3 Experiment 2: Microbial fuel cell operation

MFC operation

The framework of Experiment 2 is shown in Figure 3.9. In this experiment, the electrical equipment namely anode, cathode, PEM, wire, and external resistance were installed in the second compartment. The activated carbon cloth were attached to titanium wire and placed in the second compartment via the anode inlet. The anode were installed parallel to the cathode, and the distance between the anode and the cathode was 2 cm. 5 x 7 cm^2 silver mesh were attached to the cathode surfaces to effectively collected the electricity, which could help reduce internal resistance of the MFC. The external resistance was set to 1,000 ohms during MFC operation.

At the end of Experiment 1, the MFC systems were at steady states; therefore, the concentrations of products in the first compartment were also at steady states as well. Then, the effluent outlets at the first compartments were closed. The effluent of the first compartment would flow across the weir to the second compartment directly to generate electricity by using the oxidation processes of the products from

the first compartments. Figure 3.10 shows the MFC set up and operation in Experiment 2. In this experiment, the wastewater sampling points (Figure 3.10) consisted of 1) the influent inlet, 2) the effluent outlet in the first compartment, and 3) the effluent outlet in the second compartment. The results on parameter analysis would provide an understanding on the mechanisms in each compartment of the MFC. Parameters analyzed in this experiment were the same as in Experiment 1 as shown in Table 3.3.

The OCV and voltage across the electrodes of all the MFCs were measured over time using a multimeter (Fluke 115). The current occurred in the MFCs was calculated by the relation of Ohm's law (I=V/R). Current densities were then calculated by dividing by the anode surface area. Then, power density curves were obtained by varying the external resistance and constructing the graphs between the power densities (y axis) versus the current densities (x-axis). The maximum power density of MFC was estimated from the power density curve by polynomial regression. The polarization curves (I-V curves), which are the graphs between the voltages across the electrodes (y-axis) and the current densities (x-axis), were also constructed to estimate the voltage losses, consisting activation losses, ohmic losses, and concentration losses, in the MFCs. Polarization curves and power density curves of each MFC was constructed on day 1, 2, 4, 8, 11, 18, 21, and 24 of operation. The coulombic efficiencies of the MFCs were also calculated based on sulfide removal ($H_2S \rightarrow S_0$) according to Appendix A Table 3.5 shows the equipment for electricity measurement in this experiment.

Then, the microorganisms in the first compartments, the second compartments, and on the anode electrodes in each MFC were collected on day 33 in MFC1 and on day 40 in both MFC3 and MFC6 to analyze microbial communities in Experiment 4.

Installed the electricity equipment in the second compartment including anode, cathode, PEM, titanium wire and external resistor.

Closed the effluent outlet in the first compartment and passed the wastewater to the second compartment. Operated the systems with the same condition as Experiment 1-2.

Monitored and analyzed the wastewater quality from 3 sampling points, including 1) the influent inlet, 2) the effluent outlet in the first compartment, and 3) the effluent outlet in the second, using the standard method as described in Experiment 1.

Measured the OCV and voltage across the electrodes of MFCs and calculated coulombic efficiencies of the systems.

Varied the external resistance from 20 - 200,000 ohm to construct the polarization curves and power density curves on day 1, 2, 4, 8, 11, 18, 21, and 24.

Collected the samples of microorganisms from the first compartment, the second compartment, and the anode electrode to analyze the microbial communities in Experiment 3.

Changed the anode electrodes on day 33 in MFC1 and on day 40 in both MFC3 and MFC6.

Anode electrodes before MFC operation, used anode electrodes after rinsing with deionized water, and anode electrodes after 8 day of operation were analyzed with SEM/EDX

Figure 3.9 Framework of Experiment 2

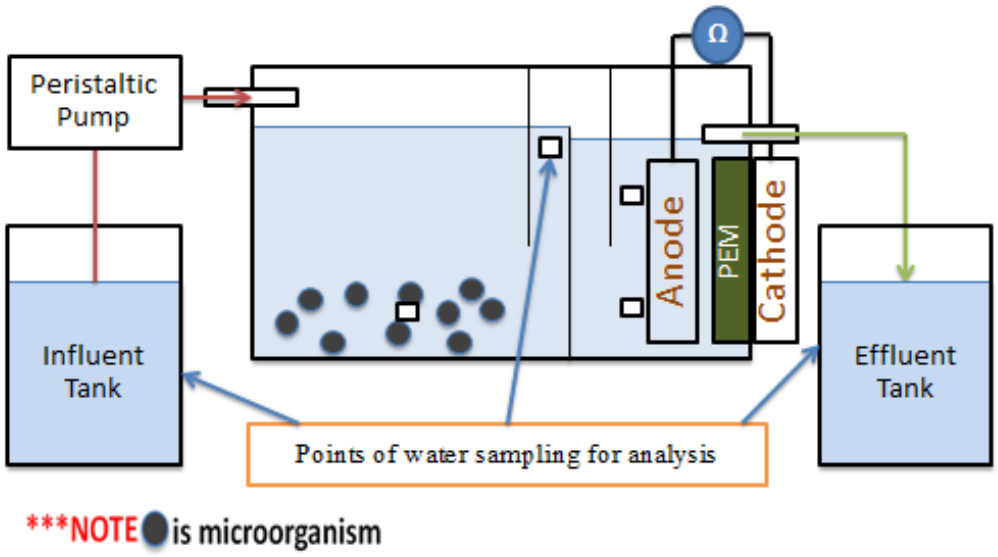


Figure 3.10 MFC set up for Experiment 2.

Table 3.5 Method and equipment for electricity measurement

Parameter	Method and equipment
Voltage (V)	Fluke 115 multimeter (Resolution: 0.1mV; Accuracy: ±0.5%+2)
External resistance (R_{ext})	Fluke 115 multimeter (Accuracy: ±0.9 % + 1)
Current (I)	Calculate with formula of I=V/R
Power density (P_d)	Calculate with formula of $P_d = V*I/A_{anode}$

MFC operation after replacing the anode electrodes

The anode electrodes in all MFCs, (MFC1, MFC3, and MFC6) were replaced by new ones, which were identical to the previous ones on day 33 in MFC1 and on day 40 in both MFC3 and MFC6. The parameters consisting COD, sulfate, sulfide, pH, OCV, and voltage across the electrode were measured every day. The polarization curves, and power densities curves were constructed on day 1, 2, 3, 4, 5, 6, and 8 of operation to estimate the maximum power densities in MFCs. The replacement of anode electrodes could help identify the effects of biofilms, sulfur accumulation, and the deterioration of the anode electrodes in MFCs.

Scanning electron microscopy (SEM)

Surfaces of the following anode electrode samples were analyzed with scanning electron microscopy (SEM) equipped with an energy dispersive X-ray (EDX) (SEM (JSM-6400)
with EDX):

1. An anode electrode before MFC operation
2. Used anode electrodes on day 33 in MFC 1 and on day 40 in MFC3 and MFC6 after rinsing with deionized water
3. New anode electrodes after 8 day of operation.

3.4 Experiment 3: Abiotic fuel cell operation

After microbial fuel cell operation, an abiotic fuel cell fed with sulfide (Figure 3.11) was operated under the same condition with the MFC operation to understand the mechanisms in the MFCs in the absence of microorganisms in the systems. Sulfide-rich wastewater with the concentrations of 99.94 ± 17.31 mgS^{2-}/L on day 1-3, 258.16 ± 45.14 mgS^{2-}/L on day 4-6, and 413.18 ± 20.68 mgS^{2-}/L on day 7-9 was prepared, which were close to the sulfide concentrations in the effluent of the first compartment of MFC6, MFC3, and MFC1, respectively. The synthetic wastewater was fed continuously to the abiotic fuel cell directly into the second compartment with the same flow rate (0.167 L/hr) and HRT (7.5 hr) as in Experiment 2. $NaHCO_3$ with the concentration of approximately 3,000 mg/L as $CaCO_3$ served as alkalinity in the system. The pH in synthetic wastewater was adjusted to 7, which was close to the average pH in the wastewater after passing the first compartment of MFCs in Experiment 2. The parameters analyzed consisted of sulfate, sulfide, OCV, and voltage across the electrodes. The samples were collected two times a day to analyze all the parameters and measure the voltage of the systems. The schematic diagram of the abiotic fuel cell in Experiment 3 can be seen in Figure 3.12.

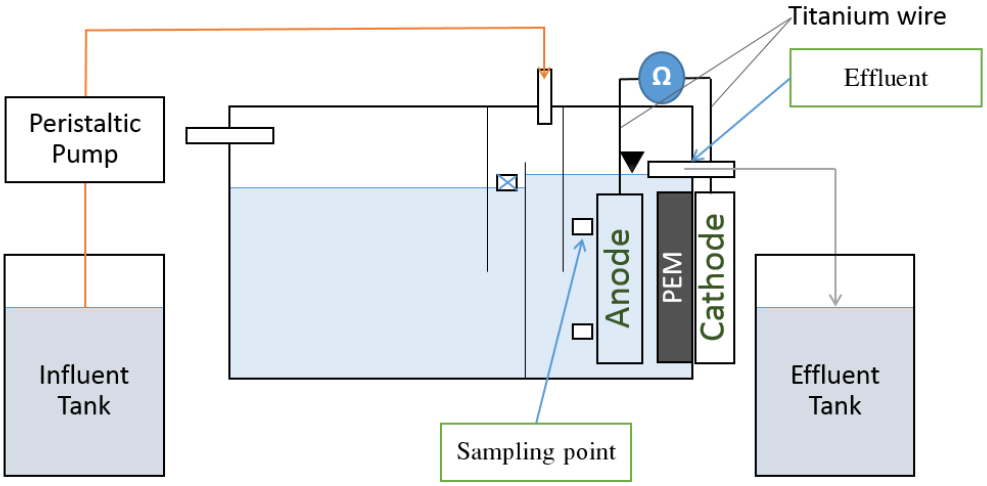


Figure 3.11 Set up of abiotic fuel cell in Experiment 3

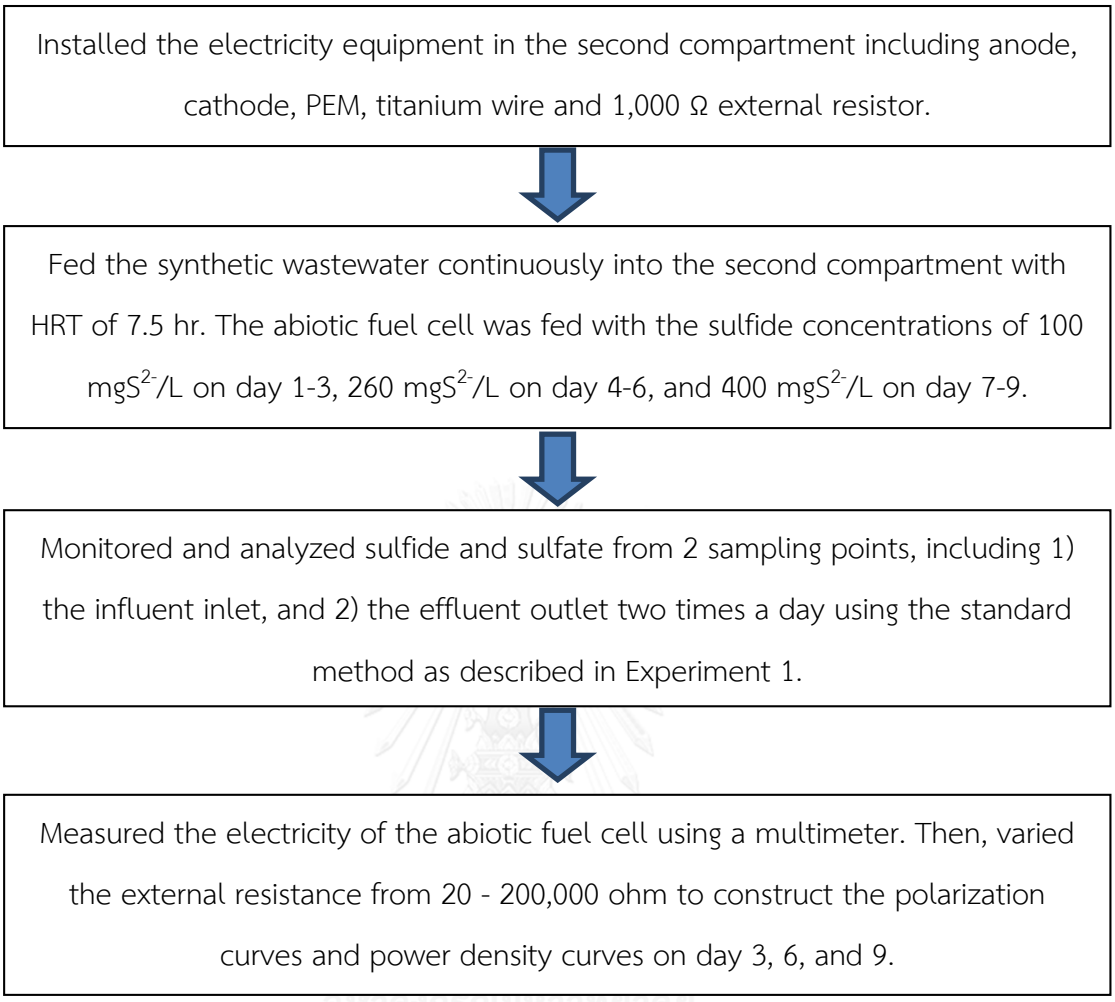


Figure 3.12 Framework of Experiment 3

3.5 Experiment 4: Microbial community analysis

The objective of this experiment was to investigate microbial communities in the MFCs at different COD:SO_4^{2-} ratio using 16S rRNA amplicon sequencing by MiSeq system (Illumina). The experimental framework can be seen in Figure 3.13. The seed sludge was stored at -20^{o}C until the analysis. Sludge in the first compartments, suspended microorganisms in the second compartments, and biofilms on the anode electrodes were collected before the replacement of the new anode electrodes on day 33 in MFC1 and on day 40 in both MFC3 and MFC6. For suspended microorganisms in the second compartment, due to the very low concentrations of suspended solids, the samples were filtered with 0.2 µm filters. The filters with the suspended solids were then used for DNA extraction. For the biofilms on the anode electrodes, deionized water was used to remove biofilms from the anode electrodes. Then, the samples were stored at -20°C before the analysis. Then, the DNA was extracted from all of the samples using FastDNA® SPIN Kit (MP Biomedicals). Microbial communities were then analyzed using 16S rRNA amplicon sequencing by MiSeq system (Illumina) (Figure 4.10), which included 6 steps as following:

1. First stage PCR: In this step, the DNA was separated from debris and 16S rRNA genes were amplified using universal primers for bacteria and archaea at the 515F-806R region of the 16S rRNA gene (Ding et al., 2015). The thermocycler (Takara, Japan) was used for PCR. Taq DNA Polymerase (Fermentus, Thermo Scientific) was used as polymerase enzyme in PCR process. The primer were as following:

   515F - Forward primer: 5′-GTGYCAGCMGCCGCGGT AA-3′

   806R - Reverse primer: 5′-GGACTACHVGGGTWTCTAAT-3′

The PCR conditions were as following:

Initial denaturing	95	Degree Celsius	3 minute	
Denaturing	95	Degree Celsius	30 second	25 rounds
Annealing	53	Degree Celsius	30 second	
Extension	72	Degree Celsius	30 second	
Final extension	72	Degree Celsius	5 minute	
End	4	Degree Celsius		

2. PCR clean-up: the DNA was separated from free primers and primer dimers using AMPure XP beads.
3. Second stage PCR: in this step, dual indices and Illumina sequencing adapters were attached to the PCR products using Nextera XT Index Kit. Then, PCR were run in a PCR machine (Takara, Japan) by using these conditions:

Initial denaturing	95	Degree Celsius	3 minute	
Denaturing	95	Degree Celsius	30 second	8 rounds
Annealing	55	Degree Celsius	30 second	
Extension	72	Degree Celsius	30 second	
Final extension	72	Degree Celsius	5 minute	
End	4	Degree Celsius		

4. PCR clean-up2: in this step, DNA was cleaned again using AMPure XP beads.
5. Library quantification and normalization: this process was to calculate the DNA concentration in nM depending on the average sizes of DNA by using a Qubit® 2.0 Fluorometer (Life Technologies), and then diluted it to 4 nM with 10 mM Tris-HCl pH 8.5. After that, each sample was aliquoted in 5 µL in one empty Eppendorf. Then, the samples were pooled as a sample library.

6. Library denaturing and MiSeq sample loading: In this step, the library was denatured with NaOH, diluted with hybridization buffer, and then heated denatured before MiSeq sequencing by Illumina MiSeq Sequencer (Illumina Inc., USA) (Figure 3.14).

After the MiSeq sequencing, the quality of data was checked by using the online FastQC application in BaseSpace (http://basespace.illumina.com). Then, the data analysis was performed as following:

1. Paired-end assemble: the sequences of each end were combined into one sequence using PANDASeq assembler (Masella et al., 2012).
2. Operational taxonomic unit (OTU): the sequences with the similarity greater than 99.7% were grouped into one operational taxonomic unit via UPARSE algorithm (Edgar, 2013).
3. Blast: The Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) was used to assign taxonomy to each OTU according to bacterial and archaeal 16S rRNA sequences from Bioprojects database from NCBI (ftp://ftp.ncbi.nlm.nih.gov/blast/db/). Then, the information on taxonomy and OTU clustering were combined to create OUT tables using our own scripts.
4. Data analysis: the results were then analyzed in MEGAN5-MetaGenome Analyzer (http://ab.inf.uni-tuebingen.de/software/megan5/).

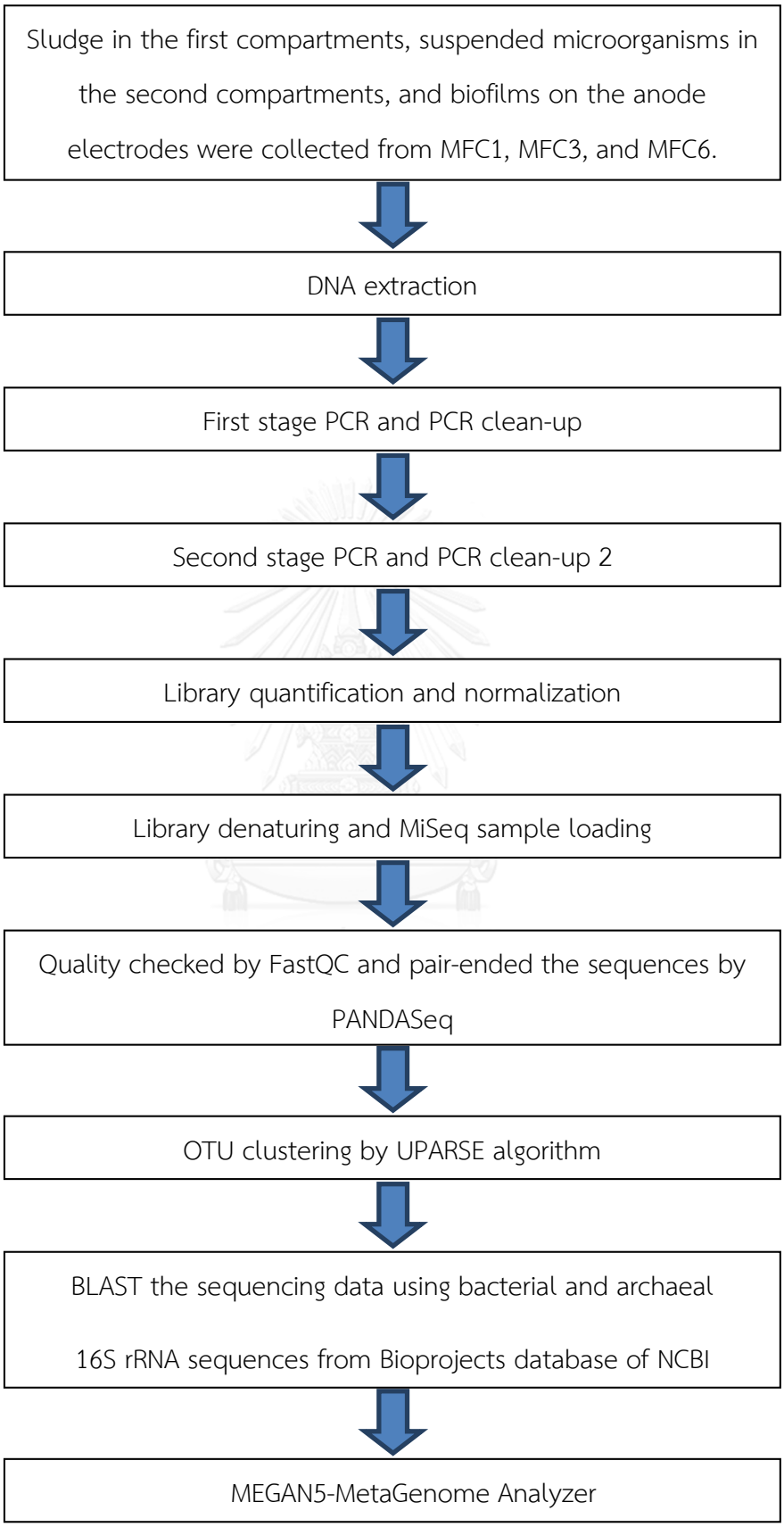


Figure 3.13 Frameworks for Experiment 3.

Figure 3.14 Illimina MiSeq system

Chapter 4
Results and Discussion

This research consists of four main parts, including 1) anaerobic bioreactor start-up, 2) MFC operation, 3) abiotic fuel cell operation, and 4) microbial community analysis. The results and discussion are presented accordingly.

4.1 Experiment 1: Anaerobic bioreactor start-up

This experiment consists of 2 parts. In Experiment 1-1, Reactor1, Reactor2, and Reactor4, were used for the enrichment of microorganisms at the COD:SO_4^{2-} ratio of 1, 2, and 4. Then, in Experiment 1-2, the sludge from Reactor1, Reactor2, and Reactor4, was transferred to the first compartments of MFC1, MFC3, and MFC6, which were operated at the COD:SO_4^{2-} ratio of 1, 3, and 6, respectively.

4.1.1 Experiment 1-1: Anaerobic bioreactor start-up in Reactor1, Reactor2, Reactor4

Three 5-L plastic bottles, Reactor1, Reactor2, and Reactor4, were used as continuous reactors for the enrichments of microorganisms that were suitable for the COD:SO_4^{2-} ratio of 1, 2, and 4, respectively. The parameters including pH, alkalinity (Alk), volatile fatty acids (VFA), COD, and sulfate were monitored in these reactors. These parameters generally indicate the overall activities of anaerobic bioreactors, which assist in controlling the stability of the systems.

pH, alkalinity, and volatile fatty acids

The pH were controlled in the range of 6.8 – 7.3, which was suitable for microorganisms in the systems, by adding $NaHCO_3$ (2,000 mg/L as $CaCO_3$) as a source of alkalinity in the influent. Figure 4.1 shows the pH values in each reactor. The average

pH of Reactor1, Reactor2, and Reactor4 were 7.08 ± 0.31, 7.07 ± 0.12, and 7.09 ± 0.20, respectively, which was suitable for all groups of microorganisms. In anaerobic bioreactors, the optimum pH for operation should be in the range of 6.6 – 7.6 (Switzenbaum et al., 1990). The effluent alkalinity of 2,214 ± 146, 2,258 ± 92, and 2,298 ± 66 mg/L as $CaCO_3$ were observed in Reactor1, Reactor2, and Reactor4, respectively, whereas, the effluent VFAs of Reactor1, Reactor2, and Reactor4 were 760 ± 67, 871 ± 28, and 733 ± 70 mg/L as CH_3COOH, respectively. Therefore, the VFA:Alk ratio in Reactor1, Reactor2, and Reactor4 were 0.343 ± 0.025, 0.386 ± 0.009, and 0.319 ± 0.031, respectively. The VFA:Alk ratio is a useful parameter for controlling conventional anaerobic treatment processes. In general, it is considered that the VFA:Alk ratio should be in the range of 0.1 – 0.35 (Switzenbaum et al., 1990), which indicates adequate alkalinity in the systems.

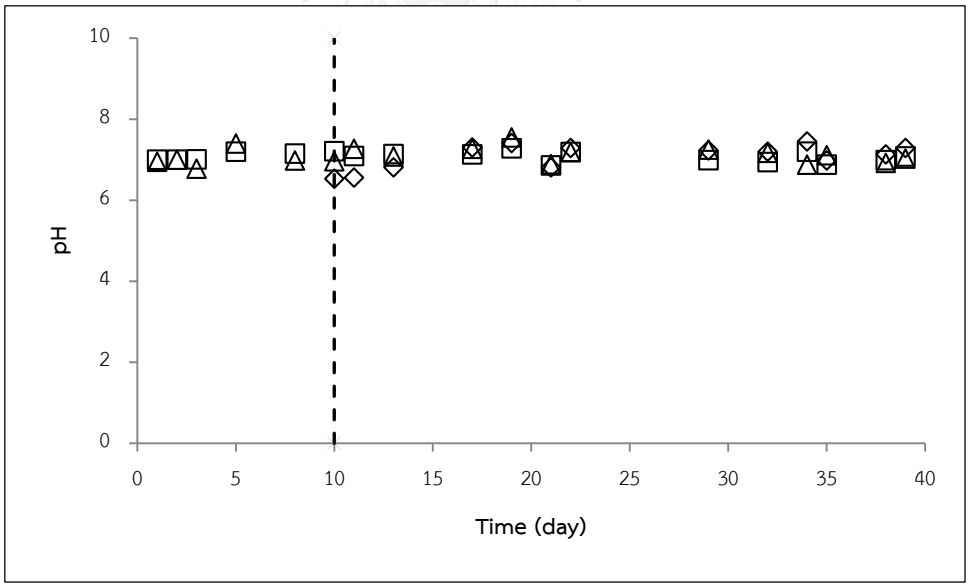


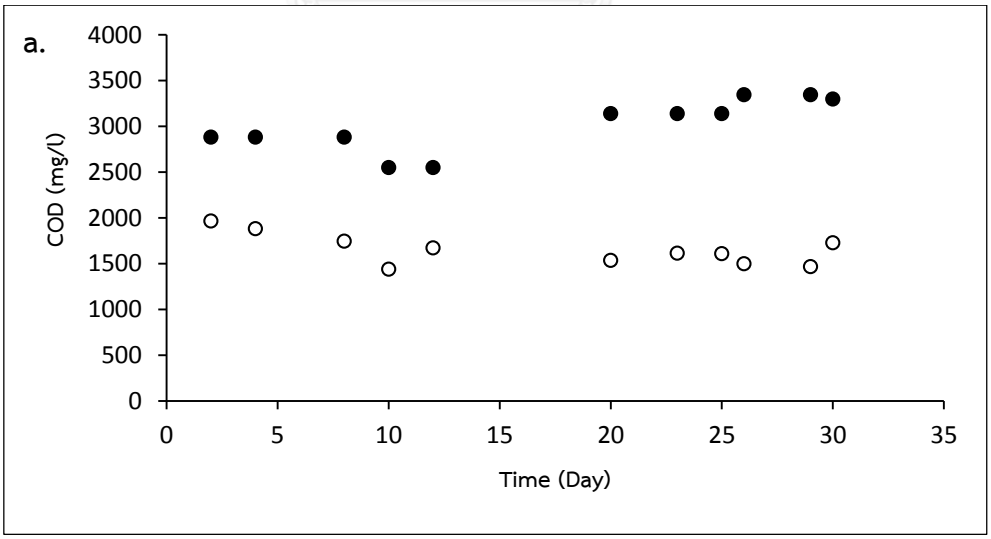
Figure 4.1 pH values in ◇ Reactor1, □ Reactor2, and △ Reactor4.

COD and sulfate removal

In Reactor1 with the COD:SO_4^{2-} ratio of 1, glucose equivalent to the COD of 3,000 mgCOD/L was added in the synthetic wastewater. On the other hand, the initial

sulfate of 750 mgSO_4^{2-}/L was added to stimulate the SRB in the system. The COD removal efficiency gradually increased. The average COD effluent concentration of 1,648 ± 169 mgCOD/L was observed (Figure 4.2a), which was equivalent to the average COD removal efficiency of 44.68 ± 8.5 %.

In the case of sulfate (Figure 4.2b), sulfate removal efficiency reached 45% on day 8 of operation. The results suggest that the microbial community in Reactor1 might have changed during this period. Sulfate concentration in the influent was then increased from 750 to 1,500 mgSO_4^{2-}/L on day 9 of operation. Then, sulfate concentration in the influent was increased again to approximately 1,800 mgSO_4^{2-}/L and 2,100 mgSO_4^{2-}/L (COD:SO_4^{2-} ratio = 1.428) on day 17 and day 26, respectively. During this phase of operation, the average sulfate removal efficiency was 58.9 ± 8.5 % with the effluent sulfate concentration of 764 ± 124 mgSO_4^{2-}/L. In this experiment, the COD:SO_4^{2-} ratio did not yet reach 1; the sludge in this system would later be transferred to the MFC in which the COD:SO_4^{2-} ratio would be further adjusted.



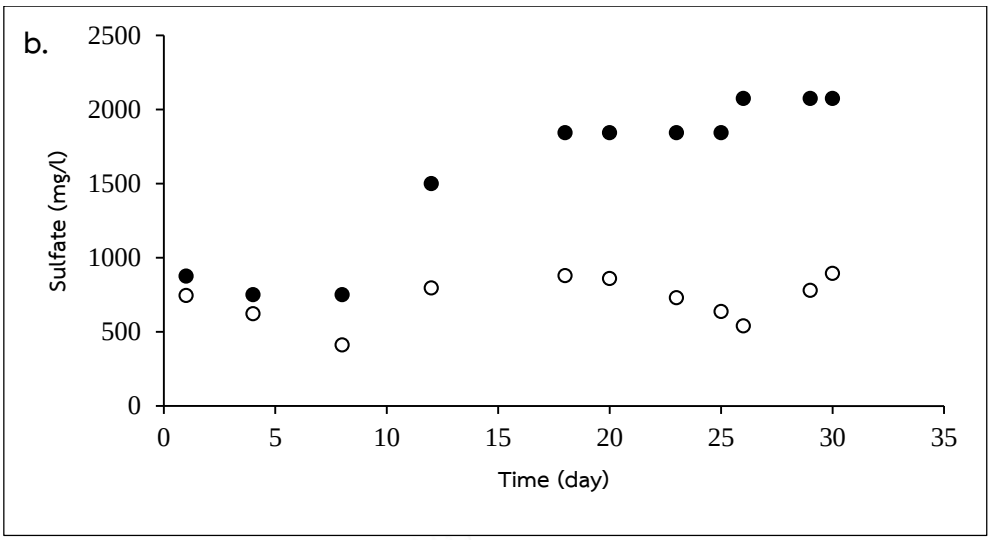


Figure 4.2 (a) COD concentrations and (b) sulfate concentrations in Reactor1: ● Influent and ○ Effluent

For Reactor2, the average glucose equivalent to 3,090 ± 303 mgCOD/L and sulfate 1,517 ± 36 mgSO_4^{2-}/L were added in the influent to stimulate microbial community at the COD:SO_4^{2-} ratio of 2 in the system. Figure 4.3a shows the COD removal in the system. The average effluent concentration of 1,604 ± 166 mgCOD/L was observed for 35 days of operation, which was equivalent to the COD removal efficiency of 46.85 ± 9.50 %.

In the case of sulfate (Figure 4.3b), the average sulfate removal efficiency was 43.41 ± 10.58 % in Reactor2. The sulfate removal efficiency slightly increased until reaching its maximum at 65% on day 10 of operation. However, the sulfate removal efficiency gradually decreased from 65% to 36% on day 34. The low sulfate removal efficiency had brought up the question whether the HRT of 1 d was enough for sulfate reduction. Therefore, in order to test the effect of HRT on sulfate reduction, the reactor was switched from a continuous mode into a batch mode for 5 days (on day 35 to day 39) as shown Figure 4.4. The results show that the COD concentrations in the reactor decreased from 3,264 mgCOD/L to 967 mgCOD/L over 5 days. On the contrary, the

sulfate concentration increased over time reaching nearly the initial concentration (1,453 mgSO_4^{2-}/L). The increase in sulfate concentrations suggests that SOB might be present especially near the sampling point on the top of the reactor, resulting in sulfide oxidation back to sulfate by SOB. Therefore, the increase in HRT appeared to promote SOB which can decrease the sulfate removal efficiency in the system; however, good performance of COD removal was achieved.

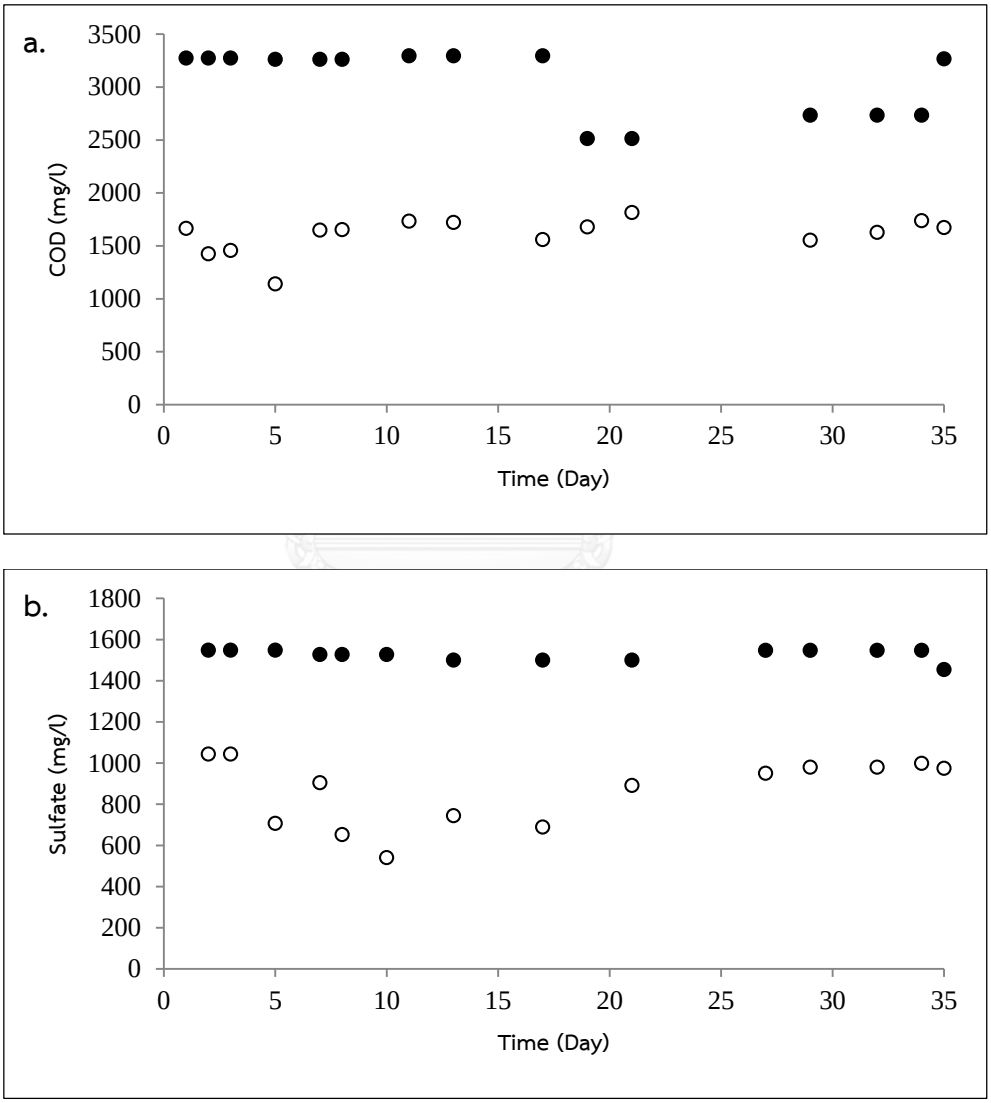


Figure 4.3 (a) COD concentrations and (b) sulfate concentration in reactor 2: ● Influent and ○ Effluent

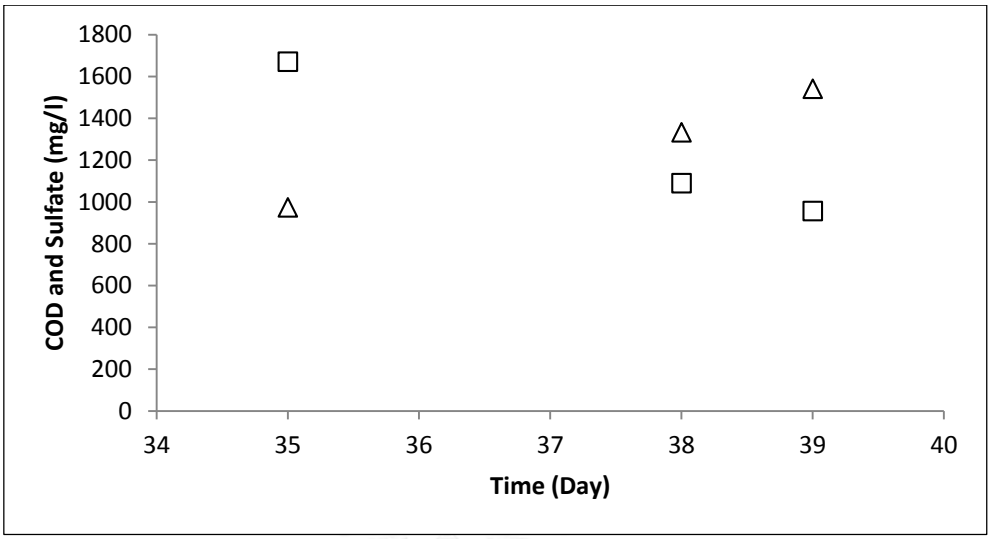


Figure 4.4 COD and sulfate concentrations in batch mode of Reactor2:
□ Effluent COD and △ Effluent sulfate

For Reactor4 (COD:SO_4^{2-} ratio of 4), the average COD concentrations in the influent was 3,107 ± 309 mgCOD/L. The average COD removal efficiency of 45.01 ± 8.50% was achieved for 40 days of operation. Figure 4.5a shows COD concentrations in Reactor4.

In the case of sulfate, the concentrations in this reactor are shown in Figure 4.5b. About 50% of sulfate was removed after 2 days of operation. Then, the sulfate concentrations in the effluent decreased to less than 100 mgSO_4^{2-}/L after 5 days of operation. The results suggest that SRB can still grow at the COD:SO_4^{2-} of 4. The average sulfate removal efficiency of 75.27 ± 17.99 % was achieved in Reactor4.

On day 32 of operation, sludge from Reactor4 with MLSS concentration of 9,000 mg/L was transferred into the first compartment of an MFC with 40%. The COD removal efficiency in the first compartment of the MFC was quite low from day 1-4 of operation. However, more than 50% of it had been removed from wastewater on day 5 of operation. In case of sulfate, the results show that, more than 90% for sulfate

removal efficiency were observed after day 6 of operation in MFC reactor. So, it can indicate that MFC reactor had been effective for treating sulfate-rich wastewater at the COD:SO_4^{2-} ratio of 4.

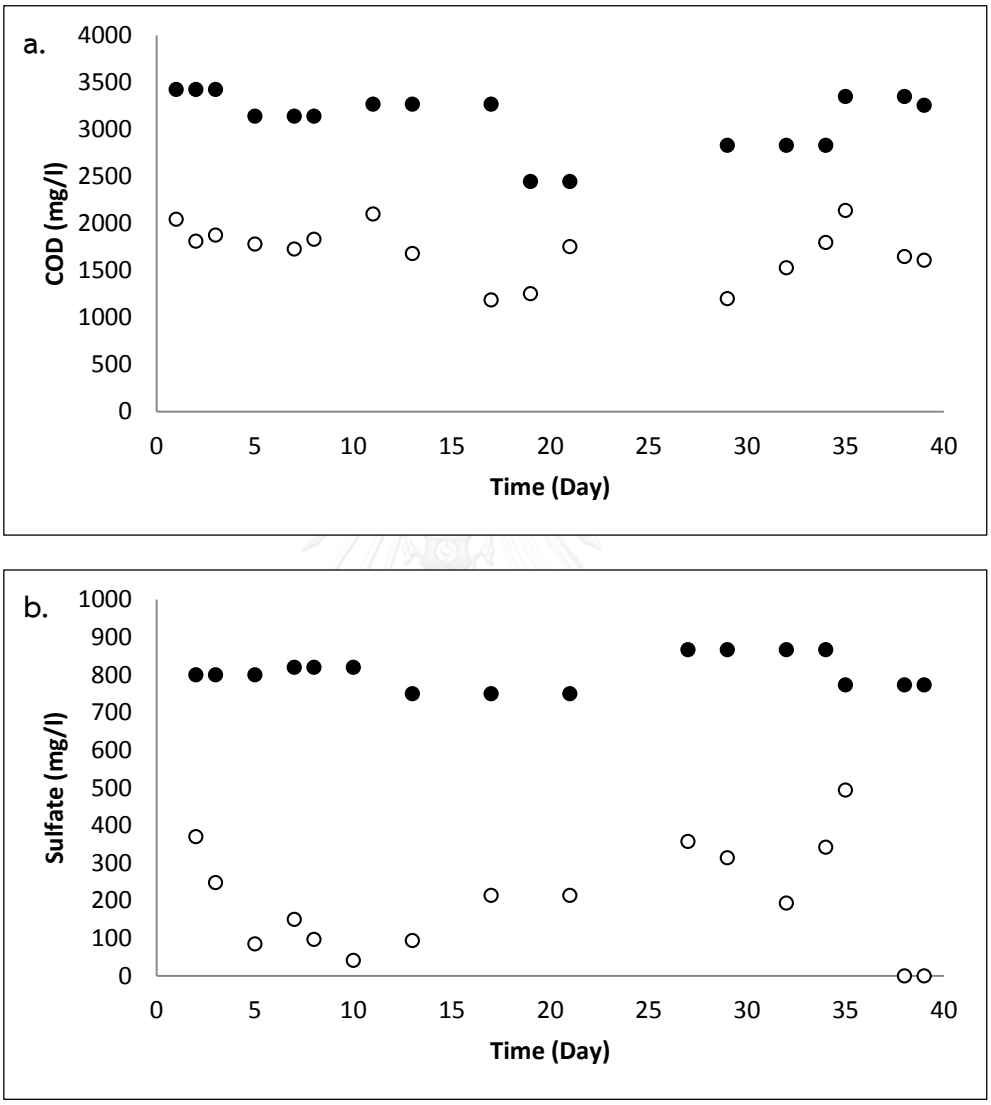


Figure 4.5 (a) COD concentrations and (b) sulfate concentration in reactor4: ● Influent and ○ Effluent

The COD removal in Reactor1, Reactor2, and Reactor4 were 1,362 ± 365, 1,460 ± 404, and 1,405 ± 321 mgCOD/L, respectively. On the other hand, the sulfate removal in Reactor1, Reactor2, and Reactor4 were 1,336 ± 365, 664 ± 158, and 597 ± 112 mgSO_4^{2-}/L, respectively. It should be noted that there was no significant difference in

both COD and sulfate removal in both Reactor2 and Reactor4. Therefore, COD:SO_4^{2-} ratio in Reactor2 and Reactor4 had been changed to 3 and 6, respectively, being the same range of predominance groups with the previous value. Table 4.1 summarizes the concentrations of COD, SO_4^{2-}, pH, and VFA:Alk in Experiment 1-1.

