Resource recovery in self-driven bio-electrochemical systems

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**RESOURCE RECOVERY IN SELF-DRIVEN BIO-
ELECTROCHEMICAL SYSTEMS**

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Interdisciplinary Graduate School

NEWRI

2019

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ELECTROCHEMICAL SYSTEMS**

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Interdisciplinary Graduate School

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A thesis submitted to the Nanyang Technological University in partial
fulfillment of the requirement for the degree of
Doctor of Philosophy

2019

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

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Authorship Attribution Statement

This thesis contains material from 2 paper(s) published in the following peer-reviewed journal(s) where I was the first author.

Chapter 5 is published as Wu D., Sun F.Q., Zhou Y.* Degradation of Chloramphenicol with Novel Metal Foam Electrodes in Bioelectrochemical Systems. *Electrochimica Acta*, 240: 136-145, (2017). DOI: <https://doi.org/10.1016/j.electacta.2017.04.059>.

The contributions of the co-authors are as follows:

- Assistant Prof. Zhou Yan provided the initial project direction and edited the manuscript drafts.
- I prepared the manuscript drafts. The manuscript was revised by Dr. Sun Faqian.
- I co-designed the study with assistant Prof Zhou Yan and performed all the laboratory work at the Interdisciplinary Graduate School and the Nanyang Environmental Water Research Institute. I also analyzed the data.
- All microscopy, including sample preparation, was conducted by me in the Facility for Analysis, Characterization, Testing.
- Dr. Sun Faqian assisted in the analysis of LCOCD data.

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The contributions of the co-authors are as follows:

- Prof. Zhou Yan helped to design the study and edited the manuscript drafts.
- I wrote the drafts of the manuscript. The manuscript was revised together with Prof. David Stuckey and Chua Fengjun, Desmond.
- I performed all the reactor operation, collected samples and analysis the performance, carried and conducted data evaluation.
- Lu Dan and Dr. Sun Faqian conducted the LCOCD determinations.
- Chua Fengjun, Desmond helped to complete the fluorescence imaging.

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Summary

There are many useful resources in the wastewater (WW) and wasted activated sludge, such as organic matters, phosphate, nitrogen, and precious metals. The cost of conventional technologies to recover them from wastewater and sludge can be very high and the efficiency is quite low. Bioelectrochemical system (BES) is considered an ideal treatment method to recover nutrients, while multiple pollutants can be degraded at the same time. Such approach can improve energy savings and reduce environmental stress, and also address the urgency to rely on fossil-fuel.

Meantime, there are large amount of industrial wastewater discharged to the environment as well. Some wastewater contains pharmaceuticals and personal care products (PPCP) which are not easy to remove using conventional bioprocess. Again, BES was introduced in recent decades to handle recalcitrant or antibiotic compounds in the wastewater. There would be certain interaction between antibiotics and biofilm in the BES reactors. However, how the presence of antibiotics would affect the BES system and the mechanisms of potential effects are not clear. Two types of antibiotics of sulfamethoxazole (SMX) and chloramphenicol (CAP) were chosen in this thesis.

The study on the effects of SMX for power generation in microbial fuel cell (MFC) was presented in Chapter 4. SMX was added in the anode to monitor the performance of power generation. We found out SMX was able to enhance the power generation as well as to extent energy duration. SMX was degraded within 36 hours operation. The degradation pathway was corresponding to the activity of three main exoelectrogenics. Meantime, SMX helped to remove competitive microorganisms in the anode and further contributed to the power generation and organic matter conversion. Notwithstanding the excellent energy recovery performance, the power produced from the MFC system cannot be collected. It would need to be utilized in-situ.

In order to improve the efficiency of CAP removal, Chapter 5 compared three different cathode materials, i.e. carbon rod (CR), copper foam (Cu), and nickel foam (NF), for CAP removal in microbial electrolysis fuel cell (MEC). The results demonstrated that Cu was the most efficient cathode for CAP degradation. With Cu electrode under a higher applied voltage (0.5 V), CAP could be degraded to nitrobenzene within 24 h. The by-product has lower toxicity for microorganisms.

Although MEC is efficient for CAP removal, it still needs external energy supply. Such energy supply can actually be provided by MFC system. In Chapter 6, a self-driven MFC-MEC system was attempted to utilize the energy generated by MFC to power MEC for CAP removal. At the same time, precious metal Ag from electroplating wastewater was also recovered in MFC cathode. Under the optimum condition, 99.8% and 98.8% of Ag(I) and CAP were recovered or removed, respectively. Scanning electron microscope (SEM), Energy Dispersive X-ray Detector (EDX) and X-ray photoelectron spectroscopy (XPS) results demonstrated the product on the electrode of MFC was pure silver.

In most of previous BES study including the self-driven system reported above, researchers commonly use acetate simulated wastewater in the anode for power generation. Considering practical application, the organic matter can be replaced with real wastewater or other waste material that contains carbon source. Chapter 7 reported an integrated sludge treatment and resource recovery system for simultaneous energy and nutrients recovery. Sludge fermented liquor (FL) was used as feed for MFC, which provided a maximum voltage of 0.477 V and power density of 8.07 W m^{-3} . A total 90.59% ammonium was removed and 42 % phosphorus was removed and stored in the biomass in the form of polyphosphate (poly-P). Nitrogen can then be recovered at the cathode and phosphorus can be recovered from the biomass in the anode.

List of publications

- (1) **Wu D.**, Lu D., Sun F.Q. Zhou Y.* (2019) Simultaneous Antibiotic Removal and Precious Metal Recovery: An Energy Neutral Process. *Science of The Total Environment*, 133914.
- (2) **Wu D.**, Sun F.Q., Chua FJD, Lu D., Stucky D., Zhou Y.* (2019) In-situ power generation and nutrients recovery from waste activated sludge – Long-term performance and system optimization. *Chemical Engineering Journal*, 361, 1207-1214. (SCI, IF: 6.087)
- (3) **Wu D.**, Sun F.Q., Zhou Y.* (2017) Degradation of Chloramphenicol with Novel Metal Foam Electrodes in Bioelectrochemical Systems. *Electrochimica Acta*, 240: 136-145. (SCI, IF: 5.116)
- (4) **Wu D.**, Sun F.Q., Chua FJD, Zhou Y.* (2019) Enhanced power generation in microbial fuel cell by an agonist of electroactive biofilm - sulfamethoxazole. (Major revision)
- (5) Sun F.Q., **Wu D.** Chua FJD, Zhu W.Y., Zhou Y.* (2018) Free nitrous acid (FNA) induced transformation of sulfamethoxazole in the enriched nitrifying culture. *Water Research*, 149, 432-439.
- (6) Sun F.Q., Xiao Y.Y., **Wu D.**, Zhu W.Y., Zhou Y.* (2017) Nitrite-driven abiotic transformation of sulfonamide micropollutants during freezing process. *Chemical Engineering Journal*, 327, 1128-1134.

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List of abbreviation

ANOVA	Analysis of variances
BES	Bioelectrochemical system
CAP	Chloramphenicol
CCD	Central composite design
CEan	Anodic coulombic efficiency
CR	Carbon rod
Cu	Copper foam
CV	Cyclic voltammetry
DI	Deionized
DOMs	Dissolved organic matters
EIS	Electrochemical impedance spectroscopy
EDX	Energy Disperse Spectroscopy
EABF	Electroactivity biofilm
EET	Extracellular electrons transfer
FESEM	Field emission scanning electron microscopy
FL	Fermented liquor
FMN	Flavin mononucleotide
HMW	High molecular weight
HSs	Humic substance
LMW	Low molecular weight
LSV	Linear sweep voltammetry
MEC	Microbial electrolysis cell
MFC	Microbial fuel cell
MW	Molecular weight
NF	Nickle foam
OCC	Open circuit condition

OFAT	One-factor-at-a-time
OTUs	Operational taxonomic units
PNs	Proteins
polyP	Polyphosphate
PPCP	Personal care products
PSs	Polysaccharide
QC	Quality control
RF	Riboflavin
RSM	Response surface method
SEM	Scanning electron microscope
sCOD	Soluble chemical oxygen demands
SMX	Sulfamethoxazole
SPE	Solid-phase extraction
tCOD	Total chemical oxygen demands
WAS	Waste activated sludge
XPS	X-ray photoelectron spectroscopy

Chapter 1 Introduction

In this chapter, the background and motivation of this thesis are firstly described in Section 1.1. In Section 1.2, the scope and contribution of this thesis are described. Four main research objectives, (1) sulfamethoxazole degradation and its influence on power generation in microbial fuel cell, (2) the mechanisms for chloramphenicol degradation in microbial electrolysis cell with different cathode materials, (3) self-driven system for chloramphenicol removal and simultaneous silver recovery and (4) in-situ power generation and nutrients recovery from waste activated sludge, are elaborated in detail. Finally, an overview of this thesis is given in Section 1.3.

1.1 Background

The increasing quantity and complicate characteristics of wastewater (WW) make it difficult and costly to deal with before releasing into the environment [1]. However, there are many useful resources in the wastewater and sludge. Some organics like short-chain fatty acid is a common and dominant product from waste activated sludge (WAS) treatment. Other organic matters, such as proteins (PNs), polysaccharides (PSs) and humic-like substances (HSs), are also commonly present in WAS [1, 2]. These organics can be converted to energy through WW treatment. In addition, phosphorus and ammonium can be released from WAS, and they are useful elements for fertilizers [3]. For industrial wastewater, it contains precious resources as well, e.g. metals with high market values [4].

Meantime, there are some emerging contaminants in the WW like antibiotics. The presence of antibiotics in the ecosystem has been known for almost 30 years. Antibiotics are used for treatment of different diseases which profoundly improved human wellbeing. These compounds were defined as chemical compounds that have functions to interfere or deactivate bacterial cells [5]. Based on the mechanism and chemical structures, the classification of antibiotics as shown in Table 1.1.

Table 1.1 Classification of antibiotics

Aminoglycosides	Typical compounds	Core structure
Beta-lactam	penicillin, cephalosporin, cefepime, flomoxef, thiomycin, cefoxitin etc.	
Aminoglycosides	streptomycin, gentamicin, kanamycin, tobramycin, small, doxycycline and amikacin etc.	

Table 1.1 Continued

Aminoglycosides	Typical compounds	Core structure
Macrolides	Erythromycin, albomycin, acetylspiramycin, midecamycin, azithromycin, luo erythromycin etc.	
Sulfonamides	Sulfadiazine, sulfamethoxazole, sulfanilamide thiazole etc.	
Nitroimidazoles	metronidazole, ornidazole etc.	
Nitrofurans	Nitrofurazone, furazolidone, nitrofurantoin etc.	
Quinolones	pipemidic acid, norfloxacin, ofloxacin, levofloxacin, ciprofloxacin etc.	
Chloramphenicols	chloramphenicol, florfenicol	
Lincosamides	clindamycin, lincomycin	
Tetracyclines	tetracycline, chlortetracycline, oxytetracycline, doxycycline etc.	

As a new type of micropollutant, widespread antibiotics in the open environment has become an emerging concern in recent years [5]. A variety of antibiotics were detected in pharmaceutical manufacturing wastewater [6]. The wastewater, once entering the environment, will impose a potential environmental risk and threaten

human health. Although the detected concentration of antibiotics in the environment were generally low ranging from a few ng/L to a few ug/L, the chemical compounds of antibiotics were considered as 'persistent pollutant' due to the difficulties in degradation by natural microorganisms [7]. The long-term and accumulative presence of antibiotics may also induce the evolution of antibiotic-resistant bacteria which may cause ineffective of these antibiotics [8].

Among the commonly used antibiotics, chloramphenicol (CAP) and sulfamethoxazole (SMX) are typical antibiotic compounds for human and animals [9, 10]. Both of them are broad-spectrum antibiotics and can effectively deactivate most of Gram-positive and Gram-negative bacteria [11, 12]. Till now, CAP and SMX are still widely used due to their excellent antimicrobial properties and low price. These compounds have been detected in surface water at concentrations varying from 0.03 to 28.36 ng/L in France, Italy, Germany, China etc. [11, 13]. Therefore, many researchers have looked into their impact on the environment and the methodologies to remove these compounds effectively. However, the efficiency of the removal is still low at this stage.

Bioelectrochemical systems (BESs) can be a useful process to directly convert the organic compounds in the WW to other resources, e.g. electrons and hydrogen [14-16]. In the anode of BESs, most organic matters could be removed and convert to electrons. In the cathode, electron acceptors (such as O_2 [66], H^+ [67] or other strong reducing substances) obtain electrons and reduced. BESs also Meantime, BES is also an efficient and novel technology to recover nutrients and remove pollutants simultaneously [106, 107].

1.2 Research objectives and scope

In this study, the overall research objectives are as follows.

(a) In chapter 4, SMX was added in the anode of a MFC to investigated the co-metabolic of SMX and acetate and its influence on power generation. The specific

objectives of this study were to: (i) Evaluate the energy generation change, and the transformation efficiency of SMX, (ii) understand the mechanisms of the influence for energy generation of SMX, (iii) analyse the microbial structure shift and its relationship with SMX degradation.

(b) Chapter 5 investigated the feasibility of bioelectrochemical systems (BESs) for degradation of representative antibiotics such as CAP in the cathode with different cathode materials and the mechanisms of removal. The specific objectives of this study were to: (i) evaluate the degradation efficiency and products of CAP under different applied voltage, (ii) understand the mechanisms and pathways of CAP degradation under different applied voltage, (iv) analyze the electrodes through X-Ray Diffraction (XRD) to explore the corrosive characters of foam metal electrode.

(c) In chapter 6, an MFC was used to power the MEC for CAP degradation and meantime precious metal Ag was also recovered. The specific objectives of this study were to: (i) Preliminary studies of different concentration of Ag(I) and CAP were conducted to explore the feasibility for the self-driven system and further used to discover the optimum condition. (ii) The morphology of product on the electrode was observed with a scanning electron microscope (SEM). (iii) Energy Disperse Spectroscopy (EDX) and X-ray photoelectron spectroscopy (XPS) were also used to analyse the characters of the products on the electrodes.

(d) Chapter 7 aimed to investigate the feasibility of simultaneous electricity generation and in-situ nutrients recovery. The long-term performance of the system under different operation phases was investigated. The specific objectives of the study were to (i) evaluate the energy and nutrients recovery efficiencies, (ii) understand the conversion and transformation of the key dissolved organic matters (DOMs) during the operation, (iii) analyse the microbial communities and reveal the relationship between the performance and microbial activities.

1.3 Technology road map

Figure 1.1 The technology roadmap

Chapter 2 Literature Review

This chapter provides recent progress of antibiotics removal within conventional technologies, highlights some drawbacks of the process which may cause environmental issues and explains the principles of bioelectrochemical systems and application. This review aims to provide some background knowledge about previous research works and knowledge gaps of this study.

2.1 Conventional methods for removal of antibiotics

Overdose of antibiotics and the existence of their residuals in natural systems have accelerated the pollution of the environment. Hence, it is crucial to treat the effluents containing antibiotics before discharging into the environment. Many studies have attempted to use advanced oxidation processes (AOPs), biodegradation and electrolysis treatment to achieve highly efficient removal of antibiotics. This section will discuss and compare these technologies.

2.1.1 AOPs for typical antibiotics removal

AOPs have been used to remove recalcitrant antibiotics [7, 17, 18]. The removal mechanism of AOPs is to use hydroxyl radical ($\bullet\text{OH}$, redox potential = 2.8 V vs SHE) as the strong oxygen-based oxidizers for reaction with organic matters. During the reaction, the generated organic free radical enters the chain reaction of $\bullet\text{OH}$ sequentially. These highly active oxygen-based oxidizers are commonly generated from O_3 or H_2O_2 along with metal, semiconductor catalyst or exposure under UV [19]. Liu et al. [7] applied AOPs combined with nanofiltration for removing trace antibiotics (Figure. 2.1). Antibiotics were removed/rejected by more than 98%, while UV_{254} did not affect the degradation of these compounds. An additional step was required to deal with the concentrate. Martins et al. [18] used Ozone/ H_2O_2 for the degradation of SMX. Results demonstrated that 200 mg L^{-1} of SMX (presented as $\text{COD} = 287 \text{ mgO}_2 \text{ L}^{-1}$ and $\text{TOC} = 116 \text{ mgC L}^{-1}$) COD and total organic carbon (TOC) removal was 76% and 32% respectively with the dosage of 20 g/m^3 of O_3 gas and $60 \text{ mg/L H}_2\text{O}_2$.

