UTS UNIVERSITY OF TECHNOLOGY SYDNEY

SPECIFIC INTEGRATED BIOCHAR – MICROBIAL FUEL CELL BIOREACTOR FOR REMOVING ANTIBIOTICS FROM SWINE WASTEWATER

by Dongle Cheng

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

under the supervision of Prof. Huu Hao Ngo

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CERTIFICATION OF ORIGINAL AUTHORSHIP

I, Dongle Cheng declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Civil and Environmental Engineering/Faculty of Engineering and Information Technology at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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LIST OF ABBREVIATIONS

Symbol	Description
A^2O	Anaerobic-anoxic-oxic process
AD	Anaerobic digestion
AFMBR	Anaerobic fluidized membrane bioreactor
AnMBRs	Anaerobic membrane bioreactors
A/O	Anaerobic/oxic process
AOB	Ammonia-oxidizing bacteria
ARB	Antibiotic resistant bacteria
ARGs	Antibiotic resistant genes
AS	Activated sludge
ASBR	Anaerobic sequencing batch reactor
BAF	Biological aerated filter
BC	Biochar
BES	Bioelectrochemical systems
BET	Brunauer-Emmett-Teller
BF-MBR	Biofilm MBR
CAP	Chloramphenicol
CEM	Cation-exchange membrane
COD	Chemical oxygen demand
CWs	Constructed wetlands
CSTR	Continuously stirred tank reactor
CTC	Chlortetracycline
DC	Doxycycline
DI water	Deionized water
DIF	Difloxacin
FAO	Food and Agriculture Organization
ECDC	European Centre for Disease Prevention and Control
EDS	Energy dispersive spectrometer
ENR	Enrofloxacin
EPS	Extracellular polymeric substances
ESI+	Electrospray positive ion mode

FTIR	Fourier transform infrared spectrometer
GAC	Granular activated carbon
HRT	Hydraulic retention time
HSF	Horizontal subsurface flow
HSSF-CWs	Horizontal subsurface flow constructed wetlands
MBRs	Membrane bioreactors
MLSS	Mixed liquor suspended solids
MRM	Multiple reaction monitoring
OC	Open-circuit mode
OTC	Oxytetracycline
PAC	Powder activated carbon
PFO	Pseudo-first-order
PSO	Pseudo-second-order
SBR	Sequencing batch reactor
SDZ	Sulfadiazine
SEM	Scanning electron microscopy
SF	Free water surface
SRT	Solids retention time
SF-CWs	Free water surface constructed wetlands
SMs	Sulfonamide antibiotics
SMX	Sulfamethoxazole
SMZ	Sulfamethazine
TC	Tetracycline
TCs	Tetracycline antibiotics
UASB	Up-flow anaerobic sludge blanket
UF	Ultrafiltration
US CDC	United State Centre for Disease Control and Prevention
VFAs	Volatile fatty acids
VSSF-CWs	Vertical subsurface flow constructed wetlands

LIST OF SYMBOLS

Symbol	Description
$C_6H_{12}O_6$	Glucose
$CaCl_2 \cdot 2H_2O$	Calcium chloride
CO_2	Carbon dioxide
CO	Carbon monoxide
$CuSO_4 \cdot 5H_2O$	Cupric sulphate
e^-	Electron
$FeCl_3$	Ferric chloride anhydrous
H^+	Proton
H_2	Hydrogen
H_2O	Water
H_2SO_4	Sulphuric acid
H_3PO_4	Phosphoric acid
K	Potassium
K_2CO_3	Potassium carbonate
K_2O	Potassium oxide
KOH	Potassium hydroxide
KH_2PO_4	Potassium dihydrogen phosphate
$MgSO_4 \cdot 7H_2O$	Magnesium sulphate
N_2	Nitrogen gas
NaN_3	Sodium azide
$NaHCO_3$	Sodium bicarbonate
$NaOH$	Sodium hydroxide
NH_3	Free ammonia
NH_3-N	Ammonia nitrogen
NH_4^+	Ionized ammonia
NH_4^+-N	Ammonium nitrogen
NH_4Cl	Ammonium chloride
NO^{2-}	Nitrite
NO^{3-}	Nitrate
O_2	Oxygen gas

OH^-	Hydroxyl
PO_4^{3-}-P	Hydrogen phosphate phosphorus
R	Resistor
$ZnSO_4 \cdot 7H_2O$	Zinc sulphate

Ph.D. DISSERTATION ABSTRACT

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Abstract

Swine wastewater is an important source of antibiotics in the environment due to their large-scale application in swine industry. High levels of antibiotics in swine wastewater have become an increasing global concern considering their potential risks to the environment, human and animal health. The integration of biochar and microbial fuel cell (MFC) is a promising technology for the treatment of swine wastewater containing antibiotics and producing electricity simultaneously. The aim of this study is to investigate the potential of a specific integrated biochar-MFC system to treat swine wastewater containing antibiotics. In this scenario, it is necessary to identify the removal process and mechanism of antibiotics in the anaerobic sludge that used in the anode chamber of MFC. Through a series of batch experiments, the results indicated that the removal of tetracycline antibiotics (TCs) in the anaerobic sludge contributed to the

biosorption of sludge, while biodegradation was responsible for the removal of sulfonamide antibiotics (SMs). The adsorption data of TCs in anaerobic sludge fitted well with the pseudo-second order kinetic and the Freundlich isotherm modes, which suggested a heterogeneous chemisorption process. Cometabolism was the main mechanism for the biodegradation of SMs and the process fitted well with the first-order kinetic model. Microbial activity in the anaerobic sludge might be curtailed due to the presence of high concentrations of SMs.

The performance of a double-chamber MFC for treating swine wastewater with the addition of different concentrations of SMs was investigated under the anode self-circulation operating condition of MFC. It is observed that chemical oxygen demand (COD) could be effectively removed (>95%) and almost not affected by the presence of SMs in MFC. A stable output of voltage was also observed. The removal efficiency of sulfamethoxazole (SMX), sulfadiazine (SDZ), and sulfamethazine (SMZ) in the MFC was in the range of 99.46% to 99.53%, 13.39% to 66. 91% and 32.84% to 67.21%, respectively, which were higher than those in a traditional anaerobic reactor with 97.45% - 98.89% for SMX, 11.96% -31.24% for SDZ and 23.85% - 33.49% for SMZ. The biodegradation process of SMs in MFC was fitted to the first-order kinetic model. Hence, MFC revealed strong resistance to antibiotic toxicity and high potential for the treatment of swine wastewater containing antibiotics.

For industrial application of the MFC in the treatment of swine wastewater containing antibiotics, the MFC was conducted in continuous operating modes under different conditions. Voltage can also be successfully generated during the continual operation with the maximum value of ~550 mv. Effective removal of COD can be achieved in both single continuous (>80%) and sequential anode-cathode (> 90%) operating modes. Nutrients can also be removed in the cathode chamber of the MFC with the maximum removal efficiency of 66.62% for NH_4^+-N and 32.1% for PO_4^{3-}-P. The removal efficiency of SMs under the sequential anode-cathode operating mode of MFC was around 49.35% - 59.37 % for SMX, 16.75% - 19.45% for SMZ and 13.98% - 16.31% for SDZ, respectively. The inhibition of SMs to pollutants' remove in both chambers of MFC was observed after SMs exposure, suggesting that SMs exert toxic effects on the microorganisms. Moreover, a positive correlation was found between the higher NH_4^+-N concentration used in this study and the removal efficiency of SMs in the cathode chamber. Results suggest that it is feasible to use the continuous anode-cathode MFC to treat swine wastewater with antibiotics, while the removal efficiency of antibiotics required to be

further improved.

The addition of biochar into the MFC is a promising method for enhancing the removal of antibiotics in continuous flow MFC. Biochar adsorption is an effective method for the removal of antibiotics from wastewater with advantages of low cost, easy production and environmentally friendly. A new pomelo peel derived biochar was developed in this study. The biochar activated by KOH displayed a large surface area (2457.37 m^2/g) and total pore volume (1.14 cm^3/g). SMs are favorable absorbed onto the heterogeneous surfaces of biochar thorough pore-filling and π-π electron donor–acceptor (EDA) interaction. The biochar's addition to a certain concentration (500 mg/L) could enhance the removal efficiency of SMX, SDZ and SMZ to 82.44% - 88.15%, 53.40% - 77.53% and 61.12% - 80.68%, respectively. Moreover, the electricity production and COD removal were increased by increasing the concentration of biochar. The improved performance of MFC could be due to the role of porous biochar as an adsorbent and biocarrier of the growth of microorganisms.

Keywords: Swine wastewater; Antibiotics; Adsorption; Biodegradation; Microbial fuel cell; Electricity generation, Organic removal; Nutrients removal.

CHAPTER 1

Introduction

1.1 Research background

With the global growth of pork consumption, conventional small pig farms are expanding rapidly into intensive large pig farms, resulting in more and more swine wastewater being discharged from pig farms. It is reported that more than 460 million tons of swine wastewater were generated in 2011 in China (Liu et al., 2016). Since the early 1950s, a variety of antibiotics have been used in livestock farms for disease control and growth promotion (Sarmah et al., 2006). In the U.S., approximately 88% of growing pigs receive antibiotics in their feed to prevent disease and promote growth, in 2011 the Food and Drug Administration reported that about 29.9 million pounds of antibiotics were used on farm animals (Leavey-Roback et al., 2016; Wang & Wang, 2016). It is reported that the consumption of antibiotics could increase by 67% globally and nearly double in Brazil, Russia, India, China, and South Africa, between 2010 and 2030 (Van Boeckel et al., 2015). However, antibiotics are poorly absorbed by pigs, and most of them are not completely metabolized; about 70% - 90% are excreted through faeces and urine in unchanged forms or as metabolites (Massé et al., 2014). As reported elsewhere, the normalized daily excretion mass of antibiotics from a pig was estimated to be 18.2 mg/day (Zhou et al., 2011). Figure 1.1 shows the pathway of antibiotics from swine farms to the environment. Thus, the swine wastewater is a significant source of antibiotics in the environment. Tetracycline and sulfonamide antibiotics are the most common antibiotics used in swine production worldwide, with the concentrations up to 685.60 and 324.4 μg/L, respectively (Kim et al., 2013).

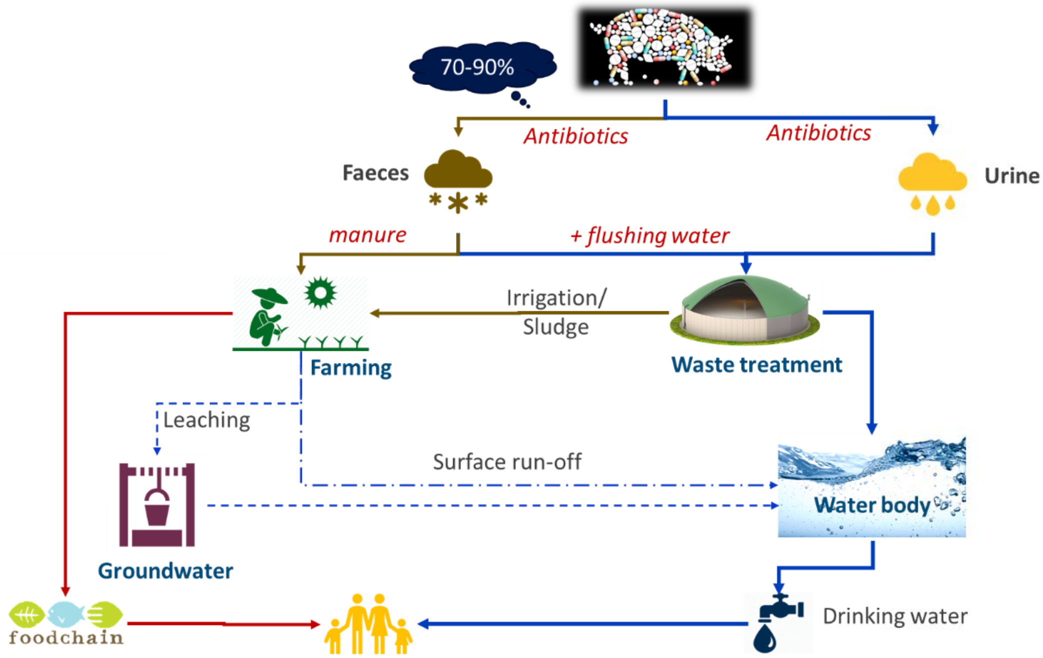

Figure 1.1 Pathway of antibiotics from swine farms to the environment

The presence of these antibiotics in the environment can cause serious risk to human health and eco-environmental security (Singh et al., 2019). Previous reports indicated that antibiotics had toxic effects on the microbial structure, growth, respiration and enzyme activity of aquatic microorganisms, including proteobacteria, cyanobacteria, algae, daphnia and fish (Brandt et al., 2015; Välitalo et al., 2017). The toxicity of antibiotics can be affected by their concentrations, exposure time, aquatic species and the co-occurrence of other antibiotics and/or other contaminants (Grenni et al., 2018). As concluded by previous researches, the antibiotics mixture could arise much stronger toxicological risk to the aquatic organisms than individual compounds (Du et al., 2017; Magdaleno et al., 2015; Välitalo et al., 2017; Yan et al., 2013). Moreover, long-term exposure to low doses of antibiotics in the environments exerts a selective pressure on autochthonous bacterial communities, which not only poses a threat on organisms, but also contributes to the development of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) (Martínez, 2008; Yan et al., 2013). ARB and ARGs are regarded as emerging pollutants, and their presence in the water environment has become an increasing global concern (Zhang et al., 2009). ARGs encoding resistance to a broad range of antibiotics have been detected in the water environment, especially in the water impacted by swine wastes (Anthony A et al., 2018; Sapkota et al., 2007; Tao et al., 2010). ARGs in surface water and soils (fertilized or irrigated by swine wastewater) can leach into groundwater

(Heuer et al., 2011; Sapkota et al., 2007). ARGs can transfer among different bacteria through horizontal gene transfer. Eventually, ARGs in the environment can easily transfer to both human and animal pathogens, creating a severe health risk to both human and animals by greatly limiting the antibiotics used to treat the infectious diseases (Ma et al., 2018). Such antibiotic resistance is a huge threat to human and animals when common infectious diseases were untreatable. As reported by the United State Centre for Disease Control and Prevention (US CDC), about 2 million people infected by antibiotic resistant-bacteria annually, resulting in at least 23000 death per year. In Europe, the death number caused by antibiotic resistance was up to 25,000 each year according to the reports by the European Centre for Disease Prevention and Control (ECDC). If antibiotic-resistant infections are not tackled, 10 million people could die every year worldwide by 2050 (O'Neill, 2014).

Therefore, the proper treatment of swine wastewater containing antibiotics is significant to protect the environment and human health. Currently, biological treatments are the most widely used technologies to treat swine wastewater, including aerobic and anaerobic processes (Liu et al., 2009). As the world's resource and energy crisis continues to intensify, it is a new trend to transform the operation of wastewater treatment plants into a sustainable model, such as low energy consumption, energy and resources recovery from wastewater. Anaerobic biological treatment is an advisable option for treating high-strength wastewater containing antibiotics in comparison with aerobic treatment. The former treatment method has certain advantages, for example, biogas production, less waste sludge production and lower energy costs (Angelidaki et al., 2003; Chen et al., 2011; Cheng et al., 2018b). The biogas generated during anaerobic digestion could serve as an attractive source of renewable energy to replace fossil fuel, while the digestate can serve as a fertilizer on farmland. However, biodegradation of antibiotics in conventional anaerobic digestion processes - even other modern high-rate anaerobic reactors like up-flow anaerobic sludge blanket (UASB) and anaerobic sequencing batch reactor (ASBR) is limited (Cheng et al., 2018b). Anaerobic membrane bioreactors (AnMBRs) are a one of the potential methods for the treatment of wastewater containing antibiotics owing to their advantages over conventional anaerobic processes, which include a high degradation capacity of anaerobic microorganisms, longer SRT, and better effluent qualities (Cheng et al., 2018b; Ozgun et al., 2013). Previous reports have indicated that AnMBRs are promising technologies for degrading common organic pollutants and emerging antibiotics in wastewater under optimal conditions (Chen et al., 2018; Xiao et al., 2017).

Nonetheless, the widespread application of AnMBRs in wastewater treatment is still restricted by membrane fouling problems. Fouling of the membrane decreases permeate flux and in fact the membrane's lifespan, and this leads to higher operating costs in regards to energy requirements in order to reduce the fouling and membrane replacement (Lin et al., 2011a; Meng et al., 2009). As reported by Pretel et al. (2014), 85% - 90% of the energy consumption in AnMBRs was related to the filtration and membrane fouling control processes. Additionally, high levels of nutrients (ammonium and phosphorous) in swine wastewater could not be removed during anaerobic processes.

Bioelectrochemical systems (BES), for example microbial fuel cells (MFCs) and microbial electrolysis cells (MECs), represent emerging technologies for wastewater treatment with recovery of the inherent energy as electricity (MFCs) or hydrogen (MECs) (Du et al., 2007; Escapa et al., 2014). MFCs utilize the presence of electrochemically-active bacteria as catalysts to convert soluble organic matter in the wastewater into useful electrical energy (Ma et al., 2016). Moreover, the degradation of refractory organic matter under anaerobic conditions could be enhanced in MFCs (Zhang et al., 2017c). Meanwhile, based on previous reports, nutrients in wastewater could be potentially removed and/or recovered from MFCs (Ye et al., 2019b). Hence, the use of MFCs provides an environmentally friendly and promising method for swine wastewater containing antibiotics.

1.2 Research motivations and scope

Although the removal of antibiotics in MFC has been studied by researchers earlier, most of previous studies only pay attention to the removal of individual antibiotic in MFC, while different antibiotics are usually present in wastewater simultaneously. It is still unclear about the removal of multiple antibiotics in MFCs and the influence of mixed antibiotics on the performance of MFCs. Furthermore, the current removal of antibiotics by MFCs was mainly conducted in batch mode, which is not effective and realistic in practical applications in the future. Moreover, less attention was paid to the feasibility of applying MFC processes to the removal of antibiotics from swine wastewater. Hence, this study mainly focus on the removal efficiency and degradation kinetics of mixed antibiotics in swine wastewater through a double-chamber MFC, and their removal in the continuous flow operation of MFC. The effect of mixed antibiotics and relative high ammonium concentration in swine wastewater on the performance of the MFC was also investigated in this research. Meanwhile, the potential recovery of nutrients from swine wastewater by the MFC was also explored. Furthermore, a new pomelo peel derived biochar was developed and integrated with MFC bioreactor to stimulate the removal of antibiotics from swine wastewater. The aim of this research is therefore to develop a specific integrated biochar-MFC system for swine wastewater treatment.

The specific objectives of this research are as follows:

1) To exhibit a comprehensive review of current studies on bioprocesses for the removal of antibiotics from swine wastewater;
2) To explore the removal mechanism of antibiotics during anaerobic treatment processes;
3) To investigate the feasibility of microbial fuel cell for treating swine wastewater with antibiotics;
4) To evaluate the performance of continuous flow MFC for removing antibiotics from swine wastewater toward its potential for practical application; and
5) To assess the performance of a specific integrated biochar - microbial fuel cell bioreactor for removing antibiotics from swine wastewater under continuous mode.

1.3. Research significance

This research can provide an effective and environmentally friendly technology to treat swine wastewater containing antibiotics with nutrients recovery and electricity production simultaneously in an integrated biochar - microbial fuel cell bioreactor. The feasibility study of MFC for removing antibiotics from swine wastewater indicated its high effectiveness for antibiotics' removal in comparison with the conventional anaerobic bioreactor. To achieve future full-scale application of MFCs, the treatment of swine wastewater containing antibiotics in a continuous flow MFC was evaluated. Furthermore, a new pomelo peel derived biochar was developed in this study to combine with MFC bioreactor to improve the removal efficiency of antibiotics in swine wastewater. Therefore, the information provided in this study is significant for evaluating the potential of MFC processes in large-scale swine wastewater treatment in future.

1.4 Organization of the thesis

The thesis includes eight chapters, and the main contributions of each chapter are as displayed in Fig. 1.2.

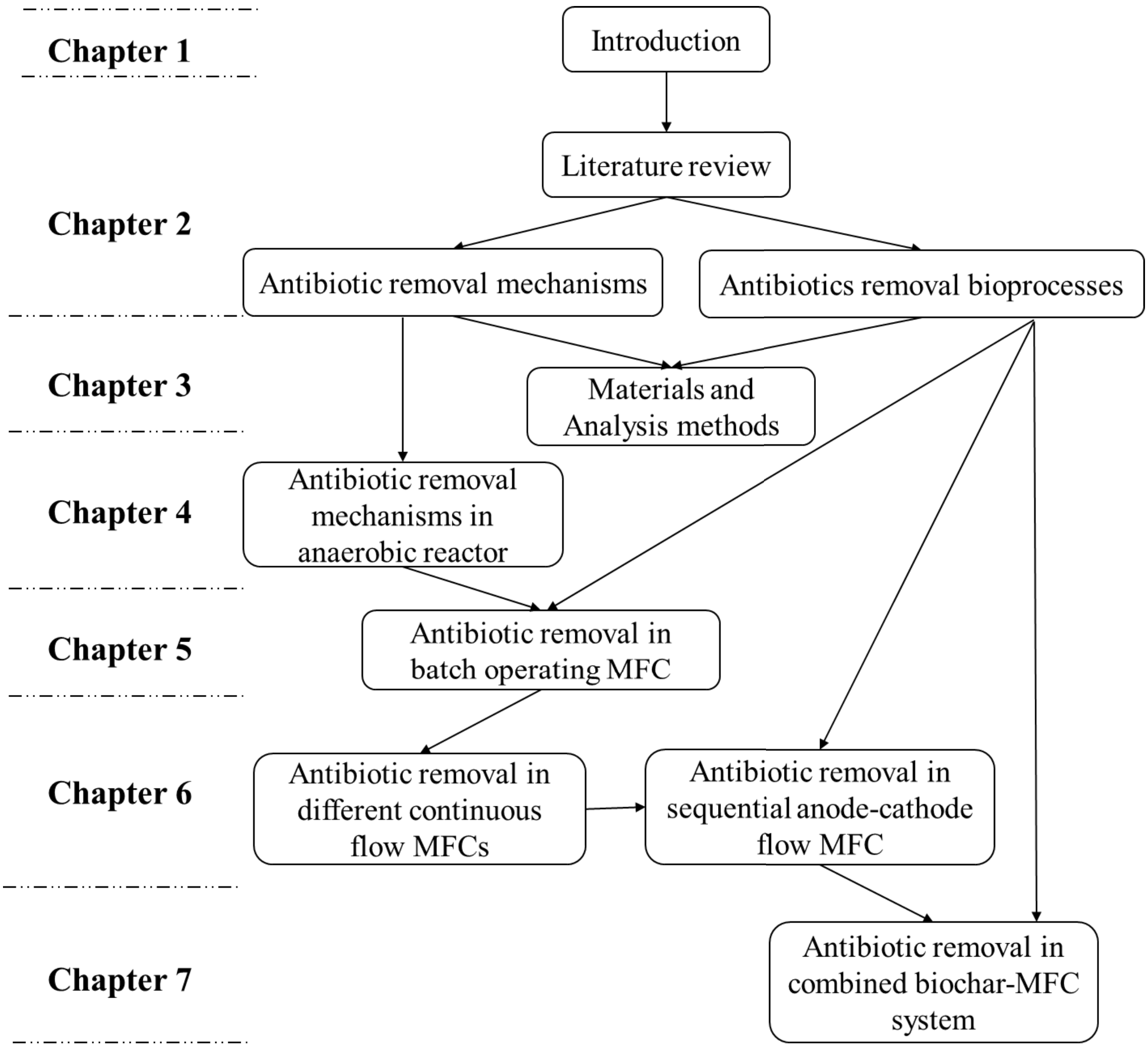

Figure 1.2 Main structure of this research

Chapter 1 generally describes the significance of antibiotics removal from swine wastewater and challenges of current treatment technologies to control swine wastewater containing antibiotics. The research motivations, scope and significance are also presented in this chapter.

Chapter 2 reviews general biological processes and removal mechanisms for removing antibiotics from swine wastewater, including conventional activated sludge, anaerobic digestion, aerobic and anaerobic membrane bioreactors, constructed wetlands and bioelectrochemical systems. Moreover, the comparison of these technologies for antibiotics removal was also given in this chapter. Finally, the promising technology and possible optimization methods for improving antibiotics' removal are provided.

Chapter 3 provides the detailed materials and methods used in the present study. It mainly includes swine wastewater composition, biochar preparation, experimental setup and operation conditions, as well as analytical methods.

Chapter 4 explores the removal mechanism of antibiotics during anaerobic treatment processes. This chapter investigates the fate of tetracycline and sulfornamide antibiotics in anaerobic sludge reactor. Additionally, the adsorption process and mechanism of tetracycline antibiotics and the biodegradation process of sulfonamide antibiotics in anaerobic bioreactor is presented in this chapter.

Chapter 5 evaluates the degradation of different concentration of mixed sulfonamide antibiotics (SMs) in a double chamber MFC. Meanwhile, the impact of these antibiotics on power generation and COD removal efficiency of MFC is also reported.

Chapter 6 indicates the removal efficiency of SMs in a continuous flow MFC under different operating modes. The performance of the MFC is also assessed in terms of electricity generation, COD removal and nutrients removal.

Chapter 7 reports the combination of biochar with MFC bioreactor for removing antibiotics from swine wastewater. This chapter mainly includes the new biochar preparation, characteristics of the biochar, biochar's capacity for adsorption of antibiotics, removal of SMs in MFC with the dosage of biochar, and performance of MFC with biochar.

Chapter 8 summarizes the works and major results of studies in this thesis, conclusions, as well as future research challenges and directions.

CHAPTER 2

Literature review

2.1 Introduction

Researches on different ways to remove antibiotics from wastewater before final release them into the environment has been carried out by scientists worldwide, especially in recent times. Advanced oxidation technology, like chlorine, ultraviolet light and ozone processes have been developed and revealed their effectiveness in removing antibiotics from swine wastewater (Ben et al., 2009; Ben et al., 2011; Qiang et al., 2006). However, such processes not only required large amounts of energy, but also produced some by-products, which can cause secondary pollution to the environment. By contrast, biological treatments are much more popular to treat swine wastewater, because they are inexpensive and simple to operate, there is no secondary pollution and therefore ecologically clean (Liu et al., 2009).

Different bioprocesses have been investigated for the treatment of tetracycline and sulfornamide antibiotics in wastewater. Biosorption and biodegradation are believed to be the main mechanism for removing antibiotics from swine wastewater by biological processes (Laurent et al., 2010). The removal via volatilization mechanism is negligible considering the low Henry's law constant (k_H) (< 10^{-6} mol /(m^3·Pa) of target antibiotics (listed in Table 2.1) (Hamid & Eskicioglu, 2012; Oliveira et al., 2016a). Removal by photo-degradation can also be ignored because of the high suspended solid concentration in swine wastewater, which blocks the penetration of sunlight in the top layer (Cheng et al., 2018b). Their removal in bioprocesses is affected by physicochemical properties of antibiotics, as well as operational parameters of wastewater, such as biomass concentration, sludge retention time, hydraulic retention time, temperature and pH (Luo et al., 2014b; Tiwari et al., 2017; Wang & Wang, 2016).

Table 2.1 Key physical-chemical properties of target antibiotics

Class	Core structure	Compounds	pKa	Solubility in water (mg/l)	Log K_{ow}	Henry's law constant (atm m^3 mol^{-1})
TCs	Amphoteric molecules having multiple ionized groups,	Tetracycline (TC)	3.3; 7.7; 9.70	231	-1.47	4.66×10^{-24}
		Oxytetracycline (OTC)	3.3; 7.3;	313	-1.501	3.971×10^{-21}

	such as hydroxyl, amino and ketone	Chlortetracycline (CTC)	9.10 3.3; 7.4; 9.30	1000	-0.325	1.7×10^{-23}
		Doxycycline (DC)	3.5; 7.7; 9.50	630	-0.54	4.66×10^{-24}
SMs	Amphoteric molecules characterized by sulfonyl and amine group at different pH	Sulfamethazine (SMZ)	2.07; 7.65	2846	0.19	1.3×10^{-14}
		Sulfamethoxazole (SMX)	1.85; 5.60	3942	0.89	6.4×10^{-13}
		Sulfadiazine (SDZ)	1.57; 6.5	77	0.76	1.6×10^{-13}

In this chapter, biological processes for treating swine wastewater containing antibiotics are critically and comprehensively reviewed. The biological processes were roughly classified into activated sludge (AS), anaerobic digestion (AD), constructed wetlands (CWs), membrane bioreactors (MBRs), MBR-based hybrid processes and bioelectrochemical systems (BES), with a discussion of their removal mechanisms, removal efficiency, as well as influencing factors. Finally, the performance of different bioprocesses for removing antibiotics from swine wastewater was compared, and discover better approaches for treating such toxicants from swine wastewater.

Major parts of Chapter 2 have been published as journal articles in ERA A-rated journal:

Cheng, D.L., Ngo, H.H., Guo, W.S., Liu, Y.W., Zhou, J.L., Chang, S.W., Nguyen, D.D., Bui, X.T. and Zhang, X.B., 2018. Bioprocessing for elimination antibiotics and hormones from swine wastewater. Science of the Total Environment, 621, pp.1664-1682.

Cheng, D., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Liu, Y., Wei, Q. and Wei, D., 2020. A critical review on antibiotics and hormones in swine wastewater: Water pollution problems and control approaches. Journal of hazardous materials, 387, p.121682.

2.2 Removal mechanisms of antibiotics during bioprocessing

2.2.1 Removal by sorption

Biosorption is a physico-chemical and metabolically-independent process that happens between organic/inorganic pollutants and biosorbents (Park et al., 2010). Biosorption mechanisms mainly include absorption, adsorption, ion exchange, surface complexation and precipitation (Fomina & Gadd, 2014). Therefore, the biosorption removal of antibiotics from wastewater highly depends on their physical-chemical properties, such as charge, solubility, hydrophobicity and chemical structures. The value of octanol–water partition coefficients (K_{OW}) is usually used by previous studies to characterize the hydrophobicity of compounds and their sorption tendency to the solid phase (Carballa et al., 2004; Yang et al., 2011a). As reviewed by (Luo et al., 2014a), the values of log K_{OW} < 2.5, between 2.5 and 4, and > 4 correspond to the low, medium and high sorption potentials of compounds, respectively.

Electrostatic interaction is another mechanism to explain the biosorption of organic compounds onto solid phase (Ahmed et al., 2015). Antibiotic compounds can exist in positive, neutral, and negative forms according to the pH condition of the solutions and pKa value of compounds, so the solution pH is critical for the electrostatic interaction between antibiotics and charged biosorbents (Cheng et al., 2018b). However, the biosorption process can be highly complex due to the different compositions of wastewater and the variety of functional groups in biomass (Fomina & Gadd, 2014). Tolls (2001) indicated that a number of hydrophobicity-independent mechanisms, such as cation exchange, cation bridging, surface complexation, and hydrogen bonding, play significant roles in biosorption removal of antibiotics from wastewater. In addition, microorganisms in biological processes can produce extracellular polymeric substances (EPS) composed of polysaccharide and protein. The EPS can facilitate the biosorption of micropollutants, due to the presence of diverse functional groups, such as carboxyl, amine and hydroxyl groups, and hydrophobic regions (Cheng et al., 2018c; Xu et al., 2013).

2.2.2 Removal by biodegradation

Biodegradation is the principal removal mechanism of micropollutants in wastewater. For example, Zheng et al. (2017) demonstrated that more than 60% of 11 veterinary antibiotics in swine wastewater were removed by biodegradation while only 24% were adsorbed by sludge, especially for SMs, whose removal almost by biodegradation (96.2%) in the reactor. According to a recent study by Chen et al. (2017), antibiotics in swine wastewater could be biodegraded under both aerobic and anaerobic conditions, and biodegradation played a more dominant role than sorption. The optimal outcome of biological technologies is to degrade pollutants by microorganism effectively (Tijani et al., 2013). Mechanisms, including metabolic and co-metabolic pathways by microorganisms, may contribute to the biodegradation removal of A&H in biological treatment processes (Cheng et al., 2018b; Tran et al., 2013). For example, Müller et al. (2013) indicated that activated sludge communities could utilize SMX as carbon and/or nitrogen source for growth, and the biodegradation was enhanced when a readily degradable energy supply (acetate) was provided which fostered metabolic activity. Other previous research also indicated that antibiotics in wastewater mainly removed by co-metabolic biodegradation, because their concentrations could be too low to serving as a sole carbon and nitrogen source for the growth of microorganisms (Fischer & Majewsky, 2014). Müller et al. (2013) demonstrated that the biodegradation of antibiotics could be enhanced by adding readily degradable carbon sources, since they provided energy for heterotrophic biomass growth and metabolic activity. The biodegradation starts when SMs have fully established sorption equilibrium with the activated sludge, or the microorganisms prefer to utilize readily biodegradable substrates before the antibiotics are degraded (Sahar et al., 2011b; Yang et al., 2012b).

Previous reports about microorganism strains responsible for the degradation of antibioitcs indicated that autotrophic ammonia oxidizers and nitrifying bacteria played a key role in cometabolizing micropollutants, while heterotrophic microbes degraded them via cometabolism and/or metabolism (Chen & Xie, 2018; Cheng et al., 2018b; Silva et al., 2012; Tran et al., 2013).

2.3 Different bioprocesses for removing antibiotics from swine wastewater

2.3.1 Activated sludge (AS) processes

As the most common biological wastewater treatment process, AS treatment can be used to treat sewage, industrial wastewater and agriculture wastewater (Suto et al., 2017; Suzuki et al., 2010). For swine wastewater, which contains high concentrations of organic matter, nutrients and suspended solids, the effluent from conventional activated sludge treatment plants is hard to meet the discharge standard (Joo et al., 2006; Sombatsompop et al., 2011). In recent years, a series of studies have started to focus on the fate and behavior of antibiotics in the AS processes. Table 2.2 summarizes studies examining the removal efficiency of antibiotics in AS processes

Table 2.2 Removal of target antibiotics during conventional aerobic sludge processes

Compounds	Wastewater source	Initial concentrations	Treatment processes	Operation conditions	Removal efficiencies / results	References
SMX	Synthetic wastewater	100 μg/L	Batch reactor	T=25°C, pH =7.0, 48 h of contact in the slurry.	92.1±2.7%	(Yang et al., 2012b)
	Swine wastewater	/	A/O	HRT=72 h for each unit	0%	(Chen et al., 2012)
SMZ	Synthetic wastewater	5 mg/L	SBR	SRT=5 and 25 d, HRT=3 h, pH= 7.0 T=30°C	45%-80%	(Huang et al., 2012b)
	Swine wastewater	/	A/O	HRT=72 h for each unit	29.6%	(Chen et al., 2012)
	Swine wastewater	100, 500 and 3000 μg/L	SBR	pH = 8.7 ± 0.2, T = 20°C, MLSS≈8000 mg/L, and SCOD≈500 mg/L	The biological activity of activated sludge was completely inhibited by the presence of SMZ.	(Ben et al., 2014)

SD	Swine wastewater	98.9 μg/L	A/O	HRT=72 h for each unit	0%	(Chen et al., 2012)
TC	Synthetic wastewater	250 μg/L	SBR	HRT=24h, SRT= 10 d	86.4 ± 8.7%, no biodegradation for TC was observed	(Kim et al., 2005a)
				HRT=7.4 h, SRT= 10 d	85.1 ± 5.4%, no biodegradation for TC was observed	
				HRT=7.4 h, SRT= 3	78.4 ± 7.1%, no biodegradation for TC was observed	
	Synthetic swine wastewater	0-87 μg/L	Lab-scale AS	T= 25 °C, 28 daerobic degradation	-28 and -35%, TC can be classified as a non-biodegradable compound	(Prado et al., 2009a)
	Swine wastewater	41.6 μg/L	A/O	HRT=72 h for each unit	27% - 97%	(Chen et al., 2012)
OTC	Swine wastewater	23.8 μg/L	A/O	HRT=72 h for each unit	94.1% - 100%	(Chen et al., 2012)
CTC	Swine wastewater	13.7 μg/L	A/O	HRT=72 h for each unit	82.8% - 90.2%	(Chen et al., 2012)

Tylosin	Synthetic swine wastewater	0-88 μg/L	Lab-scale AS	T= 25°C, 28 daerobic degradation	4 and -5%, tylosin can be classified as a non-biodegradable compound	(Prado et al., 2009a)

Generally, conventional AS treatment involves two stages: primary treatment (physicochemical) and secondary (biological) treatment; in some cases, tertiary treatment is also included to improve effluent quality and achieve water reuse purpose. Primary stage includes mechanical and flocculation-coagulation processes, and biosorption was regarded as the main removal mechanism for antibiotics in this stage, although some degradation could also occur. Thus, only those substances with higher sorption properties are expected to be eliminated in the primary stage (Luo et al., 2014b). For example, Choi et al. (2008) have shown that coagulation could remove 43% - 94% TCs from synthetic water. The study at two different full-scale swine manure-activated sludge treatment plants also demonstrated the removals of OTC, CTC and DC (71% - 76%, 75% - 80% and 95%) did partly contribute to the flocculation-coagulation process (Montes et al., 2015). For the high water solubility compounds like SMX, the removal rate through the primary treatment stage can be neglected.

By contrast, the secondary activated sludge process is the main stage for the elimination of antibiotics by both biosorption and biodegradation (Li & Zhang, 2010; Yang et al., 2011a; Zhou et al., 2013b). Biosorption onto activated sludge is believed to be the first and most rapid step and more important than the following biodegradation process (Ben et al., 2014; Yang et al., 2012b; Yang et al., 2011b). For example, sulfamethazine (SMZ) showed a rapid and high adsorption capacity in activated sludge an AS process in 6 h, although SMs were reported to be absorbed less on activated sludge (Ben et al., 2014). The high adsorption removal of SMZ in this study is mainly attributed to a large variety of organic materials and nutrients in swine wastewater, so that the acclimated activated sludge could have more carboxylic and phenolic moieties to form hydrogen bonds with the amine groups of SMZ, as well as the higher mixed liquor suspended solids (MLSS) (8000 mg/L) and longer solid retention time (SRT) (30 d). Thus, the biosorption process of antibiotics is influenced by MLSS and SRT of the wastewater.

According to the research by Chen et al. (2012) and Batt, Kim & Aga (2007), under long contact time of antibiotics with activated sludge, biosorption was found to be the principal removal mechanism of TCs in AS processes, and the effluent water from this wastewater treatment system might pose risks to the aquatic environment in the vicinity of the swine farms. In addition, the conventional AS process did not effectively contribute to the removal ARGs from wastewater, it has been reported as a hotspot for the release of ARGs into the environment (Hong et al., 2013; Rizzo et al., 2013). The proliferation of

ARGs mainly occurs in AS process, which potentially creates suitable conditions to microorganisms for selecting and spreading ARGs (Gao et al., 2015).