After 8 days of operation in MFC, the sludge in the first compartment of the MFC was transferred back to Reactor4 again in order to repaired MFC reactor for 7 days. Then, the sludge from Reactor1, Reactor2, and Reactor4 was transferred to the first compartments of MFC1, MFC3, and MFC6, respectively, after 30 days operation in Reactor1 and 40 days of operation in Reactor2 and Reactor4 with the initial volume of 40% v/v (total initial MLSS concentration of 8,000 mg/L) in the MFCs. The performances of anaerobic treatment in the first compartments of MFCs are discussed in Experiment 1-2.

Table 4.1 Concentrations of COD, SO_4^{2-}, pH, and VFA:Alk in Experiment 1-1

Parameter	Reactor1	Reactor2	Reactor4
Influent COD (mgCOD/L)	3,011 ± 287	3,090 ± 303	3,107 ± 309
Effluent COD (mgCOD/L)	1,648 ± 169	1,604 ± 166	1703 ± 285
COD removal efficiencies (%)	44.68 ± 8.5	46.85 ± 9.50	45.01 ± 8.50
Influent sulfate ($mgSO_4^{2-}$/L)	2,100	1,517 ± 36	806 ± 43
Effluent sulfate ($mgSO_4^{2-}$/L)	764 ± 124	935 ± 251	198 ± 110
Sulfate removal efficiencies (%)	58.9 ± 8.5	43.41 ± 10.58	75.27 ± 17.99
pH	7.08 ± 0.31	7.07 ± 0.12	7.09 ± 0.20
VFA:Alk ratio	0.343 ± 0.025,	0.386 ± 0.009,	0.319 ± 0.031

4.1.2 Experiment 1-2: Anaerobic bioreactor start-up in the first compartments of MFCs

After the end of Experiment 1-1, three single-chamber air-breathing microbial fuel cell (MFC) with different COD:SO_4^{2-} ratio of 1, 3, and 6 for MFC1, MFC3, and MFC6, respectively, were operated continuously with the hydraulic retention time of 1 day (24 hr). In this experiment, pH, COD, sulfate, sulfide, ORP, alkalinity, and volatile fatty acid in the influent and effluent were monitored over time. The objective of this part is to promote suitable microorganisms at different COD:SO_4^{2-} ratio.

pH, alkalinity, and volatile fatty acids

The pH values in all of the MFCs were measured over time as shown in Figure 4.6. The average pH of 7.18 ± 0.24, 7.04 ± 0.19, and 7.00 ± 0.18 were found in MFC1, MFC3, and MFC6, respectively. These neutral pH values were suitable for microorganisms (Switzenbaum et al., 1990), including methanogens, SRB, and fermentative bacteria, which were likely to be predominant groups of microorganisms in the systems. The pH in MFC1 was slightly higher than both MFC3 and MFC6 probably due a higher extent of sulfate reduction in MFC1 than in MFC3 and MFC6. Sulfate reduction process typically generated alkalinity, which can cause a pH increase in the system.

Alkalinity and volatile fatty acids (VFAs) were monitored during the first 20 days of the MFC operation. During day 1-10 of the operation, alkalinity in the influent was at approximately 2,000 mg/L as $CaCO_3$. The average alkalinity in the effluent of MFC1, MFC3, and MFC6 were 1,924 ± 105, 1892 ± 160, and 1810 ± 224 mg/L as $CaCO_3$, respectively, which were close to the influent concentrations. On the other hand, the VFA concentrations in the effluent were generated via acidogenesis process. The average VFAs in the effluent were 716 ± 47, 722 ± 48, and 716 ± 50 mg/L as CH_3COOH

for MFC1, MFC3, and MFC6, respectively which were equivalent to the VFA:Alk ratio of 0.37, 0.38, and 0.40 for MFC1, MFC3, and MFC6 respectively. These ratios were considered rather high, which could cause a pH drop in the systems. Therefore, the alkalinity in the influent was increased from 2,000 to 3,000 mg/L as $CaCO_3$ after 10 days of operation. The increase in alkalinity in synthetic wastewater could increase sulfate removal efficiencies in sulfate-reducing bioreactors (Ren et al., 2006). On day 11 – 20, the average alkalinity in the effluent of MFC1, MFC3, and MFC6 were 2,825 ± 84, 2,842 ± 145, and 2,630 ± 75 mg/L as $CaCO_3$, respectively, and the average VFA were 1,075 ± 128, 1,082 ± 140, and 880 ± 56 mg/L as CH_3COOH, respectively. These values corresponded to the VFA:Alk ratio of 0.38 ± 0.05, 0.38 ± 0.06, and 0.34 ± 0.03, which were suitable for each MFC because pH in all MFCs were around 7. Therefore, the alkalinity of influent was set to approximately 3,000 mg/L as $CaCO_3$.

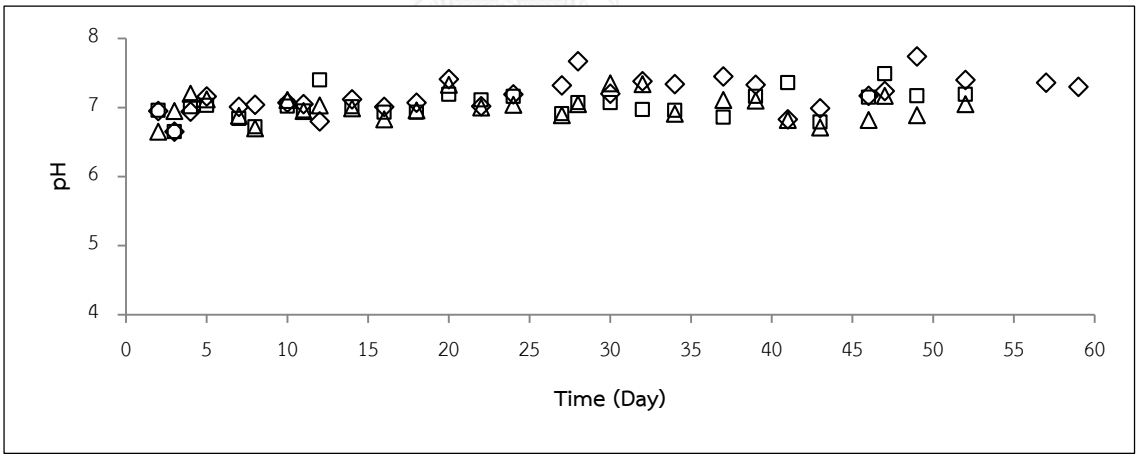


Figure 4.6 pH in the first compartment of MFC in Experiment 1-2;
◇ pH in MFC1, □ pH in MFC3, and △ pH in MFC6.

COD removal efficiency

Glucose of 3,000 mgCOD/L was added into the synthetic wastewater, which was continuously fed into MFC1, MFC3, and MFC6. The results on COD removal efficiencies of each MFC are as following:

In MFC1, the average COD concentration in the influent was 3,130 ± 195 mgCOD/L. The effluent COD was rather constant at the concentration of 1,900 ± 300 mgCOD/L, which was equivalent to the COD removal efficiency of 39.6 ± 9.8 %. Figure 4.7 shows the COD in the influent and effluent of MFC1. The operating time of MFC1 in this part was 60 days, which was longer than those in MFC3 and MFC6, because of the fluctuation of sulfate in the effluent of MFC1.

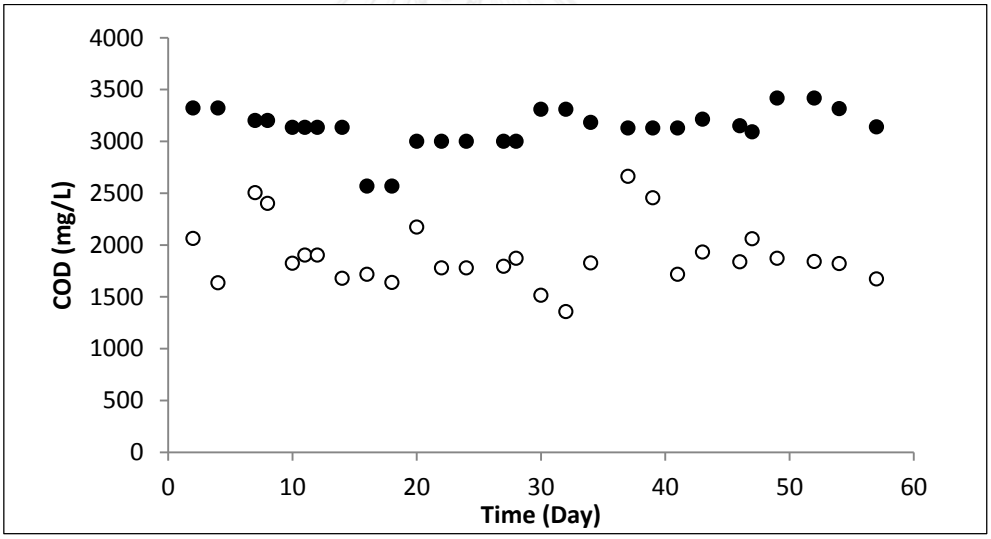


Figure 4.7 COD concentrations in MFC1 in Experiment 1-2;
● influent, ○ effluent.

In MFC3, the MFC was operated for 55 days to enrich suitable microorganisms under the COD:SO_4^{2-} ratio of 1. The average COD concentration in the influent was rather constant at 3,120 ± 250 mgCOD/L. However, the COD concentration in the effluent was fluctuated during the first 10 days of operation; after that, the values became rather constant. The average COD concentration in effluent of 1,715 ± 260

mgCOD/L was observed, which was equivalent to the COD removal efficiency of 41.3 ± 7.9 %. The COD concentrations in the influent and effluent of MFC3 are shown in Figure 4.8.

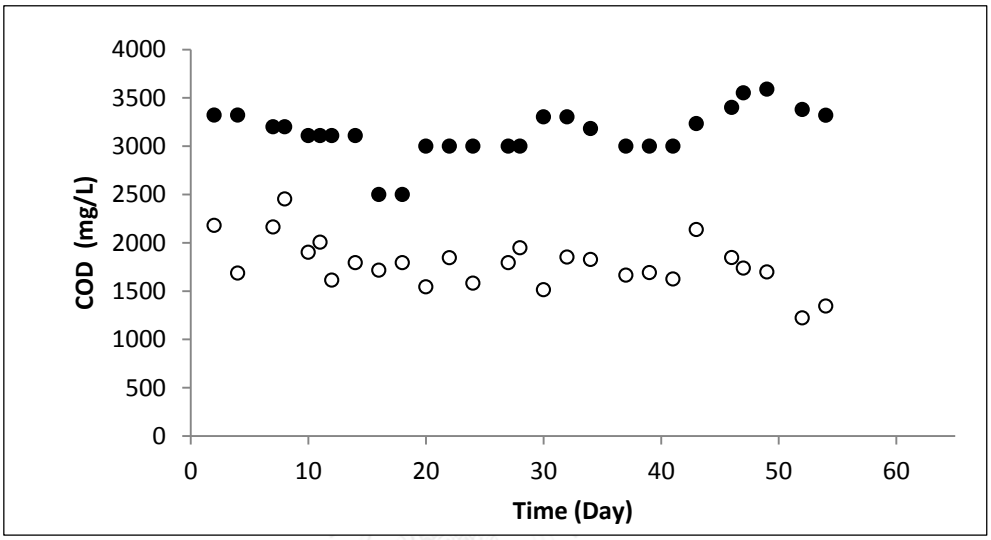


Figure 4.8 COD concentrations in MFC3 in Experiment 1-2;
● influent, ○ effluent

In MFC6, the system was operated for 55 days. The operating time was the same as in MFC3. The average COD concentration in the influent was 3,150 ± 225 mgCOD/L. However, the average COD concentration in the effluent was 1,640 ± 300 mgCOD/L, equivalent to the COD removal efficiency of 47.7 ± 9.1 %. Figure 4.9 shows COD concentrations in the influent and effluent over time. There were some peaks in effluent COD concentrations on day 7 and day 43-46. The lower COD removal could be linked to the decreasing in pH during those periods, which could have adverse effects on some groups of microorganisms such as methanogens in the systems.

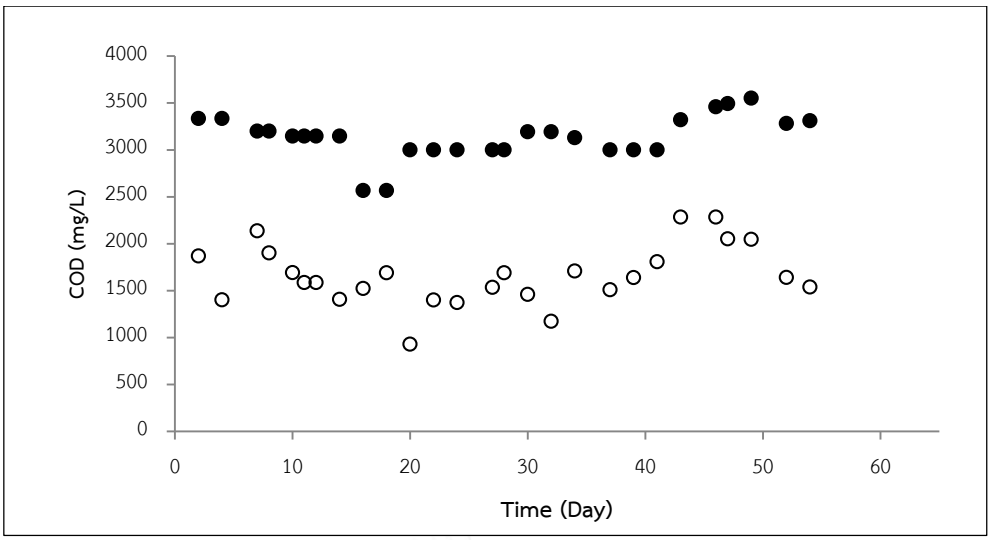


Figure 4.9 COD concentrations in MFC6 in Experiment 1-2:
● influent, ○ effluent

The results of COD concentrations indicated that the COD removal efficiencies were rather close in all of the MFCs, which were 39.6 ± 9.8 % in MFC1, 41.3 ± 7.9 % in MFC3, and 47.7 ± 9.1 % in MFC6. However, COD removal efficiencies of all MFCs were quite low compared with previous studies, which achieved high COD removal efficiencies of 70 – 90% in anaerobic bioreactors treating only COD (Araujo et al., 2008; Kosińska and Miśkiewicz, 2008). The low COD removal efficiencies could be due to not enough mixing in the first compartment of MFC, which was a large compartment. We did not provide the mixing in this compartment to avoid the loss of microorganisms from this compartment. Kosińska and Miśkiewicz (2008) reported that the increase in HRT in anaerobic bioreactors with biomass recycling could increase both COD and sulfate removal efficiencies. Therefore, the increase in hydraulic retention time might improve the COD removal efficiencies of these systems. The presence of high sulfate in synthetic wastewater might also be another factor contributing low COD removal efficiencies in the systems. Figure 4.10 shows the comparison of COD removal in MFC1, MFC3, and MFC6.

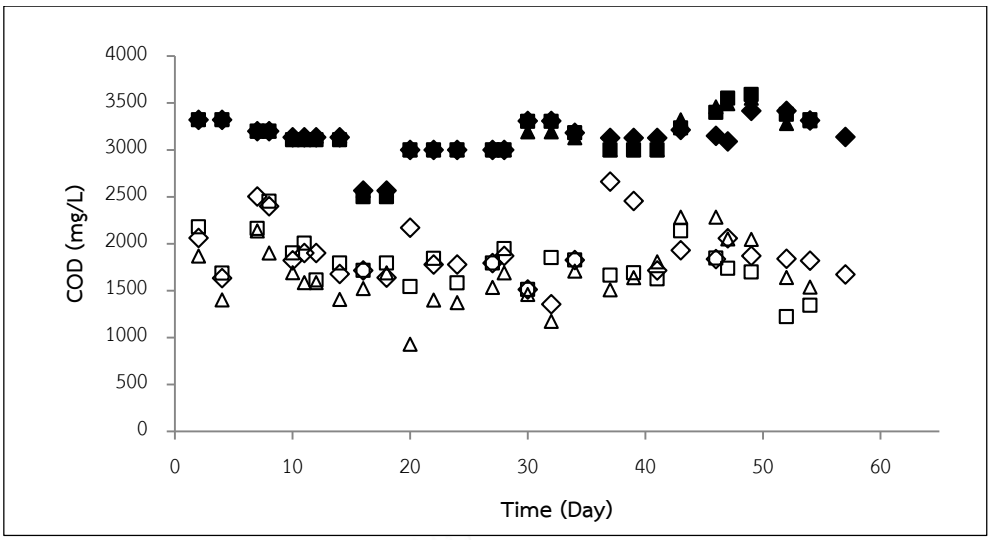


Figure 4.10 COD removal each MFC in Experiment 1-2: influent COD in ◆ MFC1, ■ MFC3, and ▲ MFC6, effluent COD in ◇ MFC1, □ MFC3, and △ MFC6.

Sulfate removal and sulfide production

The different concentrations of sulfate in MFC would affect microbial communities and the amount of each group of microorganisms, such as, SRB and methanogens in the MFCs. According to previous studies (Choi and Rim, 1991), three different COD:SO_4^{2-} ratio of 1, 3, and 6 would promote different predominant groups of microorganisms in the systems. SRB would be predominant groups at a low COD:SO_4^{2-} ratio (the ratio of 1); nevertheless, methanogens should be a predominant group at a high COD:SO_4^{2-} ratio (the ratio of 6). However, both SRB and methanogens should be able to grow together when operated with medium COD:SO_4^{2-} ratio (the ratio of 3). Sulfate concentrations of 3,000, 1,000, and 500 mgSO_4^{2-}/L were added into the synthetic wastewater in MFC1, MFC3, and MFC6 respectively, which were equivalent to COD:SO_4^{2-} of 1, 3, and 6, respectively. The results on sulfate removal of each MFC are as following:

At the COD:SO_4^{2-} ratio of 1 (MFC1), the average sulfate concentration in the influent was 3,200 ± 125 mgSO_4^{2-}/L whereas the average COD concentration was 3,130 ± 195 mgCOD/L. Therefore, the average COD:SO_4^{2-} ratio in the influent of MFC1 was 0.978. At this ratio, SRB were likely to predominate in the system (Choi and Rim, 1991; Chou et al., 2008) Figure 4.11 shows the sulfate concentrations in the influent and effluent over time. The results show that sulfate removal gradually increased over time, which might be due to a shift in microbial community in the systems toward SRB. The average sulfate concentration in the effluent was 2,225 ± 197 mgSO_4^{2-}/L, which was equivalent to 28.9 ± 4.6 %. Sulfate was removed by SRB and sulfide was generated as the final product, which could cause inhibition to microorganisms. Sulfide production (S^{2-}) was measured after day 20 of operation. The sulfide production increased over time, which had the same trend as sulfate removal. In other words, the higher sulfate removal, the higher sulfide production on that day as shown in Figure 4.11. Sulfide concentration in the last 10 days of operation was mostly higher than 250 mgS^{2-}/L, which was at the level that could inhibit microorganisms in the system including SRB (Lens et al., 1998).

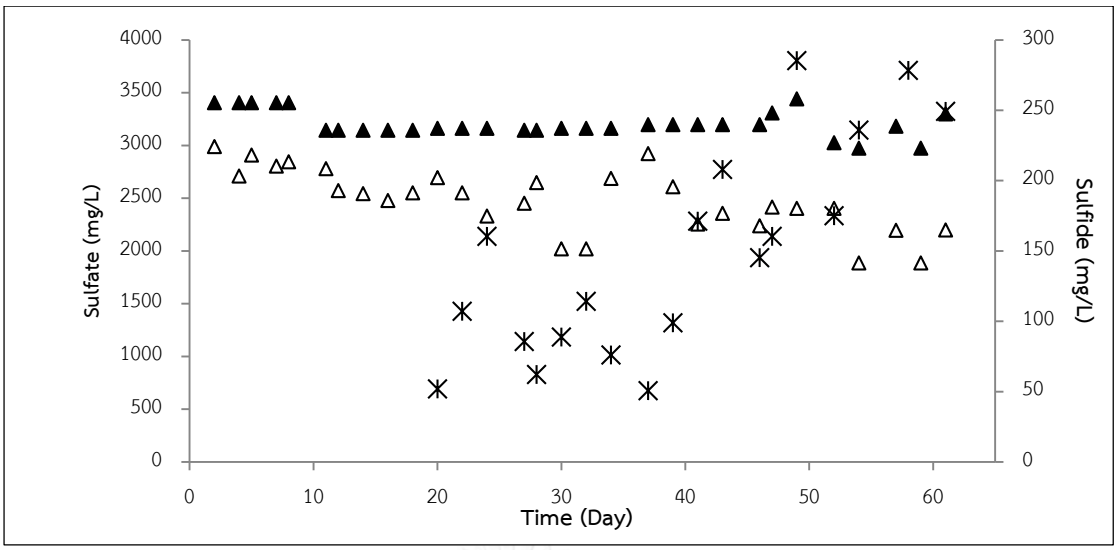


Figure 4.11 Sulfate and sulfide concentrations in MFC1 in Experiment 1-2: ▲ influent sulfate, △ effluent sulfate, and ∗ effluent sulfide

At the COD:SO_4^{2-} ratio of 3 (MFC3), the average sulfate concentration in the influent during 54 days of operation was 1,090 ± 25 mgSO_4^{2-}/L. Since the average COD concentration of MFC3 was 3,120 ± 250 mgCOD/L, the corresponding COD:SO_4^{2-} ratio in the influent of MFC3 was 2.86. At this COD:SO_4^{2-} ratio, both methanogens and SRB could co-exist (Choi and Rim, 1991). Figure 4.12 shows sulfate and sulfide concentrations in Experiment 1-2 of MFC3. The results shows that sulfate concentrations in the effluent slightly decreased over time until the concentrations getting close to 0 mgSO_4^{2-}/L on day 30 of operation. After that, sulfate was removed effectively (more than 95%) in MFC3. It should be noted that the effluent sulfate concentration on the first day of operation was higher than the influent concentration due to the remaining sulfate in the initial sludge. The higher sulfate concentration might happen due to three reasons: 1) a higher sulfate concentration remaining in Experiment 1-1, 2) the sulfur oxidation process of SOB in Reactor2 in Experiment 1-1 and 3) SRB might have lower activity to remove sulfate due to discontinuing of operation for 7 days.

Sulfide concentrations in the aqueous phase were measured after day 20 of operation. The results show that there was a fluctuation of sulfide concentrations from 40 – 180 mgS^{2-}/L in the effluent. The average sulfide concentration of 97.6 ± 39.08 mgS^{2-}/L was observed in MFC3, which was not high enough to inhibit microorganisms in the system. Nevertheless, certain amount of gaseous hydrogen sulfide (H_2S) could be lost from the MFC at the effluent valve.

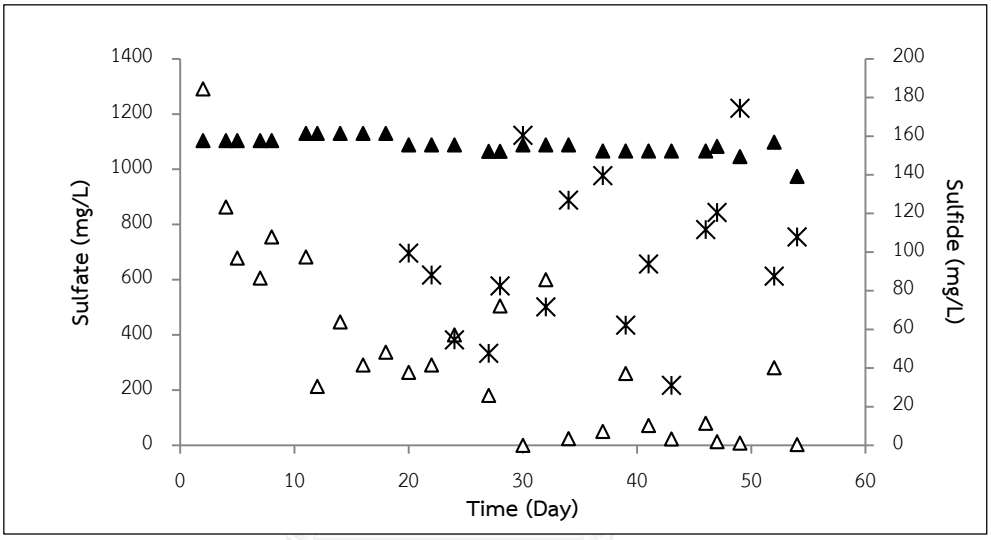


Figure 4.12 Sulfate and sulfide concentrations in MFC3 in Experiment 1-2: ▲ influent sulfate, △ effluent sulfate, and ∗ effluent sulfide

In the case of MFC6 (COD:SO_4^{2-}=6), the average sulfate concentration in the influent of 54 days of operation was rather constant at 537 ± 38 mgSO_4^{2-}/L. As the average COD concentration in the influent was 3,150 ± 225 mgCOD/L. The COD:SO_4^{2-} ratio of MFC6 was equal to 5.87, which could promote methanogens in the system (Choi and Rim, 1991). Figure 4.13 shows sulfate and sulfide concentrations in MFC6. At the beginning, the remaining sulfate in the initial sludge affected the sulfate concentrations in the effluent to be higher than that in the influent. However sulfate concentration in the effluent decreased rapidly during the first 10 days of operation. Then, the average sulfate concentration in the effluent was 110 ± 94 mgSO_4^{2-}/L after

day 10 of operation, corresponding to the sulfate removal efficiency of 82.0 ± 12.6 %. The high COD:SO_4^{2-} ratio in this MFC might not be suitable for SRB compared to the in MFC1 and MFC3, resulting in less sulfate removal in this reactor.

In the case of sulfide, sulfide concentrations in the effluent varied from 10 – 100 mgS^{2-}/L in day 20 – 58 of operation, which was considered to be in a low range and not toxic to microorganisms in the system.

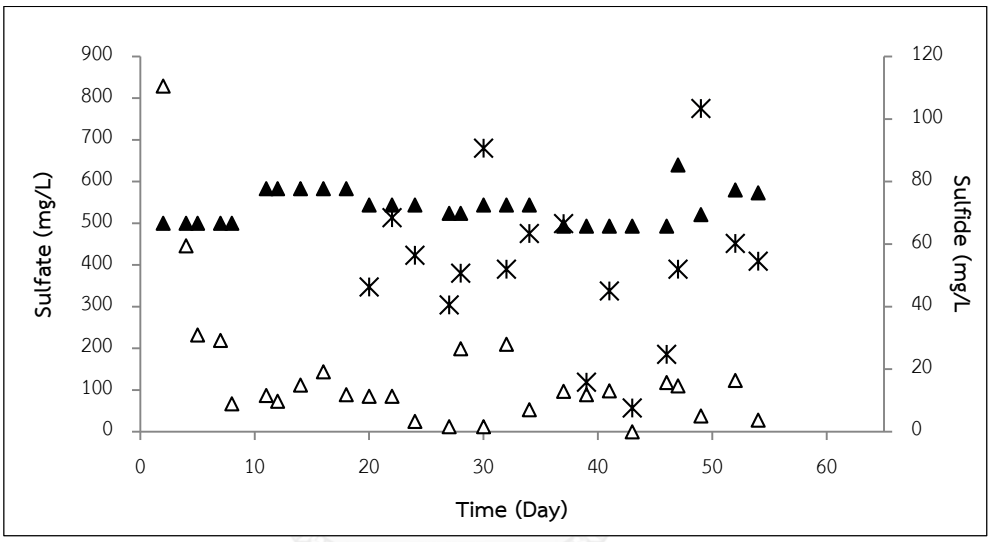


Figure 4.13 Sulfate and sulfide concentrations in MFC6 in Experiment 1-2: ▲ sulfate influent, △ sulfate effluent, and ∗ sulfide concentration

The results of Experiment 1-2 show that the COD removal efficiencies were 39.6 ± 9.8, 41.3 ± 9.4, and 47.7 ± 9.1% for MFC1, MFC3, and MFC6, respectively, which corresponded to the COD removal of 1,218 ± 360, 1,360 ± 370, and 1,410 ± 414 mgCOD/L for MFC1, MFC3, and MFC6, respectively. On the other hand, sulfate removal of 691 ± 241, 803 ± 249, and 444 ± 78 mgSO_4^{2-}/L were observed in MFC1, MFC3, and MFC6, respectively. Figure 4.11, 4.12, and 4.13 show the increase in sulfate removal in all MFCs after increasing the alkalinity from 2,000 to 3,000 mg$CaCO_3$/L on day 10 of operation, which was consistent with the previous study by Ren et al. (2006). From the results, COD:SO_4^{2-} ratio of 3 was the most effective for sulfate removal. Since the

theoretical ratio of COD:SO_4^{2-} for sulfate reduction was 0.67 assuming negligible biomass yield, the COD removal via sulfate reduction would be 415, 482, and 266 mgCOD/L for MFC1, MFC3, and MFC6, respectively, corresponding to 34.1, 35.4, and 18.9% of total COD removal in MFC1, MFC3, and MFC6, respectively. The results suggest different proportions of COD removed via sulfate reduction and methanogenesis, which might be linked to different microbial communities and populations. Although the proportion of COD removal via sulfate reduction of MFC1 was similar to that in MFC3, microbial community in MFC3 might still be different from MFC1 due to the difference in sulfate removal. However, there was a previous study suggests that methanogens can live in COD:SO_4^{2-} ratio of 1 when the system was operated at high SO_4^{2-} concentration (Hu et al., 2015); therefore, methanogens might be found in MFC1.

In addition, the results also show that the COD removal in all of the MFCs was rather constant, but sulfate removal increased over time, suggesting the shifts in microbial communities toward SRB. After SRB was promoted in the systems, hydrogen sulfide would be generated as a final product, which could cause sulfide toxicity to microorganisms in the systems.

Table 4.2 Concentrations of COD, SO_4^{2-}, pH, VFA:Alk, and sulfide in Experiment 1-2

Parameter	MFC1	MFC3	MFC6
Influent COD (mgCOD/L)	3,130 ± 195	3,120 ± 250	3,150 ± 225
Effluent COD (mgCOD/L)	1,900 ± 300	1,715 ± 260	1,640 ± 300
COD removal efficiency (%)	39.6 ± 9.8	41.3 ± 7.9	47.7 ± 9.1
Influent sulfate ($mgSO_4^{2-}/L$)	3,200 ± 125	1,090 ± 25	537 ± 38
Effluent sulfate ($mgSO_4^{2-}/L$)	2,225 ± 197	341 ± 321	110 ± 94
Sulfate removal efficiency (%)	28.9 ± 4.6	72.3 ± 24.0	82.0 ± 12.6
pH	7.18 ± 0.24	7.04 ± 0.19	7.00 ± 0.18
VFA:Alk ratio	0.38 ± 0.05	0.38 ± 0.06	0.34 ± 0.03
Sulfide (mgS^{2-}/L)	134 ± 67	98 ± 39	53 ± 24

4.2 Experiment 2: Microbial fuel cell operation

In Experiment 2, the performances of MFCs were investigated in terms of treatment efficiencies and electricity generation. After the first compartments of MFCs reached steady states in Experiment 1-2, electrical equipment including anode, cathode, PEM, titanium wire and external resistor were installed in the second compartment of the MFCs. At the end of operation (day 33 for MFC1 and day 40 for MFC3 and MFC6), anode electrode in each MFC was replaced with a new one (activated carbon cloth as same as the previous one to investigate the deterioration of the anode electrode. The suspended solids from the second compartments and biofilms on the anode electrodes were also collected for microbial community analysis before replacing the anode electrodes. In this experiment pH, COD, sulfate, sulfide, voltage across the electrodes, and OCV were measured over time. The results are as follows:

4.2.1 COD removal efficiency

Glucose equivalent to COD concentration of 3,000 mg/L was fed continuously into all of the MFCs with HRT of 24 hr, similar to Experiment 1-2. For MFC1, the COD concentrations in the influent and the effluent of the first and second compartments are shown in Figure 4.14. The average COD concentration in the influent of the first compartment was rather constant at 3,043 ± 139 mgCOD/L. On the other hand, the effluent COD concentration of the first compartment slightly decreased from approximately 1,600 mgCOD/L on day 1 - 10 of the operation to approximately 1,100 mgCOD/L on day 15 of operation. The average COD concentration in the effluent was 1,344 ± 359 mg/L, which was equivalent to COD removal efficiency of 56.06 ± 10.67 %. The effluent from the first compartment then became the influent of the second compartment. Regarding the results (Figure 4.14), the average effluent COD of the second compartment was 1,342 ± 303 mgCOD/L, corresponding to the COD removal

of only 0.15 ± 9.8 %. The results indicated that there was no COD removal in the second compartment of MFC1.

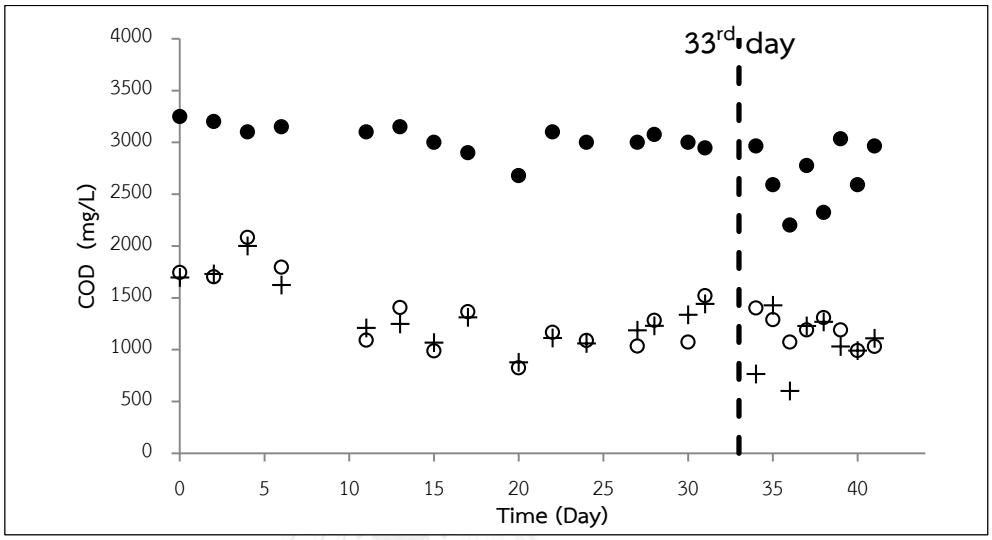


Figure 4.14 COD concentrations in MFC1 in Experiment 2

● Influent ○ Effluent from the first compartment ＋ Effluent from the second compartment

MFC3 had been operated for 40 days before replacing the anode electrode. Figure 4.15 shows the COD concentrations in MFC3. The average COD concentration in the influent was relatively stable at 2,999 ± 427 mgCOD/L. On the part of effluent, the COD concentration was also rather constant at 1,084 ± 127 mgCOD/L, corresponding to the COD removal efficiency of 62.49 ± 11.21 %. After the second compartment, COD concentrations further decreased to 994 ± 131 mgCOD/L, which was equivalent to the COD removal efficiency of 7.98 ± 10.23 %. The results show that the second compartment of MFC3 could also remove COD, suggesting the presence of microorganisms and microbial activities in this compartment.

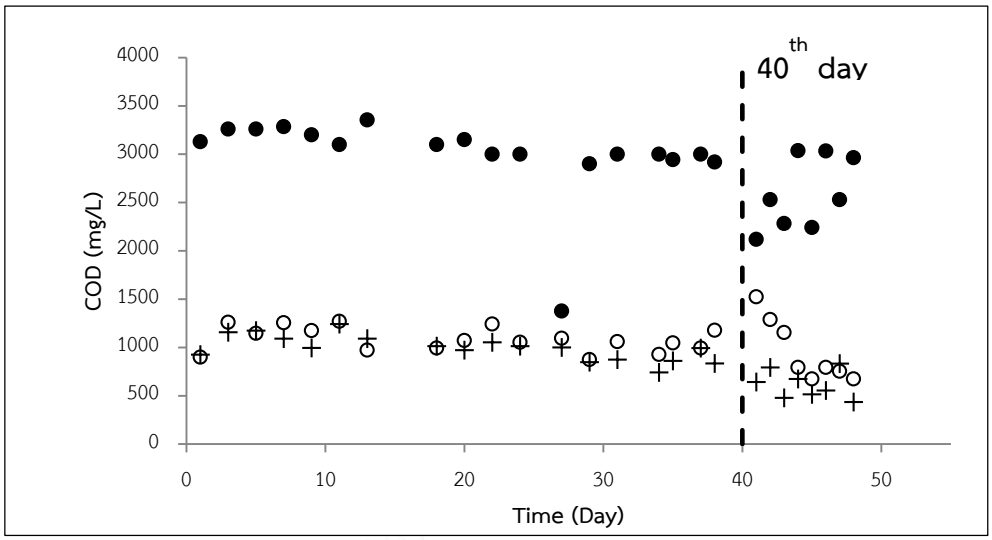


Figure 4.15 COD concentrations in MFC3 in Experiment 2

● Influent ○ Effluent from the first compartment ＋ Effluent from the second compartment

For MFC6 (Figure 4.16), the average COD concentration in the influent during the 40 days of operation was 3,033 ± 349 mgCOD/L. Effluent COD concentrations appeared to be stable at 1,500 mgCOD/L for the first 12 days. However, COD concentration rapidly dropped to nearly 1,000 mgCOD/L after day 12 of the operation, resulting in the fluctuation of effluent COD concentrations as shown in the standard deviation. The average COD concentration in the effluent of MFC6 was 1,110 ± 359 mgCOD/L, which was equivalent to the COD removal efficiency of 63.22 ± 11.57 %. The average COD concentration in the effluent of the second compartment was 1,003 ± 391 mgCOD/L, corresponding to the COD removal efficiency of 9.98 ± 16.5 %.

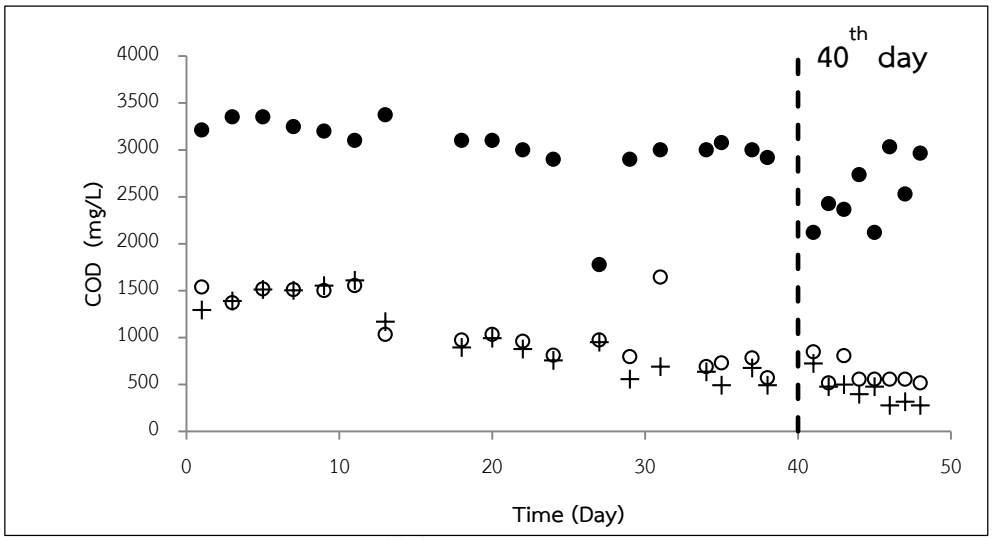


Figure 4.16 COD concentrations in MFC6 in Experiment 2

● Influent ○ Effluent from the first compartment ＋ Effluent from the second compartment

From the results, the COD removal efficiencies of 56.06 ± 10.67 %, 62.49 ± 11.21 %, and 62.49 ± 11.21 % were observed in the first compartments of MFC1, MFC3, and MFC6, respectively. The COD removal efficiencies in all MFCs in this experiment were slightly higher than those in Experiment 1-2 probably because the systems would be more air tight and less oxygen penetrated into the first compartment when both compartments were operated (Experiment 2) compared to when only the first compartment was operated (Experiment 1-2). We suspected that small amount of air might have leaked into the first compartments of MFCs via effluent tubes when only the first compartment was operated. Oxygen is generally toxic to anaerobic microorganisms (Prescott et al., 1996), such as methanogens, and could directly affect the COD removal efficiencies.

To further increase COD removal efficiencies in the first compartment, mixing, HRT, and the temperature may need to be optimized. Alternatively, anaerobic bioreactor configuration, such as anaerobic filters, UASBs and anaerobic fluidized bed

reactors, could be used instead to improve the efficiency of this compartment. Chou et al. (2008) found that 98% of initial COD concentration (1,500 mgCOD/L of acetate) was removed at the sulfate concentration of 3,000 mgSO_4^{2-}/L in an anaerobic filter. Regarding the COD removal in the second compartment, COD removal efficiencies were 0.15 ± 9.8 %, 7.98 ± 10.23 %, and 9.98 ± 16.5 % for MFC1, MFC3, MFC6, respectively, which were very low in all of the MFCs.

4.2.2 Sulfate removal and sulfide production

Samples for sulfate and sulfide analysis were collected from three different sampling points, including the influent, the effluent from the first compartment, and the effluent from the second compartment. For MFC1, the average sulfate concentration in the influent was constant at 3,036 ± 60 mgSO_4^{2-}/L, which was equivalent to COD:SO_4^{2-} ratio of 1 as expected. Figure 4.17 presents the sulfate concentration in MFC1 in Experiment 2. The results show that the effluent sulfate concentration in the first compartment slightly decreased over time from 2,200 mgSO_4^{2-}/L on the first day of operation to approximately 1,400 mgSO_4^{2-}/L at the end of the operation with the average effluent sulfate concentration of 1,736 ± 334 mgSO_4^{2-}/L (sulfate removal efficiency of 42.96 ± 10.45 %). The higher sulfate removal in this phase suggests that there were shifts in microbial community toward SRB more than that in Experiment 1-2.

Accordingly, the soluble sulfide concentration in the effluent of the first compartment was also higher than that in Experiment 1-2. The soluble sulfide concentrations (Figure 4.18) increased from 323 mgS^{2-}/L on the first day to approximately 500 mgS^{2-}/L at the end of the operation. The average soluble sulfide concentration in the first compartment was 400 ± 69 mgS^{2-}/L. Sulfide concentrations in MFC1 were in a high range that could inhibit microorganisms in the system, especially

methanogens. According to this sulfide level, methanogens were not likely to be predominant in this system.

In the case of the second compartment of MFC1, the average effluent sulfate concentration was 1,709 ± 249 mgSO_4^{2-}/L. The values were close to the influent of the second compartment (1,736 ± 334 mgSO_4^{2-}/L). The results indicated that there was no significant sulfate removal in the second compartment, which might help explain why there was no COD removal in the second compartment. In addition, the high soluble sulfide concentrations might inhibit microorganisms in the systems; therefore, there was no significant removal of both sulfate and COD in the second compartment of MFC1. For sulfide removal in the second compartment, about 60 mgS^{2-}/L was removed (sulfide removal efficiency of 14.23 ± 15.71 %), corresponding to sulfide concentration in the effluent of 342 ± 80 mgS^{2-}/L. However, the sulfide removal tended to decrease over time, which might be affected by both sulfur accumulation and biofilms on the anode electrode. Moreover, the deterioration of anode and cathode electrodes might also decrease the sulfide removal in the MFC, which will be discussed in the next section.