AOP methods for CAP and SMX removal are summarised in Table 2.1. Although AOPs could obtain high removal efficiency of these compounds, these methods need to consume a large amount of strong oxidiser, which may produce more pollutants and enter the environment. Given the characteristic of strong oxidation, AOPs have no selectivity for various types of organics [20].

Figure 2.1 Flow diagram of nanofiltration combined with ozone-based advanced oxidation Processes for removal of trace antibiotics [7]

Table 2.1 AOPs for CAP and SMX removal

Compounds	Concentration (mg L ⁻¹)	Oxidizing agent	Composition of oxidizer	Removal (%)	Reference
CAP	20 to 150	UV/H ₂ O ₂	I = 1000 μWcm ⁻² C _{H2O2} = 40 mM	50 to 41.2	[21]
CAP	100	Fenton and Photo-Fenton	12 W m ⁻²	88.4	[22]
CAP	20	UV/H ₂ O ₂	30 W, 3 mmol L ⁻¹ hydrogen peroxide	98	[23]
CAP	50	H ₂ O ₂ / pyrite	1 mM H ₂ O ₂ , 0.1 g L ⁻¹ pyrite	100	[24]
CAP	50	GLDA with H ₂ O ₂ / pyrite	100 μmol L ⁻¹ GLDA 1 mM H ₂ O ₂ , 0.1 g L ⁻¹ pyrite	83.8	[25]
SMX	200	Ozone/H ₂ O ₂	20 g/m ³ and 60 mg L ⁻¹	COD: 76 TOC: 32	[18]
SMX	30	electron beam, Ozone/UV	electrical power: 6 W 10 mM H ₂ O ₂	100	[26]

Table 2.1 Continued

Compounds	Concentration (mg L ⁻¹)	Oxidizing agent	Composite of oxidizer	Removal	Reference
SMX	100	ultrasound/ozone	ultrasound power density 600 W L ⁻¹ ozone dose 3 g h ⁻¹	100	[19]
SMX	10.13	reactive sulfate radicals	[H ₂ O ₂] = 2.4 mM	100	[27]
SMX	20	ferrioxalate complexes	UV: 500 W m ⁻² 2.5, 5.0 and 10 mg Fe ²⁺ /L same dose of H ₂ O ₂	100	[28]
SMX	50	H ₂ O ₂	UV: 250 W m ⁻² [H ₂ O ₂] = 120 mg L ⁻¹	86	[13]

2.1.2 Biodegradation for antibiotics

Biological treatment is regarded as the most common and economical approach for the contaminants in wastewater. Conventional biological wastewater treatment methods such as activity sludge processes are mainly to remove some easy-degraded substance, while certain antibiotics can also be removed in such operations.

Depending on the types of treatment processes, beta-lactam such as ampicillin and cephalirin could be removed more than 17 – 43% in AS system (Table 2.2) [29], while ampicillin removal rate can be more than 94% in membrane bioreactor (MBR) process [30]. 96% removal of cephalexin was found in an Australian wastewater treatment plant [31]. Complete amoxicillin removal was found in Italy and Switzerland [32]. The main reason for the degradation of beta-lactam antibiotics is due to the unstable beta lactam ring that can be easily degraded by microorganism [32]. Macrolides antibiotics such as roxithromycin would be removed more than 33% in a Germany wastewater treatment

plant [33]. The efficiency was higher in an MBR system (38% - 57%) [34]. The removal rate of macrolides antibiotics is proportional to the sludge retention time [35-38].

Table 2.2 Degradation of antibiotics by biological treatment

Type	Compounds	Initial concentration (ng/L)	Effluent concentration (ng/L)	Removal (%)	Reference
Beta-lactam	amoxicillin	18	0	100	[39]
	ampicillin	17	13	23.5	[29]
	cefalexin	2000	78.2	96	[31]
	penicillin V	13800	2000	86	[32]
	cloxacillin	4600	700	85	[32]
	cefaclor	980	0	100	[40]
	cefotaxime	6500	1800	71	[32]
	cephapirin	18	15	17	[29]
	oxacillin	14	8	43	[29]
Macrolides	Roxithromycin	810 ± 420	540 ± 70	33	[33]
	azithromycin	152	96	37	[41]
	tylosin	1150 ± 70	60 ± 4	95	[42]
	clarithromycin	59 - 1433	12 - 32	99	[38]
Macrolides	spiramycin	603	454	25	[39]
	erythromycin	32 - 80	---	80	[37]
Sulfonamides	SMX	246	46	81	[39]
	SMX	13 - 155	4 - 39	69 - 75	[43]
	Acetyl- SMX	2394	---	81	[44]
	sulfamerazine	2000 - 10000	---	32 - 85	[45]
	sulfathiazole	10570	180	98	[46]
	sulfadimethoxine	70	< 30	57	[47]

Table 2.2 Continued

Type	Compounds	Initial concentration (ng/L)	Effluent concentration (ng/L)	Removal (%)	Refer ence
Sulfonamides	Salazosulfapyridine	60	15	75	[40]
	Sulfadimidine	25 - 12	12 - 6	50	[48]
Quinolones	norfloxacin	339	85	75	[49]
	ciprofloxacin	1674	626	63	[50]
Quinolones	nalidixic acid	372	200	46	[38]
	pipemidic acid	54	12	78	[49]
	gatifloxacin	111	56	50	[49]
	enrofloxacin	100	20	80	[40]
Tetracyclines	acheomycin	46 - 234	16 - 38	66 - 90	[38]
	acheomycin	59.8 - 110	---	39	[30]
	aureomycin	970	40	96	[46]
	aureomycin	178	---	83	[51]
	doxycycline	210	70	67	[47]
	oxytetracycline	53.5 - 107	---	44	[52]
	minocycline	380	30	92	[46]
	demeclocycline	270	30	89	[46]
Lincosamides	jiemycin	3.9	3.7	5	[50]
	clindamycin	6.8 - 13.3	14.9 - 32.5	0	[36]
Polyether	monensin	190	10	95	[40]
Glycopeptides	vancomycin	41	40	2	[39]
Aminoglycosides	gentamicin	400 - 7600	200 - 1300	50 - 83	[53]

SMX is a common sulfonamide antibiotic. Its concentration can be reduced by 65 – 96% after activated sludge processes [43, 45, 46, 54]. However, it has been reported that part of these compounds was concentrated on sludge through adsorption [35, 36, 43]. Also, Pan et al. [55] indicated that CAP would be reduced in soil with 40 d under aerobic condition.

Conventional biologic methods would remove antibiotics without high energy and chemicals consumption. As discussed in previous studies, AOPs for antibiotics removal have high cost for reagent. Total cost of fenton process was almost 19 \$ per kg dissolved organic carbon, as well as UV/ZnO process consumed 207 \$ {Elmolla, 2010 #388}. Compared with these process, biological treatment for antibiotics are reagent free and environmental friendly method. The low cost for treatment makes biological processes widely used on a large scale. But there were still some deficiencies such as the low efficiency and further treatment of abundance sludge. These problems will require higher cost and technical support and increase environmental stress.

2.1.3 Other technologies for antibiotics removal

Electrolysis is a useful technology to remove recalcitrant pollutants. It can also achieve complete degradation of certain chemicals. Further, electro-oxidation or anodic oxidation has been a popular technology to remove pollutants.

Bocos et al. [56] used an electrochemical cell (Figure. 2.2) to generate hydroxyl radicals with granular activated carbon or carbon fiber cathodes. After 120 min, CAP could be completely removed [56]. Chen et al. [11] used a novel Al-doped PbO₂ electrode to increase hydroxyl radicals, and high electrochemical activity was demonstrated with 87.30% removal of CAP after 2.5 h operation. Lin et al. [57] conducted SMX removal in an electrochemical device with Ti/SnO₂-Sb/Ce-PbO₂ anode. With 0.5 – 40 mA/cm² applied current density, 10 – 400 mg/L SMX could be removed by 95%. Major products of SMX were found to be NH₄⁺ and SO₄²⁻ with intermediates including 3-amino-5-methylisoxazole.

Figure 2.2 Schematic diagram of electrolysis device (a) electrochemical plug flow cell (b): (1) graphite electrode, (2) BDD electrode, (3) applied voltage, (4) air supply, (5) magnetic stirrer, (6) pump, (7) influent, (8) effluent [56].

Some semiconductor materials could generate photoelectron or hydroxyl radicals under visible light or UV. These materials have been applied in the removal of recalcitrant organic matters with the combination of other AOPs process. Ngouyap et al. [58] investigated the removal of SMX under UV- and VUV-based processes, reporting that complete degradation of 10 mg/L SMX was obtained. Kim et al. [59] demonstrated that different removal efficiencies of SMX were obtained under low-pressure lamp UV (352 nm, 254 nm) and vacuum – UV (185 nm, 254 nm).

2.2 Conventional methods for sludge treatment and nutrients recovery

Conventional anaerobic sludge digestion produces biogas and convert the organic matters in WAS. The produced biogas cannot be in-situ utilized and required to convert to other forms of energy source, e.g. heat and electricity. The conversion process may have substantial energy loss and it has been estimated that only 30–35% of the energy in methane can be converted to electricity [60]. Marti et al. [61] reported a digestion system with specific biogas production rate of $0.94 \pm 0.27 \text{ L g}^{-1} \text{ TVS}_{\text{rem}}$ that contained $64 \pm 1\%$ of methane. The system had COD removal efficiency of $60 \pm 9\%$ from $40650 \text{ mg COD L}^{-1}$. The 135 L digester was operated with solids retention time of 20 days.

Based on the heat value of methane (35.8 MJ m^{-3}), the totally energy conversion from methane to electricity was estimated to be $55.72 \text{ J g}^{-1} \text{ COD d}^{-1}$.

The thermophilic anaerobic digestion can improve the methane production. With 55°C thermal alkaline condition, Chen et al. [1] reported the methane yield reached $165.80 \text{ mL g}^{-1} \text{ VSS}$. The energy conversion from methane to electricity could be $1403 \text{ J g}^{-1} \text{ COD d}^{-1}$, which was much higher than normal anaerobic process (based on 35% conversion rate). However, thermophilic anaerobic digestion requires high energy input to achieve sufficient turnover. The thermal condition almost consumed 126 kJ L^{-1} energy to maintain the high temperature (from 25°C to 55°C). Within a 6-day anaerobic digestion process with in-situ pretreatment only convert 20.3% of sCOD to methane (35% of methane converts to electricity) [1, 62].

Thus, it would make more sense to recover higher value products and directly convert the energy source to reduce the efficiency loss.

2.3 Bioelectrochemical systems

Bioelectrochemical systems (BESs) are devices which use bacteria as a catalyst in the anode to produce electrons and in the cathode to reduce pollutants [63]. Compared to the conventional electrochemical cell system, BESs use microorganisms to oxidize organics in the anode during transmission of electrons to the electrode. Electrons will be obtained by electrons accepters in the cathode after transmission through an external circuit. In MFCs, electrons were produced with anodic microorganisms from organic matters and then captured by cathodic electrons acceptors directly. Logan et al. (2005) extended the application of MFCs which used a small applied voltage to the MFC for conducting lower cathode potential. With innovation, BESs included MFCs and MECs.

In this process, microbes convert organic pollutants to electric energy directly and significantly reduced energy consumption compared to the traditional electrolysis [64] (Figure. 2.3).

Figure 2.3 Schematic drawing of a bioelectrochemical system [65]

2.3.1 Principle of BESs

BESs are generally composed of two half reactions. In the anode, the half equation is oxidation of organics under exoelectrogens action or oxidation of H₂ and sulfide, which will generate electrons and protons. Electrons are transferred through an external circuit and arrive the cathode. Meanwhile, protons are transferred from anode through the membrane between the two chambers to cathode. In the cathode, electron acceptors (such as O₂ [66], H⁺ [67] or other strong reducing substances) obtain electrons and protons to complete the half reduction reaction. Figure. 2.4 shows some common half reactions in anode and cathode.

Figure 2.4 Schematic diagram of anodic and cathodic reaction in a bio-electrochemical system [68]

If the cathode reduction reaction has higher potential than the anodic oxidation reaction (such as the reaction 2.1 and 2.2), the whole cell has a spontaneous reaction that forms a MFC system. Otherwise (as reaction 2.3), a small voltage should be applied to overcome the electrode polarization [68].

Besides the half reactions, electrons transfer from the biofilm to an electrode is also the key factor for BESs. Currently, the primary mechanisms of microbial electron transmission are shown in [Figure 2.5](#) [69]: (a) cytochrome C as electron shuttles for some reductive proteins; (b) nanowire for electrons transfer; (c) some electrons transmission mediator secreted by bacteria. However, in a real BES, the situation will be more complicated since multiple pathways can be performed simultaneously. Firstly, cytochrome C is a typical protein containing ferroheme, which is the most significant protein of electrons transmission for exoelectrogens. For example, *Shewanella. oneidensis MR-1*, a typical exoelectrogen, has more than 42 types of cytochrome C, most of which are located outside of epicyte [70]. The theory of conductive nanowire was reported by Rguera et al. (2005) where *Geobacter. Sulfurreducens* was studied for the reduction of Fe^{3+} . The conductivity of *Shewanella* nanowire is closely related cytochrome C [71]. In BES, many gram-negative bacteria (G^-) have the ability of electron shuttles secretion. For instance, riboflavin (RF) and flavin mononucleotide (FMN) secreted by exoelectrogen have been demonstrated as useful electron shuttles [72]. RF secreted by pure *Shewanella. oneidensis MR-1* would increase the electron transmission efficiency by more than 3.7 times [72]. Yang et al. (2012) indicated that, when the synthesis of cytochrome C is insufficient, the addition of RF will increase system current efficiently [73]. Although RF can be found in many microorganisms, it can be easily degraded as the carbon source, and the effect is limited [74].

Figure 2.5 Potential mechanisms for microorganisms to transfer electrons (configured from literature [69])

2.3.2 Application of BESs for removal of pollutants

Compared to conventional electrolysis process, BES has the anodic exoelectrogen that can produce electrons. The biofilm provides a lower potential to the anode (commonly -0.2 V vs SHE), and organic matters can be oxidized. At the same time, cathode would spontaneously reduce pollutants under a high potential or with a small voltage applied.

Table 2.3 Recalcitrant wastes treatment in MFCs and MECs

Acceptor	BESs	Influence Factors	Reference
oxygen	MFCs	pH, resistance, electrolyte, O_2 concentration	[75]
permanganate	MFCs	pH, initial concentration, external resistance	[76]
Cr(VI)	MFCs	pH, initial concentration, external resistance	[77]
vanadium(V)	MFCs	initial concentration, conductivity, pH, external resistance	[78]
nitrate	MFCs	O_2 concentration,	[79]

Table 2.3 Continued

Acceptor	BESs	Influence Factors	Reference
Cu(II)	MFCs	O ₂ concentration,	[80]
Cu(II)	MFCs	initial concentration, external resistance	[81]
permanganate, dichromate,	MFCs	electron acceptors, pH, O ₂ , conductivity	[82]
Fe(III)	MFCs	Initial concentration, conductivity, O ₂ concentration	[83]
Ag(I)	MFCs	pH, physical form	[84]
Hg(II)	MFCs	initial concentration, pH,	[85]
Hg(II)	MECs	initial concentration, pH, applied voltage	[86]
H ₂	MECs	applied voltage	[87]
Azo Dyes	MECs	pH, HRT, external resistance	[88]
Iodinated X-ray	MECs	initial concentration, pH, O ₂	[89]
Trichloroethylene	MECs	cathode potential	[90]
Phosphate	MECs	cathode material, applied voltage	[91]
methane	MECs	applied voltage, operational time,	[92]

Based on the different characters of pollutants, BESs would be used to convert biomass to electrical energy as well as some useful products. Compared to conventional electrolysis process, the energy consumption of BESs is quite low. As shown in Table 2.3, pollutants such as Azo Dyes [88], Iodinated X-ray [89], Trichloroethylene [90] need to be reduced under lower cathode potential in MEC. Metal ions such as Cr(VI) [77], Cu(II) [80], [81], Ag(I) [84], Fe(III) [83], vanadium(V) [78] have higher reaction potential, therefore these metal would be reduced in MFCs. However, Hg(II) [85, 86] and Co(II) [93] need to be applied a small voltage (such as 0.2 - 0.7 V) to conduct MECs. As a potential technology, BES has been used for application as rapidly pollutants removal and biomass treatments, which is an environmental friendly method for decreasing energy consumption.

BESs have excellent performance for pollutants removal, however, some factors will influence the performance of BESs like shown in table 2.3. The cathode material was the key factor to influence the internal resistance of the whole cell {Cusick, 2012 #169;Wang, 2015 #390}. In the chapter 5, the cathode materials were investigated and discussed in the MEC for antibiotic removal.