Prolonging SRT and hydraulic retention time (HRT) of AS processes can enhance the removal efficiency of antibiotics both through biosorption and biodegradation. Huang et al. (2012) reported that SMZ removal efficiency increased from 45% to 80% as SRT was increased from 5 to 20 d, and longer HRT could provide a longer period for microbes to acclimatize to SMZ. The increase of SRT could not only influence the biota, through enriching the slow growing bacteria and providing a more diverse bio-consortium, but also affect the physical nature of the floc particles, which have exopolymer coatings comprised largely of polysaccharide and proteins. Obviously, it would have an important effect on their affinity as sorbents for the adsorbent compounds (Johnson et al., 2006). Additionally, the removal efficiency of antibiotics was affected by the changes in temperature. According to the review article by Cirja et al. (2008), relatively high temperatures like those in summer season (17°C - 30°C) are positive for removing antibiotics during conventional activated sludge processes. It is evident that temperature can influence not only microbial activity, but also the adsorption equilibrium of pollutants in activated sludge. Zhou et al. (2013) demonstrated that through the AS treatment, removal percentages of SMs ranged from 83.3% - 94.8% in May of South China, but from 58.8% - 73.8% in November of that district.

Although the conventional AS process is widely used for wastewater treatment, and can achieve high organic removal efficiency, the treatment system is not sufficient for removing persistent antibiotics (Ben et al., 2011; Onesios et al., 2009). In order to remove these refractory micropollutants the optimum operating conditions, like long HRT and SPT, must be maintained. Typically, the SRT in conventional activated sludge systems is 3 - 8 d but no longer than 15 d. Yet the contact time required for the activated sludge to degrade antibiotics is longer than the HRT provided by conventional activated sludge processes. Therefore, high concentrations of antibiotics can be detected in effluent of conventional wastewater treatment plants and receiving water. As well, under short time contact of such toxicants with activated sludge, the majority of antibiotics can be removed from wastewater by biosorption on activated sludge. In that case, the adsorbed antibiotics will be introduced into the environment if no further treatments are employed to remove them from the sludge.

2.3.2 Anaerobic digestion (AD) processes

From a sustainability perspective, the anaerobic digestion (AD) process is often considered as an alternative method for swine wastewater treatment, and has been widely applied in large-scale animal farms (Cheng & Liu, 2002; Deng et al., 2006; Kim et al., 2012; Lo et al., 1994; Zhang et al., 2011b). In the AD process, biogas and digestate are generated through the degradation of organic pollutants by a complex community of anaerobic microorganisms in the absence of oxygen (Chen et al., 2008). Normally, four stages are included in the AD process (shown in Figure 2.1). The AD process has a number of advantages over the AS process for treating swine wastewater in that it needs no extra aeration equipment, less energy investment and generate less quantities of excess sludge. Moreover, the biogas generated during anaerobic digestion could serve as an attractive source of renewable energy to replace fossil fuel, while the digestate can serve as a fertilizer on farmland (Angelidaki et al., 2003; Barber & Stuckey, 1999; Cheng & Liu, 2002; Zhao et al., 2016).

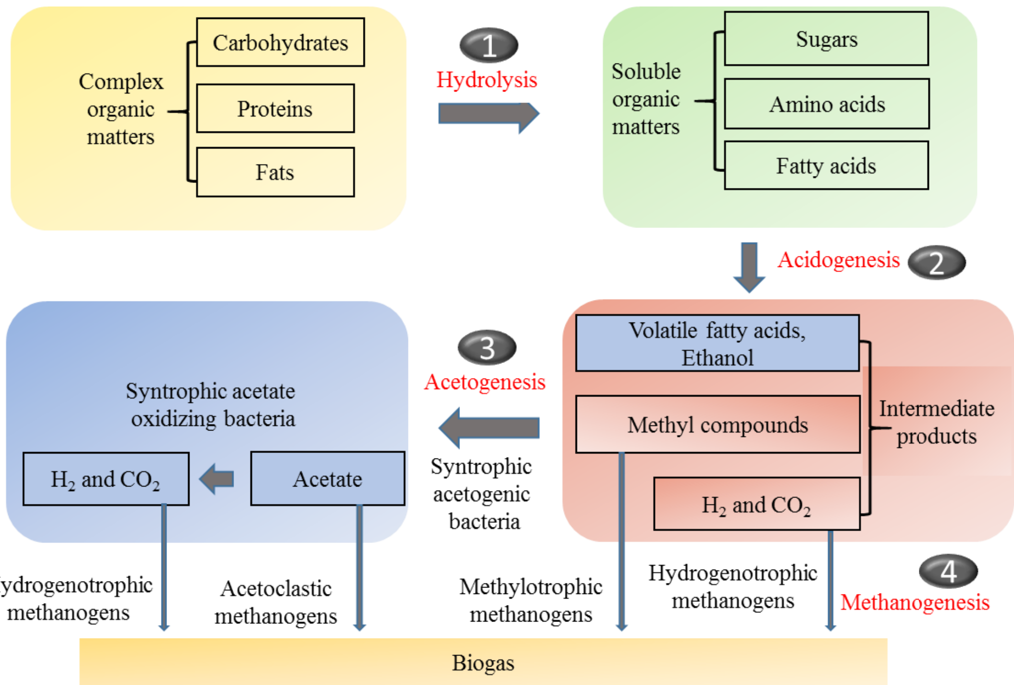

Figure 2.1 Anaerobic digestion process of organic matter

According to the review paper by Sakar et al. (2009), anaerobic treatment processes like up-flow anaerobic sludge blanket (UASB), anaerobic sequencing batch reactor (ASBR), anaerobic baffled reactors, and continuously stirred tank reactor (CSTR) can be successfully utilized for swine waste treatment in both mesophilic and thermophilic conditions. However, high concentrations of suspended solids and ammonia nitrogen in swine wastewater affect the degradation efficiency of the anaerobic reactor, the treated water from anaerobic systems still contains high concentrations of ammonia nitrogen and COD, does not meet the discharge requirement. Thus, normally, post-treatment processes are needed for digested swine wastewater (Guo et al., 2013; Zhou et al., 2016). Furthermore, antibiotics residues in digestates show that the full removal capacity cannot be guaranteed through the AD process. The digestates will introduce a high risk to the environment after their land application (Widyasari Mehta et al., 2016a). In recent years, due to the high application of AD systems in livestock wastewater treatment, researchers began investigating the removal efficiency of antibiotics from wastewater using AD processes see Table 2.3). The AD process can degrade antibiotics to various extents depending on the concentration and class of antibiotics, bioreactor types, operating conditions, etc. (Chen et al., 2012; Furuichi et al., 2006; Stone et al., 2009; Suzuki et al., 2009; Widyasari Mehta et al., 2016b).

Table 2.3 Removal of target antibiotics during conventional anaerobic processes

Compounds	Wastewater source	Initial concentrations	Treatment process	Removal rate / results	Reference
SD	Swine wastewater	98.8 μg/L	AD unit	8.3%	(Chen et al., 2012)
SMX	Swine wastewater	29 ng/L	AD unit	31.0%	(Chen et al., 2012)
	Swine wastewater	41.6 μg/L	AD unit	48.9%	(Chen et al., 2012)
TC	Liquid swine manure	/	AD unit	88.6% - 91.6%	(Tong et al., 2012)
	Synthetic wastewater	250 μg/L	ASBR	14.97% - 67.97%	(Lu et al., 2016)
OTC	Swine wastewater	23.8 μg/L	AD unit	96.7%	(Chen et al., 2012)
CTC	Liquid	/	AD unit	97.7% -	(Tong et al.,

	swine manure			98.2%	2012)
	Liquid swine manure	27 mg/L	AD unit	57%	(Stone et al., 2009)
DC	Liquid swine manure	/	AD unit	61%	(Widyasari Mehta et al., 2016b)
	Liquid swine manure	20 mg/L	Anaerobic lagoon	90%	(Kolz et al., 2005)
Tylosin	Liquid swine manure	1.6 mg/L	ASBR	>99%	(Angenent et al., 2008)
	Pharmaceutical wastewater	0-400 mg/L	UASR	95%	(Chelliapan et al., 2006)
	Pharmaceutical wastewater	600-800 mg/L	UASB	75%	(Chelliapan et al., 2006)

As shown in Table 2.3, the efficiency in removing TCs and tylosin from wastewater using AD processes was better than that of SMs. Chen et al. (2012) investigated the occurrence and elimination of 14 selected antibiotics including TCs and SMs in two swine wastewater treatment systems (AD system and A/O system) in east China. They found that the AD process can significantly degrade higher levels of TCs (48.9% for TC and 96.7% for OTC), while the removal rate of SMs was much lower, only 8.3% and 31% for SD and SMX respectively. They concluded that the efficiency of removing antibiotics with AD technology was significantly poorer than that in anoxic and aerobic biological treatments. Although large amounts of TCs were removed from the water phase, effluent and sludge from such conventional wastewater treatment systems can still pose risks to the aquatic environment in the vicinity of swine farms because of high concentrations of antibiotics remaining in effluent water (Chen et al., 2012).

The removal of TCs from liquid swine manure by the AD process also indicated high efficiency (Stone et al., 2009; Widyasari Mehta et al., 2016b). For example, when spiked OTC of 13.5, 56.9 and 95.0 mg/L appeared in swine manure, the removal efficiency of the AD process was 57.8%, 53.3%, and 67.7% respectively. CTC with initial concentrations of 9.8, 46.1 and 74.0 mg/L could be removed, respectively 82.7%, 91.3% and 89.9% (Álvarez et al., 2010b). Tong et al. (2012) indicated the degradation rates of TC and CTC were 88.6% - 91.6% and 97.7% - 98.2%, respectively, in 45 days anaerobic digestion. However, for removing TC (250 μg/L) from synthetic wastewater by a lab-scale Anaerobic Baffled Reactor (ABR), the removal rates were not as high as that from swine wastewater or liquid swine manure, ranging from 14.97% to 67.97% (Lu et al., 2016). Therefore, the large suspended solids in swine wastewater and slurry in liquid swine manure play a significant role in the adsorption removal of TCs.

The reduction efficiency of ARGs in AD processes needs more attention because of the usual land application of AD products, the copy number of ARGs could be effectively reduced by AD processes (approximately 0.21 - 1.34 logs) (Sui et al., 2016; Wang et al., 2017b). As reported, stable operational and longer SRT of AD could improve the removal of ARGs, as well, microbial community, environmental factors and nutrient level of tested samples played important roles in the abundance of ARGs along the swine waste treatment (Song et al., 2017a; Wang et al., 2016a).

Normally, a warm temperature is required for methane-forming bacteria converting VFA to biogas. As reported elsewhere, mesophilic and thermophilic conditions are more preferable for the removal of antibiotics (Carballa et al., 2007). Varel et al. (2012) indicated that CTC in swine manure can be reduced by 80% and 98% in anaerobic digesters at 38°C and 55°C, but at 22°C it could only remove 7%.

In summary, although anaerobic digestion processes are energy-efficient and environmentally friendly processes compared to conventional activated sludge processes, their treatment efficiency for high-strength and toxicant swine wastewater is limited. Like conventional AS processes, the effluent from such AD treatment plants is difficult to meet the discharge standard, not only for the traditional contaminants, but also for antibiotics. Consequently, more efficient and advanced processes are required for the removal of antibiotics from swine wastewater.

2.3.3 Constructed wetlands (CWs)

Constructed wetlands are implemented widely in rural areas to treat swine wastewaters since they are inexpensive and simple to operate compared to other market wastewater treatment technologies (Garcia-Rodríguez et al., 2014). Wastewater treatment is achieved through an integrated combination of physical, chemical, and biological interactions among vegetation, substrates, soils, microorganisms and water to remove various contaminants and improve the water quality (Wu et al., 2015).

According to the wetland hydrology (free water surface and subsurface systems) and water flow direction, CWs could be classified as: firstly, free water surface constructed wetlands (SF-CWs); secondly, horizontal subsurface flow constructed wetlands (HSSF-CWs); and thirdly, vertical subsurface flow constructed wetlands (VSSF-CWs) (Töre et al., 2012). In these CWs systems, various removal processes can take place: adsorption on the substrates, plant uptake, phytovolatilization, release of exudates, oxygen pumping into the rhizosphere, and microbial degradation (Carvalho et al., 2013). Previous studies have demonstrated that CWs can efficiently remove organics, nutrients, heavy metals, and other components from wastewater (Wu et al., 2015).

In recent years, several studies have attempted to remove antibiotics from swine wastewater by CWs, and their treatment efficiencies mainly depended on various configurations and compounds (Hsieh et al., 2015; Huang et al., 2017; Klomjek, 2016; Liu et al., 2013b; Papaevangelou et al., 2016; Shappell et al., 2007), as shown in Table 2.4. Carvalho et al. (2013) reported that removal efficiencies of TC and enrofloxacin (ENR) were at least 94% and 98%, respectively, using microcosm VSSF-CWs to treat swine wastewater containing 100 μg/L of such antibiotics. For the synthetic swine wastewater containing 40 μg/L of CTC, OTC and SMZ, the removal efficiencies by CWs were 78% - 85%, 91% - 95%, and 68% - 73%, respectively (Liu et al., 2013b). Huang et al. (2017) constructed mesocosm VSSF-CWs to treat swine wastewater with 250 μg/L OTC and difloxacin (DIF). The results revealed that the average mass removal efficiencies of OTC and DIF were higher than 90%.

Table 2.4 Removal of target antibiotics during constructed wetlands processes

Compounds	Wastewater source	Initial concentration	Treatment process	Removal rate / results	Reference
SMZ	Swine wastewater	40 μg/L	Lab-scale zeolite-medium VFCW and volcanic rock-	68% (volcanic rock), 73%	(Liu et al., 2013b)

			medium VFWC	(zeolite)	
	Synthetic swine wastewater	30 μg/L	Pilot-scale SFCW Pilot-scale HSFCW Pilot-scale VSF-LCW Pilot-scale VSF-HCW	40% - 59% 59% 87% 70%	(Liu et al., 2014)
	Swine wastewater	100 μg/L	Microcosm VSSF-CWs	94%	(Carvalho et al., 2013)
TC	Synthetic swine wastewater	30 μg/L	Pilot-scale SFCW Pilot-scale HSFCW Pilot-scale VSF-LCW Pilot-scale VSF-HCW	92% 92% 99% 98%	(Liu et al., 2014)
	Swine wastewater	40 μg/L	Lab-scale zeolite-medium VFCW and volcanic rock-medium VFWC	91% (volcanic rock), 95% (zeolite)	(Liu et al., 2013b)
OTC	Swine wastewater	250 μg/L	Mesocosm VSSF-CWs	>90%	(Huang et al., 2017)
	Livestock wastewater	217.6±166.9 ng/L	Full-scale SFCW	97%	(Hsieh et al., 2015)

For the removal of ARGs, the absolute abundances of sulfonamide resistance genes (*sul*I, *sul*II, *sul*III) and tetracycline resistance genes (*tet*O, *tet*M, *tet*W, *tet*A, *tet*X) were reduced from swine wastewater without significant difference among different types of CWs. Whereas, the relative abundances of most target genes in the CWs showed obvious increases over the treatment period (Huang et al., 2015; Liu et al., 2013a; Liu et al., 2014;

Zhang et al., 2017b). Those abundance of ARGs were developed and reduced in CWs may related to the characteristic of wastewater, operating conditions and configuration of CWs (Huang et al., 2017; Sharma et al., 2016).

Among the above mentioned three types of CWs, VSSF-CWs was the most efficient in removing antibiotics (Huang et al., 2017; Liu et al., 2014). Liu et al. (2014) operated four pilot-scale constructed wetlands (free water surface (SF), horizontal subsurface flow (HSF), vertical subsurface flows with different water level (VSF-L) and (VSF-H)) to assess their ability for removing SMZ (30 μg/L) and TC (30 μg/L) from synthetic swine wastewaters. Their results demonstrated that VSF-L and VSF-H obtained better removal efficiencies for both SMZ (87% and 70%) and TC (99% and 98%) than SF and HSF systems. This was mainly because the oxygen transfer was greater in the VSF-CWs bed than in the others, which enabled VSF-CWs to operate in unsaturated water conditions, creating a predominantly aerobic environment (Matamoros et al., 2008; Zhi & Ji, 2012). In contrast, in HSSF-CWs systems the anaerobic environment prevails because they are continuously fed and the wastewater flows slowly under the surface of the gravel wetland bed. They are also planted with plants those allow them to work in saturated water conditions. As reported earlier, aerobic pathways are generally more efficient for the biodegradation of antibiotics than anaerobic conditions (Garcia-Rodríguez et al., 2014).

In CWs, substrates are essential because they not only provide a basic environment for growth of plants and microbes, but also remove pollutants from wastewater by adsorption and biodegradation (Wu et al., 2015). However, the contribution of substrates can be influenced by their physical and chemical properties and the characteristics of pollutants. For instance, Sarmah et al. (2006) indicated the adsorption of antibiotics onto the surface of substrates was affected by hydrophobic partitioning, van der Waals interaction, electrostatic interaction, ion exchange, and surface complexation. The pH of substrates could also play an important role in their biosorption capacity due to the different ionization states of antibiotics under different pH conditions (Conkle et al., 2010; Hussain & Prasher, 2011). Liu et al. (2014) found red soil (pH=4.24) showed a higher adsorption level than oyster shell (pH=7.67) for the removal of SMZ and TC.

Substrates with high organic matter surface area and porosity could increase the removal efficiency of antibiotics. This phenomenon is attributed to the interaction between the organic groups (carboxyl and phenolic groups), ion exchange, and hydrogen bonding of the substrate matrix with the polar groups of antibiotics (Guan et al., 2017). Different substrates have been studied to compare their removal capacities. Liu et al.

(2013b) indicated that the zeolite-medium system could remove more ciprofloxacin, OTC, and SMZ than the volcanic-medium system. They concluded it was probably because of the different pH values and average pore sizes of the respective media.

Huang et al. (2017) operated both mesocosm and microcosm CWs systems to treat wastewater, and their results showed that brick-based columns had stronger OTC and DIF removal than oyster shell-based columns. It is not only due to the larger porosity and average micropore size of brick, but also because of tetracycline and quinolone compounds having complex iron, and easily being adsorbed to iron oxides and iron oxide-rich soils. Thus, the crystalline iron oxide (Fe_2O_3, 32%) in brick should be another important determinant for its higher antibiotic removal capacity. Based on all of the above, we can see the importance of substrates selection in the CWs system, to date, however, research has only focused on the removal of single classes of antibiotics. Therefore, more studies on the removal of municipal classes of antibiotics should be conducted.

Plants also play a significant role in CWs, although some research indicated that there were no significant differences between the planted and unplanted systems in removing antibiotics (Carvalho et al., 2013). For example, the study by de Carvalho (2012) documented the positive effects of Paustralis-planted beds in CWs for the elimination of veterinary pharmaceuticals from livestock and slaughterhouse industries wastewater. Xian et al. (2010) operated a constructed macrophyte floating bed system with three varieties of Italian ryegrass (Dryan, Tachimasari and Waseyutaka) to compare their removal efficiency of nutrients and veterinary antibiotics from swine wastewater. The finding indicated that Dryan performed better than Tachimasari and Waseyutaka. For Dryan, the removal rates of TN, COD, TP and sulfonamide antimicrobials were 84.0%, 90.4%, 83.4% and 91.8% - 99.5%, respectively.

In the CWs system, plants could uptake, transport and metabolize antibiotics through glycosylation and glutathione pathways to eliminate antibiotics (Carvalho et al., 2013). Liu et al. (2013b) found all three target antibiotics (CTC, OTC, and SMZ) were detected in the wetland plant leaf during the swine wastewater treatment by CWs, indicating that antibiotics can be removed by wetland pants through mass flow (in transpiration stream) and active uptake. Researchers also detected the removal of antibiotics by plants is correlative with Log Kow, water solubility and the compounds' concentration (Boonsaner & Hawker, 2010; Dettenmaier et al., 2008; Liu et al., 2013b). Compounds with LogKow ranging from 0.5 to 3.5 are identified as lipophilic compounds, which could move through the lipid bilayer of plant cell membranes, and they were water soluble enough to travel into the cell fluids of plants (Li et al., 2014). A positive correlation between the antibiotics concentrations and the accumulation levels of antibiotics inside the plants is observed (Liu et al., 2013b). In addition, both the secreting oxygen released from plant roots and other rhizodeposition products (exudates, mucigels, dead cell material, etc.) can stimulate the metabolism activity of microorganisms around the rhizosphere (Bais et al., 2006).

Temperature is also an important influencing factor in CWs systems for the removal of antibiotics. According to previous reports, temperature not only influenced the plant productivity, it also affected the activity of microbial and bacterial communities existing in CWs. This could help achieve their optimal activity and produce a beneficial outcome for the removal of antibiotics at warm temperatures in CWs (Truu et al., 2009; Zhang et al., 2011a). Liu et al. (2014) compared the removal rate of SMZ and TC in different seasonal conditions (13°C in winter and 30°C in summer), and concluded that summer conditions had a significantly positive effect on the removal rate of TC and SMZ in CWs.

In order to improve the quality of effluent from CWs system, several hybrid constructed wetlands (hybrid CWs) were developed. They are the combination of two or more wetlands or the combination of wetlands with other pond systems such as lagoons and facultative ponds in parallel or in series (Li et al., 2014). It is therefore possible to use the specific advantages of each system. For example, employing a VFCW as a first step would make it possible to nitrify the ammonia species, whereas a HFCW afterwards is able to denitrify the previously produced nitrates (Vymazal, 2013).

However, the major problem associated with CWs processes is land requirements; it is inappropriate in some regions, especially where land resources are scarce and population density is high. Moreover, the performance of CWs largely depends on local climate (Scholz & Lee, 2005). The high total suspended solid (TSS) load in swine wastewater can also result in progressive clogging occurring near the inlet. As well, the performance of CWs in the start-up period is relatively poor or unstable due to immature rhizosphere environments (Töre et al., 2012). Secondary pollution of groundwater could occur through the leaching of wetlands.

2.3.4 Membrane bioreactor (MBR)-based processes

Considering the presence of high fractions of refractory organic matter in swine wastewater, the treatment by MBRs are more efficient than conventional AS and AD processes. MBRs are the combination of adsorption, biodegradation and membrane separation processes. In MBRs, a high SRT within compact reactor volumes is achieved because it is possible to uncouple the HRT and SRT in tangential filtration, other than the traditional gravity settling in AS systems (de Cazes et al., 2014a). Compared with conventional processes, MBRs have a number of advantages, such as long SRT, flexibility in operation, compact plant structure, minimal sludge production, high nitrification performance, high biomass diversity, stable and excellent effluent quality suitable for reuse (Yang & Cicek, 2008). Thus, MBRs are considered to be a promising alternative technology for treating highly contaminated swine wastewater. The average removal efficiencies of BOD, COD, NH_4^+-N and turbidity in MBR were more than 90% (Kornboonraksa et al., 2009; Sui et al., 2014).

MBR systems functioned well for treating swine wastewater with antibiotics (see Table 2.5) (Galán et al., 2012; Liu et al., 2016; Prado et al., 2009b; Song et al., 2017b). Song et al. (2017b) indicated 83.8% of 11 typical veterinary antibiotics could be removed from digested swine wastewater in the MBR at the HRT of 5-4 d, although the removal efficiency decreased to 57.0% and 25.5% when HRT was shortened to 3-2 d and 1d, and more than 90% of COD and NH_3-N were removed. On this theme, Prado et al. (2009b) and Zhu et al. (2017) indicated that the impact of antibiotics under a certain concentration in wastewater on the performance of the MBR system was weak. Prado et al. (2009b) showed before and after TC injection the average removal efficiency of COD were 92% and 88%, respectively, and the ammonium removal efficiency stayed at 99%. As well, the removal efficiency of TC in this pilot scale MBR system was 89% as the initial concentration of 2.5 mg/L. Zhu et al. (2017) also stated that 100 μg/L of SMX and TC had no effect on the removal of pollutants in an anoxic/aerobic MBR system, may because microbial communities maintain system stability through gradual acclimation of functional bacteria and development of potential antibiotic resistance species. Such results confirmed the ruggedness and superiority of MBR over conventional bioprocesses.

Table 2.5 Removal of target antibiotics during MBR-based processes

Compounds	Wastewater source	Initial concentrations	Treatment process	Removal rate / results	Reference
SMs	Digested swine wastewater	6.27 μg/L	Lab-scale MBR	87.4%	(Song et al., 2017b)

SMs	Digested swine wastewater	6.27 μg/L	Lab-scale BF-MBR	90.3%	(Song et al., 2017b)
SMX	Municipal wastewater	/	Pilot-scale MBR	80%	(Göbel et al., 2007)
	Synthetic wastewater	/	AnMBR	95% - 98%	(Hu et al., 2016)
TCs	Digested swine wastewater	16.21 μg/L	Lab-scale MBR	86.8%	(Song et al., 2017b)
TC	Digested swine wastewater	/	Submerged MBR	94%	(Liu et al., 2016)
	Digested swine wastewater	3.83 μg/L	Lab-scale MBR	80.2%	(Song et al., 2017b)
	Swine wastewater	2.5 mg/L	Pilot-scale MBR	89%	(Prado et al., 2009b)
OTC	Digested swine wastewater	/	Submerged MBR	93.2%	(Liu et al., 2016)
	Digested swine wastewater	0.67 μg/L	Lab-scale MBR	85.1%	(Song et al., 2017b)
CTC	Digested swine wastewater	/	Submerged MBR	78.6%	(Liu et al., 2016)
	Digested swine wastewater	0.35 μg/L	Lab-scale MBR	45.7%	(Song et al., 2017b)

High removal efficiency in MBRs is attributed to stable biomass concentration and retention of particulate matter. They provide a stable scenario for the growth of a specialized microbial community efficient in the biodegradation of antibiotics. As well as better removal performance, the MBRs exhibited more stable functioning than the conventional treatment system due to faster response to variable influent concentrations and operational perturbation (De Wever et al., 2007). As well, the removal of ARGs in the MBR process was reported significantly higher than that in conventional treatment systems. Compared with the conventional AS treatment process, concentrations of ARGs (*tet*W and *tet*O) in the MBR effluent were observed to be 1 - 3 log less (Munir et al., 2011).

A submerged MBR was used to treat digested swine wastewater, with the variation of HRT. No significant difference was observed for the removal of SMZ and SMX, but the removal rates of TCs were greatly decreased as the HRT was shortened. Specifically, when the HRT was shortened from 8-12 d to 2.7 d, the removal rates of TC, OTC and CTC decreased from 94.0%, 93.2% and 78.6% to 47.6%, 61.8% and 40.5%, respectively. HRT of 3-4 d was reported to be enough for the efficient removal of COD and ammonium from digested swine wastewater, but insufficient for effectively removing antibiotics (Liu et al., 2016). Similar to conventional technologies, the treatment of swine wastewater in a semi-industrial MBR also indicated that longer SRT was beneficial for the removal of antibiotics. The removal of TC was 89% at long SRT (10 d), while it decreased to 78% at a shorter SRT (3 d). Thus, long SRT of the MBR (30 d) did enhance the adsorption of TC on the sludge surface and reduced its toxic impact (Prado et al., 2009b). Long SRT increased the growth of nitrifying bacteria, which led to large amounts of biodegradable micro-pollutants being removed.

Considering low energy input required for anaerobic technologies, anaerobic MBR (AnMBR) systems were gradually established by researchers to study their performance for removing antibiotics in wastewater (Dutta et al., 2014; Hu et al., 2017; Hu et al., 2016; Sanchez Huerta, 2016). Hu et al. (2016) investigated the performance of AnMBRs for treating antibiotics polluted wastewater, and indicated more than 90% of antibiotics were removed mainly through biological processes. Obviously, the degradation capacity of the anaerobic bacteria in AnMBR systems was improved. For example, in comparison with low removal efficiency (31%) of SMX in the conventional AD process, 95% - 98% of SMX was removed by the AnMBR system under optimal conditions after a biomass adaptation period. During the AnMBR process, seven transformation products of SMX were identified and possible degradation pathways were proposed. Moreover, stable biogas composition and methane production were achieved in the experiment (Sanchez

Huerta, 2016).

Sahar et al. (2011b) compared the removal efficiency of several macrolide and sulfonamide antibiotics from sewage by CAS coupled with ultrafiltration (UF) membrane and by a pilot MBR system. Their results showed that removing antibiotics via the MBR system was 15-42% higher than that of the CAS system, but this advantage was reduced to a maximum of 20% when the UF was added to the CAS. Based on the above results, the author hypothesized that the membrane in both systems only contributed to biosorption removal of antibiotics rather than improvement in biodegradation (Sahar et al., 2011b). However, some researchers demonstrated that the membrane in MBRs systems could not only enhance the adsorption of toxicants onto suspended sludge, but also increase its biodegradation ability. This is because the longer SRT and the sludge with higher concentrations of biomass and more effective surface in MBRs permitted sufficient adaption for heterotrophs to degrade persistent pollutants and growth of slow growers such as nitrifiers (Galán et al., 2012; Sahar et al., 2011a). For example, the stubborn TCs in swine wastewater showed an absence of biodegradability since the biodegradation rates were -28% and -35% in activated sludge systems (Prado et al., 2009a). Similarly, Göbel et al. (2007) demonstrated that the removal of SMX in MBR was significantly better than in conventional AS processes (80% and 60%, respectively), and biodegradation played a major role in the removal of SMX, while only a small portion of the removal was caused by biosorption (5% - 10%).

2.3.5 Bioelectrochemical systems (BESs)

Bioelectrochemical systems (BESs) compose of microbial metabolism and electrochemical redox reaction have attracted increased attention in recent years for pollutants removal and energy generation from wastewater simultaneously (Palanisamy et al., 2019). Microbial fuel cell (MFC) is one of the most studied BESs system, which uses organic matter in wastewater for producing bioelectricity. The basic configuration of MFCs can be roughly classified into double- or single- chamber reactors (see Figure 2.2).

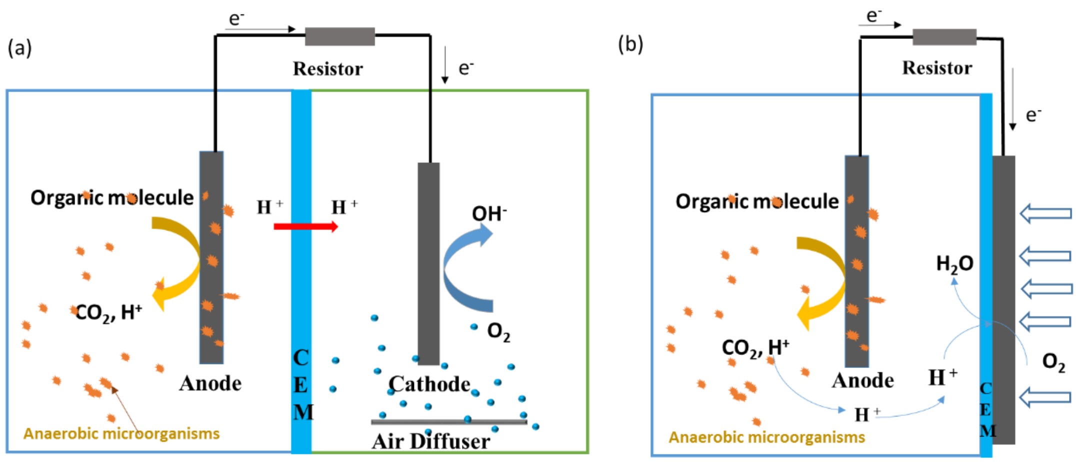

Figure 2.2 Basic configuration of MFCs: (a) Double chamber; (b) Single chamber.

In the anode chamber of MFC, organic compounds in the wastewater are oxidated by anaerobic microorganisms with the release of electrons, protons and carbon dioxide (Al Lawati et al., 2019). The anode is used as an electron acceptor for anaerobic respiration of electrogenic biofilm bacteria. The produced electrons can transfer to the cathode electrode through external electric circuit with applied resister to make the generation of electricity. Meanwhile, cations, including protons, NH_4^+ and other metal cations in wastewater pass through the cation-exchange membrane (CEM) to the cathode (Logan et al., 2006). In the cathode chamber of the double chamber MFC, the electrons are oxidized by oxygen (which externally supplied through the form of air) with the production of hydroxyls: $2H_2O + O_2 + 4e^- \rightarrow 4OH^-$ (Ye et al., 2019a).

Compared with conventional anaerobic reactors, MFCs exhibit high potential for treating wastewater containing antibiotics. Wang et al. (2015b) indicated that the removal efficiency of SMX in a double-chamber MFC was up to 99% after acclimation under batch running mode. The removal efficiency of sulfanilamide also could be reached 90% in 96 h in the anode camber of a double - chamber MFC, with was much higher than the control open-circuit reactor (58%) (Guo et al., 2016). The author also found that the antibiotic in the MFC showed positive effect on electricity generation at the studied concentration range (10 - 30 mg/L). Similar results have been found by Wu et al. (2020), who stated that SMX can be completely degraded in MFC without inhibiting the performance of MFC, meanwhile, the power density was increased by 18% by the addition of SMX (20 mg/L) in the anode chamber of MFC. The removal efficiency of tetracycline antibiotics in MFC was also higher than that achieved in a traditional anaerobic process (Yan et al., 2019). For example, Wang et al. (2017a) indicated that the degradation efficiency of TC in a dual-chamber MFC and a traditional anaerobic reactor was around 79.1% and 14.9%, respectively. The degradation rates of TC in MFCs were 31.6% higher than those in an open circuit control system. The degradation of OTC also could be enhanced in the MFCs (Yan et al., 2018).

In addition, MFCs could be possibly integrated with other technologies for the removal of antibiotics. For instance, Zhang et al. (2016b) indicated that more than 99% of SMX and TC could be removal in a MFC coupled constructed wetland systems with the initial concentration of 800 μg/L. Sun et al. (2019) concluded that the OTC removal efficiency increased by 61% in comparison with that in the open-circuit control in a self-sustained photo-bioelectrochemical fuel cell. The power generation was also increased by adding OTC to the anode chamber of the photo-bioelectrochemical fuel cell. Zhang et al. (2016a) studied the fate of SMX and TC in three-dimensional biofilm-electrode reactors and demonstrated that the removal efficiency of SMX and TC could achieve 88.9% - 93.5% and 89.3% - 95.6%, respectively. The antibiotics in MFC could be degraded to less harmful products compared to the biotoxic intermediate in traditional bioprocesses (Wang et al., 2016b).

Moreover, the MFCs might have lower risk to the production and transfer of ARGs than those found in conventional wastewater treatment plants (Xue et al., 2019). The study by Yan et al. (2018) indicated that the normalized copy numbers of total ARGs (1.7364 copies/cell) and mobile genetic elements (0.0065 copies/cell) in MFCs were significantly lower than those in the control bioreactors. However, there are still limited researches focusing on the fate of ARGs in MFCs, more investigations are required.

The biodegradation of anaerobic microorganisms coupled with electrical stimulation is a key mechanism for the biodegradation of antibiotics in MFCs (Guo et al., 2016). In the anode chamber, antibiotics act as electron donors and carbon sources for the exoelectrogenic microbes and the specific antibiotic degrading bacteria (Yan et al., 2019). Moreover, the transformation of antibiotics through microbial metabolism could be enhanced by the persistent electrical stimulation by providing electrons to the microenvironment (Wang et al., 2016b).

As reviewed by Yan et al. (2019), the effect of the initial concentration of antibiotics on their removal efficiencies in MFCs is rather complicated. The removal efficiency of antibiotics could be inhibited, enhanced or unaffected by increasing their initial concentrations, which need further investigation in the future. Electrode materials are one of the important parameters for the performance of MFCs due to the formation of biofilm on the electrode by microorganism for the transfer of electron (Jiang et al., 2018). However, the study on their effects on the removal of antibiotics is limited. More researches are necessary to investigate the connection between electrodes and microbes and the effect of electrode materials on the removal efficiency of antibiotics. The operating of MFCs under higher temperature might positive for the removal of antibiotics due to higher microbial activity. The research by Zhang et al. (2017a) explored the effect of temperature on the removal of chloramphenicol (CAP) in a MFC and concluded that the removal efficiency of CAP increased from 68.11% to 75.13% by increasing the temperature from 20℃ to 40℃.

2.4 Comparison of different bioprocesses

Table 2.6 compares the removal efficiencies of target antibiotics in different bioprocesses. Conventional treatment processes like AS and AD are widely used to eliminate traditional pollutants (e.g. COD and TN) from swine wastewater (Chen et al., 2012; Zhang et al., 2006). Yet, as shown in Table 2.6, their removal efficiencies for antibiotics are limited compared those in CWs, MBRs and MFCs. Large fluctuations of the removal efficiencies of antibiotics in AS and AD processes were observed according to different operating conditions (e.g. HRT, SRT, pH and temperature). For example, in the optimum operating conditions, like prolonging HRT and SRT in tests, a high removal efficiency (>80%) could be achieved in conventional AS processes (Kim et al., 2005a; Yang et al., 2012b). However, it is obvious that operating costs for per unit volume of wastewater will definitely increase for extending HRT and/or SRT in wastewater treatment plants, besides, unlike in MBRs processes, HRT and SRT cannot be separated completely in conventional AS processes. According to the real conditions, the common values of SRT is not enough for the growth of antibiotics - biodegrading bacterium, meaning that target antibiotics cannot be well biodegraded in the conventional AS process (Ben et al., 2014). It has been confirmed by the removal of TC and tylosin from swine wastewater, that their biodegradation efficiencies were -28% to -35% and 4% to -5%, respectively (Prado et al., 2009a). Thus, biosorption removal plays a significant role in conventional treatment processes, which entails large amounts of antibiotics remaining in the excess sludge. In that case, large amounts of money and labour should be poured into sludge treatment, otherwise, secondary pollution will be leaded after being applied to land.

Table 2.6 Comparison of target antibiotics removal from different bioprocesses

Bioprocesses	Removal rate	Advantages	Disadvantages
AS	SMs: 0-93% TCs: -35% - 100% Tylosin: -5% - 4%	1. Most widely used technology; 2. High organic removal efficiency.	1. Low biodegradability; 2. Mainly through adsorption onto sludge; 3. Secondary pollution; 4. Polishing treatment is needed.
AD	SMs: 8.3% - 31% TCs: 14.97% - 98.2% Tylosin: 75% - 99%	1. Energy investment is low; 2. Less sludge production; 3. Generating biogas.	1. Low biodegradability; 2. High concentrations in effluent.
CWs	SMs:40% - 87% TCs: 90% - 97%	1. Low costs; 2. Simple construction and operation; 3. High performance.	1. Large land requirements; 2. Highly dependent on local climate; 3. Low or unstable performance in the start-up period; 4. Secondary pollution of groundwater.
MBR-based processes	SMs:80% - 87.4% TCs: 45.7% - 94%	1. Flexibility in operation; 2. Compact plant structure; 3. Minimal sludge production; 4. High biodegradability; 5. Low energy requirement and biogas production for AnMBR.	1. Fouling and clogging of membrane; 2. High costs.

MFCs	SMs: 83.3% - 99% TCs: 70% - 99.5%	1. Low energy consumption; 2. Operated at various temperatures; 3. Less sludge generation 4. Electricity production	1. Membrane fouling 2. Still low power output

Conversely, such drawbacks in conventional AS processes can be solved in MBRs processes, in which SRT and HRT can be increased independently (De Cazes et al., 2014b). Therefore, the removal of antibiotics by biodegradation can be largely improved in MBRs. For example, 83.8% of 11 typical veterinary antibiotics could be removed from digested swine wastewater in the MBR and removal through biodegradation was the dominant mechanism (Song et al., 2017b). For most target toxicants, high and stable removal efficiencies (45.7% - 99%) are obtained in MBRs processes, especially in hybrid MBR processes (71.4% - 100%). Although the MBRs can also be influenced by the operating conditions, it is easy for MBRs to situate themselves in an ideal state. However, given that many of the world's economies are now conscious about saving energy and resources, energy dissipation and membrane fouling in MBRs are the biggest challenges, which costs lots of energy and money on aeration, membrane cleaning and replacement.