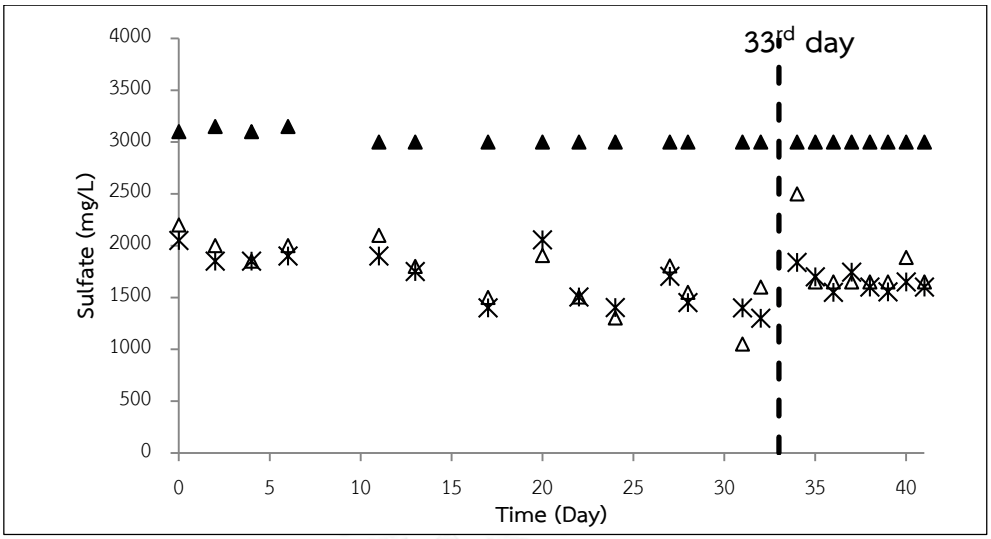


Figure 4.17 Sulfate concentrations in MFC1 in Experiment 2

▲ Influent △ Effluent from the first compartment ∗Effluent from the second compartment

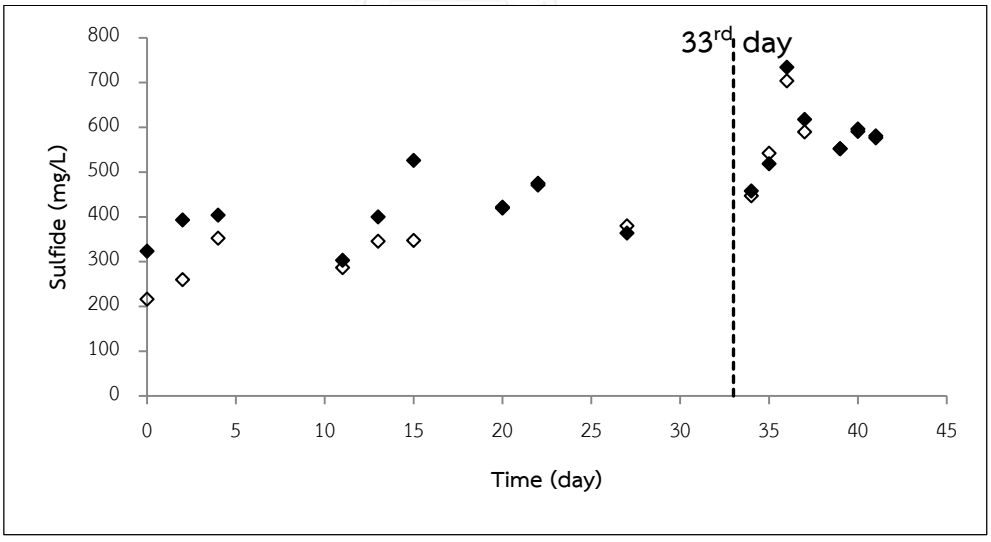


Figure 4.18 Sulfide concentrations in MFC1 in Experiment2

◆ Effluent from the first compartment ◇ Effluent from the second compartment

For MFC3, the average sulfate concentration in the influent was rather constant at 1,015 ± 29 mgSO_4^{2-}/L, which was equivalent to the COD:SO_4^{2-} ratio of 2.95. At this ratio, both methanogens and SRB were likely to coexist (Choi and Rim, 1991). Figure

4.19 and Figure 4.20 present the sulfate and soluble sulfide concentrations in MFC3. The results show that the sulfate in the effluent of the first compartment decreased rapidly from 198 mgSO_4^{2-}/L to approximately 53.8 ± 91.9 mgSO_4^{2-}/L after two days of the operation. This could indicate that the sulfate was removed successfully in this MFC. The sulfate removal in the first compartment of 95.01 ± 8.88 % was observed in MFC3.

In the case of soluble sulfide, sulfide concentrations in the first compartment fluctuated from 166 – 346 mgS^{2-}/L with the average sulfide concentration of 265 ± 59 mgS^{2-}/L. In this case, sulfide concentrations were in a medium to high range which could inhibit most of methanogens and some of SRB (Lens et al., 1998). Because of rather high sulfide production, COD removal under this condition was not as high as in previous research (Angelov et al., 2013; Hu et al., 2015).

In the case of the second compartment of MFC3, the average sulfate concentration after passing the second compartment was 59.22 ± 93.70 mgSO_4^{2-}/L, which was slightly higher than the influent of the second compartment (53.84 ± 91.86 mgSO_4^{2-}/L). The increase in sulfate in the second compartment could be due to sulfide oxidation by SOB on the anode electrode, which can use sulfide as electron donor and the anode electrode as electron acceptor. SOB have been found on anode electrodes in previous research (Cai et al., 2013; Sun et al., 2010). Sulfide could also get oxidized back to sulfate via abiotic sulfide oxidation at the anode electrode (Dutta et al., 2008; Zhang et al., 2009). The average sulfide concentration in the effluent from the second compartment was 237 ± 59 mgS^{2-}/L, which was equivalent to sulfide removal efficiency of 10.32 ± 5.01 %.

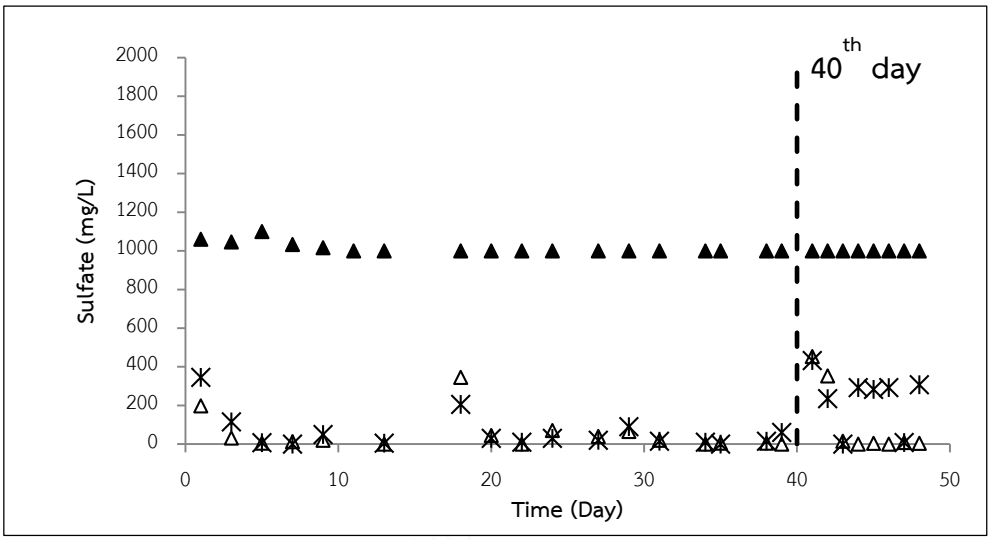


Figure 4.19 Sulfate concentrations in MFC3 in Experiment 2

▲ Influent △ Effluent from the first compartment ∗Effluent from the second compartment

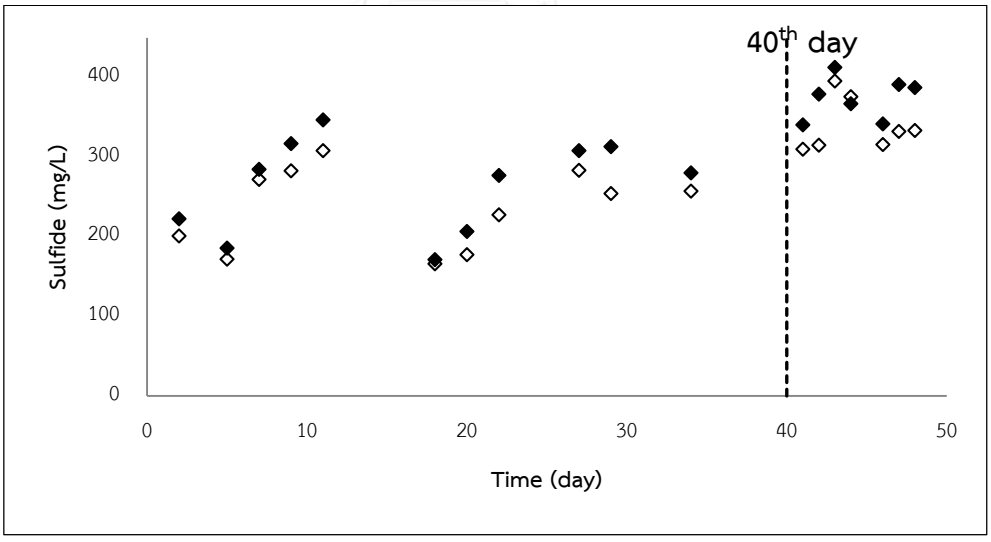


Figure 4.20 Sulfide concentrations in MFC3 in Experiment2

◆ Effluent from the first compartment ◇ Effluent from the second compartment

For MFC6 (Figure 4.21), the average sulfate concentration in the influent was rather constant at 509 ± 17 mgSO_4^{2-}/L for the first compartment of MFC6. Therefore, the COD:SO_4^{2-} ratio of the influent of MFC6 in this experiment was 5.96. At this

COD:SO_4^{2-} ratio, methanogens were likely to outcompete SRB in the system. Mostly, sulfate concentrations in the effluent of the first compartment were nearly 0 mgSO_4^{2-}/L (Figure 4.21). However, there were some points where sulfate was still detected, resulting in the average sulfate concentration of 16.85 ± 37.16 mgSO_4^{2-}/L in the effluent of the first compartment of MFC6. Therefore, the sulfate removal efficiency of the first compartment of MFC6 was 96.65 ± 7.44 %. For sulfide production in the first compartment of MFC6 (Figure 4.22), soluble sulfide concentrations were in the range of 62 and 165 mgS^{2-}/L, which should not inhibit SRB, but it might inhibit some methanogens (Lens et al., 1998). The average sulfide concentration of 119 ± 32 mgS^{2-}/L was observed in the effluent of the first compartment of MFC6.

For the second compartment of MFC6, the average sulfate concentration in the effluent was rather constant at 14.06 ± 18.28 mgSO_4^{2-}/L, which was quite close to the sulfate after passing the first compartment (16.85 ± 37.16 mgSO_4^{2-}/L). Therefore, only a slight amount of sulfate was removed in the second compartment of MFC6. However, there were some data points that the effluent sulfate concentrations were higher than those in the influent. Sulfate could also be generated from sulfide oxidation via SOB and/or abiotic oxidation process on the anode electrode as discussed earlier. On the other hand, the average sulfide concentration in the effluent of the second compartment was 100 ± 34 mgS^{2-}/L, which was equivalent to the sulfide removal efficiency of 15.69 ± 21.30 %.

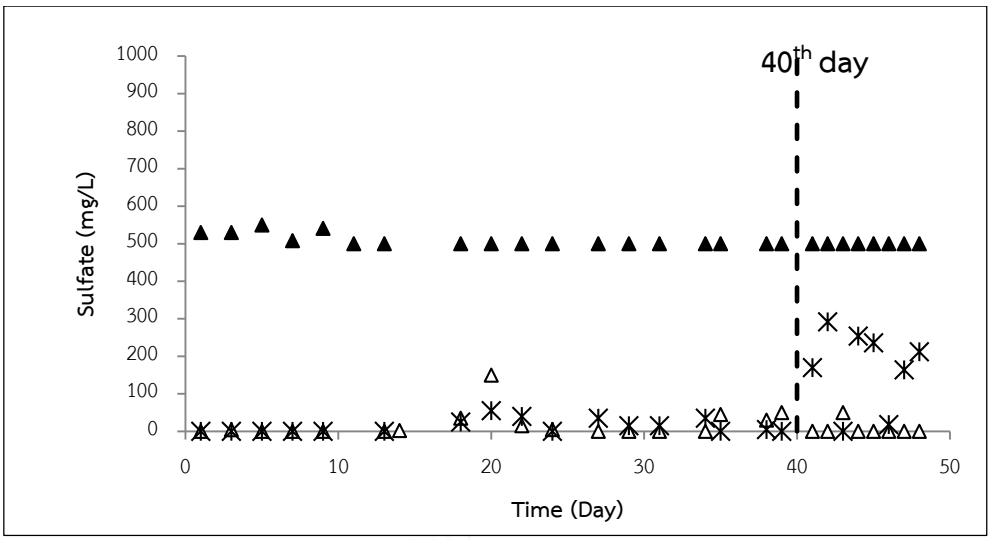


Figure 4.21 Sulfate concentrations in MFC6 in Experiment 2

▲ Influent △ Effluent from the first compartment ∗Effluent from the second compartment

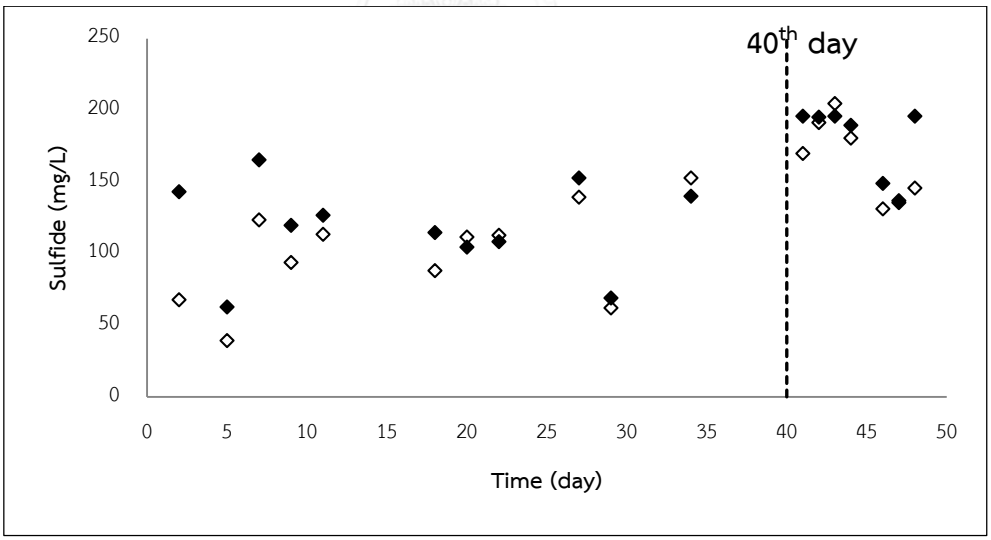


Figure 4.22 Sulfide concentrations in MFC6 in Experiment 2

◆ Effluent from the first compartment ◇ Effluent from the second compartment

The results show that the COD removal efficiencies in the first compartments were 56.06 ± 10.67, 62.49 ± 11.21, and 63.22 ± 11.57 % for MFC1, MFC3, and MFC6, respectively, corresponding to the COD removal of 1,593 ± 511, 1814 ± 610, and 1,822

± 591 mgCOD/L for MFC1, MFC3, and MFC6, respectively. On the other hand, the sulfate removal of 1,209 ± 455, 964 ± 93, and 492 ± 44 mgSO_4^{2-}/L were observed in MFC1, MFC3, and MFC6, respectively. Since the theoretical ratio of COD:SO_4^{2-} based on electron equivalents, assuming negligible biomass yield, was 0.67, the COD removal via sulfate reduction were 810, 646, and 330 mgCOD/L for MFC1, MFC3, and MFC6, respectively. These COD removal values were equivalent to 50.85, 35.61, and 18.11 % of total COD removal in the first compartment of MFC1, MFC3, and MFC6, respectively.

The different proportions of COD removal via sulfate reduction may have influence the microbial communities and populations in the systems. Previous research has clearly showed the effects of COD:SO_4^{2-} ratio on the competition between SRB and methanogens (Choi and Rim, 1991). Methanogens can usually outcompete SRB when the COD:SO_4^{2-} ratio was greater than 2; whereas, SRB tend to outcompete methanogens when the COD:SO_4^{2-} ratio was lower than 1.3 (Chou et al., 2008). However, a recent study by Hu et al. (2015) reported the coexistence of SRB and methanogens even at the COD:SO_4^{2-} ratio as low as 1 when the COD concentration was high enough (3,000 mgCOD/L), which was similar to the condition in our study. Therefore, the coexistence of COD removal via methanogenesis besides sulfate reduction at the COD:SO_4^{2-} ratio of 1 was not totally unexpected at the COD concentration used in this study (3,000 mgCOD/L). Nevertheless, SRB populations should still be the highest in MFC1 compared with MFC3 and MFC6 based on the proportions of COD removal via sulfate reduction.

However, the microorganisms in MFC1 might be affected by the high sulfide concentrations (400 ± 69 mgS^{2-}/L) that it produced. This high sulfide level could be inhibitory to many groups of anaerobic microorganisms, including SRB themselves (Lens et al., 1998). The high sulfide concentrations in MFC1 might contribute to the lower COD removal efficiencies in this MFC compared with MFC3 and MFC6, which produced

less sulfide. Angelov et al. (2013) used a microbial fuel cell based on electroactive sulfate-reducing biofilm to treat sulfate rich wastewater. The results showed that only 21.3% of COD (initial COD concentration of 8,240 mgCOD/L) was removed from this system with HRT of 24 hr and 250 mg/L of H_2S was generated. In addition, they also investigated the effect of COD removal on HRT (9 – 72 hr). The results indicated that increased HRT can improve COD removal efficiencies of MFCs.

According to the proportions of COD removal via sulfate reduction to the total COD removal (50.85, 35.61, and 18.11 % in MFC1, MFC3, and MFC6, respectively), the population of methanogens should be the highest in MFC6. However, in MFC6, sulfate could still be removed effectively, suggesting that SRB could still grow at the COD:SO_4^{2-} ratio of 6. The results suggest that although the system was operated at the high COD:SO_4^{2-} ratio, SRB could still survive in the system when sulfate was present in the wastewater. It should be noted that the COD:SO_4^{2-} ratio may not be the sole factor affecting the competition between SRB and methanogens, but the absolute concentrations of COD and sulfate could also play an important role in the competition between SRB and methanogens (Hu et al., 2015).

Wu et al. (2015) also reported that sulfate concentration in wastewater was the main parameter effecting on both COD and sulfate removal efficiencies in expanded granular sludge bed (EGSB) reactors because both COD and sulfate removal efficiencies decreased from 65% to 59.1% for COD and from 95% to 65% for sulfate when sulfate concentrations were increased from 2,000 mgSO_4^{2-}/L to 3,000 mgSO_4^{2-}/L at constant COD at 5,000 mgCOD/L, equivalent to COD:SO_4^{2-} ratio of 2.5 and 1.67, respectively. In addition, both methanogens and SRB decreased when sulfate concentrations were changed from 2,000 to 3,000 mgSO_4^{2-}/L.

In the second compartments of the MFCs, the COD removal efficiencies were 0.15 ± 9.83, 7.98 ± 10.23, and 9.98 ± 16.50 % for MFC1, MFC3, and MFC6, respectively, which corresponded to the COD removal of 1.62 ± 114.03, 85.89 ± 111.51, and 101.68 ± 229.90 mgCOD/L for MFC1, MFC3, and MFC6, respectively. On the other hand, sulfate removal in the second compartments of MFC1, MFC3, and MFC6 were 23.33 ± 140, -5.06 ± 57.07, and 3.49 ± 30.76 mgSO_4^{2-}/L, respectively. Sulfide removal in the second compartments of MFC1, MFC3, and MFC6 were 49.51 ± 57.74, 24.08 ± 13.74, and 15.69 ± 21.30 mgS^{2-}/L, respectively.

For MFC1, COD could be removed in the second compartment of the MFC. However, some of the sulfate was removed in this compartment, suggesting that SRB might grow in the second compartment. For MFC3 and MFC6, since the remaining sulfate from the first compartment was quite low, there was no significant removal of sulfate in the second compartment of both MFC3 and MFC6. However, the remaining COD concentrations from the first compartments of MFC3 and MFC6 were still high, resulting in the potentials to stimulate microbial growth in the second compartments of MFC3 and MFC6. The COD removal in the second compartments of both MFC3 and MFC6 could probably due to the activities of methanogens that could grow in the systems, which will be discussed in Section 4.4. For sulfide removal, sulfide could be removed by two pathways including 1) abiotic sulfide oxidation on the anode electrodes and 2) microbial sulfide oxidation by SOB on the anode electrodes. To further investigate these mechanisms, microbial community analysis of the biofilms on the anode electrodes and the experiment on abiotic sulfide oxidation in fuel cells were performed, which will be discussed in the Section 4.3. The results show that the pH increased after wastewater passed the second compartment probable due to the electrical generation in the system. Proton (H^+) in the second compartment could be

transferred to the cathode electrodes resulting in the increase of the pH in this compartment (Du et al., 2007).

The results in this study suggest that COD:SO_4^{2-} ratio was the parameter affecting sulfate removal, sulfide removal, and electricity generation, which were consistent with the study by Zhang et al. (2012). Zhang et al. (2012) investigated the effects of HRT and COD:SO_4^{2-} ratio on the performance of UASB-MFC system. The results suggested that the optimum COD:SO_4^{2-} and HRT for treating sulfate in wastewater were 3.7 and 55.6 hr, respectively, obtaining sulfate removal efficiency of 71.3%. However, the sulfate removal of 95.01 ± 8.88 % observed in MFC3 was higher than that in Zhang et al. (2012). Types of anaerobic reactors might also affect the removal of sulfate in wastewater.

Table 4.3 Concentrations of COD, SO_4^{2-}, pH, and sulfide in Experiment 2

Parameters	MFC1		MFC3		MFC6	
	1-1	1-2	3-1	3-2	6-1	6-2
Influent COD (mgCOD/L)	3,043 ± 139	1,344 ± 359	2,999 ± 427	1,084 ± 127	3,033 ± 349	1,110 ± 359
Effluent COD (mgCOD/L)	1,344 ± 359	1,342 ± 303	1,084 ± 127	994 ± 131	1,110 ± 359	1,003 ± 391
COD removal efficiency (%)	56.06 ± 10.67	0.15 ± 9.8	62.49 ± 11.21	7.98 ± 10.23	63.22 ± 11.57	9.98 ± 16.5
Influent sulfate ($mgSO_4^{2-}/L$)	3,036 ± 60	1,736 ± 334	1,015 ± 29	53.8 ± 91.9	509 ± 17	16.85 ± 37.16
Effluent sulfate ($mgSO_4^{2-}/L$)	1,736 ± 334	1,709 ± 249	53.8 ± 91.9	59.22 ± 93.70	16.85 ± 37.16	14.06 ± 18.28
Sulfate removal efficiency (%)	42.96 ± 10.45	N.A.	95.01 ± 8.88	N.A.	96.65 ± 7.44	N.A.
pH	7.37 ± 0.17	7.57 ± 0.32	7.08 ± 0.12	7.22 ± 0.20	7.00 ± 0.16	7.12 ± 0.20
Sulfide (mgS^{2-}/L)	400 ± 69	342 ± 80	265 ± 59	237 ± 59	119 ± 32	100 ± 34
Sulfide removal efficiency (%)		14.23 ± 15.71		10.32 ± 5.01		15.69 ± 21.30

4.2.3 Electrical production in MFCs

OCV and voltage across the electrodes over time

The OCV and voltage across the electrodes at 1,000 Ω external resistances of all MFCs decreased over time (Figure 4.23). All MFCs generated maximum value of both OCV and voltage across electrode at 1,000 Ω external resistance after the installation of electrical equipment (day 1). The maximum OCV of 635, 475, 460 mV were observed in MFC1, MFC3, and MFC6, respectively. For the voltage across the electrodes at 1,000 Ω external resistance, the maximum values of 300, 110, and 183 mV were also found on the same day as the maximum OCV values in MFC1, MFC3, and MFC6, respectively.

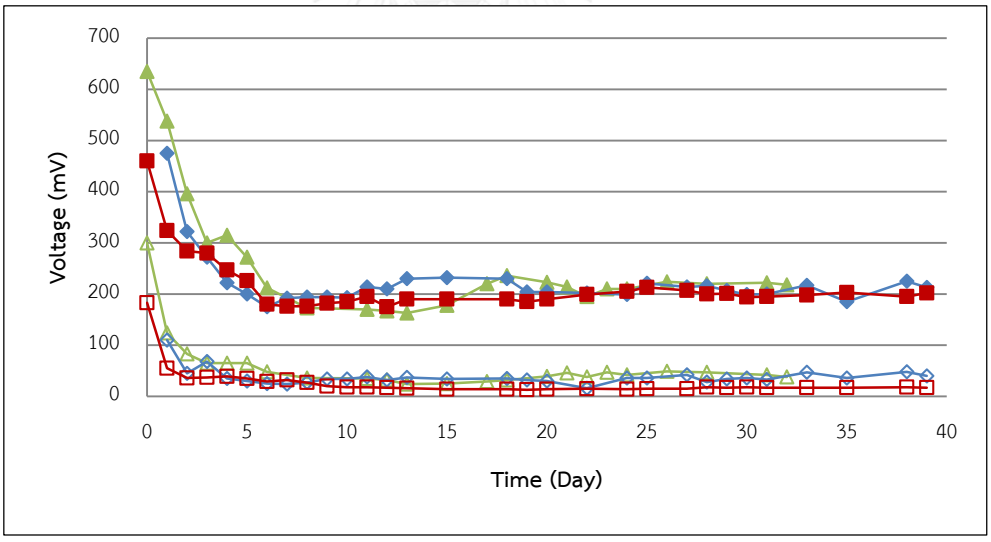


Figure 4.23 OCV in ▲MFC1, ◆MFC3, and ■MFC6 and the voltage across external resistance of 1,000Ω in △MFC1, ◇MFC3, and □MFC6

For MFC1, both OCV and voltage across the electrodes dropped rapidly from 635 to 163 mV for OCV and from 300 to 24 mV for the voltage across electrodes, respectively, on day 1-13 of operation. Then, the OCV and voltage across the

electrodes increased again to rather constant at about 220 mV on day 17 of operation for OCV and at about 40 mV on day 20 for the voltage across the electrode at 1,000 Ω external resistance.

For both MFC3 and MFC6, the OCVs decreased during the first 8 days of operation. After that the value became rather constant at 209 ± 13 and 195 ± 9 mV for MFC3, and MFC6, respectively. The voltages across the electrodes at 1,000 Ω external resistance also had the same trend as OCV, which decreased from 110 and 183 mV for MFC3 and MFC6, respectively, to 34 and 20 mV for MFC3 and MFC6, respectively, during the first 8 days of operation. Then, the values became rather constant at 35 ± 6 and 16 ± 2 mV for MFC3 and MFC6, respectively.

The decrease in OCV in all MFCs was probably due to the deterioration of the cathode electrodes (activation loss) since the replacement of anode electrodes did not improve the OCV in all MFCs. On the other hand, the decrease in voltage across the electrodes at 1,000Ω external resistances was due to the increase in the voltage losses that can be improved by the replacement of anode electrodes. These voltage losses were likely to consist of activation loss and ohmic loss, which will be discussed in the subsequent section. The higher voltage in MFC1 than those in the other MFCs was probable due to two factors: 1) higher ionic strength and 2) higher dissolved sulfide concentration in the systems.

A previous study by Liu et al. (2005) showed that the increase in solution ionic strength from 100 to 400 mM (NaCl) could increase the power density of the MFC from 720 to 1330 mW/m^2. The increase in ionic strength in MFCs can improve the proton transfers, thereby decreasing the ohmic loss of the systems. In our study, MFC1 has the highest ionic strength since the highest amount of sulfate was added into the system. On the other hand, the increase in sulfide concentration could increase the

power densities in abiotic fuel cells fed with sulfide. The voltage losses also decreased with increasing sulfide concentrations.

Electricity power generation and voltage losses

In Experiment 2, polarization curves (I-V curve) and power density curves were constructed on day 1, 2, 4, 11, 18, 21, and 24 of operation by varying the external resistances from 47 Ω to 150,000 Ω (Figure 4.24). Table 4.4 summarizes the estimated parameters from polarization curves and power density curves of MFC1, MFC3, and MFC6. The maximum power density of each MFC on each day was estimated from the power density curve using polynomial regression. The maximum power densities of MFC1, MFC3, and MFC6 on day 1 were 9.33, 1.79, and 1.41 mW/m^2, respectively, which were estimated from the power density curves (Figure 4.24a). The power densities obtained in this study were in the same range with the power density reported in Chou et al. (2013) using a dual-chamber MFC treating sulfate wastewater (1-13 mW/m^2 on day 1 of operation). However, there were previous studies such as Zhang et al. (2012) and Angelov et al. (2013) that obtained high power densities (989.6 ± 22.9 mW/m^2 and 680 mW/m^2, respectively) using MFC treating sulfate rich wastewater under continuous operation.

However, the power densities during the MFC operation at 1,000 Ω external resistances in MFC1, MFC3, and MFC6 were 7.40, 1.04, and 0.96 mW/m^2, respectively. It should be noted that the operating condition of MFC1 (1,000 Ω external resistance) was near the maximum power density which occurred at the external resistance of 2,000 Ω. On the other hand, the maximum power densities of MFC3 and MFC6 occurred at the external resistance of 3,000 and 5,000 Ω, respectively, which were far from operating condition (1,000 Ω external resistances). Therefore, to obtain more power

densities from MFC3 and MFC6, the external resistors should be adjusted to 3,000 and 5,000 Ω for MFC3 and MFC6, respectively.

The polarization curves of MFCs can be used to estimate the voltage loss in the systems. Figure 4.24b, 4.24d, 4.24f, 4.24h, 4.24j, 4.24l, and 4.24n show the polarization curves in this study. The results suggest that both activation losses and ohmic losses could occur in all MFCs. However, the concentration losses were not found in all MFCs. The activation losses and ohmic losses in these systems could be affected by many factors as following:

Activation losses:

1) Sulfur accumulation and biofilm formation of non-exoelectrogenic microorganisms on the anode electrodes, which can decrease the active surface areas of the anode electrodes, thereby increasing the activation losses.
2) The deterioration of the cathode electrodes, which could increase the activation loss of the systems, resulting in the decrease in both OCV and voltage across the electrodes.
3) Initial sulfide concentrations. High concentration of sulfide should increase the driving force of the reactions on the anode electrodes, resulting in less activation loss.

Ohmic losses:

1) Solution ionic strength. The higher the ionic strength is, the less in ohmic loss could occur.
2) Sulfur accumulation and biofilm formation of non-exoelectrogenic microorganisms on the anode electrodes, which can also increase

the ohmic losses by obstructing the electron transfers in the systems.

3) The biofilm formation on PEM, which could impede the proton transfers from the anode chamber to cathode chamber, resulting in the increase in ohmic losses in the systems.

The estimation of voltage losses in MFCs, which consist of only activation loss and ohmic loss, commonly can be calculated using Tafel equation combined with ohmic loss equation ($V = Voc_v - (a + b \log |j| + jR)$. However, most of the polarization curves observed in this study appeared to be straight lines. Therefore, the slope of each graph was used to estimate the total losses in the systems.

Liu et al. (2005) reported that the distances between the anode and cathode electrodes and ionic conductivity of the electrolytes were the factors directly affecting ohmic losses. The increase in ionic strength by NaCl from 100 mM to 400 mM could increase power output from 720 to 1,330 mW/m^2(Liu et al., 2005). Since the distances between the anode and cathode electrodes of all MFCs were 2 cm, the initial ohmic loss should be the same. However, ionic conductivity of the electrolyte of each MFC was different. Ionic conductivity is generally related to total dissolved solids. Since MFC1 had higher remaining sulfate in the second compartment than MFC3 and MFC6; therefore, the ohmic loss of MFC1 should be lower than those in MFC3 and MFC6.

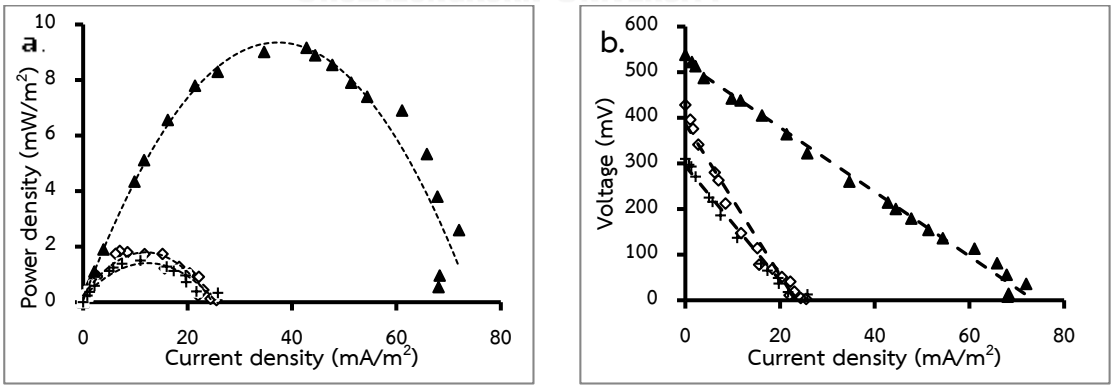
Moreover, the decrease in voltage losses, which were likely to be activation losses, with increasing sulfide concentrations. The higher sulfide concentration in MFC1 could also contribute to the lower voltage losses in MFC1 compared with the other MFCs.

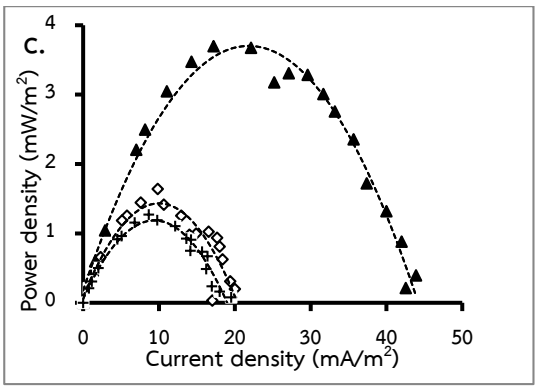
Table 4.4 shows the slope of polarization curves and maximum power densities on day 1, 2, 4, 11, 18, 21, and 24 of operation. For MFC1, the slope was rather constant

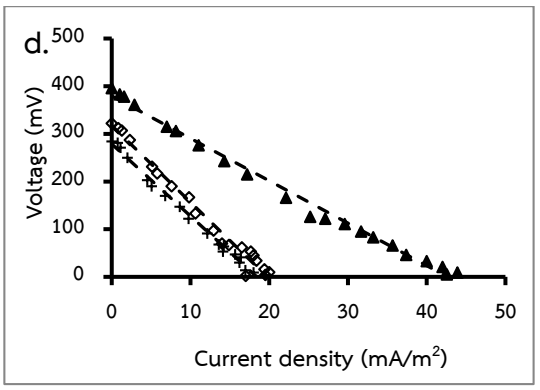
at about 9 Ω·m^2, excepting on day 11 and day 18. According to the high slope on these days, the voltages across the 1,000 Ω external resistance on these days were also at the low values.

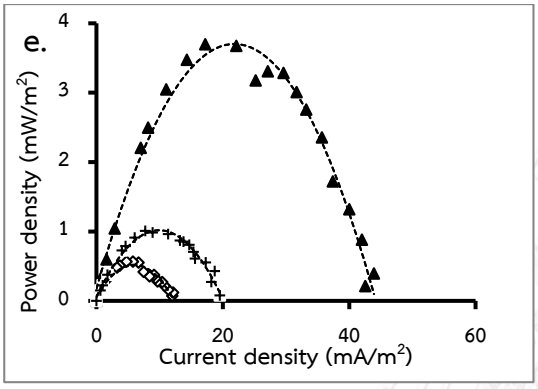
For MFC3, the slope was in the range of 12.5 to 17.4 Ω·m^2. However, the slope increased to 26.30 Ω·m^2 on day 21. Large amount of sludge in the first compartment was lost into the second compartment on day 20, and it might obstruct the electron flows in the system, resulting in the increase in slope on day 21. Then, the sludge and remaining wastewater in the second compartment was drained out. As a result, the decrease in the slope on polarization curve after the sludge draining was observed on day 24 of operation.

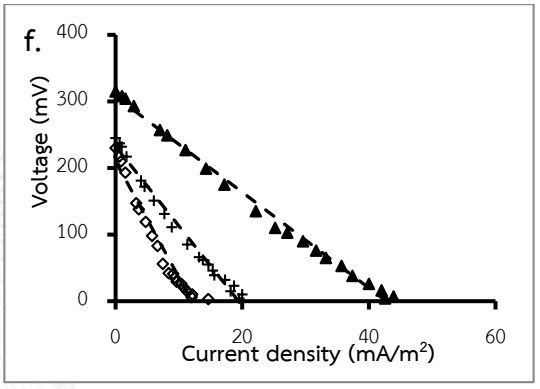
For MFC6, the slope increased over time from approximately 12.5 to 30 Ω·m^2. The increases in sulfur and non-exoelectrogenic microorganisms on the anode electrode might increase the activation loss in the MFCs by decreasing the active surface areas for reactions on anode electrodes. In addition, sulfur and non-exoelectrogenic microorganisms on the anode could also impede the electron transfers in the systems, resulting in the increase in ohmic loss over time.

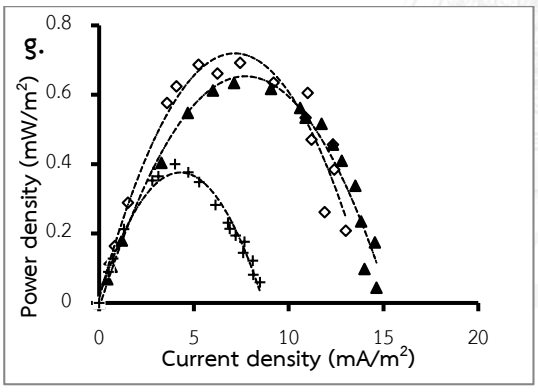


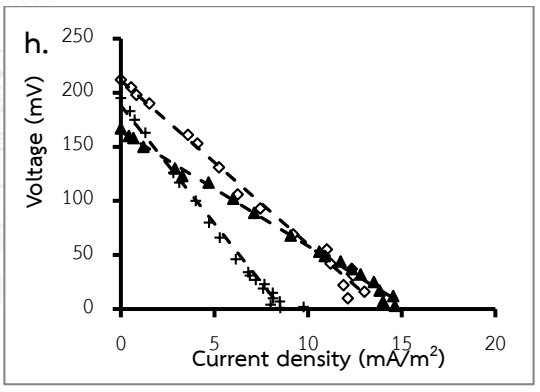

c.
Power density (mW/m2)
Current density (mA/m2)

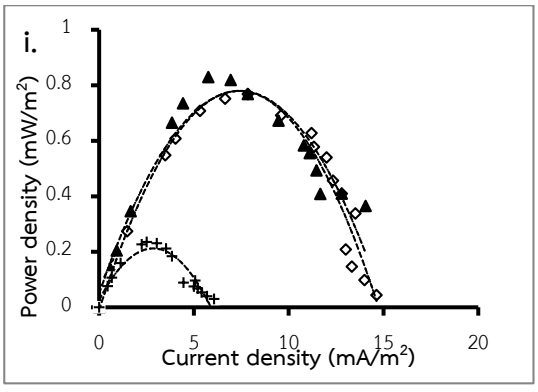

d.
Voltage (mV)
Current density (mA/m2)

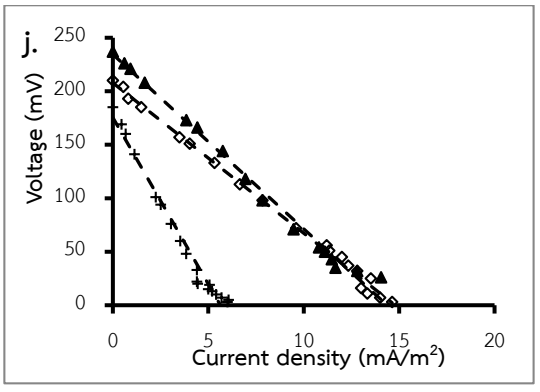

e.
Power density (mW/m2)
Current density (mA/m2)


f.
Voltage (mV)
Current density (mA/m2)


g.
Power density (mW/m2)
Current density (mA/m2)


h.
Voltage (mV)
Current density (mA/m2)


i.
Power density (mW/m2)
Current density (mA/m2)


j.
Voltage (mV)
Current density (mA/m2)

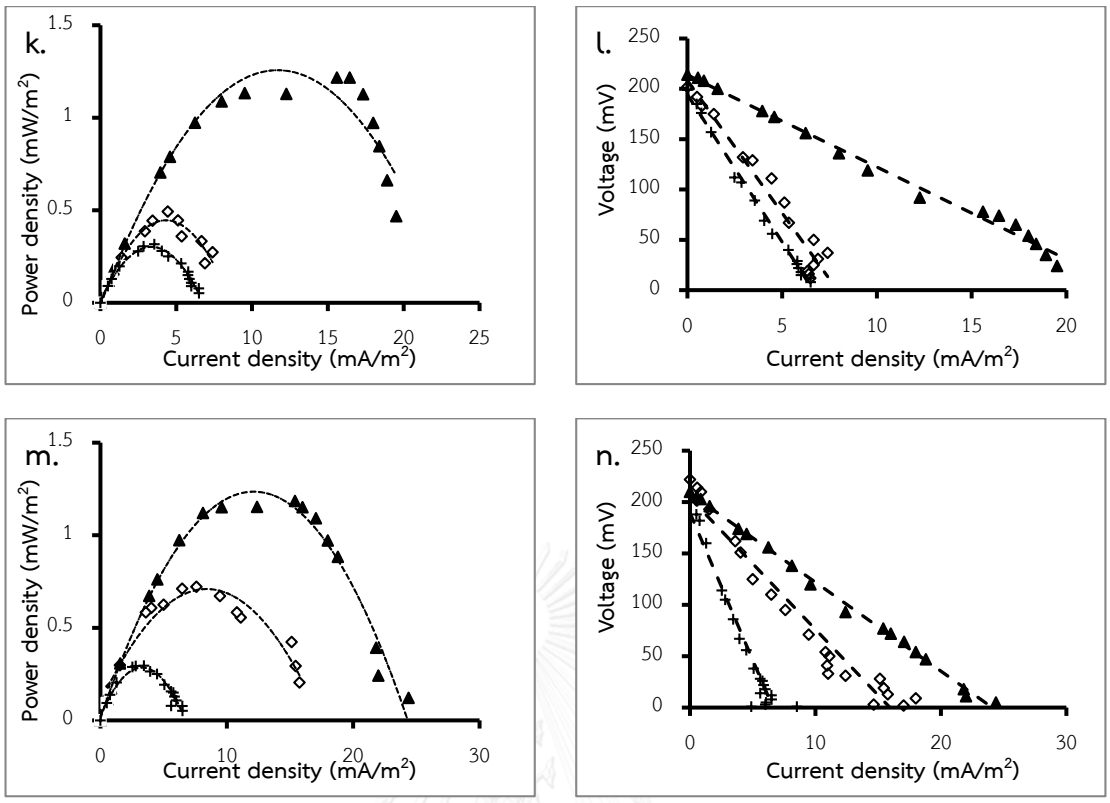


Figure 4.24 Polarization curve on day (a) 1, (c) 2, (e) 4, (g) 11, (i) 18, (k) 21, and (m) 24 and Power density curve on day (b) 1, (d) 2, (f) 4, (h) 11, (j) 18, (l) 21, and (n) 24 of operation:

▲ MFC1, ◇ MFC3, and + MFC6

Table 4.4 Electricity parameters estimated from the polarization and power density curves of MFC1, MFC3, and MFC6.