2.3.3 Application of BESs for removal of antibiotic

The chlorinated nitroaromatic antibiotic could be removed by a biological method with a long sludge retention time. However, the intermediate products were normally accumulated and long sludge retention time may also lead to sludge accumulation [34]. Although electrolysis can reduce CAP efficiently and completely, high energy consumption is a major concern [11]. Since CAP cannot be reduced in a higher potential (-0.3 V for 1 g/L acetate under standard condition vs SHE, MFC), MECs with applied voltage have been explored for CAP reduction as shown in Table 2.4. In these studies, conventional carbon-based materials were selected as cathode including carbon rod, carbon fiber, graphite felt, carbon cloth, as well as, graphite blush. Liang et al. [94] indicated that under 0.5 V applied voltage, CAP would be rapidly degraded to amine products in 36 h as cathodic electrons acceptor. With glucose as co metabolism substrate, the biofilm on the cathode would contribute to the degradation efficiency.

In addition to CAP, Wang et al. [97] used SMX as the carbon source for the anode in MFC and almost 85% of initial 20 ppm SMX was degraded within 12 h. Miran et al. {Miran, 2018 #385} used MFC to remove different concentrations of SMX from 0.04 to 0.79 mM. The specific removal rates of 0.67 to 13.36 $\mu\text{M h}^{-1}$ were obtained. Zhang et al. {Zhang, 2017 #386} investigated wetland-coupled MFC to drive a BES for removal of SMX. Results indicated that stable electricity of 0.84 to 1.01 V was obtained by the coupled MFC and supported the BES for rapid SMX degradation. Within 32 h, 30 mg L⁻¹ of SMX could be completely removed in the coupled system. In another study, different concentration of SMX (2000 to 4000 $\mu\text{g L}^{-1}$) were tested for removal in

the coupled MFC-constructed wetland system. Results indicated the excellent SMX removal of 99.29% was obtained {Zhang, 2018 #387}.

Kong et al. [95] reduced nitrofurans antibiotic furazolidone in the cathode of MEC and obtained 3-amino-2-oxazolidinone as products. Song et al. [96] tried to use a different concentration of metronidazole as fuels in an anode of MFC. The results demonstrated that 10 mg/L of metronidazole would be efficiently degraded to 1.46 mg/L in 24 h and achieved power density as high as 99.23 mW m⁻². All these previous studies have shown the feasibility of degradation of antibiotic in BESs.

Table 2.4 CAP degradation in BESs

BESs	Applied voltage (V)	Cathode/catalyst	Removal efficiency (%)	Initial concentration of CAP (mg L ⁻¹)	Products	Reference
MEC	0.5	carbon cloth/bacteria	96	32	amine product (AMCl)	[94]
MEC	0.5	carbon cloth/bacteria	86.3	30	amine product (AMCl)	[9]
MEC	0.5	graphite fiber brush/bacteria	98	30	amine product (AMCl)	[98]
MEC	Cathode potential (-0.15 to -1.25 V)	Graphite fiber brush	0 – 99.86	50	3-amino-2- oxazolidinone	[99]
MEC	0.5	Graphite fiber brush/bacteria	99.5	30	amine product (AMCl)	[100]

2.3.4 Application of BESs for sludge treatment and nutrients recovery

MFC or MEC can be a useful process to directly convert the compounds in the FL to other resources, e.g. electrons and hydrogen [14-16]. Chen et al. [101] used the FL

of WAS and kitchen waste as the feed for a MFC which generated 0.556 V of maximum voltage power. Xu et al. [102] reported the highest energy recovery efficiency of $169 \pm 0.1\%$ and the maximum soluble chemical oxygen demands (sCOD) removal efficiency of $51.4 \pm 0.6\%$ were obtained with 0.8 V external applied voltage in a MEC system. Hence, the complex organics from the FL could be used as the carbon source for a MFC and converted to electrical energy directly [103-105].

BESs have also been used for nitrogen and phosphorus removal and/or recovery. For example, nitrification [106, 107] and denitrification [108] could occur in the anode or single chamber air cathode of MFC. The form of $\text{NH}_4^+/\text{NH}_3$ and the chemical status of phosphorus can be affected by the pH of electrolyte [109]. Therefore, they can be recovered at high pH conditions [91]. Although previous studies showed BES was a promising application in nitrogen and phosphate recovery, there still are several challenges to overcome. For example, phosphate precipitates are normally formed on the cathode electrode. The collection of those precipitates and replacement/regeneration of the cathode electrode will be a great challenge [110]. To date, most of the organics, nitrogen and phosphorus removal/recovery processes are carried out in separated systems due to the various requirements on pH values, redox potential, reaction locations, and external energy. This requires specific design of the system to target each individual compound, which likely increases the overall capital and operating costs.

As discussed in section 2.3, BESs was a potential and novel technology to remove different kinds of pollutants and recovery nutrients which decreased energy consumption and increased efficiency.

Chapter 3 Materials and methods

This chapter provides detail information of the reactor construction and setup in section 3.1. In section 3.2, the inoculation and operation of bioelectrochemical system were described. Finally, chemicals and equipment used in all studies were listed and introduced. Electrochemical analysis methods used in the study were also discussed.

3.1 Reactor setup

In this study, all MFCs and MECs were made of dual cubic chambers (Figure 3.1), which were separated by a cation exchange membrane (Ultrex CMI-7000, Membranes International, Ringwood, NJ, USA). The working volume of each chamber was 28 mL. A carbon rod (Chijiu Duratight Carbon Co., China) and graphite fiber (1.5×1.5×1.0 cm, Sanye Co., Beijing, China) were used as the anode. Meanwhile, the carbon rod was used as cathode electrode during the acclimation period.

Figure 3.1 The dual chamber BES reactor and structure diagram

3.2 Inoculation and operation

Table 3.1 Composition in the anolyte

Purpose	Composite	Concentration (g/L)
carbon source	CH_3COONa	1.00
nitrogen	$(\text{NH}_4)_2\text{SO}_4$	0.39
source	K_2SO_4	0.15
buffer	$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	3.31
	$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$	10.25
trace element		12.5 (mL/L)
vitamins		12.5 (mL/L)

For all MFC and MEC used in these experiments, the anodes were inoculated from a bioelectrochemical reactor running with acetate as feed [77, 111]. The composition of anolyte is shown in Table 3.1 [63, 93, 112]. The anolyte was dispersed with N₂ for 15 min before transferred to the anode. During the anode biofilm acclimation period, DI water with dissolved oxygen was used as the electron acceptor [113] with an external resistor of 1000 Ω [63].

3.3 Electrochemical test

An automatic data collection system (PISO-813, Hongge Co., Taiwan) was used for collecting potential of anode and cathode (Figure. 2.1). The power density of microbial cells was tested with a potentiostat (SP-150, BioLogic, Singapore) and normalized with the formula in equation 3.3 (W/m^3) using an anode as counter electrode and a cathode as a working electrode. Before the liner sweep voltammetry measurement, the cell was in open circuit mode until the potential was invariable and then performed from 0.0 V to the open circuit condition (OCC) at a scan rate of 0.1 mV/s [63].

EIS and CV tests for cathode were conducted with the same potentiostat and a three-electrode system using cathode electrode as working electrode, an Ag/AgCl reference (195 mV vs SHE) near the working electrode. In chapter 4 and 6, a Pt filament was used as a counter electrode to replace the anode with a biofilm, in order to avoid any effect of anode bacteria on the analysis of the cathode. The cathode was working electrode to test different electrochemical performance of different materials. The anolyte was 50 mM PBS buffer solution while the catholyte was CAP solution [111]. EIS analysis was conducted under polarized conditions which were close to MFC cathodic operating potentials of -0.1 V and 0 V (vs. SHE, for different cathodes) with a frequency range of 100 kHz to 1 mHz with a sinusoidal perturbation of 10 mV reference amplitude [114]. The equivalent circuit and exact value of different resistances were conducted through Zsimpwin software [115]. Otherwise, in chapter 6 and 7, the anode was a counter electrode with Pt filament as electrode.

3.4 Chemicals and Analytical Methods

Samples were collected with centrifuged at 12000 g for 10 min and the supernatant was filtered through a 0.45 μm membrane filter for further analysis. Total chemical oxygen demand (tCOD) and ammonium were measured according to the standard methods [116]. TOC was measured by a TOC analyser (TOC-5000A, Shimadzu) using the combustion-infrared method. Soluble PNs and HSs was determined by the lowry-Folin method [117] with bovine serum albumin and humic acid as standards at 760 nm. The COD in the anolyte was tested with standard method [118].

Experimental reagents used in this study can be found in [Table 3.2](#).

Table 3.2 Experimental reagents

Compounds	Purity	Company
Chloramphenicol	analytical reagent	Sigma Aldrich
Sulfamethoxazole	analytical reagent	Sigma Aldrich
Methanol	chromatographic grade	Sigma Aldrich
Acetonitrile	chromatographic grade	Sigma Aldrich
Formic acid	analytical reagent	Sigma Aldrich

3.4.1 Analytical methods for CAP and SMX

Solid-phase extraction (SPE) (Phenomenex Co. Singapore) was conducted for the purification of transformation products of CAP and SMX. After filtration through a 0.22 μm filter, the purified samples were analyzed through a High-Performance Liquid Chromatography (HPLC 1260, Agilent) to obtain the concentration of CAP and SMX. The separation of these compounds was conducted with a C18 column (Gemini-NX 3 μ , 110A, 100 $\times$ 2.0 mm, Phenomenex) and parameters are shown in Table 3.3.

Table 3.3 Methods of CAP and SMX by HPLC analysis

Parameters	Target compounds	
	CAP	SMX
Mobial phase	methanol: water (55:45)	methanol: water (55:45)
Injection volume (μL)	10 μL	10 μL
Temperature ($^{\circ}\text{C}$)	30	30
Determine wavelength (nm)	275	263
Velocity of flow (mL/min)	0.3	0.3

3.4.2 Analytical determination for transformation products

Transformation intermediates of SMX and CAP were analyzed by LC-MS/MS (G6460C, Agilent), equipped with an electrospray ionization source were used. The LC/MS was operated under the positive/negative (for SMX) and negative (for CAP) polarity mode. Separation was conducted with the same C18 column under the conditions below (Table 3.4). The scanned range was normally from 30 m/z (mass to charge) to 600 m/z.

Table 3.4 Methods of CAP and SMX for LCMS/MS analysis

Parameters	Target compounds	
	CAP	SMX
Mode	ESI, negative	ESI, positive/negative
Fragment voltage (V)	120	110
Mobile phase	A, 0.1 % formic acid in distilled water	A, 0.1 % formic acid in distilled water
	B, 0.1 % formic acid in acetonitrile	B, 0.1 % formic acid in acetonitrile
Gradient program	1. 5.00 min: A, 20.00 %	1. 0.1 min: A, 40.00 % B, 60.00 %
	B, 80.00 %	2. 3.00 min: A, 25.00 % B, 75.00 %
		3. 5.00 min: A, 15.00 % B, 85.00 %

	2. 5.50 min: A, 20.00 % B, 80.00 %	4. 5.50 min: A, 15.00 % B, 85.00 % 5. 5.60 min: A, 90.00 % B, 10.00 %
	3. 5.60 min: A, 90.00 % B, 10.00 %	6. 10.00 min: A, 90.00 % B, 10.00 %
	4. 8.60 min: A, 90.00 % B, 10.00 %	
Flow rate (mL/min)	0.25	0.2
Injection volume (μ L)	5	5
Scan range (amu)	60-400	60-500

3.4.3 Analytical determination for VFAs

VFAs were measured through a gas chromatograph (GC7890A, Agilent, USA) with a flame ionization detector. The composition of VFAs was analyzed with the equipped DB-FFAP fused-silica capillary column. 1 mL sample was acidified with 10% (V/V) formic acid and filtered with 0.45 μ m membrane filter for further analysis in a 2 mL gas chromatograph (GC) vial.

3.4.4 Analytical determination for dissolved organic matters

The dissolved organic matters (DOMs) were characterized through the LC-OCD-OND system (DOC-LABOR, Karlsruhe, Germany) which include biopolymers (high molecular weight of protein and carbohydrate), humic substances, building blocks, low molecular weight (LMW) acid, LMW neutrals and hydrophobic organic carbon. The quantification of DOMs was through the software program (ChromCALC, DOC-LABOR, Karlsruhe, Germany) [119].

3.4.5 Visualization of polyphosphate (polyP) granules

For better detection of polyphosphate granules, an improved DAPI staining procedure was used in this study. Centrifuge 1 mL sample at 2350 g for 5 minutes and discard the supernatant. Wash the cell pellet with Mellvaine's buffer and fix with 200 μ L of 4% formaldehyde. After incubating for 30 minutes, the cells were permeable by treating them with 200 μ L of 0.3% (v/v) Triton-X-100 solution for 5 minutes. Finally mixed the cells on the slides with 200 μ L DAPI solution, incubated for 30 minutes and dried at room temperature in the dark. The polyphosphate granules could be observed under confocal microscopy [120].

3.4.6 DNA extraction and high-throughput sequencing

The anode biofilm was collected at day 0, 14, 21, 35 and 45, respectively. The DNA of the microbial community was extracted using the fast DNA extraction Kit for soil (MP Biomedicals, Singapore) and then stored at -20 °C before sequencing. Primer of 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGG-TATCTAAT-30') were used for high-throughput sequencing which targeting V3 and V4 regions of rRNA genes [1]. The low-quality sequences and artificial replicate sequences were removed by quality control (QC) assessment (NovogeneAIT Genomics Singapore Pte Ltd). The QC passed sequences were clustered into operational taxonomic units (OTUs) at 97% similarity.

3.4.7 Characterization of electrodes and products in BES

At the end of operation under optimum conditions, electrodes in MFC and MEC electrodes were taken out and vacuum dried, and then visualized under scanning electron microscope (FESEM, JSM-7600F JOEL, Japan) with an associated electron dispersive EDX. The chemical composition of the outer and inner surface of electrodes were investigated by an XPS (AXIS Supra, Kratos Analytical, UK) using monochromatic Al K α excitation source at 1486.7 eV. The C 1s peak with the corrected

energy at 284.5 eV was used as reference for spectra calibration. All XPS spectra for elements were analyzed by the CasaXPS software.

XRD patterns were taken on a D8-Advance Bruker-AXS diffractometer using Cu K α irradiation operated at 40 kV and 30 mA. Microwave Plasma Atomic Emission Spectroscopy (4200 MP-AES, Agilent) was used to analyze the dissolved metal ions in the catholyte.

3.5 Calculation

3.5.1 System current, current density, power density and coulombic efficiency

$$I = \frac{U}{R} \quad (3.1)$$

where: I —circuit current (A)

U —voltage of external resistance (V)

R —external resistance (Ω)

Based on the area of membrane, the calculation of current density and power density :

$$I_{ca} = \frac{I}{V} \quad (3.2)$$

$$P_{ca} = \frac{U \times I}{V} \quad (3.3)$$

where: U —voltage (V)

I_{ca} —current density (A/m³)

P_{ca} —power density (W/m³)

V —effect volume of cathode (m³)

Anodic coulombic efficiency (CE_{an}) is defined as the ratio of total circuit electricity quantity and the electrons generated from the oxidation of organic in the anodic chamber which is calculated based on the consumption of chemical oxygen demand (ΔCOD_{an}).

$$CE_{an} = \frac{\sum_{i=1}^n I_i \Delta t}{F \times \frac{4 \times \Delta COD_{an} \times V_{an}}{M_{O_2}}} \times 100\% \quad (3.4)$$

where: V_{an} —the volume of anolyte in MFCs (L)

ΔCOD_{an} —the consumption of chemical oxygen demand in anode (g/L)

I —circuit current (A)

Δt —the interval of current collection (s)

M_{O_2} —molecular weight (MW) of O_2 (g/mol)

F —Faraday's constant (96485 C/mol e^-)

4—electrons obtained by one mol O_2 (mol/mol)

3.5.2 Yield of products

The ratio of the removal amount of CAP or SMX and the consumption of COD in the anode.

$$Y_{CAP} = \frac{\Delta[CAP] \times V_{MEC,ca} \times M_{O_2}}{\Delta COD_{MEC,an} \times V_{MEC,an} \times M_{CAP}} \quad (3.5)$$

$$Y_{SMX} = \frac{\Delta[SMX] \times M_{O_2}}{\Delta COD_{MFC,an} \times M_{SMX}} \quad (3.6)$$

where: $\Delta[CAP]$ —the concentration change of CAP in MEC cathode (g/L)

$\Delta[SMX]$ —the concentration change of SMX in the MFC anode (g/L)

$V_{MFC, an}$ —the volume of anolyte in MFC (L)

$V_{MEC, an}$ —the volume of anolyte in MEC (L)

$V_{MEC, ca}$ —the volume of catholyte in MEC (L)

$\Delta COD_{MFC,an}$ —the consumption of COD in anode of MFC (g/L)

$\Delta COD_{MEC,an}$ —the consumption of COD in anode of MEC (g/L)

M_{CAP} and M_{SMX} —MW of CAP and SMX (g/mol)

3.5.3 Reaction rate constant

The kinetics of CAP alteration was evaluated through follow first-order reaction model.

$$C = C_0 e^{-kt} \quad (3.7)$$

where: C —the concentration change of CAP in MEC cathode at time t (g/L)

C_0 —initial concentration of CAP in MEC cathode (g/L)

The rate constant k (1/h) was fitted by SigmaPlot v.11.0 software analysis [94]. The half-life time ($t_{1/2}$) of CAP was calculated:

$$t_{1/2} = 0.693 / k \quad (3.8)$$

Chapter 4 Rapid degradation of Sulfamethoxazole and its influences of power generation in microbial fuel cells

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Sulfamethoxazole is widely used in medical areas. Chapter 4 investigated SMX degradation and its influence on power generation in the anode of MFC. Specific method and materials were shown in section 4.3. The detailed results are discussed in section 4.4. A summary of this study is shown in section 4.5.

