Improving sulfonamide antibiotics removal from swine wastewater by supplying a new pomelo peel derived biochar in an anaerobic membrane bioreactor

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Abstract

Sulfonamide antibiotics **(**SMs), as a class of antibiotics commonly used in swine industries, pose a serious threat to animal and human health. This study aims to evaluate the performance of an anaerobic membrane bioreactor (AnMBR) with and without supplying a new pomelo peel derived biochar to treat swine wastewater containing SMs. Results shows that 0.5 g/L biochar addition could increase more than 30% of sulfadiazine (SDZ) and sulfamethazine (SMZ)) removal in AnMBR. Approximately 95% of chemical oxygen demand (COD) was removed in the AnMBR at an influent organic loading rate （OLR） of 3.27 kg COD/(m^3·d) while an average methane yield was 0.2 L/g $COD_{removed}$ with slightly change at a small dose 0.5 g/L biochar addition. SMs inhibited the COD removal and methane production and increased membrane fouling. The addition of biochar could reduce the membrane fouling by reducing the concentration of SMP and EPS.

Keywords: Anaerobic membrane bioreactor; Swine wastewater; Sulfonamide antibiotics; Biochar; Membrane fouling

1. Introduction

Given that it is the most consumed meat worldwide, global pork production was approximately 118.8 million metric tons in 2018 (Cheng et al., 2016). The growth of world's population means that more pork production was required to meet human consumption requirements for proteins. Concentrated swine feeding operations have been developed to enhance swine productivity. Under these circumstances, increasing amounts of swine wastewater (around 4 - 8 L/d per pig), including manure, urine and washing wastewater, were discharged from swine farms (García et al., 2017). Moreover, antibiotics are usually applied in swine industries to promote growth, and curtail infectious disease treatment and prevention in pigs. However, due to the poor absorption capacity of pigs, more than 70% of antibiotics

were extracted through their urine or faeces (Cheng et al., 2018b). Hence, swine wastewater contains not only high concentrations of organic matter and nutrients, but also veterinary antibiotics. Without proper treatment, swine wastewater containing antibiotics can pose adverse effects on ecological safety and human health.

Globally, anaerobic biological treatment has been widely applied in swine wastewater treatment. Comparison to aerobic processes, anaerobic technologies have the advantages of renewable bioenergy production, valuable organic fertilizer production, and low energy requirement (Cheng et al., 2018b). Based on previous analyses, the treatment of antibiotics in swine wastewater by conventional anaerobic system is limited, especially for sulfonamide antibiotics (SMs). The review paper by Cheng et al. (2018b) demonstrated that SMs removal from swine wastewater in the conventional anaerobic process was poorer than that of tetracycline antibiotics and tylosin. This less than satisfactory removal of SMs under anaerobic conditions was also reported by Zhao et al. (2018). These authors investigated the removal efficiency of nine SMs in a membrane bioreactor system, and concluded that the anaerobic reactor made a negligible contribution to the total removal of SMs. This may be attributed to aromatic rings and double bond functional groups in SMs, which are strong and highly resistant to biodegradation (Sarmah et al., 2006). Nevertheless, SMs are one of the most widely used antibiotics in swine farms due to their low cost and broad-spectrum antimicrobial activity. Their poor removal efficiency in the conventional anaerobic processes increases their existence in the natural environment and in turn poses a risk to animal and human health (Michael et al., 2013). Hence, advanced treatment technologies are urgently required to improve the removal of SMs from swine wastewater.

The extension of sludge retention time (SRT) may potentially improve the degradation of antibiotics, and therefore, anaerobic membrane bioreactors (AnMBRs) have been considered as promising alternatives to conventional anaerobic processes (Cheng et al.,

2018b). AnMBRs which combined the benefits of anaerobic processes and complete retention of biomass by membrane filtration, thereby enhance the growth of poorer growing microorganisms and degradation of refractory antibiotics in swine wastewater (Huang et al., 2018). An investigation on the effectiveness of AnMBRs was conducted for eliminating pharmaceuticals from wastewater. For instance, Dutta et al. (2014) observed a high removal efficiency of pharmaceuticals in fluidized AnMBRs. Monsalvo et al. (2014) looked at the removal efficiency of 38 trace organics from domestic wastewater in an AnMBR, and concluded that only 9 compounds were removed effectively (>90%) while others were removed in smaller amounts (<50%). Nonetheless, only a few studies focused on removing SMs in swine wastewater used by AnMBRs, even though high concentrations of SMs (over 300 μg/L) were detected in swine wastewater. One possible reason might be the restriction of membrane fouling issues, which can increase total operating costs. It is necessary to study the performance of the AnMBR for removing SMs from swine wastewater and to find a solution to mitigate membrane fouling.