Day	slope ($\Omega \cdot m^2$)			Maximum power density (mW/m^2)		
	MFC1	MFC3	MFC6	MFC1	MFC3	MFC6
1	7.05	16.57	12.54	9.33	1.79	1.41
2	9.00	16.06	15.12	3.39	1.43	1.19
4	9.10	17.41	11.96	3.70	1.02	0.52
11	10.34	14.93	21.81	0.65	0.71	0.38
18	16.30	13.57	31.13	0.83	0.78	0.24
21	9.14	26.30	29.27	1.24	0.44	0.30
24	9.16	12.88	28.65	1.18	0.49	0.32

Effects of COD removal on electrical production

In MFC1, COD removal of 1.62 ± 114.0 mgCOD/L was observed in the second compartment, which was considered very low and negligible. However, this MFC generated the highest power density in comparison to the other MFCs. The average power density of MFC1 was 0.98 ± 0.28 mW/m^2. Therefore, it can be concluded that the COD was not the main energy source for electrical production in MFC1. In addition, exoelectrogenic microorganisms, which can transfer electrons directly to anode electrodes (Du et al., 2007; Logan, 2008), were not found in this system. The results on microbial community analysis will be presented and discussed in the next section.

On the other hand, COD removal of 85.9 ± 111.5 and 101.7 ± 229.9 mgCOD/L were observed in the second compartment in MFC3 and MFC6, respectively. Certain amount of COD was removed in both MFC3 and MFC6. Three possible pathways for COD removal in these MFCs include: 1) COD removal by methanogenesis, 2) COD removal via sulfate-reduction by SRB, and 3) COD removal by exoelectrogenic microorganisms on the anode electrode. Among these 3 pathways, only the third

pathway by exoelectrogenic microorganisms can convert chemical energy (COD) into electrical energy. The COD removed via methanogen and SRB could not generate electricity. The average power densities of 0.60 ± 0.16 and 0.31 ± 0.06 mW/m^2 were observed in MFC3 and MFC6, respectively. Exoelectrogenic microorganisms on the anode electrode could generate electrical energy in the systems. However, this process was not likely to be the main process to generate electricity in MFC3 and MFC6. According to the microbial community analysis, exoelectrogenic microorganisms were not significantly observed on the anode electrodes. In contrast, methanogens were found on the anode electrodes of both MFC3 and MFC6, suggesting that COD removal observed in these MFCs were likely due to methanogenesis.

In addition, the formation of biofilms on the anode electrode by non-exoelectrogenic microorganisms might obstruct the electron transfers, resulting in low power density production. Due to the high dissolved sulfide concentrations in the wastewater in all MFCs, the conditions may not be suitable for exoelectrogenic microorganisms, which were different from previous studies (Sun et al., 2010). The presence of large amount of SOB and exoelectrogenic microorganisms on the anode electrode obtained in Sun et al. (2010) should be a key factor encouraging the voltage generated from biofilm on anode to be higher and more persistent than the voltage generated from the abiotic process only.

Types of substrates may also contribute to both microbial communities and electrical production in MFCs. Many types of exoelectrogenic microorganisms prefer small organic compounds, such as acetate, lactate, and butyrate, to large organic compounds, such as glucose. Therefore, the complete fermentation of glucose to VFAs in the first compartment would be essential for the electricity generation in the second compartment.

The organic loading rates might be another factor affecting biofilm formation on anode electrodes. Gil et al. (2003) suggested that lower organic loading rates can generate higher current densities than higher organic loading rates. It should be noted that high organic loading rate might be more favorable to fermenters than exoelectrogenic microorganisms in mixed cultures of microorganisms (Kim et al., 2004; Moon et al., 2006; Rabaey et al., 2003). Therefore, increase in HRT not only help improve fermentation in the first compartment, but it might also stimulate the growth of exoelectrogenic microorganisms on anode electrodes.

Effects of sulfide removal on electrical production.

Sulfide, the product of sulfate reduction process, was slightly removed in the second compartment of all MFCs. The dissolved sulfide concentrations of 49.51 ± 57.74, 24.08 ± 13.74, and 15.69 ± 21.30 mgS^{2-}/L were removed in MFC1, MFC3, and MFC6, respectively. Sulfide can be used for electricity generation by two pathways, including 1) abiotic sulfide oxidation on anode electrodes, and 2) microbial sulfide oxidation via SOB (Zhang et al., 2013a). Zhang et al. (2013a) indicated that abiotic sulfide oxidation (50%) on anode electrodes was the main mechanism when using sulfide and glucose as the initial substrates. In addition, 25% of sulfide was removed by microbial sulfide oxidation via SOB and the last 25% was removed by adsorption and volatilization.

The results of microbial community analysis show that there was certain amount of SOB, such as *Dyella thiooxydans* (Anandham et al., 2011), on the anode electrodes of all MFCs. Therefore, microbial sulfide oxidation could also be one mechanism to generate electricity in all MFCs. The amount of SOB in MFC1 appear to be greater than those in MFC3 and MFC6, which could be due to higher amount of sulfide in MFC1. Nevertheless, since very small amounts of SOB were observed in all

MFCs, microbial sulfide oxidation should not be the main mechanism of electricity generation in the MFCs. Other processes, such as abiotic sulfide oxidation on the anode electrodes was likely to be the main mechanism of electricity generation, which will be further discussed in the following section.

In addition, the average voltage across the electrodes at 1,000 Ω external resistance in MFC1, MFC3, and MFC6 were 38.17 ± 7.95, 35.14 ± 6.57, and 16.28 ± 1.84, respectively. The voltages across the electrodes had the same trend as sulfide removal in the MFCs, which were 58.20 ± 67.89, 28.30 ± 16.16, and 18.45 ± 25.04 mgS^{2-}/L in MFC1, MFC3, and MFC6, respectively. Moreover, the results of abiotic sulfide oxidation in fuel cells suggest that there could be a relationship between sulfide removal and electricity production. Therefore, sulfide was likely to play an important role on electricity generation in these MFCs.

However, sulfur production and accumulation on anode electrodes could be one of the factors hindering sulfide oxidation by decreasing the active areas and electrical conductivity of the anode electrodes. The deterioration of MFCs by sulfide accumulation on the anode electrode has been previously reported in Sangcharoen et al. (2015). The decreases in voltages across the electrodes and power densities over time could be due to the accumulation of sulfur on the anode electrodes. Besides sulfur accumulation on anode electrodes, biofilm formation of non-exoelectrogenic microorganisms on the anode electrodes and biofilm formation on PEM could also impede sulfide oxidation and proton diffusion, respectively, which might decrease the power densities in the MFCs.

Columbic efficiencies

Since the main mechanism of electrical production in these MFCs was likely to be sulfide oxidation, the columbic efficiencies were calculated based on sulfide

removal in the Equation CE = $C_p/C_t \times 100$. The columbic efficiencies of 2.80 ± 4.51, 1.34 ± 1.60, and 0.84 ± 0.53 % were observed in MFC1, MFC3, and MFC6, respectively. The columbic efficiencies of all MFC observed in this study were in the range of 0.7 – 8.1%, which were observed in the study by He et al. (2005) using an upflow microbial fuel fed with artificial organic wastewater containing fermentable substrates with alternative electron acceptors. High slope of polarization curves in these MFCs might be the main factor contributing to the low columbic efficiencies in all MFCs. Therefore, to improve the columbic efficiencies in the MFCs, the voltage loss, which consisted of activation loss and ohmic loss, should be reduced to a minimum value.

4.2.4 MFC operation after replacing the anode electrodes

The anode electrodes in all MFCs were replaced with the new ones, which were identical to the previous ones, on day 33 in MFC1 and on day 40 in MFC3 and MFC6 of operation. The COD, sulfate, and sulfide were analyzed in this experiment. Figure 4.26, 4.27, and 4.28 show the COD and sulfate concentrations in the second compartments of MFC1, MFC3, and MFC6, respectively. For the electrical production, the OCV and voltage across the electrodes at 1,000 Ω external resistances were measured over time (Figure 4.25). The polarization curves and power densities curves were constructed on day 1, 2, 3, 4, 5, 6, and 8 of operation.

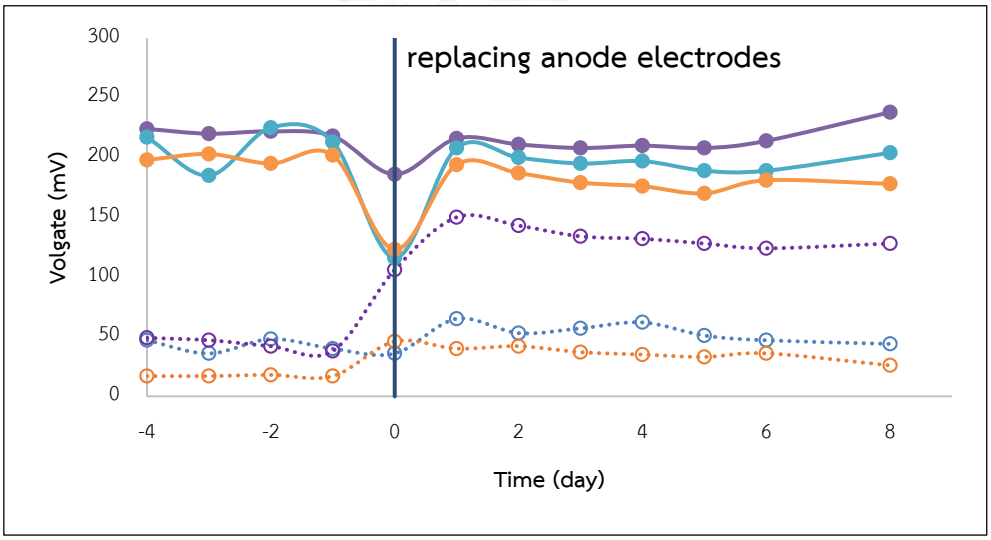


Figure 4.25 OCV and voltage across the electrodes at 1,000 Ω external resistances: OCV in ●MFC1, ● MFC3, and ● MFC6 and voltage across the electrodes at 1,000Ω external resistances in ○ MFC1, ○ MFC3, and ○ MFC6

For MFC1, the average COD concentrations in the influent and the effluent (Figure 4.26) were 1,184 ± 145 and 1,053 ± 270 mgCOD/L, respectively, which corresponded to the COD removal of 131 ± 279 mgCOD/L. On the other hand, the average sulfate in the influent and effluent were 1,786 ± 300 and 1,654 ± 100 mgSO_4^{2-}/L, respectively, which was equivalent to the sulfate removal of 131 ± 236 mgSO_4^{2-}/L.

The results indicate that the second compartment of MFC1 could remove a small extent of both COD and sulfate. However, the average sulfide removal was only 6.43 ± 18.83 mgS^{2-}/L. The removal of sulfate commonly generates sulfide as a final product. The sulfide produced from sulfate reduction might result in apparently low sulfide removal in the second compartment of MFC1. In the case of electricity generation, the OCV did not change after the installation of new anode electrode, unlike the voltage across the electrodes at 1,000 Ω external resistances. The voltage across the electrodes at 1,000 Ω external resistances suddenly increased from 38 mV to 106 mv after changing the anode electrode. Similar observation was reported in Sangcharoen et al. (2015). The average voltages were 134 ± 9 mV which was higher than the voltage in Experiment 2 (38.2 ± 7.9 mV).

It should be noted that the OCV decreased over time in MFC1 in Experiment 2. No improvement in the OCV after replacing the anode electrodes suggests that the cathode electrodes might be the main problem contributing to the decrease in OCV in Experiment 2. Because the new anode electrode had no sulfur accumulation, the active surface area was higher than that in the old one, resulting in higher power density production after replacing the anode electrode. The slope of polarization curves of all MFCs (Table 4.5) were also lower than those observed in Experiment 2. The accumulation of elemental sulfur on the anode could increase the slope of polarization curves over time, resulting in the decrease in maximum power densities over time as shown in Table 4.5. Sulfur accumulation on the anode was observed by SEM-EDX, which will be discussed in details in the following section.

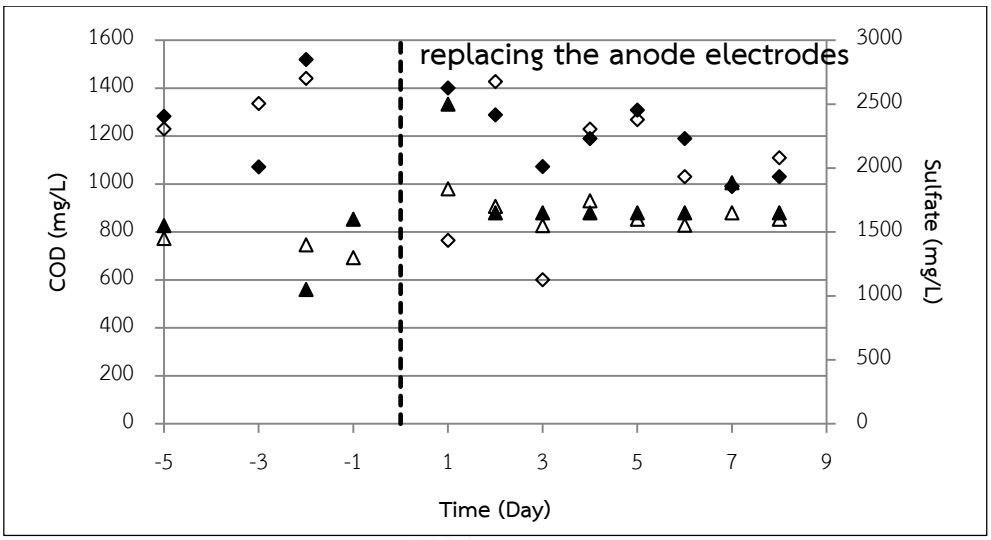


Figure 4.26 COD and sulfate concentrations in the second compartment after replacing the anode electrode in MFC1:
◆ influent COD, ◇ effluent COD, ▲ influent sulfate, and △ effluent sulfate

For MFC3, the average COD concentration in the influent and the effluent (Figure 4.27) were 957 ± 322 and 616 ± 145 mgCOD/L, respectively, corresponded to the COD removal of 341 ± 318 mgCOD/L. In contrast, sulfate was increased from 105 ± 186 mgSO_4^{2-}/L to 231 ± 151 mgSO_4^{2-}/L in the second compartment, which was equivalent to the sulfate production of 126 ± 180 mgSO_4^{2-}/L (about 1.31 mM). However, sulfide concentration of 34 ± 26 mgS^{2-}/L (about 1.06 mM) was removed from the second compartment of this MFC. Sulfate production was slightly higher than sulfide removal in the system. Sulfate production could occur by three possible pathways consisting of 1) microbial sulfide oxidation via SOB, 2) abiotic sulfide oxidation on anode electrode, and 3) microbial sulfur oxidation via SOB. It might be possible that hydrogen sulfide in the gas phase might dissolve into wastewater and get oxidized to sulfate via the possible pathways mentioned above. In addition, the remaining elemental sulfur in the second compartment from the previous experiment might be oxidized to sulfate via SOB (Zhang et al., 2008).

For electrical production of MFC3, the voltage across the electrodes at 1,000 Ω external resistances rapidly increased from 36 mV to 65 mV after one day of operation. The higher voltage after replacing the anode electrode might be caused by three factors including: 1) the lower slope on polarization curves after replacing the anode electrode, 2) greater sulfide removal after replacing the anode electrode, and 3) sulfate was the final product instead of sulfur. Oxidation number of sulfate is 6 whereas oxidation number of elemental sulfur is 0. Therefore, the anode electrode could receive more electrons when sulfide is oxidized to sulfate compared with sulfur. Besides higher voltage generation, the production of sulfate instead of sulfur could alleviate the problems related to sulfur accumulation on the anode electrode. However, sulfate production in MFC was not desirable because one of the objectives was to remove sulfate from wastewater.

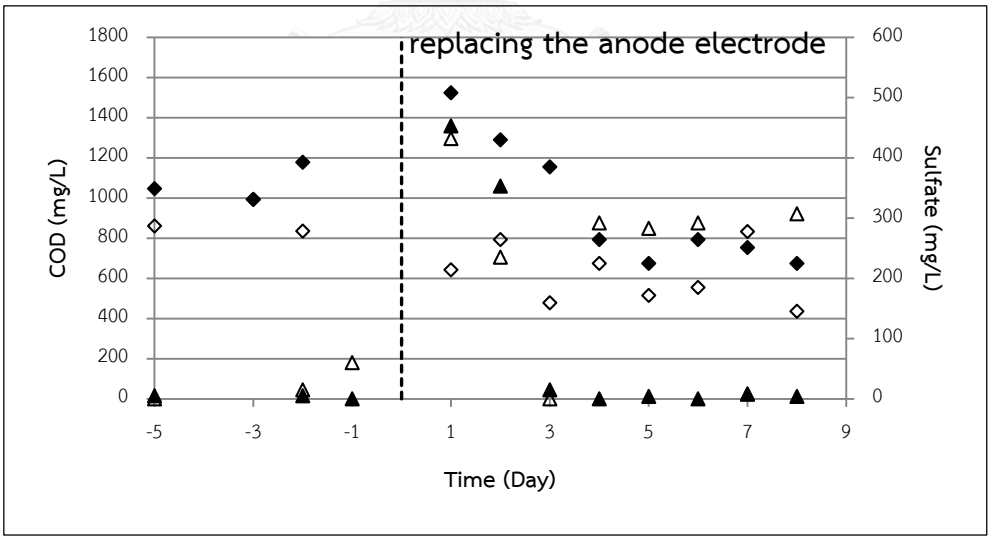


Figure 4.27 COD and sulfate concentrations in the second compartment after replacing the anode electrode in MFC3:

◆ influent COD, ◇ effluent COD, ▲ influent sulfate, and △ effluent sulfate

For MFC6, the average COD concentration in the influent and the effluent (Figure 4.28) were 613 ± 133 and 430 ± 149 mgCOD/L, respectively, corresponding to the COD removal of 183 ± 97 mgCOD/L. On the contrary, the average sulfate of 162 ± 119 mgSO_4^{2-}/L (1.69 mM) of were generated in the MFC6. The removal of COD suggests that microorganisms, such as methanogens might be present in the second compartment of MFC6. The average sulfide of 14 ± 20 mgS^{2-}/L (0.44 mM) was removed in the second compartment of MFC6. The sulfide removal was not balanced with the sulfate production in the second compartment of MFC6 based on the mass balance of sulfur. Sulfide in the gas phase and the remaining elemental sulfur in the second compartment from Experiment 2 might also serve as a substrate for sulfate production in the system. For electrical generation, the average voltage across the electrodes at 1,000 Ω external resistances was 35.6 ± 5.2 mV, which was higher than those in MFC6 in Experiment 2. The increase in voltage could be caused by several factors as mentioned above. The polarization curves and power density curves were constructed every day except day 7 as shown in Figure 4.29.

In conclusion, replacing the anode electrodes could reduce the slopes of polarization curves in the MFCs, resulting in the higher voltage across the electrodes and higher power density generation. The slopes of MFC1 were lower than those in MFC3 and MFC6. It was probably due to the higher ionic strength and higher sulfide in MFC1 than those in MFC3 and MFC6 as discussed earlier. However, the activation losses as observed in the OCV still remained after the replacement of anode electrodes. Therefore, these activation losses were likely to be due to the cathode electrodes and/or PEM rather than the anode electrodes. These activation losses could be derived from biofilm formation on PEM, the deterioration of platinum on cathode electrodes, and the decrease in proton permeability of PEM (Logan and Rabaey, 2012). On the other hand, the sulfate was generated in the systems. To reduce sulfate generation,

the prevention of oxygen leakage into the MFCs should be considered for limiting sulfide oxidation via SOB in suspended solids.

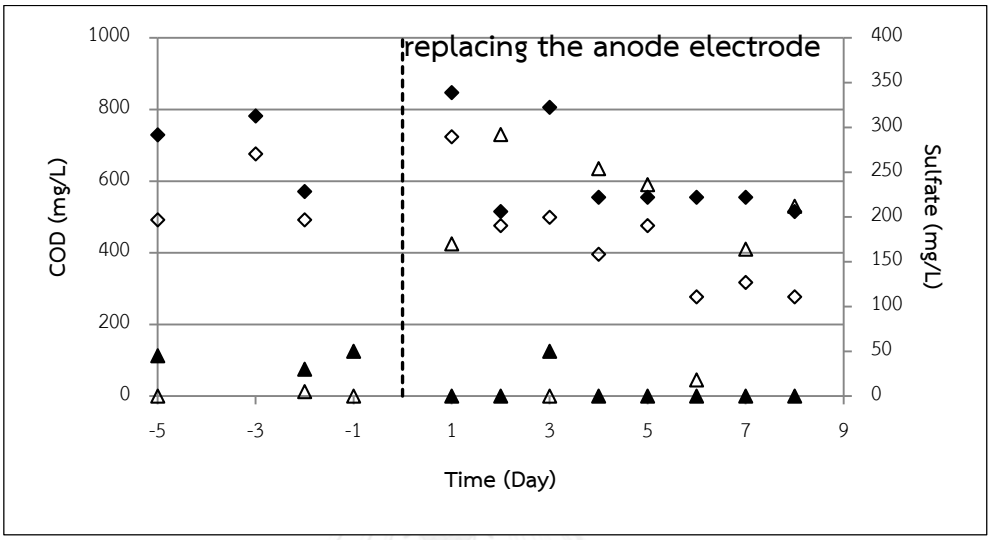
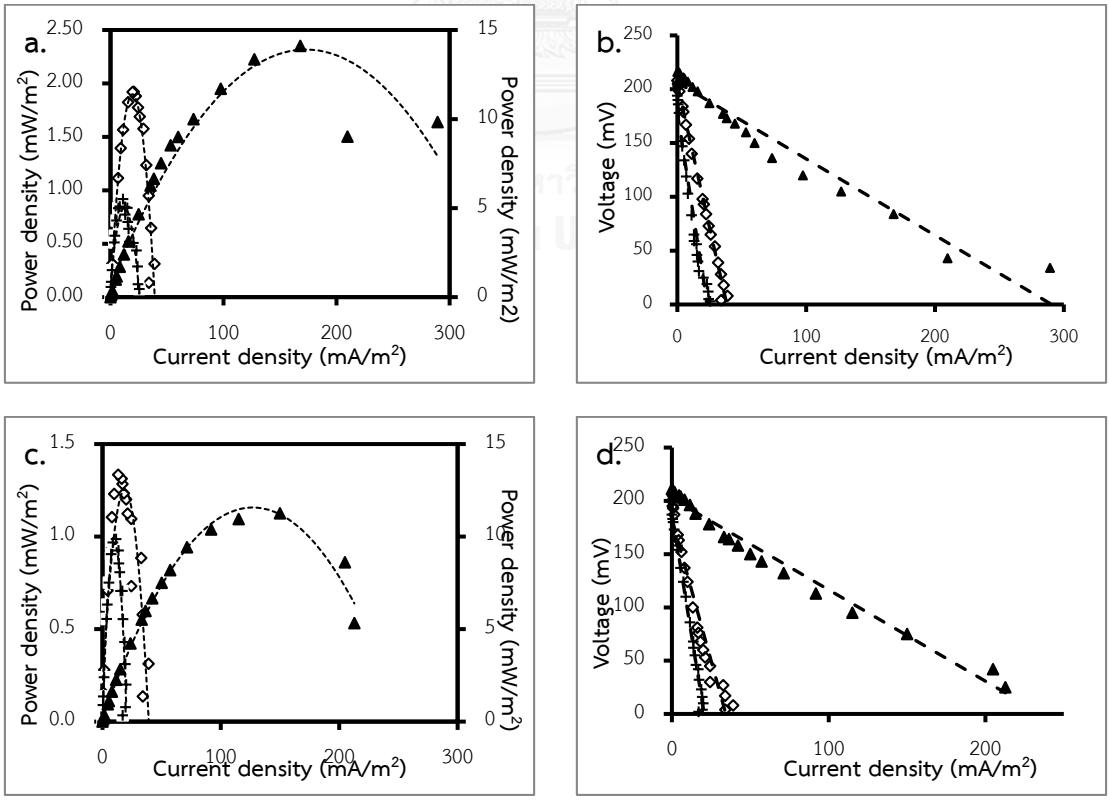


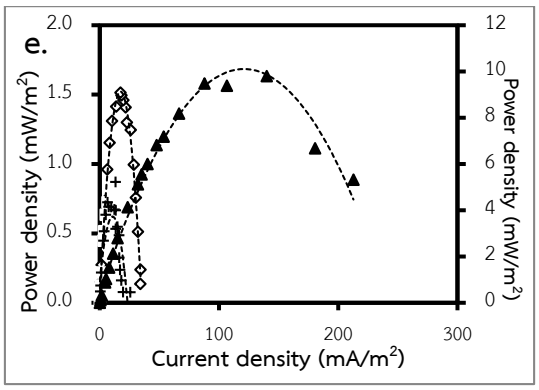
Figure 4.28 COD and sulfate concentrations in the second compartment after replacing the anode electrode in MFC6:

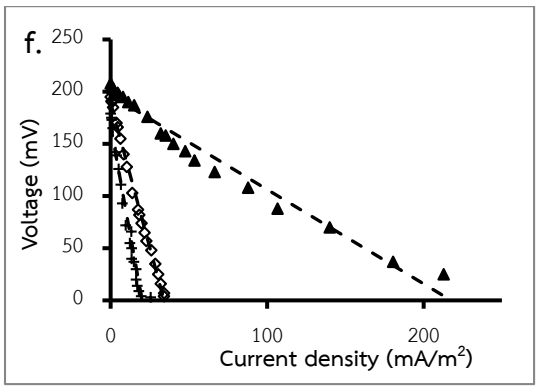
◆ influent COD, ◇ effluent COD, ▲ influent sulfate, and △ effluent sulfate

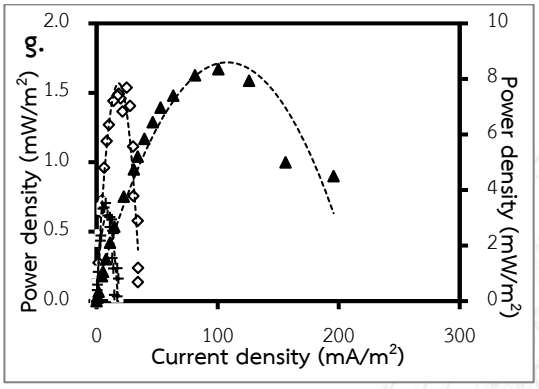
Table 4.5 Maximum power densities and slope of polarization curves in MFC1, MFC3, and MFC6 after replacing the anode electrodes

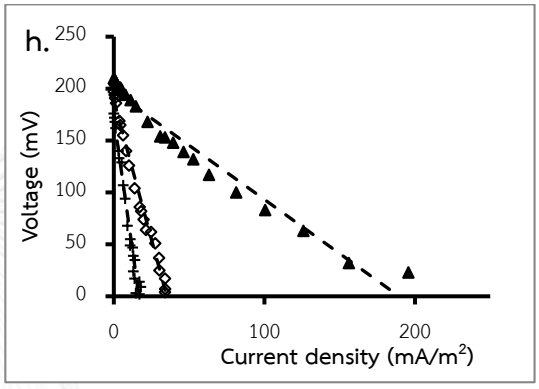
Day	MFC1		MFC3		MFC6	
	Maximum power densities (mW/m^2)	Slope ($\Omega \cdot m^2$)	Maximum power densities (mW/m^2)	Slope ($\Omega \cdot m^2$)	Maximum power densities (mW/m^2)	Slope ($\Omega \cdot m^2$)
1	15.3	0.71	1.9	5.32	0.83	7.60
2	11.66	0.86	1.26	5.33	0.96	9.17
3	9.68	0.90	1.53	5.54	0.62	8.32
4	8.63	1.04	1.56	5.36	0.62	10.26
5	8.04	1.00	1.23	6.54	0.6	9.48
6	8.17	1.10	1.09	6.85	0.58	10.36
8	7.4	1.76	1.03	8.11	0.42	14.60

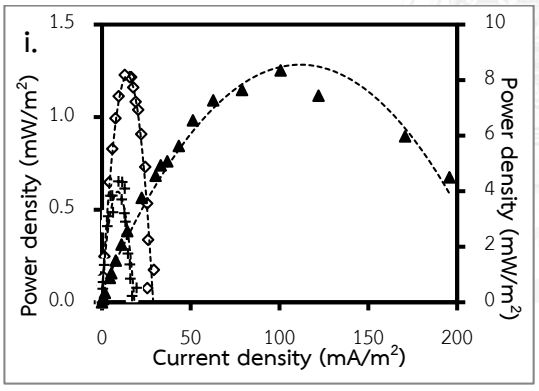


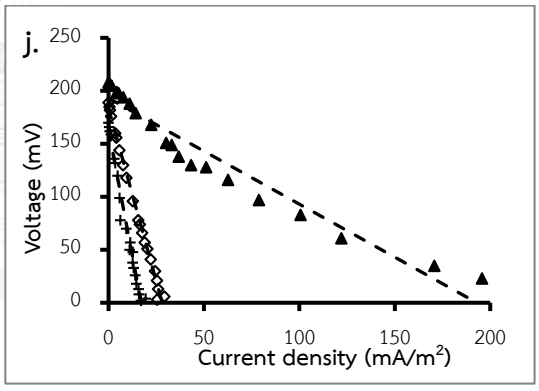

e.
Power density (mW/m2)
Power density (mW/m2)
Current density (mA/m2)
0.0
0.5
1.0
1.5
2.0
0
2
4
6
8
10
12
0
100
200
300

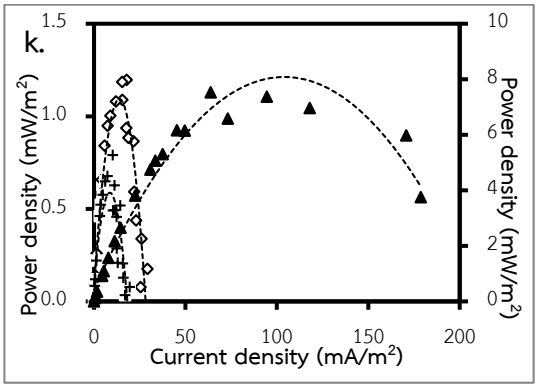

f.
Voltage (mV)
Current density (mA/m2)
0
50
100
150
200
250
0
100
200

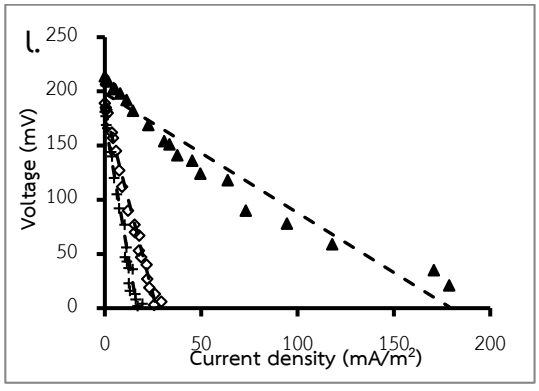

g.
Power density (mW/m2)
Power density (mW/m2)
Current density (mA/m2)
0.0
0.5
1.0
1.5
2.0
0
2
4
6
8
10
0
100
200
300


h.
Voltage (mV)
Current density (mA/m2)
0
50
100
150
200
250
0
100
200


i.
Power density (mW/m2)
Power density (mW/m2)
Current density (mA/m2)
0.0
0.5
1.0
1.5
0
2
4
6
8
10
0
50
100
150
200


j.
Voltage (mV)
Current density (mA/m2)
0
50
100
150
200
250
0
50
100
150
200


k.
Power density (mW/m2)
Power density (mW/m2)
Current density (mA/m2)
0.0
0.5
1.0
1.5
0
2
4
6
8
10
0
50
100
150
200


l.
Voltage (mV)
Current density (mA/m2)
0
50
100
150
200
250
0
50
100
150
200

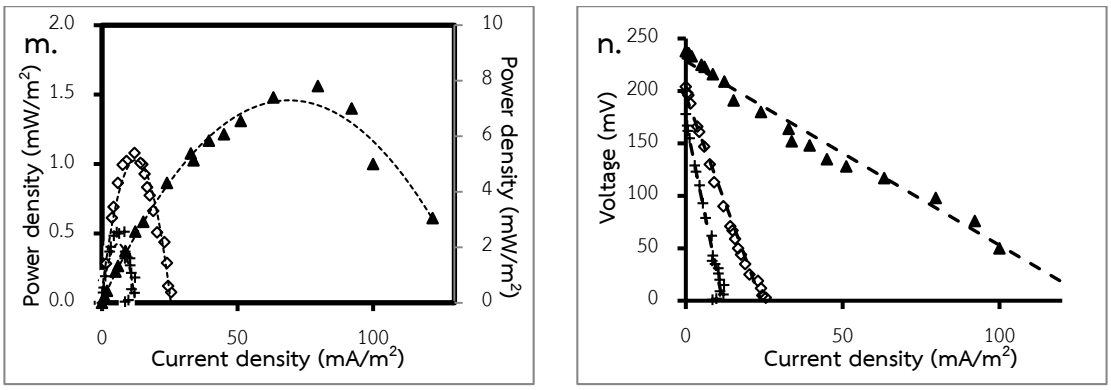


Figure 4.29 Power density curves and polarization curves after replacing the anode electrodes: power density curves on day (a) 1, (c) 2, (e) 3, (g) 4, (i) 5, (k) 6, and (m) 8 and polarization curves on day (b) 1, (d) 2, (f) 3, (h) 4, (j) 5, (l) 6, and (n) 8 of operation.:

▲ MFC1, ◇ MFC3, and + MFC6, Note: MFC1, use secondary axis (right axis) for power density curves.

4.2.5 Surface analysis of anode electrodes via scanning electron microscopy

The surfaces of anode electrodes before and after MFC operation were analyzed by scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX) to investigate the elements attached and accumulated on the anode electrodes. Figure 4.30 shows the characteristics of the surface and the elemental analysis of an activated carbon cloth before using as the anode electrode in all of the MFCs. The results show that carbon was the main element in carbon cloth. On the other hand, elemental sulfur was rarely observed.

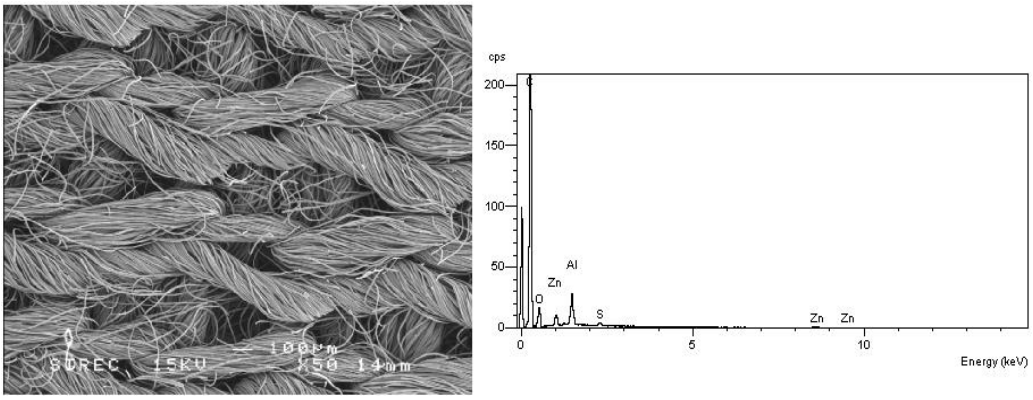


Figure 4.30 SEM/EDX analysis of an activated carbon cloth before using as the anode electrode

The anode electrodes used in MFC1, MFC3, and MFC6 were analyzed after day 8 of operation (the end of MFC operation in Experiment 2). Figure 4.31a, 4.31b, and 4.31c show the SEM/EDX results of the anode electrodes after day 8 of operation in MFC1, MFC3, and MFC6, respectively. The results indicate that elemental sulfur was formed and accumulated on the anode electrodes. It should be noted that sulfide oxidation on anode electrodes occurred, resulting in simultaneous sulfide removal and electricity generation in the MFCs. However, accumulation of elemental sulfur could cause several adverse effects on the electricity generation in MFCs. Elemental sulfur on anode electrodes not only decreased the active surface areas of the electrodes but they might also prevent the accumulation of EEM biofilm on anode electrodes. In addition, sulfur accumulation can also impede electron transfers on the anode electrodes due to low electrical conductivity of sulfur. As a results, sulfur accumulation on the anode electrodes could lead to low efficiencies in electricity generation, sulfide removal, and COD removal in MFCs. Future research on material coating of anode electrodes to prevent sulfur accumulation might be interesting for the improvement of anode life cycles.

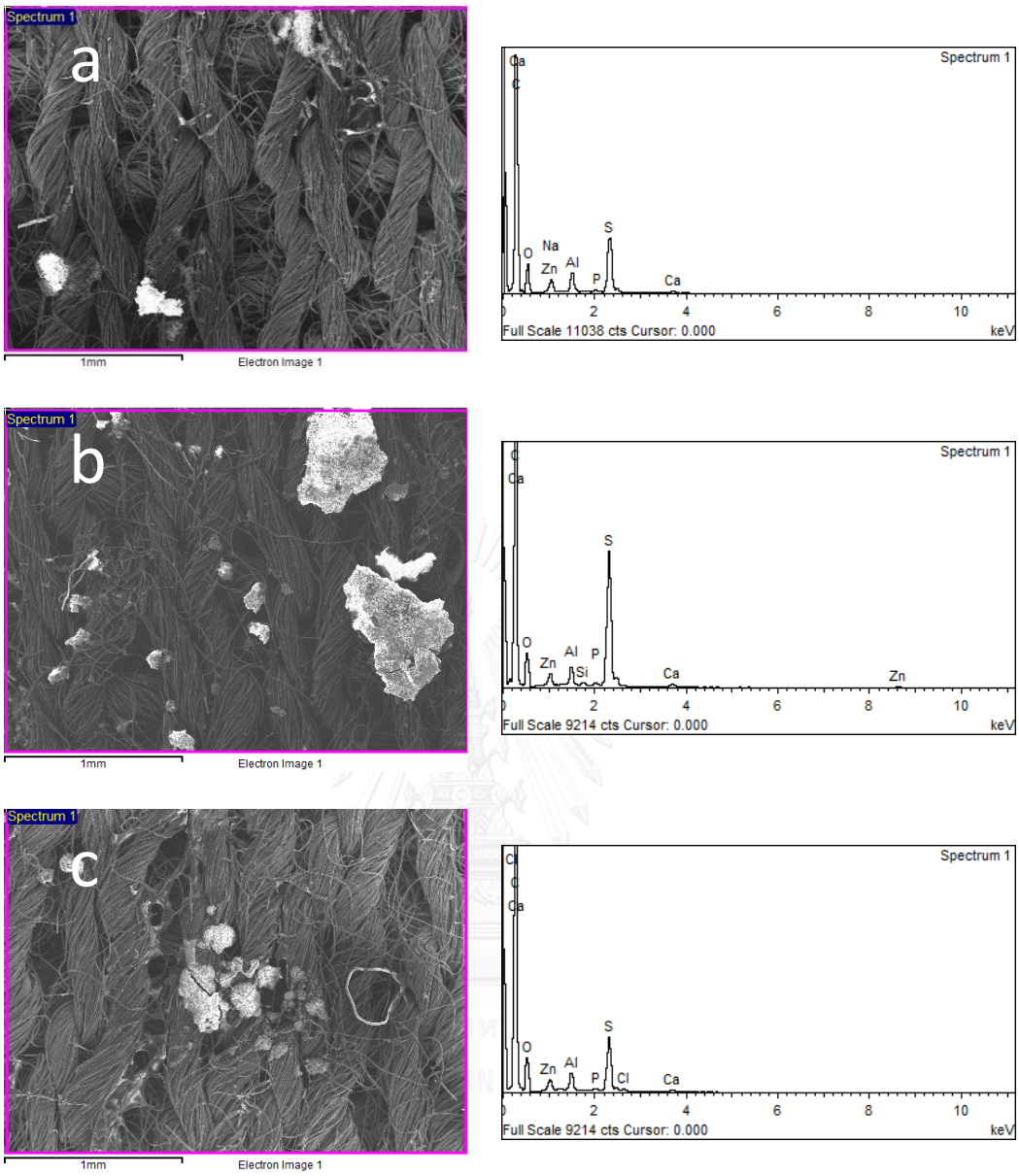


Figure 4.31 SEM/EDX results of the anode electrodes after day 8 of operation in a) MFC1, b) MFC3, and c) MFC6

The used anode electrodes after day 33 in MFC1 and day 40 in both MFC3 and MFC6 of operation at the end of Experiment 2 were rinsed with deionized water to remove loosely attached biofilm and elemental sulfur on the surfaces. Figure 4.32a, 4.32b, and 4.32c show the SEM/EDX results of the used anode electrodes in MFC1, MFC3, and MFC6, respectively. The elemental analysis shows that most elemental sulfur could be removed after rinsing with deionized water. Therefore, the anode

electrode might be reused as the electrode again after removing loosely attached biofilm and elemental sulfur by rinsing with water. However, the reuse of anode electrodes still requires further studies.