As an energy-efficient and environmentally friendly technology, AD processes are commonly used for treating wastewater originating from livestock farms. However, they are not efficient for treating high-strength and toxicant swine wastewater. As stated previously, the biodegradable removal of toxicants in anaerobic conditions is less efficient than in aerobic conditions, possibly due to the toxicity of antibiotics. For the hard adsorption removal compounds, SMs, only 8.3% - 31% were removed from swine wastewater in the AD process. The AnMBR process is a good alternative to the conventional AD processes and aerobic MBR process, since relatively low energy consumed and highly improved degradation capacity of the anaerobic bacteria in such a process. In contrast, 95% - 98% of SMX was removed from synthetic wastewater via the AnMBR system under optimal conditions and after the biomass adaptation period.

Nonetheless, the widespread application of AnMBR in wastewater treatment is still restricted by membrane fouling problems. Fouling of the membrane decreases permeate flux and in fact the membrane's lifespan, and this leads to higher operating costs in regards to energy requirements in order to reduce the fouling and membrane replacement (Lin et al., 2011a; Meng et al., 2009). As reported by Pretel et al. (2014), 85% - 90% of the energy consumption in AnMBR was related to the filtration and membrane fouling control processes. In both aerobic membrane bioreactors (MBRs) and AnMBRs, membrane fouling is caused by the undesirable deposition and accumulation of microorganisms, colloids, solutes, and cell debris in the pores and on the surface of the membrane (Guo et al., 2012; Le-Clech et al., 2006; Lin et al., 2013). Although the same membrane module is used in aerobic MBR and AnMBR systems, the latter system usually encounters more severe membrane fouling problems. Not only are the higher biomass concentrations and longer biomass retention times required in the AnMBRs, they work at lower membrane fluxes than the aerobic MBRs as well (Lin et al., 2013). For example, as reported by Di Bella et al. (2007), the membrane foulants in the AnMBRs are more difficult to remove than that in the aerobic compartment because of the different sludge properties. Lin et al. (2011b) found that the cake thickness in a submerged AnMBR was much higher than was reported in the aerobic MBR systems.

Moreover, the presence of antibiotics in AnMBRs could accelerate the membrane fouling rate and shorten the membrane fouling cycle due to the effect of antibiotics on anaerobic sludge and the microbial communities in AnMBRs (Li et al., 2017; Zhu et al., 2018b). For example, Zhu et al. (2018b) indicated that the membrane fouling cycle decreased from 25 days to 8 days with the addition of sulfamethoxazole (SMX) and tetracycline (TC) each at 100 μg/L, and further decreased to 4 days when the concentration of SMX and TC rose to 1000 μg/L in the reactor. As similar results have been confirmed by Li et al. (2017), the membrane fouling cycle was obviously short due to the presence of antibiotics (50 mg/L benzothiazole) in the feed wastewater in an integrated anaerobic fluidized-bed membrane bioreactor (AFMBR). In addition, the membrane fouling layer became denser and more compact as the level of antibiotic concentrations increased (Zhu et al., 2018b). Therefore, higher concentrations of antibiotics in the AnMBRs possibly result in higher operation and maintenance costs.

Compared with the above market technologies, several authors reported that CWs processes are promising treatment technologies for removing antibiotics from swine wastewater because of their low cost, simple operation and high performance in removing conventional and toxic pollutants and pathogens. Choosing suitable substrate, plants, and CWs types is important for the proper functioning of CWs processes. VSSF-CWs systems were regarded as the most efficient systems among three types of CWs. The high removal rate (>70%) of initially large concentrations of antibiotics can be obtained in such systems. Substrates, like red soil, zeolite, and brick were reported as being more suitable for the removal of antibiotics than oyster shell and volcanic rock. However, most research focused only on single classes of antibiotics, so further studies about their function on municipal classes of antibiotics should be conducted. In addition, drawbacks associated with CWs processes, such as large land requirements, high dependence on local climate and secondary pollution to groundwater cannot be neglected. Besides these issues, clogging may also occur near the inlet due to the high total suspended solid (TSS) load in swine wastewater.

MFCs are regarded as an environmentally friendly and promising technology for wastewater treatment. Through the integration of microbial metabolism and continuous electrical stimulation, MFCs showed high potential for the treatment of swine wastewater containing antibiotics. Compared with conventional processes, the enhanced removal efficiencies of antibiotics was achieved in MFCs. Moreover, as reported by previous studies, MFCs have high effectiveness for recovering nutrients from wastewater, which is also significant for swine wastewater treatment (with high levels of nutrients).

2.5 Future perspectives

The risk of residual antibiotics in the environment has generated global concerns and this risk will continue due to the endless use of veterinary medicines on pigs. There are furthermore still no clear guidelines for utilizing veterinary medicines and management of swine wastewater treatment. Governments must establish the guidelines and discharge standards as soon as possible.

In biological treatment processes, sorption and biodegradation simultaneously contributed to the removal of antibiotics from swine wastewater. However, for different classes of antibiotics, the contributions of sorption and biodegradation vary. It is closely related to their own physicochemical characteristics, operating conditions, adopted technologies, etc. Other studies have not clearly demonstrated the ratios of antibiotics removed by sorption and biodegradation. The toxicants removed by sorption still remain in the sludge, and can cause secondary pollution after sludge enter the environment. In order to decrease such secondary pollution, more studies are urgently required to clarify the contribution of sorption and biodegradation, respectively.

In addition, as the most important removal mechanism of toxicants, the specific degradation pathways and intermediates of biodegradation should be fully investigated in the future. As mentioned above, only a small fraction of antibiotics was completely oxidized into water and carbon dioxide. The majority of them were simply transformed into intermediates. Some research has reported that such intermediates are more harmful than their original forms. In order to improve the removal of toxicants from wastewater, the role and function of microorganisms in bioprocesses should also be considered.

Although the biodegradation and detoxification of antibiotics and their intermediates could be enhanced in MFCs, the application of MFCs in swine wastewater containing antibiotics is limited in current research. The removal capacities and mechanisms, as well as the impact of antibiotics, ammonium and various environment factors on the performance of MFCs for treating swine wastewater should be further investigated in the future. Moreover, most of current researches only focus on the removal of individual antibiotics in the batch operating of MFCs. The research on the removal of multiple antibiotics in continuous flow MFCs is quite necessary for their large-scale application. As discussed in section 2.4, the combination of MFCs with other technologies should be a promising method for optimizing the removal of antibiotics in MFCs, which also requires being studied in the future research.

2.6 Conclusion

Swine wastewater has become a major pollution source of antibiotics because of the huge demand for pork and the high extraction rate through swine manure and urine. In biological treatment processes, such micro-pollutants are mainly removed through sorption and biodegradation, and biodegradation is the most important mechanism. The physicochemical characteristics of various antibiotics correlate with their degradation profile. TCs are relatively easily absorbed on activated sludge through electrostatic interactions and hydrophobic interactions. In contrast, SMs were mainly removed by biodegradation because of their low log Kow value (log Kow<2) and less electrostatic interaction with the activated sludge's negatively charged surface. Co-metabolism by microorganisms is the major pathway for the biodegradation of antibiotics. Some microorganism strains have been isolated from sludge for the biodegradation of antibiotics.

Conventional treatment processes are never complete and sorption is the major removal pathway for most antibiotics, which means that large amounts of toxicants remain in the sludge. With particular reference to AD processes, the biodegradability of anaerobic bacteria needs to be improved. Although CWs processes do have several advantages and are more efficient than conventional treatment processes, their limits and drawbacks for wide application must be recognized. MBRs demonstrate much better performance and practicability than other technologies. Conversely, the membrane fouling, energy consumption and cost in conventional MBRs have to be considered. MFCs showed high and stable removal efficiencies of antibiotics in wastewater, which has been considered as a promising technology for treating swine wastewater containing antibiotics. Therefore, more studies on the performance of MFCs for the removal of antibiotics from swine wastewater and the optimization of this system required to be conducted in the future.

CHAPTER 3

Experimental investigation

3.1 Introduction

This chapter consists of materials, the compositions of synthetic swine wastewater, experimental design and setup, as well as analysis methods of samples in the series of experiments (Chapter 4 - 7) during the whole research period. In addition, the raw material, production method, and characterization methods for the specific biochar used in MFCs (in Chapter 7) were presented in this chapter.

3.2 Materials

3.2.1 Synthetic wastewater and anaerobic sludge

Synthetic swine wastewater was prepared and used in the experiment of this research. In this study, glucose ($C_6H_{12}O_6$) was used as an organic carbon source, ammonium chloride (NH_4Cl) and potassium phosphate (KH_2PO_4) which containing nitrogen and phosphorus were used as source of nutrients. The synthetic swine wastewater mainly contained 3000 mg/L of chemical oxygen demand (COD) (provided by glucose), 223 or 446 mg/L of NH_4Cl, 66 or 132 mg/L of KH_2PO_4, as well as trace elements (as shown in Table 3.1). A stock solution was prepared for 5-days use and stored in a refrigerator at 5 ± 0.5 °C, in which the synthetic wastewater was obtained through diluting the stock solution with distilled water (DI water).

Anaerobic sludge employed in this study was collected from the Cronulla wastewater treatment plant in New South Wales, Australia, and acclimated in an upflow anaerobic sludge blanket reactor with synthetic swine wastewater without the addition of antibiotics. The feeding solution was purged with nitrogen gas for 15 min and then adjusted to pH 7.5 ± 0.1 by employing $NaHCO_3$ and H_2SO_4 solutions prior to pumping into the anode chamber.

Table 3.1 Components of the synthetic swine wastewater

Compounds	Chemical formula	Molecular weight (g/mol)	Concentration (mg/L)
Organics and nutrients			
Glucose	$C_6H_{12}O_6$	180.0	2804
Ammonium chloride	NH_4Cl	53.5	223/446
Potassium phosphate	KH_2PO_4	136.1	66/132
Trace nutrients			

Magnesium sulphate	$MgSO_4 \cdot 7H_2O$	246.5	54.3
Calcium chloride	$CaCl_2 \cdot 2H_2O$	147.0	4.0
Zinc sulphate	$ZnSO_4 \cdot 7H_2O$	287.5	4.7
Ferric chloride anhydrous	$FeCl_3$	162.2	7.8
Cupric sulphate	$CuSO_4 \cdot 5H_2O$	249.7	4.2
Yeast extract	-	-	321.4

3.2.2 Targeted antibiotics and organic solvents

Target antibiotics, including tetracycline (TC), oxytetracycline (OTC) chlortetracycline (CTC), sulfamethoxazole (SMX), sulfamethazine (SMZ) and sulfadiazine (SDZ) were purchased from Sigma-Aldrich, Australia. LC-MS grade acetonitrile and methanol used for sample preparation and liquid chromatography analysis were also obtained from Sigma-Aldrich, Australia. Stock solutions of TCs and SMs (1 g/L) were prepared by dissolving each compound in methanol, and stored at −15 °C in a refrigerator before use. The experimental solution was obtained by diluting the stock solution into the required concentrations.

3.3 Experimental setup and operating conditions

3.3.1 Batch experiments

In this study, a series of batch experiments were conducted to investigate removal mechanisms of the selected antibiotics in anaerobic processes in 150 ml glass bottles with non-sterile and sterile sludge (0.15 g sodium azide (NaN_3) was added into each bottle to inhibit the activity of anaerobic microorganisms). The design of this experiment is summarized in Table 3.2. The selected two classes of antibiotics (TCs and SMs) were spiked into the bottle separately with the initial concentrations of 300 μg/L and 100 μg/L, respectively. Following this, the glass bottles were completely sealed with rubber plugs and N_2 was sparged for 2 min in each bottle to displace any oxygen present. The bottles were shaken on a thermostatic rotary shaker at 125 rpm and at room temperature (~25 °C). The MLSS concentration was around 5000 mg/L in the reactor and pH = 7.5 ± 0.1. Based on experimental results derived from the first stage, the experiment on the biosorption and biodegradation mechanisms of antibiotics would be conducted in the following step. Control experiments, TCs and SMs solution without the addition of anaerobic sludge, and anaerobic sludge without TCs and SMs were conducted to avoid their photodegration/adsorption on containers and their residue in the sludge. All experiments were conducted in duplicate. The sample (2 ml) collected from the bottle at each sampling time was centrifuged at a speed of 3500 rpm for 5 min. The supernatant was then filtered through a syringe filter (0.2 μm) before analysis.

Table 3.2. Batch experiment design for behavior of antibiotics in anaerobic sludge

Reactor	Anaerobic sludge	Wastewater	Antibiotics	NaN_3
R1/R1'	+	+	TCs/SMs	-
R2/R2'	+	+	TCs/SMs	+
R3/R3'	-	+	TCs/SMs	+
R4	+	+	-	-

“R (1,2,3)” and “R’(1,2,3)” represented the reactor with the addition of TCs and SMs, respectively. “+” indicated “with”, “-” indicated “without”.

3.3.2 MFC system

A double-chamber MFC made of plexiglass material was employed in this study, and the anode and cathode chamber have the same effective volume of 0.35 L. A cylindrical graphite felt (3 cm in diameter and 6 cm thickness) and a carbon-fiber brush (3 cm diameter and 3 cm length) served as the anode and cathode of MFC, respectively. Two chambers were separated by a cation exchange membrane (CEM) (CMI7000, Membranes International Inc., USA) and connected by a copper wire via a resistor of 1000 Ω. At the start-up period, the anode chamber was inoculated by anaerobic and fed with synthetic swine wastewater without the addition of antibiotics until the COD removal efficiency was up to 90% and the voltage production was stable. In this research, the MFC was operated under self - circulating, single continual, and sequential anode-cathode modes, respectively (see Figure 3.1).

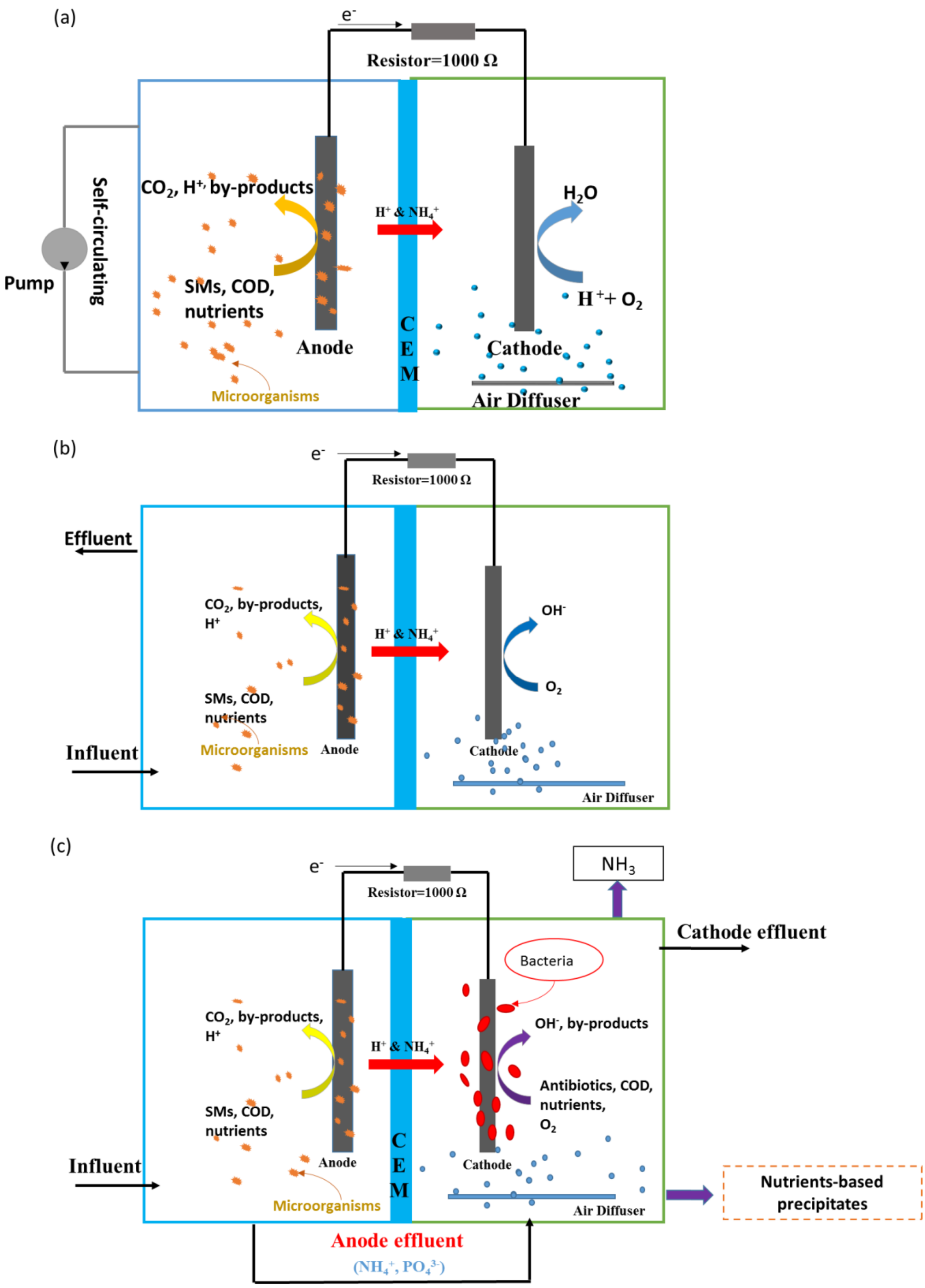

Figure 3.1 The schematic of MFC operating under: (a) Self-circulating; (b) Single continual; and (c) Sequential anode-cathode modes

Under the self-circulating operating mode of MFC, reactors were conducted in closed-circuit mode (MFC) (Figure 3.1 (a)) and open-circuit mode (OC) simultaneously.

The OC disconnects the anode and cathode chamber, which was regarded as the conventional anaerobic reactor. All reactors were conducted in batch running mode at room temperature (around 25°C). Synthetic swine wastewater was pumped into the anode chamber by a peristaltic pump and then self-circulated at a flow rate of 20 mL/min, which was replayed after each running circle (120 h). After the reactor achieved stable COD removal and voltage generation, 100 μg/L, 200 μg/L and 300 μg/L of SMs (SMX, SMZ and SDZ) were added to the synthetic swine wastewater and pumped into the anode chamber in the consecutive operating circles.

In the continual running mode of MFC, the double-chamber MFC was operated under single continual mode (mode 1) (Figure 3.1 (b)) and sequential anode-cathode mode (mode 2) (Figure 3.1 (c)), respectively. In the single continuous operating mode (mode 1), the synthetic swine wastewater continuously flowed through the anode chamber via a peristaltic pump (Model 77202-60, Masterflex, Illinois, United States). Under this operating mode, DI water was supplied in the cathode compartment as catholyte. To further purify effluents of the anode chamber, the sequential anode-cathode mode (mode 2) was conducted. Under this operating method, effluent from the anode chamber served as a continuous influent of the cathode chamber to be further treated under aerobic conditions. Considering the large nutrients content in swine wastewater, the concentrations of NH_4Cl and KH_2PO_4 were increased from 223 to 446 mg/L and 66 to 132 mg/L, respectively, in the later operating mode (mode 3), to study their effect on antibiotics' removal efficiency and MFC performance. The whole experiment was conducted at the HRT of 24 h and at room temperature (~25 °C).

3.3.3 Biochar-MFC system

A. Biochar production and modification

Pomelo peel wastes were washed with distilled water and dried in an oven at 80 °C for 24 h, and following this they were crushed into small pieces prior to use. Prepared pomelo peels were pyrolyzed at 400 °C and 600 °C, respectively, for 2 h with a heating rate of 10 °C min^{-1} in a muffle furnace. The resulting sample was designated as BC-400 and BC-600. The BC-400 was mixed with KOH (1:4), followed by carbonization at 600 °C for 2 h in a muffle furnace with a heating rate of 10°C min^{-1}. Biochar obtained from this activation was designated as BC-KOH, which was washed with 35% HNO_3 for 24 h and washed with distilled water until the pH value of the filtrate reaches 7.0 ± 0.2. Finally, the produced biochar was oven-dried at 80 °C for 24h. The particle sizes of BC-

400, BC-600 and BC-KOH were sieved (<75 μm) for further use.

B. Biochar adsorption experiments

The simultaneous adsorption behavior of SMX, SDZ and SMZ onto BC-KOH were performed through batch experiments. The BC-KOH (100 mg/L) were added to flasks (50 ml) with the SMs concentration of 100 μg/L for each, and samples were collected at different time intervals until the adsorption reaches equilibrium (12h). To investigate the adsorption isotherm, the concentrations of SMs were varied from 50 μg/L to 500 μg/L. After reaching the adsorption equilibrium (56 h), the residual concentrations of SMs were measured. Flasks for all the above experiments were shaken at 120 rpm in a temperature controlled orbital shaker at 25 ℃, and all the solution pH was kept at 7.0 ± 0.5. All experiments were performed in duplicate and the average values were calculated.

C. Biochar – MFC system

The biochar - MFC system was operated under the sequential anode-cathode mode of MFC with the working volume of 350 ml for the anode and cathode chamber, respectively. SMX, SMZ and SDZ (100 μg/L for each) were directly added into the synthetic swine wastewater after the COD removal efficiency was up to 90% or more and the voltage production was stable. During the whole experiment, the mixed liquor suspended solids (MLSS) concentration was around 5000 mg/L and 750 mg/L in the anode and cathode chamber, respectively, the HRT was kept at 24 h under room temperature (around 25 °C). Different concentrations of biochar were added into the anode chamber in phase 1 (100 mg/L), phase 2 (200 mg/L) and phase 3 (500 mg/L), respectively. After each phase (7 days), the addition of SMs into the feeding swine wastewater were stopped for two days. The entire experimental process was operated under the same condition.

3.4 Analytical methods

3.4.1 Antibiotics, organics, nutrients, pH, DO, electricity

Samples collected from the influent/effluent of MFCs and batch experiments were filtered through a syringe filter (0.2 μm) which are made by hydrophilic polytetrafluoroethylene (Merck Millipore, Burlington, USA) to remove fine suspended material and any biomass, and stored in the refrigerator before analysing.

A Phenomenex C18 column (Luna, 3.0 × 100 mm, 3 μm) was used at a constant temperature of 28°C to separate the antibiotics. Water and acetonitrile with 0.1% (V/V) formic acid served as mobile phase A and mobile phase B, respectively. The LC gradient started with 30% of mobile phase B, which was retained for 7 min. Thereafter, the concentration of B increased to 95% and held for an equilibration time of 3 min. It was returned back to 30% over 3 min until the next injection. The flow rate was 0.4 mL/min, and the injection volume was 1 μL. Electrospray positive ion mode (ESI+) was used for the mass spectrometry operation. The multiple reaction monitoring (MRM) mode with two mass transitions was selected for the quantitation. The interface voltage was set at 4.0 kV. The nebulizing gas and heating gas were using a flow rate of 3.0 and 10.0 L/min, respectively. The interface temperature was held at 300 ℃.

The concentrations of COD, NH_4^+-N and PO_4^{3-}-P concentration in the influent and effluent samples were determined based on the Standard Methods, by using the test kit HI93754B-25 (Hanna Instruments Australia, Melbourne, Australia) for COD, 100683 and114848 (Merck Millipore, Burlington, USA) for NH_4^+-N and PO_4^{3-}-P, respectively. A DO meter (OM-51, Horiba, Tokyo, Japan) was employed to check the DO concentration in the cathode chamber (maintained at 6 mg/L of DO during the operation). The cell voltage (U) generated during the experiment periods was detected by a universal digital meter (VC86E, Shenzhen City Station Win Technology Co. Ltd., Shenzhen, China). The solution pH during the experimental period was detected by a pH meter (HI9025, Hanna Instruments, Limena, Italy).

3.4.2 Biochar characterization

Surface morphology and elemental compositions of the prepared biochar were investigated using a scanning electron microscopy (SEM) and an energy dispersive spectrometer (EDS) (Zeiss Evo-SEM). Renishaw inVia Raman spectrometer (Gloucestershire, UK) was used to analyze the Raman spectra of the produced biochar. The functional groups present in the biochar were determined by Fourier transform infrared (FTIR) spectrometer (Miracle-10, Shimadzu) in the 4000 - 400 cm^{-1} range. Brunauer-Emmett-Teller (BET) analyzer (Quantachrome Autosorb IQ, USA) was applied to determine the surface area and pore size distribution of the biochar via adsorption/desorption isotherm of nitrogen at 77 K. Before adsorption measurements were taken, degassing of the sample was conducted under vacuum at 473 K for 6 h.

3.4.3 Data calculation

The amount of antibiotics adsorbed on biochar at time t (q_t, mg/g) and equilibrium (q_e, mg/g) was calculated by Eq. (3.1 - 3.2):

$$q_t = \frac{(C_0 - C_t) \times V}{m} \tag{3.1}$$

$$q_e = \frac{(C_0 - C_e) \times V}{m} \tag{3.2}$$

where C_0, C_t and C_e are the concentrations of antibiotics at initial, at time t and equilibrium (mg/L); V is the volume of TCs solution (L); and m (g) is the amount of biochar used in study.

For this study, the following adsorption kinetic models, i.e. pseudo-first-order (PFO), pseudo-second-order (PSO) (Eq. (3.3 - 3.4) were selected to assess the adsorption mechanisms between the interaction of the biochar and antibiotics. The equations and relevant parameters can be expressed as follows:

$$\mathrm{q}_t = \mathrm{q}_e(1 - \exp(-k_1 t)) \tag{3.3}$$

$$q_t = \frac{k_2 q_e^2 t}{1 + k_2 q_e t} \tag{3.4}$$

where, q_t and q_e (mg/g) are the amount of antibiotics adsorbed at time t (min) and equilibrium; k_1 and k_2 are the rate constants of the pseudo-first-order and pseudo-second-order, respectively.

Two classic adsorption models, Freundlich and Langmuir models, were utilized to fit the adsorption isotherms, which are written as follows (Eq. (3.5 - 3.6):

$$q_e = K_F c_e^{1/n} \tag{3.5}$$

$$q_e = \frac{q_m K_L c_e}{1 + K_L c_L}, R_L = \frac{1}{1 + K_L c_L} \tag{3.6}$$

Where, q_e is the adsorption capacity (mg/g) at equilibrium time; c_e is the equilibrium concentration (mg/L) of TCs in solution; K_F ($mg^{(1-n)}L^n/g$) is Freundlich affinity coefficient indicating adsorption capacity; 1/n presents the adsorption intensity; q_m is the maximum adsorption capacity (mg/g); $c_L$is the lowest initial concentration (mg/L); K_L (L/mg) is the Langmuir sorption coefficient related to the bonding force of adsorption; and R_L is a dimensionless constant separation factor.

CHAPTER 4

Removal process and mechanism of antibiotics during anaerobic treatment of swine wastewater

4.1 Introduction

Conventional small-scale swine husbandry has in recent decades been transformed into an intensive swine industry due to people's increasing demand for meat (Feng et al., 2017). To maintain the swine health and limit disease transmission and ensure that pigs can be kept in a high-density and closed system, veterinary antibiotics are widely used in swine farms to treat and prevent diseases (Sarmah et al., 2006). Moreover, antibiotics are usually used as feed additives to improve the growth rate and efficiency of pigs. However, antibiotics are poorly absorbed by pig guts, and around 70% - 90% of them are excreted via urine and faeces based on the used antibiotics' compounds (Cheng et al., 2018b). Tetracycline antibiotics (TCs) and sulfonamide antibiotics (SMs) are the most widely used antibiotics on swine farms due to their low costs and broad range of activity (Hruska & Franek, 2012; Koike et al., 2007). As reviewed by Cheng et al. (2018b), TCs and SMs have been frequently detected in swine wastewater at concentrations of up to 316.5 μg/L and 685.6 μg/L, respectively. Therefore, swine wastewater is a significant source for the spread of TCs and SMs into the environment.

The increasing presence of antibiotics in the environment could cause adverse outcomes for people's health and ecological safety, which has become a major concern worldwide (Richardson & Ternes, 2005). Reports by previous researchers have stated that antibiotics can affect the composition, growth, respiration and enzyme activity of aquatic and terrestrial microorganisms (Brandt et al., 2015; Välitalo et al., 2017; Zhou et al., 2013a). In addition, long-term exposure of antibiotics will generate antibiotic-resistant bacteria and antibiotic-resistant genes (ARGs), which have been considered as emerging contaminants (Zhang et al., 2009). In the water environment, ARGs can easily transfer to both human and animal pathogens through horizontal gene transfer, creating a severe health risk to humans and animals by greatly limiting the efficacy of antibiotics that have been developed to treat infectious diseases (Ma et al., 2018). For this reason, removing antibiotics from swine wastewater is now critical if the adverse effects of antibiotics and ARGs on the environment and human health are to be mitigated.

The anaerobic treatment process is one of the mostly widely used technologies for high-strength swine wastewater, considering it is characterized by low power consumption and high energy recovery potential (Sakar et al., 2009). Although anaerobic treatment processes have been considered able to remove antibiotics to various extents, based on their type and concentration as well as operating conditions of the process, most prior studies mainly considered the removal of antibiotics under aerobic conditions (Cheng et al., 2018b). Moreover, studies about the removal of antibiotics in anaerobic wastewater treatment processes only focused on their removal efficiency, while information about the removal mechanisms of different classes of antibiotics is still limited. Biosorption and biodegradation have been suggested as the two main mechanisms influencing the removal of antibiotics during biological wastewater treatment processes (Cheng et al., 2018b; Li & Zhang, 2010). Considering the further reuse of the effluent and waste sludge from anaerobic treatment processes of swine wastewater, it is essential to move from merely monitoring the removal efficiencies to understanding the bioadsorption and biodegradation of antibiotics during anaerobic treatment processes. Therefore, the objective of the present study was to: 1) investigate the fate of TCs and SMs during anaerobic treatment processes; and 2) determine the biosorption and biodegradation mechanism of selected antibiotics.

A major part of Chapter 4 has been published as a journal article in ERA A-rated journal:

Cheng, D., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Liu, Y., Shan, X., Nghiem, L.D. and Nguyen, L.N., 2020. Removal process of antibiotics during anaerobic treatment of swine wastewater. Bioresource Technology, 300, p.122707.

4.2 Materials and methods

4.2.1 Materials

The synthetic swine wastewater, anaerobic sludge and target antibiotics used in this study is described in Chapter 3, Section 3.2

4.2.2 Experimental setup and operating conditions

Batch experiments conducted in this study has been given in Chapter 3, Section 3.3.1.

4.2.3 Analytical method

Analytical method is described in Chapter 3, Section 3.4.1.

4.3 Results and discussion

4.3.1 Removal of TCs and SMs in anaerobic sludge reactors

The concentration variation of the selected antibiotics in the anaerobic reactor during the 120 h experimental period is presented in Figure 4.1 (a), which reveals that a similar removal trend and efficiency was found for TCs in the anaerobic reactor with non-sterilized and sterilized sludge. All of TCs can be rapidly and significantly removed in reactors with activated and sterilized sludge. Thus, the major removal route for TCs in the anaerobic reactor was adsorption rather than biodegradation. TCs were adsorbed onto the anaerobic sludge immediately after they made contact with the anaerobic sludge (>90% in the first 30 min), possibly due to the abundance of active sites on the adsorbent's surface. The rapid and strong adsorption of TCs onto solid matter during anaerobic digestion of animal manure or aerobic sludge has been reported by other researchers (Álvarez et al., 2010a). For instance, Huang et al. (2012a), Prado et al. (2009a) and Kim et al. (2005b) found no biodegradation of TCs in the aerobic activated sludge system, and sorption contributed to be the principal removal mechanism. As shown in Figure 4.1 (a) and (c), the removal mechanism and efficiency of TC, CTC and OTC were quite similar in the anaerobic reactor, although they belong to different subclasses of TCs.

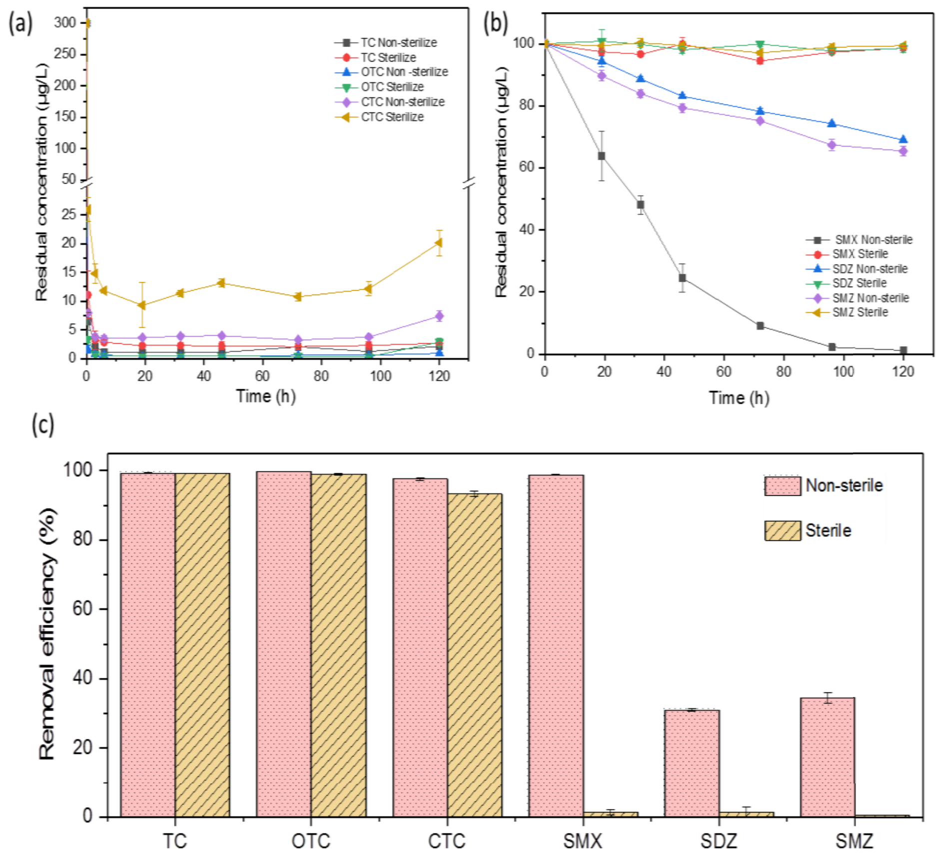

Figure 4.1 The concentration variation of TCs (a) and SMs (b); and their removal efficiency (c) in the reactor with non-sterile and sterile anaerobic sludge.

The concentration of SMs in the reactor with the activated sludge deceased gradually while the change in the concentration was almost negligible in the reactor with the sterilized sludge (Figure 4.1(b)). This outcome reflected the fact that the continual reduction of SMs is attributed to biodegradation by anaerobic microorganisms. Similar results have been found for the biodegradation of SMs in aerobic sludge processes in other studies (Yang et al., 2011a; Yang et al., 2012a). They explained that SMs with low n-octanolewater distribution coefficients (log Kow) have high water solubility and their adsorption onto activated sludge was negligible. According to the pK_{a1} and pK_{a2} values of SMs (1.85, 5.6 for SMX, 2.07, 7.65 for SMZ, 1.57, 6.5 for SDZ, respectively), the predominant species of SMs would be in the form of anion at the study pH of 7.5. Thus, SMs adsorb less due to electrostatic repulsion by the negatively charged surface of the anaerobic sludge (Cheng et al., 2018b; Oberoi et al., 2019). Similar to SMs, TCs also have low log K_{ow} and high-water solubility, so that their adsorption onto the activated sludge was not caused by hydrophobic interactions. Conversely, based on the pK_a values of TCs (3.32, 7.78, 9.58 for TC, 3.22, 7.46, 8.94 for OTC, and 3.33, 7.55, 9.33 for CTC, respectively), they could exist in a neutral form at pH 7.5 that was more amenable to adsorptive removal via electrostatic interactions between the zwitterionic species and negatively charged surface of biological sludge (Oliveira et al., 2019; Wang et al., 2015a).

4.3.2 Adsorption process of TCs onto anaerobic sludge

To investigate the adsorption process of TCs onto anaerobic sludge, a series of batch adsorption experiments were conducted in 150 ml glass bottles with 100 ml sterile sludge to avoid the biodegradation of TCs. The glass bottles were shaken in an orbital shaker at 125 rpm. An experiment for the kinetics study was done by using 300 μg/L of TCs adsorbed onto different amounts of anaerobic sludge (MLSS = 1000, 2000 and 3000 mg/L). The variation in adsorption capacity of anaerobic sludge at different times is presented in Figure 4.2. For isotherm studies, experiments with varying initial concentrations (50, 100, 200, 300, and 500 μg/L) were conducted. Control experiments at the same initial TCs concentration without the addition of sludge were also prepared under the same laboratory conditions and no significant loss was documented. Based on the results of the kinetics experiments, the biosorption of TCs on anaerobic sludge could reach equilibrium within 12 h, the aqueous TCs' concentrations changed very little once adsorption equilibrium had been achieved.

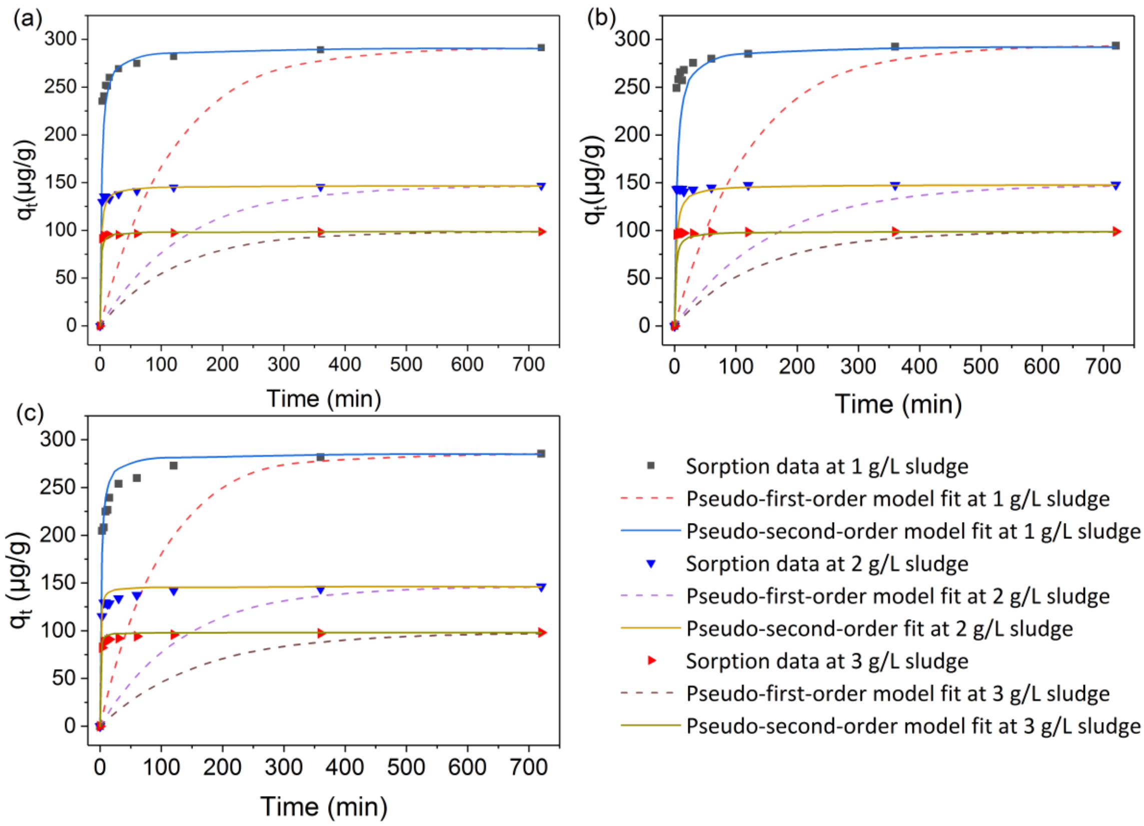

Figure 4.2 Adsorption kinetics data and fitted modes of tetracycline (TC) (a), chlortetracycline (CTC) (b) and oxytetracycline (OTC) (c) onto different concentrations of anaerobic sludge

In the present study, pseudo-first-order and pseudo-second-order equations were separately used for the regression of the adsorption process of TCs onto anaerobic sludge. The experimental results are summarized in Figure 4.2 and Table 4.1, which fit well to the pseudo-second-order equation with higher correlation coefficients than the pseudo-first-order model. Meanwhile, the theoretical values of q_e calculated from the pseudo-second-order model correspond well with the experimental q_e values. Thus, the pseudo-second-order model is more suitable to describe the behavior of the adsorption process than the pseudo-first-order kinetic model. This is consistent with the results of previous studies that investigated the adsorption of TCs onto anaerobic and aerobic sludge (Huang et al., 2012a; Li et al., 2013). Such results suggested that: firstly, chemisorption may be the rate-limiting step; and secondly, the sorption capacity was proportional to the number of available active sites on the sorbent. The process involves exchange or sharing of electrons mainly between cation and functional groups (hydroxyl and carboxyl groups) of the biomass cell (Michalak et al., 2013). Moreover, the increase of the pseudo-second-order rate constant (k_2) was observed when the sludge concentration changed from 1000 mg/L to 3000 mg/L, which might due to the available adsorption sites increased with increasing amount of adsorbent. However, the equilibrium adsorption capacity of anaerobic sludge decreased when the initial sludge concentrations at the same initial concentration of TCs were increased (Figure 4.2). A possible explanation for this is that the increase in adsorption sites resulted in unsaturated adsorption surfaces at a constant amount of TCs (Mihciokur & Oguz, 2016).