Adding biocarriers (e.g., activated carbon) in these AnMBRs is regarded as a potential strategy for membrane fouling control. Moreover, compared with activated carbon, biochar is considered an emerging and low-cost adsorbent from industrial and agricultural wastes (Ahmed et al., 2015). Therefore, a study on the role of biochar in membrane fouling mitigation and antibiotics removal from swine wastewater in AnMBR is crucial. Based on one of our previous study, a new biochar derived from pomelo peel was used in the current research due to its high capacity for the adsorption of SMs from wastewater (Cheng et al., 2020a). This study is therefore to investigate SMs removal in an AnMBR with and without adding the biochar. The main objectives are to: (1) investigate the removal of SMs (sulfamethoxazole (SMX), sulfadiazine (SDZ) and sulfamethazine (SMZ)) in swine wastewater by an AnMBR without adding biochar; (2) determine the effects of SMs on

organic matter removal, methane production, and microbial community of the AnMBR; (3) study the impact of SMs on and membrane fouling of the AnMBR; and (4) investigate influences of the pomelo peel derived biochar on the performance of the AnMBR and membrane fouling.

2. Materials and methods

2.1. Materials

Sulfonamide antibiotics, acetonitrile, methanol, formic acid and other chemicals used in current research were purchased from Sigma-Aldrich, Australia. The main compounds in synthetic swine wastewater are glucose, NH_4Cl, KH_2PO_4, $MgSO_4 \cdot 7H_2O$ and $CaCl_2 \cdot 2H_2O$, with concentrations of 3000 mg/L glucose COD, 223 mg/L, 66 mg/L, 54 mg/L and 4 mg/L, respectively. The anaerobic sludge inoculated in the AnMBR was collected from a local water treatment plant (Cronulla water treatment plant, Sydney).

2.2. Experimental setup and operation

A submerged upflow AnMBR (3.5 L) with a polyvinylidene fluoride (PVDF) hollow - fibre membrane was used in this study. The membrane used has a surface area of 0.08 m^2, pore size of 0.07 - 0.1 μm. Synthetic swine wastewater was continuously pumped into the AnMBR from the bottom of the reactor using a peristaltic pump. The pH of the wastewater was adjusted to 7.5 ± 0.1 by $NaHCO_3$ and HCl prior to being fed into the reactor. A peristaltic pump was used to circulate the mixed liquor from the top to the bottom of the reactor at a rate of 20 ml/min. Permeate from the membrane module was extracted by another peristaltic pump in an intermittent suction cycle with 8 min on and 2 min off, to slow down the membrane fouling. During the operation period, the organic loading rate (OLR) was kept at 3.27 kg COD/($m^3 \cdot$ d) by keeping the HRT at 22 h. The temperature was also kept constant at 22 ± 1 °C in a temperature-controlled room. The mixed liquor suspended solid (MLSS)

concentration in the reactor was measured regularly, and the value was maintained at 8 ± 0.2 g/L during the experiment. A digital pressure gauge recorded changes in pressure and then calculated TMP. The membrane was changed when TMP reached approximately 30 kPa. A sampling gasbag was used to collect the biogas produced in the AnMBR.

At the initial stage, synthetic swine wastewater without the addition of SMs was fed into the AnMBR and operated for 50 days. 100 μg/L each of SMX, SMZ and SDZ was added to the wastewater until stable COD removal was obtained (from day 51). After three weeks of operation with the presence of SMs in the reactor, a pomelo peel derived biochar (0.5 g/L), which was activated by KOH, was added into the reactor. The production, activation and characteristics of a newly developed pomelo peel derived biochar have been described previously (Cheng et al., 2020c). In short, the surface area and total pore volume of this activated biochar is up to 2457.37 m^2/g and 1.14 cm^3/g.

2.3. Analytical methods

The MLSS and COD of the sample were measured based on the Standard Methods. The volume of biogas was determined by a liquid displacement device. Biogas composition was measured by using Geotech potable biogas analyser (Biogas 5000, Geotech, UK). A triple quadrupole mass spectrometer LCMS-8060 (Shimadzu) served to detect the concentrations of SMX, SMZ and SDZ in the sample. A detailed description has been given in our previous research (Cheng et al., 2020b). The method about the extraction of extract soluble microbial products (SMP) and extracellular polymeric substances (EPS) in mixed liquor was described by Deng et al. (2014). The concentrations of SMP and EPS were determined by analysing polysaccharide (modified Lowry method), and protein concentrations (Anthrone-sulfuric acid method).