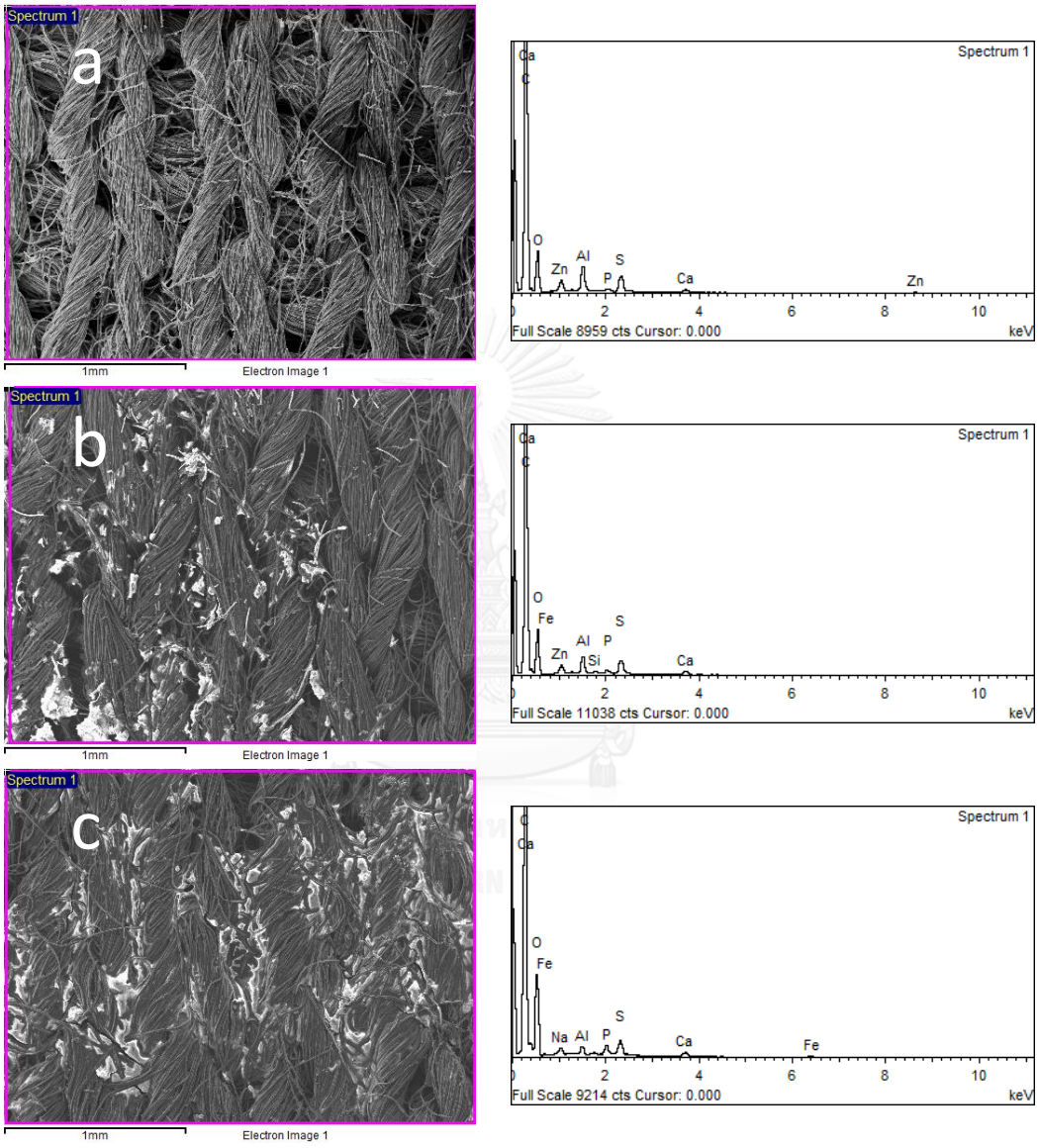


Figure 4.32 SEM/EDX results of the used anode electrodes after day 33 for MFC1 and day 40 for MFC3 and MFC6 of operation in a) MFC1, b) MFC3, and c) MFC6 after rinsing with deionized water to remove loosely attached biofilm and elemental sulfur.

4.3 Experiment 3: Abiotic fuel cell operation

An abiotic fuel cell fed with sulfide was operated for 9 days at three different sulfide concentrations, including 99.94 ± 17.31 mgS^{2-}/L on day 1-3, 258.16 ± 45.14 mgS^{2-}/L on day 4-6, and 413.18 ± 20.68 mgS^{2-}/L on day 7-9. These sulfide concentrations were similar to the average sulfide concentrations in the first compartments of MFC1, MFC3, and MFC6 in Experiment 2, respectively. The abiotic fuel cell had the hydraulic retention time of 7.5 hr, identical to the second compartment of the MFCs in Experiment 2. $NaHCO_3$ was also added into the synthetic wastewater as a pH buffer in the system. The pH in synthetic wastewater was adjusted to 7. Sulfide, OCV, and the voltage across electrodes at 1,000Ω external resistance were measured over time.

On day 1-3, the average sulfide concentration of 99.94 ± 17.31 mgS^{2-}/L was fed into the MFC. The average sulfide in the effluent was 28.47 ± 15.21 mgS^{2-}/L, which was equivalent to the sulfide removal of 71.47 ± 14.72 mgS^{2-}/L and the sulfide removal efficiency of 72.20 ± 12.64 %. Figure 4.33 shows sulfide concentrations in this Experiment. On the other hand, both the OCV and voltage across electrodes at 1,000Ω external resistances (Figure 4.34) were rapidly decreased from 856 mV to 475 mV and 214 mV to 78 mV, respectively. The results indicate that sulfide can be used to generate electricity via the abiotic process in fuel cells.

However, the decrease in voltage over time could be derived from the deterioration of the cathode and anode electrodes. The deterioration of cathode electrodes which decreased the voltage over time was also found in Experiment 2 with similar trends. Sulfur accumulation on the anode electrodes could increase both activation loss and ohmic loss as discussed in the previous section. The OCV and voltage across electrodes at 1,000 Ω external resistances were suddenly increased after increasing the sulfide concentrations on day 3 and day 6.

On day 4-6, the influent sulfide concentrations were changed from 99.94 ± 17.31 mgS^{2-}/L to 258.16 ± 45.14 mgS^{2-}/L, which were equivalent to the sulfide concentrations in the first compartment of MFC3. Then, sulfide concentration in the effluent increased to 203.12 ± 6.67 mgS^{2-}/L, which was equivalent to the sulfide removal of 63.50 ± 20.08 mgS^{2-}/L and the sulfide removal efficiency of 25.08 ± 5.88 %. The results show that the extent of sulfide removal at a higher sulfide concentration (day 4-6) did not significantly differ from that at a lower sulfide concentration (day 1-3). In other words, higher dissolved sulfide concentrations in wastewater might not directly increase the extent of sulfide oxidation on the anode electrodes.

It should be noted that the difference in sulfide concentrations in this range did not significantly affect the theoretical voltage electrochemical potentials (E) of these MFCs. Electrical power generation appeared to relate primarily to the extent of sulfide removal in the abiotic fuel cell. However, the deterioration of the anode electrode due to sulfur accumulation could be a major problem in abiotic fuel cell operation when using sulfide as a fuel. Figure 4.35 shows sulfide removal and voltage across electrodes at 1,000Ω external resistances over time.

On day 7-9, the influent sulfide concentrations were increased from 258.16 ± 45.14 mgS^{2-}/L to 413.18 ± 20.68 mgS^{2-}/L, which were equivalent to the sulfide concentrations in the first compartment of MFC6. Sulfide concentrations in the effluent suddenly increased to the average of 351.26 ± 29.16 mgS^{2-}/L within 10 hr after changing the sulfide concentrations. The average sulfide removal were 66.82 ± 21.22 mgS^{2-}/L (16.03 ± 5.24%), which was close to the sulfide removal in the previous sulfide concentration. The results confirm that the increase in sulfide concentrations did not increase the sulfide oxidation in the abiotic fuel cell. In this case, it was possible that the active surface areas of the electrodes might be limited; therefore, the increase in sulfide concentrations did not appear to increase the electricity generation. To improve

electrical power generation and sulfide oxidation, longer hydraulic retention time and/or improvements on electrodes might be required.

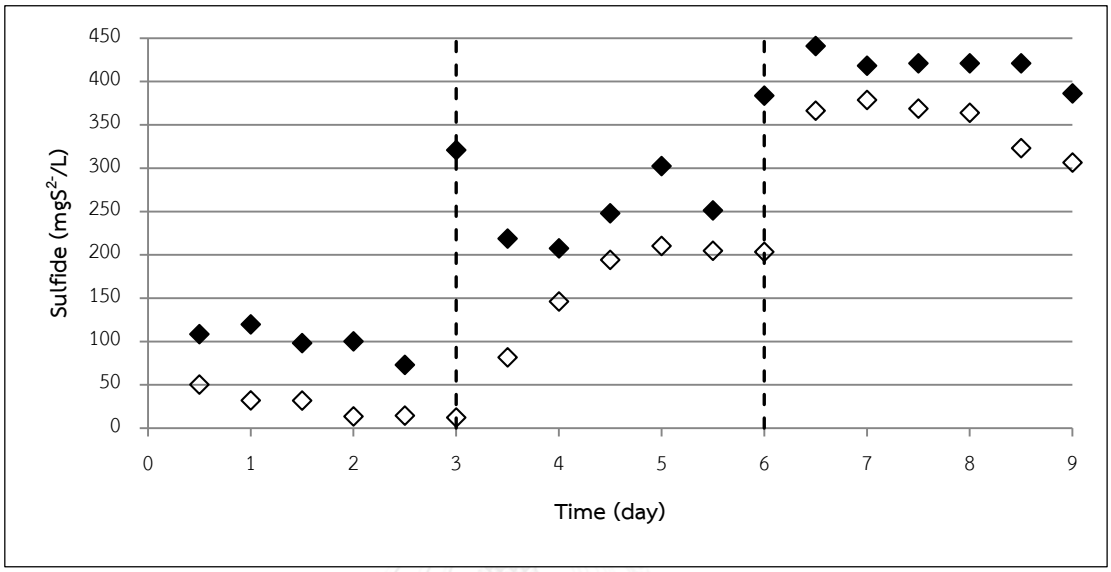


Figure 4.33 Sulfide concentrations in the abiotic fuel cell:
◆ Influent and ◇ Effluent

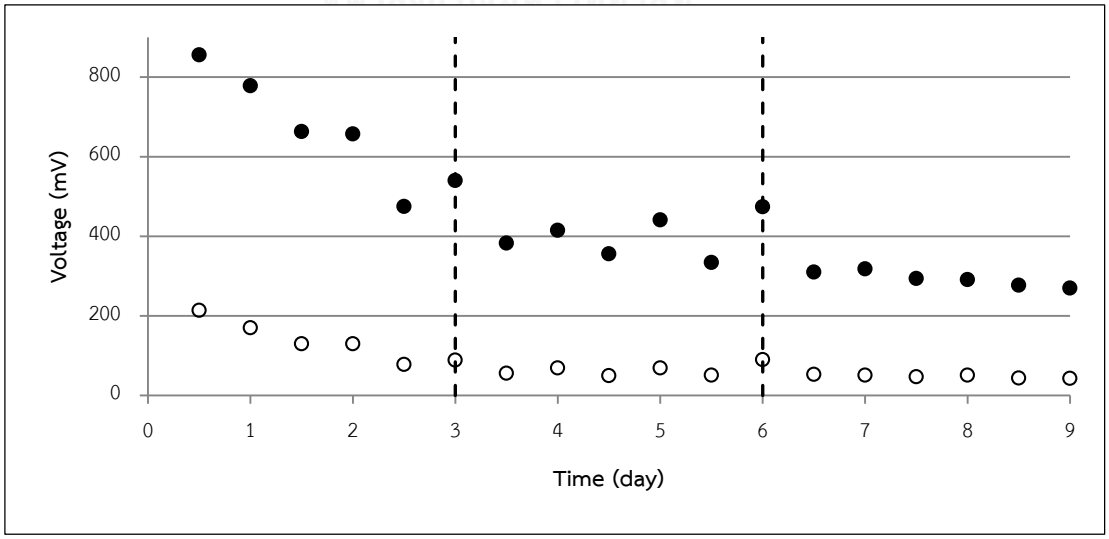


Figure 4.34 Voltages in the abiotic fuel cell:
● OCV and ○ voltage across electrodes at 1,000Ω external resistances

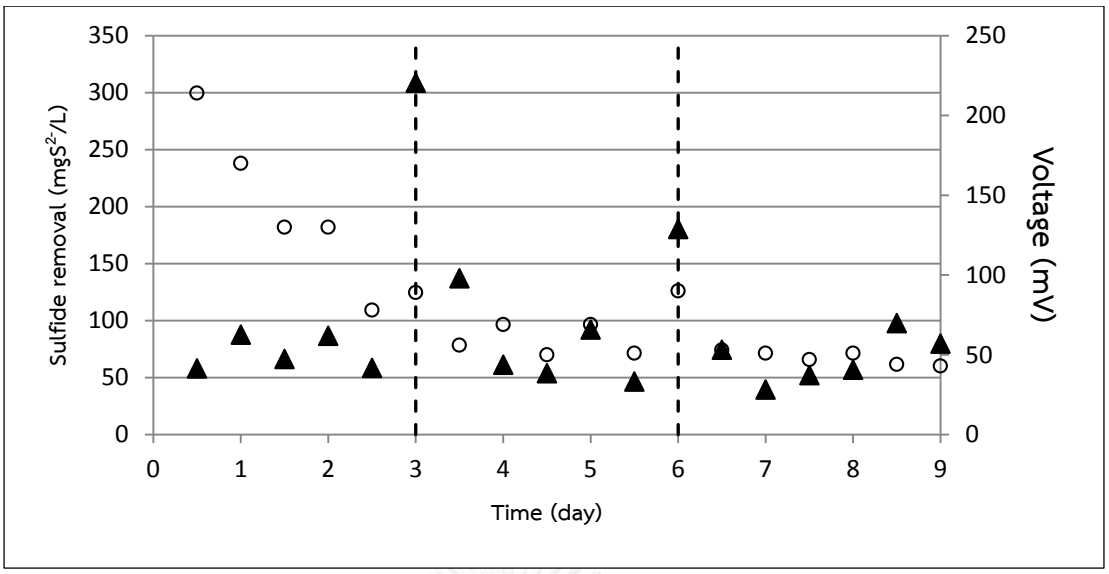


Figure 4.35 ▲ Sulfide removal and ○ Voltage across electrodes at 1,000Ω external resistances

Power density curves were constructed on day 3, 6, and 9 of the abiotic fuel cell operation. The maximum power densities of 6.80, 3.48, and 1.39 mW/m^2 were observed on day 3, day 6, and day 9, respectively. The results show the decrease in power densities in the abiotic fuel cell over time (Figure 4.36). The power density generation on day 9 was the lowest comparing with those on day 3 and day 6 although the influent sulfide concentrations were at the highest. In addition, the voltages across electrodes at 1,000Ω external resistances were rather constants at 56.17 ± 13.51 mV after day 3 of operation. However, the OCV in this experiment slightly decreased over time probably due to the activation loss. In addition, the polarization curves (Figure 4.36b) had the slopes of 10.45, 11.92, and 12.19 Ω·m^2 on day 3, day 6, and day 9, respectively. The rather constant slopes also suggest that the decrease in OCV in MFCs should come from the activation losses in the systems.

From these results, both sulfide removal and voltage across electrodes at 1,000 Ω external resistances in the abiotic fuel cell were close to those in MFC1 in the Experiment 2. The results suggest that the main mechanism of electricity generation

in MFC1 was likely to be the abiotic sulfide oxidation. However, the OCV and voltage across electrodes at 1,000 Ω external resistances in the abiotic fuel cell were higher than that in MFC3 and MFC6. The main mechanism of electricity generation in MFC3 and MFC6 could still be the abiotic sulfide oxidation, but there might be some interferences, such as biofilms of non- exoelectrogenic microorganisms on the anode electrodes that decreased the electricity generation in both MFC3 and MFC6.

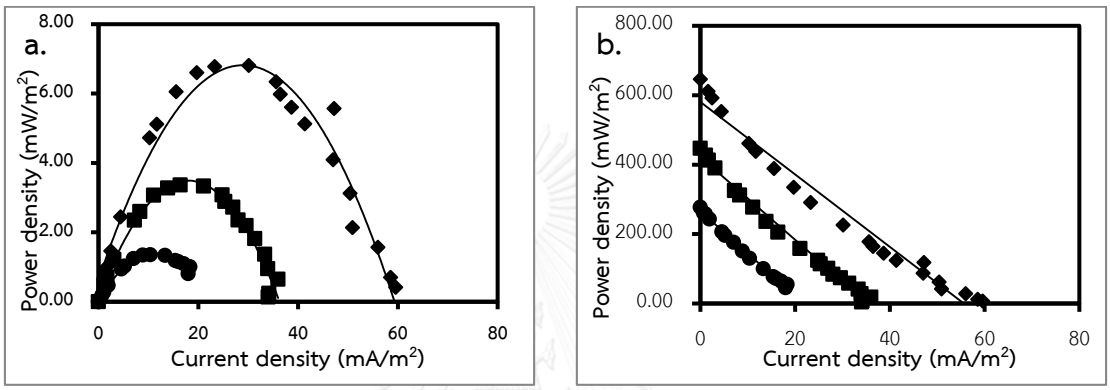


Figure 4.36 a) Power densities curves and b) polarization curves in the abiotic fuel cell with influent sulfide of ◆ 100, ■ 258, and ● 413 mgS^{2-}/L

4.4 Experiment 4: Microbial community analysis

Seed sludge, sludge in the first compartment, suspended solids in the second compartment, and biofilms on the anode electrodes in all MFCs were analyzed using 16S rRNA gene amplicon sequencing by MiSeq system (Illumina) using universal primers for bacteria and archaea, 505F 5′-GTGYCAGCMGCCGCGGTAA-3’ and 806R 5′-GGACTACHVGGGTWTCTAAT-3′ (Ding et al., 2015). The results on microbial communities can reveal the overall activities and mechanisms in different parts of the MFCs at different COD:SO_4^{2-} ratio. Figure 4.37 shows the PCR products of the samples consisting of:

1.) Seed sludge (S1)
2.) Suspended sludge in the first compartment of MFC1 (MFC1-1)
3.) Suspended solids in the second compartment of MFC1 (MFC1-2)
4.) Anode-attached biofilm in MFC1 (MFC A1)
5.) Suspended sludge in the first compartment of MFC3 (MFC3-1)
6.) Suspended solids in the second compartment of MFC3 (MFC3-2)
7.) Anode-attached biofilm in MFC3 (MFC A3)
8.) Suspended sludge in the first compartment of MFC6 (MFC6-1)
9.) Suspended solids in the second compartment of MFC6 (MFC6-2)
10.)Anode-attached biofilm in MFC6 (MFC A6)

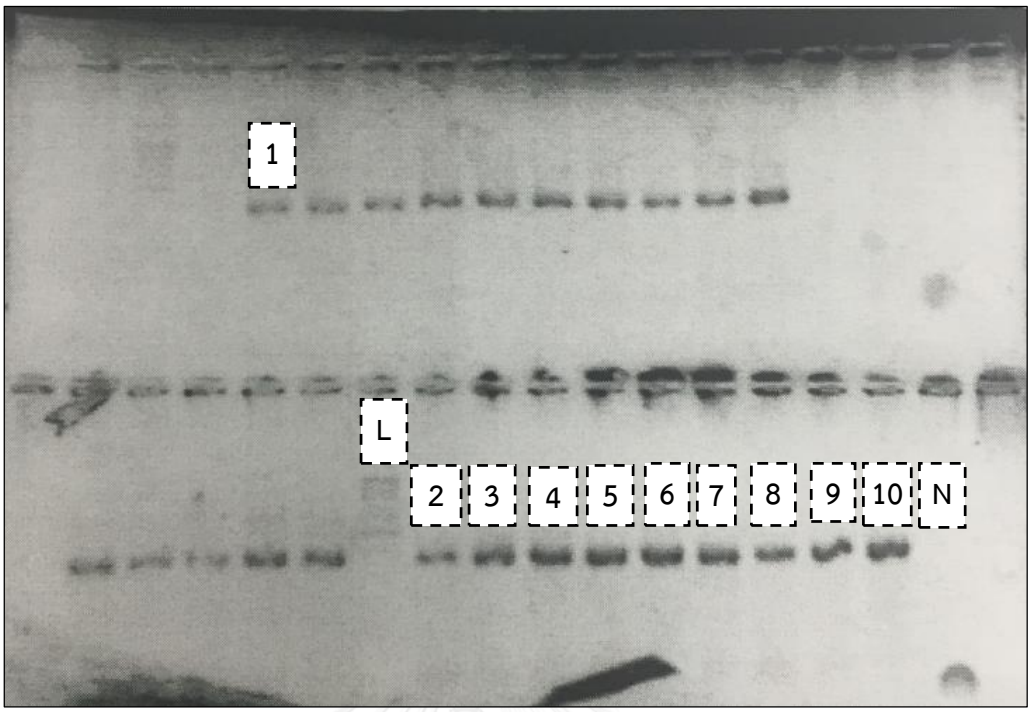
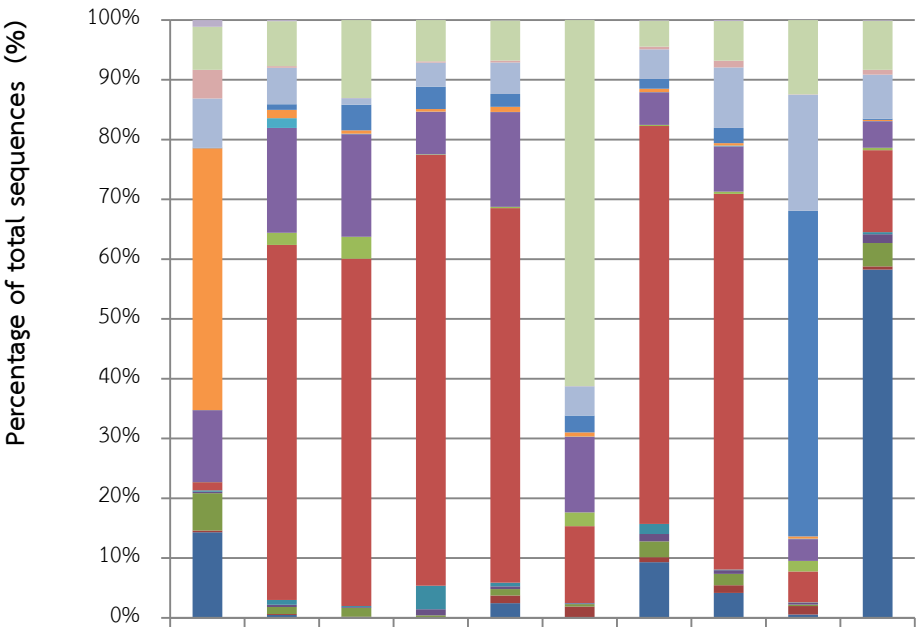


Figure 4.37 PCR products of the samples

1) S1, 2) MFC1-1, 3) MFC1-2, 4) MFC A1, 5) MFC3-1, 6) MFC3-2, 7) MFC A3, 8) MFC6-1, 9) MFC6-2, 10) MFC A6, N) Negative control, and L) DNA ladder



	S1	1-1	1-2	A1	3-1	3-2	A3	6-1	6-2	A6
Actinobacteria	1.134	0.197	0.004	0.002	0.066	0.004	0.094	0.165	0.019	0.106
Bacteroidetes	7.198	7.515	13.045	6.925	6.712	61.236	4.361	6.651	12.410	8.242
Chloroflexi	4.798	0.286	0.015	0.201	0.320	0.019	0.456	1.112	0.047	0.778
Firmicutes	8.227	6.099	1.107	4.005	5.280	4.907	4.947	10.125	19.454	7.377
Lentisphaerae	0.101	0.922	4.281	3.750	2.132	2.805	1.627	2.538	54.453	0.214
Proteobacteria (alpha)	43.790	1.400	0.556	0.428	0.879	0.706	0.573	0.406	0.365	0.175
Proteobacteria (beta)	0.010	1.646	0.098	0.049	0.012	0.052	0.014	0.146	0.086	0.032
Proteobacteria (delta)	12.040	17.530	17.162	7.096	15.862	12.617	5.468	7.574	3.644	4.456
Proteobacteria (epsilon)	0.004	2.022	3.649	0.122	0.200	2.316	0.199	0.367	1.763	0.384
Proteobacteria (gamma)	1.356	59.380	58.099	72.044	62.662	12.838	66.544	62.835	5.171	13.712
Proteobacteria (unclassified)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Spirochaetes	0.246	0.814	0.185	3.936	0.603	0.063	1.680	0.115	0.066	0.367
Synergistetes	0.242	0.372	0.129	1.003	0.444	0.149	1.245	0.613	0.316	1.450
Other bacteria	6.244	1.171	1.481	0.327	1.093	0.389	2.637	1.887	0.241	3.914
Unclassified bacteria	0.285	0.177	0.185	0.095	1.285	1.803	0.840	1.287	1.409	0.531
Archeae	14.32	0.47	0.002	0.02	2.45	0.09	9.32	4.18	0.56	58.26

Figure 4.38 Microbial communities shown as percent relative abundance of microbial phyla

Figure 4.38 shows microbial communities as percent relative abundance of microbial phyla of the samples obtained in this study. The results show great differences in the microbial community of the seed sludge from the other samples, suggesting the selection of microbial communities after the MFC operation.

4.4.1 Seed sludge (S1)

The predominant genus in the seed sludge was *Rhodovibrio* spp. (43.59 % of total sequences) which was a phototrophic purple non-sulfur bacteria. Both SRB (6.74 % of total sequences) and methanogens (14.15 % of total sequences) were found in the seed sludge. Table 4.6 shows the top 5 of most abundant genera in the initial sludge.

Table 4.6 Dominant genera in the initial sludge

Dominant genera	Relative abundance (% of total sequences)
Rhodovibrio	43.59
Methanosaeta	6.47
Methanolinea	5.68
Alkaliflexus	5.12
Smithella	2.46

4.4.2 Microbial communities in MFC1

Microbial community in the first compartment

For the first compartment in MFC1, *Tolumonas* spp. were the main species in this compartment. *Tolumonas* spp. were fermentative bacteria, which can grow on glucose and produce acetate, ethanol, and formate as fermentation product (Fischer-Romero et al., 1996). *Desulfovibrio* spp., the second dominant genus, were also found in this system. *Desulfovibrio* was one genus of SRB, which use acetate, pyruvate, and hydrogen as electron donors and uses sulfate as an electron accepter. *Klepsiella* spp., *Desulforhabdus* spp., and *Vallitalea* spp. which were fermentative bacteria, SRB, and

fermentative bacteria, respectively, which were also found in the top 5 of most abundant genera in the system as shown in Table 4.7.

MFC1 (COD:SO_4^{2-} ratio=1) was fed with the influent with the average COD and sulfate concentrations of 3,043 ± 139 mgCOD/L and 3,036 ± 60 mgSO_4^{2-}/L, respectively. Since glucose was a large molecule that cannot be used by most SRB, fermentative bacteria could grow in this system by fermenting glucose into smaller organic compounds such as acetate, ethanol, and formate. *Tolumonas* spp., the fermenters which appeared to be able to tolerate high sulfide in this system, were found as predominant species. The products of fermentation could be used by SRB (*Desulfovibrio* spp. and *Desulforhabdus* spp.) as electron donors while sulfate in wastewater was used as an electron acceptor to generate hydrogen sulfide as a final product (Madigan et al., 2003). Hydrogen sulfide was a toxicity substance for many microorganisms. Methanogens were not found in this system, probably due to the high sulfide concentrations in this system, which were in the range that was toxic to methanogens (Lens et al., 1998).

Table 4.7 Dominant genera in the first compartment of MFC1

Dominant genera	Relative abundance (% of total sequences)
Tolumunas	49.36
Desulfovibrio	10.20
Klebsiella	7.47
Desulforhabdus	4.09
Vallitalea	4.04

Microbial community in the second compartment

For suspended microorganisms in the second compartment of MFC1, the results of microbial analysis show that *Klebsiella* spp. became the predominant

species in this compartment. Since high concentration of sulfate still remained in the second compartment of MFC1 (1,736 ± 334 mgSO_4^{2-}/L), SRB such as *Desulfovibrio* spp. were found as one of the dominant species in the system. Fermentative bacteria, such as *Bacteroides* spp., *Tolumonas* spp., and *Victivallis* spp., were also found in this system as shown in Table 4.8.

The remaining substrates and products from the first compartment could affect microbial communities in the second compartment, including the suspended microorganisms and the biofilm on the anode electrode. *Klebsiella* spp. were the dominant species which were found in the suspended solids of the second compartment of MFC1. It should be noted that organic and inorganic substances in wastewater might affect microbial community. Due to the high sulfide concentrations in the second compartment, methanogens was not found in this compartment.

Table 4.8 Dominant genera in the second compartment of MFC1

Dominant genera	Relative abundance (% of total sequences)
Klebsiella	49.81
Desulfovibrio	16.45
Bacteroides	11.64
Tolumonas	4.81
Victivallis	4.28

Microbial community on the anode electrode

For the biofilm on the anode electrode in MFC1, *Klebsiella* spp. were the dominant species. On the other hand, *Victivallis* spp. and *Desulfovibrio* spp., noticeably decreased in their relative abundances on the anode electrode compared with the suspended microorganisms. The lower abundance of *Desulfovibrio* spp. on the anode electrode might suggest that SRB preferred to grow as suspended microorganisms compared with as biofilm on the anode electrode. Similar findings were reported in

previous studies (Sangcharoen et al., 2015; Sun et al., 2009). *Dyella* spp., SOB, were also observed on the anode electrode. The presence of SOB on the anode electrode of the MFC treating sulfide has been observed in many studies (Sangcharoen et al., 2015; Sun et al., 2009; Zhang et al., 2012). Sun et al. (2009) also reported that an MFC treating sulfide with the presence of microorganisms generated a higher persistent current density than an abiotic fuel cell fed with sulfide. *Dyella* spp. observed in this study could also contribute to electricity generation in the system. However, their relative abundances were very low. Table 4.9 shows the top 5 of most abundant genera on the anode electrode.

From the microbial community comparison (Figure 4.42), the microbial community of the suspended microorganisms in the second compartment of MFC1 was rather similar to the microbial community of the biofilm on the anode electrode in MFC1. It was possible that the microorganisms on the anode electrode might originally come from the suspended solids in the second compartment. On the other hand, the biofilm on the anode electrode might slough off and became suspended microorganisms in the second compartment. However, the presence of SOB on the anode electrode suggests that dissolved sulfide might be converted into elemental sulfur and/or sulfate as a final product during electrical generation via SOB.

Table 4.9 Dominant genera in the biofilm on the anode electrode of MFC1

Dominant genera	**Relative abundance (% of total sequences)**
Klebsiella	64.76
Victivallis	3.75
Desulfovibrio	3.23
Dyella	2.93
Vallitalea	2.92

Comparison of mechanisms and microbial communities in MFC1

For fermentative bacteria, *Tolumunas* spp. were predominant species in the first compartment; however, their relative abundances decreased in the second compartment and on the anode electrode. On the other hand, the relative abundances of *Klebsiella* spp. increased in the second compartment and on the anode electrode.

For SRB, the percentages of SRB to total sequences of 17.32, 16.78, and 6.49 % were observed for the first compartment, the second compartment, and the anode electrode, respectively, as shown in Figure 4.39. The results suggest that SRB were preferentially found in soluble as suspended microorganisms rather than on the anode electrode as biofilm. The remaining sulfate after passing the first compartment might stimulate SRB in the second compartment both as suspended microorganisms and biofilm on the anode electrode.

For methanogens, they were not found in MFC1 (Figure 4.40). Methanogens appeared to be out-competed by SRB in this MFC at the COD:SO_4^{2-} ratio of 1, which were consistent with previous studies (Choi and Rim, 1991; Chou et al., 2008).

4.4.3 Microbial communities in MFC3

In the first compartment, *Tolumunas* spp. (59.62 % of total sequences) were predominant species for fermentative bacteria in the system. *Bacteroides* spp. (4.07 % of total sequences) and *Victivallis* spp. (2.13 % of total sequences) were also fermentative bacteria found in the top 5 of most abundant genera as shown in Table 4.10. Total SRB of 15.64 % of total sequences were observed. The dominant genus of SRB was *Desulfovibrio* (12.92 % of total sequences). Although the synthetic wastewater had a high concentration of sulfate (1,000 mgSO_4^{2-}/L), methanogens about 2.43% of total sequences could still exist in the system.

The average COD and sulfate concentrations in MFC3 were 2,999 ± 427 mgCOD/L and 1,015 ± 29 mgSO_4^{2-}/L, respectively. Glucose had to be fermented to smaller organic compounds by fermentative bacteria, such as *Tolumunas* spp., *Bacteroides* spp., and *Victivallis* spp. Then, SRB, such as *Desulfovibrio* spp. and *Desulforhabdus* spp., could use the fermentation products namely acetate, propionate, and butyrate as electron donors while sulfate served as an electron acceptor. Since sulfate concentration in the influent of MFC3 was lower than that in MFC1, sulfide production in MFC3 was also lower than in MFC1, resulting in less sulfide toxicity in MFC3 than in MFC1. On the other hand, methanogens in the families of *Methanosaetaceae* and *Methanoregulaceae* were found in the first compartment of MFC3. Therefore, it should be noted that these families of methanogens could still grow even at a high sulfide concentration (265 ± 59 mgS^{2-}/L). Table 4.10 shows the dominant genera in the first compartment of MFC3.

Table 4.10 Dominant genera in the first compartment of MFC3

Dominant genera	Relative abundance (% of total sequences)
Tolumunas	59.62
Desulfovibrio	12.92
Bacteroides	4.07
Victivallis	2.13
Desulforhabdus	2.11

For the second compartment of MFC3, the predominant genus of fermentative bacteria was *Bacteroides* (59.62 % of total sequences). The possible fermentative products of *Bacteroides* spp. were acetate, propionate, pyruvate, succinate, formate, and lactate. Other fermenters found in this system were *Klebsiella* spp., *Tolumonas* spp., and *Victivallis* spp. For SRB, *Desulfovibrio* spp. about 12.32 % of total sequences were mainly found as the only genus of SRB in the second compartment of MFC3.

Table 4.11 shows dominant genera in the second compartment of MFC3. Methanogens were not found in this system.

Due to the remaining of high concentration of COD, many fermentative bacteria could grow under this condition. The remaining sulfate concentration was approximately 53.84 ± 91.86 mgSO_4^{2-}/L, SRB (12.41 % of total sequences) were observed in the system. Although, SRB were found in the suspended solids in the second compartment, sulfate concentrations did not significantly decrease in this compartment. It was possible that sulfate reduction might still occur but the production of sulfate via sulfide oxidation on the anode electrode might cause the sulfate concentration to be apparently the same. Moreover, the amount of suspended microorganisms in the second compartment was very low, resulting in low activities of SRB.

Table 4.11 Dominant genera in the second compartment of MFC3

Dominant genera	Relative abundance (% of total sequences)
Bacteroides	59.82
Desulfovibrio	12.32
Klebsiella	7.82
Tolumonas	3.71
Victivallis	2.81

For the biofilm on the anode electrode in MFC3, *Tolumonas* spp. approximately 56.14 % of total sequences were observed. The presences of *Tolumonas* spp. and other fermenters, such as *Klebsiella* spp. suggest that glucose might still remain in the second compartment. *Methanosaeta* spp. of ~8.63 % of total sequences were observed on the anode electrode. This genus can produce methane

from acetate. SRB, consisting of *Desulfovibrio* spp. and *Desulforhabdus* spp., were also found in the top 5 of the most abundant genera.

Although methanogenesis has been shown to occur in many MFCs, until now there was only one previous study by (He et al., 2005) that actually observed methanogens on the anode electrode as most of the study investigated only bacteria, not archaea, on the anode electrodes (Logan et al., 2006). The presence of methanogens on the anode electrode of MFC3 suggests that the COD removal in the second compartment of MFC3 could be derived from methanogenesis. The COD was not likely to be used for electrical production in this MFC because only 0.17% of total sequences of known exoelectrogenic microorganisms (*Actinobacteria* spp. and *Geobacter* spp.) were observed. Negligible amount of exoelectrogenic microorganisms on the anode electrode not only suggested that the biofilm did not generate electricity but also suggested that it might in fact increased the voltage loss of the MFC. It was found that the voltage across the electrodes at 1,000 Ω external resistances was higher after changing the anode electrode. For SRB, SRB of only ~4.71 % of total sequences were observed on the anode biofilm. Their relative abundances were less than those as suspended microorganisms in the second compartment.

Table 4.12 Dominant genera in the biofilm on the anode electrode of MFC3

Dominant genera	**Relative abundance (% of total sequences)**
Tolumonas	56.14
Klebsiella	9.05
Methanosaeta	8.63
Desulfovibrio	2.58
Desulforhabdus	1.75

Comparison of mechanisms and microbial communities in MFC3

For fermentative bacteria, *Tolumunas* spp. were the predominant species in both the first compartment (59.62 % of total sequences) and the biofilm on the anode electrode (56.14 % of total sequences). Nevertheless, only 3.71 % of total sequences of *Tolumunas* spp. were found in the second compartment. On the other hand, *Bacteroides* spp. (59.82 % of total sequences) became dominant species in the second compartment.

For SRB (Figure 4.39), *Desulfovibrio* was the main genus of SRB observed in MFC3. However, the relative abundance of SRB was the highest in the first compartment (15.64 % of total sequences) and the lowest in the biofilm on the anode electrode (4.71 % of total sequences). Due to the low sulfate concentration in the second compartment, the relative abundance of SRB in the second compartment was lower than that in the first compartment. The relative abundance of SRB in the suspended solids in the second compartment was higher than that in the biofilm on the anode electrode, confirming that SRB was preferentially found as suspended microorganisms rather than as biofilm as discussed earlier.

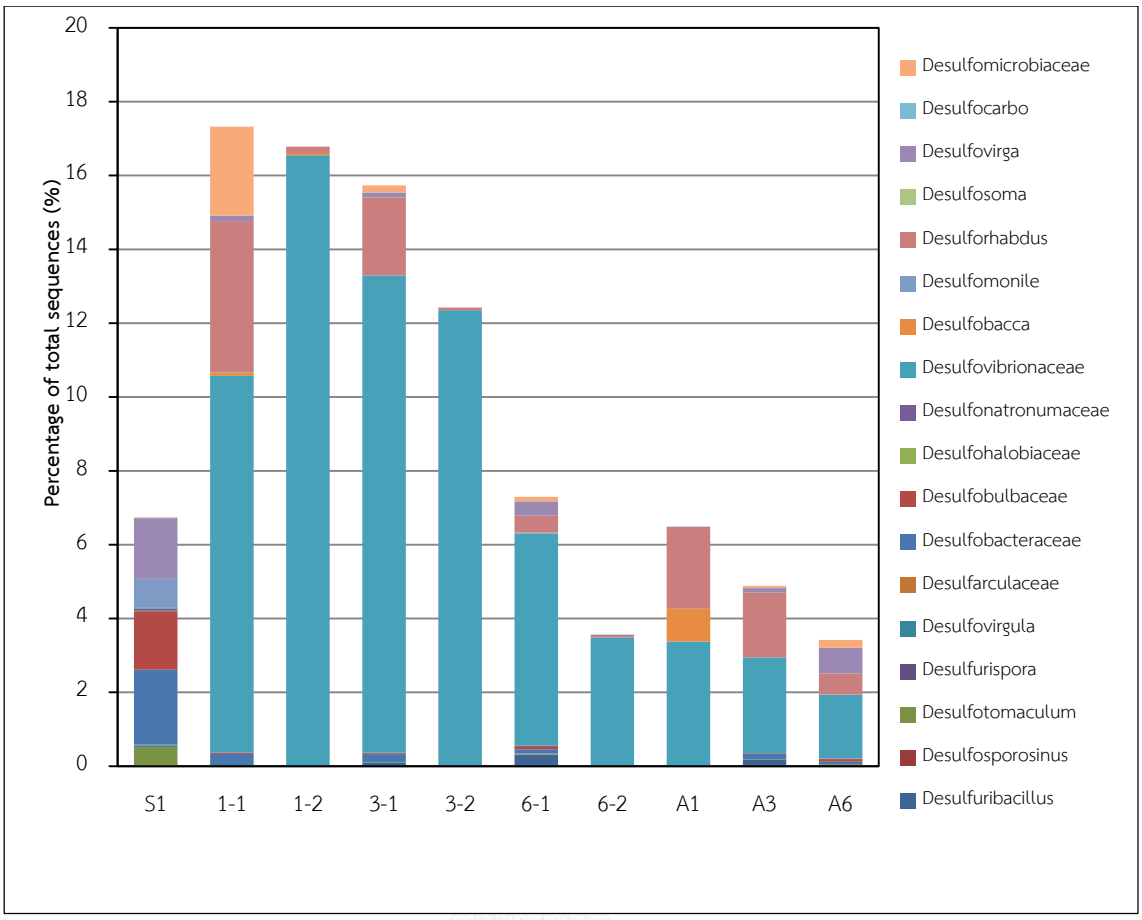


Figure 4.39 SRB communities shown as percent relative abundance of genera and families

Methanogens of only 2.43 % of total sequences were found in the first compartment as shown in Figure 4.40. This observation could suggest that the COD removal in the first compartment could occur via 2 pathways, including 1) COD removal by SRB via sulfate reduction and 2) COD removal by methanogens via methanogenesis. In the second compartment, methanogens were not found in suspended solids; however, a number of methanogens were observed in the biofilm on the anode electrode. The difference in the relative abundances of methanogens in the suspended solids and on the biofilm on the anode electrode suggested that methanogens might preferentially grow as biofilm rather than as suspended microorganisms.

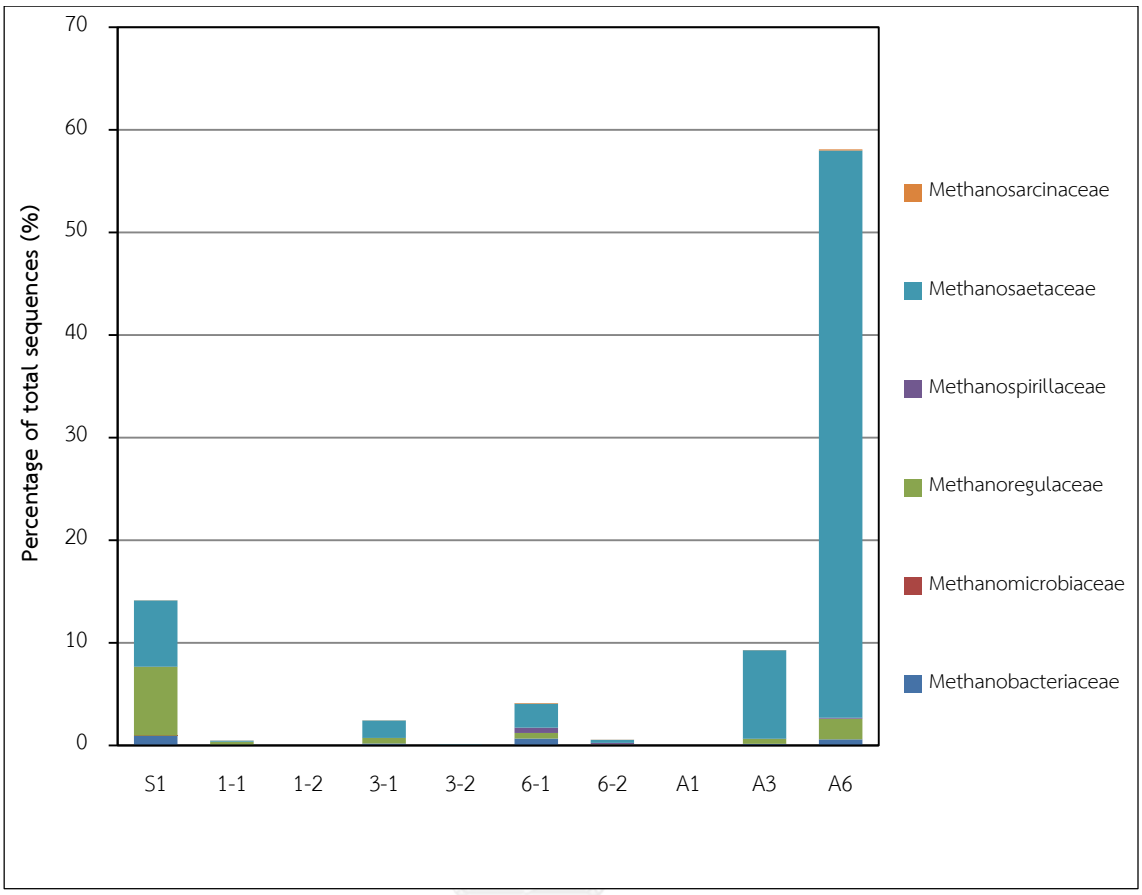


Figure 4.40 Methanogen communities shown as percent relative abundance of families

4.4.4 Microbial communities in MFC6

In the first compartment of MFC6, *Tolumunas* spp. were the predominant species of fermentative bacteria. In addition *Trichococcus* spp., *Bacteroides* spp., and *Victivallis* spp. were also found in the top 5 of the most abundant genera in this system. On the other hand, *Desulfovibrio* were the main genus of SRB found in the first compartment of MFC6. Table 4.13 shows the dominant genera in the first compartment of MFC6. From these results, it should be noted that methanogens were not found in the top 5 of the most abundant genera in this system. Methanosaetaceae was the most abundant family of methanogens observed in this system.