4.1 Introduction

SMX, as an antibiotic, is widely applied in medical areas and produced by the pharmaceutical industry. It is mainly used for bacterial infections such as urinary tract infections, bronchitis, and prostatitis and is effective against both gram negative and positive bacteria such as *Listeria monocytogenes* and *E. coli*. However, while it has achieved profound influences in medical treatments, it also brought side effects to the environment as manufacturing facilities do not thoroughly treat them before being discharged.

Previous studies have effectively reduced such hazardous effects by treating sludge containing SMX with MFCs. It is a bio-electrochemical device that use bacteria as the catalysts to convert organic substrates directly into electrical energy using redox reactions [97]. Based on the results from our study, in 36 hours, 20 ppm SMX is degraded into 3-amino-5-methylisoxazole. While repeating the same experiment, it was found that SMX can enhance power generation. After days of acclimation, electroactive biofilm (EABF) inside the MFCs can completely adapt to the medium in the anode and generate power by transferring electrons to electrodes.

4.2 Experimental outline

Figure 4.1 Experimental procedures for SMX degradation in BESs

4.3 Experiment operation

For batch study, two reactors were operated in parallel to measure the biodegradation of SMX in the anode of MFC. During the acclimation period, MFC operating conditions were applied and DI water with oxygen was used as electron acceptor in cathode chamber [25] with an external resistor of 1000 Ω [22]. When stable voltage output was achieved, 100 mM $K_3[Fe(CN)_6]$ was used to replace the catholyte to have a stable cathode. Once the MFC achieved stable performance with new cathode, 20 mg L⁻¹ of SMX was added together with 1 g L⁻¹ HAc in the anode to investigate the impact of SMX on the system. Biofilm samples were taken from HAc as sole substrate stage and co-substrate stage for DNA extraction and microbial community analysis.

In addition, two control reactors were set up for baseline study. The first control reactor was operated in OCC to examine changes of SMX in the absence of current generation. The second reactor was operated without microbial culture in the anode under OCC to analyze the redox behavior without biocatalyst and electron flow (abio-OCC). The two control experiments were carried out for 3 times respectively to obtain reproducible results. During the batch tests and control tests, nine liquid samples were obtained from anodes within a 48 h cycle.

The two batch reactors were then selected for long-term study in parallel. These reactors were operated for 48 days in a cyclic feed-draw manner, which was divided into two operating stages (stage I and stage II). In stage I, the reactors were operated with HAc as sole carbon source for 24 days to achieve stable performance. There were four phases in stage II (1, 2, 3, 4). The acclimation phase was named as phase 1, in which 20 mg L⁻¹ of SMX and 1 g L⁻¹ HAc were used as co-substrate. The performance was deteriorated in phase 2 after phase 1 operation. Phase 3 was a recovery phase with HAc as the sole carbon source. Following phase 3, the reactors were fed with co-substrate again and this phase was named as phase 4. Detailed performance and stage/phase categorization can be found in results section.

4.4 Results and discussion

4.4.1 Start-up and electrochemical performance

Figure 4.2 (A) Potential profile of anode and cathode as feed; (B) SMX concentration as a function of operation time

The electricity generation of MFCs was stable after acclimation with HAc feed and power generation duration was 24 h to 36 h (Figure 4.2A). With the addition of 20 mg L⁻¹ SMX, it was found that the overall potential increased to 0.607 V (Figure 4.2A). Meanwhile, the duration of power generation also prolonged from 41.5 h to 103 h. This result indicates that SMX contributed to the additional power generation. Based on the circuit current, the electricity recovered from the co-substrate increased from 54 coulombs to 167 coulombs, which was almost 3 times improvement. However, 20 mg L⁻¹ SMX alone, if 100% mineralized and fully converted to electricity, can only contribute 52.1 coulombs (TOC = 9 mg L⁻¹). Thus, it can be concluded that SMX had a positive effect on the EABFs extracellular electrons transfer (EET) and metabolism.

Within 48 h reaction, 98% SMX was degraded in the anode (Figure 4.2B). Compared with the removal efficiency of 47% under open circuit control, SMX removal efficiency was more than doubled, indicating the electrons contribution to the SMX degradation. Meanwhile, the abiotic OCC just achieved 21% removal, suggesting the EABFs catalyst function played a role in the anode for SMX degradation (Figure 4.2B).

4.4.2 Electrochemical activity of EABFs analysis

To investigate the activity of EABFs, LSV was used to analyze the power generation of the whole MFC. The open circuit potential increased from 0.691 V to 0.728 V after addition of SMX and the power density improved by 18% compared to the absence of SMX case (Figure 4.3A). The result proves that by dosing SMX, higher power generation achieved, which is consistent with the voltage generation (Figure 4.3A).

Figure 4.3 (A) Linear sweep voltammetry test with presence and absence of SMX; (B) Cyclic voltammetry analyses of MFC anode under different conditions (CV, scan rate: 10 mV/s); (C) Current is presented as a function of scan rate; (D) Plot of peak current vs scan rate of CV.

No redox peaks were detected in the PBS and abiotic controls (Figure 4.3B). In contrast, multiple peaks were observed in EABFs attached electrode with acetate as the sole organic substrate, reflecting that neither an exogenous nor an endogenous soluble redox mediator was involved in current generation in the MFCs. The CV peak current of acetate as feed shows the highest oxidation peak current at 0.35 V, suggesting the oxidation reaction occurred on the electrode (Figure 4.3B). While a stronger reductive

peak current of P1 (0.22 mA) and P2 (0.24 mA) were observed at 0.47 V and 0.32 V respectively with the addition of SMX (Figure 4.3B), which is likely correlated with the removal of SMX (Figure 4.2B). The appearance of multiple reducing peaks were likely due to the electron donation by in-situ bacterial electron mediators or electroactive enzymes located within the outer cell wall [122]. The redox peaks of P2 and P5 would most likely correspond to outer cytochrome C protein [123], reflecting the electricity generation was linked to cytochrome-C protein. Previous studies applied cyclic voltammetry to investigate the electron transfer of *Geobacter* biofilms and reported a major electron transfer redox system around -0.376 V vs. Ag/AgCl (0.224 V vs. SHE). With the addition of SMX, the cyclic voltammetry curve exhibited higher catalytic current at P6 at 0.54 V of 0.27 mA and the high peak P6 may obscure the signal of P5 at the same position with SMX [123, 124]. Based on the height of peak current, the oxidation peak P6 appeared to contribute more to electricity generation than other redox systems. These results imply that the oxidation peak P6 was likely to be the dominant redox system functioning within the anode EABFs with the presence of SMX. Thus, the addition of SMX also changed the redox system of EABFs for power generation.

To investigate the mechanism of electron transfer between EABFs and electrode, cyclic voltammetry analysis under different scan rates was performed on the MFC with SMX and acetate as co-substrate. In the anode, the peak current of the main reductive system P2 and oxidative P6 were directly proportional to the scan rate (V) for all the scan rate examined (0.1-15 mV S⁻¹) (Figure 4.3C and Figure 4.3D). Such linear correlation between peak height and scan rate is typically considered as “thin-film behavior”, where the “film” released (or accepted) electrons as a single entity. It has been commonly reported that thin-film behavior is due to the adsorption of redox proteins onto electrode surface [125]. For example, with regard to microbial electrochemical system, submonolayer film of *S. oneidensis* cells that formed on electrode surface have shown thin-film behavior without both electron donors and electron shuttles [126]. Thus, it is suggested that, with the presence of SMX, the anodic

EABFs behaved like a thin film in electrode reactions. It was also found that the current was not limited by diffusion and showed a linear dependency to the scan rate (Figure 4.3D). Such EABFs structure may allow solute diffusion through biofilm at a relatively greater speed and extent [127].

4.4.3 Long-term performance of co-substrate MFC

Figure 4.4 The current profile of MFC under different feed (stage I, stage II)

Although the detailed performance of the two reactors were not exactly identical, the trends and overall performance were highly similar. Reactors were operated with HAc as sole substrate for 24 days in Stage I. The performance was relatively stable (Figure 4.4A). In Stage II phase 1, co-substrate was introduced. The presence of SMX

clearly enhanced the circuit current and extended the power generation period (Figure 4.4B). Nevertheless, in phase 2, the effect of SMX disappeared and the performance with co-substrate was similar as that with sole substrate (HAc). To explore if the effect of SMX is repeatable, the feed was resumed to acetate in phase 3 and changed back to co-substrate in phase 4. Four recovery cycles were carried out in phase 3. The performance was comparable with stage I. In phase 4, the performance of power generation was immediately improved again once SMX was added (Figure 4.4B). These results confirm the presence of SMX affected the activity of EABFs metabolism, and such effect may vanish when the community adapted to the conditions. Interestingly, the adaption capability would disappear once the feed was changed. Microbial community change in anodic EABFs will be discussed later.

Generally, SMX, as an antibiotic, may affect the microbial energy metabolism and microbial activity in three mechanisms. Firstly, SMX changed the redox system of anodic EABFs. This was observed in Figure 2B where the obvious peak of P6 appeared. The secreted extracellular polymer, redox proteins in particular, can act as electrons shuttles or electroactive enzymes (P2 and P5 in Figure 4.3B). Secondly, SMX is an inhibitor on p-amino benzoic acid production in the folic acid metabolic cycle, thus further inhibits the multiplication of microorganisms [128]. As such, it has a decoupling effect that reduced the energy requirement for microbial growth and diverted the electrons to the energy output. Thirdly, SMX may alter the microbial community in anodic EABFs [129]. This will be discussed in the next section.

4.4.4 Microbial analysis

Operational taxonomic units (OTUs) number of microbial community in anodic EABFs of HAc stage (367) was greater than that of co-substrate stage (253), reflecting that SMX reduced EABFs diversity. At the phyla level, *Proteobacteria* was the dominant phylum in both HAc and co-sub stages (77.48% and 79% respectively), suggesting that *Proteobacteria* may contribute greatly to electron transfer and power

generation [130]. Second abundant phylum in two stages was *Bacteroidetes* (13.98% and 12.63%). There was no *archaea* or *methanogens* detected.

Figure 4.5 The change of relative abundances of microbial communities which presented in taxonomic phylum (Top 20), Taxa represented occurrence at >1% frequency in at least one sample with additional of SMX (★ indicated the improved culture with additional of SMX)

Figure 4.5 shows the relative abundance change of top 20 microbial genus after SMX was added. It was observed some genera largely increased when the reactor was fed with SMX. In particular, the relative abundance of *Rhodopseudomonas*, *Geobacter*, and *Aquamicrobium* increased by 0.3-, 1- and 25-folds, respectively. It should be noted that these three genera are well-known exoelectrogenic population [130-133]. Notwithstanding, *Rhodococcus*, which was not in the top 20 genera, increased by 80% after addition of SMX. These species are beneficial to the mineralization of SMX and transformation of some by-products. The function of *Rhodococcus* will be discussed later. On the other hand, some genera like *Alcaligenes* and *Nitrosomonas* decreased by about 90% with the shock of substrate shift. These types of microorganisms consume

organic carbon or nitrogen for their growth and reproduction, as such they may be involved in organic carbon removal as well [134]. The results suggest SMX repelled certain organic matter competing population and improved exoelectrogenic community in EABFs simultaneously, thus contributed to the additional energy generation.

4.4.5 SMX degradation metabolites and pathways

Table 4.1 Metabolites of SMX degradation in co-sub MFC

Product ID	[M+H ⁺] (m/z)	Molecular formula	Proposed structure
SMX	254	C ₁₀ H ₁₁ O ₃ N ₃ S	
M1	256	C ₁₀ H ₁₃ N ₃ O ₃ S	
M2	171	C ₆ H ₈ O ₂ N ₂ S	
M3	156	C ₆ H ₇ O ₂ NS	
M4	92	C ₆ H ₇ N	
M5	254	C ₁₀ H ₁₀ O ₄ N ₂ S	
M6	108	C ₆ H ₇ NO	
M7	128	C ₆ H ₆ O ₃	

To investigate the degradation pathway of SMX, the mixed liquor samples, taken from the batch tests at 0 h, 24 h and 48 h, were analyzed with LC-MS/MS. The proposed chemical structures of the degradation intermediates are listed in Table 4.1. The results indicate SMX was partially degraded in the anode of MFC through three possible pathways as a function of EABFs (Figure 4.5).

Figure 4.5 Proposed microbial degradation pathways of SMX

3-amino-5-methyl-isoxazole and 4-aminobenzenesulfonate were reported to be the main stable biodegradation products of SMX [135, 136]. However, neither of them was detected in this study. This could be attributed to different cultures and environment in the anodic EABFs of MFC. It has been reported the type of SMX-degrading bacteria directly affected the degradation pathways [137]. In this study, three main species in anode may contribute to three different pathways. The dominant pathway is proposed via sulfate reducing bacteria metabolism. *Rhodopseudomonas*, as a typical sulfate reducing bacteria and electrogenes [138], were likely responsible for SMX degradation in this pathway (Figure 4.5 – pathway A). In pathway A, most intermediates exhibited an altered isoxazole ring which indicates the attack site was in the isoxazole ring and the result was in agreement with the latter case [139]. Briefly, the ring cleavage may start from the bond of N and O through hydrogenation under the reductive environment and form SMX⁺. This step can be catalyzed by NADH-dependent reductase [140]. Then SMX⁺ can be transformed via pathway A to compound M1. Following which, M2 was formed from the side chain broken of M1

and it could be further degraded through side chain broken. Through pathway A, SMX was degraded to stable end-product M4 (aniline).

Gauthier et al. [141] reported M5 (Hydroxy-N-(5-methyl-3-isoxazole) benzene-1-sulfonamide) was a main intermediate from SMX transformation. M5 was also detected in this study. The presence of *Rhodococcus* likely converted SMX into M5 via pathway B (Figure 4.5). Using pure culture of *Achromobacter*, Reis et al. [142] and Nguyen et al. [143] demonstrated the main intermediate of SMX degradation was 3-amino-5-methoxazole, which was then further transformed into simpler products. In this study, 3-amino-5-methoxazole was not detected. However, its downstream degradation intermediates M6 (4-aminophenol) and M7 (trihydroxybenzene) were identified [137] in the pathway C.

4.4.6 Energy analysis

Table 4.2 energy analysis of different feed for MFC

Parameters	HAc substrate	Co-sub substrate
Initial COD in anode (mg L ⁻¹)	483.5	512
Effluent COD in anode (mg L ⁻¹)	103.7	89
Consumption COD in anode (ΔCOD , mg L ⁻¹)	379.8	423
Electrons in circuit (coulombs)	54	167
Columbic efficiency (%) ^a	13	36
Energy efficiency (KW·h kg ⁻¹ COD)	3.57	9.6
Energy duration (h)	41.5	103
Energy output (per m ³ wastewater, KW·h)	1.4	4.17

The energy output performance of SMX stimulating EABF was examined in MFC tests. The maximum power density was improved by 18% to 40 W m⁻³ (Figure 4.3A). Meantime, the overall columbic efficiency increased from 13% to 36% based on the consumed COD in the anode, reflecting SMX contributed to the energy conversion

efficiency (Table 4.2). In addition, the energy efficiency with the presence of SMX was also improved from 3.57 KW·h kg⁻¹ COD to 9.6 KW·h kg⁻¹. Apparently, these results demonstrated SMX significantly enhanced the performance of EABF in the anode of MFC and improved the energy conversion from substrate.