Table 4.1 Kinetic and isotherm models and parameters for the adsorption of tetracycline antibiotics onto anaerobic sludge

Model	Parameter	TCs		
		TC	OTC	CTC
Pseudo-first-order kinetics	q_e	38.59 [a], 11.32 [b], 4.51 [c]	32.27 [a], 4.57 [b], 1.88 [c]	56.89 [a], 17.34 [b], 9.04 [c]
	k_1	0.0085 [a], 0.0074 [b], 0.0081 [c]	0.01 [a], 0.0075 [b], 0.0063 [c]	0.0082 [a], 0.0064 [b], 0.0073 [c]
	R^2	0.9138 [a], 0.8063 [b], 0.9294 [c]	0.9644 [a], 0.6324 [b], 0.8069 [c]	0.8959 [a], 0.8015 [b], 0.8688 [c]
Pseudo-second-order kinetics	q_e	285.5 [a], 147.06 [b], 99.01 [c]	294.12 [a], 147.06 [b], 99.01 [c]	285.71 [a], 147.06 [b], 98.04 [c]
	k_2	0.0015 [a], 0.005 [b], 0.0132 [c]	0.002 [a], 0.0113 [b], 0.0276 [c]	0.001 [a], 0.003 [b], 0.0064 [c]
	R^2	1.0 [a], 1.0 [b], 1.0 [c]	1.0 [a], 1.0 [b], 1.0 [c]	0.9999 [a], 0.9999 [b], 1.0 [c]
Langmuir	K_L	0.34	0.49	0.28
	q_m	169.49	185.19	185.19

	R^2	0.9446	0.9598	0.9676
	K_F	36.3	52.34	35.47
Freundlich	1/n	0.707	0.703	0.690
	R^2	0.9760	0.9902	0.9933

a: 1000 mg/L MLSS, b: 2000 mg/L MLSS; c: 3000 mg/L MLSS.

Langmuir and Freundlich isotherm models were used to evaluate the adsorption data of TCs onto anaerobic sludge. The Langmuir equation assumes that the adsorption covers the homogeneous surface of adsorbent and the adsorbate molecules are non-interactive, while the Freundlich isotherm is suitable for adsorption on a heterogeneous surface, which assumes that the adsorption occurs at available sites on the surface with a different free energy (Ayawei et al., 2017). As shown in Table 4.1 and Figure 4.3, the Freundlich model with a larger correlation coefficient (R^2=0.976 - 0.993) fits better to the experimental data than the Langmuir model (R^2=0.945 - 0.968), suggesting that the adsorption of TCs onto the anaerobic sludge is a complex heterogeneous surface adsorption. The heterogeneous structure of extracellular polymeric substances (EPS) produced by activated sludge may affect the adsorption process (Song et al., 2014). As well, the 1/n values obtained by the Freundlich model are lower than 1.0, which means that TCs' adsorption on anaerobic sludge is a favorable process (Ahmed, 2017).

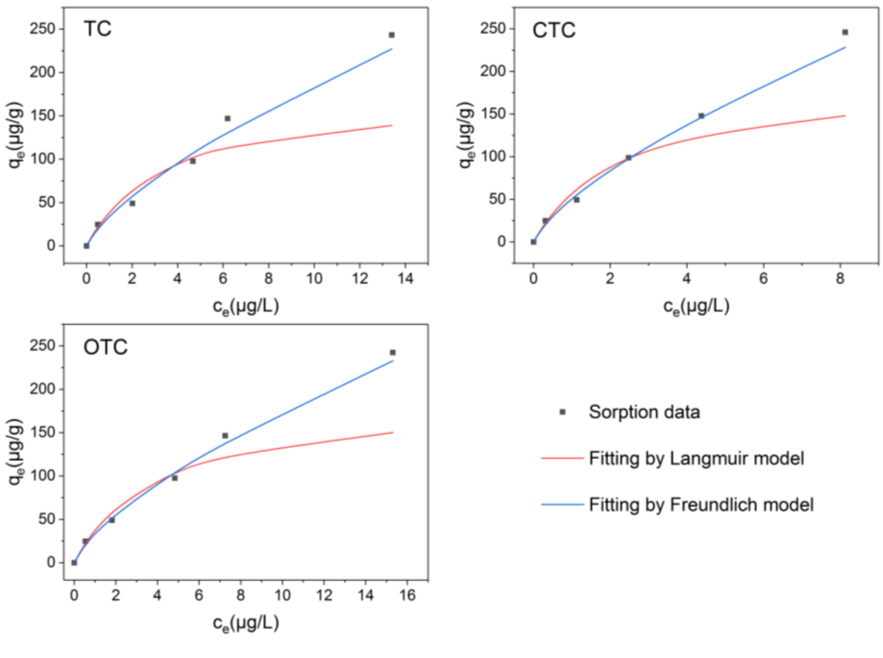

Figure 4.3 The adsorption isotherms of tetracycline (TC), chlortetracycline (CTC), and oxytetracycline (OTC) onto anaerobic sludge.

4.3.3 Degradation of SMs in anaerobic sludge

As shown in Figure 4.1 (b), the removal of SMs in the anaerobic sludge is due to the role of biodegradation. The experiment on the biodegradation kinetics of SMs was also explored in batch experiments. The initial SMs concentrations were 100, 200 and 300 μg/L, respectively, under the pH of 7.5 ± 0.1 and at room temperature for 120 h. According to the removal efficiency vs. time profiles (shown in Figure 4.4 (a)), the concentration of SMX, SDZ and SMZ decreased steadily in the first 72 h of the experiment, with the removal efficiencies being 84.2% - 91.0%, 5.5% - 21.1% and 18.3% - 25.3% in 72 h, respectively. During this experimental period, the degradation ratio of SMs in anaerobic sludge was in the order of SMX>SMZ>SDZ, with the values of 97.4% - 98.9%, 12.0% - 31.2% and 23.9% - 33.5%, respectively.

The biodegradation data of SMX, SDZ and SMZ in anaerobic sludge were analysed by using the first-order kinetic model, as shown in the following kinetic formula:

$$\frac{dC}{dt} = -k_1 \cdot C \leftrightarrow C_t = C_0 \cdot e^{-k_1 \cdot t}$$

Where, C_0 is initial concentration of the antibiotic added in the sludge; C_t is concentration of the antibiotic at time t; and k is the degradation rate constant. Using this equation, half-lives, $t_{1/2}$ can be calculated as (DT50=ln 2/k).

The degradation of SMX, SDZ and SMZ in anaerobic sludge fitted well with the first-order reaction kinetic model, with all R^2 values ranging from 0.84 - 0.99, as presented in Figure 4.4 and Table 4.2.

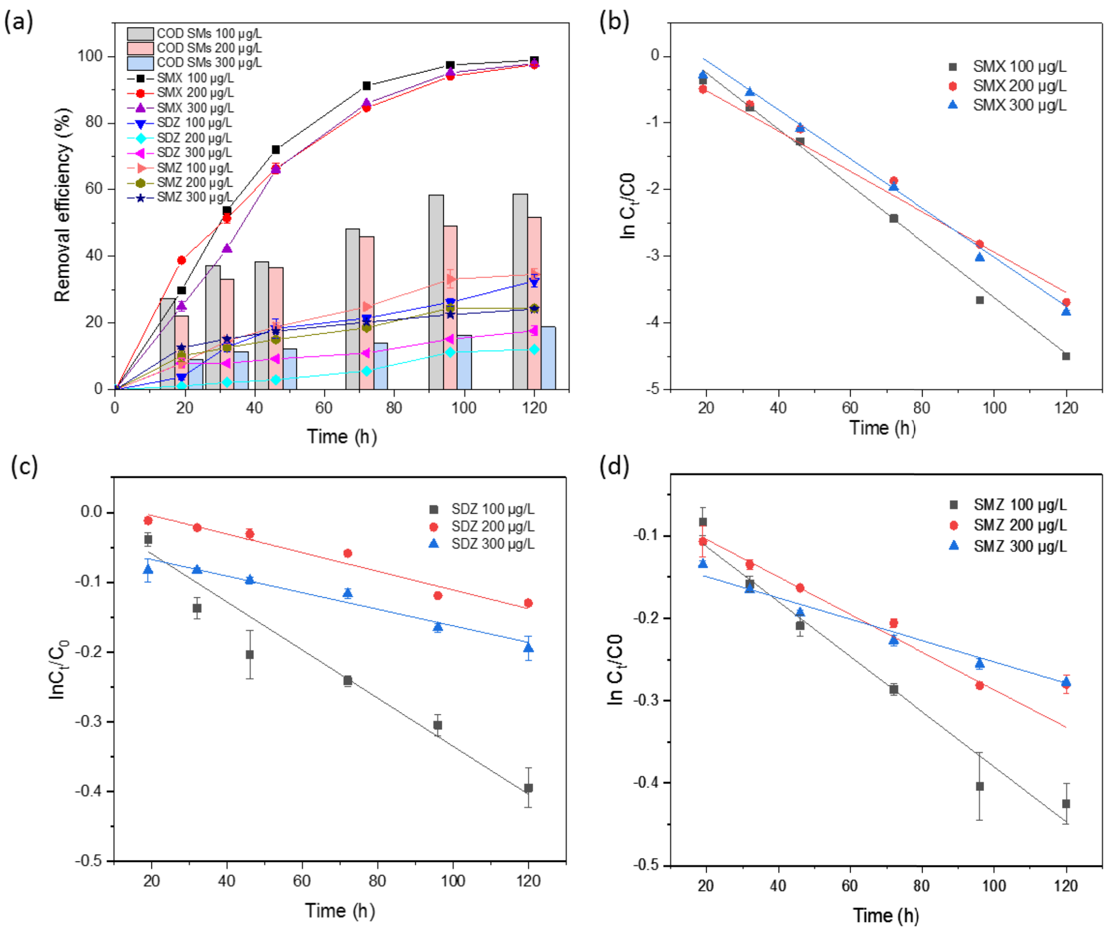

Figure 4.4 (a) Removal efficiencies of sulfonamide antibiotics and COD in the anaerobic reactor; First-order biodegradation kinetic model of sulfamethoxazole (SMX) (b), sulfadiazine (SDZ) (c) and sulfamethazine (SMZ) (d).

Table 4.2 Degradation rate constants (k1) and half-lives (t1/2) of the three sulfonamide antibiotics in anaerobic reactor

Antibiotic	Initial concentration (μg/L)	$k_1(h^{-1})$	R^2	DT50 (h)
	100	0.0397	0.9861	17.45963
SMX	200	0.0306	0.9881	22.65187
	300	0.0337	0.983	20.56817
	100	0.0031	0.9826	223.5959
SDZ	200	0.0012	0.9352	577.6227
	300	0.0013	0.8659	533.1901

	100	0.0034	0.9695	203.8668
SMZ	200	0.0021	0.8946	330.0701
	300	0.0020	0.8357	346.5736

Comparatively, the degradation rate of SMX appeared to be much faster than that of SDZ and SMZ, of which more than 50% can be degraded in less than 23 h. SDZ and SMZ showed a persistent ability to be degraded in anaerobic sludge with the DT50 values of 223.6 - 577.6 h and 203.9 - 346.6 h, respectively, with the initial concentration of 100-300 μg/L. The fast and large removal of SMX also has been detected in previous research studies. For example, Feng et al. (2017) and Mohring et al. (2009) concluded that the SMX in swine manure was almost 100% degraded and rapidly. Larcher and Yargeau (2012) also indicated that the removal rate of SMX could achieve > 99% with both low and high initial SMX concentrations. Feng et al. (2017) found no biodegradation for SDZ during anaerobic digestion of swine manure. The persistence of SMZ during anaerobic fermentation was also found by Mohring et al. (2009), who discovered that 100% of the initially measured concentration of sulfamethazine (SMZ) could still be detected after a 34 - day fermentation period. The different functional group of SMX, SMZ and SDZ may contribute to their varying degradation rates. Yang et al. (2016) explained that functional groups may contribute electronegativity effects that inhibit the degradation of SMZ and SDZ by influencing their interaction with the microbes.

The initial concentration of antibiotics wielded some effects on the degradation rate of SMs, and the degradation would be slower at a higher exposure level (Shen et al., 2018). In the present study, all of these three antibiotics with the initial concentration of 100 μg/L revealed the lowest DT50 values, whereas higher concentrations of antibiotics (200 μg/L) caused a lower degradation rate and longer persistence. The review paper by Cheng et al. (2018a) indicated that higher dosages of antibiotics showed more inhibition of microbial activity which in turn inhibited the degradation of SMs. Yang et al. (2016) also suggested that degradation kinetics of SMs depended on the initial concentrations and removal rates would be slower at a higher concentration. However, the removal efficiency and degradation rate of SMs only indicated a slight change when increasing the concentration from 200 to 300 μg/L, which means the microbial community in anaerobic sludge could adapt to the presence of SMs. As well, microorganisms in anaerobic sludge that are able to degrade SMs might be enriched by increasing the concentration of SMs (Cycoń et al., 2019).

Co-metabolism is regarded as an important mechanism for the biodegradation of antibiotics in the biological wastewater treatment process (Cheng et al., 2018b; Oliveira et al., 2016b). In this study, batch experiments were conducted with different initial concentrations of COD to investigate the effect of COD on the biodegradation of SMs in anaerobic reactors. Different COD concentrations in the reactor were obtained by diluting the stock solution of synthetic swine wastewater to 1500, 1800, 2700 and 4500 mg/L, respectively. The experiment was run under the same conditions with the above experiment by using 100 μg/L of SMX, SMZ and SDZ, respectively. As shown in Figure 4.5, the enhanced removal of SMs has been observed by increasing the COD concentration from 1500 to 2700 mg/L. By the end of 120 hours reaction, the efficiencies in removing SMX, SMZ and SDZ rose, respectively, from 76.06% to 98.69%, 10.58% to 32.53%, and 11.17% to 30.65%. Thus, an increasing trend was observed for the biodegradation of SMs in the anaerobic reactor by increasing COD concentrations, although a slight decline was found when the COD concentration further increased to 4500 mg/L. This finding indicated that the presence of easily biodegradable substrates, such as glucose used in this study, could enhance the biodegradation of SMs, which suggested the degradation mechanism of cometabolism. What was observed in this study agrees with previous recent analyses by Oliveira et al. (2016b) and Oliveira et al. (2019), who demonstrated that the addition of readily available organic matter enhanced the removal efficiency of SMZ in anaerobic treatment processes.

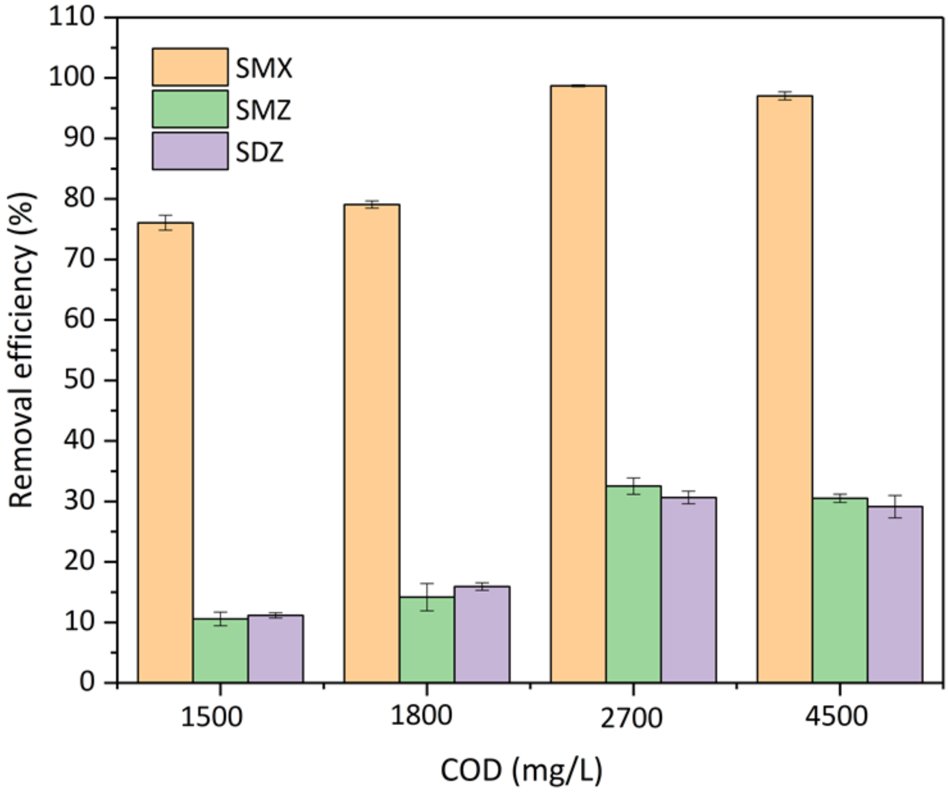

Figure 4.5 Removal efficiencies of sulfamethoxazole (SMX), sulfadiazine (SDZ) and sulfamethazine (SMZ) in the anaerobic reactor with different concentrations of COD.

Additionally, a clear correlation between COD consumption and SMs removal can be observed from Figure 4.4 (a). The removal rate of SMs is positively correlated with the consumption of COD, and more COD was consumed when achieving higher removal efficiencies of SMs. Oliveira et al. (2017) and Alvarino et al. (2014) also observed a linear relationship between COD removal rate and the biodegradation rate of SMs during anaerobic processes. These results also reflected the cometabolic biodegradation of SMs in anaerobic processes. As displayed in Fig 4.4 (a), the removal efficiency of COD fell from 58.76% to 51.65% by increasing the initial concentration of SMs from 100 to 200 μg/L, which dropped to only 18.82% when 300 μg/L of SMs was added to the reactor. The degradation of organic pollutants in anaerobic treatment processes is reliant on the synergistic cooperation of various microbial groups forming a metabolic network (Stams, 1994). Thus, the decline in the COD removal efficiency may result from the inhibition effect of SMs on microbial activity under higher concentrations. However, the removal efficiency of SMs was not limited by raising their initial concentrations from 200 to 300 μg/L. This finding indicated that the cometabolic biodegradation of SMs was determined specifically by cometabolism instead of the overall metabolism, which was caused by specific groups of microorganisms. Similar results were concluded by Barret et al. (2010), and these authors demonstrated that the cometabolic biodegradation of pharmaceutical compounds would be mainly affiliated with specific metabolic stages of the whole biodegradation process. Oliveira et al. (2017) also indicated that the micropollutant transformation is expected to be associated with particular metabolic pathways. This is consistent with a cometabolic transformation caused by the non-specificity of specific enzymes that occasionally convert the micropollutant along with its main substrate.

4.4 Conclusion

In this chapter, the removal mechanism of TCs and SMs in anaerobic sludge was investigated and found that TCs were removed through adsorption of anaerobic sludge while SMs were eliminated through biodegradation. Meanwhile, the adsorption kinetics and isotherms of TCs, and the degradation kinetics of SMs in anaerobic sludge was explored in present study. The adsorption of TCs onto the anaerobic sludge fitted well with the pseudo-second kinetic mode and the Freundlich isotherm, suggesting the importance of a heterogeneous chemisorption process. The degradation of SMs in anaerobic processes fitted well to the first-order kinetic model. SMX was the most easily biodegradable antibiotic with the lowest DT50 values. The degradation of SMs occurred via the cometabolism triggered by specific microbial communities. Higher levels of SMs exposure would inhibit microbial activity in anaerobic sludge to prolong their persistence.

CHAPTER 5

Feasibility of microbial fuel cell for removing antibiotics from swine wastewater

5.1 Introduction

The occurrence and accumulation of antibiotics in the environment has attracted widespread attention worldwide, due to their harmful effects on the ecosystem and contribution to cause antibiotic resistance (Singh et al., 2019). Such resistance can reduce or eliminate the effectiveness of antibiotics to treat infectious diseases, since multidrug-resistant bacteria with strong resistance to various antibiotics could lead to untreatable diseases and endanger human health (Ma et al., 2018). The discovery of multidrug-resistant bacteria in the environment has reported earlier (Lee et al., 2018). Antibiotics, as the contributor to the development of antibiotic resistance, have positive effects on the accumulation and spread of ARGs in the environment (Cheng et al., 2019b).

Swine wastewater is an important source of antibiotics in the environment, owing to large amounts of antibiotics used as drugs and feed additives in swine industries. Sulfonamides (SMs) are one of the oldest and most widely used antibiotics in swine farms considering their low cost and relative efficacy in some common bacterial diseases (Broll et al., 2004). As the increase of the global population and the demand of pig products, the consumption of SMs will continue to increase in future. Therefore, a variety of treatment technologies, including biological, physiochemical and bioelectrochemical systems, have been conducted by researchers to remove antibiotics from wastewaters (Homem & Santos, 2011). Among these technologies, the microbial fuel cell (MFC) is receiving increasing attention due to its advantages of effective removal of organic matters, moderate operating condition, low sludge production, and power production (Lovley, 2008). The effectiveness of MFC for enhancing the removal of refractory organic pollutants such as pesticides, toluene, phenol, indole, and azo dye from wastewater has been proved previously (Huang et al., 2011).

Although all SMs have the same mechanism of action, there are significant differences in activity and antibacterial spectrum due to the various physiochemical characteristics of SMs. Therefore, sulfonamide combinations are usually used as feed ingredients in swine production instead of individual sulfonamide, resulting in the residual of sulfonamide combinations in swine wastewater. Unfortunately, most of current researches only pay attention to the removal of individual sulfornamide in MFC, few studies focused on the removal and degradation kinetic of the simultaneous sulfamethoxazole (SMX), sulfamethazine (SMZ) and sulfadiazine (SDZ) from swine wastewater in MFC (Wang et al., 2018a; Wu et al., 2020). Moreover, the combined antibiotics may show more serious inhibition to the performance of bioreactors than the individual one (Cheng et al., 2018a). Hence, this study aimed to explore the effect of different concentrations of sulfonamide combinations on the electricity generation and organic matters removal in a double-chamber MFC. The removal efficiency and degradation kinetics of sulfonamide combinations (SMX, SMZ and SDZ) in the MFC were also studied in the present study.

A major part of Chapter 5 has been published as a journal article in ERA A-rated journal:

Cheng, D., Ngo, H.H., Guo, W., Lee, D., Nghiem, D.L., Zhang, J., Liang, S., Varjani, S. and Wang, J., 2020. Performance of microbial fuel cell for treating swine wastewater containing sulfonamide antibiotics. Bioresource Technology, p.123588.

5.2 Materials and methods

5.2.1 MFC construction and inoculation

The double - chamber MFC used in this study is described in Chapter 3, Section 3.3.2. The system was operated under the self-circulating operating mode of MFC. The anode chamber was inoculated by anaerobic sludge collected from a pilot scale anaerobic digester and fed with synthetic swine wastewater (3000 mg/L COD, 223 mg/L NH_4Cl, 66 mg/L of KH_2PO_4, 54 mg/L $MgSO_4 \cdot 7H_2O$ and 4 mg/L $CaCl_2 \cdot 2H_2O$). The synthetic swine wastewater was adjusted to pH 7.5 ± 0.1 and purged with N_2 gas for 15 minutes before fed to the anode chamber. Meanwhile, the cathode chamber was filled with distilled water and purged with air continuously to maintain the dissolved oxygen (DO) concentration of 6 mg/L.

5.2.2 Experimental design and operation

In order to investigate the removal efficiency of SMs in MFC and compare the different from open-circuit mode, reactors in this study were conducted in closed-circuit mode (MFC) and open-circuit mode (OC) simultaneously, as described in Chapter 3, Section 3.3.2. Samples from the MFC and OC were collected respectively at different operating times, and filtered by syringe filter (0.2μm) before testing.

5.2.3 Data analysis

Data analysis is described in Chapter 3, Section 3.4.1.

5.3 Results and discussion

5.3.1 Impacts of SMs on power generation of MFC

The voltage generation under different initial concentrations of SMs is presented in Figure 5.1. The stable voltage output was achieved before SMs were added into MFC with the average value of 551.1 mV, which indicates the enrichment of exoelectrogenic bacteria on the anode surface and the successful start-up of the MFC. From Figure 5.1, the average voltage was 555.1 mV and 536.4 mV after the injection of 100 μg/L and 200 μg/L of SMs into the MFC in successive operating cycles. Stable voltage production was observed during the operating period, which reflected that microorganisms in the anode chamber have a strong tolerance to SMs. A slight increase of the voltage production (average of 583.6 mV) was observed by further increasing the initial concentration of SMs to 300 μg/L. Wu et al. (2020)'s research also found that the presence of SMX in the anode of MFC enhanced the abundance of exoelectrogens, and increased the power density by 18%. The study by Wen et al. (2011) also revealed that the addition of ceftriaxone in MFC had positive effects on the production of electricity.

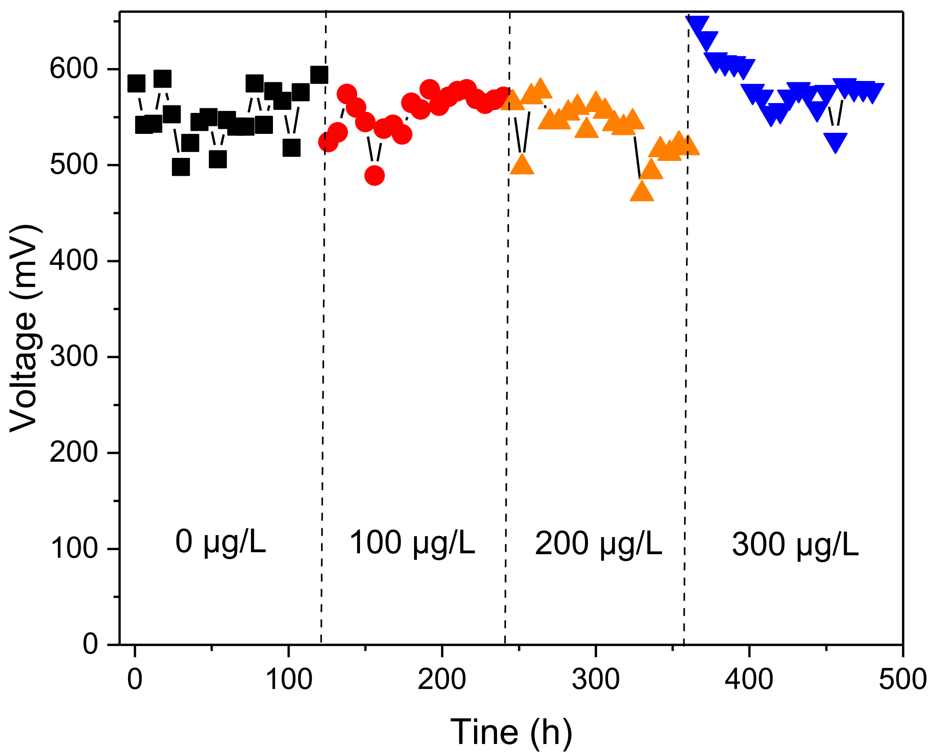

Figure 5.1 The voltage generation under different initial concentrations of SMs in MFC

It is clear that the electricity production in the MFC system was through the oxidation of organic matters by the biocatalysis of microorganisms. During this process, the produced electrons were transferred from the cell to the anode electrode and then flowed to the cathode through an external circuit to produce electricity. Therefore, the electricity generation in MFC was determined by the activity of exoelectrogenic bacteria and the transfer of electron between bacterial cells and electrode. Based on such mechanism of electricity production, it is suggested that the addition of SMs to 300 μg/L in the MFC might improve the activity of exoelectrogenic bacteria and or the ability of electrons transfer from microbe cell to the anode.

5.3.2 Impacts of SMs on COD removal in MFC

As for practical application, the performance of MFC on the removal of organic matters from swine wastewater and the effect of antibiotics on their removal were also important. Hence, the removal efficiency of COD in MFC and OC under different SMs concentrations was monitored and the results were displayed in Figure 5.2. As observed from Figure 5.2 (a), the degradation rate of COD decreased by increasing the initial concentration of SMs, which reflected that higher concentrations of antibiotics showed more inhibition to the anaerobic microbes. This finding is consistent with previous reports (Cheng et al., 2018a). High COD removal efficiencies were achieved in both MFC (98.85%) and OC (94.21%) before the addition of SMs into the reactor (Figure 5.2 (b)). By adding 100, 200 and 300 μg/L of SMs into the reactor, the overall removal efficiency of COD in MFC was quite stable (95.28% - 98.66%) while its removal in OC greatly reduced to 58.72%, 51.65% and 18.82%, respectively. The high and stable degradation efficiency of organic matters in MFC systems was also found by the addition of other types of antibiotics in MFC (Wang et al., 2018a; Wen et al., 2011; Zhou et al., 2018). This result indicated that MFC could eliminate the toxic of SMs to microorganism, which has great potential to be used for the treatment of wastewater containing antibiotics. The high and stable removal efficiency of COD in MFC with the addition of SMs was consistent with the stable electricity production.

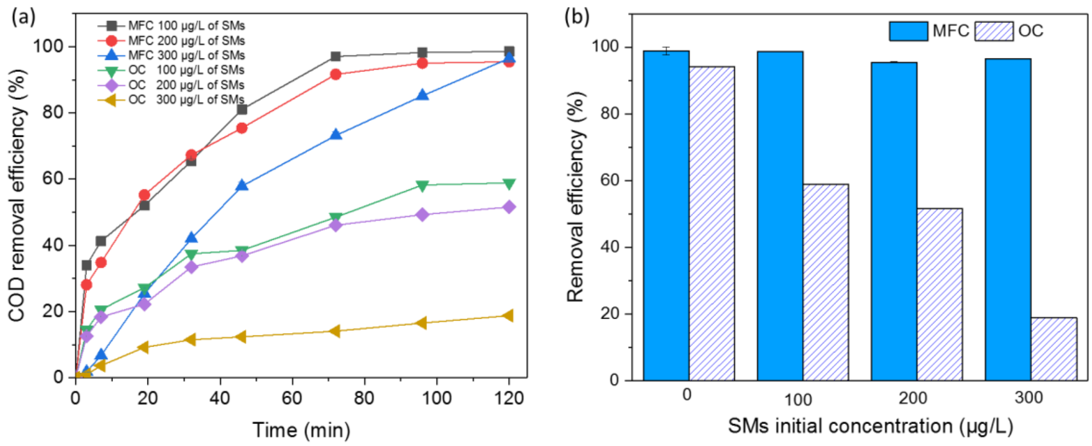

Figure 5.2 The removal efficiency of COD in MFC and OC under different SMs concentrations

5.3.3 Degradation of SMs in MFC

The concentration change and removal efficiency of SMX, SMZ and SDZ in MFC and OC under the initial concentration of 100, 200 and 300 μg/L were presented in Figure 5.3. It is observed that MFC revealed higher and faster removal for all the SMs than those in OC under all the initial concentrations. Xue et al. (2019) and Song et al. (2018) also found the rapid removal of SMX and SDZ in MFC and their low residual concentrations in MFC effluent in comparison with the effluent from OC. The possible reason is that the stimulation of electron transfer could enhance the growth of microorganisms and the microbial metabolisms in the anode of MFC (Cao et al., 2015; Zhang et al., 2017a). Based on the study previously, the removal of SMs in anaerobic reactors is attributed to the biodegradation of microorganisms (Cheng et al., 2020). The degradation of SMX, SDZ and SMZ in MFC followed the first-order kinetic reaction model and the parameter was summarized in Table 5.1.

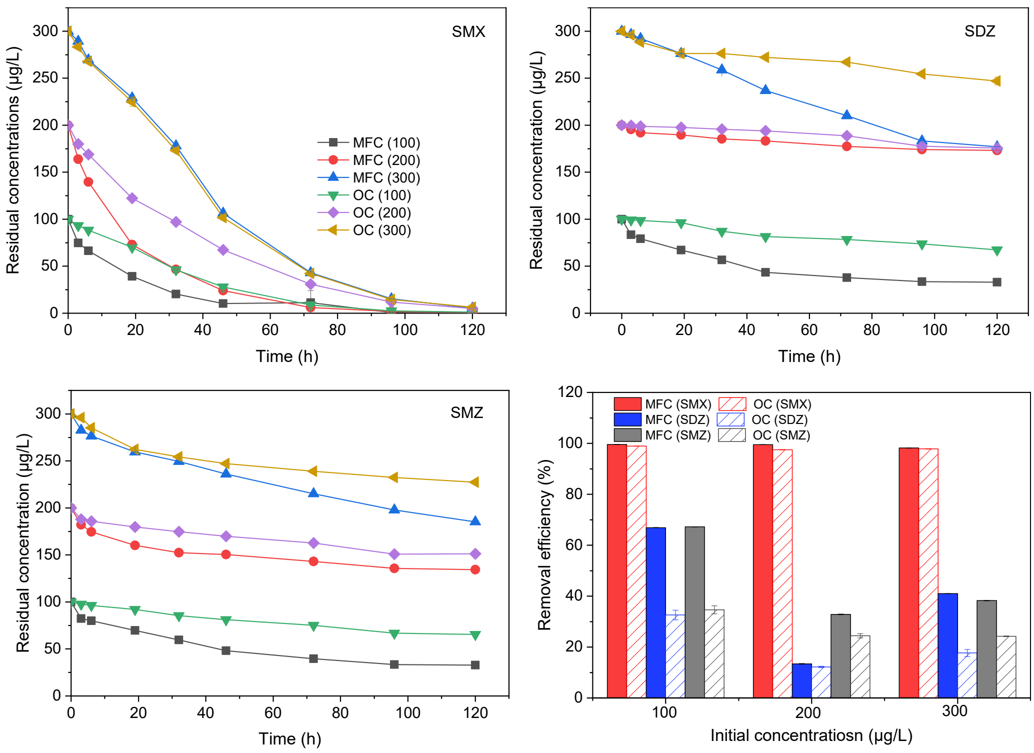

Figure 5.3 The concentration change and removal efficiency of SMs in MFC and OC at different initial concentrations.

Table 5.1 Fitting Results of SMX, SDZ and SMZ degradation in MFC using the first-order kinetic model

Kinetic formula	Antibiotic	Initial concentration (µg/L)	K	R^2	DT50 (h)
$\frac{dC}{dt} = -k_1 \cdot C \leftrightarrow C_t = C_0 \cdot e^{-k_1 \cdot t}$	SMX	100	0.045	0.999	15.79
		200	0.045	0.996	15.44
		300	0.033	0.953	20.88
	SDZ	100	0.0053	0.864	129.81
		200	0.001	0.906	693.15
		300	0.0049	0.993	142.04
	SMZ	100	0.00889	0.975	78.68
		200	0.00309	0.970	228.76
		300	0.00399	0.992	177.28

High removal efficiency of SMX can be achieved in MFC (>99%) at all tested concentrations, and its degradation rate was faster than the degradation of SDZ and SMZ, with much higher degradation rate constant (K) and lower time for the degradation of 50% SMs (DT50) (Table 5.1). The effective performance of MFC for removing SMX was also reported by the study of Xue et al. (2019) and Wu et al. (2020), the author indicated that SMX could be completely degraded into less harmful byproducts without affecting the performance of MFC and its removal was less affected by the initial concentration. Comparatively, the removal efficiency of SDZ and SMZ was in the range of 13.39% to 66.91% and 32.84% to 67.21% at the studied concentrations. The research by Harnisch et al. (2013) also found the complete removal of SMX and only part removal of SDZ in MFC. This phenomenon demonstrated that the degradation of SMs by microorganisms in MFC has substance-specific properties. With the increase of the initial concentration of SMs from 100 to 200 μg/L, the removal efficiency of SDZ and SMZ in MFC decreased from 66.91% to 13.39% and 67.21% to 32.84%, and the DT50 increased from 129.81 to 693.1h and 78.68 to 228.77 h, respectively. This phenomenon might due to the reduced bioactivity of the degrading microorganisms in the anode compartment. Whereas, the SDZ removal efficiency was recovered to 40.1% by further increasing the addition concentration to 300 μg/L, and a slight increase for SMZ (38.25%) was also observed. This result suggested that microorganisms in MFC might have gradually adapted to the presence of SMs to some extent and became more active after a period of acclimation. Wang et al. (2016b) also stated that the ability of microbes to degrade recalcitrant chemicals could be enhanced by a long acclimation period. This result is also agree with the improved voltage generation at 300 μg/L of SMs. Compared to the removal of individual SDZ in MFC reported previously, its removal efficiency in this study was quite low (Wang et al., 2018a). Probably due to sulfonamide combinations are more toxic to their degrading microorganisms than the individual antibiotic (Cheng et al., 2018a).

5.4 Conclusion

Compared with the conventional anaerobic reactor, MFC revealed high potential for the treatment of swine wastewater with antibiotics. Organic matters in swine wastewater could be highly removed in MFC and was little affected by the presence of SMs. Meanwhile, stable voltage was generated continuously in MFC by feeding with synthetic swine wastewater. The addition of SMs might increase the electricity production through the improved activity of exoelectrogenic bacteria after a period of domestication and or the enhanced ability of electrons transfer from microbe cell to the anode. The simultaneous removal of SMX, SDZ and SMZ in MFC was higher than those in the conventional anaerobic reactor.