In MFC6 operated at the COD:SO_4^{2-} ratio of 6, the average COD and sulfate concentration were 3,033 ± 349 mgCOD/L and 509 ± 17 mgSO_4^{2-}/L, respectively. Although MFC6 was operated at a high COD:SO_4^{2-} ratio in which methanogens should be predominant in the system (Choi and Rim, 1991; Chou et al., 2008), methanogens (4.13%) did not appear to be predominant in this study. Since methanogens could mainly use acetate and hydrogen to generate methane, fermentation of glucose and acetogenesis were necessary.

Due to the high concentrations of COD, substrates were likely to be sufficient for both methanogens and SRB to co-exist in the systems. The results also show that the number of sequences related to SRB was higher than that of methanogens although direct comparison on the abundance of these microorganisms cannot be made. SRB could have several competitive advantages over methanogens including 1) the growth yields of SRB are higher than methanogens, 2) SRB could use various types of substrates, namely acetate, propionate, and butyrate, but methanogens can use only acetate and hydrogen, and 3) SRB generally have higher affinity (lower K_s) to acetate and hydrogen than methanogens (Rabus et al., 2006), and 4) the maximum specific growth of SRB are higher than that in methanogens (Oude Elferink and Jansen, 1994).

Table 4.13 Dominant genera in the first compartment of MFC6

Dominant genera	Relative abundance (% of total sequences)
Tolumunas	60.93
Desulfovibrio	5.75
Trichococcus	3.27
Bacteroides	2.99
Victivallis	2.54

In the second compartment in MFC6, fermentative bacteria were also predominant in this system. The relative abundances of 54.45, 16.05, 10.57, and 2.35 % of total sequences of *Victivallis* spp., *Trichococcus* spp., *Bacteroides* spp., and *Tolumonas* spp. were observed, respectively. SRB were also found in this compartment. It should be noted that the remaining of low concentration of sulfate (16.85 ± 37.16 mgSO_4^{2-}/L) could still stimulate SRB in the system. However, methanogens were not found in the second compartment of MFC6, similarly to the second compartment of MFC3. Table 4.14 shows the genera in the second compartment of MFC6.

The major substrate fed into the second compartment was COD. The high relative abundance of fermentative bacteria in the second compartment suggests that the remaining substrate from the first compartment might still be large molecular compounds (e.g. glucose). The major fermentative products of *Victivallis* spp. are acetate, ethanol, and H_2 when using glucose as an initial substrate. Overall, fermentation is considered to be the main mechanism in this compartment.

Table 4.14 Dominant genera in the second compartment of MFC6

Dominant genera	Relative abundance (% of total sequences)
Victivallis	54.45
Trichococcus	16.05
Bacteroides	10.57
Desulfovibrio	3.46
Tolumonas	2.35

For the biofilm on the anode electrode in MFC6, *Methanoseata* spp. were the predominant species growing on the anode electrode. Others methanogens found in the system were *Methanoregulaceae* (2.01 % of total sequences),

Methanobacteriaceae (0.59 % of total sequences), *Methanosarcinaceae* (0.11% of total sequences), and *Methanospirillaceae* (0.09 % of total sequences). *Tolumonas* spp. (12.54 % of total sequences), *Alkaliflexus* spp. (3.66 % of total sequences), and *Trichococcus* spp. (2.74 % of total sequences) were the fermentative bacteria, which were also observed on the anode electrode. SOB such as *Dyella* spp. (0.54 % of total sequences). *Sulfurovum* spp. (0.22 % of total sequences), and *Sulfuricurvum* spp. (0.09 % of total sequences) could also grow on anode electrode. Table 4.15 shows the dominant genera in the biofilm on the anode electrode of MFC6.

The presence of methanogens on the anode electrode suggests that methanogens might be responsible for the COD removal in the second compartment of MFC6 while generating CH_4 as a final product. This COD removal would not contribute to electricity generation. Moreover, large amount of methanogens on the anode electrode might impede the electron transfers to the anode electrode, resulting in the decrease in power density of MFC. Due to the low abundances of SOB (0.86 % of total sequences) and EEM (0.25 % of total sequences), electricity generation via microorganisms appeared to be low.

Table 4.15 Dominant genera in the biofilm on the anode electrode of MFC6

Dominant genera	Relative abundance (% of total sequences)
Methanoseata	55.29
Tolumonas	12.54
Alkaliflexus	3.66
Trichococcus	2.74
Lutibacter	2.14

Comparison of mechanisms and microbial communities in MFC6

For fermentative bacteria, *Tolumunas* was the dominant genus in the first compartment (60.93 % of total sequences) and in the biofilm on the anode electrode (12.54 % of total sequences). However, *Victivallis* spp. (54.45 % of total sequences) were predominant in the second compartment. The results show that *Victivallis* spp. of only 2.54 % of total sequences were observed in the first compartment, but they became predominant in suspended microorganisms of the second compartment. The amount of fermentative bacteria on the anode electrode was extremely low while methanogens became dominant. It was likely that methanogenesis rather than fermentation was the primary biological process in the second compartment of MFC6, which appeared to occur at a greater extent in MFC6 than in MFC3 and MFC1.

For SRB, *Desulfovibrio* was the main genus of SRB still found in MFC6 as shown in Figure 4.39. Because of the highest sulfate concentration in the influent, SRB was the most abundance in the first compartment compared with the second compartment and on the anode electrode.

For methanogens (Figure 4.40), their relative abundances were only 4.13% of total sequences in the first compartment. MFC6 had a high COD:SO_4^{2-} ratio in which methanogens should be predominant in the system (Choi and Rim, 1991; Chou et al., 2008). However, the results did not agree with this hypothesis and the previous findings. It should be noted that all these previous studies were conducted at a lower range of COD concentrations (about 1,500 mgCOD/L). At the high concentration of COD (3,000 mg/L) in this current study, organic compounds may not be the limiting substrate for SRB and methanogen competition, resulting in the co-existence of SRB and methanogens. Co-existence of SRB and methanogens at the COD:SO_4^{2-} ratio of 6 was

previously reported in the study by Hu et al. (2015) while the COD concentration was around 3,000 mg/L, similarly to the COD concentrations in this study.

Moreover, high sulfide concentration in the system (119 ± 32 mgS^{2-}/L) might also inhibit methanogens in the first compartment of MFC6. In the second compartment, methanogens (0.55 % of total sequences) were rarely found in suspended solids; however, they were abundant on the anode electrode (58.09 % of total sequences). The results suggest that methanogens might prefer to grow as a biofilm rather than as suspended microorganisms.

4.4.5 Comparison of microbial communities in the first compartment of all MFCs

In the first compartment, only sulfate concentrations in the influent of each MFC were different, which were 3,000, 1,000, and 500 mgSO_4^{2-}/L, in MFC1, MFC3, and MFC6, respectively. *Tolumonas* was the predominant genus in all MFCs. Since glucose was the sole organic substrate in the influent, fermentative bacteria were necessary to ferment glucose into VFAs and acetate, which can be further used by methanogens and SRB in the system. The difference on sulfate concentrations appears to affect the relative abundance of SRB in the systems. The results show that the percentages of SRB to total sequences in MFC1, MFC3, and MFC6 were 17.32 %, 15.64 %, and 6.98 % of total sequences, respectively. In addition, sulfide concentrations in all MFCs were in a high range (400 ± 69, 265 ± 59, and 119 ± 32 mgS^{2-}/L in MFC1, MFC3, and MFC6, respectively) even in MFC6 with the lowest sulfate in the influent. Sulfide production in these systems might also be another important factor affecting microbial communities in the MFCs.

4.4.6 Comparison of microbial communities of suspended solids in the second compartment of all MFCs

In the second compartment, the remaining substrates, consisting of COD, SO_4^{2-}, and sulfide, should be the main important factors for the enrichment of suitable microorganisms in the systems. Figure 4.42 shows the comparison of microbial communities of all samples using principal component analysis. Samples 1-2, 3-2, and 6-2 were the microbial communities of suspended microorganisms in the second compartment of MFC1, MFC3, and MFC6, respectively. The results show that there were close similarities among these microbial communities in all MFCs. However, the predominant genera were completely different in each MFC. *Klebsiella* was a predominant genus in MFC1 whereas *Bacteroides* and *Victivallis* were predominant genera in MFC3 and MFC6, respectively. Microbial diversity in the second compartment of MFC1 (1-2) appears to be less compared to the other MFCs, probably due to a very high sulfide concentration in MFC1. *Klebsiella* spp., which were dominant in the second compartment of MFC1, might be the fermentative bacteria which could tolerate high sulfide concentration compared to others. For MFC3, the medium range of sulfide concentrations was observed. In the second compartment of this MFC, *Bacteroides* spp. were predominant.

For SRB, MFC1 still had high sulfate concentration in the wastewater after passing the first compartment, resulting in a greater abundance of SRB in MFC1 than in MFC3 and MFC6. Accordingly, since the sulfate concentration in MFC3 (53.8 ± 91.9 $mgSO_4^{2-}/L$) was higher than that in MFC6 (16.85 ± 37.16 $mgSO_4^{2-}/L$), the percentage of SRB to total sequences in MFC3 was also higher than that in MFC6. Despite the presence of low sulfate concentrations in MFC3 and MFC6, SRB still grew in the second compartment of both MFCs. Moestedt et al. (2013) also found SRB in industrial anaerobic digesters at low sulfate concentrations (100 $mgSO_4^{2-}/L$). SRB could still grow

at low sulfate concentrations probably because they can use various types of substrates such as lactate, fumarate, and ethanol to produce propionate, acetate, CO_2 and H_2 (Oude Elferink and Jansen, 1994; Plugge et al., 2011)

4.4.7 Comparison of microbial communities of the biofilms on the anode electrodes in all MFCs

Figure 4.42 shows the comparison of microbial communities of the biofilms on the anode electrode of all MFCs. Samples A1, A3, and A6 were the biofilms on the anode electrodes of MFC1, MFC3, and MFC6, respectively. The results indicate that the microbial communities of the biofilms on the anode electrode of these MFCs were completely different. For MFC1, *Klebsiella* spp., fermentative bacteria, were found to be predominant on the anode electrode. *Dyella* spp, SOB, were found in the top 5 of most abundant genera on the anode electrode. SOB could convert sulfide to sulfate using anode electrodes as electron acceptors (Sun et al., 2009), resulting in electricity generation in MFCs. Other SOB (Vidyalakshmi et al., 2009) such as Sulfurovum *spp*. and Sulfuricurvum *spp*. were also found in MFC1.

On the other hand, the sulfate concentration in the second compartment of MFC3 was rather low but the sulfide concentration was still high. In this MFC, *Methanosaetaceae* about 8.63% of total sequences were observed on the anode electrode. For MFC6, sulfate and sulfide concentrations in the second compartment were the lowest compared to those in MFC1 and MFC3. Because of the low sulfate and sulfide concentrations, methanogens could become predominant on the anode electrode of MFC6. The presences of methanogens in both MFC3 and MFC6 suggest that COD removal in the second compartment of these MFCs might be derived from methanogenesis. Ishii et al. (2012) reported that the increase in *Bacteroidetes* on anode electrodes after long term operation might have a relationship with the efficiencies of biofilm function in MFCs. However, Figure 4.41 shows low amount of known

exoelectrogenic microorganisms on the anode electrodes. The results show that known exoelectrogenic microorganisms of only 0.07, 0.17, and 0.25 % of total sequences were observed in MFC1, MFC3, and MFC6, respectively. High sulfide concentrations in these MFCs might be unfavorable for the growth of exoelectrogenic microorganisms.

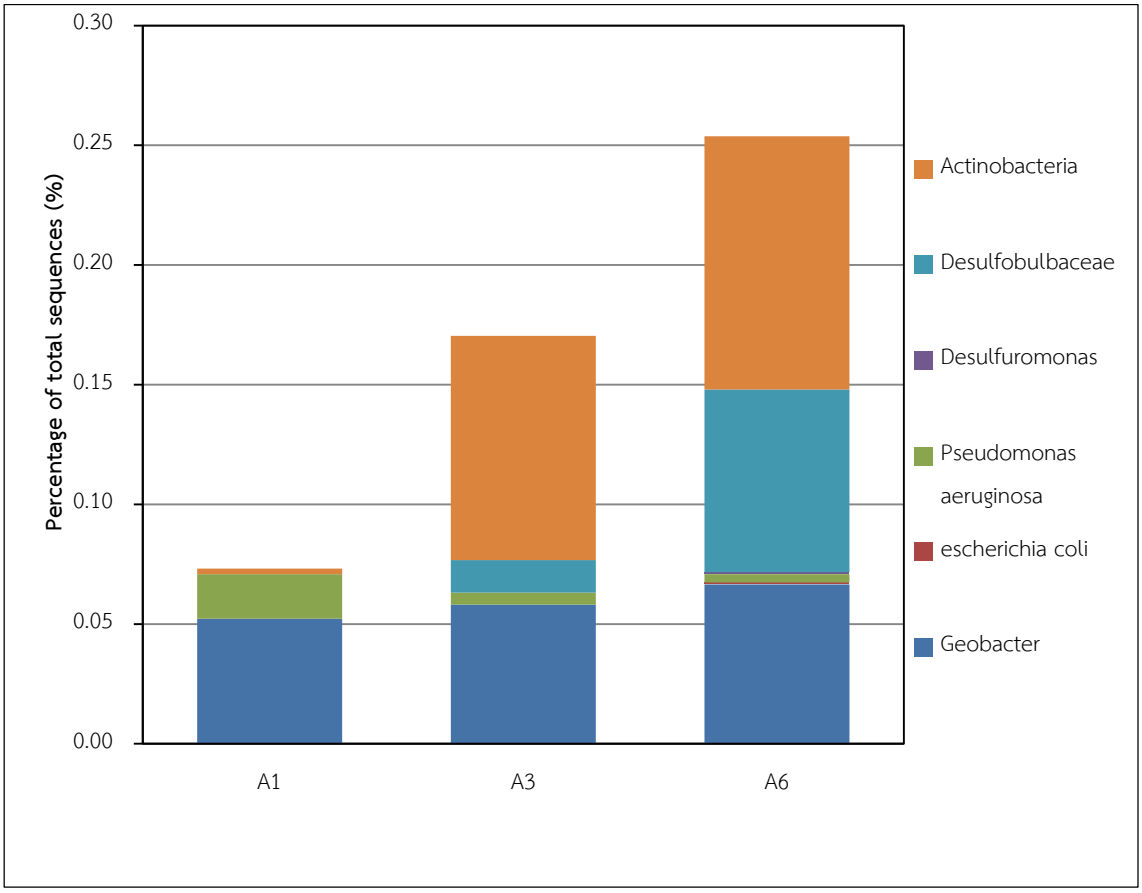


Figure 4.41 Exoelectrogenic microorganisms communities on anode electrodes

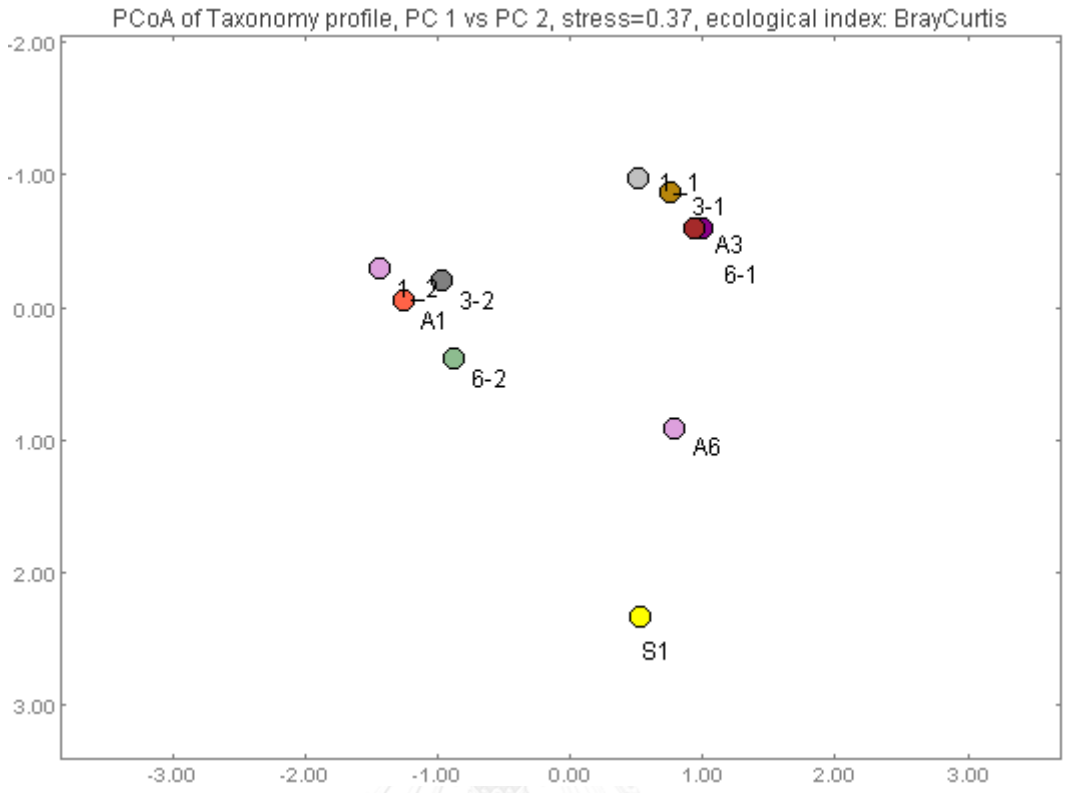


Figure 4.42 Comparison of microbial communities by principal component analysis among the samples:

1) S1, 2) MFC1-1, 3) MFC1-2, 4) MFC A1, 5) MFC3-1, 6) MFC3-2, 7) MFC A3, 8) MFC6-1, 9) MFC6-2, and 10) MFC A6

4.5 Mechanism analysis in MFCs

4.5.1 Mechanisms in the first compartments of MFCs

Figure 4.43 shows possible mechanisms in the first compartments of MFCs. Since glucose was a large molecule that cannot be used directly by SRB and methanogens, fermentative bacteria could grow in the systems as a predominant group in all MFCs. *Tolumonas* spp. were the predominant species observed in the first compartments of all MFC, which could ferment [1] glucose into small organic molecules such as VFAs and acetate.

Then, SRB could use the fermentation products including acetate, propionate, and butyrate as electron donors [2.2] while sulfate served as an electron acceptor [3.1]. In this process, both COD and SO_4^{2-} were removed from the wastewater whereas sulfide [4.1], acetate, CO_2 and H_2O were the final products. Simultaneously, methanogens could also remove COD by using acetate as an electron donor [2.1] whereas CO_2 served as an electron acceptor to produce methane as a final product [5.1].

COD:SO_4^{2-} ratio was an important parameter affecting the competition between SRB and methanogens in the systems. The relative abundances of SRB were higher when operated at low COD:SO_4^{2-} ratio (COD:SO_4^{2-}=1) whereas the relative abundances of methanogens were higher when operated at high COD:SO_4^{2-} ratio (COD:SO_4^{2-}=6). The COD removal efficiencies were rather close in all of the MFCs, which were 56.06 ± 10.67 % in MFC1, 62.49 ± 11.21 % in MFC3, and 62.49 ± 11.21 % in MFC6. On the other hand, the sulfate removals were different in all of the MFCs, which were 1,209 ± 455, 964 ± 93, and 492 ± 44 mgSO_4^{2-}/L in MFC1, MFC3, and MFC6, respectively, reflecting the different portions of COD removal via sulfate reduction at different COD:SO_4^{2-} ratio. Therefore, COD removal via sulfate reduction in MFC1 was the highest than those in MFC3 and MFC6.

Moreover, sulfide and methane could be produced from other processes which used hydrogen as an electron donor. The results on microbial community analysis also indicated that there were hydrogenotrophic methanogens, such as *methanoregulaceae*, and hydrogen-consuming SRB such as *Desulfovibrio vulgaris* (Hu et al., 2015) in all of the MFCs. Therefore, sulfide [4.2] and methane [5.2] generation by using hydrogen as an initial substrate could occur in these systems.

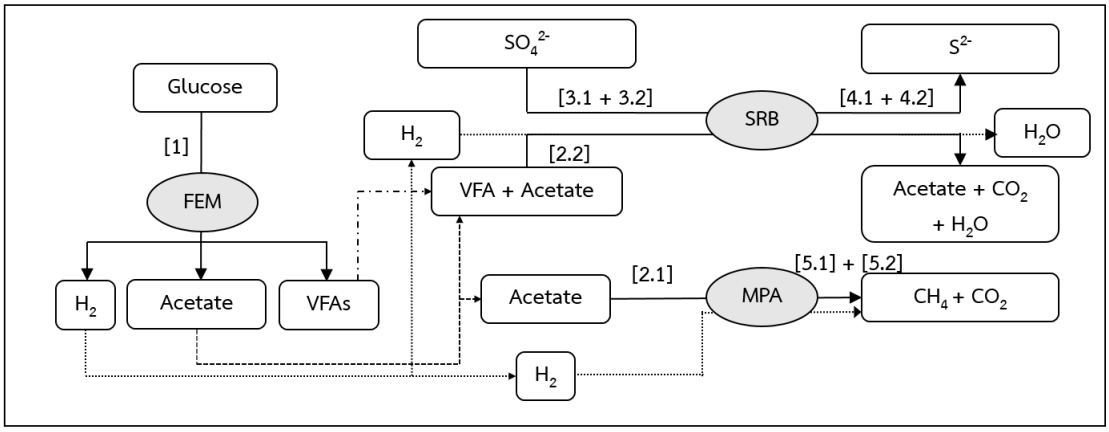


Figure 4.43 Possible mechanisms in the first compartments in MFCs: FEM = fermentative bacteria, SRB = sulfate-reducing bacteria, and MPA = methane-producing archaea

Where; [1] = Ferment via fermentative bacteria

[2] = COD removal [2.1] COD removal from methanogen [2.2] COD removal from SRB [2.3] COD removal from EEM

[3] = Sulfate removal [3.1] Sulfate removal from organic [3.2] Sulfate removal from Hydrogen

[4] = Sulfide production [4.1] Sulfide production from organic [4.2] Sulfide production from hydrogen

[5] = Methane production [5.1] Methane production from acetate [5.2] Methane production from hydrogen

4.5.2 Mechanisms in the second compartments of MFCs

In the second compartments of MFCs, microorganisms in suspended solids and biofilms on the anode electrodes should be responsible for COD and sulfate removals in the second compartments of the MFCs. Since high concentrations of COD still remained in the second compartments, fermentative bacteria consisting of *Klebsiella, Bacteroides,* and *Victivallis* were the predominant genera in suspended microorganisms in MFC1, MFC3, and MFC6, respectively. These microorganisms could ferment glucose into small organic molecules [1].

SRB, *Desulfovibrionaceae* spp., were also found in the second compartments of all MFCs. The presence of SRB in the systems should remove the remaining sulfate in the wastewater. However, only small amounts of microorganisms were observed in suspended solids in the second compartments of all MFCs. Therefore, very low removal of both COD [2.2] and sulfate [3.1] [3.2] from suspended microorganisms were observed.

In addition, methanogens were not found in the suspended solids of the second compartments; therefore, the methane production should not occur from the suspended microorganisms. The possible mechanisms in the suspended microorganisms were shown in Figure 4.44 (blue boxes).

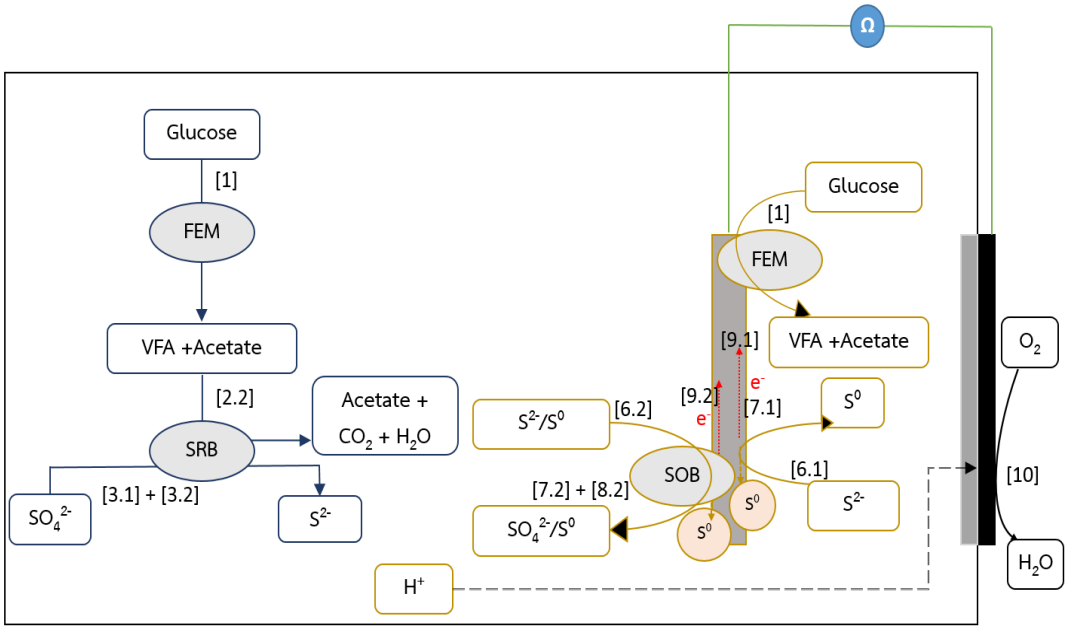


Figure 4.44 Possible mechanisms in the second compartment in MFC1

Where; [1] = Ferment via fermentative bacteria

[2] = COD removal [2.1] COD removal from methanogen [2.2] COD removal from SRB [2.3] COD removal from EEM

[3] = Sulfate removal [3.1] Sulfate removal from organic [3.2] Sulfate removal from Hydrogen

[4] = Sulfide production [4.1] Sulfide production from organic [4.2] Sulfide production from hydrogen

[5] = Methane production [5.1] Methane production from acetate [5.2] Methane production from hydrogen

[6] = Sulfide removal [6.1] Abiotic [6.2] biotic

[7] = Sulfur production [7.1] Abiotic [7.2] biotic

[8] = Sulfate production [8.1] Abiotic [8.2] biotic

[9] = Electrical production [9.1] Abiotic [9.2] biotic

[10] = Oxidation reduction at the cathode electrodes

The mechanisms on the anode electrodes were shown in Figure 4.44 (yellow boxes). There were two main mechanisms including: 1) substrate removal and 2) electricity generation.

Figure 4.44 shows the possible mechanisms in the second compartment of MFC1. The results on microbial community analysis show that *Klebsiella* spp., fermentative bacteria, were the predominant species observed on the anode electrode in MFC1. Therefore, the fermentation process [1] should occur in MFC1 via *Klebsiella* spp. SOB about 3.06% of total sequences were also found on the anode electrode of MFC1, which could oxidize sulfide and sulfur [6.2] to elemental sulfur [7.1] and sulfate [8.1] and simultaneously generate electricity [9.2]. In addition, sulfide could be removed [6.1] via abiotic oxidation on the anode electrode and could generate elemental sulfur [7.1] as a final product. The abiotic sulfide oxidation was considered to be the primary mechanism for electricity generation in MFC1 as previously shown and discussed in Experiment 3. Moreover, exoelectrogenic microorganisms were not found on the anode electrode, suggesting that the electricity generation mainly occurred via sulfide oxidation process.

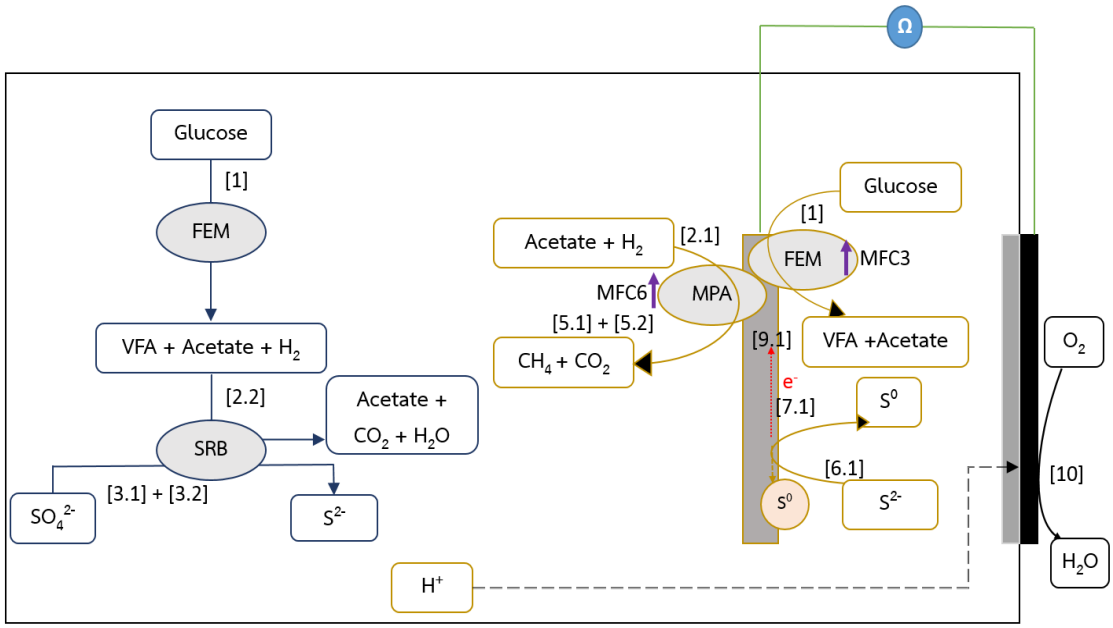


Figure 4.45 Possible mechanisms in the second compartment in MFC3 and MFC6

Figure 4.45 shows possible mechanisms in the second compartments of MFC3 and MFC6. For MFC3, *Tolumonas* spp. and *Klebsiella* spp. were fermentative bacteria observed as predominant species in the systems. Due to the lower concentrations of sulfate and sulfide compared with those in MFC1, methanogens could grow on the anode electrode of MFC3 and remove COD [2.1]. However, exoelectrogenic microorganisms were not found on the anode electrode in MFC3. Therefore, the COD removal in the second compartment of MFC3 was not likely to generate electricity to the system. For sulfide removal, SOB of only 0.76% of total sequences were observed on the anode electrode, suggesting negligible microbial sulfide oxidation [6.2]. The abiotic sulfide oxidation [6.1] should be the primary mechanism of sulfide removal and electricity generation in MFC3 as previously discussed in Experiment 3, which resulted in elemental sulfur [7.1] as a final product.

For MFC6, *Methanoseata*, a methanogen family, was a predominant family on the anode electrode. Methanogens could remove COD [2.1] and produce methane [5.1] via methanogenesis. In addition, *Methanoregulaceae*, which produced methane

from hydrogen [5.2], were also found on the anode electrode. *Tolumonas* spp. (12.54%), which could ferment glucose into small organic molecule via fermentation process [1], were also observed. The presences of large amount of non-exoelectrogenic microorganisms on the anode electrode could increase the voltage losses in the MFC systems. For sulfide removal and electricity generation, abiotic sulfide oxidation [6.1] was considered to be the main mechanism, resulting in elemental sulfur as a final product [7.1]. The mechanisms in MFC6 were similar to those in MFC3, but the relative abundances of fermenters and methanogens were different.

Chapter 5
Conclusion

Summary

Three identical two-compartment single chamber air-breathing microbial fuel cells were used to study the effects of COD:SO_4^{2-} ratio (1, 3, 6) on MFC performances under continuous mode of operation. Glucose equivalent to 3,000 mgCOD/L was used as the organic substrate.

In the first compartments of MFC1, MFC3, and MFC6, the COD removal efficiencies were 56.06 ± 10.67, 62.49 ± 11.21, and 63.22 ± 11.57%, respectively. The sulfate removal efficiencies of 42.96 ± 10.45, 95.01 ± 8.88, and 96.65 ± 7.44% were observed in the first compartments of MFC1, MFC3, and MFC6, respectively, which were equivalent to the sulfate removal of 1,209 ± 455, 964 ± 93, and 492 ± 44 mgSO_4^{2-}/L in MFC1, MFC3, and MFC6, respectively. As a result, dissolved sulfide were 400 ± 69, 265 ± 59, and 119 ± 32 mgS^{2-}/L in MFC1, MFC3, and MFC6, respectively.

From the results on microbial community analysis using 16S rRNA gene amplicon sequencing (MiSeq, Illumina), *Tolumunas* spp. were the predominant species found in the first compartment of all MFCs. *Tolumunas* spp. are the fermenters which can ferment glucose to VFAs and acetate being suitable for obligate anaerobic microorganisms. On the other hand, *Desulfovibrio* spp. were the predominant SRB observed in all MFCs. The percentage of SRB of 17.32%, 15.64%, and 6.98% were observed in MFC1, MFC3, and MFC6, respectively. For methanogens, methanogens of only 0.46, 2.43, and 4.13% of total sequences were observed in the first compartments of MFC1, MFC3, and MFC6, respectively. Methanoregulaceae and Methanosaetaceae were the predominant families obtained in this compartment. It should be noted that

COD:SO_4^{2-} ratio was a factor affecting the treatment efficiencies and microbial communities in the anaerobic bioreactor.

For the second compartment, the COD removal efficiencies were 0.15 ± 9.83, 7.98 ± 10.23, and 9.98 ± 16.50 % for MFC1, MFC3, and MFC6, respectively. On the other hand, there was no significant removal of sulfate in the second compartment. Sulfide removal efficiencies were 49.51 ± 57.74, 24.08 ± 13.74, and 15.69 ± 21.30 mgS^{2-}/L in MFC1, MFC3, and MFC6, respectively. The maximum power densities of 9.33, 1.79, and 1.41 mW/m^2 in MFC1, MFC3, and MFC6, respectively, were observed on day 1 of operation. The decrease of OCV and voltage across the electrodes at 1,000Ω external resistances might be due to the increase in voltage losses consisting of activation losses and ohmic losses. The accumulation of sulfur and non exo-electrogenic biofilm forming on both the anode electrodes and PEM could contribute to the voltage losses in this study. The primary mechanism for electrical generation in all MFCs was abiotic sulfide oxidation. The results of SEM/EDX indicated that there was elemental sulfur accumulated on the anode electrodes after 7 days of operation.

The results on microbial community analysis show that *Klebsiella* spp., *Bacteroides* spp., and *Victivallis* spp. were the fermenters, which were the predominant species in suspended solids in the second compartment of MFC1, MFC3, and MFC6, respectively. *Desulfovibrio* was the predominant genus of SRB in suspended solids in the second compartments of all MFCs. However, methanogens was not found in the suspended solids in the second compartment of all MFCs. On the other hand, *Methanoseata* was predominant on the anode electrode in MFC6 whereas *Klebsiella* and *Tolumonas* were the predominant genera on the anode electrode in MFC1 and MFC3, respectively. Exo-electrogenic microorganisms were not found in all MFCs; therefore, the COD removal in the second compartments was not likely to contribute to electricity generation. However, small amounts of SOB such as *Dyella* were found

on the anode electrodes of all MFCs, which could also contribute to electricity generation in the MFCs.

Suggested areas of future research

1. Since high sulfide remained in the wastewater after passed the second compartment of MFC which had the potential to generate more electricity, many MFCs should be connected in series for removing sulfide remaining in the systems simultaneously with electricity generation and recover elemental sulfur from this process.

2. The increase in COD in the synthetic wastewater should be investigated. The remaining COD after passed MFCs might be high enough to convert to methane in another anaerobic reactor, such as UASB, EGSB, anaerobic fluidize bed reactor, and anaerobic digester.

3. MFCs could be used for hydrogen sulfide removal in biogas to increase the methane content in the biogas. The mass transfer of hydrogen sulfide into liquid phase and sulfide oxidation on anode electrodes should be further studied to investigate the feasibility of these processes. Therefore, separation of hydrogen sulfide from methane with simultaneous electricity generation and elemental sulfur recovery is one of the interesting areas of research.

4. The results showed the accumulation of elemental sulfur on anode electrodes. The materials coating on the surface areas of the anode electrodes for preventing the accumulation of elemental sulfur should be studied and developed. The less sulfur accumulation on anode electrodes is, the longer lifetime of anode electrodes will be.

Engineering significance

This study shows that COD, sulfate, and sulfide could be removed in two-compartment single chamber air-breathing microbial fuel cells (MFCs). These types of MFCs can be applied to treat sulfate-rich wastewater at different COD:SO_4^{2-} ratio simultaneously with electricity generation as energy recovery in the systems. Although the amount of SRB was likely to be higher than methanogens resulting in less amount of methane production, the electricity production could become an alternative energy replacing the loss of methane for this type of wastewater.

Since, the major mechanism generating electrical power in all of the MFCs was sulfide oxidation via abiotic process on the anode electrodes, the MFCs can be applied to treat sulfide-rich wastewater and simultaneously provide electrical energy as the valuable final product. Besides electricity production, the removal of sulfide from wastewater can be applied to control many adverse effects from sulfide, such as odor problems and corrosion in concrete pipes.

Moreover, since the major product on the anode electrodes was elemental sulfur, MFCs might be applied to sulfur recovery from other species of sulfur, such as sulfate, sulfite, and sulfide in wastewater via microbial reduction, microbial oxidation, and abiotic oxidation. Elemental sulfur from this process could be used as an initial substance to produce sulfate for commercial use.

Sulfate concentration in wastewater could be one factor affecting methanogens in the systems. High concentrations of sulfate could decrease the amount of methane generation in the systems. In addition, high sulfate concentration (3,000 mgSO_4^{2-}/L) can also generate sulfide in a high range, which are toxic to methanogens in the systems as observed in MFC1. Therefore, the pre-treatment and sulfide removal process could be applied before passing wastewater into anaerobic bioreactors for generating methane as a final product.

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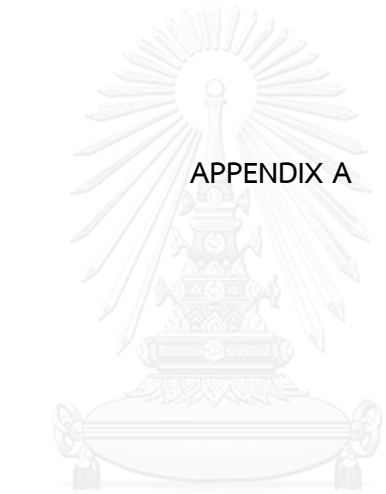
APPENDIX

APPENDIX A



Columbic efficiency using sulfide as a fuel

From CE = (Cp/Ct) x 100

From Ct = (F x bi x Si x v) / M

Where; F = 96,458 C/mol

b = 2 mol

Q = 2.025 L/Day

T = 1 Day

S = Sulfide (inf) - Sulfide (eff) mg/L

M = 32,000 mg

When, the sulfide removal was 18.44 mg/L

Therefore, Ct = (32,000 x 2 x 18.44 x 2.025 x 1)/32,000

= 225.11

From Cp = I x t

When, V = 16.28 Volt at external resistances of 1,000 Ω

Where, t = 86,400

second

I = 0.00001628 A

Therefore, Cp = 86,400 x 0.00001628

= 1.406

Therefore CE = (Cp/Ct) x 100

= (1.406/225.11) x 100

= 0.624 %

The ratio of COD removal from SRB

Since the theoretical ratio of COD:SO_4^{2-} for sulfate reduction is 0.67 assuming negligible biomass yield, the COD removal via sulfate reduction can be calculated from

COD removal from SRB = Sulfate removal x 0.67

Therefore,

The ratio of COD removal = (COD removal from SRB/total COD removal) x 100

Example

If total COD = 1,500 mgCOD/L and sulfate removal = 1,000 mg/L, the COD removal from SRB is COD removal from SRB = 1,000 x 0.67

= 670 mgCOD/L

The ratio of COD removal = (670/1,500) x 100

= 44.67 %

APPENDIX B

Table B1 COD concentration in Experiment 1-1

Day	Reactor1		Day	Reactor2		Reactor4	
	Inf COD (mg/L)	Eff COD (mg/L)		Inf COD (mg/L)	Eff COD (mg/L)	Inf COD (mg/L)	Eff COD (mg/L)
			1	3272	1664	3424	2044
			2	3272	1424	3424	1812
			3	3272	1456	3424	1876
			5	3260	1140	3140	1780
			7	3260	1648	3140	1728
			8	3260	1652	3140	1832
2	2880	1964	11	3293	1732	3267	2100
4	2880	1880	13	3293	1720	3267	1680
8	2880	1744	17	3293	1558	3267	1186
10	2548	1438	19	2512	1677	2446	1253
12	2548	1670	21	2512	1813	2446	1754
20	3137	1533	29	2733	1552	2830	1200
23	3137	1613	32	2733	1626	2830	1529
25	3137	1606	34	2733	1736	2830	1798
26	3343	1496	35	3265	1671	3349	2138
29	3343	1466	38	3265	1090	3349	1648
30	3295	1725	39	3308	957	3256	1609
Average	3,011	1,648		3,090	1,604	3,107	1703
SD	287	169		303	166	309	285
Removal efficiencies	44.68 ± 8.5			46.85 ± 9.50		45.01 ± 8.50	

Table B2 Sulfate concentration in Experiment 1-1

Date	Reactor1		Date	Reactor2		Reactor4	
	Inf SO_4^{2-} (mg/L)	Eff SO_4^{2-} (mg/L)		Inf SO_4^{2-} (mg/L)	Eff SO_4^{2-} (mg/L)	Inf SO_4^{2-} (mg/L)	Eff SO_4^{2-} (mg/L)
			2	1548	1043	800	370
			3	1548	1043	800	248
			5	1548	707	800	85
			7	1527	904	820	150
			8	1527	652	820	97
1	876	745	10	1527	540	820	41
4	750	622	13	1500	744	750	94
8	750	411	17	1500	689	750	214
12	1500	796	21	1500	891	750	214
18	1843	879	27	1547	950	867	357
20	1843	859	29	1547	980	867	314
23	1843	730	32	1547	980	867	194
25	1843	637	34	1547	998	867	342
26	2074	540	35	1454	974	773	494
29	2074	779	38	1454	1333	773	0
30	2074	894	39	1454	1542	773	0
Average	**-**	**-**		**1,517**	**935**	**806**	**198**
SD	**-**	**-**		**36**	**251**	**43**	**110**
Removal efficiencies	**58.9 ± 8.5**			**43.41 ± 10.58**		**75.27 ± 17.99**	

Table B3 pH, VFAs, and alkalinity concentration in Experiment 1-1

Date	Reactor1			Date	Reactor2			Reactor4		
	pH	Alk	VFA		pH	Alk	VFA	pH	Alk	VFA
				1	7.00	2420	920	6.95	2300	600
				2	7.00	2300	880	7.00	2300	750
				3	7.01	-	-	6.78	-	-
				5	7.20	2140	840	7.40	2240	820
				8	7.15	2200	890	6.98	2400	780
1	6.53	2000	660	10	7.21	2250	850	6.96	2200	700
2	6.56	2200	750	11	7.09	2300	870	7.27	2350	730
4	6.81	2400	880	13	7.14	2200	850	7.08	2300	750
8	7.30	2200	750	17	7.13	-	-	7.27	-	-
10	7.41	2400	730	19	7.28	-	-	7.55	-	-
12	6.81	2100	800	21	6.86	-	-	6.89	-	-
13	7.29	2200	750	22	7.19	-	-	7.19	-	-
20	7.23	-	-	29	6.99	-	-	7.25	-	-
23	7.20	-	-	32	6.94	-	-	7.17	-	-
25	7.45	-	-	34	7.20	-	-	6.88	-	-
26	6.98	-	-	35	6.88	-	-	7.11	-	-
29	7.13	-	-	38	6.99	-	-	6.92	-	-
30	7.29	-	-	39	7.08	-	-	7.03	-	-
Average	**7.08**	**2214**	**760**		**7.07**	**2258**	**871**	**7.09**	**2298**	**733**
SD	**0.31**	**146**	**63**		**0.12**	**92**	**28**	**0.20**	**66**	**70**
VFA:Alk	**0.348 ± 0.026**				**0.386 ± 0.009**			**0.313 ± 0.074**		

Table B4 COD concentrations in Experiment 1-2

Date	MFC1		MFC3		MFC6	
	Inf COD (mg/L)	Eff COD (mg/L)	Inf COD (mg/L)	Eff COD (mg/L)	Inf COD (mg/L)	Eff COD (mg/L)
2	3321	2062	3321	2179	3334	1868
4	3321	1634	3321	1686	3334	1401
7	3200	2504	3200	2163	3200	2137
8	3200	2400	3200	2452	3200	1901
10	3134	1822	3108	1901	3147	1691
11	3134	1901	3108	2006	3147	1586
12	3134	1901	3108	1613	3147	1586
14	3134	1677	3108	1793	3147	1406
16	2567	1716	2500	1716	2567	1522
18	2567	1638	2500	1793	2567	1690
20	3000	2171	3000	1543	3000	928
22	3000	1778	3000	1844	3000	1399
24	3000	1778	3000	1582	3000	1373
27	3000	1793	3000	1793	3000	1535
28	3000	1870	3000	1948	3000	1690
30	3309	1513	3303	1513	3193	1460
32	3309	1356	3303	1852	3193	1173
34	3182	1826	3182	1826	3130	1708
37	3128	2662	3000	1664	3000	1509
39	3128	2455	3000	1690	3000	1638
41	3128	1716	3000	1625	3000	1807
43	3213	1930	3233	2136	3321	2284
46	3150	1836	3400	1846	3459	2284
47	3091	2059	3551	1737	3493	2052
49	3416	1870	3590	1698	3551	2046
52	3416	1840	3378	1222	3281	1640

Table B4 COD concentrations in Experiment 1-2 (Cont.)