Such findings can be applied in energy recovery from wastewater or waste. Generally, the wastewater treatment plants always require high energy consumption for COD removal. For example, Monteith et al. (2007) reported that the energy consumption was 0.405 KW·h m⁻³ in wastewater treatment plants [144]. In fact, the energy, in the form of COD, in the wastewater, can be harvested directly using MFC. Compared with conventional wastewater treatment processes, MFC with the presence of SMX recovered more than 10 times of energy.

4.5 Conclusion

The study on the effects of SMX for power generation in MFC has been investigated, 20 mg/L of SMX was added in the anode to monitor the performance of power generation, long-term performance and microbial culture shift. Results show that 20 mg/L SMX was degraded in the MFC anode after 36 h and enhanced the power generation as well as the extension the duration of energy production. Electrochemical tests indicated that the power density was improved and the anodic reactions exhibited multiple redox peaks. Other than acetate feed, the rest reflected that the redox system of anode became diversified. There were three main degradation pathways in the anode of MFC, corresponding to three main exoelectrogenic cultures.

Chapter 5 Comparison of Chloramphenicol degradation by three different cathodes in microbial electrolysis cells

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In this chapter, three different metal foam materials were used in the MEC cathodes for CAP degradation. The performance of CAP degradation, efficiency and degradation products were studied. Section 5.1 describes the research gap and main challenge, followed by the proposed outline in section 5.2. The detailed results and discussion are presented in section 5.4. Finally, a summary of this study is given in the section 5.4.

5.1 Introduction

In chapter 2, I discussed the feasibility of BES for antibiotics degradation. CAP can exhibit reduction peak current when the potential is below -0.4 V in electrochemical test [94]. Hence MECs with a 0.5 V applied voltage have been explored for CAP reduction [9, 94, 98-100]. Lower applied voltage has not been tested according to our best knowledge. In most of these studies, conventional carbon-based materials, e.g. carbon rod, carbon fiber, graphite felt, carbon cloth, graphite blush, were selected as cathodes. In spite of non-corrosive and biocompatibility characteristics of carbon materials, the conductivity is not as good as metal materials [113]. Metal materials are commonly used in some conventional electrolysis process to enhance electrochemical catalytic activity [145, 146]. Compared to ordinary metal electrodes, metals foam has highly open porous walls and larger specific surface area [147, 148]. The unique characteristics of metal foams can accept the electrochemical deposition. The excellent performance of copper nanofoams in the CO₂ reduction in an electrochemical cell indicated that the high surface roughness, hierarchical porosity, and confinement of reactive species of the material contributed to both final products and faradaic efficiencies [148]. Nickel foam as the cathode was studied in an electrocoagulation device for boron removal and results revealed nickel foam removed 99.2% of 10 mg L⁻¹ boron acid in 120 min at particular onset potential of 0.8 V [149]. Nickel foam has also been considered as the best cathode material for hydrogen generation [150].

In this study, for the first time, the metal foam was used as the cathode to degrade toxic and recalcitrant organic compounds in a BES. CAP was chosen as a model antibiotic to investigate the efficiency of carbon rod, copper foam and nickel foam as electrodes. The effect of applied voltage and electrochemical activity of these materials were elucidated by CV test and EIS. The degradation mechanism of CAP in the electrochemical oxidation system was analyzed, and a possible degradation pathway of CAP was proposed.

5.2 Experimental outline

Three reactors were operated in parallel to compare the different materials as cathodes (carbon rod (CR), copper foam (Cu) and nickel foam (NF)). Different voltages (0 V, 0.3 V and 0.5 V) were applied to each reactor. Initial concentration of CAP was 32 mg/L at pH = 6.0 under room temperature. The pH of catholyte was adjusted with 1 M HCl to 6.03 ± 0.10 . The experiment procedure is shown in Figure 5.1.

Figure 5.1 Experimental procedures for CAP reduction in BESs

5.3 Operation for CAP degradation

The reactor was acclimated under MFC mood and air cathode for to achieve stable power generation. Once anode reactor was stable, the CAP reactor was applied with a 0.3 V voltage as a MEC. At the same time, a cathode was replaced by 32 ppm CAP with different cathode materials. Different foam metallic materials (Jiashide Co. Suzhou, China) which had the same surface area of 4 cm^2 were used as the cathode to test the performance of CAP degradation. Cathodic materials were firstly polished using abrasive papers and then steeped with ethanol and deionized (DI)water before installation of them in the devices [93, 151]. Aqueous CAP was sparged with N_2 for 15 min to remove dissolved oxygen influence on CAP removal [152]. Three reactors with

identical operating conditions were operated in parallel. All reactors were operated in fed-batch mode under room temperature ($25 \pm 3^\circ\text{C}$).

5.4 Results and discussion

5.4.1 Acclimation of anode of MFC

At the acclimation period, a cathode of MFC was filled with deionized water and O_2 as the cathodic electrons acceptor. Fresh medium was mixed with the effluent discharged from the anode in the first five enrichment cycles to ensure a faster acclimation process, in which, the potential in MFC experienced a gradual decrease (Figure. 5.2A). Feed of anode was then changed to fresh medium (Table 3.1) afterwards. The potential of anode then decreased from -0.25 V to -0.3 V. This value is frequently observed in other MFC systems with 1 g/L acetate as anodic fuel. Hence, the development of biofilms on the anode electrode and acclimation for anodic microorganisms were completed after five to six cycles. There was a trend of increase in open circuit potential of MFC from 0.45 to 0.64 V (Figure. 5.2C) during the 30 d acclimation, and the power density of the whole cell increased from 0.85 W/m^3 to 4.61 W/m^3 (Figure. 5.2D). The cell voltage and current dropped rapidly leading to a doubling back of the power density curve which was observed on 7 d, 14 d and 21 d (Figure. 5.2B). This 'M' type of power overshoot often appears at the initial stage of acclimation due to the immaturity of anode biofilm and reflected electron transfer limitation during the first 21 d acclimation [153].

The polarization curve displays the different resistance of the whole cell which includes activation resistance, ohmic resistance and concentration polarization [63]. In the linear region of the curve, a slope of trend line represents the ohmic resistance of the cell. In this study, the ohmic resistance decreased with the prolonged acclimation at 14 d, 21 d and 28 d, achieving values of 1062.8, 469.84 and 439.31Ω respectively. This decrease of resistance in the whole cell indicates the formation of biofilm on the anode progressively [154, 155].

Figure 5.2 (A) MFC acclimation, (B) power density, (C) polarization curve, (D) ohmic internal resistance periodically during acclimation (arrows indicate when anode was fed by fresh medium)

5.4.2 Comparison of CR, Cu and NF reactor performance under 0.3 V applied voltage

During acclimation period, reactors were in MFC mode, where dissolved O_2 was cathodic electrons acceptor. In first five cycles, feed medium was mixed with anode effluent from a stable MFC reactor to accelerate the enrichment of exoelectrogens. After several cycles, the potential in MFC experienced a gradual decrease (Figure 5.3A). From day ten onwards, feed medium only was used, and the potential of anode further decreased from -0.25 V to -0.3 V. These values are frequently observed with 1 g L^{-1} acetate as anodic fuel in MFC [80]. The results demonstrated the development of biofilms on anode electrode was successful and the biofilms were matured after 5-6 cycles. From day 18, DI water in cathode chamber was replaced with $32.57 \pm 0.62 \text{ mg L}^{-1}$ CAP solution, and the reactor was converted to MEC with applied 0.3 V voltage. As such, a sharp decrease in the cathode potential was observed (Figure 5.3A). The potential appeared stable in the following three cycles' operation (Figure 5.3A), demonstrating the suitability of the MEC for further experiments.

In the open circuit test, only around 7.8% of CAP was removed during 120 h operation regardless of cathode type (Figure 5.3B). Therefore, electrons generated from the anode and applied voltage can possibly enhance the degradation efficiency (Figure 5.3B). With 0.3 V applied voltage, 88.68% of CAP was removed in MEC using NF cathode after 120 h. The circuit current density of NF cathode was $4.91 \pm 1.82 \text{ A m}^{-3}$ (Figure 5.3D). The average cathode potential of NF was about -0.37 V (Figure 5.3C), therefore hydrogen evolution may contribute as a electrons competitor in such system [150]. Different from NF performance, CR completely degraded CAP in 36 h with η_{CAP} of $0.36 \text{ mol CAP mol}^{-1} \Delta\text{COD}$ and Cu completely removed CAP in 24 h with η_{CAP} of $0.54 \text{ mol CAP mol}^{-1} \Delta\text{COD}$ respectively (Table 5.1). The higher current density of $11.01 \pm 4.07 \text{ A m}^{-3}$ with Cu (Figure 5.3D) may contribute to the higher removal efficiency. In addition, the rate constant, k , of Cu cathode was 1.75 and 16.13 times to that of CR and NF cathodes (Table 5.1), indicating that copper foam electrode had better degradation performance. Besides, a higher CE_{an} of Cu further confirmed that Cu was a more efficient material for CAP degradation. It was also understandable the more negative Cu cathode potentials of -0.52 V to -0.59 V (Figure 5.3C) led to higher CAP degradation. These results demonstrated the importance of cathode materials selection on effective CAP reduction in MEC.

Figure 5.3 (A) MEC acclimation, (B) CAP concentration, (C) cathode potential, (D) circuit current density in MEC as a function of operation time

5.4.3 Comparison of CR, Cu and NF reactor performance under 0.5 V applied voltage

It was clear that the reduction efficiency of CAP by CR, Cu and NF cathodes increased with higher applied voltage (Table 5.1). Notwithstanding, the effectiveness of NF cathode was always lower than the other two materials with the consequence of the lower current density (Figure 5.4C). With a higher applied voltage of 0.5 V, CAP degradation by NF cathode increased to 94.88% in 120 h, higher than the removal efficiency of 0.3 V condition (Table 5.1). The higher removal efficiency of CAP under 0.5 V was due to the lower cathode potential and higher current density at higher applied voltage (Figure 5.4). However, as an excellent cathode material for hydrogen evolution, nickel foam can benefit from the more negative cathode potentials at a higher applied voltages for hydrogen evolution rather than CAP degradation [150, 156]. In addition, there was a general increasing trend of the cathodes potential for these three cathodes under higher voltage (Figure 5.4B), consistent with the tendency of circuit current

(Figure 5.4C). These results were in agreement with the previous study that higher the circuit current higher the overpotential for hydrogen ion reduction [64, 157].

Figure 5.4 (A) CAP concentration, (C) circuit current density, (D) cathode potential in MEC as a function of operation time

The three cathodes exhibited different overall efficiency (η_{CAP}). CE_{an} increased with the increase of corresponding circuit current in most cases (Table 5.1), mainly ascribed to the trend of anodic COD consumption (Eqs.3.2). The copper foam had higher overall efficiency at both applied voltage (Table 5.1). After 6 h, there was only $1.46 \pm 0.28 \text{ mg L}^{-1}$ CAP left in the effluent from Cu reactor under 0.5 V. However, lower anodic coulmbic efficiency was observed for Cu and as a consequence of high COD consumption under higher applied voltage [150, 158]. At the same applied voltage of 0.5 V, the copper foam had a higher rate constant of 0.571 than that of carbon material based bio-cathode [94]. In addition, the XRD curves confirmed that CR and NF electrodes were very stable after 120 h operation, while a tiny area of Cu electrode was oxidised to CuO likely due to the natural oxidation in the atmosphere during sample transportation and analysis (Figure 5.10). Hence, there is a potential for copper foam to replace conventional bio-cathode for CAP degradation due to its lower cost, better performance and greater stability.

Table 5.1 The efficiency for the CAP degradation (operation time: 120 h)

Parameters		CAP removal	k / h^{-1}	R^2	$t_{1/2}$	CE_{an} (%)	η_{CAP} (mol CAP/mol ΔCOD)
AP = 0.3	CR	100 ± 0.22	0.148	0.97	4.7	18.09	0.36
V	Cu	100 ± 0.19	0.258	0.98	2.69	19.56	0.54
	NF	88.68 ± 0.12	0.016	0.90	43.59	10.23	0.06
AP = 0.5	CR	100 ± 0.1	0.314	0.96	2.21	33.93	0.28
V	Cu	100 ± 0.02	0.571	0.97	1.21	59.32	0.44
	NF	94.88 ± 0.08	0.022	0.95	31.94	26.59	0.06

Noted : AP: applied voltage

5.4.4 Voltammetric characteristics of cathodes using CV

The CV curves for these electrodes were recorded between -1 to 0.5 V (vs SHE) at a scan rate of 10 mV s^{-1} . In the catholyte of BESs, oxygen, due to the high redox potential, can easily obtain electrons from the electrode. Oxygen reduction reaction (ORR) involves a series of electrons transfer processes which depend on the type of electrodes materials. In general, the reduction of oxygen in the electrolysis process occurs through either two electrons or four electrons pathway [159]. The oxygen molecules approach electrode through the diffusion transport, so adsorption preference of electrode materials towards oxygen may affect the preponderance of these two pathways [160]. CV tests can provide an effective way to analyze the ORR on the three electrodes and further reveal the mechanism of CAP degradation in BESs [161].

In the presence of oxygen without CAP, Cu and NF electrodes showed double reduction peaks at -0.12 V and -0.43 V respectively which demonstrated the oxygen was reduced through these two pathways on these metal foam electrodes (Figure 5.5B). The two electron pathway is beneficial for the further formation of radicals [160]. However, CR as cathode only displayed one reduction peak near -0.20 V (Figure 3B) that indicated the oxygen reduction was carried out through the four electron pathway

with conventional carbon electrode [161]. Under the same electrolyte conditions, Cu electrode had a higher reduction peak current (Figure 5.5B) which demonstrated this material had more catalytic characters for the ORR [162]. In the absence of oxygen, compared with the blank control, CAP exhibited reduction peak on these three electrodes at -0.40 V (Figure 5.5A and Figure 5.5B) which indicated the CAP could obtain electrons through electrodes directly [94].

In the presence of both oxygen and CAP, these three electrodes showed ORR peak near -0.12 V and CAP degradation peak near -0.40 V (Figure 5.5A), which suggested during the CAP degradation, oxygen was involved in the two electrons pathway and producing radicals [159]. This supposed mechanism also corresponded with the observed hydrolysis product that had a molecular mass of 339 (Figure 5.7). As shown in Figure 3A, when 32 mg L⁻¹ CAP co-existed with the dissolved oxygen, a sharp reduction current peak of -10.74 mA for copper foam was observed at -0.606 V (Figure 5.5A). Compared to CR and NF electrodes, Cu exhibited higher reduction current. NF had the lowest reduction current at about -0.52 V, corresponding to the relatively poor performance for CAP degradation. The results strongly suggested that the removal efficiency of CAP was highly dependent on the materials reduction current. Moreover, NF, as an excellent material for hydrogen generation, exhibited a hydrogen reduction peak near -0.79 V (Figure 5.5A). This hydrogen generation process competed for electrons with CAP degradation, leading to the lowest performance of NF (Figure 5.3A) [150].

To further investigate the limiting factor of the electrolysis, copper foam as the most efficient cathode in this study was chosen as the electrode to test CV under different scan rates varying from 1 mV s⁻¹ to 10 mV s⁻¹. The effect of different scan rates on the voltammetric response of Cu electrode was investigated, and the results are shown in Figure 5.5C. It is clear that the redox couple in all cyclic voltammograms exhibited an increased current response with the increase of scan rate from 1 to 10 mV s⁻¹. Figure 5.5D showed that the peak currents were proportional to the square root of the scan

rates. The peak current was in linear with the square root of scan rate, reflecting that the redox reactions on the Cu electrodes were the typical electrons acceptor diffusion-controlled electrochemical processes [11]. Such diffusion limited the speed of electron transfer, as evidenced by the strong correlation between soluble CAP concentration and anodic current [163]. Diffusion limits the contribution of electron transfer by this mechanism, as shown by the strong relationship between soluble CAP concentration and anodic current [163].

Figure 5.5 Cyclic voltammetry tests carried out on the various cathodes with the (A) presence or (B) absence of CAP in the catholyte.