CHAPTER 6
Performance of continuous flow microbial fuel cell for antibiotics removal from swine wastewater

6.1 Introduction

An increase in the demand for pork worldwide has resulted in the conversion of small-scale pig farms into large-scale and intensive pig industries. As reported earlier, the world pig production increased by 456.6% from 1890 to 2014 (Roser, 2017). A consequence of the mass production of pigs was the discharge of large amounts of swine wastewater from intensive pig industries. Moreover, veterinary antibiotics are commonly used in swine industries as feed additives to prevent infectious diseases and enhance growth rates of pigs (Cheng et al., 2018b; Sarmah et al., 2006). Lou et al. (2018) reported that the annual antibiotics consumption in China's pig production industry rose by 97000 tons. However, the low digestibility of antibiotics in animals' guts produced high concentrations of antibiotics released into animal wastewater through animal faeces and urine (Cheng et al., 2018b). Zhang et al. (2015b) indicated that around 50000 tons of antibiotics were released by animal wastes every year. Therefore, swine wastewater not only contains high concentrations of organic matter and nutrients, but also has been recognized as the major source of antibiotics in the environment (Cheng et al., 2018b). Consequently, the direct discharge or irrigation of swine wastewater can pose a high threat to the environment due to: firstly, the toxic effects of antibiotics on environmental biology; and secondly, the development of antibiotic resistant genes (ARGs). Sulfonamide antibiotics (SMs) have been reported as the most widely used antibiotic classes in the swine industry and the dominant antibiotic components detected in swine wastewater (Han et al., 2020). According to the review paper by Cheng et al. (2018b), the detected concentration of SMs in swine wastewater was mainly in the 0.44 to 324.40 μg/L range. Hence, when effective the removal of SMs from swine wastewater will significantly eliminate their dangerous effects on the environment and human health.

Anaerobic treatment technologies are commonly recommended for the treatment of swine wastewater, due to their cost effectiveness and high concentrations of organic matter in swine wastewater. Nevertheless, the widely used anaerobic processes in current wastewater treatment plants are mainly devised to remove general pollutants, yet have been poor in their antibiotics removal efficiency (Cheng et al., 2018b). As well, nutrients contained in swine wastewater could not be effectively removed by single anaerobic processes. Many studies have been conducted on the removal of antibiotics in some advanced wastewater treatment processes (Leng et al., 2019; Pei et al., 2019). For example, microbial fuel cell (MFC) has emerged as a promising and 'green' technology for improving the degradation of refractory pollutants from wastewater and generating electricity simultaneously through microorganisms on the anode (Lovley, 2008; Zhou et al., 2018). The effective removal of SMs from wastewater by MFCs has been reported in recent studies, mainly because of the enhanced microbial metabolism in the presence of anode. For instance, Wang et al. (2016b) and Wu et al. (2020) demonstrated that sulfamethoxazole (SMX) can be rapidly and completely degraded by MFC and the power generation was enhanced due to the presence of SMX.

Another sulfonamide antibiotic, sulfadiazine (SDZ), can also be degraded into 2-aminopyrimidine, 2-amino-4-hydroxypyrimidine and benzenesulfinic acid under the behavior of microorganisms in MFCs (Wang et al., 2018a). The effective removal or recovery of nutrients from wastewater by MFCs also has been reported previously (Ye et al., 2019b). However, most previous studies only focused on the removal of individual antibiotics by MFCs, while different antibiotics are usually present in wastewater simultaneously. It is still unclear about the removal of multiple antibiotics in MFCs and the influence of mixed antibiotics on the performance of MFCs. Furthermore, the removal of antibiotics by MFCs was mainly done in batch mode so far, which is not effective and realistic in practical applications in the future. Moreover, less attention was paid to the feasibility of applying MFC processes to the removal of antibiotics from swine wastewater.

Therefore, a continuous flow operation of MFC was conducted in this study, which is the first to investigate simultaneously removing sulfamethoxazole (SMX), sulfamethazine (SMZ) and sulfadiazine (SDZ) from swine wastewater. The removal efficiency of the selected antibiotics was examined in single continual and sequential anode-cathode continual mode, respectively. The effect of higher ammonium concentration in swine wastewater on their removal efficiencies was also analyzed in this research. Furthermore the performance of the MFC in removing organic matter, ammonium and phosphorus from swine wastewater, as well as the generation of electricity was investigated.

6.2 Materials and methods

6.2.1 Experimental design and set up

The MFC design and setup are described in Chapter 3, Section 3.3.2. In addition, anaerobic sludge was used to inoculate the anode by mixing it with synthetic swine wastewater to make a concentration of mixed liquor suspended solids (MLSS) of 5000 mg/L. The synthetic swine wastewater mainly contained glucose (3 g/L of COD), NH_4Cl (0.223 and 0.446 g/L), KH_2PO_4 (0.066 and 0.132 g/L), $MgSO_4{\cdot}7H_2O$ (0.054 g/L) and $CaCl_2{\cdot}2H_2O$ (0.004 g/L). After voltage stabilization, SMX, SMZ and SDZ were added to the synthetic swine wastewater with the initial concentration of 100 μg/L.

6.2.2 Experimental operation

In this study, the MFC was operated under single continual mode and sequential anode-cathode mode. The operation process of this study has been descried in Chapter 3, Section 3.3.2.

6.2.3 Analytical methods

The sample analysis is described in Chapter 3, Section 3.4.2.

6.3 Results and discussion

6.3.1 SMs removal in continuous flow MFC systems

The simultaneous removals of SMX, SMZ and SDZ in different operation modes of the continuous flow MFC are displayed in Figure 6.1. In the mode 1 (single continuous mode), removal efficiencies of SMs in the anode chamber of MFC and the open-circuit control were compared. From Figure 6.1, it is observed that higher removal efficiencies of SMs were achieved in the MFC than those in its open-circuit control system. Similar results have been concluded by earlier studies for the removal of individual SMX and SDZ in MFC systems (Miran et al., 2018; Song et al., 2018). Accordingly, the removal of SMs in both closed and open-circuit MFC systems was due to the role of biodegradation in comparison to adsorption (Cheng et al., 2020; Harnisch et al., 2013). In the MFC system, degradation products of SMX and SDZ, their degradation mechanisms, as well as the functional microorganism that contributed to their removal have been successfully identified by previous researchers (Wang et al., 2018a; Xue et al., 2019). The relatively higher efficiency in removing SMs in the MFC system was mainly possible because the enhanced metabolic rate of bacteria in the anode chamber was caused by the electron transfer to the cathode via the external circuit. This could also result in faster oxidation of the co-substrate to produce more electrons for SMs degradation leading to accelerated degradation of SMX (Aghababaie et al., 2015). The better removal efficiency of COD in the MFC was also observed in this study, which will be discussed in the following section. Research by Wang et al. (2016b) demonstrated that the ATP level of the microorganisms in MFC was nearly three times higher than that in the open-circuit controls, which may be linked to the rapid degradation of antibiotics in MFCs.

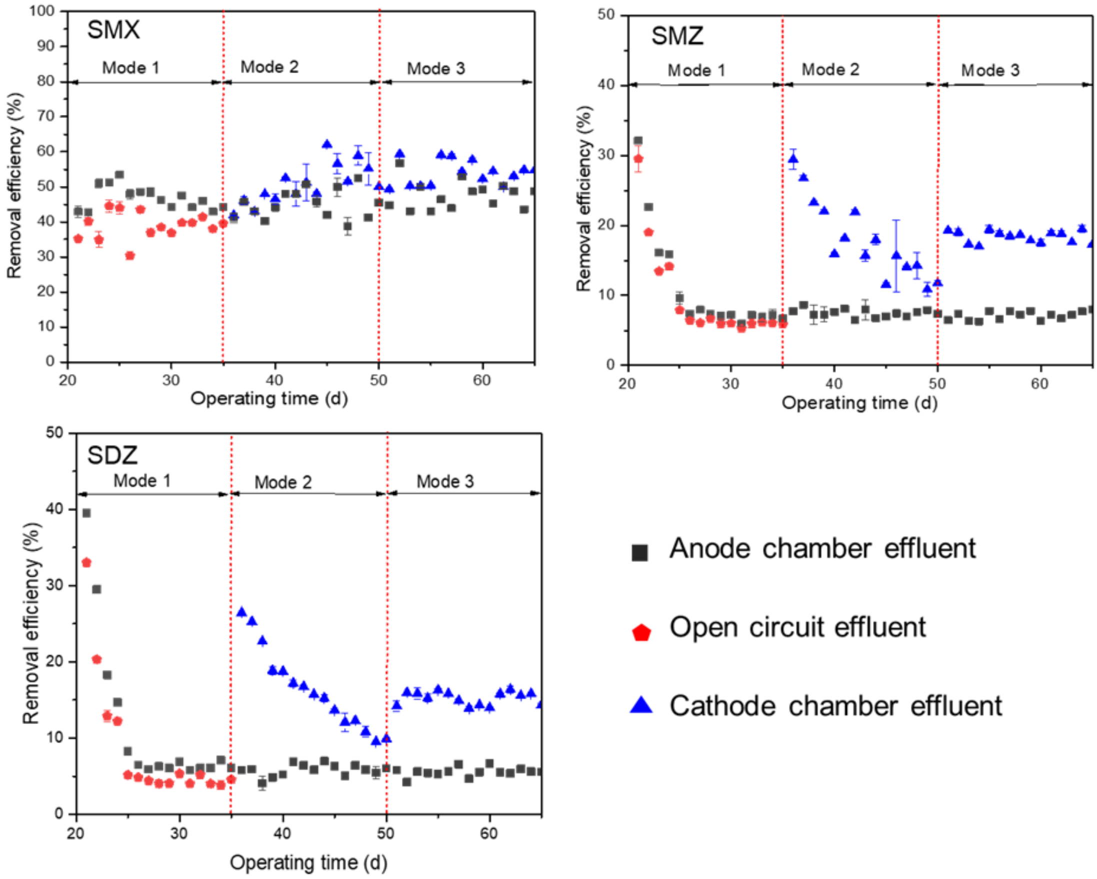

Figure 6.1 Removal efficiencies of SMs during the continuous operation of MFC under different scenarios: (a) SMX removal efficiency; (b) SMZ removal efficiency; (c) SDZ removal efficiency.

Compared with the high removal efficiency of individual SMX and SDZ in MFC systems under self-loop batch operating conditions (Miran et al., 2018; Wang et al., 2018a; Xue et al., 2019), the efficiency in the simultaneous removal of SMX, SMZ and SDZ was quite low under the single continuous operation mode of MFC in this study. As shown in Figure 6.1, the removal efficiency of SMX, SDZ and SMZ in mode 1 was in the 42.63% - 53.43%, 6.37% - 32.07% and 5.8% - 39.52% range, respectively. The biodegradation of SMX in this study was easier than SDZ and SMZ in the MFC. A similar result was concluded by Harnisch et al. (2013), and these authors found SMX was completely removed but only some SMZ was removed in the batch operating MFC in 7 days. Moreover, a decline was observed for the removal efficiency of SMs in the first few days after continuous pumping of SMs into the MFC, which reflected the toxic effect of SMs and/or their degradation intermediates on the microorganisms responsible for this degradation. In addition to the different operating conditions in comparison to a previous study, the poor removal efficiency of SMs in this study may also result from the increased toxicity of the coexistence of SMX, SDZ and SMZ to the microorganisms in MFC. The review paper by Cheng et al. (2018a) has indicated that combined antibiotics revealed more inhibition than the individual antibiotic. It is clear that effluent from the anode chamber of the MFC still contains a high concentration of SMs, so further treatment is necessary to reduce their emission into the environment.

To increase the removal efficiency of SMs in the continuous operation of MFC, the sequential anode–cathode mode was conducted in the present study. During this mode, the effluent from the anode compartment was used as a continuous feed for the cathode chamber, which enabled aerobic bacteria to grow in the cathode part (Freguia et al., 2008a). Therefore, the double-chamber MFC can be operated as a combination of the anaerobic (anode chamber) and aerobic (cathode chamber) processes, in which the pollutants in the anode effluent may be further degraded by aerobic bacteria activities. An obvious improvement was observed for the removal of SMZ and SDZ in the cathode effluent compared to their removal in the effluent from the anode chamber, although their removal also decreased gradually as this operation progressed (see Figure 6.1).

By contrast, the removal efficiency of SMX experienced only a slight improvement in this mode although the biodegradability of SMX under anaerobic and aerobic conditions has been extensively concluded by earlier studies (Müller et al., 2013; Wang & Wang, 2018a). The possible reason might be the different operating conditions, varied microbial species and feeding wastewater components in the cathode reactor. For example, the solution pH is an important factor that can affect the degradation of SMs by influencing the composition of the microbial community (Maspolim et al., 2015). As monitored in this experiment, the pH value was over 8 in the cathode compartment due to the produced OH^- from the oxygen reduction reaction, which is higher than that in the common aerobic reactor (around neutral pH values). Additionally, the relatively high efficiency in removing SMX in the anode chamber may limit its biodegradation rate in the following cathode chamber, possibly due to the low bioavailability of SMX when its concentration was too small (Wang & Wang, 2018a; Wang & Wang, 2018b). In contrast, the high residual concentration of SDZ and SMZ in the anode effluent may make them much more biodegradable in the cathode chamber. Collectively, the sequential anode–cathode operating mode of MFC can enhance the removal efficiency of SMs from swine wastewater.

Considering the large ammonium concentration in swine wastewater and the influence of ammonium on the microorganisms in MFC, the NH_4Cl concentration in the synthetic swine wastewater was increased from 223 mg/L to 446 mg/L in the third mode of this experiment. This was done to investigate its effect on the removal of SMs and the performance of MFC. As observed in Figure 6.1, the removal efficiency of SMs was increased in the cathode chamber effluent when the NH_4^+-N concentration was doubled in the feeding wastewater. Based on previous studies, the increased removal of SMs may be linked to the improved activities of ammonia-oxidizing bacteria (AOB) in the MFC by increasing the NH_4^+-N concentration. As reported by He et al. (2009), the AOB and denitrifying bacteria were found on both the anode and cathode electrodes of a MFC. The AOB can use NH_4^+-N as the growth substrate, and therefore the NH_4^+-N level is a major factor for the abundance and distribution of AOB. Ye and Zhang (2011) demonstrated that the AOB increased largely through increasing NH_4^+-N concentrations, which enabled the AOB to be a major player in the nitrification reactors. A positive relationship between micropollutants elimination and AOB activity has been reported in other analyses (Kumwimba & Meng, 2019; Xu et al., 2016). The biodegradation of micropollutants by AOB was mainly due to its non-specific enzyme ammonia monooxygenase (AMO), which is able to degrade various kinds of micropollutants through cometabolic biodegradation (Helbling et al., 2012; Roh et al., 2009; Xu et al., 2016). Hence, an inherent connection can be found between the removal of SMs and the concentration of NH_4^+-N in MFC (Delgadillo-Mirquez et al., 2011; Tran et al., 2016; Tran et al., 2018). It can be assumed that the increase in the growth substrate NH_4^+-N to some extent can improve the cometabolic degradation of SMs, which has been confirmed by the experimental results of the present study.

Overall, the final removal efficiencies of SMX, SMZ and SDZ in this sequential anode-cathode double-chamber MFC were around 49.35% - 59.37 %, 16.75% - 19.45% and 13.98% - 16.31%, respectively. Based on the above discussion, such poor removal efficiency of SMs in this MFC system was mainly due to the toxic effect of SMs and or degradation byproducts on the microbial community in both the anode and cathode chambers. However, this study also discovered that the activity of the microorganism that was responsible for degrading the SMs in the MFC could be recovered gradually after stopping exposure to SMs. After 5 days stopping the addition of SMs to the influent of MFC, the SMs removal efficiencies reached 78.22% for SMX, 51.87% for SMZ and 49.54% for SDZ. This leads to the conclusion that sequential anode-cathode double-chamber MFC configuration can be used for removing multiple antibiotics from swine wastewater, while further research is required to reduce the toxic effect of antibiotics on microorganisms to improve the removal efficiency.

6.3.2 Electricity generation

Electricity generation was evaluated by monitoring the voltage production in the present study. Figure 6.2 describes the variations in voltage production at different operating modes of MFC for treating swine wastewater. In the acclimatization process, the wastewater was continuously pumped into the anode compartment of the double-chamber MFC, and the MFC was conducted under open circuit mode for 30 days to form the biofilm on the anode electrode's surface. Thereafter, the closed circuit mode was conducted until the stable operation of the MFC with the voltage generation ranged from 630 mv to 720 mv, indicating the electrochemically active biofilms were formed on the anode of MFC (Zhou et al., 2018). Little change was observed for the voltage generation after the injection of SMs (see Figure 6.2). This is consistent with the study by Harnisch et al. (2013), who found that the power output remained constant after exposure to SMs in the MFC.

Interestingly, some researchers even detected an increase in power generation after the addition of a single antibiotic (SMX or OTC) and multiple antibiotics under low concentrations in the MFC (Sun et al., 2019; Wu et al., 2020; Zhou et al., 2018). Wu et al. (2020) explained that the increased abundance of exoelectrogens in MFC was due to the decline in the competitive population in the anode caused by the presence of SMX. Zhou et al. (2018) demonstrated that although the MFC's output voltage could be inhibited by adding antibiotics into the reactor, it subsequently improved when the concentration of antibiotics shrunk. The present result of this study may explain why the inhibition of SMs (100 μg/L) to exoelectrogenic bacteria was less when compared to other competitive organisms in the MFC's anode chamber. In addition, the degradation products of SMX, SMZ and SDZ may serve as mediators to facilitate the electron transfer from bacteria to the anode, thereby compensating for their inhibition (Sun et al., 2019).

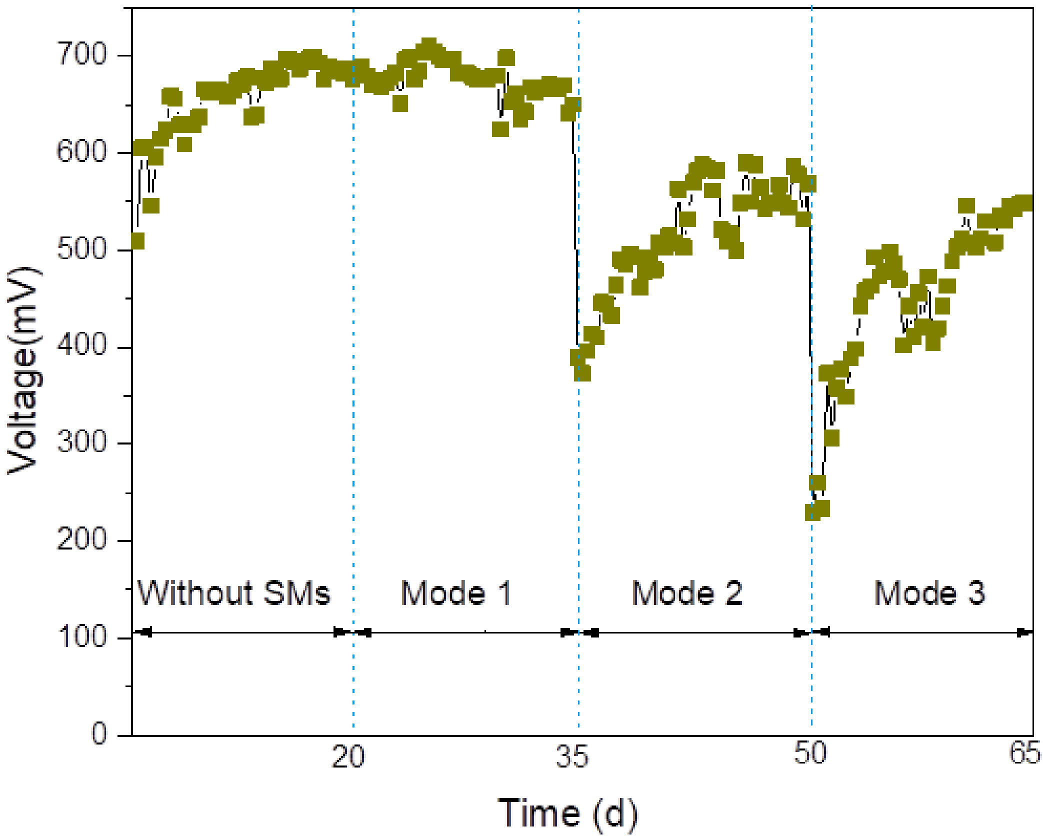

Figure 6.2 Voltage generation when the MFC is operating under different modes with and without SMs present.

It can be seen that the voltage generation decreased in the sequential anode–cathode mode. Generally, the generation of electricity in the MFC mainly depends on: 1) the oxidation of organic substrates in anode, 2) the transfer of electrons from microbes to anode, 3) transfer of protons through the membrane, and 4) the concentration of oxygen in the cathode chamber (Amari et al., 2015; Gil et al., 2003). Compared with the single continuous step, the catholyte in this mode was changed from DI water to the continuous supply of anode effluent. The residual organic matter in the anode effluent enhances the growth of aerobic heterotrophs in the cathode compart, leading to the reduction of oxygen concentration in the cathode chamber (Freguia et al., 2008a). This has been confirmed by the further removal of COD in the cathode section. In this study, oxygen served as the final electron accepter in the cathode portion, and the competitive consumption of oxygen can limit the voltage generation (Amari et al., 2015; Najafgholi et al., 2015). The other possible reason is due to the permeation of oxygen from the anode to the cathode camber through what connects them. This may limit the activity of electron bacteria and decrease the amount of power being generated (Najafgholi et al., 2015).

The voltage generation fell to around 230 mv after the sudden increase in the NH_4^+-N concentration in the influent (Figure 6.2). The negative effect of ammonium on electricity generation in MFC systems was also reported by other researchers (Hiegemann et al., 2018; Kim et al., 2011; Tice & Kim, 2014; Ye et al., 2019a). For instance, Ye et al. (2019a) investigated the impact of ammonium on power generation in a double-chamber MFC and indicated that the voltage generation decreased gradually by increasing the NH_4^+-N concentration from 5 to 40 mg/L. This may result from the inhibition of NH_4^+-N on the activity of exoelectrogenic bacteria in microbial fuel cells (MFCs) (Liu et al., 2017). Conversely as can be seen from Figure 6.2, the power generation recovered gradually with the operating time, suggesting the adaptation of bacteria to the high ammonium concentration through continuous exposure (Kim et al., 2011). Therefore, the electricity generated by the MFC may not be affected by the high ammonium concentration in swine wastewater under long-term continuous flow operation.

6.3.3 COD removal

The efficiency of the MFC in this study for removing COD from swine wastewater was investigated under different operating modes (Figure 6.3). As can be seen from Figure 6.3, COD can be effectively removed (90.86% - 93.51%) in the anode chamber of the MFC when operations remain stable. It was better than that in its open-circuit control system (84.97% - 88.30%) in mode 1. This finding confirmed that the exoelectrogenic bacteria activities were responsible for COD removal in the MFC anode chamber. A slight fall in the COD removal efficiency (81.56% - 87.73%) did occur after adding SMs into the feeding wastewater (Cheng et al., 2018d). According to the review by Cheng et al. (2018d), the reduced removal of COD might due to the stimulation of antibiotics to fermentative or acid-forming bacteria and the inhibition of antibiotics to volatile fatty acids (VFAs) degrading bacteria and acetoclastic methanogens. The MFC demonstrated better buffering capacity and adaptability to SMs than the conventional anaerobic reactor, since a greater reduction in COD removal efficiency was observed in the open-circuit system than in the MFC after the injection of SMs. Wang et al. (2018b) also indicated that the biotoxicity of SMs might be effectively eliminated in the MFC.

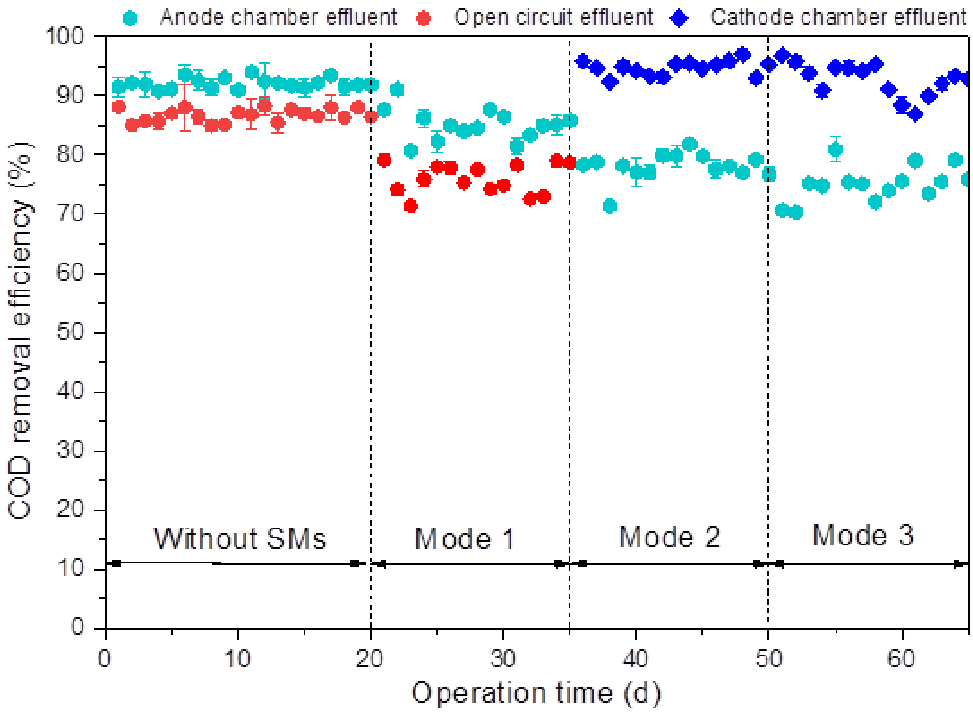

Figure 6.3 COD removal from swine wastewater as MFC operates under different modes with and without SMs present.

In the sequential anode–cathode scenario, COD removal efficiencies in the anode chamber (71.46% - 81.89%) were relatively low in comparison with the previous mode, but their further increase was evident in the following cathode chamber (92.48% - 97.02%). The slight decrease in COD removal in the anode might be attributed to the intrusion of oxygen into the anode chamber, which restricted the activity of anaerobic microorganisms. The overall large removal of COD in the sequential anode-cathode configuration of the MFC was consistent with what previous studies have reported. They stated that COD could be further removed at the cathode compartment by heterotrophic bacteria (Freguia et al., 2008b; Wen et al., 2010). Hence, the enhanced removal of COD in the cathode chamber of MFC in this study confirmed the presence of aerobic heterotrophs.

Although electricity generation was influenced by the sudden increase in NH_4^+-N concentration in the feeding wastewater, the COD removal efficiency was only minimally affected. It maintained a similar range to the last mode in both the anode and cathode chambers (see Figure 6.3). Research by other groups has found similar results. For instance, Ye et al. (2019a) did not observe any reduction in COD removal efficiency in the MFC because the influent concentration of NH_4^+-N increased from 5 to 40 mg/L. Liu et al. (2017) also found little change in COD removal efficiency (from 95.82% to 92.21%) when the NH_4^+-N concentration rose from 45 mg/L to 600 mg/L. It is understood that the effect of NH_4^+-N on COD removal efficiency mainly depends on the concentration of NH_4^+-N, COD/ NH_4^+-N (C/N) ratio and other operating parameters (Kocaturk & Erguder, 2016; Li et al., 2016; Tice & Kim, 2014; Yenigun & Demirel, 2013). As reported by Tice and Kim (2014), COD removal efficiency only started to decline for MFCs when the NH_4^+-N concentration exceeded 1000 mg/L. Yadu et al. (2018) maintained that a substantial removal efficiency of COD (>90%) could be achieved with a high C/N ratio (7–30). Therefore, considering the relatively low concentration of NH_4^+-N (150 mg/L) and high C/N (15:1) ratio in the synthetic swine wastewater used in this study, it is reasonable to observe that only a small fluctuation in COD removal efficiency occurred.

6.3.4 Nutrients removal

Figure 6.4 illustrates the removal of NH_4^+-N and PO_4^{3-}-P in the double-chamber MFC for different operating conditions. Before the injection of SMs into the feeding wastewater, the removal efficiency of NH_4^+-N in the anode chamber of MFC was around 35.13%-37.11% with the initial concentration of 75 mg/L. Previous reports have demonstrated that the potential mechanism for the removal of NH_4^+-N in the anode chamber of MFC was the joint action of electrochemical and biological processes (Kim et al., 2008; Min et al., 2005b). It is obvious that one aspect of ammonium reduction was to synthesize new biomass in the anode chamber. Meanwhile, NH_4^+in the anode chamber can transfer to the cathode through a cation exchange membrane (CEM), which was further converted into NH_3 under alkaline pH and air-breathing conditions in the cathode chamber (Zhou et al., 2015). As reported earlier, the removal of NH_4^+-N in the anode compartment might also occur through nitrification/denitrification and/or the Anammox process (He et al., 2009; Kim et al., 2016). However, NO_3-N or NO_2-N was not detected in the anode chamber of this study, suggesting that the reaction of nitrification/denitrification or Anammox was impossible due to the anaerobic conditions.

This result agrees with the previous report by Tao et al. (2014), who indicated that no nitrifying or anammox bacteria existed in the anode chamber. The removal of PO_4^{3-}-P in this mode was around 37.66% - 44.23% with the initial concentration of 15 mg/L, which was mainly through absorption by the microbial organisms. After the presence of SMs in the anode chamber, a decline in NH_4^+-N and PO_4^{3-}-P removal efficiency in the anode chamber became evident (Figure 6.4). Similar to the effect of SMs on COD removal, the inhibition of SMs to the microorganisms could reduce their uptake to these nutrients. It is notable that the constant voltage generation following the addition of SMs could confirm the stable movement of NH_4^+ between the two chambers (Kim et al., 2008). Therefore, the toxic effect of SMs on the microorganism was the main explanation for the reduced removal of both NH_4^+ and PO_4^{3-}-P in the anode chamber.

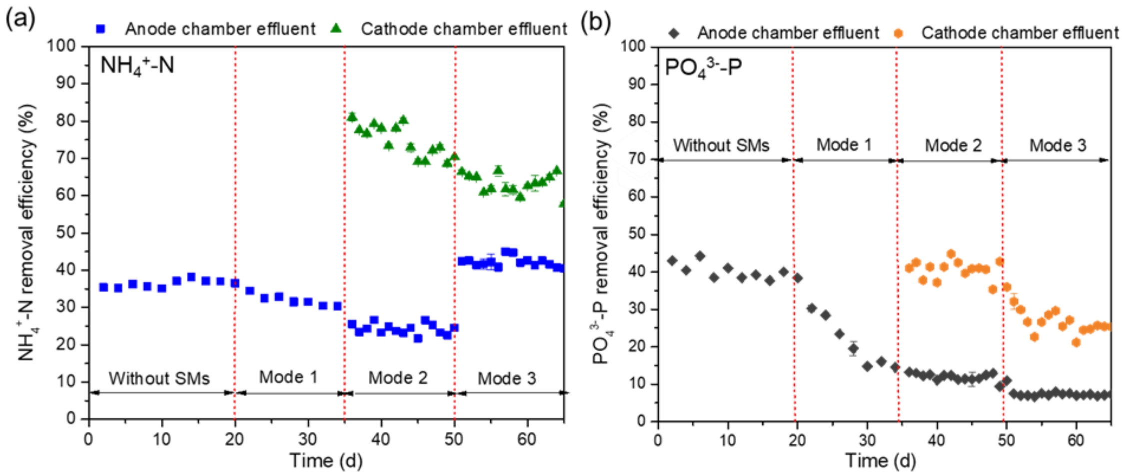

Figure 6.4 Nutrients removal efficiencies as the MFC operated under different modes with and without SMs present: (a) NH_4^+-N removal efficiency; (b) PO_4^{3-}-P removal efficiency.

A decreasing trend was observed for the removal efficiency of NH_4^+-N (21.71% - 26.64%) and PO_4^{3-}-P (9.36% - 13.28%) in the anode chamber under the sequential anode-cathode running mode. According to the above discussion, this may result from the relatively low microbial activities in the anode compartment in comparison with the previous stage. Additionally, the NH_4^+ concentration gradient between the anode and cathode chambers was lower than the last stage due to the residual NH_4^+ in the anode effluent, which limited the ammonium diffusion to the cathode compartment (Ye et al., 2019b). It can be seen from Figure 6.4 that the removal efficiencies of NH_4^+-N and PO_4^{3-}-P in the following cathode chamber further rose to 68.62% - 81.02% and 35.34% - 44.79%, respectively.

It is clear that part of NH_4^+-N and PO_4^{3-}-P could be used as a substrate to maintain the activity of heterotrophic bacteria in the cathode chamber. As well, NH_4^+-N can be reduced by the activity of nitrifiers and AOB, which was suggested by the detected NO^{3-} (1.0-1.6 mg/L) and NO^{2-} (0.06-0.1 mg/L) in the cathode effluent. In addition, the reaction between oxygen and the electrons can form hydroxyl ions, resulting in an increase in the pH value in the cathode compartment. Previous reports have documented that NH_4^+-N and PO_4^{3-}-P can be recovered from swine wastewater by struvite crystallization when the pH rises to between 8 and 10 (Kim et al., 2017; Suzuki et al., 2007). As monitored in this study, the pH value in the cathode chamber ranged from 8.2 to 8.6, which facilitated the removal/recovery of NH_4^+-N and PO_4^{3-}-P through precipitation with magnesium and/or calcium ions in the wastewater (Ye et al., 2019b). Air stripping also contributed to the higher removal efficiency of NH_4^+-N when the aeration condition and high pH value in the cathode chamber were taken into consideration (Ye et al., 2019b).

Interestingly, the removal efficiency of NH_4^+-N in the anode chamber increased to >40% when the initial concentration also increased (see Figure 6.4). This outcome agrees with the study by Ye et al. (2019a), who also found the increased amount of NH_4^+-N being removed was made possible by increasing the NH_4^+-N concentration. On one hand, the more NH_4^+ there was in the anode chamber increased the ammonium concentration gradient between the two chambers, which enhanced its diffusion to the cathode chamber. On the other hand, the high NH_4^+-N concentration might stimulate the microorganisms to take up more NH_4^+-N for their growth. However, the PO_4^{3-}-P removal efficiency in the anode compartment decreased by increasing its initial concentration to 30 mg/L, possibly due to the low absorption capacity of bacteria. As for their removal in the cathode compartment, the simultaneous reduction for the removal of ammonium and phosphate was mainly possible due to the deficiency of magnesium and/or calcium ions in the synthetic swine wastewater, which limited their recovery by precipitation (Kim et al., 2017).

6.4 Conclusion

In this chapter, a continuous flow double - chamber MFC was designed for simultaneously removing antibiotics, organic matter and nutrients from swine wastewater. Results indicated that the removal efficiency of SMs and COD in the MFC was greater than in the open-circuit control system. The sequential anode-cathode operation mode of the MFC performed better than the single continuous mode in terms of pollutants' removal from swine wastewater. A decrease was observed for the removal of pollutants in both anode and cathode chambers in the first few days after the exposure of SMs, which indicated their toxic effect on the microorganisms. Meanwhile, electricity was successfully generated by pumping synthetic swine wastewater to the anode chamber continuously. In contrast, generating electricity under the single continuous mode was more successful than during the sequential anode-cathode mode. The presence of SMs in the MFC revealed little effect on the voltage generation. Interesting, a higher NH_4^+-N concentration could improve the removal of SMs in the cathode chamber, possibly due to the enhanced activity of AOB. Hence, this study found that the continuous flow MFC is feasible for treating swine wastewater that contains antibiotics. Further research is necessary to understand how: firstly, the toxicity of antibiotics can be reduced; and secondly, their removal efficiencies can be improved.

CHAPTER 7

Antibiotics removal from swine wastewater in microbial fuel cell with a new biochar

7.1 Introduction

As one of the common classes of veterinary medicine, sulfonamide antibiotics (SMs) are widely utilized in the swine industry to prevent diseases and promote the growth of pigs (Cheng et al., 2018b). However, high levels of SMs (70% - 90%) were excreted by pigs in unchanged form and/or their metabolites through their faeces and urine (Cheng et al., 2018b). It is no doubt that swine wastewater now constitutes an important source of antibiotics in the environment. As reviewed recently by Cheng et al. (2018b), the concentration of SMs in swine wastewater worldwide was in the 0.005 - 325 μg/L range. In addition, high levels of antibiotics have been detected in the aquatic and terrestrial environments near the swine farms. Occurrence and accumulation of antibiotics in the environment can cause the generation of antibiotic-resistant bacteria and antibiotic-resistant genes, which could seriously endanger the health of people and the eco-environment (Cheng et al., 2019a). Thus, it is urgent to develop appropriate methods to eliminate antibiotics from swine wastewater.

A series of methods including biological treatment, chlorination, ozonation, membrane filtration and adsorption have been investigated for removing SMs from swine wastewater (Cheng et al., 2019a). For practical applications, biological treatment is a preferred technology due to its advantages of being environmentally friendly and cost effective (Grandclément et al., 2017). In recent years, microbial fuel cell (MFC) technology has become a research favorite due to its wastewater treatment ability and simultaneous bioelectricity production. Organic pollutants in wastewater can be converted into electricity by utilizing microorganisms as biocatalysts (Logan et al., 2006). Higher removal efficiency of SMs in closed circuit MFC system than in its corresponding open circuit system has been reported recently (Hu et al., 2020). Although high removal efficiencies of SMs could be achieved in batch running mode of the MFC, their removal in the continuous flow operation of the MFC was limited. According to our pre-experiment, the sequential anode-cathode double-chamber MFC could enhance the removal efficiency of SMs by further aerobic degradation in the cathode chamber. In contrast, the average removal efficiencies for SMX, SDZ and SMZ during the running period were only 53.95%, 15.23% and 18.34%, respectively. The predicated reason for the low removal efficiencies of SMs in the continuous flow MFC system was mainly attributed to: firstly, the inhibition of SMs to the specific SMs degrading microorganisms in both the anode and cathode chambers; and secondly, the retransformation of their degradation products to the parent substance (Tran et al., 2016). For the practical application of the MFC in swine wastewater treatment, it is critical to reduce the antibiotics' effect on the performance of the MFC and enhance the removal efficiency of SMs in the MFC system's continuous flow.

Adsorption is a promising and better choice for the removal of antibiotics from wastewater, due to its advantages of effectiveness, low cost, easy operation and the adsorption process does not produce intermediate products. Several adsorption materials have been investigated for removing antibiotics from aqueous solution, such as activated carbon, carbon nanotubes, natural clay materials, ion exchange materials, and biochar (BC) (Ahmed et al., 2015). Of these materials, BC derived from agricultural wastes has been the subject of enormous research focus, because of its advantages of originating from a wide range of raw materials, low-cost preparation, being environmentally friendly and having adsorption properties (Ahmad et al., 2014; Enders et al., 2012). To date, BC has been used to adsorb antibiotics from wastewater very efficiently (Chen et al., 2019; Dai et al., 2019).

Fruit peels, generated in significant amounts, can be used as raw materials for conversion into biochar through pyrolysis instead of being disposed as waste through landfilling, open burning or composting. Greenhouse gases emission, such as carbon dioxide and methane, from conventional disposal of these wastes is an important issue for global warming. The conversion of such waste to reusable resources is an alternative method for waste management, which achieved net energy production and avoided greenhouse gas emissions. For instance, Sial et al. (2019) indicated that the greenhouse gas emission was significantly reduced by converting orange peels waste to biochar. Pomelo is a very popular fruit and is planted around the world. According to the report by Food and Agriculture Organization (FAO), the global production of grapefruit (including pomelos) was around 9.37 million metric tons in 2018. Pomelo peels which account for nearly 45% of the total weight, are generally treated as agricultural waste, which is not only a waste of resources but also harms the environment (Liang et al., 2014). Compared to most other citrus fruits, pomelo peels are deemed to be promising raw materials for BC production. In addition to their high output, the white floc layer of the pomelo peel contains cellulose and semi-fiber with three-dimensional network structures and various active functional groups, which make the pomelo peel a promising raw material of biochar adsorbent (Song et al., 2019; Zhang et al., 2020). Moreover, the adsorption ability of pomelo peel BC can be enhanced through chemical activation (Romero - Cano et al., 2017).