Date	MFC1		MFC3		MFC6	
	Inf COD (mg/L)	Eff COD (mg/L)	Inf COD (mg/L)	Eff COD (mg/L)	Inf COD (mg/L)	Eff COD (mg/L)
54	3314	1820	3320	1344	3310	1537
57	3139	1672	-	-	-	-
59	3255	1763	-	-	-	-
61	3255	1751	-	-	-	-
Average	**3,130**	**1,900**	**3,120**	**1,715**	**3,150**	**1,640**
SD	**195**	**300**	**250**	**260**	**255**	**300**
Removal efficiencies	**39.6 ± 9.8**		**41.3 ± 7.9**		**47.7 ± 9.1**	

Table B5 sulfate concentrations in Experiment 1-2

Date	MFC1		MFC3		MFC6	
	Inf COD (mg/L)	Eff COD (mg/L)	Inf COD (mg/L)	Eff COD (mg/L)	Inf COD (mg/L)	Eff COD (mg/L)
2	3406	2990	1104	1291	500	829
4	3406	2710	1104	863	500	446
5	3406	2910	1104	678	500	232
7	3406	2805	1104	606	500	219
8	3406	2845	1104	755	500	67
11	3145	2781	1130	682	583	87
12	3145	2572	1130	213	583	73
14	3145	2544	1130	447	583	112
16	3145	2478	1130	291	583	144
18	3145	2551	1130	337	583	89
20	3163	2696	1088	264	544	85
22	3163	2551	1088	291	544	85
24	3163	2332	1088	400	544	25
27	3145	2452	1065	181	524	12
28	3145	2649	1065	505	524	199
30	3163	2021	1088	0	544	12
32	3163	2021	1088	600	544	210
34	3163	2688	1088	24	544	53
37	3198	2923	1066	50	493	97
39	3198	2609	1066	260	493	89
41	3198	2256	1066	72	493	98
43	3198	2357	1066	23	493	0
46	3198	2238	1066	80	493	118
47	3309	2416	1083	13	640	110
49	3442	2404	1046	8	521	38
52	3027	2404	1098	281	580	123

Table B5 sulfate concentrations in Experiment 1-2

Date	MFC1		MFC3		MFC6	
	Inf COD (mg/L)	Eff COD (mg/L)	Inf COD (mg/L)	Eff COD (mg/L)	Inf COD (mg/L)	Eff COD (mg/L)
54	2975	1886	974	3	573	28
57	3182	2197	-	-	-	-
59	2975	1886	-	-	-	-
61	3300	2200	-	-	-	-
Average	**3,200**	**2,225**	**1,090**	**341**	**537**	**110**
SD	**250**	**197**	**25**	**321**	**38**	**94**
Removal efficiencies	**28.9 ± 4.6**		**72.3 ± 24.0**		**82.0 ± 12.6**	

Table B6 sulfide concentrations in Experiment 1-2

Date	MFC1 (mgS^{2-}/L)	MFC3 (mgS^{2-}/L)	MFC6 (mgS^{2-}/L)
20	52	99	46
22	107	88	68
24	160	55	56
27	86	48	41
28	62	82	51
30	89	160	91
32	114	72	52
34	76	127	63
37	51	140	67
39	99	62	16
41	171	94	45
43	208	31	8
46	145	112	25
47	160	120	52
49	285	174	103
52	175	88	60
54	236	108	55
58	278	-	-
61	249	-	-
Average	**134**	**98**	**53**
SD	**67**	**39**	**24**

Table B7 Alkalinity and volatile fatty acids in Experiment 1-2

Date	MFC1		MFC3		MFC6	
	Alk (mg/L as $CaCO_3$)	VFA (mg/L as CH_3COOH)	Alk (mg/L as $CaCO_3$)	VFA (mg/L as CH_3COOH)	Alk (mg/L as $CaCO_3$)	VFA (mg/L as CH_3COOH)
3	1740	640	1620	650	1600	640
4	1980	740	2040	740	1800	720
5	1950	750	1950	730	1900	720
7	2000	750	1950	780	1900	780
8	1950	700	1900	710	1850	720
Change influent concentration from 2,000 to 3,000 mg/L						
12	2800	1000	2850	1050	2650	880
14	2820	1220	2700	1160	2660	920
16	2940	940	3040	900	2690	800
18	2740	1140	2780	1220	2520	920
Average	**2825**	**1075**	**2842**	**1082**	**2630**	**880**
SD	**84**	**128**	**145**	**141**	**75**	**56**
VFA:Alk	**0.38 ± 0.05**		**0.38 ± 0.06**		**0.34 ± 0.03**	

Table B8 pH in Experiment 1-2

Day	MFC1	MFC3	MFC6
2	6.95	6.96	6.65
3	6.65	6.65	6.95
4	6.94	7.01	7.2
5	7.16	7.03	7.12
7	7.01	6.84	6.88
8	7.04	6.72	6.7
10	7.07	7.02	7.11
11	7.05	6.95	6.95
12	6.8	7.4	7.03
14	7.12	7.01	6.99
16	7.01	6.93	6.83
18	7.07	6.93	6.96
20	7.41	7.19	7.33
22	7.02	7.11	7
24	7.19	7.16	7.04
27	7.32	6.91	6.89
28	7.67	7.07	7.05
30	7.2	7.07	7.35
32	7.38	6.97	7.34
34	7.34	6.96	6.91
37	7.45	6.86	7.11
39	7.33	7.16	7.1
41	6.83	7.36	6.82
43	6.99	6.79	6.71
46	7.17	7.15	6.82
47	7.24	7.49	7.17
49	7.74	7.17	6.89
52	7.4	7.19	7.05

Table B8 pH in Experiment 1-2

Day	MFC1	MFC3	MFC6
57	7.36	-	-
59	7.3	-	-
61	7.46	-	-
Average	**7.18**	**7.04**	**7.00**
SD	**0.24**	**0.19**	**0.18**

Table B9 COD concentrations in Experiment 2

Day	MFC1			Day	MFC3			MFC6		
	Inf COD	Eff1 COD	Eff2 COD		Inf COD	Eff1 COD	Eff2 COD	Inf COD	Eff1 COD	Eff2 COD
				1	3129	900	926	3212	1537	1293
				3	3260	1261	1158	3352	1370	1389
				5	3260	1146	1174	3352	1517	1513
0	3248	1744	1696	7	3285	1256	1091	3248	1510	1503
2	3200	1703	1730	9	3200	1174	995	3200	1500	1554
4	3100	2082	2001	11	3100	1270	1242	3100	1554	1609
6	3150	1795	1624	13	3354	973	1091	3373	1032	1170
11	3100	1091	1209	18	3100	993	1013	3100	973	895
13	3150	1406	1249	20	3150	1072	973	3100	1032	993
15	3000	987	1068	22	3000	1243	1054	3000	960	878
17	2900	1365	1312	24	3000	1054	1014	2900	811	757
20	2678	824	878	27	1376	1095	1000	1776	973	951
22	3100	1166	1113	29	2900	875	848	2900	795	557
24	3000	1087	1060	31	3000	1060	875	3000	1644	689
27	3000	1034	1186	34	3000	928	742	3000	689	636
28	3076	1283	1230	35	2943	1046	861	3076	729	492
30	3000	1072	1336	37	3000	993	993	3000	782	676
31	2945	1520	1441	38	2918	1178	835	2918	571	492

Table B9 COD concentrations in Experiment 2

Day	MFC1			Day	MFC3			MFC6		
	Inf COD	Eff1 COD	Eff2 COD		Inf COD	Eff1 COD	Eff2 COD	Inf COD	Eff1 COD	Eff2 COD
Average	3,043	1,344	1,342		2,999	1,084	994	3,033	1,110	1,003
SD	139	359	303		427	127	131	349	359	391
Removal efficiencies		56.06	0.15			62.49	7.98		63.22	9.98
SD		10.67	9.8			11.21	10.23		11.57	16.5

Table B10 sulfate concentrations in Experiment 2

Day	MFC1			Day	MFC3			MFC6		
	Inf SO_4^{2-}	Eff1 SO_4^{2-}	Eff2 SO_4^{2-}		Inf SO_4^{2-}	Eff1 SO_4^{2-}	Eff2 SO_4^{2-}	Inf SO_4^{2-}	Eff1 SO_4^{2-}	Eff2 SO_4^{2-}
				1	1060	198	345	530	0	0
				3	1046	31	115	530	4.32	0
				5	1100	5	7.5	550	0	0
0	3100	2200	2050	7	1033	12.5	0	508	0	0
2	3150	2000	1850	9	1016	20	50	541	0	0
4	3100	1850	1850	11	-	-	-	-	-	-
6	3150	2000	1900	13	1000	0	5	500	0	0
11	3000	2100	1900	18	1000	345	205	500	35	25
13	3000	1800	1750	20	1000	45	30	500	150	55
15	-	-	-	22	1000	0	10	500	15	40
17	3000	1500	1400	24	1000	70	30	500	5	0
20	3000	1905	2056	27	1000	40	20	500	0	35
22	3000	1504	1504	29	1000	65	90	500	0	15
24	3000	1303	1403	31	1000	20	15	500	0	15
27	3000	1805	1704	34	1000	0	10	500	0	35
28	3000	1550	1450	35	1000	5	0	500	45	0
31	3000	1050	1400	38	1000	5	15	500	30	5
32	3000	1600	1300	39	1000	0	60	500	50	0

Table B10 sulfate concentrations in Experiment 2

Day	MFC1			Day	MFC3			MFC6		
	Inf SO_4^{2-}	Eff1 SO_4^{2-}	Eff2 SO_4^{2-}		Inf SO_4^{2-}	Eff1 SO_4^{2-}	Eff2 SO_4^{2-}	Inf SO_4^{2-}	Eff1 SO_4^{2-}	Eff2 SO_4^{2-}
Average	3,036	1,736	1,709		1,015	58.8	59.2	509	16.8	14.06
SD	60	334	249		29	91.9	93.7	17	37.2	18.28
Removal efficiencies		42.96	N.A.			95.01	N.A.		96.65	N.A.
SD		10.45	N.A.			8.88	N.A.		7.44	N.A.

Table B11 sulfide concentrations in Experiment 2

Day	MFC1		Day	MFC3		MFC6	
	1-1	1-2		3-1	3-2	6-1	6-2
			2	222.59	201.02	143.32	67.85
			5	185.80	171.85	62.78	39.32
0	323.41	216.24	7	284.73	272.05	165.51	123.66
2	393.17	260.00	9	317.07	282.83	119.85	93.85
4	403.95	352.59	11	346.88	308.20	126.83	113.51
11	303.12	286.63	18	171.22	166.15	114.78	88.15
13	400.15	345.61	20	206.73	177.56	104.63	111.61
15	526.34	347.51	22	277.12	227.66	108.44	112.88
20	421.71	419.80	27	308.20	283.46	152.83	139.51
22	471.17	474.98	29	313.27	254.29	69.12	62.15
27	364.00	379.85	34	280.29	257.46	140.15	152.83
Average	**400**	**342**		**265**	**237**	**119**	**100**
SD	**69**	**80**		**59**	**59**	**32**	**34**
Removal	**14.23 ± 15.71**			**10.32 ± 5.01**		**15.69 ± 21.30**	

Table B12 pH in Experiment 2

Day	MFC1		Day	MFC3		MFC6	
	1-1	**1-2**		**3-1**	**3-2**	**6-1**	**6-2**
			1	7.33	7.63	6.97	7.17
			3	7.11	7.21	6.89	7.35
			5	7.12	7.26	6.75	7.25
0	7.22	7.66	7	7.2	7.4	6.83	7.27
2	7.32	7.86	9	7.15	7.2	6.91	6.94
4	7.15	7.43	11	7.07	7.17	6.89	6.81
11	7.25	7.42	18	7	7.11	7	7.08
13	7.32	7.6	20	7.14	7.3	6.93	7.15
15	7.42	7.07	22	6.94	7.23	7.04	6.98
17	7.2	7.39	24	7.02	7.19	7.01	7.18
20	7.6	7.82	27	7.14	7.35	7.09	7.23
22	7.6	8.08	29	7.12	7.48	7.3	7.39
24	7.61	7.95	31	7.09	7.28	7.13	7.29
27	7.48	7.2	34	7.07	6.98	7.39	7.01
28	7.49	7.14	35	6.93	6.81	6.96	6.94
31	7.2	7.75	38	6.85	6.93	6.92	6.98
32	6.78	7.06	39	6.73	6.96	6.92	7.02
Average	**7.37**	**7.57**		**7.08**	**7.22**	**7.00**	**7.12**
SD	**0.17**	**0.32**		**0.12**	**0.20**	**0.16**	**0.20**

Table B13 OCV and voltage across the electrodes at 1,000Ω external resistances in Experiment 2

Day	MFC1		Day	MFC3		MFC6	
	OCV	Voltage		OCV	Voltage	OCV	Voltage
			0	475	110	324	55
			1	322	45	284	36
			2	272	68	280	37
			3	222	35	247	39
			4	200	30	226	35
			5	175	25	180	29
			6	192	24	176	32
0	635	300	7	194	27	176	27
1	538	124	8	194	34	182	20
2	396	83	9	193	34	185	18
3	300	65	10	214	38	195	18
4	315	65	11	210	32	175	17
5	272	65	12	230	37	190	16
6	212	48	13	232	34	190	14
8	173	36	15	225	35	187	14
11	170	35	18	204	32	185	13
12	167	32	19	202	30	190	14
13	163	24	20	202	16	199	15
15	178	25	22	199	36	205	14
17	220	29	24	221	36	213	15
18	236	32	25	214	42	207	15
20	223	39	27	216	28	200	18
21	214	46	28	207	34	201	17
22	195	38	29	199	36	194	18
23	210	47	30	200	33	195	17
24	210	42	31	217	47	198	17

Table B13 OCV and voltage across the electrodes at 1,000Ω external resistances in Experiment 2

Day	MFC1		Day	MFC3		MFC6	
	OCV	Voltage		OCV	Voltage	OCV	Voltage
26	224	49	33	185	36	203	17
28	220	47	35	225	48	195	18
31	222	42	38	213	40	202	17
32	218	38	39	116	36	123	46
Average	203.2	38.2		209.6	35.1	194.8	16.3
SD	23.7	7.9		12.8	6.6	9.2	1.8

Table B14 Power densities and current densities adjusted by external resistances in Experiment 2

Day	MFC1			MFC3			MFC6		
	Vol (mV)	Current density (mA/m2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)
1	8	68.09	0.54	3	25.53	0.08	5	42.55	0.21
	14	68.29	0.96	5	24.39	0.12	8	39.02	0.31
	36	72.00	2.59	11	22.00	0.24	12.9	25.80	0.33
	56	67.88	3.80	19	23.03	0.44	17.9	21.70	0.39
	81	65.85	5.33	41	22.16	0.91	36.5	19.73	0.72
	113	61.08	6.90	51	20.40	1.04	49	19.60	0.96
	136	54.40	7.40	69	18.40	1.27	65	17.33	1.13
	154	51.33	7.91	78	15.60	1.22	80	16.00	1.28
	179	47.73	8.54	114	15.20	1.73	137	10.96	1.50
	200	44.44	8.89	147	11.76	1.73	186	7.44	1.38
	214	42.80	9.16	212	8.48	1.80	216	5.76	1.24
	260	34.67	9.01	263	7.01	1.84	225	5.00	1.13
	322	25.76	8.29	280	6.22	1.74	271	2.17	0.59
	364	21.41	7.79	341	2.73	0.93	293	1.26	0.37
	405	16.20	6.56	376	1.62	0.61	297	0.79	0.24
	438	11.68	5.12	396	1.06	0.42	310	0.00	0.00
	442	9.82	4.34	428	0.00	0.00			
	487	3.90	1.90						
	513	2.14	1.10						
	522	1.39	0.73						
	538	0.00	0.00						
2	5	42.55	0.21	2	17.02	0.03	3	25.53	0.08
	9	43.90	0.40	4	19.51	0.08	4	19.51	0.08
	21	42.00	0.88	10	20.00	0.20	9	18.00	0.16
	33	40.00	1.32	16	19.39	0.31	14	16.97	0.24
	46	37.40	1.72	34	18.38	0.62	30	16.22	0.49

Table B14 Power densities and current densities adjusted by external resistances in Experiment 2

Day	MFC1			MFC3			MFC6		
	Vol (mV)	Current density (mA/m2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)
	66	35.68	2.35	45	18.00	0.81	41	16.40	0.67
	83	33.20	2.76	53	17.67	0.94	47	15.67	0.74
	95	31.67	3.01	62	16.53	1.03	53	14.13	0.75
	111	29.60	3.29	67	14.89	1.00	64	14.22	0.91
	122	27.11	3.31	70	14.00	0.98	68	13.60	0.92
	126	25.20	3.18	97	12.93	1.25	91	12.13	1.10
	166	22.13	3.67	133	10.64	1.42	122	9.76	1.19
	215	17.20	3.70	167	9.82	1.64	147	8.65	1.27
	243	14.29	3.47	190	7.60	1.44	170	6.80	1.16
	276	11.04	3.05	217	5.79	1.26	190	5.07	0.96
	306	8.16	2.50	231	5.13	1.19	203	4.51	0.92
	315	7.00	2.21	287	2.30	0.66	250	2.00	0.50
	361	2.89	1.04	307	1.28	0.39	271	1.13	0.31
	378	1.58	0.60	312	0.83	0.26	281	0.75	0.21
	383	1.02	0.39	322	0.00	0.00	284	0.00	0.00
	396	0.00	0.00						
4	4	34.04	0.14	1	8.51	0.01	3	25.53	0.08
	7	34.15	0.24	3	14.63	0.04	4	19.51	0.08
	16	32.00	0.51	6	12.00	0.07	10	20.00	0.20
	26	31.52	0.82	10	12.12	0.12	15	18.18	0.27
	38	30.89	1.17	14	11.38	0.16	23	18.70	0.43
	53	28.65	1.52	20	10.81	0.22	32	17.30	0.55
	65	26.00	1.69	26	10.40	0.27	39	15.60	0.61
	76	25.33	1.93	29	9.67	0.28	46	15.33	0.71
	90	24.00	2.16	35	9.33	0.33	55	14.67	0.81
	103	22.89	2.36	41	9.11	0.37	62	13.78	0.85

Table B14 Power densities and current densities adjusted by external resistances in Experiment 2

Day	MFC1			MFC3			MFC6		
	Vol (mV)	Current density (mA/m2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)
	110	22.00	2.42	42	8.40	0.35	66	13.20	0.87
	135	18.00	2.43	56	7.47	0.42	85	11.33	0.96
	175	14.00	2.45	83	6.64	0.55	111	8.88	0.99
	199	11.71	2.33	98	5.76	0.56	131	7.71	1.01
	227	9.08	2.06	119	4.76	0.57	151	6.04	0.91
	249	6.64	1.65	138	3.68	0.51	172	4.59	0.79
	257	5.71	1.47	147	3.27	0.48	181	4.02	0.73
	293	2.34	0.69	193	1.54	0.30	217	1.74	0.38
	304	1.27	0.39	210	0.88	0.18	232	0.97	0.22
	308	0.82	0.25	217	0.58	0.13	237	0.63	0.15
	315	0.00	0.00	230	0.00	0.00	245	0.00	0.00
11	2	17.02	0.03	2	17.02	0.03	1	8.51	0.01
	3	14.63	0.04	4	19.51	0.08	2	9.76	0.02
	7	14.00	0.10	7	14.00	0.10	4	8.00	0.03
	12	14.55	0.17	10	12.12	0.12	7	8.48	0.06
	17	13.82	0.23	16	13.01	0.21	10	8.13	0.08
	25	13.51	0.34	22	11.89	0.26	15	8.11	0.12
	32	12.80	0.41	31	12.40	0.38	19	7.60	0.14
	37	12.33	0.46	37	12.33	0.46	23	7.67	0.18
	44	11.73	0.52	42	11.20	0.47	27	7.20	0.19
	49	10.89	0.53	49	10.89	0.53	31	6.89	0.21
	53	10.60	0.56	55	11.00	0.61	34	6.80	0.23
	68	9.07	0.62	69	9.20	0.63	46	6.13	0.28
	89	7.12	0.63	93	7.44	0.69	66	5.28	0.35
	102	6.00	0.61	106	6.24	0.66	80	4.71	0.38
	117	4.68	0.55	131	5.24	0.69	100	4.00	0.40

Table B14 Power densities and current densities adjusted by external resistances in Experiment 2

Day	MFC1			MFC3			MFC6		
	Vol (mV)	Current density (mA/m2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)
	123	3.28	0.40	153	4.08	0.62	117	3.12	0.37
	130	2.89	0.38	161	3.58	0.58	126	2.80	0.35
	150	1.20	0.18	190	1.52	0.29	163	1.30	0.21
	158	0.66	0.10	198	0.83	0.16	175	0.73	0.13
	160	0.43	0.07	205	0.55	0.11	183	0.49	0.09
	167	0.00	0.00	212	0.00	0.00	195	0.00	0.00
18	1	8.51	0.01	2	17.02	0.03	1	8.51	0.01
	2	9.76	0.02	3	14.63	0.04	1	4.88	0.00
	3	6.00	0.02	7	14.00	0.10	3	6.00	0.02
	8	9.70	0.08	11	13.33	0.15	5	6.06	0.03
	12	9.76	0.12	16	13.01	0.21	7	5.69	0.04
	26	14.05	0.37	25	13.51	0.34	10	5.41	0.05
	32	12.80	0.41	32	12.80	0.41	13	5.20	0.07
	35	11.67	0.41	37	12.33	0.46	15	5.00	0.08
	43	11.47	0.49	45	12.00	0.54	19	5.07	0.10
	50	11.11	0.56	51	11.33	0.58	20	4.44	0.09
	54	10.80	0.58	56	11.20	0.63	22	4.40	0.10
	71	9.47	0.67	72	9.60	0.69	33	4.40	0.15
	98	7.84	0.77	98	7.84	0.77	48	3.84	0.18
	118	6.94	0.82	113	6.65	0.75	60	3.53	0.21
	144	5.76	0.83	133	5.32	0.71	76	3.04	0.23
	166	4.43	0.73	151	4.03	0.61	94	2.51	0.24
	173	3.84	0.67	157	3.49	0.55	101	2.24	0.23
	208	1.66	0.35	185	1.48	0.27	141	1.13	0.16
	221	0.92	0.20	193	0.81	0.16	160	0.67	0.11
	226	0.60	0.14	204	0.54	0.11	169	0.45	0.08

Table B14 Power densities and current densities adjusted by external resistances in Experiment 2

Day	MFC1			MFC3			MFC6		
	Vol (mV)	Current density (mA/m2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)
	237	0.00	0.00	210	0.00	0.00	185	0.00	0.00
21	2	17.02	0.03	1	8.51	0.01	1	8.51	0.01
	4	19.51	0.08	1	4.88	0.00	1	4.88	0.00
	9	18.00	0.16	2	4.00	0.01	3	6.00	0.02
	15	18.18	0.27	4	4.85	0.02	5	6.06	0.03
	24	19.51	0.47	5	4.07	0.02	8	6.50	0.05
	35	18.92	0.66	12	6.49	0.08	12	6.49	0.08
	46	18.40	0.85	16	6.40	0.10	15	6.00	0.09
	54	18.00	0.97	19	6.33	0.12	18	6.00	0.11
	65	17.33	1.13	25	6.67	0.17	22	5.87	0.13
	74	16.44	1.22	31	6.89	0.21	26	5.78	0.15
	78	15.60	1.22	37	7.40	0.27	29	5.80	0.17
	92	12.27	1.13	50	6.67	0.33	40	5.33	0.21
	119	9.52	1.13	67	5.36	0.36	56	4.48	0.25
	136	8.00	1.09	87	5.12	0.45	69	4.06	0.28
	156	6.24	0.97	111	4.44	0.49	89	3.56	0.32
	172	4.59	0.79	129	3.44	0.44	107	2.85	0.31
	178	3.96	0.70	132	2.93	0.39	112	2.49	0.28
	200	1.60	0.32	175	1.40	0.25	157	1.26	0.20
	208	0.87	0.18	183	0.76	0.14	176	0.74	0.13
	211	0.56	0.12	192	0.51	0.10	185	0.49	0.09
	214	0.00	0.00	202	0.00	0.00	199	0.00	0.00
24	2	17.02	0.03	2	17.02	0.03	1	8.51	0.01

Table B14 Power densities and current densities adjusted by external resistances in Experiment 2

Day	MFC1			MFC3			MFC6		
	Vol (mV)	Current density (mA/m2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)
	5	24.39	0.12	3	14.63	0.04	1	4.88	0.00
	11	22.00	0.24	9	18.00	0.16	3	6.00	0.02
	18	21.82	0.39	13	15.76	0.20	5	6.06	0.03
	21	17.07	0.36	19	15.45	0.29	8	6.50	0.05
	32	17.30	0.55	28	15.14	0.42	12	6.49	0.08
	47	18.80	0.88	31	12.40	0.38	14	5.60	0.08
	54	18.00	0.97	33	11.00	0.36	18	6.00	0.11
	64	17.07	1.09	41	10.93	0.45	22	5.87	0.13
	72	16.00	1.15	50	11.11	0.56	26	5.78	0.15
	77	15.40	1.19	54	10.80	0.58	28	5.60	0.16
	93	12.40	1.15	71	9.47	0.67	38	5.07	0.19
	120	9.60	1.15	95	7.60	0.72	56	4.48	0.25
	138	8.12	1.12	110	6.47	0.71	67	3.94	0.26
	156	6.24	0.97	125	5.00	0.63	86	3.44	0.30
	169	4.51	0.76	151	4.03	0.61	105	2.80	0.29
	174	3.87	0.67	162	3.60	0.58	114	2.53	0.29
	196	1.57	0.31	193	1.54	0.30	160	1.28	0.20
	203	0.85	0.17	210	0.88	0.18	182	0.76	0.14
	205	0.55	0.11	214	0.57	0.12	188	0.50	0.09
	210	0.00	0	222	0.00	0.00	205	0.00	0.00

Table B15 COD concentration after replacing the anode electrodes

Day	MFC1			MFC3			MFC6		
	Inf COD mg/L	Eff1 COD mg/L	Eff2 COD mg/L	Inf COD mg/L	Eff1 COD mg/L	Eff2 COD mg/L	Inf COD mg/L	Eff1 COD mg/L	Eff2 COD mg/L
1	2964	1401	765	2119	1524	642	2119	847	724
2	2591	1289	1428	2529	1289	793	2427	515	476
3	2201	1073	601	2283	1155	478	2365	806	499
4	2776	1190	1229	3036	793	674	2735	555	396
5	2324	1309	1269	2242	674	515	2119	555	476
6	3033	1190	1031	3033	793	555	3033	555	277
7	2591	991	991	2529	753	833	2529	555	317
8	2964	1031	1110	2964	674	436	2964	515	277
Average	**2680**	**1184**	**1050**	**2592**	**957**	**616**	**2536**	**613**	**430**
SD	**308**	**145**	**270**	**374**	**322**	**145**	**350**	**133**	**149**
Removal Efficiency		**55.3**	**10.5**		**61.4**	**31.0**		**75.1**	**30.3**
SD		**7.1**	**22.3**		**17.4**	**22.7**		**8.1**	**16.4**

Table B16 sulfate concentration after replacing the anode electrodes

Day	MFC1			MFC3			MFC6		
	Inf SO_4^{2-} mg/L	Eff1 SO_4^{2-} mg/L	Eff2 SO_4^{2-} mg/L	Inf SO_4^{2-} mg/L	Eff1 SO_4^{2-} mg/L	Eff2 SO_4^{2-} mg/L	Inf SO_4^{2-} mg/L	Eff1 SO_4^{2-} mg/L	Eff2 SO_4^{2-} mg/L
1	3000	2500	1838	1000	453	432	500	0	170
2	3000	1650	1700	1000	353	235	500	0	292
3	3000	1650	1550	1000	15	0	500	50	0
4	3000	1650	1744	1000	0	292	500	0	254
5	3000	1650	1600	1000	4	283	500	0	236
6	3000	1650	1554	1000	0	292	500	0	18
7	3000	1886	1650	1000	8	8	500	0	164
8	3000	1650	1600	1000	4	307	500	0	212
Average	3000	1786	1654	1000	105	231	500	6.2	168
SD	0	300	100	0	186	156	0	17.7	107
Removal Efficiency		40.5	6.0		89.5	N.A.		98.8	N.A.
SD		10.0	10.0		18.6	N.A.		3.5	N.A.

Table B17 sulfide concentration after replacing the anode electrodes

Day	MFC1		MFC3		MFC6	
	1-1	1-2	3-1	3-2	6-1	6-2
1	458	447	341	310	196	170
2	519	542	379	315	195	192
3	734	704	413	396	196	205
4	618	590	367	376	190	181
6	552	552	342	316	149	131
7	591	596	391	332	136	137
8	581	576	387	334	196	146
Average	**579**	**572**	**374**	**340**	**180**	**166**
SD	**86**	**76**	**26**	**33**	**26**	**28**
Removal	**6.4 ± 18.8**		**34.5 ± 26.2**		**13.8 ± 19.8**	

Table B18 pH after replacing the anode electrodes

Day	MFC1		MFC3		MFC6	
	1-1	1-2	3-1	3-2	6-1	6-2
1	6.80	6.98	6.79	7.00	6.85	7.00
2	7.11	7.33	6.91	6.98	7.04	7.01
3	7.10	7.61	6.85	6.95	7.07	7.13
4	7.33	7.60	7.03	6.99	7.05	7.02
6	7.26	7.57	7.00	7.15	6.90	6.99
7	7.28	7.58	6.95	7.05	7.52	7.59
8	7.33	7.51	7.04	7.28	7.05	7.12
Average	**7.19**	**7.48**	**6.95**	**7.07**	**7.06**	**7.12**
SD	**0.18**	**0.22**	**0.09**	**0.11**	**0.20**	**0.20**

Table B19 OCV and voltage across the electrodes at 1,000 Ω external resistances after replacing the anode electrodes

Day	MFC1		MFC3		MFC6	
	OCV (mV)	Voltage (mV)	OCV (mV)	Voltage (mV)	OCV (mV)	Voltage (mV)
0	186	106	116	36	123	46
1	216	150	208	65	194	40
2	211	143	200	53	187	42
3	208	134	195	57	179	37
4	210	132	197	62	176	35
5	208	128	189	51	170	33
6	214	124	189	47	181	36
8	238	128	204	44	178	26
Average	**215**	**134**	**197**	**54**	**181**	**36**
SD	**11**	**9**	**7**	**8**	**8**	**5**

Table B20 Power densities and current densities adjusted by external resistances after replacing the anode electrodes

Day	MFC1			MFC3			MFC6		
	Vol (mV)	Current density (mA/m2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)
1	34	289.36	9.84	4	34.04	0.14	3	25.53	0.08
	43	209.76	9.02	8	39.02	0.31	5	24.39	0.12
	84	168.00	14.11	18	36.00	0.65	12	24.00	0.29
	105	127.27	13.36	28	33.94	0.95	19	23.03	0.44
	120	97.56	11.71	39	31.71	1.24	25	20.33	0.51
	136	73.51	10.00	54	29.19	1.58	31	16.76	0.52
	150	60.00	9.00	65	26.00	1.69	40	16.00	0.64
	160	53.33	8.53	73	24.33	1.78	46	15.33	0.71
	168	44.80	7.53	84	22.40	1.88	56	14.93	0.84
	173	38.44	6.65	93	20.67	1.92	59	13.11	0.77
	177	35.40	6.27	98	19.60	1.92	65	13.00	0.85
	187	24.93	4.66	117	15.60	1.83	83	11.07	0.92
	198	15.84	3.14	140	11.20	1.57	103	8.24	0.85
	202	11.88	2.40	154	9.06	1.40	119	7.00	0.83
	207	8.28	1.71	167	6.68	1.12	134	5.36	0.72
	210	5.60	1.18	179	4.77	0.85	147	3.92	0.58
	211	4.69	0.99	184	4.09	0.75	152	3.38	0.51
	215	1.72	0.37	198	1.58	0.31	178	1.42	0.25
	216	0.90	0.20	203	0.85	0.17	186	0.78	0.14
	216	0.58	0.12	205	0.55	0.11	190	0.51	0.10
	216	0.00	0.00	208	0.00	0.00	194	0.00	0.00
2	25	212.77	5.32	4	34.04	0.14	2	17.02	0.03
	42	204.88	8.60	8	39.02	0.31	4	19.51	0.08
	75	150.00	11.25	17	34.00	0.58	10	20.00	0.20
	95	115.15	10.94	27	32.73	0.88	16	19.39	0.31

Table B20 Power densities and current densities adjusted by external resistances after replacing the anode electrodes

Day	MFC1			MFC3			MFC6		
	Vol (mV)	Current density (mA/m2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)
	113	91.87	10.38	30	24. 39	0.73	23	18.70	0.43
	132	71.35	9.42	45	24.32	1.09	32	17.30	0.55
	143	57.20	8.18	53	21.20	1.12	42	16.80	0.71
	150	50.00	7.50	60	20.00	1.20	46	15.33	0.71
	158	42.13	6.66	68	18.13	1.23	55	14.67	0.81
	164	36.44	5.98	76	16.89	1.28	62	13.78	0.85
	166	33.20	5.51	81	16.20	1.31	68	13.60	0.92
	178	23.73	4.22	100	13.33	1.33	86	11.47	0.99
	188	15.04	2.83	124	9.92	1.23	110	8.80	0.97
	196	11.53	2.26	137	8.06	1.10	124	7.29	0.90
	201	8.04	1.62	152	6.08	0.92	137	5.48	0.75
	204	5.44	1.11	163	4.35	0.71	154	4.11	0.63
	205	4.56	0.93	168	3.73	0.63	158	3.51	0.55
	208	1.66	0.35	187	1.50	0.28	173	1.38	0.24
	209	0.87	0.18	193	0.81	0.16	180	0.75	0.14
	210	0.56	0.12	195	0.52	0.10	183	0.49	0.09
	211	0.00	0.00	200	0.00	0.00	187	0.00	0.00
3	25	212.77	5.32	4	34.04	0.14	3	25.53	0.08
	37	180.49	6.68	7	34.15	0.24	4	19.51	0.08
	70	140.00	9.80	16	32.00	0.51	9	18.00	0.16
	88	106.67	9.39	25	30.30	0.76	14	16.97	0.24
	108	87.80	9.48	35	28.46	1.00	20	16.26	0.33
	123	66.49	8.18	48	25.95	1.25	30	16.22	0.49
	134	53.60	7.18	57	22.80	1.30	37	14.80	0.55
	143	47.67	6.82	65	21.67	1.41	40	13.33	0.53

Table B20 Power densities and current densities adjusted by external resistances after replacing the anode electrodes

Day	MFC1			MFC3			MFC6		
	Vol (mV)	Current density (mA/m2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)
	150	40.00	6.00	74	19.73	1.46	50	13.33	0.67
	158	35.11	5.55	82	18.22	1.49	55	12.22	0.67
	160	32.00	5.12	87	17.40	1.51	66	13.20	0.87
	176	23.47	4.13	103	13.73	1.41	72	9.60	0.69
	187	14.96	2.80	128	10.24	1.31	93	7.44	0.69
	190	11.18	2.12	140	8.24	1.15	111	6.53	0.72
	195	7.80	1.52	155	6.20	0.96	126	5.04	0.64
	197	5.25	1.03	166	4.43	0.73	139	3.71	0.52
	199	4.42	0.88	170	3.78	0.64	142	3.16	0.45
	203	1.62	0.33	185	1.48	0.27	165	1.32	0.22
	204	0.85	0.17	190	0.79	0.15	173	0.72	0.13
	205	0.55	0.11	191	0.51	0.10	175	0.47	0.08
	208	0.00	0.00	195	0.00	0.00	179	0.00	0.00
4	23	195.74	4.50	4	34.04	0.14	1	8.51	0.01
	32	156.10	5.00	7	34.15	0.24	2	17.02	0.03
	63	126.00	7.94	17	34.00	0.58	3	14.63	0.04
	83	100.61	8.35	25	30.30	0.76	9	18.00	0.16
	100	81.30	8.13	37	30.08	1.11	14	16.97	0.24
	117	63.24	7.40	51	27.57	1.41	17	13.82	0.23
	132	52.80	6.97	62	24.80	1.54	24	12.97	0.31
	139	46.33	6.44	64	21.33	1.37	35	14.00	0.49
	148	39.47	5.84	74	19.73	1.46	39	13.00	0.51
	153	34.00	5.20	82	18.22	1.49	47	12.53	0.59
	154	30.80	4.74	86	17.20	1.48	49	10.89	0.53
	168	22.40	3.76	104	13.87	1.44	55	11.00	0.61

Table B20 Power densities and current densities adjusted by external resistances after replacing the anode electrodes

Day	MFC1			MFC3			MFC6		
	Vol (mV)	Current density (mA/m2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)
	183	14.64	2.68	126	10.08	1.27	68	9.07	0.62
	189	11.12	2.10	140	8.24	1.15	94	7.52	0.71
	194	7.76	1.51	155	6.20	0.96	107	6.29	0.67
	199	5.31	1.06	165	4.40	0.73	129	5.16	0.67
	201	4.47	0.90	169	3.76	0.63	133	3.55	0.47
	206	1.65	0.34	186	1.49	0.28	140	3.11	0.44
	208	0.87	0.18	191	0.80	0.15	162	1.30	0.21
	209	0.56	0.12	194	0.52	0.10	168	0.70	0.12
	210	0.00	0.00	197	0.00	0.00	172	0.46	0.08
5	23	195.74	4.50	3	25.53	0.08	2	17.02	0.03
	35	170.73	5.98	6	29.27	0.18	4	19.51	0.08
	61	122.00	7.44	13	26.00	0.34	8	16.00	0.13
	83	100.61	8.35	21	25.45	0.53	13	15.76	0.20
	97	78.86	7.65	30	24.39	0.73	18	14.63	0.26
	116	62.70	7.27	41	22.16	0.91	26	14.05	0.37
	128	51.20	6.55	51	20.40	1.04	33	13.20	0.44
	130	43.33	5.63	57	19.00	1.08	38	12.67	0.48
	138	36.80	5.08	66	17.60	1.16	48	12.80	0.61
	149	33.11	4.93	74	16.44	1.22	50	11.11	0.56
	151	30.20	4.56	78	15.60	1.22	57	11.40	0.65
	168	22.40	3.76	96	12.80	1.23	70	9.33	0.65
	179	14.32	2.56	118	9.44	1.11	78	6.24	0.49
	188	11.06	2.08	130	7.65	0.99	99	5.82	0.58
	194	7.76	1.51	144	5.76	0.83	120	4.80	0.58
	198	5.28	1.05	156	4.16	0.65	132	3.52	0.46

Table B20 Power densities and current densities adjusted by external resistances after replacing the anode electrodes

Day	MFC1			MFC3			MFC6		
	Vol (mV)	Current density (mA/m2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)
	199	4.42	0.88	160	3.56	0.57	136	3.02	0.41
	205	1.64	0.34	176	1.41	0.25	159	1.27	0.20
	205	0.86	0.18	182	0.76	0.14	162	0.68	0.11
	207	0.55	0.11	185	0.49	0.09	166	0.44	0.07
	208	0.00	0.00	189	0.00	0.00	170	0.00	0.00
6	21	178.72	3.75	3	25.53	0.08	2	17.02	0.03
	35	170.73	5.98	6	29.27	0.18	4	19.51	0.08
	59	118.00	6.96	13	26.00	0.34	8	16.00	0.13
	78	94.55	7.37	19	23.03	0.44	13	15.76	0.20
	90	73.17	6.59	27	21.95	0.59	16	13.01	0.21
	118	63.78	7.53	40	21.62	0.86	23	12.43	0.29
	124	49.60	6.15	47	18.80	0.88	36	14.40	0.52
	136	45.33	6.17	53	17.67	0.94	37	12.33	0.46
	141	37.60	5.30	67	17.87	1.20	43	11.47	0.49
	151	33.56	5.07	70	15.56	1.09	47	10.44	0.49
	154	30.80	4.74	77	15.40	1.19	56	11.20	0.63
	169	22.53	3.81	90	12.00	1.08	77	10.27	0.79
	182	14.56	2.65	112	8.96	1.00	92	7.36	0.68
	192	11.29	2.17	127	7.47	0.95	105	6.18	0.65
	198	7.92	1.57	145	5.80	0.84	120	4.80	0.58
	202	5.39	1.09	157	4.19	0.66	140	3.73	0.52
	203	4.51	0.92	162	3.60	0.58	144	3.20	0.46
	209	1.67	0.35	180	1.44	0.26	166	1.33	0.22
	210	0.88	0.18	183	0.76	0.14	169	0.71	0.12
	211	0.56	0.12	185	0.49	0.09	177	0.47	0.08

Table B20 Power densities and current densities adjusted by external resistances after replacing the anode electrodes

Day	MFC1			MFC3			MFC6		
	Vol (mV)	Current density (mA/m2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)	Vol (mV)	Current density (mA/m^2)	Power density (mW/m^2)
	214	0.00	0.00	189	0.00	0.00	181	0.00	0.00
8	19	161.70	3.07	3	25.53	0.08	1	8.51	0.01
	25	121.95	3.05	5	24.39	0.12	2	9.76	0.02
	50	100.00	5.00	12	24.00	0.29	6	12.00	0.07
	76	92.12	7.00	19	23.03	0.44	9	10.91	0.10
	98	79.67	7.81	25	20.33	0.51	15	12.20	0.18
	117	63.24	7.40	35	18.92	0.66	20	10.81	0.22
	128	51.20	6.55	44	17.60	0.77	26	10.40	0.27
	135	45.00	6.08	50	16.67	0.83	31	10.33	0.32
	148	39.47	5.84	59	15.73	0.93	35	9.33	0.33
	152	33.78	5.13	67	14.89	1.00	38	8.44	0.32
	164	32.80	5.38	71	14.20	1.01	43	8.60	0.37
	180	24.00	4.32	90	12.00	1.08	62	8.27	0.51
	191	15.28	2.92	113	9.04	1.02	79	6.32	0.50
	209	12.29	2.57	130	7.65	0.99	93	5.47	0.51
	216	8.64	1.87	147	5.88	0.86	110	4.40	0.48
	223	5.95	1.33	161	4.29	0.69	123	3.28	0.40
	225	5.00	1.13	166	3.69	0.61	129	2.87	0.37
	233	1.86	0.43	188	1.50	0.28	155	1.24	0.19
	236	0.99	0.23	196	0.82	0.16	162	0.68	0.11
	236	0.63	0.15	198	0.53	0.10	167	0.45	0.07
	238	0.00	0.00	204	0.00	0.00	178	0.00	0.00

Table B21 Power densities and current densities adjusted by external resistances in abiotic fuel cell

Day	Voltage (mV)	Current density (mA/m^2)	Power density (mW/m^2)
3	7	59.574	0.417
	12	58.54	0.70
	28	56.00	1.57
	42	50.91	2.14
	62	50.41	3.13
	87	47.03	4.09
	118	47.20	5.57
	124	41.33	5.13
	145	38.67	5.61
	164	36.44	5.98
	178	35.60	6.34
	226	30.13	6.81
	291	23.28	6.77
	335	19.71	6.60
	389	15.56	6.05
	438	11.68	5.12
	461	10.24	4.72
	553	4.42	2.45
	592	2.47	1.46
	611	1.63	1.00
	646	0.00	0.00
6	4	34.04	0.14
	7	34.15	0.24

Table B21 Power densities and current densities adjusted by external resistances in abiotic fuel cell

Day	Voltage (mV)	Current density (mA/m^2)	Power density (mW/m^2)
	18	36.00	0.65
	28	33.94	0.95
	41	33.33	1.37
	58	31.35	1.82
	74	29.60	2.19
	84	28.00	2.35
	101	26.93	2.72
	114	25.33	2.89
	124	24.80	3.08
	158	21.07	3.33
	205	16.40	3.36
	236	13.88	3.28
	277	11.08	3.07
	312	8.32	2.60
	325	7.22	2.35
	390	3.12	1.22
	413	1.73	0.71
	427	1.14	0.49
	447	0.00	0.00
9	1	8.51	0.01
	3	14.63	0.04
	5	10.00	0.05
	11	13.33	0.15

Table B21 Power densities and current densities adjusted by external resistances in abiotic fuel cell

Day	Voltage (mV)	Current density (mA/m^2)	Power density (mW/m^2)
	18	14.63	0.26
	20	10.81	0.22
	45	18.00	0.81
	55	18.33	1.01
	64	17.07	1.09
	72	16.00	1.15
	77	15.40	1.19
	100	13.33	1.33
	130	10.40	1.35
	151	8.88	1.34
	176	7.04	1.24
	196	5.23	1.02
	206	4.58	0.94
	243	1.94	0.47
	258	1.08	0.28
	261	0.70	0.18
	277	0.00	0.00

APPENDIX C

Table C1 Microbial community in seed sludge

Domain	Phylum	Class	No. of read	%
Bacteria			214077	85.675
	Actinobacteria		2834	1.134
	Aquificae		6	0.002
	Bacteroidetes		17986	7.198
	Chlorobi		37	0.015
	Ignavibacteriae		561	0.225
	Caldiserica		3879	1.552
	Lentisphaerae		252	0.101
	Verrucomicrobia		2119	0.848
	Chloroflexi		11988	4.798
	Cyanobacteria		62	0.025
	Deferribacteres		2	0.001
	Deinococcus-Thermus		4	0.002
	Dictyoglomi		2	0.001
	Acidobacteria		130	0.052
	Fibrobacteres		9	0.004
	Firmicutes		20557	8.227
	Fusobacteria		14	0.006
	Nitrospirae		5450	2.181
	Plactomycetes		711	0.285
	Proteobacteria		142926	57.200
		Alphaproteobacteria	109417	43.790
		Betaproteobacteria	25	0.010
		Deltaproteobacteria	30085	12.040
		Epsilonproteobacteria	10	0.004
		Gammaproteobacteria	3389	1.356

Table C1 Microbial community in seed sludge (Cont.)