5.4.5 Electrochemical analysis of cathodes using EIS

Figure 5.6 (A) Nyquist plots of EIS spectra by different three electrodes in 32 mg/L CAP solution. (B) fit with the equivalent circuit

Based on the same anode, EIS was used to explore the main reason for the different performance of these three materials. EIS spectra were fitted to equivalent circuits (Figure 5.6A) to identify the components of the internal resistance of the cathode material. After EIS test, the fitting circuit Nyquist data were fitted using the electrical circuit given in Figure 5.6A, with characteristic parameters for the polarization and ohmic resistances, which delineate the resistance of materials [164]. As described in Table 5.2 of the fitting results, copper foam electrode exhibited a lower electrode ohmic resistance (37.6 Ω) and electron transfer resistance (45.4 Ω) than CR and NF. The lowest resistance of the Cu electrode showed the highest circuit current (Figure 5.2D and Figure 5.3B) which contributed to the higher degradation efficiency of CAP.

Table 5.2 Component analysis of internal resistance for different material cathodes

	Ohmic resistance (Ω)	Electron transfer resistance (Ω)
CR	57.89	45.38
Cu	37.6	45.4
NF	66.74	300

5.4.6 Degradation pathway of CAP

Figure 5.7 The LC–MS profiles of the CAP degradation products by (A, B, C) Cu and CR electrodes, (D, E) NF under 0.3 V applied voltage

Figure 5.8 The LC–MS profiles of the CAP degradation products under 0.5 V

Products of CAP degradation were identified by LC-MS. The major intermediates products were mono- and di-hydroxyl derivatives of CAP (Figure 5.9), which are the typical products from electrolysis or photodegradation processes [11, 165]. It is speculated that electrochemical degradation of organic pollutants was recognized as a free-radical mechanism where hydroxyl radicals ($\cdot\text{OH}$) or $\cdot\text{O}_2^-$ formed on the electrode. On the cathode, the electrons generated by the anode transferred to the surface of the material and then scavenged by dissolved oxygen, facilitating the hole–electron separation. Meanwhile, the holes leaving from the valence band of the cathode to form hydroxyl radicals or directly oxidise various organic compounds [166].

According to the detected products at different sampling points, CAP degradation pathways with different cathodes were proposed. Under 0.3 V applied voltage, there were two possible pathways for CAP degradation using Cu and CR as cathodes, whereas the pathway using NF was straightforward (Figure 5.9). For NF electrode, the starting change was the hydroxylation of the head-end carbon with a nitro group, yielding the compound M1, ion with m/z 309 $[\text{M}-\text{H}]^-$, which was consistent to the

addition of mass to CAP. The degradation ceased in the form of M1 due to the lower current and high cathode potential (Figure 5.3C and Figure 5.3D). For CR and Cu cathodes, the initial degradation started from the hydroxylation of the terminal carbon that contains two chlorine atoms to form compound M2. The presence of M3 was due to the reaction of M2 who accepted six electrons [94]. Two side chains of M3 were detached and benzene ring formed via hydroxyl radicals attack. Then the further hydroxyl radicals attack would result in the formation of CO₂ and H₂O. For these three materials, M9 was also detected as the common hydrolysate.

After changing the applied voltage to 0.5 V, potential below -0.6 V was achieved for all three cathodes. The degradation started directly from the bond breaking of the terminal carbon that contains two chlorine atoms, yielding the compound M3 with a m/z of 237/239 [M-H]⁻. Further oxidation of the amino and hydroxyl groups of M3 led to the production of M5. Subsequent oxidation of the lateral groups of M5 broke the side chain of the methyl to produce nitrobenzene. The denitration of nitrobenzene yielded M7 by obtaining six electrons. This compound was ultimately converted to CO₂ and H₂O with further hydroxyl radicals attack. Similar behavior was observed during the photolytic degradation of the antibiotic sulfamethoxazole [165].

The results confirmed that different cathode materials exhibited different cathode potential and circuit current, which in turn affected the degradation pathways and removal efficiency of CAP. Compared with the conventional semiconductor heterojunction photocatalysts or electrolysis processes, copper foam as the cathode of BES achieved high CAP removal efficiency in situ with the electrons generated from the anode and also this non-coated material can greatly reduce the cost. With the Cu cathode, the relatively lower cathode potential would be sufficient to achieve complete degradation of CAP.

Figure 5.9 Possible initial pathway for CAP degradation

Under the applied voltage condition, the cathode potential for NF and Cu is -0.35 V and -0.52 V respectively [80, 86]. Hence, under the 0.3 V applied voltage condition, copper and nickel ions, if any, could be easily reduced to pure metal and attached on the cathode electrode. Hence, the metal cathodes were protected in this study. As to the possible oxidation of the surface of electrodes, XRD was carried out to analyze the surface properties of different electrodes before and after the operation, and the result is shown in the XRD analysis (Figure 5.10). The results of XRD showed that CR and NF electrodes were stable after 120 h operation. However, Cu as cathode electrode will be oxidized to CuO. Through the analysis, the oxidation area was small during long operation likely due to the natural oxidation in the atmosphere during sample

transportation and analysis. In addition, no metal ions were detected in the catholyte during the analysis. Hence, the electrodes could be considered stable in the process.

Figure 5.10 XRD analyses on electrodes of (A) Cu (B) NF and (C) CR before and after 120 h operation

5.5 Conclusions

In this study, bioelectrochemical degradation of CAP in aqueous solution was investigated using carbon rod, copper foam and nickel foam as cathodes. Based on the comparison of degradation performance, applied voltage, electrochemical activity of these materials, it could be concluded that: The copper foam had the best performance for CAP degradation and the electrochemical degradation of CAP on Cu electrode followed pseudo-first-order kinetics. Copper foam is a promising material as the cathode for recalcitrant organics degradation in wastewater treatment. The applied voltage was the most critical factor in determining degradation and mineralization efficiency. The increase of applied voltage contributed to the higher circuit current and

complete degradation of CAP. The degradation mechanism was proposed based on cyclic voltammetry tests, and it was deduced that electrons generated from anode microorganism and the hydroxyl radicals generated in the cathode played an important role in reducing CAP. A possible degradation pathway including radical reaction, ring open and mineralization was proposed.

Chapter 6 Removal of chloramphenicol with simultaneous silver recovery in a self-driven bio-electrochemical system

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Based on Chapter 5, copper foam is an efficient cathode material for CAP degradation. In this chapter, copper foam was used in the MEC for CAP degradation, a silver recovery MFC was applied as external energy supply for MEC. The specific method and material used were shown in section 6.3. In section 6.4, different concentrations of Ag(I) and CAP were tested, and the response surface methodology model was performed to investigate the optimum condition of system. Finally, the detailed results are discussed. A summary of this study is in section 6.5.

6.1 Introduction

Last chapter described the feasibility of MEC for completely removal of CAP with copper foam electrodes. Although the MEC has high removal efficiency for CAP, the high applied voltage needs high energy input during operation. These problems will cause higher operation cost and require intensive technical support.

Previous studies proved the feasibility of self-driven systems for hydrogen production (Sun et al. 2009), dye decolourization (Li et al. 2016), reduction of carbon dioxide (Zhao et al. 2012) and heavy metal recovery (Li et al. 2017, Shen et al. 2015, Wu et al. 2015a, Wu et al. 2015b). These systems can improve energy savings, reduce environmental stress, and also address the urgency of over reliance on fossil fuel. However, there has no special attention been paid on how to fully utilize the self-driven system. In most of previous studies, only one cathode chamber was used for pollutants removal or recovery, and the other one was left without actual function. Thus, it will make more sense to further develop such system to maximize its usage and application.

Wastewater generated from electroplating industries contains high concentration of heavy metals, such as, Cr(VI), Cu(II), Ni(II), Zn(II) and Ag(I). These metals once enter the environment, will impose a potential environmental risk. In fact, such types of wastewater can be a potential good candidate for the MFC catholyte. Among these metals, silver is highly toxic but precious element. It is clear that the Ag(I) contained wastewater represents both a substantial environmental hazard and a considerable loss of expensive reagents. As a result, recovery of silver from electroplating wastewater is one of the primary objectives in the wastewater treatment and resource recovery practice. Therefore, Ag(I) was chosen for MFC catholyte in this study.

A system consisted of a MFC using Ag(I) as catholyte for Ag recovery and power production and a MEC for CAP degradation, was designed and set-up. As the concentration of Ag(I) and CAP can influence the system performance [17], preliminary studies of different concentration of Ag(I) and CAP were conducted to

explore the feasibility of the self-driven system. One-factor-at-a-time (OFAT) and response surface method (RSM) were used to optimize the concentration selection. The performance of the system, in terms of power generation, Ag recovery efficiency, CAP removal efficiency, as well as yield and morphology of products, was closely monitored.

6.2 Experimental outline

Figure 6.1 Experimental procedures for silver recovery and CAP removal in BESs

6.3 Methods and material

6.3.1 System operation

To acclimate biofilm on anodes, MFC mode was applied for all reactors during the acclimation period and DI water with dissolved oxygen was used as electrons acceptor in cathode chambers [113] with an external resistor of $1000\ \Omega$ [63]. After eight cycles operation, the performance of all reactors was stabilized. Catholyte for designed MFC and MEC was replaced by various concentration of Ag(I) and CAP, respectively. The conductivity of each catholyte was adjust to $5\ \text{mS cm}^{-1}$ with $1\ \text{M NaCl}$ solution [167]. Ag(I) concentration in MFC was $300, 400$ and $500\ \text{mg L}^{-1}$, while CAP

concentration in MEC was 20, 25 and 30 mg L⁻¹ (Table 6.1). The pH of MFC catholyte was adjusted to 2 with 1 M H₂SO₄. The established MFC and MEC were connected using titanium wire and operated for 15 days. A low resistance of 10 Ω was used in the circuit in order to detect the circuit current. One control experiment was also carried out. The system was operated in OCC to examine of the baseline adsorption of Ag(I) and CAP in the absence of current. All reactors were operated in fed-batch mode and maintained at room temperature. All experiments were carried out in duplicates.

6.3.2 Calculation

Reduction efficiency of Ag(I) and CAP in MFC and MEC, and the yield of silver and CAP (mol Ag mol⁻¹ ΔCOD and mol CAP mol⁻¹ ΔCOD) were calculated using Eqs. (6.1) - (6.4):

$$\text{CAP reduction (\%)} = \frac{CAP_i - CAP_t}{CAP_i} \times 100\% \quad (6.1)$$

$$\text{Ag(I) reduction (\%)} = \frac{Ag(I)_i - Ag(I)_t}{Ag(I)_i} \times 100\% \quad (6.2)$$

$$Y_{CAP} = \frac{[CAP_i - CAP_t] \times V_{MEC,ca} \times M_{O_2}}{\Delta COD_{MEC,an} \times V_{MEC,an} \times M_{CAP}} \quad (6.3)$$

$$Y_{Ag} = \frac{[Ag(I)_i - Ag(I)_t] \times V_{MFC,ca} \times M_{O_2}}{\Delta COD_{MFC,an} \times V_{MFC,an} \times M_{Ag}} \quad (6.4)$$

$$CE_{an} = \frac{\int_0^t Idt}{\frac{4 \cdot \Delta COD_{an} \cdot V_{an} \cdot F}{M_{O_2}}} \times 100\% \quad (6.5)$$

6.4 Result and discussion

6.4.1 Preliminary study using OFAT method

The effects of different concentration of Ag(I) and CAP were investigated by OFAT method in the preliminary study. Overall, the results show increase of Ag(I) concentration in the cathode of MFC contributed to the increase of CAP removal in the

cathode of MEC (Table 6.1), which suggests that the applied voltage from MFC was the key factor for MEC performance. While Ag(I) concentration was 300 mg L⁻¹, CAP cannot be completely removed. The increase of Ag(I) concentration improved the output power of MFC. However, high concentration of Ag(I) again cannot remove CAP completely because most of the electrons were used for Ag(I) reduction in the MFC.

In conventional BES, the CAP degradation was in proportion with the MEC applied voltage. The current density could be increased by increasing input voltage [168]. However, for the self-driven system, the applied voltage of MEC is not the key condition for good performance. When cathodic reaction in MEC is suppressed, the internal resistance increases and thus more voltage is distributed to the MEC which leads to the low current density in the circuit [169]. Therefore, a higher CAP degradation efficiency could be achieved at a higher circuit current. Therefore, it is essential to optimize the matching concentration of Ag(I) and CAP to balance the system for the highest removal efficiency for both.

The results in Table 6.2 were used to set the range and level factors for RSM (Table 6.2). The level of each factor was coded as -1, 0, or 1. The concentration of Ag(I) and CAP was coded as A or B respectively. With the model, central composite design (CCD) provided the solution for optimization of self-driven system for maximum removal of both Ag(I) and CAP.

The experimental data from design expert 8.0.7.1 was fitted to seven types of model. Based on the established criteria, it was concluded that the quadratic model was the best polynomial model to describe the relationship between the variables and the response. All the responses of Ag(I) and CAP removal were analyzed via analysis of variances (ANOVA). Figure 6.2 show that the predicted values generally match against the experimental data. This implies that the predicted values were very close to the actual experimental values and hence, supports the quadratic models given by the ANOVA. Similar results are presented in Figure 6.2C and Figure 6.2D where the normal % probability plot of residuals also fell on a straight line, implying that the

errors were normally distributed thus supporting the adequacy of the model. Therefore, the empirical model is adequate to describe the removal of Ag(I) and CAP in the self-driven system.

Table 6.1 Results for the preliminary study using one factor at a time (OFAT)

Varying parameter		Removal efficiency (%)		Recovery yield (mol/mol COD)	
Ag(I) (mg L ⁻¹)	CAP (mg L ⁻¹)	Ag(I)	CAP	silver	CAP
300	20	98.11 ± 0.38	96.79 ± 0.78	0.93 ± 0.01	0.02 ± 0.00
	25	99.14 ± 0.33	88.51 ± 4.20	1.02 ± 0.04	0.02 ± 0.00
	30	94.7 ± 0.70	80.2 ± 1.15	0.88 ± 0.02	0.02 ± 0.00
400	20	93.89 ± 0.11	100 ± 0	1.18 ± 0.03	0.02 ± 0.00
	25	97.8 ± 0.13	100 ± 0	1.22 ± 0.02	0.03 ± 0.00
	30	93.39 ± 0.1	92.75 ± 0.19	1.16 ± 0.03	0.03 ± 0.00
500	20	96.27 ± 0.19	100 ± 0	1.49 ± 0.03	0.02 ± 0.00
	25	98.36 ± 0.05	98.7 ± 0.13	1.35 ± 0.02	0.03 ± 0.00
	30	95.22 ± 0.04	85.56 ± 1.54	1.5 ± 0.02	0.03 ± 0.00

Table 6.2 Levels of variables chosen via RSM-CCD for optimization of silver and CAP removal

Factor	Variables	Coded variable level		
		Low	Centre	High
		-1	0	+1
A	Ag(II) concentration	300	400	500
B	CAP concentration	20	25	30

Figure 6.2 Comparison between experimental and predicted values for (A) Silver recovery efficiency, (B) CAP removal efficiency. Normal probability plot of residual for (C) silver recovery, (D) CAP removal.

The response surface plot and contour plot for interaction between the Ag(I) concentration and CAP concentration creates a circular display (Figure 6.3). This pattern indicates that the center of the interaction to the point of maximum removal of CAP. These results are consistent with the OFAT method, where CAP removal increased with increase of the concentration of Ag(I) (Table 6.1). It is because higher concentration of Ag(I) provided high external voltage to power the MEC for CAP degradation in the cathode [170]. It is clear that the highest CAP removal achieved at the Ag(I) concentration of 320 mg L^{-1} and CAP concentration approaching 10 mg L^{-1} , respectively. The removal efficiency declined beyond these values.

Figure 6.3 Response surface and contour plot of CAP removal as a function of Ag(I) concentration and CAP concentration.

6.4.2 Optimum range of process parameters and validation of models

Table 6.3 Optimum condition suggested by “Design-Expert”

Parameters	Unit	Value
Concentration of Ag(I)	mg/L	509
Concentration of CAP	mg/L	24
Response 1: Recovery efficiency	%	98.74
Response 2: Removal efficiency	%	97.61

The optimum conditions of maximum removal efficiency for both Ag(I) and CAP, as suggested by the model, are shown in Table 6.3. Under the optimum conditions, $510.1 \pm 3.81 \text{ mg L}^{-1}$ of Ag(I) was used in the MFC cathode for powering MEC to degrade $24.88 \pm 0.79 \text{ mg L}^{-1}$ of CAP. Within the first 6 hours operation, Ag(I) was rapidly removed by 86.2%, and 99.8% removal was recorded at the end of 24 hours operation (Figure 6.4A). Concomitantly, CAP was removed by 66.9% within 6 h and by 98.8% at the end of operation (Figure 6.4B). The performance was very close to the predicted values by model (Table 6.4) with standard deviation less than 1. In OCC, only 15.41% of Ag(I) and 22.9% of CAP were removed (Figure 6.4A and Figure 6.4B) likely due to the electrodes adsorption.