The surface area and pore size are two important characteristics of biochar, which influence its adsorption capacity to organic pollutants pollutants (Ahmed et al., 2017a; Cheng & Li, 2018). According to previous reports, the carbon having enough pore size was required to create adsorption sites in pores for organic matter (Zhang et al., 2014). However, the surface area and pore size of the originally generated biochar are limited, so further activations are required to increase their surface area and porosity. Thermal treatment and KOH activation are common methods for the production of porous biochar (Zhu et al., 2018a).

Additionally, biochar could be used to support the growth of microorganisms in the bioreactor owing to the porous structure and specific surface area (Shanmugam et al., 2018). The conductive property of biochar makes them a sustainable electrode material in the MFC to enhance electricity production (Huggins et al., 2014). However, a study on the application of biochar in the MFC bioreactor set out to stimulate the removal of SMs for purifying swine wastewater has yet to be done. In the present research, pomelo peel derived biochar was prepared and added to the continuous flow MFC to enhance the removal of SMs from swine wastewater. The characteristics of the produced biochar and the adsorption capacity for SMs were investigated. Moreover, adsorption kinetics and isotherm modes were applied to analyze the adsorption process between the biochar and SMs. Finally, different biochar dosages were added into the anode chamber of the MFC to investigate their potential for improving SMs removal in a continuous flow MFC system. The effect of adding biochar to the MFC in terms of electricity production, COD and nutrients removal from swine wastewater was also examined.

Major parts of Chapter 7 have been published as journal articles in ERA A-rated journal:

Cheng, D., Ngo, H.H., Guo, W., Chang, W.S., Nguyen, D.D., Zhang, X., Varjani, S. and Liu, Y., 2020. Feasibility study on a new pomelo peel derived biochar for tetracycline antibiotics removal in swine wastewater. *Science of The Total Environment*, p.137662.

Cheng, D., Ngo, H.H., Guo, W., W. Chang, S., Nguyen, D.D., Li, J., Ly, W.Q., Nguyen, T.A.N., Tran, V.S., 2020. Applying a new pomelo peel derived biochar in microbial fell cell for enhancing sulfonamide antibiotics removal in swine wastewater, *Bioresource Technology*, p.123886.

7.2 Materials and methods

7.2.1 Materials

The used raw materials of biochar production, chemicals, synthetic swine wastewater and anaerobic sludge in this study is described in Chapter 3, Section 3.2.1 and 3.2.2.

7.2.2 Biochar preparation and modification

Processes and methods applied for biochar preparation and modification is described in Chapter 3, Section 3.3.3 (A).

7.2.3 Experimental setup and operation

Experiments conducted to investigate the adsorption behavior of SMX, SDZ and SMZ onto biochar simultaneously and the application of the biochar in MFC system is described in Chapter 4, Section 3.3.3 (B) and (C).

7.2.4 Analytical methods

The method used for analysing the biochar characteristic is described in Chapter3, Section 3.4.2. The sample analysis and calculation are described in Chapter 3, Section 3.4.1 and 3.4.3, respectively.

7.3. Results and discussion

7.3.1 Characterization of biochar

The biochar yield derived from pomelo peel was 35.93% at 400 ℃ and 32.78% at 600℃, respectively (Table 7.1). Based on a previous review paper, the value is comparable with the yield of biochar produced from other raw materials (Yang et al., 2019b). Like previous reports, a slight decline in the biochar yield was observed by increasing the pyrolysis temperature, which is explained by the loss of volatiles and condensation of aliphatic compounds (Zhao et al., 2017). The high biochar yield is generally considered to be an important factor in practical applications.

Table 7.1 Physicochemical properties of biochars produced under different conditions.

Sample	C (wt %)	O (wt %)	Yield (%)	BET surface area (m^2/g)	Total pore volume (cm^3/g)
BC-400	66.1	14.89	35.93	3.278	0.003
BC-600	72.21	14.01	32.78	27.501	0.021

BC-KOH	86.18	11.81	31.65	2457.367	1.14

The analytical results of EDS indicated that carbon was a dominant element in the produced biochar from pomelo peel. By increasing the pyrolysis temperature and KOH activation, the carbon content increased from 66.1% to 86.18% (by weight), while the oxygen content decreased (Table 7.1). The decrease of oxygen content indicated less oxygen-containing functional groups on the biochar surface, which can be proved by the FTIR analysis results. The FTIR peak of biochars produced in the present study is illustrated in Figure 7.1(A), and the FTIR spectrum curve displays peaks at about 2348, 1560, 1372 and 1168 cm^{-1} for BC-400 and 2348,1623, 1397,1006 863, 831 and 695 cm^{-1} for BC-600. The peak at around 2348 and 2320 cm^{-1} may belong to C ≡C or C ≡N. The peak at approximately 1560 and 1623 cm^{-1} indicates the presence of C=C or C=O stretching in aromatic groups, while the peak at around 1397 and 1372 cm^{-1} may relate to the methyl C-H bending vibration in alkanes and alkyl groups (Uchimiya et al., 2011). The presence of a peak at 1168 and 1064 cm^{-1} possibly contributes to C-O, C-O-C or C-C stretching modes, while peaks at 900-695 cm^{-1} may belong to aromatic C-H bending vibration (Komnitsas & Zaharaki, 2016). Yet, most of the peaks disappeared after KOH activation, which confirmed the finding that functional groups' reduction on the surface of biochar after alkaline modification occurred (Yang et al., 2019b).

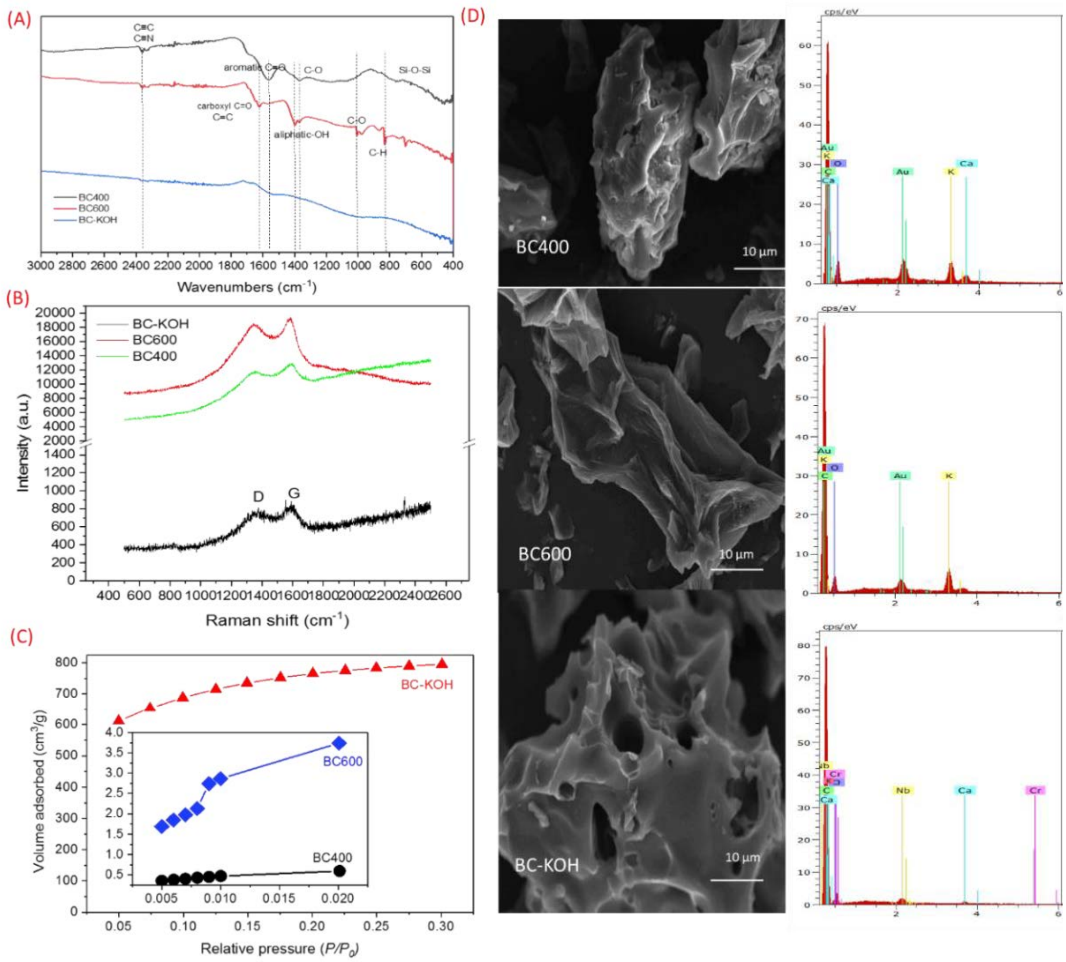

Figure 7.1 Characteristics of biochar produced at 400 ℃ (BC-400), 600 ℃ (BC-600) and activated by KOH (BC-KOH): (A) FTIR spectrum; (B) Raman spectrum; (C) BET surface area isotherm; (D) SEM-EDS spectrum.

Raman spectrum of the BC-400, BC-600 and BC-KOH are depicted in Figure 7.1 (B). The Raman spectrum of the biochars consists of two prominent peaks at 1350 cm^{-1} (D) and 1590 cm^{-1} (G), which represented the graphitic lattice vibration mode and disorder in the graphitic structure of the biochar (Zhu et al., 2014). Specifically, peak D refers to disordered sp^2-hybridized carbon atoms with vacancies and impurities, while peak G causes from the stretching of sp^2 atomic pairs in the carbon atom ring or carbon chain. It can be assumed that the carbon atom is bonded to a sp^2 hybridized covalent bond, while electrons which are not involved in hybridization form a π bond (Fan et al., 2016).

The BET surface area and total pore size volume of the produced biochar are shown in Table 7.1 and Figure 7.1(C). An increase in the BET surface area and total pore volume were observed by increasing the pyrolysis temperature from 400 to 600 ℃. A similar result has been reported in other recent studies (Zhao et al., 2018; Zhao et al., 2017). They explained that the increase in the surface area and pore volumes might be caused by the quick release of H_2 and CH_4 and the reaction of aromatic condensation when the temperature rose from 400 to 600 ℃. As well, when the temperature increased, the formation of more pores and a larger surface area was due to the release of more volatiles from the biomass surface (Cheng & Li, 2018). When the BC-400 was further activated by KOH, a huge increase in the surface area (2457.367 m^2/g) and total pore volumes (1.14 cm^3/g) was obtained. This finding was consistent with the observation of the SEM analysis.

The microstructure of BC-400, BC-600 and BC-KOH studied by SEM is displayed in Figure 7.1 (D). It can be observed that the BC-400 and BC-600 have a relatively smooth and dense surface. A clear pore structure can be seen on the surface of the BC-KOH, indicating an enhancement in exposed surface area and pore size. As mentioned earlier, the interaction between alkali compounds and carbon promotes the formation of a large surface area (Otawa et al., 1990). For instance, Zhang et al. (2015a) produced a highly porous activated carbon from petroleum coke via the KOH activation process at 800 ℃, with a large surface area (2800 - 2900 m^2/g) and total pore volume (1.4 - 2.1 cm^3/g). Similarly, Yang et al. (2019a) employed KOH to activate willow branch-derived biochar at 850 ℃ and achieved a large surface area (3342 m^2/g) and total pore volume (1.912 cm^3/g). The increase in the surface area and pore size of biochar by KOH activation is a synergistic effect of several factors (Cheng & Li, 2018). The chemical reaction for the process of KOH activation is shown in Eq. (7.1 - 7.4):

$$6KOH + 2C \rightarrow 2K + 3H_2 \uparrow + 2K_2CO_3 \tag{7.1}$$

$$6KOH + CO_2 \rightarrow K_2CO_3 + H_2O \uparrow \tag{7.2}$$

$$K_2CO_3 + 2C \rightarrow K_2O + 2CO \uparrow \tag{7.3}$$

$$K_2O + CO \rightarrow 2K + CO \uparrow \tag{7.4}$$

Through the above chemical reactions, the biochar can be activated through the etching process by KOH and its intermediates K_2CO_3 and K_2O. The H_2 and CO produced from chemical reactions can promote the formation of microporosity and macropores on the biochar (Yang et al., 2019a). Furthermore, the produced alkali metal can insert into the biochar matrix to extend the biochar lattice and then enlarge the existing pores (Cheng & Li, 2018). It is noted that the adsorption capacity of biochar is positive with the surface area/total pore volume. Therefore, BC-KOH emerged as the best biochar for adsorbing antibiotics and subsequently chosen for further research.

7.3.2 Adsorption behavior of SMs onto biochar

The adsorption capacities of SMX, SMZ and SDZ onto BC-KOH are displayed in Figure 7.2 (a). As shown in Figure 7.2 (a), the adsorption can reach equilibrium in 540 min, and a rapid adsorption could occur in 60 min. The equilibrium adsorption capacities of SMX, SMZ and SDZ onto the biochar were around 831.85, 857.39 and 671.32 μg/g, respectively, which proved the effective removal of SMs from water solution by biochar adsorption. By comparing the adsorption kinetic parameters, the pseudo-second order mode emerged as being more appropriate for describing the adsorption process with: firstly, a high correlation coefficients value (R^2=1); and secondly, consistency between the calculated (qe^c) and experimental adsorption capacities (qe^b) for SMs onto the biochar (Figure 7.2 (a) and Table 7.2). These agreed with previous studies on the adsorption of SMs onto other types of biochar (Ahmed et al., 2017b). It further indicated that the adsorption rate was determined by the square of the adsorption site vacancies on the biochar surface and the adsorption process was controlled by chemisorptive mechanisms.

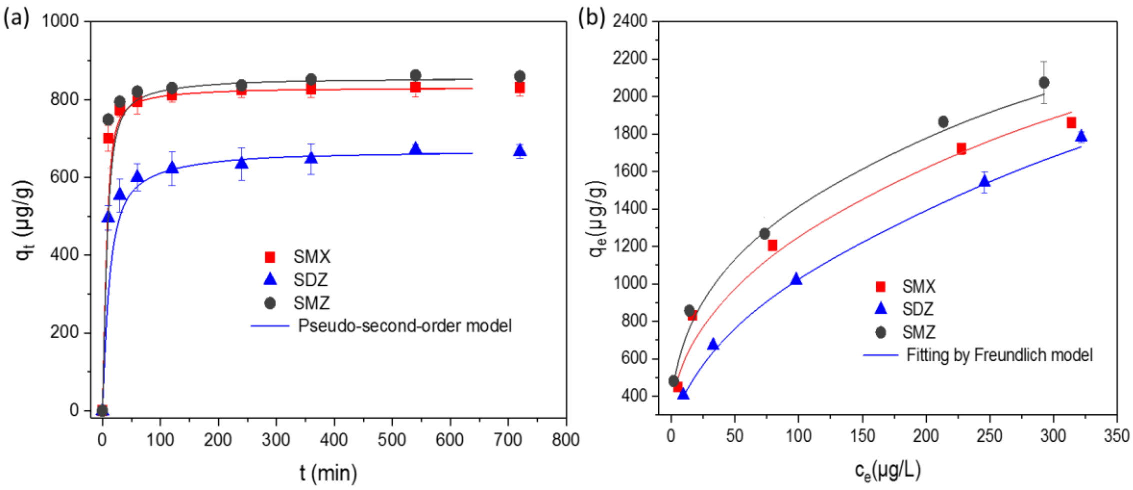

Figure 7.2 The adsorption kinetic (a) and isotherm (b) of SMX, SMZ and SDZ onto biochar

Table 7.2 Sorption coefficients of SMs onto biochar evaluated by Pseudo second-order and Freundlich models

	SMX	SDZ	SMZ
q_e^b	831.85	671.32	857.39
Pseudo second-order model			
		$q_t = \frac{k_2 q_e^2 t}{1 + k_2 q_e t}$	
q_e^c	833.95	673.58	864.01
K_2	4.69×10^{-4}	1.73×10^{-4}	3.09×10^{-4}
R^2	1	1	1
Freundlich model			
		$q_e = K_F c_e^{1/n}$	
K_f	288.04	157.17	396.35
1/n	0.33	0.42	0.29
R^2	0.981	0.998	0.996

Figure 7.1 (b) shows the adsorption isotherm of SMs onto the BC-KOH. Comparatively, the experimental data exhibited a higher correlation with the Freundlich mode with the correlation coefficients R^2 ranging from 0.981 to 0.998, indicating that the adsorption process probably took place on the biochar's heterogeneous surfaces. As shown in Table 7.1, the 1/n value (<1) suggested that the adsorption of SMs onto the biochar was a favorable process. Considering the high surface area and pore volume of BC-KOH used in the present study, the favorable adsorption between SMs and the biochar could be caused by the pore-filling effect. According to the Raman analysis results, graphitic layers did exist in the structure of the biochar, which can function as a π-π acceptor (Jin et al., 2014). The π-π electron donor acceptor (EDA) interaction was also a potential mechanism for the biochar's adsorption of SMs, due to the graphitic surface of the biochar and the amino functional group of SMs (Peiris et al., 2017).

7.3.3 Economic feasibility of the biochar application in the MFC

To assess the large-scale application of biochar on the adsorption removal of antibiotics from swine wastewater, the economic performance of biochar compared with activated carbon was considered in this study. The production cost of biochar mainly relates to the cost of feedstock materials, production cost and other additional cost, which can be estimated and calculated by Eq. (7.5):

$$\text{Total biochar production cost} = \text{feedstock cost} + \text{production cost} \quad (7.5)$$

Based on this study, the total cost of producing 1 kg pomelo peel derived biochar (BC-KOH) was estimated as follows:

The feedstock cost is USD $ 5.69 (including pomelo peels collection cost and KOH cost). The production cost is USD$ 2.42 (including the cost of electricity consumption for drying and carbonization). The cost for others such as washing, grinding and transporting is USD $ 1.71. Therefore, the total cost for generating per kg BC-KOH was USD $ 9.82, which is cheaper than commercial activated carbon (up to USD $45.71/kg) as sold in Henan Huasheng Charcoal Industry Ltd. (http://hnhsty.com/).

Commercial activated carbons are commonly produced from expensive and non-renewable materials, such as natural coal, wood, peat and petroleum residues, by pyrolysis and the following physical activation at high temperatures (700 - 1100℃) (Allen et al., 1998). From this point of view, using pomelo peel wastes as the raw material to produce biochar in this study not only save the cost of raw materials but also save the cost of waste disposal. From the perspective of energy saving, chemical activation is commonly chosen for activating the carbon material due to the lower temperature and shorter time requirement than that of physical activation (Mohammad-Khah & Ansari, 2009). The common method of chemical activation was using chemicals such as H_3PO_4, KOH, or NaOH followed by heating under a gas (nitrogen) flow at the temperature between 450 and 900◦C (Gupta et al., 2009). Thus, less energy would be consumed for the production of the biochar in this study at lower temperature.

7.3.4 Removal of SMs in the MFC with the biochar dosage

The overall removal of SMs during the MFC operating period with the addition of different concentrations of biochar is depicted in Figure 7.3 (a). According to the pre-experiment, the removal efficiencies of SMX, SDZ and SMZ in the MFC without the addition of biochar were 49.35% - 59.37 %, 13.98% - 16.31% and 16.75% - 19.45%, respectively. By adding 100 and 200 mg/L of biochar in the anode chamber, the SMX removal efficiencies rose to 55.06% - 68.73% and 71.58% - 85.53% respectively. In contrast, a higher and more stable removal efficiency of SMX (82.44 - 88.15%) could be achieved when a further increase in the biochar concentration to 500 mg/L occurred, which was comparable with SMX removal in other biological processes. For instance, as reviewed by Chen and Xie (2018), the removal efficiency of SMX was around -463 - 72% in various full-scale wastewater treatment plants, 90% - 100% in MBR, 0 - 75% in lab-scale activated sludge systems, and 34.4% in a sulfate-reducing up-flow sludge bed reactor.

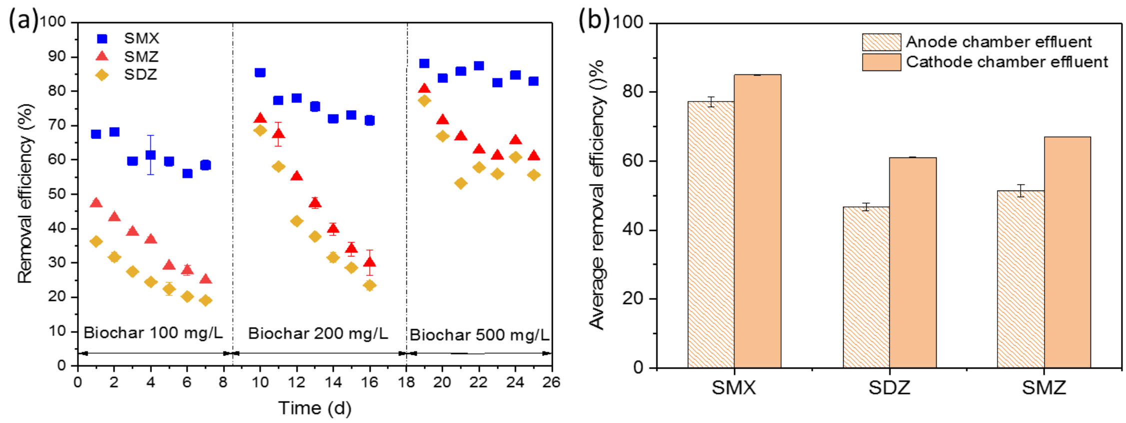

Figure 7.3 (a) the removal efficiency of SMs in MFC with the addition of different concentrations of biochar; (b) the average removal efficiency of SMs in the anode and cathode chamber of MFC, respectively (500 mg/L of biochar).

In addition, the removal efficiency of SDZ and SMZ did largely improve in the present study (Figure 7.3 (a)), although their removal efficiencies also gradually decreased as the system ran by adding 100 and 200 mg/L of biochar. It is noted that the accumulation of SDZ and SMZ in the reactor was alleviated by the dosage of 500 mg/L biochar, with relatively high removal efficiencies for SDZ (53.40% - 77.53%) and SMZ (61.12% - 80.68%). This result suggested that the removal efficiency of SMs in the MFC could be greatly improved when the addition of biochar in the anode chamber reached a certain level. The enhanced removal of SMs in the MFC with biochar added was partly due to the adsorption of SMs onto the biochar, considering the favorable adsorption process as mentioned in section 3.1. Additionally, the porous biochar used in this study could act as a microbial carrier, which could form a biofilm on the surface of biochar as time passes (Sunyoto et al., 2016). This phenomenon could enhance the activity of microorganisms and then improve the removal of SMs through biodegradation (Mumme et al., 2014). The inhibitive effect of SMs on microorganisms can be reduced due to the fast adsorption of SMs onto the biochar as observed in the above batch adsorption experiment. Consequently, it is important to investigate the relationship between the biochar and the activity of microorganisms.

During the stable operating period of the MFC with the dosage of biochar (500 mg/L), the respective contribution of the anode and cathode compartments of the MFC to the removal of SMs from swine wastewater was monitored and presented in Figure 7.3 (b). As observed in Figure 7.3 (b), SMs could be degraded in both the anode and cathode chambers. In the cathode chamber, the removal of SMs through air stripping and volatilization was impossible due to their polarity and very low Henry constants, suggesting that the further removal of SMs in the cathode chamber was attributed to aerobic degradation (Li & Zhang, 2010). This finding agrees with previous analyses on the biodegradability of SMs in both aerobic and anaerobic conditions (Chen & Xie, 2018). In the present study, the average removal efficiencies of SMX, SDZ and SMZ in the anode chamber with the addition of biochar (500 mg/L) were 77.21%, 46.7% and 51.78%, respectively, which accounts for the major part of their total removal. These figures confirmed the positive effect of adding biochar for removing SMs in the MFC.

7.3.5 Performance of the MFC with biochar addition

The average daily voltage generation in the MFC with different concentrations of biochar has been presented in Figure 7.4 (a). It was observed that the voltage production was stable during the operational period and enhanced by increasing the biochar concentration from 100 mg/L to 500 mg/L. The highest daily average voltage generated in in this study was 650 mv when the biochar concentration reached 500 mg/L in the MFC, which was comparable to previous reports about the electricity produced from swine wastewater in MFCs (Min et al., 2005a). Any improvement in electricity generation in the MFC with the presence of biochar might be due to biofilm formation on the surface of biochar and the increase in conductivity of electrodes' surfaces in the MFC (Ayyappan et al., 2018; Hejazi et al., 2019).

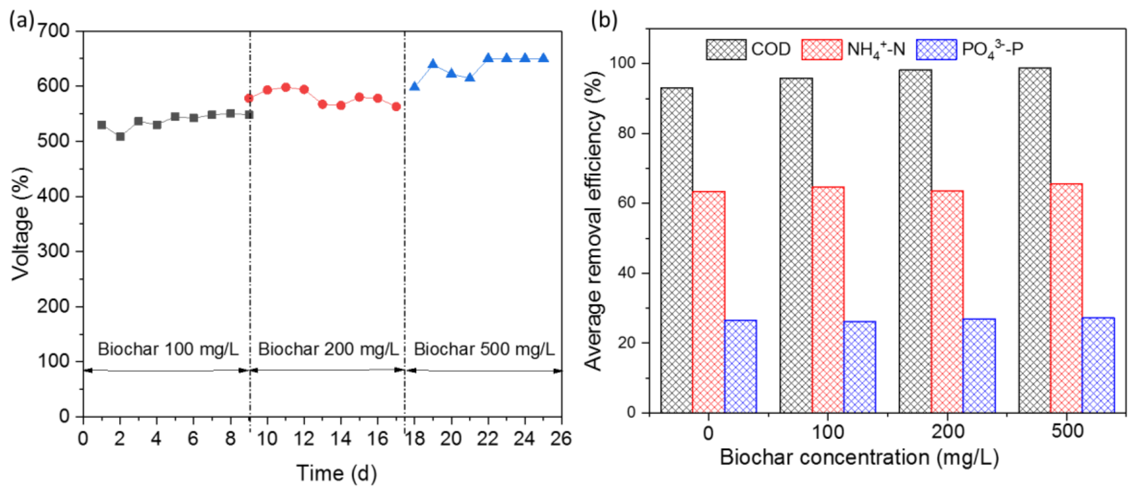

Figure 7.4 (a) the average daily voltage generation and (b) COD and nutrients removal efficiency in MFC with different concentrations of biochar.

The organic matter and nutrients removal efficiency was monitored during the

MFC's operating period. As shown in Figure 7.4 (b), COD could be effectively removed and the average removal efficiency of COD rose from 93.08% to 95.88% by dosing 100 mg/L of biochar in the reactor, which was further increased to > 98% when 200 mg/L and 500 mg/L of biochar were added. By contrast, the removal of nutrients in the MFC revealed little change when the concentrations of biochar were 100 mg/L and 200 mg/L in the reactor, which then slightly increased after the dosage of biochar increased to 500 mg/L. From Figure 7.4 (b), it is observed that the average removal efficiencies of NH_4^+-N and PO_4^{3-}-P increased from 63.33% to 65.67% and 26.08% to 27.19% after the addition of 500 mg/L of biochar. This small increase in the removal of COD and nutrients could be attributed to the absorption removal of microbial inhibitors (SMs), which further enhanced the ability of microorganisms to absorb more carbon sources and nutrients (Masebinu et al., 2019). The similarity of adding biochar to the performance of bioprocesses was also noted by dosing the apple tree-derived biochar into activated sludge processes (Kim et al., 2020). Accordingly, the removal of NH_4^+-N and PO_4^{3-}-P in the sequential anode-cathode operating mode of the MFC was mainly due to the recovery through air stripping and struvite crystallization under an alkaline environment (pH = 8.2 - 8.6) in the cathode chamber (Ye et al., 2019b). It is found in this study that the addition of biochar in the MFC system has little effect on the recovery of nutrients from swine wastewater.

7.4 Conclusion

The pomelo peel-derived biochar employed in this study is a cost-effective and promising adsorbent and biocarrier in the continuous flow MFC to efficiently enhance the removal of SMs from swine wastewater. Adsorption kinetic and isotherm results suggested that SMs were absorbed on the heterogeneous surfaces of the biochar thorough pore-filling and π-π EDA interaction mechanisms. The removal efficiency of SMs can be improved by the dosage of biochar in the MFC, probably due to the combined effect of adsorption and biodegradation. The essential performance of the MFC remained stable and improved slightly with a direct dose of biochar into the MFC.

CHAPTER 8

Conclusions and recommendations

8.1 Conclusions

To minimize adverse effects of antibiotics in swine wastewater on the environment and human health, a series of experiments was carried out for: 1) the removal process and mechanism of antibiotics in anaerobic sludge that used in the anode chamber of MFC; 2) the feasibility of a double-chamber MFC for removing antibiotics from swine wastewater; 3) the performance of a continual flow MFC for treating swine wastewater containing antibiotics; and 4) the optimization of the continual flow MFC for enhancing antibiotics' removal were conducted in this study. The key conclusions are as follows:

In the reactor with anaerobic sludge, tetracycline antibiotics (TCs) were removed by the adsorption of the sludge, while the removal of sulfonamide antibiotics (SMs) were mainly attributed to biodegradation. The adsorption of TCs could be described by the pseudo-second kinetic mode and the Freundlich isotherm mode, which indicated that the adsorption is a heterogeneous chemisorption process. Among the studied SMs (sulfamethoxazole (SMX), sulfamethazine (SMZ) and sulfadiazine (SDZ)), SMX showed the fastest and highest removal in the anaerobic reactor. The degradation of these SMs were better fitted by the first-order kinetic model. The presence of certain concentrations of the easily degradable organic matter in the anaerobic reactor could enhance the removal efficiency of SMs, suggesting the cometabolism removal mechanism of SMs in anaerobic processes. Specific microbial communities in anaerobic sludge were responsible for the degradation of SMs. The activity of anaerobic microorganisms might be inhibited by increasing the concentration of SMs from 100 μg/L to 300 μg/L, while the tolerance of anaerobic sludge with the exposure of antibiotics increased by prolonging the contact time.

Higher and faster removal of SMs and COD were achieved in the MFC in comparison with their removal in the conventional anaerobic reactor, indicating that the MFC has high potential for treating swine wastewater containing antibiotics. Moreover, the removal efficiency of COD in the MFC was little affected by the presence of SMs, which revealed that the MFC system has a higher tolerance to toxicants in the wastewater. Meanwhile, electricity could be generated stably by feeding with synthetic swine wastewater in the anode chamber of MFC. Further increasing of the voltage output was observed with the addition of SMs in the wastewater, due to the improvement of the activity of exoelectrogenic bacteria after a period of domestication and or the enhanced ability of electrons transfer from microbe cell to the anode.

For large-scale application, the performance of a continuous flow double-chamber MFC was exploded in terms of simultaneous removal of antibiotics, organic matter and nutrients from swine wastewater. The MFC system was operated under single continuous mode and sequential anode-cathode operation mode, respectively. In the sequential anode-cathode operating mode of the MFC, higher removal efficiencies of the above mentioned pollutants was achieved than those in the single continuous MFC, due to their further removal in the cathode chamber. The toxic effect of SMs on the microorganism was observed in the first few days after adding SMs in the MFC. Whereas, the electricity generated in the single continuous operating MFC was higher in comparison with the generation in the sequential anode-cathode mode, which was little effected by the presence of SMs. Moreover, the relative high concentration of NH_4^+-N in the cathode chamber of the sequential anode-cathode operating of the MFC showed positive effect on the removal of SMs by enhancing the activity of ammonia-oxidizing bacteria. Therefore, the MFC operated under the sequential anode-cathode operation mode has high potential for treating swine wastewater, but the removal efficiency of antibiotics has to be further improved.

To improve the removal efficiency of antibiotics from swine wastewater in the continuous flow MFC, a new pomelo peel derived biochar was produced in this study and added in the anode chamber of the MFC as a promising adsorbent and biocarrier. The biochar has large surface area (2457.37 m^2/g) and total pore volume (1.14 cm^3/g) by the activation of KOH. The adsorption results indicated that SMs could be effectively absorbed on the heterogeneous surfaces of the biochar thorough the mechanism of pore-filling and π-π EDA interaction. The addition of biocahr in the MFC could enhance the removal efficiency of SMs to 82.44 - 88.15%, 53.40 - 77.53% and 61.12 - 80.68%, respectively, probably due to the combined effect of adsorption and biodegradation. Meanwhile, the electricity production, COD and nutrients removal of the MFC was stable and slightly improved with the direct dosage of biochar into MFC. Hence, the combination of biochar with MFC was an effective method for the treatment of swine wastewater with antibiotics.

8.2 Recommendations

The application of the integrated system of biochar and MFC for treating swine wastewater containing antibiotics has been comprehensively investigated in this study. Some recommendations for the future study are summarized below:

1) Identify the specific microorganisms in both anode and cathode chambers of the biochar - MFC which are responsible for the degradation of antibiotics to enrich and domesticate the special strains to further improve the removal efficiency of antibiotics;

2) Investigate the co-existence of antibiotics and other contaminants in swine wastewater, such as heavy metals, hormones and other types of antibiotics which may affect the removal efficiency of antibiotics in the biochar - MFC system;

3) Explore the accumulation and transfer of antibiotic resistance genes (ARGs) in the biochar - MFC system, as well as the concentration of ARGs in the effluent of the bioahcr - MFC.

4) Optimize the production process to further reduce production costs of the biochar;

5) Develop biochar based electrode to improve antibiotic removal from swine wastewater; and

6) Conduct the pilot-scale MFC experiments using real swine wastewater to achieve the practical application.

References

References

1) Aghababaie, M., Farhadian, M., Jeihanipour, A., Biria, D. 2015. Effective factors on the performance of microbial fuel cells in wastewater treatment–a review. *Environmental Technology Reviews*, **4**(1), 71-89.
2) Ahmad, M., Rajapaksha, A.U., Lim, J.E., Zhang, M., Bolan, N., Mohan, D., Vithanage, M., Lee, S.S., Ok, Y.S. 2014. Biochar as a sorbent for contaminant management in soil and water: a review. *Chemosphere*, **99**, 19-33.
3) Ahmed, M.B., Zhou, J.L., Ngo, H.H., Guo, W. 2015. Adsorptive removal of antibiotics from water and wastewater: progress and challenges. *Science of the Total Environment*, **532**, 112-126.
4) Ahmed, M.B., Zhou, J.L., Ngo, H.H., Guo, W., Johir, M.A.H., Belhaj, D. 2017a. Competitive sorption affinity of sulfonamides and chloramphenicol antibiotics toward functionalized biochar for water and wastewater treatment. *Bioresource Technology*, **238**, 306-312.
5) Ahmed, M.B., Zhou, J.L., Ngo, H.H., Guo, W., Johir, M.A.H., Sornalingam, K. 2017b. Single and competitive sorption properties and mechanism of functionalized biochar for removing sulfonamide antibiotics from water. *Chemical Engineering Journal*, **311**, 348-358.
6) Ahmed, M.J. 2017. Adsorption of quinolone, tetracycline, and penicillin antibiotics from aqueous solution using activated carbons. *Environmental Toxicology and Pharmacology*, **50**, 1-10.
7) Al Lawati, M.J., Jafary, T., Baawain, M.S., Al-Mamun, A. 2019. A mini review on biofouling on air cathode of single chamber microbial fuel cell; prevention and mitigation strategies. *Biocatalysis and Agricultural Biotechnology*, 101370.
8) Allen, S., Whitten, L., McKay, G. 1998. The production and characterisation of activated carbons: a review. *Developments in Chemical Engineering and Mineral Processing*, **6**(5), 231-261.
9) Álvarez, J.A., Otero, L., Lema, J., Omil, F. 2010a. The effect and fate of antibiotics during the anaerobic digestion of pig manure. *Bioresource Technology*, **101**(22), 8581-8586.
10) Álvarez, J.A., Otero, L., Lema, J.M., Omil, F. 2010b. The effect and fate of antibiotics during the anaerobic digestion of pig manure. *Bioresource Technology*, **101**(22), 8581-8586.

11) Alvarino, T., Suarez, S., Lema, J., Omil, F. 2014. Understanding the removal mechanisms of PPCPs and the influence of main technological parameters in anaerobic UASB and aerobic CAS reactors. *Journal of Hazardous Materials*, **278**, 506-513.
12) Amari, S., Vahdati, M., Ebadi, T. 2015. Investigation into effects of cathode aeration on output current characteristics in a tubular microbial fuel cell. *International Journal of Environmental Science and Technology*, **12**(12), 4037-4042.
13) Angelidaki, I., Ellegaard, L., Ahring, B.K. 2003. Applications of the anaerobic digestion process. in: *Biomethanation II*, Springer, pp. 1-33.
14) Angenent, L.T., Mau, M., George, U., Zahn, J.A., Raskin, L. 2008. Effect of the presence of the antimicrobial tylosin in swine waste on anaerobic treatment. *Water Research*, **42**(10), 2377-2384.
15) Anthony A, A., Adekunle C, F., Thor A, S. 2018. Residual antibiotics, antibiotic resistant superbugs and antibiotic resistance genes in surface water catchments: Public health impact. *Physics and Chemistry of the Earth*, **105**, 177-183.
16) Ayawei, N., Ebelegi, A.N., Wankasi, D. 2017. Modelling and interpretation of adsorption isotherms. *Journal of Chemistry*, **2017**, Article ID 3039817.
17) Ayyappan, C.S., Bhalambaal, V., Kumar, S. 2018. Effect of biochar on bio-electrochemical dye degradation and energy production. *Bioresource Technology*, **251**, 165-170.
18) Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, **57**, 233-266.
19) Barber, W.P., Stuckey, D.C. 1999. The use of the anaerobic baffled reactor (ABR) for wastewater treatment: a review. *Water Research*, **33**(7), 1559-1578.
20) Barret, M., Barcia, G.C., Guillon, A., Carrère, H., Patureau, D. 2010. Influence of feed characteristics on the removal of micropollutants during the anaerobic digestion of contaminated sludge. *Journal of Hazardous Materials*, **181**(1-3), 241-247.
21) Ben, W., Qiang, Z., Pan, X., Chen, M. 2009. Removal of veterinary antibiotics from sequencing batch reactor (SBR) pretreated swine wastewater by Fenton's reagent. *Water Research*, **43**(17), 4392-4402.
22) Ben, W., Qiang, Z., Pan, X., Nie, Y. 2011. Degradation of veterinary antibiotics

by ozone in swine wastewater pretreated with sequencing batch reactor. *Journal of Environmental Engineering*, **138**(3), 272-277.

23) Ben, W., Qiang, Z., Yin, X., Qu, J., Pan, X. 2014. Adsorption behavior of sulfamethazine in an activated sludge process treating swine wastewater. *Journal of Environmental Sciences*, **26**(8), 1623-1629.

24) Boonsaner, M., Hawker, D.W. 2010. Accumulation of oxytetracycline and norfloxacin from saline soil by soybeans. *Science of the Total Environment*, **408**(7), 1731-1737.