Domain	Phylum	Class	No. of read	%
		Unclassified proteobacteria	0	0.000
	Spirochaetes		615	0.246
	Synnergistetes		605	0.242
	Tenericutes		489	0.196
	Thermotogae		2126	0.851
	Unclassified bacteria		713	0.285
Archeae			35793	14.325
	Crenarchaeota		148	0.059
	Euryarchaeota		35591	14.244
	Thaumarchaeota		54	0.022
Total			**249870**	**100.000**

Table C2 Sulfate-reducing bacteria abundant in seed sludge

Type	Dominant family/genus	No. of read	%
Genus	Desulfosporosinus	6	0.00
Genus	Desulfotomaculum	1281	0.51
Genus	Desulfurispora	2	0.00
Genus	Desulfovirgula	130	0.05
Family	Desulfarculaceae	36	0.01
Family	Desulfobacteraceae	5075	2.03
Family	Desulfobulbaceae	3967	1.59
Family	Desulfohalobiaceae	14	0.01
Family	Desulfomicrobiaceae	50	0.02
Family	Desulfonatronumaceae	149	0.06
Genus	Desulfovibrionaceae	91	0.04
Genus	Desulfobacca	7	0.00
Genus	Desulfomonile	1926	0.77
Genus	Desulforhabdus	0	0.00
Genus	Desulfosoma	2	0.00
Genus	Desulfovirga	4107	1.64
Genus	Desulfocarbo	4	0.00
	Total	16847	6.74

Table C3 Sulfur-reducing bacteria abundant in seed sludge

Type	Dominant family/genus	No. of read	%
Genus	Dethiobacter	30	0.01
Genus	Desulrella	0	0.00
Family	Desulfuromonadaceae	14	0.01
Genus	Sulfurospirillum	0	0.00
Family	Desulfurococcaceae	136	0.05
	Total	180	0.07

Table C4 Methanogen abundant in seed sludge

Type	Dominant family/genus	No. of read	%
Family	Methanobacteriaceae	2378	0.95
Family	Methanomicrobiaceae	2	0.00
Family	Methanoregulaceae	16775	6.71
Family	Methanospirillaceae	3	0.00
Family	Methanosaetaceae	16171	6.47
Family	Methanosarcinaceae	16	0.01
	Total	35345	14.15

Table C5 Sulfur-oxidizing bacteria abundant in seed sludge

Type	SOB	No. of read	%
Genus	Sulfuricurvum	0	0.00
Species	Wolinella succinogens	0	0.00
Genus	Sulfurovum	10	0.00
Genus	Thioalkalivibrio	52	0.02
Genus	Halothiobacillus	2	0.00
Genus	Thioalkalibacter	0	0.00
Genus	Thioprofundum	7	0.00
Genus	Thiohalobacter	0	0.00
Genus	Thiohalorhabdus	1163	0.47
Species	Dyella thiooxydans	0	0.00
	Total	1234	0.49

Table C6 Microbial community in the first compartment of MFC1

Domain	Phylum	Class	No. of read	%
Bacteria			75230	99.529
	Actinobacteria		149	0.197
	Bacteroidetes		5680	7.515
	Ignavibacteriae		17	0.022
	Caldiserica		242	0.320
	Lentisphaerae		697	0.922
	Verrucomicrobia		23	0.030
	Chloroflexi		216	0.286
	Deferribacteres		82	0.108
	Deinococcus-Thermus		4	0.005
	Acidobacteria		5	0.007
	Firmicutes		4610	6.099
	Nitrospirae		225	0.298
	Plactomycetes		15	0.020
	Proteobacteria		61963	81.977
		Alphaproteobacteria	1058	1.400
		Betaproteobacteria	1244	1.646
		Deltaproteobacteria	13250	17.530
		Epsilonproteobacteria	1528	2.022
		Gammaproteobacteria	44883	59.380
		Unclassified proteobacteria	0	0.000
	Spirochaetes		615	0.814
	Synnergistetes		281	0.372
	Tenericutes		56	0.074
	Thermotogae		216	0.286
	Unclassified bacteria		134	0.177
Archeae			356	0.471

Table C6 Microbial community in the first compartment of MFC1

Domain	Phylum	Class	No. of read	%
	Crenarchaeota		4	0.005
	Euryarchaeota		352	0.466
Total			75586	100.000

Table C7 Sulfate-reducing bacteria abundant in the first compartment of MFC1

Type	Dominant family/genus	No. of read	%
Genus	Desulfosporosinus	0	0.00
Genus	Desulfotomaculum	12	0.02
Genus	Desulfurispora	0	0.00
Genus	Desulfovirgula	2	0.00
Family	Desulfarculaceae	0	0.00
Family	Desulfobacteraceae	238	0.31
Family	Desulfobulbaceae	30	0.04
Family	Desulfohalobiaceae	0	0.00
Family	Desulfomicrobiaceae	1820	2.41
Family	Desulfonatronumaceae	0	0.00
Genus	Desulfovibrionaceae	7710	10.20
Genus	Desulfobacca	66	0.09
Genus	Desulfomonile	9	0.01
Genus	Desulforhabdus	3093	4.09
Genus	Desulfosoma	0	0.00
Genus	Desulfovirga	114	0.15
Genus	Desulfocarbo	0	0.00
	Total	13094	17.32

Table C8 Sulfur-reducing bacteria abundant in the first compartment of MFC1

Type	Dominant family/genus	No. of read	%
Genus	Dethiobacter	0	0.00
Genus	Desulrella	26	0.03
Family	Desulfuromonadaceae	0	0.00
Genus	Sulfurospirillum	449	0.59
Family	Desulfurococcaceae	4	0.01
	Total	479	0.63

Table C9 Methanogen abundant in the first compartment of MFC1

Type	Dominant family/genus	No. of read	%
Family	Methanobacteriaceae	47	0.06
Family	Methanomicrobiaceae	0	0.00
Family	Methanoregulaceae	218	0.29
Family	Methanospirillaceae	0	0.00
Family	Methanosaetaceae	82	0.11
Family	Methanosarcinaceae	3	0.00
	Total	350	0.46

Table C 10 Sulfur-oxidizing bacteria abundant in the first compartment of MFC1

Type	SOB	No. of read	%
Genus	Sulfuricurvum	8	0.01
Species	Wolinella succinogens	1012	1.34
Genus	Sulfurovum	0	0.00
Genus	Acidithiobacillus	5	0.01
Genus	Thioalkalivibrio	6	0.01
Genus	Halothiobacillus	0	0.00
Genus	Thioalkalibacter	0	0.00
Genus	Thioprofundum	0	0.00
Genus	Thiohalobacter	0	0.00
Genus	Thiohalorhabdus	6	0.01
Species	Dyella thiooxydans	422	0.56
	Total	1459	1.93

Table C11 Microbial community in the second compartment of MFC1

Domain	Phylum	Class	No. of read	%
Bacteria			90391	99.998
	Actinobacteria		4	0.004
	Bacteroidetes		11792	13.045
	Ignavibacteriae		44	0.049
	Lentisphaerae		3870	4.281
	Verrucomicrobia		14	0.015
	Chloroflexi		14	0.015
	Cyanobacteria		4	0.004
	Deferribacteres		1124	1.243
	Firmicutes		1001	1.107
	Nitrospirae		8	0.009
	Proteobacteria		71920	79.564
		Alphaproteobacteria	503	0.556
		Betaproteobacteria	89	0.098
		Deltaproteobacteria	15513	17.162
		Epsilonproteobacteria	3298	3.649
		Gammaproteobacteria	52517	58.099
		Unclassified proteobacteria	0	0.000
	Spirochaetes		167	0.185
	Synnergistetes		117	0.129
	Tenericutes		46	0.051
	Thermotogae		99	0.110
	Unclassified bacteria		167	0.185
Archeae			2	0.002
	Euryarchaeota		2	0.002
Total			90393	100.000

Table C12 Sulfate-reducing bacteria abundant in the second compartment of MFC1

Type	Dominant family/genus	No. of read	%
Genus	Desulfosporosinus	0	0.00
Genus	Desulfotomaculum	0	0.00
Genus	Desulfurispora	0	0.00
Genus	Desulfovirgula	0	0.00
Family	Desulfarculaceae	0	0.00
Family	Desulfobacteraceae	9	0.01
Family	Desulfobulbaceae	0	0.00
Family	Desulfohalobiaceae	0	0.00
Family	Desulfomicrobiaceae	0	0.00
Family	Desulfonatronumaceae	0	0.00
Genus	Desulfovibrionaceae	14951	16.54
Genus	Desulfobacca	53	0.06
Genus	Desulfomonile	0	0.00
Genus	Desulforhabdus	156	0.17
Genus	Desulfosoma	0	0.00
Genus	Desulfovirga	0	0.00
Genus	Desulfocarbo	0	0.00
	Total	15169	16.78

Table C13 Sulfur-reducing bacteria abundant in the second compartment of MFC1

Type	Dominant family/genus	No. of read	%
Genus	Dethiobacter	0	0.00
Genus	Desulrella	330	0.37
Family	Desulfuromonadaceae	0	0.00
Genus	Sulfurospirillum	1461	1.62
Family	Desulfurococcaceae	0	0.00
	Total	1791	1.98

Table C14 Methanogen abundant in the second compartment of MFC1

Type	Dominant family/genus	No. of read	%
Family	Methanobacteriaceae	0	0.00
Family	Methanomicrobiaceae	0	0.00
Family	Methanoregulaceae	0	0.00
Family	Methanospirillaceae	0	0.00
Family	Methanosaetaceae	0	0.00
Family	Methanosarcinaceae	2	0.00
	Total	2	0.00

Table C15 Sulfur-oxidizing bacteria abundant in the second compartment of MFC1

Type	SOB	No. of read	%
Genus	Sulfuricurvum	0	0.00
Species	Wolinella succinogens	1451	1.61
Genus	Sulfurovum	0	0.00
Genus	Acidithiobacillus	3	0.00
Genus	Thioalkalivibrio	8	0.01
Genus	Halothiobacillus	14	0.02
Genus	Thioalkalibacter	2	0.00
Genus	Thioprofundum	0	0.00
Genus	Thiohalobacter	0	0.00
Genus	Thiohalorhabdus	0	0.00
Species	Dyella thiooxydans	1791	1.98
	Total	3269	3.62

Table C16 Microbial community on the anode electrode of MFC1

Domain	Phylum	Class	No. of read	%
Bacteria			86120	99.985
	Actinobacteria		2	0.002
	Armatimonadetes		2	0.002
	Bacteroidetes		5965	6.925
	Ignavibacteriae		42	0.049
	Caldiserica		6	0.007
	Lentisphaerae		3230	3.750
	Verrucomicrobia		21	0.024
	Chloroflexi		173	0.201
	Deferribacteres		10	0.012
	Firmicutes		3450	4.005
	Nitrospirae		178	0.207
	Proteobacteria		68682	79.739
		Alphaproteobacteria	369	0.428
		Betaproteobacteria	42	0.049
		Deltaproteobacteria	6112	7.096
		Epsilonproteobacteria	105	0.122
		Gammaproteobacteria	62054	72.044
		Unclassified proteobacteria	0	0.000
	Spirochaetes		3390	3.936
	Synnergistetes		864	1.003
	Tenericutes		4	0.005
	Thermotogae		19	0.022
	Unclassified bacteria		82	0.095
Archeae			13	0.015
	Euryarchaeota		13	0.015

Table C16 Microbial community on the anode electrode of MFC1

Domain	Phylum	Class	No. of read	%
Total			86133	100.000

Table C17 Sulfate-reducing bacteria abundant on the anode electrode of MFC1

Type	Dominant family/genus	No. of read	%
Genus	Desulfuribacillus	0	0.00
Genus	Desulfosporosinus	0	0.00
Genus	Desulfotomaculum	2	0.00
Genus	Desulfurispora	0	0.00
Genus	Desulfovirgula	0	0.00
Family	Desulfarculaceae	0	0.00
Family	Desulfobacteraceae	16	0.02
Family	Desulfobulbaceae	0	0.00
Family	Desulfohalobiaceae	0	0.00
Family	Desulfomicrobiaceae	0	0.00
Family	Desulfonatronumaceae	0	0.00
Genus	Desulfovibrionaceae	2891	3.36
Genus	Desulfobacca	764	0.89
Genus	Desulfomonile	0	0.00
Genus	Desulforhabdus	1913	2.22
Genus	Desulfosoma	0	0.00
Genus	Desulfovirga	2	0.00
Genus	Desulfocarbo	0	0.00
	Total	5588	6.49

Table C18 Sulfur-reducing bacteria abundant on the anode electrode of MFC1

Type	Dominant family/genus	No. of read	%
Genus	Dethiobacter	0	0.00
Genus	Desulrella	479	0.56
Family	Desulfuromonadaceae	0	0.00
Genus	Sulfurospirillum	14	0.02
Genus	Dethiosulfovibrio	0	0.00
Family	Desulfurococcaceae	0	0.00
	Total	493	0.57

Table C19 Methanogen abundant on the anode electrode of MFC1

Type	Dominant family/genus	No. of read	%
Family	Methanobacteriaceae	3	0.00
Family	Methanomicrobiaceae	0	0.00
Family	Methanoregulaceae	10	0.01
Family	Methanospirillaceae	0	0.00
Family	Methanosaetaceae	0	0.00
Family	Methanosarcinaceae	0	0.00
	Total	13	0.02

Table C20 Sulfur-oxidizing bacteria abundant on the anode electrode of MFC1

Type	SOB	No. of read	%
Genus	Sulfuricurvum	3	0.00
Species	Wolinella succinogens	85	0.10
Genus	Thioalkalivibrio	6	0.01
Genus	Halothiobacillus	15	0.02
Species	Dyella thiooxydans	2527	2.93
	Total	2636	3.06

Table C21 Microbial community in the first compartment of MFC3

Domain	Phylum	Class	No. of read	%
Bacteria			279224	97.550
	Actinobacteria		189	0.066
	Bacteroidetes		19211	6.712
	Chlorobi		3	0.001
	Ignavibacteriae		339	0.118
	Caldiserica		1234	0.431
	Lentisphaerae		6102	2.132
	Verrucomicrobia		148	0.052
	Chloroflexi		917	0.320
	Deferribacteres		33	0.012
	Dictyoglomi		2	0.001
	Acidobacteria		24	0.008
	Firmicutes		15112	5.280
	Fusobacteria		7	0.002
	Nitrospirae		240	0.084
	Plactomycetes		19	0.007
	Proteobacteria		227889	79.616
		Alphaproteobacteria	2517	0.879
		Betaproteobacteria	34	0.012
		Deltaproteobacteria	45403	15.862
		Epsilonproteobacteria	573	0.200
		Gammaproteobacteria	179362	62.662
		Unclassified proteobacteria	0	0.000
	Spirochaetes		1726	0.603
	Synnergistetes		1272	0.444
	Tenericutes		53	0.019
	Thermotogae		1027	0.359

Table C21 Microbial community in the first compartment of MFC3

Domain	Phylum	Class	No. of read	%
	Unclassified bacteria		3677	1.285
Archeae			7012	2.450
	Crenarchaeota		12	0.004
	Euryarchaeota		7000	2.446
Total			286236	100.000

Table C22 Sulfate-reducing bacteria abundant in the first compartment of MFC3

Type	Dominant family/genus	No. of read	%
Genus	Desulfuribacillus	276	0.10
Genus	Desulfosporosinus	0	0.00
Genus	Desulfotomaculum	32	0.01
Genus	Desulfurispora	2	0.00
Genus	Desulfovirgula	20	0.01
Family	Desulfarculaceae	2	0.00
Family	Desulfobacteraceae	612	0.21
Family	Desulfobulbaceae	75	0.03
Family	Desulfohalobiaceae	6	0.00
Family	Desulfomicrobiaceae	558	0.19
Family	Desulfonatronumaceae	27	0.01
Genus	Desulfovibrionaceae	36968	12.92
Genus	Desulfobacca	2	0.00
Genus	Desulfomonile	45	0.02
Genus	Desulforhabdus	6030	2.11
Genus	Desulfosoma	0	0.00
Genus	Desulfovirga	380	0.13
Genus	Desulfocarbo	4	0.00
	Total	44763	15.64

Table C23 Sulfur-reducing bacteria abundant in the first compartment of MFC3

Type	Dominant family/genus	No. of read	%
Genus	Dethiobacter	0	0.00
Genus	Desulrella	118	0.04
Family	Desulfuromonadaceae	0	0.00
Genus	Sulfurospirillum	373	0.13
Family	Desulfurococcaceae	4	0.00
	Total	495	0.17

Table C24 Methanogen abundant in the first compartment of MFC3

Type	Dominant family/genus	No. of read	%
Family	Methanobacteriaceae	549	0.19
Family	Methanomicrobiaceae	0	0.00
Family	Methanoregulaceae	1522	0.53
Family	Methanospirillaceae	5	0.00
Family	Methanosaetaceae	4866	1.70
Family	Methanosarcinaceae	19	0.01
	Total	6961	2.43

Table C25 Sulfur-oxidizing bacteria abundant in the first compartment of MFC3

Type	SOB	No. of read	%
Genus	Sulfuricurvum	0	0.00
Species	Wolinella succinogens	63	0.02
Genus	Sulfurovum	106	0.04
Genus	Acidithiobacillus	0	0.00
Genus	Thioalkalivibrio	22	0.01
Genus	Halothiobacillus	6	0.00
Genus	Thioalkalibacter	0	0.00
Genus	Thiohalophilus	2	0.00
Genus	Thioprofundum	2	0.00
Genus	Thiohalobacter	0	0.00
Genus	Thiohalorhabdus	0	0.00
Species	Dyella thiooxydans	743	0.26
	Total	944	0.33

Table C26 Microbial community in the second compartment of MFC3

Domain	Phylum	Class	No. of read	%
Bacteria			185974	99.906
	Actinobacteria		8	0.004
	Bacteroidetes		113990	61.236
	Ignavibacteriae		81	0.044
	Caldiserica		38	0.020
	Lentisphaerae		5222	2.805
	Verrucomicrobia		26	0.014
	Chloroflexi		36	0.019
	Deferribacteres		375	0.201
	Firmicutes		9135	4.907
	Nitrospirae		3	0.002
	Plactomycetes		2	0.001
	Proteobacteria		53106	28.529
		Alphaproteobacteria	1314	0.706
		Betaproteobacteria	96	0.052
		Deltaproteobacteria	23487	12.617
		Epsilonproteobacteria	4311	2.316
		Gammaproteobacteria	23898	12.838
		Unclassified proteobacteria	0	0.000
	Spirochaetes		118	0.063
	Synnergistetes		277	0.149
	Tenericutes		74	0.040
	Thermotogae		126	0.068
	Unclassified bacteria		3357	1.803
Archeae			175	0.094
	Euryarchaeota		175	0.094

Table C26 Microbial community in the second compartment of MFC3

Domain	Phylum	Class	No. of read	%
Total			186149	100.000

Table C27 Sulfate-reducing bacteria abundant in the second compartment of MFC3

Type	Dominant family/genus	No. of read	%
Genus	Desulfuribacillus	38	0.02
Genus	Desulfosporosinus	0	0.00
Genus	Desulfotomaculum	0	0.00
Genus	Desulfurispora	0	0.00
Genus	Desulfovirgula	0	0.00
Family	Desulfarculaceae	0	0.00
Family	Desulfobacteraceae	19	0.01
Family	Desulfobulbaceae	4	0.00
Family	Desulfohalobiaceae	0	0.00
Family	Desulfomicrobiaceae	15	0.01
Family	Desulfonatronumaceae	0	0.00
Genus	Desulfovibrionaceae	22932	12.32
Genus	Desulfobacca	0	0.00
Genus	Desulfomonile	2	0.00
Genus	Desulforhabdus	119	0.06
Genus	Desulfosoma	0	0.00
Genus	Desulfovirga	9	0.00
Genus	Desulfocarbo	4	0.00
	Total	23104	12.41

Table C28 Sulfur-reducing bacteria abundant in the second compartment of MFC3

Type	Dominant family/genus	No. of read	%
Genus	Dethiobacter	0	0.00
Genus	Desulrella	210	0.11
Family	Desulfuromonadaceae	0	0.00
Genus	Sulfurospirillum	3676	1.97
Genus	Dethiosulfovibrio	2	0.00
Family	Desulfurococcaceae	0	0.00
	Total	3888	2.09

Table C29 Methanogen abundant in the second compartment of MFC3

Type	Dominant family/genus	No. of read	%
Family	Methanobacteriaceae	15	0.01
Family	Methanomicrobiaceae	0	0.00
Family	Methanoregulaceae	19	0.01
Family	Methanospirillaceae	6	0.00
Family	Methanosaetaceae	133	0.07
Family	Methanosarcinaceae	0	0.00
	Total	173	0.09

Table C30 Sulfur-oxidizing bacteria abundant in the second compartment of MFC3

Type	SOB	No. of read	%
Genus	Sulfuricurvum	5	0.00
Species	Wolinella succinogens	45	0.02
Genus	Sulfurovum	0	0.00
Genus	Acidithiobacillus	99	0.05
Genus	Thioalkalivibrio	0	0.00
Genus	Halothiobacillus	15	0.01
Genus	Thioalkalibacter	0	0.00
Genus	Thioprofundum	2	0.00
Genus	Thiohalobacter	0	0.00
Genus	Thiohalorhabdus	0	0.00
Species	Dyella thiooxydans	1279	0.69
	Total	1445	0.78

Table C31 Microbial community on the anode electrode of MFC3

Domain	Phylum	Class	No. of read	%
Bacteria			180918	90.684
	Actinobacteria		187	0.094
	Bacteroidetes		8701	4.361
	Chlorobi		4	0.002
	Ignavibacteriae		2921	1.464
	Caldiserica		1336	0.670
	Lentisphaerae		3245	1.627
	Verrucomicrobia		153	0.077
	Chloroflexi		910	0.456
	Acidobacteria		71	0.036
	Deferribacteres		20	0.010
	Firmicutes		9869	4.947
	Fusobacteria		2	0.001
	Nitrospirae		160	0.080
	Planctomycetes		18	0.009
	Proteobacteria		145236	72.799
		Alphaproteobacteria	1144	0.573
		Betaproteobacteria	28	0.014
		Deltaproteobacteria	10909	5.468
		Epsilonproteobacteria	398	0.199
		Gammaproteobacteria	132757	66.544
		Unclassified proteobacteria	0	0.000
	Spirochaetes		3352	1.680
	Synnergistetes		2483	1.245
	Tenericutes		130	0.065
	Thermotogae		443	0.222

Table C31 Microbial community on the anode electrode of MFC3

Domain	Phylum	Class	No. of read	%
	Unclassified bacteria		1675	0.840
Archeae			18586	9.316
	Crenarchaeota		2	0.001
	Euryarchaeota		18584	9.315
Total			199504	100.000

Table C32 Sulfate-reducing bacteria abundant on the anode electrode of MFC3

Type	Dominant family/genus	No. of read	%
Genus	Desulfuribacillus	361	0.18
Genus	Desulfosporosinus	0	0.00
Genus	Desulfotomaculum	22	0.01
Genus	Desulfurispora	0	0.00
Genus	Desulfovirgula	7	0.00
Family	Desulfarculaceae	0	0.00
Family	Desulfobacteraceae	287	0.14
Family	Desulfobulbaceae	27	0.01
Family	Desulfohalobiaceae	4	0.00
Family	Desulfomicrobiaceae	128	0.06
Family	Desulfonatronumaceae	10	0.01
Genus	Desulfovibrionaceae	5152	2.58
Genus	Desulfobacca	2	0.00
Genus	Desulfomonile	12	0.01
Genus	Desulforhabdus	3495	1.75
Genus	Desulfosoma	2	0.00
Genus	Desulfovirga	241	0.12

Table C32 Sulfate-reducing bacteria abundant on the anode electrode of MFC3

Type	Dominant family/genus	No. of read	%
Genus	Desulfocarbo	0	0.00
	Total	9389	4.71

Table C 33 Sulfur-reducing bacteria abundant on the anode electrode of MFC3

Type	Dominant family/genus	No. of read	%
Genus	Dethiobacter	0	0.00
Genus	Desulrella	162	0.08
Family	Desulfuromonadaceae	0	0.00
Genus	Sulfurospirillum	298	0.15
Genus	Dethiosulfovibrio	0	0.00
Family	Desulfurococcaceae	2	0.00
	Total	462	0.23

Table C34 Methanogen abundant on the anode electrode of MFC3

Type	Dominant family/genus	No. of read	%
Family	Methanobacteriaceae	321	0.16
Family	Methanomicrobiaceae	0	0.00
Family	Methanoregulaceae	978	0.49
Family	Methanospirillaceae	2	0.00
Family	Methanosaetaceae	17212	8.63
Family	Methanosarcinaceae	30	0.02
	Total	18543	9.29

Table C35 Sulfur-oxidizing bacteria abundant on the anode electrode of MFC3

Type	SOB	No. of read	%
Genus	Sulfuricurvum	29	0.01
Species	Wolinella succinogens	6	0.00
Genus	Sulfurovum	55	0.03
Genus	Acidithiobacillus	11	0.01
Genus	Thioalkalivibrio	2	0.00
Genus	Halothiobacillus	8	0.00
Genus	Thioalkalibacter	2	0.00
Genus	Thioprofundum	4	0.00
Genus	Thiohalobacter	2	0.00
Genus	Thiohalorhabdus	10	0.01
Species	Dyella thiooxydans	1379	0.69
	Total	1508	0.76

Table C36 Microbial community in the first compartment of MFC6

Domain	Phylum	Class	No. of read	%
Bacteria			314474	95.820
	Actinobacteria		540	0.165
	Aquificae		13	0.004
	Bacteroidetes		21828	6.651
	Chlorobi		22	0.007
	Ignavibacteriae		713	0.217
	Caldiserica		2786	0.849
	Lentisphaerae		8328	2.538
	Verrucomicrobia		369	0.112
	Chloroflexi		3649	1.112
	Cyanobacteria		9	0.003
	Deferribacteres		30	0.009
	Deinococcus-Thermus		2	0.001
	Dictyoglomi		2	0.001
	Elusimicrobia		2	0.001
	Acidobacteria		149	0.045
	Firmicutes		33230	10.125
	Fusobacteria		10	0.003
	Gemmatimonadetes		2	0.001
	Nitrospirae		481	0.147
	Plactomycetes		141	0.043
	Proteobacteria		234092	71.328
		Alphaproteobacteria	1334	0.406
		Betaproteobacteria	478	0.146
		Deltaproteobacteria	24858	7.574
		Epsilonproteobacteria	1204	0.367
		Gammaproteobacteria	206218	62.835

Table C36 Microbial community in the first compartment of MFC6

Domain	Phylum	Class	No. of read	%
		Unclassified proteobacteria	0	0.000
	Spirochaetes		378	0.115
	Synnergistetes		2012	0.613
	Tenericutes		38	0.012
	Thermotogae		1423	0.434
	Unclassified bacteria		4225	1.287
Archeae			13717	4.180
	Crenarchaeota		50	0.015
	Euryarchaeota		13667	4.164
Total			328191	100.000

Table C37 Sulfate-reducing bacteria abundant in the first compartment of MFC6

Type	Dominant family/genus	No. of read	%
Genus	Desulfuribacillus	1024	0.31
Genus	Desulfosporosinus	4	0.00
Genus	Desulfotomaculum	78	0.02
Genus	Desulfurispora	0	0.00
Genus	Desulfovirgula	17	0.01
Family	Desulfarculaceae	0	0.00
Family	Desulfobacteraceae	387	0.12
Family	Desulfobulbaceae	277	0.08
Family	Desulfohalobiaceae	0	0.00
Family	Desulfomicrobiaceae	401	0.12
Family	Desulfonatronumaceae	23	0.01
Genus	Desulfovibrionaceae	18879	5.75
Genus	Desulfobacca	2	0.00
Genus	Desulfomonile	95	0.03
Genus	Desulforhabdus	1494	0.46
Genus	Desulfosoma	2	0.00
Genus	Desulfovirga	1259	0.38
	Total	22918	6.98

Table C38 Sulfur-reducing bacteria abundant in the first compartment of MFC6

Type	Dominant family/genus	No. of read	%
Genus	Dethiobacter	12	0.00
Genus	Desulrella	20	0.01
Family	Desulfuromonadaceae	0	0.00
Genus	Sulfurospirillum	196	0.06
	Total	228	0.07

Table C39. Methanogen abundant in the first compartment of MFC6

Type	Dominant family/genus	No. of read	%
Family	Methanobacteriaceae	2194	0.67
Family	Methanomicrobiaceae	0	0.00
Family	Methanoregulaceae	1795	0.55
Family	Methanospirillaceae	1679	0.51
Family	Methanosaetaceae	7713	2.35
Family	Methanosarcinaceae	162	0.05
	Total	13543	4.13

Table C40 Sulfur-oxidizing bacteria abundant in the first compartment of MFC6

Type	SOB	No. of read	%
Genus	Sulfuricurvum	2	0.00
Species	Wolinella succinogens	12	0.00
Genus	Thiobacillus	26	0.01
Genus	Sulfurovum	981	0.30
Genus	Acidithiobacillus	0	0.00
Genus	Thioalkalivibrio	10	0.00
Genus	Halothiobacillus	0	0.00
Genus	Thioalkalibacter	0	0.00
Genus	Thiohalophilus	2	0.00
Genus	Thioprofundum	2	0.00
Genus	Thiohalobacter	0	0.00
Genus	Thiohalorhabdus	32	0.01
Species	Dyella thiooxydans	18	0.01
	Total	1085	0.33

Table C41 Microbial community in the second compartment of MFC6

Domain	Phylum	Class	No. of read	%
Bacteria			340168	99.444
	Actinobacteria		65	0.019
	Bacteroidetes		42451	12.410
	Ignavibacteriae		80	0.023
	Caldiserica		128	0.037
	Lentisphaerae		186268	54.453
	Verrucomicrobia		102	0.030
	Chloroflexi		160	0.047
	Cyanobacteria		7	0.002
	Deferribacteres		47	0.014
	Elusimicrobia		19	0.006
	Acidobacteria		25	0.007
	Firmicutes		66546	19.454
	Fusobacteria		51	0.015
	Nitrospirae		22	0.006
	Plactomycetes		29	0.008
	Proteobacteria		37725	11.028
		Alphaproteobacteria	1248	0.365
		Betaproteobacteria	293	0.086
		Deltaproteobacteria	12465	3.644
		Epsilonproteobacteria	6029	1.763
		Gammaproteobacteria	17690	5.171
		Unclassified proteobacteria	0	0.000
	Spirochaetes		226	0.066
	Synnergistetes		1082	0.316
	Tenericutes		183	0.053
	Thermotogae		133	0.039

Table C41 Microbial community in the second compartment of MFC6 (Cont.)

Domain	Phylum	Class	No. of read	%
	Unclassified bacteria		4819	1.409
Archeae			1902	0.556
	Crenarchaeota		2	0.001
	Euryarchaeota		1900	0.555
Total			342070	100.000

Table C42 Sulfate-reducing bacteria abundant in the second compartment of MFC6

Type	Dominant family/genus	No. of read	%
Genus	Desulfuribacillus	47	0.01
Genus	Desulfosporosinus	4	0.00
Genus	Desulfotomaculum	12	0.00
Genus	Desulfurispora	0	0.00
Genus	Desulfovirgula	0	0.00
Family	Desulfarculaceae	0	0.00
Family	Desulfobacteraceae	12	0.00
Family	Desulfobulbaceae	17	0.00
Family	Desulfohalobiaceae	0	0.00
Family	Desulfomicrobiaceae	46	0.01
Family	Desulfonatronumaceae	2	0.00
Genus	Desulfovibrionaceae	11823	3.46
Genus	Desulfobacca	0	0.00
Genus	Desulfomonile	4	0.00
Genus	Desulforhabdus	136	0.04
Genus	Desulfosoma	0	0.00
Genus	Desulfovirga	119	0.03
	Total	12175	3.56

Table C43 Sulfur-reducing bacteria abundant in the second compartment of MFC6

Type	Dominant family/genus	No. of read	%
Genus	Dethiobacter	2	0.00
Genus	Desulrella	22	0.01
Family	Desulfuromonadaceae	0	0.00
Genus	Sulfurospirillum	2204	0.64
Family	Desulfurococcaceae	2	0.00
	Total	2230	0.65

Table C44 Methanogen abundant in the second compartment of MFC6

Type	Dominant family/genus	No. of read	%
Family	Methanobacteriaceae	81	0.02
Family	Methanomicrobiaceae	0	0.00
Family	Methanoregulaceae	213	0.06
Family	Methanospirillaceae	555	0.16
Family	Methanosaetaceae	1015	0.30
Family	Methanosarcinaceae	23	0.01
	Total	1887	0.55

Table C45 Sulfur-oxidizing bacteria abundant in the second compartment of MFC6

Type	SOB	No. of read	%
Genus	Sulfuricurvum	22	0.01
Species	Wolinella succinogens	108	0.03
Genus	Sulfurovum	130	0.04
Genus	Acidithiobacillus	6	0.00
Genus	Thioalkalivibrio	0	0.00
Genus	Halothiobacillus	7	0.00
Genus	Thioalkalibacter	0	0.00
Genus	Thiofaba	2	0.00
Genus	Thioprofundum	2	0.00
Genus	Thiohalobacter	0	0.00
Genus	Thiohalorhabdus	0	0.00
Species	Dyella thiooxydans	359	0.10
	Total	636	0.19

Table C46 Microbial community on the anode electrode of MFC6

Domain	Phylum	Class	No. of read	%
Bacteria			97857	41.737
	Actinobacteria		248	0.106
	Bacteroidetes		19324	8.242
	Chlorobi		2	0.001
	Ignavibacteriae		4059	1.731
	Caldiserica		2876	1.227
	Lentisphaerae		501	0.214
	Verrucomicrobia		339	0.145
	Chloroflexi		1823	0.778
	Cyanobacteria		7	0.003
	Deferribacteres		2	0.001
	Acidobacteria		140	0.060
	Firmicutes		17297	7.377
	Fusobacteria		4	0.002
	Nitrospirae		260	0.111
	Planctomycetes		246	0.105
	Proteobacteria		43981	18.758
		Alphaproteobacteria	410	0.175
		Betaproteobacteria	74	0.032
		Deltaproteobacteria	10448	4.456
		Epsilonproteobacteria	900	0.384
		Gammaproteobacteria	32149	13.712
		Unclassified proteobacteria	0	0.000
	Spirochaetes		860	0.367
	Synnergistetes		3400	1.450
	Tenericutes		297	0.127
	Thermotogae		946	0.403

Table C46 Microbial community on the anode electrode of MFC6 (Cont.)

Domain	Phylum	Class	No. of read	%
	Unclassified bacteria		1245	0.531
Archeae			136606	58.263
	Crenarchaeota		17	0.007
	Euryarchaeota		136587	58.255
	Thaumarchaeota		2	0.001
Total			234463	100.000

Table C47 Sulfate-reducing bacteria abundant on the anode electrode of MFC6

Type	Dominant family/genus	No. of read	%
Genus	Desulfuribacillus	60	0.03
Genus	Desulfosporosinus	4	0.00
Genus	Desulfotomaculum	60	0.03
Genus	Desulfurispora	0	0.00
Genus	Desulfovirgula	4	0.00
Family	Desulfarculaceae	0	0.00
Family	Desulfobacteraceae	151	0.06
Family	Desulfobulbaceae	179	0.08
Family	Desulfohalobiaceae	2	0.00
Family	Desulfomicrobiaceae	489	0.21
Family	Desulfonatronumaceae	23	0.01
Genus	Desulfovibrionaceae	4052	1.73
Genus	Desulfobacca	2	0.00
Genus	Desulfomonile	15	0.01
Genus	Desulforhabdus	1340	0.57
Genus	Desulfosoma	0	0.00
Genus	Desulfovirga	1634	0.70
	Total	7955	3.39

Table C48 Sulfur-reducing bacteria on the anode electrode of MFC6

Type	Dominant family/genus	No. of read	%
Genus	Dethiobacter	23	0.01
Genus	Desulrella	11	0.00
Family	Desulfuromonadaceae	2	0.00
Genus	Sulfurospirillum	167	0.07
Family	Desulfurococcaceae	17	0.01
	Total	220	0.09

Table C49 Methanogen abundant on the anode electrode of MFC6

Type	Dominant family/genus	No. of read	%
Family	Methanobacteriaceae	1382	0.59
Family	Methanomicrobiaceae	0	0.00
Family	Methanoregulaceae	4710	2.01
Family	Methanospirillaceae	217	0.09
Family	Methanosaetaceae	129636	55.29
Family	Methanosarcinaceae	263	0.11
	Total	136208	58.09

Table C 50 Sulfur-oxidizing bacteria abundant on the anode electrode of MFC6

Type	SOB	No. of read	%
Genus	Sulfuricurvum	210	0.09
Species	Wolinella succinogens	5	0.00
Genus	Sulfurovum	508	0.22
Genus	Acidithiobacillus	2	0.00
Genus	Thioalkalivibrio	8	0.00
Genus	Halothiobacillus	13	0.01
Genus	Thioalkalibacter	0	0.00
Genus	Thioprofundum	8	0.00
Genus	Thiohalobacter	2	0.00
Genus	Thiohalorhabdus	3	0.00
Species	Dyella thiooxydans	1262	0.54
	Total	2021	0.86

Phylogenic tree

1) https://drive.google.com/file/d/0Bxg3ddooKg7kd2QwNW1NOWpickk/view?usp=sharing

2) https://www.dropbox.com/s/ymbo3c1kv7atd6p/sample%20comparison-cmp.jpg?dl=0

VITA

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1) Niyom, W., Komolyothin, D., and Suwannasilp, B.B. "Performances of Microbial Fuel Cells Treating Organic Wastewater at Various COD: sulfate ratio". Oral presentation in the 3rd Interational Conference on Biological, Chemical and Environmental Sciences (BCES-2015), Kuala Lumpur, Malaysia, September 21-22, 2015.

2) Sangcharoen, A., Niyom, W., Suwannasilp, B.B. (2015) "A microbial fuel cell treating organic wastewater containing high sulfate under continuous operation: Performance and microbial community," Process Biochemistry 50(10): 1648-1655.