Figure 6.4 (A) Ag(I) concentration, (B) CAP concentration, (C) potential of cathode in MFC and MEC, (D) applied voltage and circuit current in MEC as a function of operation time.

Figure 6.4C shows the change of cathode potential for both MFC and MEC. It is obvious the cathode potential of MFC decreased with the reduction of Ag(I) concentration and further influenced the cathode potential of MEC which increased from -0.5 V to -0.38 V (Figure 6.4C). Therefore, the applied voltage from MFC to MEC also sharply decreased from 0.8 V to 0.48 V during the first 6 h (Figure 6.4D). The applied voltage from MFC continuously kept the cathode potential of MEC below -0.4 V, which generated hydroxyl radicals for CAP degradation [168]. During the 24 h operation, the self-driven system displayed 0.1 – 0.2 mA current (Figure 6.4D), demonstrating the successful establishment of the integrated MFC - MEC system.

Figure 6.5 (A) Cyclic voltammetry test with presence and absence of Ag(I), (B) Cathode theoretical potential for half-reactions of Ag(I) to Ag in MFC at different pH.

Cyclic voltammetry analysis on the carbon rod electrode of Ag(I) shows the presence of one strong reduction peak of 0.21 V (Figure 6.5A). Compared with the case without of Ag(I), the induction peak confirms the reducing reaction of Ag(I) on the surface of electrode [170]. As Ag(I) was reduced on the electrode, the product formed on the cathodes mainly depended on substrate concentration and pH [171]. In this study, theoretical potential of cathode at different pH conditions were calculated using Nernst equations (Figure 6.5B). The initial pH of Ag(I) contained catholyte was 2.0 and the effluent was changed by 1–2 pH units. Considering the slight change of pH, Ag(I) was reductively changed to Ag(0) (Figure 6.5B).

6.4.3 Final products property and profile in cathodes

In order to investigate the final products in integrated system, the cathode electrodes in MFC and the effluent from cathode in MEC were carefully examined. First of all, the morphology of two cathode electrodes in MFC and MEC were studied. Carbon rod from MFC and copper foam electrode from MEC were subjected to SEM, EDX and XPS analysis. Clusters of irregular crystals were observed on the surface of carbon rod (Figure 6.6A, B) in MFC cathode. EDX analysis shows the material was Ag (Figure 6.6C). There was negligible oxygen peak detected and the ratio of Ag and O was much higher than 2 (Ag₂O), implying most of Ag(I) was reduced to pure silver

[172]. Further XPS results (Figure 6.7A) demonstrate the crystals on the surface was pure silver.

Figure 6.6 SEM image of (A) new carbon rod, (B) surface of carbon rod after one cycle operation in MFC; EDX results on (C) carbon rod after one cycle operation in MFC; SEM image of (D) new copper foam, (E) surface of copper foam after one cycle operation in MEC; EDX results on (F) copper foam in MEC after one cycle operation.

As to copper foam electrode in MEC, a rougher surface was found compared to the blank electrode (Figure 6.6D and Figure 6.6E). In the MEC, it is known electrons generated in anode are transferred to the surface of the cathode electrode and then scavenged by dissolved oxygen at the electrode surface, facilitating the hole–electron separation. Meanwhile, the holes leaving from the valence band of the cathode to form hydroxyl radicals or directly oxidize various organic compounds [166]. In this study, a small quantity of Cu(II) may dissolve into the catholyte from the cathode electrode and can be reduced back and attached onto the cathode electrode under -0.4 V cathode potential (Figure 6.6C) [171]. The formation of small copper crystals was observed on the rough surface of the copper foam electrode (Figure 6.6D), reflecting the reduction of Cu(II) dissolved in the catholyte. EDX analysis identified the crystals on the rough surface to be pure copper (Figure 6.6F). Meanwhile, the ratio of copper and oxygen was much higher than 2, which indicated the product was not Cu₂O but pure copper [171].

XPS results confirm the surface roughness change was due to the reduction Cu(II) and reformation of Cu at the electrode surface. It was also noted that the copper peak increased after one cycle, consisting with the reformation of new copper on the electrode (Figure 6.7B).

Figure 6.7 XPS tests on (A) carbon rod, (B) copper foam after one cycle operation and software fitting.

With the applied voltage from MFC, the cathode potential of MEC was below -0.6 V for copper foam electrode. As shown in Figure 6.8, CAP was converted from the bond breaking of the terminal carbon that contains two chlorine atoms to form the M1 with m/z 237/239 $[M-H]^-$. The further oxidation of amino and hydroxyl groups of M1 produced M2. Then the lateral groups and side chain of methyl of M2 was further oxidized to produce M4. Subsequent denitration of nitrobenzene yielded M5 through obtaining six electrons [173]. This compound was ultimately converted to CO_2 and H_2O with further hydroxyl radicals attack (Figure 6.8). Similar behavior was also observed during the photolytic degradation of CAP [165].

Figure 6.8 Proposed degradation pathway of CAP in the cathode of MEC

6.5 Conclusions

This study, for the first time, reported a self-driven bioelectrochemical system for precious metal recovery and recalcitrant organic removal from wastewater. OFAT method and RSM model was used to identify the optimum operation condition for maximum efficiency of both silver and CAP removal. Multiple characterization methods were used to analyse the final products. Completely removal of Ag(I) and CAP were obtained in the integrated MFC-MEC system under optimal conditions, which were close to the model predicted results. SEM, EDX and XPS analysis confirmed the product on the cathode electrode in MFC was pure silver.

Chapter 7 In-situ power generation and nutrients recovery from waste activated sludge - long-term performance and system optimization

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Previous studies used acetate as the anode electrons donor. In chapter 7, real waste activated sludge was used to provide electron donors in anodic feed. Section 7.3 introduces detailed methods for the pretreatment method of waste activated sludge and operation of MFC. Section 7.4 describes the nutrients recovery performance in MFC with fermenter liquor as feed. The detailed results are discussed in section 7.4. A summary of this study is listed in section 7.5.

7.1 Introduction

Biological wastewater treatment plants discharge a large amount of WAS. Its increasing quantity and complicate characteristics make it difficult and costly to treat before releasing into the environment [1]. Conventional sludge treatment employs anaerobic digester to convert organic matter in the sludge to biogas and further to electricity or heat. However, the efficiency of such energy conversion is relatively low ($47.01 \text{ J (g COD} \cdot \text{d)}^{-1}$) [60, 61]. Thus, it would make more sense to recover higher value products and directly convert the energy source to reduce the efficiency loss.

Prior to any resource recovery step, sludge solubilization is crucial to transfer the compounds of interests into the soluble form. Thermophilic alkaline treatment has been reported to be a very efficient method to solubilize the sludge. For instance [1], treated WAS under thermophilic alkaline conditions for short-chain fatty acid generation. Besides VFAs, other organic matter such as PNs, PSs and HSs are commonly present in the FL [1, 2]. The concentrations of phosphorus and ammonium are also relatively high in the FL. Thus, organic matters and nutrients co-exist in the fermented liquid. In order to reduce the recovery efficiency loss, one-step or simultaneous resource recovery would be preferred compared to sequential recovery.

Although previous studies showed a promising prospect for application of BES in nitrogen and phosphate recovery (section 2.3.4), there are still several challenges to overcome. For example, phosphate precipitates are normally formed on the cathode electrode. Collection of those precipitates and replacement/regeneration of the cathode electrode will be a great challenge [110]. To date, most of the organic, nitrogen and phosphorus removal or recovery processes are carried out in separated systems due to the various requirements on pH values, redox potential, reaction locations, and external energy. That needs a specific design of the system to target each compound, which likely increases the overall capital and operating costs.

This study aimed to investigate the feasibility of simultaneous electricity generation and in-situ nutrients recovery. The long-term performance of the system under different operation phases was investigated. The specific objectives were to (i) evaluate the energy and nutrients recovery efficiencies, (ii) understand the conversion and transformation of the key dissolved organic matters (DOMs) during the operation, (iii) analyze the microbial communities and reveal the relationship between the performance and microbial activities.

7.2 Experimental outline

Figure 7.1 Experimental procedures for nutrients recovery in BESs

7.3 Methods and material

7.3.1 Sludge pretreatment

The feed sludge was mixed primary and secondary sludge (w/w: 1:1) from a local water reclamation plant. The fermentation of sludge was conducted in a 7 L fermenter with 3 L working volume at 55°C and pH 9.9 for VFA production. The detailed operation condition can be found in Chen et al. (2017) [1]. Before entering the anode of MFC, the FL was separated from the remaining solids, and the pH was adjusted to 7.0 ± 0.2 with 1 M HCl. The characteristics of the FL are displayed in Table 7.1. Total organic carbon was around $1247.75 \text{ mg L}^{-1}$, with 47.6% as VFAs. A high content of HSs was found.

Phosphorus concentration was much higher than that of ammonium due to the association of high phosphorus content in the primary and secondary sludge. The performance of the fermenter and characteristics of the FL were relatively stable during the operation period.

Table 7.1 Characteristics of FL

Parameter	Units	FL value
pH		7.0 ± 0.2
Total organic carbon (TOC)	g L^{-1}	1.25 ± 0.04
Ammonium	mg L^{-1}	277.01 ± 69.79
Humic like substance (HSs)	g L^{-1}	1.68 ± 0.14
Soluble Proteins (PNs)	mg L^{-1}	427.63 ± 18.48
Soluble polysaccharide (PSs)	mg L^{-1}	219.19 ± 14.79
Phosphate	mg L^{-1}	695.61 ± 35.17
Acetic	mM	17.18 ± 0.63
Propionic	mM	4.05 ± 0.24
iso-Butyric	mM	0.97 ± 0.02
n-Butyric	mM	1.75 ± 0.25

7.3.2 MFC reactor setup and operation

After one month operation, power output from all the reactors was relatively stable. Two randomly selected reactors were operated under OCC mode as the control. The feed of another three reactors was changed to FL. It should be noted that there are many other complex substances in the FL besides VFA, e.g. HSs, which may enhance electrons transfer [174]. To understand if the complex organics can enhance power production, synthetic FL that only contained VFA but with the same COD concentration as real FL was used to act as a control in another reactor. Composition of VFA (acetic, propionic, iso-Butyric and n-Butyric) followed the proportion reported in Table 7.1, amounting to a total of $7852 \text{ COD mg L}^{-1}$ which was the same as that of the FL.

The systems fed with FL were operated for almost 300 days in a cyclic feed-draw manner. Each cycle lasted for around five days, and the new feed was fed into the

anodes at the beginning of each cycle. There were two operation stages during the whole operation period (stage I and II). In stage I, there were three phases (A, B, C), while stage II had multiple phases A and B. The acclimation phases were named phase A, during which the systems may encounter feed changes or biomass withdrawal. Phase B in all the stages represented the stably performed cycles. The phase C in stage I showed deteriorated performance due to the overgrowth of biomass. Detailed performance and stage/phase categorization can be found in [Figure 7.5](#).

7.4 Results and discussion

7.4.1 Cyclic performance

Figure 7.2 The performance of organic matter and nutrients removal in the anode of MFC as the function of operation time in one typical cycle (A) concentration of TOC, HSs, PNs, PSs, ammonium and phosphate; (B) concentration of VFAs.

The FL contained a large amount of organic matter from the solubilisation of sludge. PNs, PSs, HSs, and VFAs were the dominant organic compounds. In one cycle, TOC concentration could be reduced from $1247.25 \pm 42.07 \text{ mg L}^{-1}$ to $252.75 \pm 5.3 \text{ mg L}^{-1}$ in 120 h with a removal efficiency of 79.74% ([Figure 7.2A](#)). Correspondingly, soluble protein and polysaccharide degradation followed first-order kinetic reduction. The degradation rate of polysaccharide was higher than that of protein, indicating that polysaccharide was probably a preferred carbon source compared to protein for power generation, and the final removal efficiency was higher than that of protein (68.34% v.s

48.26%). Notwithstanding, the degradation rate of protein in the MFC reactors was still higher than that in a conventional anaerobic process ($41.27 \text{ mg (L}\cdot\text{d)}^{-1}$ v.s. $36.92 \text{ mg (L}\cdot\text{d)}^{-1}$) [175, 176].

It is believed that HSs are non-biodegradable in a traditional biological system and their degradation could only be carried out with chemical oxidation. For example, Katsumata et al. [177] reported the 10 mg L^{-1} HSs can be completely degraded by H_2O_2 and Fe(II) after 8 hours reaction. In this study, nearly half of the HSs (800 mg L^{-1}) was reduced within a 120 hours cycle (Figure 7.2A). The degradation rate of HSs was calculated as $6.77 \text{ mg (L}\cdot\text{h)}^{-1}$, which were 4.41 times faster than the Photo-Fenton process. The dark brown color of FL was decolored steeply during the operation time and became clear at the end of the cycle.

VFAs were rapidly utilized in the anode of MFC (Figure 7.2B). The degradability and conversion of different VFAs were dependent on the carbon chain length of the VFAs [15]. The higher removal rates were found with the simpler VFA structures. The degradability of the VFAs was in the following order: Acetic acid > Propionic acid > n-Butyric acid > iso-Butyric acid. Within a cycle, 92.5% of acetic acid, 79.3% of propionic acid, 72.6% of n-butyric acid and 36.1% of iso-butyric acid could be converted.

Figure 7.3 (A) DAPI staining of polyphosphate granules in the biomass, (B) Nile red stained PHA present in the biomass from the anode of MFC

It should be noted that the systems also achieved good performance concerning in-situ nutrients separation and recovery (Figure 7.2A). 80% - 90% of ammonium in the FL was recovered in the cathode chamber, which was due to the function of cationic exchange membrane [178]. This enabled the concentration and recovery of ammonium in the cathode. It is interesting to note that around 42 % of phosphorus was also removed in every cycle. It was proved that there was no precipitation in the anode chamber ($\text{pH } 6.7 \pm 0.2$). Instead, poly-P granules and PHAs were found in the biomass discharged in the effluent (Figure 7.3A). The presence of poly-P granules indicated the existence of polyphosphate-accumulating organisms in the reactors, which will be discussed further later in the chapter.

Along with the accumulation of phosphate, microbial accumulation of PHAs was also observed in the biomass (Figure 7.3B). Early studies indicated that ATP from poly-P degradation was the primary source of energy used by polyphosphate-accumulating organisms for substrate uptake, PHAs synthesis and cell maintenance under anaerobic conditions [179]. The observation of PHAs and poly-P evidenced the active metabolism of polyphosphate-accumulating microorganisms in the system. It is suggested the polyphosphate-accumulating organisms removed phosphate and stored it as poly-P in the cell and possibly used it as the energy source for the synthesis of PHAs. However, further research is needed to assess the mechanistic pathway of biological phosphate removal in the MFC.

In a typical cycle, the reactor generated the highest voltage of 0.477 V and an average of 0.388 V (Figure 7.4A). The anode achieved average anode potential of -0.185 V which was close to that from a reactor with acetate as feed (-0.23 V, 1 g L⁻¹ of sodium acetate). The result demonstrated the acetate enriched exoelectrogenic culture can utilize complex organics to generate electrons. An increase of anode potential after 30 hours was observed, and was corresponding to the rapid degradation of VFA (Figure 7.2B) and other organic matters, e.g. polysaccharide, during the first 24 hours (Figure 7.2A).

Figure 7.4 (A) Anode and cathode potential change; (B) LC-OCD chromatogram in UV254-signal as a function of operation time in one cycle

As shown in Figure 7.4B, the detected DOMs were mainly divided into HI DOC, biopolymers (>20 kDa, including HMW PSs, HMW PNs and amino-sugars), HSs building blocks (300-500 Da), LMW acid (<350 Da) and LMW neutrals (<350 Da, including alcohols, aldehydes, ketones and mono-oligosaccharides) in terms of respective properties and molecular weight. The results present the detailed transformation of organic matter within a cycle. The hydrophilic dissolved organic carbon (HI DOC; the sum of building blocks, LMW neutrals, LMW acids, HMW protein and HMW polysaccharide) decreased by 67.63% from 1139.87 mg L⁻¹ in 120 hours (Table 7.2). The removal of hydrophilic organic compounds was consistent with TOC results (Figure 7.2A). However, biopolymers which include high molecular (HWM) PNs and HWM PSs exhibited a decreasing trend at the beginning of the cycle and then increased at the later stage (Figure 7.4B). The degradation of HMW PNs was also only observed during the first 48 hours of power generation (Figure 7.4B). As shown in Table 7.2, the HMW PNs was removed by 46.7% in 48 hours and then gradually increased to the original level at the end of the cycle. Meanwhile, the HMW PSs followed the similar trend (Table 7.2). 94.9 % removal of low molecular weight (LMW) acids was observed. However, a slight increase of LMW acid was also detected at the end of the cycle. It suggested that most organics were consumed in the first 30 hours for power generation and biomass growth, while the hydrolysis and acidogenesis continued in the reactor anode that further released organic matters which may be less

biodegradable (Figure 7.4A). Thus, the power generation was slowed down with the organic matter still available.