25) Brandt, K.K., Amézquita, A., Backhaus, T., Boxall, A., Coors, A., Heberer, T., Lawrence, J.R., Lazorchak, J., Schönfeld, J., Snape, J.R. 2015. Ecotoxicological assessment of antibiotics: a call for improved consideration of microorganisms. *Environment International*, **85**, 189-205.

26) Broll, S., Kietzmann, M., Bettin, U., Kreienbrock, L. 2004. The use of sulfonamides and sulfonamide/trimethoprim combinations as animal feed drugs for pigs in Schleswig-Holstein. *Berliner und Munchener Tierarztliche Wochenschrift*, **117**(9-10), 392-397.

27) Cao, X., Song, H.-l., Yu, C.-y., Li, X.-n. 2015. Simultaneous degradation of toxic refractory organic pesticide and bioelectricity generation using a soil microbial fuel cell. *Bioresource Technology*, **189**, 87-93.

28) Carballa, M., Omil, F., Lema, J.M., Llompart, M.a., García-Jares, C., Rodríguez, I., Gomez, M., Ternes, T. 2004. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. *Water Research*, **38**(12), 2918-2926.

29) Carballa, M., Omil, F., Ternes, T., Lema, J.M. 2007. Fate of pharmaceutical and personal care products (PPCPs) during anaerobic digestion of sewage sludge. *Water Research*, **41**(10), 2139-2150.

30) Carvalho, P.N., Araújo, J.L., Mucha, A.P., Basto, M.C.P., Almeida, C.M.R. 2013. Potential of constructed wetlands microcosms for the removal of veterinary pharmaceuticals from livestock wastewater. *Bioresource Technology*, **134**, 412-416.

31) Chelliapan, S., Wilby, T., Sallis, P.J. 2006. Performance of an up-flow anaerobic stage reactor (UASR) in the treatment of pharmaceutical wastewater containing macrolide antibiotics. *Water Research*, **40**(3), 507-516.

32) Chen, J., Liu, Y.S., Zhang, J.N., Yang, Y.Q., Hu, L.X., Yang, Y.Y., Zhao, J.L., Chen, F.R., Ying, G.G. 2017. Removal of antibiotics from piggery wastewater by

biological aerated filter system: Treatment efficiency and biodegradation kinetics. *Bioresource Technology*, **238**, 70-77.

33) Chen, J., Xie, S. 2018. Overview of sulfonamide biodegradation and the relevant pathways and microorganisms. *Science of the Total Environment*, **640**, 1465-1477.

34) Chen, Y., Cheng, J.J., Creamer, K.S. 2008. Inhibition of anaerobic digestion process: a review. *Bioresource Technology*, **99**(10), 4044-4064.

35) Chen, Y., Shi, J., Du, Q., Zhang, H., Cui, Y. 2019. Antibiotic removal by agricultural waste biochars with different forms of iron oxide. *RSC Advances*, **9**(25), 14143-14153.

36) Chen, Y., Zhang, H., Luo, Y., Song, J. 2012. Occurrence and dissipation of veterinary antibiotics in two typical swine wastewater treatment systems in east China. *Environmental Monitoring and Assessment*, **184**(4), 2205-2217.

37) Chen, Z., Su, H., Hu, D., Jia, F., Li, Z., Cui, Y., Ran, C., Wang, X., Xu, J., Xiao, T. 2018. Effect of organic loading rate on the removal of DMF, MC and IPA by a pilot-scale AnMBR for treating chemical synthesis-based antibiotic solvent wastewater. *Chemosphere*, **198**, 49-58.

38) Chen, Z., Wang, H., Chen, Z., Ren, N., Wang, A., Shi, Y., Li, X. 2011. Performance and model of a full-scale up-flow anaerobic sludge blanket (UASB) to treat the pharmaceutical wastewater containing 6-APA and amoxicillin. *Journal of Hazardous Materials*, **185**(2-3), 905-913.

39) Cheng, D., Ngo, H., Guo, W., Chang, S., Nguyen, D., Kumar, S.M., Du, B., Wei, Q., Wei, D. 2018a. Problematic effects of antibiotics on anaerobic treatment of swine wastewater. *Bioresource Technology*, **263**, 642-653.

40) Cheng, D., Ngo, H., Guo, W., Liu, Y., Zhou, J., Chang, S., Nguyen, D., Bui, X., Zhang, X. 2018b. Bioprocessing for elimination antibiotics and hormones from swine wastewater. *Science of the Total Environment*, **621**, 1664-1682.

41) Cheng, D., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Liu, Y., Shan, X., Nghiem, L.D., Nguyen, L.N. 2020. Removal process of antibiotics during anaerobic treatment of swine wastewater. *Bioresource Technology*, **300**, 122707.

42) Cheng, D., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Liu, Y., Wei, Q., Wei, D. 2019a. A critical review on antibiotics and hormones in swine wastewater: Water pollution problems and control approaches. *Journal of Hazardous Materials*, 121682.

43) Cheng, D., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Liu, Y., Zhang, X.,

Shan, X., Liu, Y. 2019b. Contribution of antibiotics to the fate of antibiotic resistance genes in anaerobic treatment processes of swine wastewater: a review. *Bioresource Technology*, 122654.

44) Cheng, D., Ngo, H.H., Guo, W., Liu, Y., Chang, S.W., Nguyen, D.D., Nghiem, L.D., Zhou, J., Ni, B. 2018c. Anaerobic membrane bioreactors for antibiotic wastewater treatment: performance and membrane fouling issues. *Bioresource Technology*,

45) Cheng, D.L., Ngo, H.H., Guo, W.S., Chang, S.W., Nguyen, D.D., Kumar, S.M., Du, B., Wei, Q., Wei, D. 2018d. Problematic effects of antibiotics on anaerobic treatment of swine wastewater. *Bioresource Technology*, **263**, 642-653.

46) Cheng, F., Li, X. 2018. Preparation and application of biochar-based catalysts for biofuel production. *Catalysts*, **8**(9), 346.

47) Cheng, J., Liu, B. 2002. Swine wastewater treatment in anaerobic digesters with floating medium. *Transactions of the ASAE*, **45**(3), 799-805.

48) Choi, K.J., Kim, S.G., Kim, S.H. 2008. Removal of antibiotics by coagulation and granular activated carbon filtration. *Journal of Hazardous Materials*, **151**(1), 38-43.

49) Conkle, J.L., Lattao, C., White, J.R., Cook, R.L. 2010. Competitive sorption and desorption behavior for three fluoroquinolone antibiotics in a wastewater treatment wetland soil. *Chemosphere*, **80**(11), 1353-1359.

50) Cycoń, M., Mrozik, A., Piotrowska-Seget, Z. 2019. Antibiotics in the Soil Environment—Degradation and Their Impact on Microbial Activity and Diversity. *Frontiers in Microbiology*, **10**(338).

51) Dai, Y., Zhang, N., Xing, C., Cui, Q., Sun, Q. 2019. The adsorption, regeneration and engineering applications of biochar for removal organic pollutants: A review. *Chemosphere*, **223**, 12-27.

52) de Carvalho, P.N. 2012. Implementation of methodologies for removal of veterinary pharmaceuticals residues from WWTPs effluents of the livestock industry[PhD]. *University of Porto, Porto*.

53) de Cazes, M., Abejón, R., Belleville, M.-P., Sanchez-Marcano, J. 2014a. Membrane bioprocesses for pharmaceutical micropollutant removal from waters. *Membranes*, **4**(4), 692-729.

54) De Cazes, M., Belleville, M.P., Petit, E., Llorca, M., Rodríguez-Mozaz, S., De Gunzburg, J., Barceló, D., Sanchez-Marcano, J. 2014b. Design and optimization

of an enzymatic membrane reactor for tetracycline degradation. *Catalysis Today*, **236**, 146-152.

55) De Wever, H., Weiss, S., Reemtsma, T., Vereecken, J., Müller, J., Knepper, T., Rörden, O., Gonzalez, S., Barcelo, D., Hernando, M.D. 2007. Comparison of sulfonated and other micropollutants removal in membrane bioreactor and conventional wastewater treatment. *Water Research*, **41**(4), 935-945.

56) Delgadillo-Mirquez, L., Lardon, L., Steyer, J.-P., Patureau, D. 2011. A new dynamic model for bioavailability and cometabolism of micropollutants during anaerobic digestion. *Water Research*, **45**(15), 4511-4521.

57) Deng, L.W., Zheng, P., Chen, Z.A. 2006. Anaerobic digestion and post-treatment of swine wastewater using IC–SBR process with bypass of raw wastewater. *Process Biochemistry*, **41**(4), 965-969.

58) Dettenmaier, E.M., Doucette, W.J., Bugbee, B. 2008. Chemical hydrophobicity and uptake by plant roots. *Environmental Science & Technology*, **43**(2), 324-329.

59) Di Bella, G., Durante, F., Torregrossa, M., Viviani, G., Mercurio, P., Cicala, A. 2007. The role of fouling mechanisms in a membrane bioreactor. *Water Science and Technology*, **55**(8-9), 455-464.

60) Du, J., Zhao, H., Liu, S., Xie, H., Wang, Y., Chen, J. 2017. Antibiotics in the coastal water of the South Yellow Sea in China: Occurrence, distribution and ecological risks. *Science of the Total Environment*, **595**, 521-527.

61) Du, Z., Li, H., Gu, T. 2007. A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy. *Biotechnology Advances*, **25**(5), 464-482.

62) Dutta, K., Lee, M.Y., Po, W.W., Lee, C.H., Lin, A.Y.C., Lin, C.F., Lin, J.W. 2014. Removal of pharmaceuticals and organic matter from municipal wastewater using two-stage anaerobic fluidized membrane bioreactor. *Bioresource Technology*, **165**, 42-49.

63) Enders, A., Hanley, K., Whitman, T., Joseph, S., Lehmann, J. 2012. Characterization of biochars to evaluate recalcitrance and agronomic performance. *Bioresource Technology*, **114**, 644-653.

64) Escapa, A., San-Martín, M.I., Morán, A. 2014. Potential use of microbial electrolysis cells in domestic wastewater treatment plants for energy recovery. *Frontiers in Energy Research*, **2**, 19.

65) Fan, H.-T., Shi, L.-Q., Shen, H., Chen, X., Xie, K.-P. 2016. Equilibrium, isotherm,

kinetic and thermodynamic studies for removal of tetracycline antibiotics by adsorption onto hazelnut shell derived activated carbons from aqueous media. *RSC Advances*, **6**(111), 109983-109991.

66) Feng, L., Casas, M.E., Ottosen, L.D.M., Møller, H.B., Bester, K. 2017. Removal of antibiotics during the anaerobic digestion of pig manure. *Science of the Total Environment*, **603**, 219-225.

67) Fischer, K., Majewsky, M. 2014. Cometabolic degradation of organic wastewater micropollutants by activated sludge and sludge-inherent microorganisms. *Applied Microbiology and Biotechnology*, **98**(15), 6583-6597.

68) Fomina, M., Gadd, G.M. 2014. Biosorption: current perspectives on concept, definition and application. *Bioresource Technology*, **160**, 3-14.

69) Freguia, S., Rabaey, K., Yuan, Z., Keller, J. 2008a. Sequential anode–cathode configuration improves cathodic oxygen reduction and effluent quality of microbial fuel cells. *Water Research*, **42**(6-7), 1387-1396.

70) Freguia, S., Rabaey, K., Yuan, Z.G., Keller, J. 2008b. Sequential anode-cathode configuration improves cathodic oxygen reduction and effluent quality of microbial fuel cells. *Water Research*, **42**(6-7), 1387-1396.

71) Furuichi, T., Kannan, K., Suzuki, K., Tanaka, S., Giesy, J.P., Masunaga, S. 2006. Occurrence of estrogenic compounds in and removal by a swine farm waste treatment plant. *Environmental Science & Technology*, **40**(24), 7896-7902.

72) Galán, M.J.G., Díaz-Cruz, M.S., Barceló, D. 2012. Removal of sulfonamide antibiotics upon conventional activated sludge and advanced membrane bioreactor treatment. *Analytical and Bioanalytical Chemistry*, **404**(5), 1505-1515.

73) Gao, P., He, S., Huang, S., Li, K., Liu, Z., Xue, G., Sun, W. 2015. Impacts of coexisting antibiotics, antibacterial residues, and heavy metals on the occurrence of erythromycin resistance genes in urban wastewater. *Applied Microbiology and Biotechnology*, **99**(9), 3971-3980.

74) Garcia-Rodríguez, A., Matamoros, V., Fontàs, C., Salvadó, V. 2014. The ability of biologically based wastewater treatment systems to remove emerging organic contaminants—a review. *Environmental Science and Pollution Research*, **21**(20), 11708-11728.

75) Gil, G.-C., Chang, I.-S., Kim, B.H., Kim, M., Jang, J.-K., Park, H.S., Kim, H.J. 2003. Operational parameters affecting the performannce of a mediator-less microbial fuel cell. *Biosensors and Bioelectronics*, **18**(4), 327-334.

76) Göbel, A., McArdell, C.S., Joss, A., Siegrist, H., Giger, W. 2007. Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. *Science of the Total Environment*, **372**(2), 361-371.
77) Grandclément, C., Seyssiecq, I., Piram, A., Wong-Wah-Chung, P., Vanot, G., Tiliacos, N., Roche, N., Doumenq, P. 2017. From the conventional biological wastewater treatment to hybrid processes, the evaluation of organic micropollutant removal: a review. *Water Research*, **111**, 297-317.
78) Grenni, P., Ancona, V., Caracciolo, A.B. 2018. Ecological effects of antibiotics on natural ecosystems: A review. *Microchemical Journal*, **136**, 25-39.
79) Guan, Y.D., Wang, B., Gao, Y.X., Liu, W., Zhao, X.L., Huang, X.F., Yu, J.H. 2017. Occurrence and Fate of Antibiotics in the Aqueous Environment and Their Removal by Constructed Wetlands in China: A review. *Pedosphere*, **27**(1), 42-51.
80) Guo, W., Ngo, H.-H., Li, J. 2012. A mini-review on membrane fouling. *Bioresource Technology*, **122**, 27-34.
81) Guo, W., Song, H., Zhou, L., Sun, J. 2016. Simultaneous removal of sulfanilamide and bioelectricity generation in two-chambered microbial fuel cells. *Desalination and Water Treatment*, **57**(52), 24982-24989.
82) Guo, X.S., Liu, J.X., Xiao, B.Y. 2013. Bioelectrochemical enhancement of hydrogen and methane production from the anaerobic digestion of sewage sludge in single-chamber membrane-free microbial electrolysis cells. *International Journal of Hydrogen Energy*, **38**(3), 1342-1347.
83) Gupta, V.K., Carrott, P., Ribeiro Carrott, M., Suhas. 2009. Low-cost adsorbents: growing approach to wastewater treatment—a review. *Critical Reviews in Environmental Science and Technology*, **39**(10), 783-842.
84) Hamid, H., Eskicioglu, C. 2012. Fate of estrogenic hormones in wastewater and sludge treatment: A review of properties and analytical detection techniques in sludge matrix. *Water Research*, **46**(18), 5813-5833.
85) Han, Y., Yang, L., Chen, X., Cai, Y., Zhang, X., Qian, M., Chen, X., Zhao, H., Sheng, M., Cao, G. 2020. Removal of veterinary antibiotics from swine wastewater using anaerobic and aerobic biodegradation. *Science of the Total Environment*, **709**, 136094.
86) Harnisch, F., Gimkiewicz, C., Bogunovic, B., Kreuzig, R., Schröder, U. 2013. On the removal of sulfonamides using microbial bioelectrochemical systems. *Electrochemistry Communications*, **26**, 77-80.

87) He, Z., Kan, J., Wang, Y., Huang, Y., Mansfeld, F., Nealson, K.H. 2009. Electricity production coupled to ammonium in a microbial fuel cell. *Environmental Science & Technology*, **43**(9), 3391-3397.

88) Hejazi, F., Ghoreyshi, A., Rahimnejad, M. 2019. Simultaneous phenol removal and electricity generation using a hybrid granular activated carbon adsorption-biodegradation process in a batch recycled tubular microbial fuel cell. *Biomass and Bioenergy*, **129**, 105336.

89) Helbling, D.E., Johnson, D.R., Honti, M., Fenner, K. 2012. Micropollutant biotransformation kinetics associate with WWTP process parameters and microbial community characteristics. *Environmental Science & Technology*, **46**(19), 10579-10588.

90) Heuer, H., Schmitt, H., Smalla, K. 2011. Antibiotic resistance gene spread due to manure application on agricultural fields. *Current Opinion in Microbiology*, **14**(3), 236-243.

91) Hiegemann, H., Lubken, M., Schulte, P., Schmelz, K.G., Gredigk-Hoffmann, S., Wichern, M. 2018. Inhibition of microbial fuel cell operation for municipal wastewater treatment by impact loads of free ammonia in bench- and 45 L-scale. *Science of the Total Environment*, **624**, 34-39.

92) Homem, V., Santos, L. 2011. Degradation and removal methods of antibiotics from aqueous matrices–a review. *Journal of Environmental Management*, **92**(10), 2304-2347.

93) Hong, P.-Y., Al-Jassim, N., Ansari, M.I., Mackie, R.I. 2013. Environmental and public health implications of water reuse: antibiotics, antibiotic resistant bacteria, and antibiotic resistance genes. *Antibiotics*, **2**(3), 367-399.

94) Hruska, K., Franek, M. 2012. Sulfonamides in the environment: a review and a case report. *Veterinarni Medicina*, **57**(1), 1-35.

95) Hsieh, C.Y., Liaw, E.T., Fan, K.M. 2015. Removal of veterinary antibiotics, alkylphenolic compounds, and estrogens from the Wuluo constructed wetland in southern Taiwan. *Journal of Environmental Science and Health, Part A*, **50**(2), 151-60.

96) Hu, D., Min, H., Wang, H., Zhao, Y., Cui, Y., Wu, P., Ge, H., Luo, K., Zhang, L., Liu, W. 2020. Performance of an up-flow anaerobic bio-electrochemical system (UBES) for treating sulfamethoxazole (SMX) antibiotic wastewater. *Bioresource Technology*, **305**, 123070.

97) Hu, D., Xu, J., Chen, Z., Wu, P., Wang, Z., Wang, P., Xiao, T., Su, H., Li, X., Wang, H., Zhang, Y. 2017. Performance of a pilot split-type anaerobic membrane bioreactor (AnMBR) treating antibiotics solvent wastewater at low temperatures. *Chemical Engineering Journal*, **325**, 502-512.

98) Hu, D.X., Tian, Y., Wang, Z.J., Wu, P., Wang, P., Chen, Z.B., Cui, Y.B., Ge, H. 2016. The operational efficiency of a novel AnMBR treating antibiotic solvent wastewater in start-up stage. *Journal of Water Reuse and Desalination*, jwrd2016064.

99) Huang, L., Cheng, S., Chen, G. 2011. Bioelectrochemical systems for efficient recalcitrant wastes treatment. *Journal of Chemical Technology and Biotechnology*, **86**(4), 481-491.

100) Huang, M.-h., Yang, Y.-d., Chen, D.-h., Chen, L., Guo, H.-d. 2012a. Removal mechanism of trace oxytetracycline by aerobic sludge. *Process Safety and Environmental Protection*, **90**(2), 141-146.

101) Huang, M.H., Tian, S.X., Chen, D.H., Zhang, W., Wu, J., Chen, L. 2012b. Removal of sulfamethazine antibiotics by aerobic sludge and an isolated Achromobacter sp. S-3. *Journal of Environmental Sciences*, **24**(9), 1594-1599.

102) Huang, X., Liu, C., Li, K., Su, J., Zhu, G., Liu, L. 2015. Performance of vertical up-flow constructed wetlands on swine wastewater containing tetracyclines and tet genes. *Water Research*, **70**, 109-117.

103) Huang, X., Zheng, J.L., Liu, C.X., Liu, L., Liu, Y.H., Fan, H.Y. 2017. Removal of antibiotics and resistance genes from swine wastewater using vertical flow constructed wetlands: Effect of hydraulic flow direction and substrate type. *Chemical Engineering Journal*, **308**, 692-699.

104) Huggins, T., Wang, H., Kearns, J., Jenkins, P., Ren, Z.J. 2014. Biochar as a sustainable electrode material for electricity production in microbial fuel cells. *Bioresource Technology*, **157**, 114-119.

105) Hussain, S.A., Prasher, S.O. 2011. Understanding the sorption of ionophoric pharmaceuticals in a treatment wetland. *Wetlands*, **31**(3), 563-571.

106) Jiang, Z., Zhang, D., Zhou, L., Deng, D., Duan, M., Liu, Y. 2018. Enhanced catalytic capability of electroactive biofilm modified with different kinds of carbon nanotubes. *Analytica Chimica Acta*, **1035**, 51-59.

107) Jin, H., Capareda, S., Chang, Z., Gao, J., Xu, Y., Zhang, J. 2014. Biochar pyrolytically produced from municipal solid wastes for aqueous As (V) removal:

adsorption property and its improvement with KOH activation. *Bioresource Technology*, **169**, 622-629.

108) Johnson, A., Williams, R., Matthiessen, P. 2006. The potential steroid hormone contribution of farm animals to freshwaters, the United Kingdom as a case study. *Science of the Total Environment*, **362**(1-3), 166-178.

109) Joo, H.-S., Hirai, M., Shoda, M. 2006. Piggery wastewater treatment using Alcaligenes faecalis strain No. 4 with heterotrophic nitrification and aerobic denitrification. *Water Research*, **40**(16), 3029-3036.

110) Kim, D., Min, K.J., Lee, K., Yu, M.S., Park, K.Y. 2017. Effects of pH, molar ratios and pre-treatment on phosphorus recovery through struvite crystallization from effluent of anaerobically digested swine wastewater. *Environmental Engineering Research*, **22**(1), 12-18.

111) Kim, D.G., Choi, D., Cheon, S., Ko, S.-O., Kang, S., Oh, S. 2020. Addition of biochar into activated sludge improves removal of antibiotic ciprofloxacin. *Journal of Water Process Engineering*, **33**, 101019.

112) Kim, D.P., Saegerman, C., Douny, C., Dinh, T.V., Xuan, B.H., Vu, B.D., Hong, N.P., Scippo, M.-L. 2013. First survey on the use of antibiotics in pig and poultry production in the Red River Delta region of Vietnam. *Food and Public Health*, **3**(5), 247-256.

113) Kim, H.W., Nam, J.Y., Shin, H.S. 2011. Ammonia inhibition and microbial adaptation in continuous single-chamber microbial fuel cells. *Journal of Power Sources*, **196**(15), 6210-6213.

114) Kim, J.R., Zuo, Y., Regan, J.M., Logan, B.E. 2008. Analysis of ammonia loss mechanisms in microbial fuel cells treating animal wastewater. *Biotechnology and Bioengineering*, **99**(5), 1120-1127.

115) Kim, S., Eichhorn, P., Jensen, J.N., Weber, A.S., Aga, D.S. 2005a. Removal of antibiotics in wastewater: Effect of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. *Environmental Science &Technology*, **39**(15), 5816-5823.

116) Kim, S., Eichhorn, P., Jensen, J.N., Weber, A.S., Aga, D.S. 2005b. Removal of antibiotics in wastewater: effect of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. *Environmental Science & Technology*, **39**(15), 5816-5823.

117) Kim, T., An, J., Lee, H., Jang, J.K., Chang, I.S. 2016. pH-dependent

ammonia removal pathways in microbial fuel cell system. *Bioresource Technology*, **215**, 290-295.

118) Kim, W., Shin, S.G., Cho, K., Lee, C., Hwang, S. 2012. Performance of methanogenic reactors in temperature phased two-stage anaerobic digestion of swine wastewater. *Journal of Bioscience and Bioengineering*, **114**(6), 635-639.

119) Klomjek, P. 2016. Swine wastewater treatment using vertical subsurface flow constructed wetland planted with Napier grass. *Sustainable Environment Research*, **26**(5), 217-223.

120) Kocaturk, I., Erguder, T.H. 2016. Influent COD/TAN ratio affects the carbon and nitrogen removal efficiency and stability of aerobic granules. *Ecological Engineering*, **90**, 12-24.

121) Koike, S., Krapac, I., Oliver, H., Yannarell, A., Chee-Sanford, J., Aminov, R., Mackie, R.I. 2007. Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine production facilities over a 3-year period. *Applied and Environmental Microbiology*, **73**(15), 4813-4823.

122) Kolz, A., Moorman, T., Ong, S., Scoggin, K., Douglass, E. 2005. Degradation and metabolite production of tylosin in anaerobic and aerobic swine-manure lagoons. *Water Environment Research*, **77**(1), 49-56.

123) Komnitsas, K.A., Zaharaki, D. 2016. Morphology of modified biochar and its potential for phenol removal from aqueous solutions. *Frontiers in Environmental Science*, **4**, 26.

124) Kornboonraksa, T., Lee, H.S., Lee, S.H., Chiemchaisri, C. 2009. Application of chemical precipitation and membrane bioreactor hybrid process for piggery wastewater treatment. *Bioresource Technology*, **100**(6), 1963-1968.

125) Kumwimba, M.N., Meng, F. 2019. Roles of ammonia-oxidizing bacteria in improving metabolism and cometabolism of trace organic chemicals in biological wastewater treatment processes: A review. *Science of the Total Environment*, **659**(419-441.

126) Laurent, J., Casellas, M., Dagot, C. 2010. Heavy metals biosorption on disintegrated activated sludge: Description of a new equilibrium model. *Chemical Engineering Journal*, **164**(1), 63-69.

127) Le-Clech, P., Chen, V., Fane, T.A. 2006. Fouling in membrane bioreactors used in wastewater treatment. *Journal of Membrane Science*, **284**(1-2), 17-53.

128) Leavey-Roback, S.L., Krasner, S.W., Suffet, I.H. 2016. Veterinary

antibiotics used in animal agriculture as NDMA precursors. *Chemosphere*, **164**, 330-338.

129) Lee, J.Y., Monk, I.R., da Silva, A.G., Seemann, T., Chua, K.Y., Kearns, A., Hill, R., Woodford, N., Bartels, M.D., Strommenger, B. 2018. Global spread of three multidrug-resistant lineages of Staphylococcus epidermidis. *Nature microbiology*, **3**(10), 1175-1185.

130) Leng, L., Wei, L., Xiong, Q., Xu, S., Li, W., Lv, S., Lu, Q., Wan, L., Wen, Z., Zhou, W. 2019. Use of microalgae-based technology for the removal of antibiotics from wastewater: A review. *Chemosphere*, 124680.

131) Li, B., Zhang, T. 2010. Biodegradation and adsorption of antibiotics in the activated sludge process. *Environmental Science & Technology*, **44**(9), 3468-3473.

132) Li, J.Z., Meng, J., Li, J.L., Wang, C., Deng, K.W., Sun, K., Buelna, G. 2016. The effect and biological mechanism of COD/TN ratio on nitrogen removal in a novel upflow microaerobic sludge reactor treating manure-free piggery wastewater. *Bioresource Technology*, **209**, 360-368.

133) Li, K., Ji, F., Liu, Y., Tong, Z., Zhan, X., Hu, Z. 2013. Adsorption removal of tetracycline from aqueous solution by anaerobic granular sludge: equilibrium and kinetic studies. *Water Science and Technology*, **67**(7), 1490-1496.

134) Li, Y., Hu, Q., Chen, C.-H., Wang, X.-L., Gao, D.-W. 2017. Performance and microbial community structure in an integrated anaerobic fluidized-bed membrane bioreactor treating synthetic benzothiazole contaminated wastewater. *Bioresource Technology*, **236**, 1-10.

135) Li, Y.F., Zhu, G.B., Ng, W.J., Tan, S.K. 2014. A review on removing pharmaceutical contaminants from wastewater by constructed wetlands: Design, performance and mechanism. *Science of the Total Environment*, **468–469**(908-932.

136) Liang, Q., Ye, L., Huang, Z.-H., Xu, Q., Bai, Y., Kang, F., Yang, Q.-H. 2014. A honeycomb-like porous carbon derived from pomelo peel for use in high-performance supercapacitors. *Nanoscale*, **6**(22), 13831-13837.

137) Lin, H., Liao, B.-Q., Chen, J., Gao, W., Wang, L., Wang, F., Lu, X. 2011a. New insights into membrane fouling in a submerged anaerobic membrane bioreactor based on characterization of cake sludge and bulk sludge. *Bioresource Technology*, **102**(3), 2373-2379.

138) Lin, H., Peng, W., Zhang, M., Chen, J., Hong, H., Zhang, Y. 2013. A

review on anaerobic membrane bioreactors: applications, membrane fouling and future perspectives. *Desalination*, **314**, 169-188.

139) Lin, H., Wang, F., Ding, L., Hong, H., Chen, J., Lu, X. 2011b. Enhanced performance of a submerged membrane bioreactor with powdered activated carbon addition for municipal secondary effluent treatment. *Journal of Hazardous Materials*, **192**(3), 1509-1514.

140) Liu, L., Liu, C., Zheng, J., Huang, X., Wang, Z., Liu, Y., Zhu, G. 2013a. Elimination of veterinary antibiotics and antibiotic resistance genes from swine wastewater in the vertical flow constructed wetlands. *Chemosphere*, **91**(8), 1088-1093.

141) Liu, L., Liu, C.X., Zheng, J.Y., Huang, X., Wang, Z., Liu, Y.H., Zhu, G.F. 2013b. Elimination of veterinary antibiotics and antibiotic resistance genes from swine wastewater in the vertical flow constructed wetlands. *Chemosphere*, **91**(8), 1088-1093.

142) Liu, L., Liu, Y.-h., Wang, Z., Liu, C.-x., Huang, X., Zhu, G.-f. 2014. Behavior of tetracycline and sulfamethazine with corresponding resistance genes from swine wastewater in pilot-scale constructed wetlands. *Journal of Hazardous Materials*, **278**, 304-310.

143) Liu, R., Chen, L.J., Song, X.Y., Wei, D., Zheng, W., Qiu, S.K., Zhao, Y. 2016. Treatment of digested piggery wastewater with a membrane bioreactor. *Environmental Engineering & Management Journal* **15**(10),

144) Liu, S.X., Li, L., Li, H.Q., Wang, H., Yang, P. 2017. Study on ammonium and organics removal combined with electricity generation in a continuous flow microbial fuel cell. *Bioresource Technology*, **243**, 1087-1096.

145) Liu, Z.-h., Kanjo, Y., Mizutani, S. 2009. Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment—physical means, biodegradation, and chemical advanced oxidation: a review. *Science of the Total Environment*, **407**(2), 731-748.

146) Lo, K., Liao, P., Gao, Y. 1994. Anaerobic treatment of swine wastewater using hybrid UASB reactors. *Bioresource Technology*, **47**(2), 153-157.

147) Logan, B.E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K. 2006. Microbial fuel cells: methodology and technology. *Environmental Science & Technology*, **40**(17), 5181-5192.

148) Lou, Y., Ye, Z.-L., Chen, S., Ye, X., Deng, Y., Zhang, J. 2018. Sorption behavior of tetracyclines on suspended organic matters originating from swine wastewater. *Journal of Environmental Sciences*, **65**, 144-152.

149) Lovley, D.R. 2008. The microbe electric: conversion of organic matter to electricity. *Current Opinion in Biotechnology*, **19**(6), 564-571.

150) Lu, M.Q., Niu, X.J., Liu, W., Zhang, J., Wang, J., Yang, J., Wang, W.Q., Yang, Z.Q. 2016. Biogas generation in anaerobic wastewater treatment under tetracycline antibiotic pressure. *Scientific Reports*, **6**

151) Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C. 2014a. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total Environment*, **473**, 619-641.

152) Luo, Y.L., Guo, W.S., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C. 2014b. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total Environment*, **473–474**, 619-641.

153) Ma, D., Jiang, Z.-H., Lay, C.-H., Zhou, D. 2016. Electricity generation from swine wastewater in microbial fuel cell: Hydraulic reaction time effect. *International Journal of Hydrogen Energy*, **41**(46), 21820-21826.

154) Ma, Z., Wu, H., Zhang, K., Xu, X., Wang, C., Zhu, W., Wu, W. 2018. Long-term low dissolved oxygen accelerates the removal of antibiotics and antibiotic resistance genes in swine wastewater treatment. *Chemical Engineering Journal*, **334**(630-637.

155) Magdaleno, A., Saenz, M., Juárez, A., Moretton, J. 2015. Effects of six antibiotics and their binary mixtures on growth of Pseudokirchneriella subcapitata. *Ecotoxicology and Environmental Safety*, **113**, 72-78.

156) Martínez, J.L. 2008. Antibiotics and antibiotic resistance genes in natural environments. *Science*, **321**(5887), 365-367.

157) Masebinu, S., Akinlabi, E., Muzenda, E., Aboyade, A. 2019. A review of biochar properties and their roles in mitigating challenges with anaerobic digestion. *Renewable and Sustainable Energy Reviews*, **103**, 291-307.

158) Maspolim, Y., Zhou, Y., Guo, C., Xiao, K., Ng, W.J. 2015. The effect of pH on solubilization of organic matter and microbial community structures in sludge fermentation. *Bioresource Technology*, **190**, 289-298.

159) Massé, D.I., Saady, N.M.C., Gilbert, Y. 2014. Potential of biological processes to eliminate antibiotics in livestock manure: an overview. *Animals*, **4**(2), 146-163.

160) Matamoros, V., García, J., Bayona, J.M. 2008. Organic micropollutant removal in a full-scale surface flow constructed wetland fed with secondary effluent. *Water Research*, **42**(3), 653-660.

161) Meng, F., Chae, S.-R., Drews, A., Kraume, M., Shin, H.-S., Yang, F. 2009. Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material. *Water Research*, **43**(6), 1489-1512.

162) Michalak, I., Chojnacka, K., Witek-Krowiak, A. 2013. State of the art for the biosorption process—a review. *Applied Biochemistry and Biotechnology*, **170**(6), 1389-1416.

163) Mihciokur, H., Oguz, M. 2016. Removal of oxytetracycline and determining its biosorption properties on aerobic granular sludge. *Environmental Toxicology and Pharmacology*, **46**, 174-182.

164) Min, B., Kim, J., Oh, S., Regan, J.M., Logan, B.E. 2005a. Electricity generation from swine wastewater using microbial fuel cells. *Water Research*, **39**(20), 4961-4968.

165) Min, B., Kim, J.R., Oh, S.E., Regan, J.M., Logan, B.E. 2005b. Electricity generation from swine wastewater using microbial fuel cells. *Water Research*, **39**(20), 4961-4968.

166) Miran, W., Jang, J., Nawaz, M., Shahzad, A., Lee, D.S. 2018. Biodegradation of the sulfonamide antibiotic sulfamethoxazole by sulfamethoxazole acclimatized cultures in microbial fuel cells. *Science of the Total Environment*, **627**, 1058-1065.

167) Mohammad-Khah, A., Ansari, R. 2009. Activated charcoal: preparation, characterization and applications: a review article. *Int J Chem Tech Res*, **1**(4), 859-864.

168) Mohring, S.A., Strzysch, I., Fernandes, M.R., Kiffmeyer, T.K., Tuerk, J., Hamscher, G. 2009. Degradation and elimination of various sulfonamides during anaerobic fermentation: a promising step on the way to sustainable pharmacy? *Environmental Science & Technology*, **43**(7), 2569-2574.

169) Montes, N., Otero, M., Coimbra, R.N., Mendez, R., Martin-Villacorta, J. 2015. Removal of tetracyclines from swine manure at full-scale activated sludge

treatment plants. *Environmental Technology*, **36**(15), 1966-1973.

170) Müller, E., Schüssler, W., Horn, H., Lemmer, H. 2013. Aerobic biodegradation of the sulfonamide antibiotic sulfamethoxazole by activated sludge applied as co-substrate and sole carbon and nitrogen source. *Chemosphere*, **92**(8), 969-978.

171) Mumme, J., Srocke, F., Heeg, K., Werner, M. 2014. Use of biochars in anaerobic digestion. *Bioresource Technology*, **164**, 189-197.

172) Munir, M., Wong, K., Xagoraraki, I. 2011. Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan. *Water Research*, **45**(2), 681-693.

173) Najafgholi, Z., Rahimnejad, M., Najafpour, G. 2015. Effect of Electrolyte Conductivity and Aeration on Performance of Sediment Microbial Fuel Cell.

174) O'Neill, J. 2014. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. *Rev. Antimicrob. Resist*, **20**, 1-16.

175) Oberoi, A.S., Jia, Y., Zhang, H., Khanal, S.K., Lu, H. 2019. Insights into the Fate and Removal of Antibiotics in Engineered Biological Treatment Systems: A Critical Review. *Environmental Science & Technology*, **53**(13), 7234-7264.

176) Oliveira, B.M., Zaiat, M., Oliveira, G.H. 2019. The contribution of selected organic substrates to the anaerobic cometabolism of sulfamethazine. *Journal of Environmental Science and Health, Part B*, **54**(4), 263-270.

177) Oliveira, D.A.d., Pinheiro, A., Veiga, M.d., Alves, T.C. 2016a. Occurrence and mobility of antimicrobials and hormones in Oxisol with application of swine slurry. *RBRH*, **21**(2), 391-400.

178) Oliveira, G.H.D.d., Santos-Neto, A.J.d., Zaiat, M. 2016b. Evaluation of sulfamethazine sorption and biodegradation by anaerobic granular sludge using batch experiments. *Bioprocess and Biosystems Engineering*, **39**(1), 115-124.

179) Oliveira, G.H.D.d., Santos-Neto, A.J.d., Zaiat, M. 2017. Removal of the veterinary antimicrobial sulfamethazine in a horizontal-flow anaerobic immobilized biomass (HAIB) reactor subjected to step changes in the applied organic loading rate. *Journal of Environmental Management*, **204**, 674-683.

180) Onesios, K.M., Jim, T.Y., Bouwer, E.J. 2009. Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review. *Biodegradation*, **20**(4), 441-466.

181) Otawa, T., Yamada, M., Tanibata, R., Kawakami, M. 1990. Preparation,

pore analysis and adsorption behavior of high surface area active carbon from coconut shell. *Proceeding of the international symposium on gas separation technology. Antwerp (Belgium): Elsevier*. pp. 263-70.

182) Ozgun, H., Dereli, R.K., Ersahin, M.E., Kinaci, C., Spanjers, H., van Lier, J.B. 2013. A review of anaerobic membrane bioreactors for municipal wastewater treatment: integration options, limitations and expectations. *Separation and Purification Technology*, **118**, 89-104.

183) Palanisamy, G., Jung, H.Y., Sadhasivam, T., Kurkuri, M.D., Kim, S.C., Roh, S.H. 2019. A comprehensive review on microbial fuel cell technologies: Processes, utilization, and advanced developments in electrodes and membranes. *Journal of Cleaner Production*, **221**, 598-621.

184) Papaevangelou, V.A., Gikas, G.D., Tsihrintzis, V.A., Antonopoulou, M., Konstantinou, I.K. 2016. Removal of endocrine disrupting chemicals in HSF and VF pilot-scale constructed wetlands. *Chemical Engineering Journal*, **294**, 146-156.

185) Park, D., Yun, Y.-S., Park, J.M. 2010. The past, present, and future trends of biosorption. *Biotechnology and Bioprocess Engineering*, **15**(1), 86-102.

186) Pei, M., Zhang, B., He, Y., Su, J., Gin, K., Lev, O., Shen, G., Hu, S. 2019. State of the art of tertiary treatment technologies for controlling antibiotic resistance in wastewater treatment plants. *Environment International*, **131**, 105026.

187) Peiris, C., Gunatilake, S.R., Mlsna, T.E., Mohan, D., Vithanage, M. 2017. Biochar based removal of antibiotic sulfonamides and tetracyclines in aquatic environments: a critical review. *Bioresource Technology*, **246**, 150-159.

188) Prado, N., Ochoa, J., Amrane, A. 2009a. Biodegradation and biosorption of tetracycline and tylosin antibiotics in activated sludge system. *Process Biochemistry*, **44**(11), 1302-1306.

189) Prado, N., Ochoa, J., Amrane, A. 2009b. Biodegradation by activated sludge and toxicity of tetracycline into a semi-industrial membrane bioreactor. *Bioresource Technology*, **100**(15), 3769-3774.

190) Pretel, R., Robles, A., Ruano, M., Seco, A., Ferrer, J. 2014. The operating cost of an anaerobic membrane bioreactor (AnMBR) treating sulphate-rich urban wastewater. *Separation and Purification Technology*, **126**, 30-38.

191) Qiang, Z., Macauley, J.J., Mormile, M.R., Surampalli, R., Adams, C.D.

2006. Treatment of antibiotics and antibiotic resistant bacteria in swine wastewater with free chlorine. *Journal of Agricultural and Food Chemistry*, **54**(21), 8144-8154.

192) Richardson, S.D., Ternes, T.A. 2005. Water analysis: emerging contaminants and current issues. *Analytical Chemistry*, **77**(12), 3807-3838.

193) Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M., Michael, I., Fatta-Kassinos, D. 2013. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. *Science of the Total Environment*, **447**, 345-360.

194) Roh, H., Subramanya, N., Zhao, F., Yu, C.-P., Sandt, J., Chu, K.-H. 2009. Biodegradation potential of wastewater micropollutants by ammonia-oxidizing bacteria. *Chemosphere*, **77**(8), 1084-1089.

195) Romero-Cano, L.A., González-Gutiérrez, L.V., Baldenegro-Pérez, L.A., Carrasco-Marín, F. 2017. Grapefruit peels as biosorbent: characterization and use in batch and fixed bed column for Cu (II) uptake from wastewater. *Journal of Chemical Technology and Biotechnology*, **92**(7), 1650-1658.

196) Roser, H.R.a.M. 2017. Meat and Seafood Production & Consumption, Vol. 2019, Our World in Data. https://ourworldindata.org/meat-and-seafood-production-consumption.

197) Sahar, E., Ernst, M., Godehardt, M., Hein, A., Herr, J., Kazner, C., Melin, T., Cikurel, H., Aharoni, A., Messalem, R. 2011a. Comparison of two treatments for the removal of selected organic micropollutants and bulk organic matter: conventional activated sludge followed by ultrafiltration versus membrane bioreactor. *Water Science and Technology*, **63**(4), 733-740.

198) Sahar, E., Messalem, R., Cikurel, H., Aharoni, A., Brenner, A., Godehardt, M., Jekel, M., Ernst, M. 2011b. Fate of antibiotics in activated sludge followed by ultrafiltration (CAS-UF) and in a membrane bioreactor (MBR). *Water Research*, **45**(16), 4827-4836.

199) Sakar, S., Yetilmezsoy, K., Kocak, E. 2009. Anaerobic digestion technology in poultry and livestock waste treatment—a literature review. *Waste Management & Research*, **27**(1), 3-18.

200) Sanchez Huerta, C. 2016. Removal and Degradation Pathways of Sulfamethoxazole Present in Synthetic Municipal Wastewater via an Anaerobic Membrane Bioreactor.

201) Sapkota, A.R., Curriero, F.C., Gibson, K.E., Schwab, K.J. 2007. Antibiotic-resistant enterococci and fecal indicators in surface water and groundwater impacted by a concentrated swine feeding operation. *Environmental Health Perspectives*, **115**(7), 1040-1045.

202) Sarmah, A.K., Meyer, M.T., Boxall, A.B. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere*, **65**(5), 725-759.

203) Scholz, M., Lee, B.h. 2005. Constructed wetlands: a review. *International Journal of Environmental Studies*, **62**(4), 421-447.

204) Shanmugam, S.R., Adhikari, S., Nam, H., Sajib, S.K. 2018. Effect of bio-char on methane generation from glucose and aqueous phase of algae liquefaction using mixed anaerobic cultures. *Biomass and Bioenergy*, **108**, 479-486.

205) Shappell, N.W., Billey, L.O., Forbes, D., Matheny, T.A., Poach, M.E., Reddy, G.B., Hunt, P.G. 2007. Estrogenic activity and steroid hormones in swine wastewater through a lagoon constructed-wetland system. *Environmental Science & Technology*, **41**(2), 444-450.

206) Sharma, V.K., Johnson, N., Cizmas, L., McDonald, T.J., Kim, H. 2016. A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. *Chemosphere*, **150**, 702-714.

207) Shen, G., Zhang, Y., Hu, S., Zhang, H., Yuan, Z., Zhang, W. 2018. Adsorption and degradation of sulfadiazine and sulfamethoxazole in an agricultural soil system under an anaerobic condition: Kinetics and environmental risks. *Chemosphere*, **194**, 266-274.

208) Sial, T.A., Lan, Z., Khan, M.N., Zhao, Y., Kumbhar, F., Liu, J., Zhang, A., Hill, R.L., Lahori, A.H., Memon, M. 2019. Evaluation of orange peel waste and its biochar on greenhouse gas emissions and soil biochemical properties within a loess soil. *Waste Management*, **87**, 125-134.

209) Silva, C.P., Otero, M., Esteves, V. 2012. Processes for the elimination of estrogenic steroid hormones from water: a review. *Environmental Pollution*, **165**, 38-58.

210) Singh, R., Singh, A.P., Kumar, S., Giri, B.S., Kim, K.-H. 2019. Antibiotic resistance in major rivers in the world: a systematic review on occurrence, emergence, and management strategies. *Journal of Cleaner Production*, **234**, 1484-1505.

211) Sombatsompop, K., Songpim, A., Reabroi, S., Inkong-ngam, P. 2011. A comparative study of sequencing batch reactor and movingbed sequencing batch reactor for piggery wastewater treatment. *Maejo International Journal of Science and Technology*, **5**(2), 191-203.

212) Song, C., Sun, X.F., Xing, S.F., Xia, P.F., Shi, Y.J., Wang, S.-G. 2014. Characterization of the interactions between tetracycline antibiotics and microbial extracellular polymeric substances with spectroscopic approaches. *Environmental Science and Pollution Research*, **21**(3), 1786-1795.

213) Song, H.L., Li, H., Zhang, S., Yang, Y.L., Zhang, L.M., Xu, H., Yang, X.L. 2018. Fate of sulfadiazine and its corresponding resistance genes in up-flow microbial fuel cell coupled constructed wetlands: effects of circuit operation mode and hydraulic retention time. *Chemical Engineering Journal*, **350**, 920-929.

214) Song, W., Wang, X., Gu, J., Zhang, S., Yin, Y., Li, Y., Qian, X., Sun, W. 2017a. Effects of different swine manure to wheat straw ratios on antibiotic resistance genes and the microbial community structure during anaerobic digestion. *Bioresource Technology*, **231**, 1-8.

215) Song, X.Y., Pan, G.X., Bai, Y.W., Liang, F., Xing, J.J., Gao, J., Shi, F.N. 2019. Preparation and electrochemical properties of biochar from pyrolysis of pomelo peel via different methods. *Fullerenes, Nanotubes and Carbon Nanostructures*, **27**(5), 453-458.

216) Song, X.Y., Liu, R., Chen, L.J., Kawagishi, T. 2017b. Comparative experiment on treating digested piggery wastewater with a biofilm MBR and conventional MBR: simultaneous removal of nitrogen and antibiotics. *Frontiers of Environmental Science & Engineering*, **11**(2), Article number: 11 (2017).

217) Stams, A.J. 1994. Metabolic interactions between anaerobic bacteria in methanogenic environments. *Antonie Van Leeuwenhoek*, **66**(1-3), 271-294.

218) Stone, J.J., Clay, S.A., Zhu, Z.W., Wong, K.L., Porath, L.R., Spellman, G.M. 2009. Effect of antimicrobial compounds tylosin and chlortetracycline during batch anaerobic swine manure digestion. *Water Research*, **43**(18), 4740-4750.

219) Sui, Q., Zhang, J., Chen, M., Tong, J., Wang, R., Wei, Y. 2016. Distribution of antibiotic resistance genes (ARGs) in anaerobic digestion and land application of swine wastewater. *Environmental Pollution*, **213**, 751-759.

220) Sui, Q.W., Liu, C., Dong, H.M., Zhu, Z.P. 2014. Effect of ammonium

nitrogen concentration on the ammonia-oxidizing bacteria community in a membrane bioreactor for the treatment of anaerobically digested swine wastewater. *Journal of Bioscience and Bioengineering*, **118**(3), 277-283.

221) Sun, J., Xu, W., Yang, P., Li, N., Yuan, Y., Zhang, H., Wang, Y., Ning, X., Zhang, Y., Chang, K. 2019. Enhanced oxytetracycline removal coupling with increased power generation using a self-sustained photo-bioelectrochemical fuel cell. *Chemosphere*, **221**, 21-29.

222) Sunyoto, N.M., Zhu, M., Zhang, Z., Zhang, D. 2016. Effect of biochar addition on hydrogen and methane production in two-phase anaerobic digestion of aqueous carbohydrates food waste. *Bioresource Technology*, **219**, 29-36.

223) Suto, R., Ishimoto, C., Chikyu, M., Aihara, Y., Matsumoto, T., Uenishi, H., Yasuda, T., Fukumoto, Y., Waki, M. 2017. Anammox biofilm in activated sludge swine wastewater treatment plants. *Chemosphere*, **167**, 300-307.

224) Suzuki, K., Tanaka, Y., Kuroda, K., Hanajima, D., Fukumoto, Y., Yasuda, T., Waki, M. 2007. Removal and recovery of phosphorous from swine wastewater by demonstration crystallization reactor and struvite accumulation device. *Bioresource Technology*, **98**(8), 1573-1578.

225) Suzuki, K., Waki, M., Yasuda, T., Fukumoto, Y., Kuroda, K., Sakai, T., Suzuki, N., Suzuki, R., Matsuba, K. 2010. Distribution of phosphorus, copper and zinc in activated sludge treatment process of swine wastewater. *Bioresource Technology*, **101**(23), 9399-9404.

226) Suzuki, Y., Kubota, A., Furukawa, T., Sugamoto, K., Asano, Y., Takahashi, H., Sekito, T., Dote, Y., Sugimoto, Y. 2009. Residual of 17β-estradiol in digestion liquid generated from a biogas plant using livestock waste. *Journal of Hazardous Materials*, **165**(1–3), 677-682.

227) Tao, Q.Q., Luo, J.J., Zhou, J., Zhou, S.Q., Liu, G.L., Zhang, R.D. 2014. Effect of dissolved oxygen on nitrogen and phosphorus removal and electricity production in microbial fuel cell. *Bioresource Technology*, **164**, 402-407.

228) Tao, R., Ying, G.G., Su, H.C., Zhou, H.W., Sidhu, J.P. 2010. Detection of antibiotic resistance and tetracycline resistance genes in Enterobacteriaceae isolated from the Pearl rivers in South China. *Environmental Pollution*, **158**(6), 2101-2109.

229) Tice, R.C., Kim, Y. 2014. Influence of substrate concentration and feed frequency on ammonia inhibition in microbial fuel cells. *Journal of Power*

Sources, **271**, 360-365.

230) Tijani, J.O., Fatoba, O.O., Petrik, L.F. 2013. A review of pharmaceuticals and endocrine-disrupting compounds: sources, effects, removal, and detections. *Water, Air, & Soil Pollution*, **224**(11), 1770.

231) Tiwari, B., Sellamuthu, B., Ouarda, Y., Drogui, P., Tyagi, R.D., Buelna, G. 2017. Review on fate and mechanism of removal of pharmaceutical pollutants from wastewater using biological approach. *Bioresource Technology*, **224**, 1-12.

232) Tolls, J. 2001. Sorption of veterinary pharmaceuticals in soils: a review. *Environmental Science & Technology*, **35**(17), 3397-3406.

233) Tong, Z., Liu, Y., Hu, Z., Yuan, S. 2012. Anaerobic digestion of animal manure contaminated by tetracyclines. *Huan jing ke xue= Huanjing kexue/[bian ji, Zhongguo ke xue yuan huan jing ke xue wei yuan hui" Huan jing ke xue" bian ji wei yuan hui.]*, **33**(3), 1028-1032.

234) Töre, G.Y., Meriç, S., Lofrano, G., De Feo, G. 2012. Removal of Trace Pollutants from Wastewater in Constructed Wetlands. in: *Emerging Compounds Removal from Wastewater*, Springer, pp. 39-58.

235) Tran, N.H., Chen, H., Reinhard, M., Mao, F., Gin, K.Y.-H. 2016. Occurrence and removal of multiple classes of antibiotics and antimicrobial agents in biological wastewater treatment processes. *Water Research*, **104**, 461-472.

236) Tran, N.H., Reinhard, M., Gin, K.Y.H. 2018. Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. *Water Research*, **133**, 182-207.

237) Tran, N.H., Urase, T., Ngo, H.H., Hu, J., Ong, S.L. 2013. Insight into metabolic and cometabolic activities of autotrophic and heterotrophic microorganisms in the biodegradation of emerging trace organic contaminants. *Bioresource Technology*, **146**, 721-731.

238) Truu, M., Juhanson, J., Truu, J. 2009. Microbial biomass, activity and community composition in constructed wetlands. *Science of the Total Environment*, **407**(13), 3958-3971.

239) Uchimiya, M., Wartelle, L.H., Klasson, K.T., Fortier, C.A., Lima, I.M. 2011. Influence of pyrolysis temperature on biochar property and function as a heavy metal sorbent in soil. *Journal of Agricultural and Food Chemistry*, **59**(6), 2501-2510.

240) Välitalo, P., Kruglova, A., Mikola, A., Vahala, R. 2017. Toxicological impacts of antibiotics on aquatic micro-organisms: a mini-review. *International Journal of Hygiene and Environmental Health*, **220**(3), 558-569.

241) Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant, A., Laxminarayan, R. 2015. Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*, **112**(18), 5649-5654.

242) Varel, V., Wells, J., Shelver, W., Rice, C., Armstrong, D., Parker, D. 2012. Effect of anaerobic digestion temperature on odour, coliforms and chlortetracycline in swine manure or monensin in cattle manure. *Journal of Applied Microbiology*, **112**(4), 705-715.

243) Vymazal, J. 2013. The use of hybrid constructed wetlands for wastewater treatment with special attention to nitrogen removal: a review of a recent development. *Water Research*, **47**(14), 4795-4811.

244) Wang, H., Yao, H., Sun, P., Pei, J., Li, D., Huang, C.-H. 2015a. Oxidation of tetracycline antibiotics induced by Fe (III) ions without light irradiation. *Chemosphere*, **119**, 1255-1261.

245) Wang, J., Ben, W., Yang, M., Zhang, Y., Qiang, Z. 2016a. Dissemination of veterinary antibiotics and corresponding resistance genes from a concentrated swine feedlot along the waste treatment paths. *Environment International*, **92**(317-323.

246) Wang, J., He, M.-F., Zhang, D., Ren, Z., Song, T.-s., Xie, J. 2017a. Simultaneous degradation of tetracycline by a microbial fuel cell and its toxicity evaluation by zebrafish. *RSC Advances*, **7**(70), 44226-44233.

247) Wang, J., Wang, S. 2018a. Microbial degradation of sulfamethoxazole in the environment. *Applied Microbiology and Biotechnology*, **102**(8), 3573-3582.

248) Wang, J.L., Wang, S.Z. 2016. Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: a review. *Journal of Environmental Management*, **182**, 620-640.

249) Wang, L., Liu, Y., Ma, J., Zhao, F. 2016b. Rapid degradation of sulphamethoxazole and the further transformation of 3-amino-5-methylisoxazole in a microbial fuel cell. *Water Research*, **88**, 322-328.

250) Wang, L., Wu, Y., Zheng, Y., Liu, L., Zhao, F. 2015b. Efficient degradation of sulfamethoxazole and the response of microbial communities in microbial fuel

cells. *RSC Advances*, **5**(69), 56430-56437.

251) Wang, L., You, L., Zhang, J., Yang, T., Zhang, W., Zhang, Z., Liu, P., Wu, S., Zhao, F., Ma, J. 2018a. Biodegradation of sulfadiazine in microbial fuel cells: reaction mechanism, biotoxicity removal and the correlation with reactor microbes. *Journal of Hazardous Materials*, **360**, 402-411.

252) Wang, L., You, L.X., Zhang, J.M., Yang, T., Zhang, W., Zhang, Z.X., Liu, P.X., Wu, S., Zhao, F., Ma, J. 2018b. Biodegradation of sulfadiazine in microbial fuel cells: Reaction mechanism, biotoxicity removal and the correlation with reactor microbes. *Journal of Hazardous Materials*, **360**, 402-411.

253) Wang, R., Chen, M., Feng, F., Zhang, J., Sui, Q., Tong, J., Wei, Y., Wei, D. 2017b. Effects of chlortetracycline and copper on tetracyclines and copper resistance genes and microbial community during swine manure anaerobic digestion. *Bioresource Technology*, **238**, 57-69.

254) Wang, S., Wang, J. 2018b. Biodegradation and metabolic pathway of sulfamethoxazole by a novel strain Acinetobacter sp. *Applied Microbiology and Biotechnology*, **102**(1), 425-432.

255) Wen, Q., Kong, F., Zheng, H., Yin, J., Cao, D., Ren, Y., Wang, G. 2011. Simultaneous processes of electricity generation and ceftriaxone sodium degradation in an air-cathode single chamber microbial fuel cell. *Journal of Power Sources*, **196**(5), 2567-2572.

256) Wen, Q., Wu, Y., Wang, G.L., Zhao, L.X., Sun, Q.A., Kong, F.Y. 2010. Microbial Fuel Cell Using Sequential Anode-cathode to Treat Brewery Wastewater. *Chemical Journal of Chinese Universities-Chinese*, **31**(6), 1231-1234.

257) Widyasari Mehta, A., Hartung, S., Kreuzig, R. 2016a. From the application of antibiotics to antibiotic residues in liquid manures and digestates: a screening study in one European center of conventional pig husbandry. *Journal of Environmental Management*, **177**, 129-137.

258) Widyasari Mehta, A., Suwito, H.R.K.A., Kreuzig, R. 2016b. Laboratory testing on the removal of the veterinary antibiotic doxycycline during long-term liquid pig manure and digestate storage. *Chemosphere*, **149**, 154-160.

259) Wu, D., Sun, F., Chua, F.J.D., Zhou, Y. 2020. Enhanced power generation in microbial fuel cell by an agonist of electroactive biofilm–Sulfamethoxazole. *Chemical Engineering Journal*, **384**, 123238.

260) Wu, H.M., Zhang, J., Ngo, H.H., Guo, W.S., Hu, Z., Liang, S., Fan, J.l., Liu, H. 2015. A review on the sustainability of constructed wetlands for wastewater treatment: Design and operation. *Bioresource Technology*, **175**, 594-601.

261) Xian, Q.M., Hu, L.X., Chen, H.C., Chang, Z.Z., Zou, H.X. 2010. Removal of nutrients and veterinary antibiotics from swine wastewater by a constructed macrophyte floating bed system. *Journal of Environmental Management*, **91**(12), 2657-2661.

262) Xiao, Y., Yaohari, H., De Araujo, C., Sze, C.C., Stuckey, D.C. 2017. Removal of selected pharmaceuticals in an anaerobic membrane bioreactor (AnMBR) with/without powdered activated carbon (PAC). *Chemical Engineering Journal*, **321**, 335-345.

263) Xu, J., Sheng, G.-P., Ma, Y., Wang, L.-F., Yu, H.-Q. 2013. Roles of extracellular polymeric substances (EPS) in the migration and removal of sulfamethazine in activated sludge system. *Water Research*, **47**(14), 5298-5306.

264) Xu, Y., Yuan, Z., Ni, B.-J. 2016. Biotransformation of pharmaceuticals by ammonia oxidizing bacteria in wastewater treatment processes. *Science of the Total Environment*, **566**, 796-805.

265) Xue, W., Li, F., Zhou, Q. 2019. Degradation mechanisms of sulfamethoxazole and its induction of bacterial community changes and antibiotic resistance genes in a microbial fuel cell. *Bioresource Technology*, **289**, 121632.

266) Yadu, A., Sahariah, B.P., Anandkumar, J. 2018. Influence of COD/ammonia ratio on simultaneous removal of NH4+-N and COD in surface water using moving bed batch reactor. *Journal of Water Process Engineering*, **22**(66-72.

267) Yan, C., Yang, Y., Zhou, J., Liu, M., Nie, M., Shi, H., Gu, L. 2013. Antibiotics in the surface water of the Yangtze Estuary: occurrence, distribution and risk assessment. *Environmental Pollution*, **175**, 22-29.

268) Yan, W., Guo, Y., Xiao, Y., Wang, S., Ding, R., Jiang, J., Gang, H., Wang, H., Yang, J., Zhao, F. 2018. The changes of bacterial communities and antibiotic resistance genes in microbial fuel cells during long-term oxytetracycline processing. *Water Research*, **142**, 105-114.

269) Yan, W., Xiao, Y., Yan, W., Ding, R., Wang, S., Zhao, F. 2019. The effect of bioelectrochemical systems on antibiotics removal and antibiotic resistance

genes: a review. *Chemical Engineering Journal*, **358**, 1421-1437.

270) Yang, C.-W., Hsiao, W.-C., Chang, B.-V. 2016. Biodegradation of sulfonamide antibiotics in sludge. *Chemosphere*, **150**, 559-565.

271) Yang, J., Dai, J., Wang, L., Ge, W., Xie, A., He, J., Yan, Y. 2019a. Ultrahigh adsorption of tetracycline on willow branche-derived porous carbons with tunable pore structure: Isotherm, kinetics, thermodynamic and new mechanism study. *Journal of the Taiwan Institute of Chemical Engineers*, **96**, 473-482.

272) Yang, S.F., Lin, C.F., Lin, A.Y.C., Hong, P.K.A. 2011a. Sorption and biodegradation of sulfonamide antibiotics by activated sludge: experimental assessment using batch data obtained under aerobic conditions. *Water Research*, **45**(11), 3389-3397.

273) Yang, S.F., Lin, C.F., Wu, C.J., Ng, K.K., Lin, A.Y.C., Hong, P.-K.A. 2012a. Fate of sulfonamide antibiotics in contact with activated sludge–sorption and biodegradation. *Water Research*, **46**(4), 1301-1308.

274) Yang, S.F., Lin, C.F., Wu, C.J., Ng, K.K., Lin, A.Y.C., Hong, P.K.A. 2012b. Fate of sulfonamide antibiotics in contact with activated sludge–Sorption and biodegradation. *Water Research*, **46**(4), 1301-1308.

275) Yang, S.F., Lin, C.F., Yu C. L., A., Andy Hong, P.K. 2011b. Sorption and biodegradation of sulfonamide antibiotics by activated sludge: Experimental assessment using batch data obtained under aerobic conditions. *Water Research*, **45**(11), 3389-3397.

276) Yang, W., Cicek, N. 2008. Treatment of swine wastewater by submerged membrane bioreactors with consideration of estrogenic activity removal. *Desalination*, **231**(1–3), 200-208.

277) Yang, X., Zhang, S., Ju, M., Liu, L. 2019b. Preparation and modification of biochar materials and their application in soil remediation. *Applied Sciences*, **9**(7), 1365.

278) Ye, L., Zhang, T. 2011. Ammonia-oxidizing bacteria dominates over ammonia-oxidizing archaea in a saline nitrification reactor under low DO and high nitrogen loading. *Biotechnology and Bioengineering*, **108**(11), 2544-2552.

279) Ye, Y., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Liu, Y., Ni, B.-j., Zhang, X. 2019a. Microbial fuel cell for nutrient recovery and electricity generation from municipal wastewater under different ammonium concentrations. *Bioresource Technology*, **292**, 121992.

280) Ye, Y., Ngo, H.H., Guo, W., Liu, Y., Chang, S.W., Nguyen, D.D., Ren, J., Liu, Y., Zhang, X. 2019b. Feasibility study on a double chamber microbial fuel cell for nutrient recovery from municipal wastewater. *Chemical Engineering Journal*, **358**, 236-242.

281) Yenigun, O., Demirel, B. 2013. Ammonia inhibition in anaerobic digestion: A review. *Process Biochemistry*, **48**(5-6), 901-911.

282) Zhang, B., Wu, Y., Cha, L. 2020. Removal of methyl orange dye using activated biochar derived from pomelo peel wastes: performance, isotherm, and kinetic studies. *Journal of Dispersion Science and Technology*, **41**(1), 125-136.

283) Zhang, D., Yin, J., Zhao, J., Zhu, H., Wang, C. 2015a. Adsorption and removal of tetracycline from water by petroleum coke-derived highly porous activated carbon. *Journal of Environmental Chemical Engineering*, **3**(3), 1504-1512.

284) Zhang, D.Q., Tan, S.K., Gersberg, R.M., Sadreddini, S., Zhu, J.F., Tuan, N.A. 2011a. Removal of pharmaceutical compounds in tropical constructed wetlands. *Ecological Engineering*, **37**(3), 460-464.

285) Zhang, L., Lee, Y.W., Jahng, D. 2011b. Anaerobic co-digestion of food waste and piggery wastewater: Focusing on the role of trace elements. *Bioresource Technology*, **102**(8), 5048-5059.

286) Zhang, M., Li, A., Zhou, Q., Shuang, C., Zhou, W., Wang, M. 2014. Effect of pore size distribution on tetracycline adsorption using magnetic hypercrosslinked resins. *Microporous and Mesoporous Materials*, **184**, 105-111.

287) Zhang, Q.Q., Ying, G.G., Pan, C.G., Liu, Y.S., Zhao, J.L. 2015b. Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modeling, and linkage to bacterial resistance. *Environmental Science & Technology*, **49**(11), 6772-6782.

288) Zhang, Q., Zhang, Y., Li, D. 2017a. Cometabolic degradation of chloramphenicol via a meta-cleavage pathway in a microbial fuel cell and its microbial community. *Bioresource Technology*, **229**, 104-110.

289) Zhang, S., Song, H.L., Yang, X.L., Huang, S., Dai, Z.Q., Li, H., Zhang, Y.-Y. 2017b. Dynamics of antibiotic resistance genes in microbial fuel cell-coupled constructed wetlands treating antibiotic-polluted water. *Chemosphere*, **178**, 548-555.

290) Zhang, S., Song, H.L., Yang, X.L., Yang, K.Y., Wang, X.Y. 2016a. Effect

of electrical stimulation on the fate of sulfamethoxazole and tetracycline with their corresponding resistance genes in three-dimensional biofilm-electrode reactors. *Chemosphere*, **164**, 113-119.

291) Zhang, S., Song, H.L., Yang, X.L., Yang, Y.L., Yang, K.Y., Wang, X.Y. 2016b. Fate of tetracycline and sulfamethoxazole and their corresponding resistance genes in microbial fuel cell coupled constructed wetlands. *RSC Advances*, **6**(98), 95999-96005.

292) Zhang, S., Yang, X.L., Li, H., Song, H.L., Wang, R.C., Dai, Z.Q. 2017c. Degradation of sulfamethoxazole in bioelectrochemical system with power supplied by constructed wetland-coupled microbial fuel cells. *Bioresource Technology*, **244**, 345-352.

293) Zhang, X.-X., Zhang, T., Fang, H.H. 2009. Antibiotic resistance genes in water environment. *Applied Microbiology and Biotechnology*, **82**(3), 397-414.

294) Zhang, Z.J., Zhu, J., King, J., Li, W.H. 2006. A two-step fed SBR for treating swine manure. *Process Biochemistry*, **41**(4), 892-900.

295) Zhao, B., O'Connor, D., Zhang, J., Peng, T., Shen, Z., Tsang, D.C., Hou, D. 2018. Effect of pyrolysis temperature, heating rate, and residence time on rapeseed stem derived biochar. *Journal of Cleaner Production*, **174**, 977-987.

296) Zhao, C., Shao, Q., Ma, Z., Li, B., Zhao, X. 2016. Physical and chemical characterizations of corn stalk resulting from hydrogen peroxide presoaking prior to ammonia fiber expansion pretreatment. *Industrial Crops and Products*, **83**, 86-93.

297) Zhao, S.X., Ta, N., Wang, X.D. 2017. Effect of temperature on the structural and physicochemical properties of biochar with apple tree branches as feedstock material. *Energies*, **10**(9), 1293.

298) Zheng, W., Zhang, Z.Y., Liu, R., Lei, Z.F. 2017. Removal of veterinary antibiotics from anaerobically digested swine wastewater using an intermittently aerated sequencing batch reactor. *Journal of Environmental Sciences*,

299) Zhi, W., Ji, G.D. 2012. Constructed wetlands, 1991–2011: a review of research development, current trends, and future directions. *Science of the Total Environment*, **441**, 19-27.

300) Zhou, L.J., Ying, G.-G., Liu, S., Zhang, R.Q., Lai, H.J., Chen, Z.F., Pan, C.-G. 2013a. Excretion masses and environmental occurrence of antibiotics in typical swine and dairy cattle farms in China. *Science of the Total Environment*,

444, 183-195.

301) Zhou, L.J., Ying, G.G., Liu, S., Zhao, J.L., Yang, B., Chen, Z.F., Lai, H.J. 2013b. Occurrence and fate of eleven classes of antibiotics in two typical wastewater treatment plants in South China. *Science of the Total Environment*, **452–453**, 365-376.

302) Zhou, L.J., Ying, G.G., Zhao, J.L., Yang, J.F., Wang, L., Yang, B., Liu, S. 2011. Trends in the occurrence of human and veterinary antibiotics in the sediments of the Yellow River, Hai River and Liao River in northern China. *Environmental Pollution*, **159**(7), 1877-1885.

303) Zhou, L.J., Zhao, M., Feng, L., Wang, X., Xu, X.Y., Xia, S.Q. 2016. Performance and bacterial community structure of an anoxic/oxic membrane bioreactor treating anaerobically digested piggery wastewater. *Desalination and Water Treatment*, **57**(59), 28581-28591.

304) Zhou, X., Qu, Y., Kim, B.H., Du, Y., Wang, H., Li, H., Dong, Y., He, W., Liu, J., Feng, Y. 2015. Simultaneous current generation and ammonia recovery from real urine using nitrogen-purged bioelectrochemical systems. *RSC Advances*, **5**(86), 70371-70378.

305) Zhou, Y., Zhu, N., Guo, W., Wang, Y., Huang, X., Wu, P., Dang, Z., Zhang, X., Xian, J. 2018. Simultaneous electricity production and antibiotics removal by microbial fuel cells. *Journal of Environmental Management*, **217**, 565-572.

306) Zhu, X., Li, C., Li, J., Xie, B., Lü, J., Li, Y. 2018a. Thermal treatment of biochar in the air/nitrogen atmosphere for developed mesoporosity and enhanced adsorption to tetracycline. *Bioresource Technology*, **263**, 475-482.

307) Zhu, X., Liu, Y., Qian, F., Zhou, C., Zhang, S., Chen, J. 2014. Preparation of magnetic porous carbon from waste hydrochar by simultaneous activation and magnetization for tetracycline removal. *Bioresource Technology*, **154**, 209-214.

308) Zhu, Y., Wang, Y., Zhou, S., Jiang, X., Ma, X., Liu, C. 2018b. Robust performance of a membrane bioreactor for removing antibiotic resistance genes exposed to antibiotics: Role of membrane foulants. *Water Research*, **130**, 139-150.

309) Zhu, Y.J., Wang, Y.Y., Jiang, X.X., Zhou, S., Wu, M., Pan, M., Chen, H. 2017. Microbial community compositional analysis for membrane bioreactor treating antibiotics containing wastewater. *Chemical Engineering Journal*, **325**, 300-309.

Appendix

LIST OF PUBLICATIONS

A. Peer-Reviewed Journal articles

1) **Cheng, D. L**., Ngo, H. H., Guo, W. S., Chang, S. W., Nguyen, D. D., & Kumar, S. M. (2019). Microalgae biomass from swine wastewater and its conversion to bioenergy. Bioresource Technology, 275, 109-122 (IF: **7.539**; SJR: **Q1**).

2) **Cheng, D.L.,** Ngo, H.H., Guo, W.S., Liu, Y.W., Zhou, J.L., Chang, S.W., Nguyen, D.D., Bui, X.T. and Zhang, X.B., 2018. Bioprocessing for elimination antibiotics and hormones from swine wastewater. Science of the Total Environment, 621, 1664-1682 (IF: **6.551**; SJR: **Q1**).

3) **Cheng, D**., Ngo, H. H., Guo, W. S., Chang, S. W., Nguyen, D. D., Kumar, M., ... & Wei, D. (2018). Problematic effects of antibiotics on anaerobic treatment processes in swine wastewater. Bioresource technology, 263, 642-653 (IF: **7.539**; SJR: **Q1**)

4) **Cheng, D**., Ngo, H. H., Guo, W., Liu, Y., Chang, S. W., Nguyen, D. D., ... & Ni, B. (2018). Anaerobic membrane bioreactors for antibiotic wastewater treatment: performance and membrane fouling issues. Bioresource technology, 267, 714-724 (IF: **7.539**; SJR: **Q1**)

5) **Cheng, D.**, Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Liu, Y., Zhang, X., Shan, X. and Liu, Y., 2019. Contribution of antibiotics to the fate of antibiotic resistance genes in anaerobic treatment processes of swine wastewater: a review. Bioresource Technology, p.122654 (IF: **7.539**; SJR: **Q1**).

6) **Cheng, D.,** Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Liu, Y., Wei, Q. and Wei, D., 2020. A critical review on antibiotics and hormones in swine wastewater: Water pollution problems and control approaches. Journal of Hazardous Materials, 387, p.121682 (IF: **9.038**; SJR: **Q1**).

7) **Cheng, D.**, Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Liu, Y., Shan, X., Nghiem, L.D. and Nguyen, L.N., 2020. Removal process of antibiotics during anaerobic treatment of swine wastewater. Bioresource Technology, 300, p.122707 (IF: **7.539**; SJR: **Q1**).

8) **Cheng, D.**, Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Zhang, X., Varjani, S. and Liu, Y., 2020. Feasibility study on a new pomelo peel derived biochar for tetracycline antibiotics removal in swine wastewater. Science of The Total Environment, p.137662 (IF: **6.551**; SJR: **Q1**).
9) **Cheng, D**., Ngo, H.H., Guo, W., Lee, D., Nghiem, D.L., Zhang, J., Liang, S., Varjani, S. and Wang, J., 2020. Performance of microbial fuel cell for treating swine wastewater containing sulfonamide antibiotics. Bioresource Technology, p.123588. (IF: **7.539**; SJR: **Q1**).
10) **Cheng**, **D**., Liu, Y., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Zhang, S., Luo, G. and Liu, Y., 2020. A mini review on application of enzymatic bioprocesses in animal wastewater and manure treatment. Bioresource Technology, p.123683. (IF: **7.539**; SJR: **Q1**)
11) **Cheng, D.,** Ngo, H.H., Guo, W., W. Chang, S., Nguyen, D.D., Li, J., Ly, W.Q., Nguyen, T.A.N., Tran, V.S., 2020. Applying a new pomelo peel derived biochar in microbial fell cell for enhancing sulfonamide antibiotics removal in swine wastewater, Bioresource Technology, p.123886. (IF: **7.539**; SJR: **Q1**)
12) **Dongle Cheng**, Huu Hao Ngo, Wenshan Guo, Soon Woong Chang, Dinh Duc Nguyen, Yiwen Liu, Yi Liu; Lijuan Deng; Zhuo Chen. Feasibility of a continuous flow microbial fuel cell as supplementary in a bioreactor for swine wastewater treatment (Water Research, under review)
13) Yi Liu[1], **Dongle Cheng[1]**, Huu Hao Ngo*, Wenshan Guo, Soon Woong Chang, Dinh Duc Nguyen, Shicheng Zhang, Gang Luo, Xinbo Zhang. Bioprocesses in animal fat and protein wastes management: biodegradation and bio-utilization (STOTEN, under review)
14) **Dongle Cheng**, Huu Hao Ngo, Wenshan Guo, Soon Woong Chang, Dinh Duc Nguyen, Quynh Anh Nguyen, Jian Zhang, Shuang Liang (Water Research, under review).

B. Book chapters

1. Guo, W. S., **Cheng, D.,** Ngo, H. H., Chang, S. W., Nguyen, D. D., Nguyen, D. P. and Bui, X. T. (2020). Chapter 9: Anaerobic membrane bioreactors for antibiotic wastewater treatment, In Current Developments in Biotechnology and Bioengineering: Advanced Membrane Separation Processes for Sustainable Water

and Wastewater Management – Anaerobic Membrane Bioreactor Processes and Technologies, Ngo, H. H., Guo, W. S., Ng, H. Y., Mannina, G. and Pandey A. (Eds.), Elsevier, 219-239. (ISBN: 9780128198520, 9780128198537)

2. Ngo, H. H., **Cheng, D.,** Guo, W. S., Pandey A., Lee, D. J. and Deng, L. (2020). Chapter 8: Biotransformation of organic micro-pollutants in biological wastewater, In Current Developments in Biotechnology and Bioengineering: Emerging Organic Micro-pollutants, Varjani, S., Pandey A., Tyagi, R., **Ngo, H. H.** and Larroche, C. (Eds.), Elsevier, 185-204. (ISBN: 9780128195949, 9780128213094)
3. Huu Hao Ngo, **Cheng, D.**, Wenshan Guo, Chapter 20: Approaches towards resource recovery from breeding wastewater, In Resource and Energy Recovery and Reuse, Guo, W. S., Ngo, H. H., Rao Y. Surampalli, Tian C. Zhang (Eds.), Wiley, (In press).

C. Contributions to scientific forums

1) **D. L. Cheng,** H. H. Ngo, W. S. Guo , J. Zhang, S. Liang, Y. Liu, L. D. Nghiem, B. J. Ni. 2018. Identification of mechanisms for removing antibiotics and hormones from swine wastewater in anaerobic bioreactors (Oral presentation). Bioresource Technology for Bioenergy, Bioproducts & Environmental Sustainability (BIORESTECH) . Sitges, Barcelona, Spain, 16 - 19 September.
2) **D. L. Cheng**, H. H. Ngo, W. S. Guo, S. W. Chang, D. D. Nguyen, Y. Liu, S. M. Kumar. 2019. Fate of antibiotics during anaerobic treatment of swine wastewater (Oral presentation). The 2nd International Conference on Non point Source Pollution Control and Aquatic Ecosystem Protection (NPAE 2019). Wuhan, China, 19 - 23 September.
3) **D.L. Cheng**, H. H. Ngo, W. S. Guo, S. W. Chang, D. D. Nguyen, X.B. Zhang. 2019. Adsorption behaviour and mechanism of a new pomelo peel derived biochar for removing tetracycline antibiotics from swine wastewater. Green Technologies for Sustainable Water (GTSW). Ho Chi Minh City, Vietnam, 1 - 5, December.

D. Awards during a period of PhD study

1) 2017 HDR Students Publication Award from Faculty of Engineering and Information Technology (FEIT), University of Technology, Sydney (UTS) for publishing in high-quality journals.
2) 2019, 1st Oral Presentation Award in The 2nd International Conference on Non-point Source Pollution Control and Aquatic Ecosystem Protection (NPAE 2019) in Wuhan, China held on 19-23 September.