Figure 7.5 (A) Linear sweep voltammetry of FL and VFA fed reactors; (B) Cyclic voltammetry tests carried out on the FL and VFA fed reactors.

Previous studies reported that VFAs used as the organic substrate and electron donor could produce stable power in MFC [14, 15]. To investigate the influence of other compounds in FL on power generation, the synthetic FL with the same COD concentration was prepared using VFA. Interestingly, the maximum power density obtained with the FL as the feed was 5.5 times than that with VFA feed although VFA should be the preferred carbon source (Figure 7.5A). Concomitant with power density, the open circuit potential with FL feed was also higher. The anode in FL fed reactor exhibited distinct oxidation peak at 0.75 V (Figure 7.5B), whereas anode in VFA fed reactor did not show electrochemical signal. The results suggest that certain organic compounds in the FL significantly contributed to the power generation. It has been reported that the HA in the FL can alter the propensity of reactor performance and play an essential role in electron transfer efficiency as electron mediator [174, 180]. Given the high concentration of HA in the FL fed reactors, it is likely that HA may enhance the electron transfer and the overall power generation performance.

Table 7.2 Quantification of dissolved organic matters (mg L⁻¹)

Operation time	HI DOC	HMW PSs	HMW PNs	Bio polymers	Building blocks	LMW neutrals	LMW acids
Feed	1139.87	76.16	114.23	190.39	422.35	178.9	549.56
24 h	508.78	79.15	89.26	168.41	318.28	99.66	153.6
48 h	535.14	60.93	60.93	121.86	301.41	80.61	109.14
72 h	378.38	76.44	62.54	138.98	293.95	53.93	71.24
96 h	568.92	105	79.21	184.21	275.13	83.13	9.75
120 h	368.92	100.13	112.91	213.04	218.01	40.83	28.00

7.4.2 Long term performance of the system

Figure 7.6 The potential profile of anode and cathode of FL as feed in MFC (phase A, B and C)

The potential profile of anode and cathode during the 300 days operation can be found in Figure 7.6. Phases A represented the acclimation period of the system when the system for the first time received the fermented liquid or the period after the biomass withdrawal. Phases B represented the system performance at the stable stage. Phase C showed system deterioration by the overgrowth of biomass. From the potential profile, the whole operation period can be separated into 2 stages, where stage 1 (first phases

A, B, C) experienced acclimation, stabilization and deterioration, and stage 2 (second, third and fourth phases A, B) having acclimation and stabilization phases.

During Phase A, anode potential was relatively low (- 0.27 V) while high overall potential of 0.537 V was observed due to the change of feed or biomass amount. Generally, Phase A lasted for two cycles, and then the reactor entered the stable power generation Phase B, which produced 0.424 – 0.477 V voltage. It is noteworthy that the performance in Phase B was not as good as Phase A in terms of organic matters removal as well as power generation. This is likely due to the microbial community shifts which will be discussed later. Phase B generally sustained for 10 to 12 cycles before the voltage decreased (Figure 7.6).

In stage I, it was observed that a large amount of biomass was accumulated in the anode as well as in the effluent. Although the biomass would be discharged together with the effluent, the discharge rate seemed to be slower than the growth rate. During entire Phase C, the system was unable to resume its performance as Phase A or B. At the end of Phase C, 20 % of the biomass was withdrawn manually from the anode, and the performance was immediately resumed in the stage 2 Phase A (Figure 7.6). After 2 cycles of operation, the system was stabilized in Phase B. In stage II, biomass was withdrawn regularly at the end of each Phase B as soon as the power generation was reduced. This confirms that amount of biomass should be controlled and regularly removed from the system to ensure the stable system performance.

The removal efficiencies of organic compounds coincided with the power generation performance. Table 7.3 displays the main organic matter removal efficiencies during the operation. Average TOC removal efficiency was the highest in Phases A (70%), followed by Phases B and then C. Protein, polysaccharides, HSs, VFAs had similar degradation trends as TOC. Phosphate removal, on the other hand, showed better performance at the latter phase. As chemical phosphate removal was not observed, it was hypothesized that the biological P removal occurred in the system. Hence, the system would need to take some time to build up the P-removal microbial

community. Ammonium removal was relatively consistent other than Phase C where the potential was low.

Conventional anaerobic sludge digestion produces biogas from the organic matters in sludge. The produced biogas cannot be in-situ utilized and required to convert to other forms of energy source, e.g. heat and electricity. The conversion process may have substantial energy loss, and it has been estimated that only 30% – 35% of the energy in methane can be converted to electricity [60]. Marti et al. [61] reported a digestion system with a specific biogas production rate of $0.94 \pm 0.27 \text{ L (g TVS}_{\text{rem}})^{-1}$ that contained $64 \pm 1\%$ of methane. The system had COD removal efficiency of $60 \pm 9\%$ from $40650 \text{ mg COD L}^{-1}$. The 135 L digester was operated with a solid retention time of 20 days. Based on the heat value of methane (35.8 MJ m^{-3}), the total energy conversion from methane to electricity was estimated to be $55.72 \text{ J (g COD}\cdot\text{d)}^{-1}$. Based on the performance of the MFC in this study, the system fed with FL was able to produce 167.4 C electrons and an average of 0.388 V voltage for 120 hours (Figure 7.4A), which was $224.4 \text{ J (g COD}\cdot\text{d)}^{-1}$ equivalent electricity. Hence, compared with the conventional anaerobic digestion, the energy efficiency of MFC was 19.82 times higher. The thermophilic anaerobic digestion can improve the methane production. With 55°C thermal alkaline condition, Chen et al. [1] reported the methane yield reached $165.80 \text{ mL (g VSS)}^{-1}$. The energy conversion from methane to electricity could be $1403 \text{ J (g COD}\cdot\text{d)}^{-1}$, which was much higher than normal anaerobic process (based on 35% conversion rate). However, thermophilic anaerobic digestion requires high energy input to achieve sufficient turnover. The thermal condition roughly consumed 126 kJ L^{-1} energy to maintain the high temperature (From 25°C to 55°C). Within a 5-day cycle (phase B), MFC converted almost 45.02 % carbon source to electricity (based on anodic coulombic efficiency) while a 6-day anaerobic digestion process with in-situ pretreatment only convert 20.3% of sCOD to methane (35% of methane converts to electricity) [1, 62]. Compared with these studies, the conversion of organics to electricity of MFC was 6.63 times more efficient than the thermophilic anaerobic process.

Table 7.3 Average removal efficiency of nutrients and some key organic matters in different phases

Phase	Removal efficiency (%)							
	TOC	Phosphate	Ammonium	Acetic	Propionic	HSs	PNs	PSs
A	70.10 ± 6.87	49.00 ± 0.55	85.28 ± 8.21	89.24 ± 3.05	57.15 ± 23.28	60.46 ± 4.31	55.56 ± 12.15	72.51 ± 12.87
B	48.60 ± 17.19	57.71 ± 6.67	87.36 ± 4.93	78.95 ± 19.46	49.50 ± 16.14	27.55 ± 40.77	40.17 ± 17.26	62.12 ± 15.23
C	21.20 ± 3.33	61.56 ± 9.48	69.21 ± 3.62	60.49 ± 8.44	8.12 ± 10.64	24.31 ± 4.02	6.69 ± 2.24	14.17 ± 9.85

7.4.3 Microbial community and diversity

Figure 7.7 Distribution of bacterial level at phylum (A) and heat map of genera (B). Taxa represented occurrence at >1% frequency in at least one sample under different operation cycles (1, 2, 14, 30 and 45)

Figure 7.7 describes the microbial community in different cycles. At the phylum level, *proteobacteria* were the most abundant (84%), followed by *bacteroidetes* (8%) and *firmicutes* (0.8%) in the first cycle (Figure 7.7A). It has been reported that *proteobacteria* play an important role in anaerobic hydrolysis and provide highly effective electrogenesis [181, 182]. It was noted *proteobacteria* was reduced from 83% to 34% due to the change of feed from acetate acid to FL (Figure 7.7A). In contrast, *bacteroidetes* and *firmicutes* were increased to 27% and 22%, respectively. The results suggest although *proteobacteria* decreased along the operation time, it still occupied the major phyla which contributed to power generation (Figure 7.6). *Firmicutes* is well known to produce proteases, cellulases, lipases, and other extracellular enzymes that are closely involved in organic compounds degradation [183]. *Bacteroidetes* was always notable in various sewage treatment process for metabolizing a variety of complex carbohydrates [184]. The long-term operation with FL as feed improved the abundance of the population for organic compounds degradation. Such degradation may continue in the system even the power generation was slowed down (Figure 7.6, Table 7.3).

The presence of *Alphaproteobacteria* was reported to be beneficial for the degradation of carbohydrate and electrons generation [185, 186]. After the first cycle, *Alphaproteobacteria* decreased from the high abundance of 56% to 16% and remained stable, due to the adaptation of electrogenesis to the FL feed. Nevertheless, *Bacteroidia* increased from 7% to 27% indicating the improvement of microbial degradation ability for protein and polysaccharide [187].

At the genus level, *Rhodopseudomonas* and *Geobacter*, as exoelectrogenic bacteria, displayed a high abundance of 42% and 19% in the first cycle and sharply dropped to 7% and 1% respectively once the feed was changed (Figure 7.7B). However, other exoelectrogenic bacteria such as *Desulfovibrio* was not affected by the change of feed and stayed with the 2% abundance. The ammonia-oxidizing bacterium *Nitrosomonas* was found on the anode electrode with 5% abundance. Meantime,

anaerobic denitrification species *Alcaligenes* was found to be 8% in the anode. The presence of above two types of nitrogen converting bacteria explains the ammonium loss from the anode chamber due to nitrification and denitrification. But most of the ammonium was lost to cathode chamber across through the membrane [108].

Notably, *Rhodocyclaceae* included *unidentified_Rhodocyclaceae* (0.2%), *thauera* (0.2%), *azovibrio* (0.4%) and *Dechlorobacter* (0.002%) were also found in the anode. These species have been reported as the highly efficient phosphate remover [40, 41]. Even though *Rhodocyclaceae* was not the dominant population, its function for phosphate removal and polyphosphate accumulation was observed (Figure 7.2A and Figure 7.3). *Aquamicrobium*, which is a chemoheterotrophic microorganism, was the subdominant population in this system. Organic carbon and nitrogen may be used as the carbon source and nitrogen source for their growth and reproduction, as such they may involve in organic matter and nitrogen removal as well [134].

The microbial community results suggest that the exoelectrogenic microbes remained as the dominant community and played an important role for extracting and transferring electrons from organic substrates in FL. Meanwhile, a large number of functional microorganisms that favoring for ammonium and phosphate removal was also enriched with the FL as feed. At this stage, it was hypothesized that phosphorus stored in the biomass can be released and recovered in an off-line set-up since the biomass was withdrawn from the system regularly.

7.5 Conclusions

This study, for the first time, reported an integrated system for energy and nutrients recovery from the pretreated waste sludge. Long-term performance of the system, major dissolved organic matter transformation and microbial community change were closely monitored. MFC with FL feed can achieve a maximum voltage of 0.477 V and power density of 8.07 W m⁻³. Both high and low molecular weight protein and polysaccharides, and HSs were converted and contributed to power generation. Certain unknown

compounds in FL could enhance the power generation by 5.5 times than VFA feed. A total of 90.59% ammonium was removed with the majority being done in the cathode. Phosphorus was removed and stored in the biomass as a form of poly-P. Exoelectrogenic was the dominant community while *Aquamicrobium*, *Nitrosomonas*, *Achromobacter* and *Rhodocyclaceae* were also enriched in the anode with the FL as feed.

Chapter 8 Conclusions and recommendations

In this chapter, major findings of this thesis are described in Section 8.1. In Section 8.2, the contribution of this thesis and future work are described.

8.1 Overall conclusions

This thesis presents development of BES for PPCPs removal and resources recovery from WW and sludge. SMX as a widely used antibiotic was degraded in the anode of a MFC and its presence induced the development of electroactive biofilm (Chapter 4). In order to improve the treatment efficiency of antibiotics in BES process, a MEC was constructed with a novel copper foam electrode in the cathode for rapid CAP removal (Chapter 5). Even though BES can achieve high removal of antibiotics, the external energy input was still necessary. The self-driven system was then conducted to monitor the performance of nutrients recovery and antibiotic removal without external energy consumption (Chapter 6). Finally, the real fermented liquor of WAS was used in the anode of MFC to demonstrate the feasibility in practice for the application of BES (Chapter 7). The major findings and achievements of this thesis are listed as follows:

(1) The presence of SMX enhanced the power generation as well as extended the duration of energy production. The power density was improved by 18.09% with the presence of SMX. Cyclic voltammetry exhibited multiple redox peaks. There were three main degradation pathways in the anode of MFC, which were corresponding to three main exoelectrogenic cultures. Within these electrogenes, SMX could degraded to a simple product, aniline.

(2) The copper foam was identified as the best cathode for CAP degradation. The electrochemical degradation of CAP on Cu electrode followed pseudo-first-order kinetics. The degradation mechanism was proposed based on cyclic voltammetry tests, and it was deduced that electrons generated from anode microorganism and the hydroxyl radicals generated in the cathode played an important role in reducing CAP.

(3) 99.8% and 98.8% removal efficiency of Ag(I) and CAP were obtained in the integrated MFC-MEC system under optimal conditions, which were close to the model predicted results. Pure silver was recovered in MFC. (4) Fermented liquor can be used

to replace acetate in anode of MFC. The system can achieve a maximum voltage of 0.477 V and power density of 8.07 W m^{-3} , which is comparable to a system fed with acetate acid. Protein and polysaccharides, and HSs were converted and contributed to power generation. Certain unknown compounds in FL could enhance the power generation by 5.5 times than VFA feed. A total of 90.59% ammonium was removed with the majority being in the cathode. Phosphorus was removed and stored in the biomass as a form of poly-P.

8.2 Recommendation for future research

SMX was demonstrated as an agonist of EABFs in chapter 4. In co-sub MFC, the energy output increased from 33.84 W m^{-3} to 39.96 W m^{-3} upon the addition of SMX, indicating the interference of SMX with the MFC did not inhibit overall MFC functionality. Although SMX applied in this study did not have an inhibitory effect on EABFs, it is not clear if the long-term exposure would inhibit the microbial community. Further research is required to look at the metabolic responses of the EABFs in the presence of SMX.

In chapter 5 and chapter 6, CAP was successfully degraded in the MEC with copper foam electrode. Meanwhile, the self-driven system achieved the simultaneously CAP removal and silver recovery without any energy consumption. The optimized condition of the self-driven system was successfully identified. To establish a larger-scale sustainable system, more efforts have to be made to improve the performance and resilience of this integrated system. Firstly, silver reduction was at rather fast rate resulting in the fluctuation of the circuit current and further affected on the CAP degradation. Therefore, efforts should be made to increase the long-term stability of this MEC-MFC coupled system. For instance, Ag (I) concentration in the MFC cathode can be maintained constantly through a continuous feeding operation. To improve the CAP degradation, reactor designs [188, 189], novel electrodes [190] and membranes [191] can be explored. Secondly, more factors should be considered to optimize the system, such as, ionic strength, pH and distance between electrodes [192]. Finally, the

integrated system will not be limited for silver and CAP removal only. Some other metals such as Cu(II), Cr(VI) can also be considered in the MFC cathode and used to power the MEC for more types of recalcitrant organic compounds removal.

Results in chapter 7 demonstrated the feasibility of using FL for energy generation. A future study shall consider to release and concentrate the phosphorus in-situ by forcing the biomass releasing the phosphorus back to the solution via shock conditions. The lowest concentration level of phosphorus that the system can achieve should be also investigated. Ammonium in the cathode can be recovered using air stripping method. The role of the organic matters that can potentially improve the power generation will be investigated further. Finally, employing FL in the anode of a self-driven system should be investigate in the future work.

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