2.4. Microbial communities of sludge in the AnMBR

2.4.1. DNA extraction and quality testing

Duplicate samples of the reactor content were collected three times, one prior to the addition of antibiotics and two after antibiotics introduced for the profile of microbial community. The collected sample was mixed with 100% v/v ethanol (1:1 v/v) and then stored at -20 ^{o}C before proceeding to DNA extraction. In this study, QIAamp DNA Stool Mini Kit (Qiagen) was selected for genomic DNA extraction. NanoDrop® spectrophotometer evaluated the DNA integrity, purity, and concentration. DNase/Pyrogen-Free Water was used to normalize the concentration of DNA to 20 ng/µl for all samples prior to the sequencing analysis.

2.4.2. Amplicon sequencing and bioinformatics analysis

The universal primer set Pro341F (5'-CCTACGGGNBGCASCAG-3') and Pro805R (5'-GACTACNVGGGTATCTAATCC-3') was used to target both bacterial and archaeal 16S rRNA V3 – V4 regions for characterization of the entire microbial community. Paired-end amplicon sequencing (2 x 300 bp) was performed on the Illumina MiSeq platform (Australian Genome Research Facility, Melbourne, Australia). The raw sequence data was generated by Illumina *bcl2fastq* pipeline (version 2.20.0.422).

Raw reads were imported into Quantitative Insights into Microbial Ecology (QIIME) 2 (version 2019.10) for computational analysis. Quality filtering, denoising (primer and read trimming), paired-end reads merging, dereplication, chimera filtering and amplicon sequence variants (ASVs) clustering (≥ 97% similarity) were conducted using the *q2-dada2 denoise-paired* plugin (Callahan et al., 2016). Reverse reads sequences were truncated at position 260 in the 3' end due to a decline in quality. Reads were mapped back to ASVs with a minimum identity of 97% to obtain the number of reads in each ASV.

Taxonomy was assigned to ASVs using the *q2-feature-classifier classify-sklearn* Naïve Bayes taxonomy classifier against the SILVA database (release 132) with a confidence of 0.7. All ASVs were aligned with MAFFT and used to construct phylogenetics tree with FastTree 2 via the *q2-phylogeny align-to-tree-mafft-fasttree* pipeline. Alpha-diversity metrics including observed ASVs and Shannon index, beta diversity metrics including weighted UniFrac, unweighted UniFrac, Jaccard distance, and Bray-Curtis dissimilarity were estimated using the *q2-diversity core-metrics-phylogenetic* pipeline. This occurred after the samples were rarefied (subsampled without replacement) to 85,000 sequences per sample.

3. Results and discussion

3.1. SMs removal in the AnMBR and BC-AnMBR

The removal efficiency of three SMs including SMX, SMZ and SDZ in the AnMBR system was tracked and displayed in Fig. 1 (a). It is observed that the AnMBR had different removal capacities for these three SMs. From day 51 to day 72, the total SMX removal efficiency ranged from 87.33 ± 0.46% to 91.7 ± 0.83%, which was the highest and most stable removal among these three antibiotics. The removal efficiency of SMX in this study was comparable to previous results although it was slightly lower than them, which may be due to the different types of wastewater and operating conditions. For example, it was found that the removal efficiency of SMX in an AnMBR treated sewage was only 67.8 ± 13.9% (Xiao et al., 2017) while 97.1% SMX was removed from municipal wastewater by AnMBR at the temperature of 35 ± 1 °C (Wei et al., 2019). In addition, compared with SMX alone, the coexistence of SMX, SMZ and SDZ might have a greater inhibitory effect on anaerobic microorganisms (Cheng et al., 2018a). By contrast, the removal efficiency of SMZ and SDZ was relatively low and showed a decreasing trend, between 43.72 ± 0.41% to 20.57 ± 2.32% and 46.78 ± 1.14% to 22.37 ± 0.27%, respectively. Feng et al. (2017) noted a rapid

degradation of SMX and recalcitrance of sulfamethizole, and SDZ in anaerobic processes, as SMX was quantitatively removed (close to 100%) in one day, but no removal was observed for sulfamethizole and SDZ in a 40-day period. The resistant degradation of SDZ by anaerobic microorganisms was also found in a recent study during anaerobic digestion processes (Tang et al., 2019). This difference indicated that the performance of the AnMBR on antibiotics' removal was substance dependent. Based on a previous report, SMX requires relatively low reduction potential in the process of initiating ipso-hydroxylation and subsequent fragmentation (a common degradation pathway of SMs) in comparison with SMZ, making it easier to biodegrade (Han et al., 2020).

Insert Fig. 1

Although only less than 50% of SMZ and SDZ could be removed in the AnMBR, their removal efficiencies were higher than those in conventional anaerobic reactors. For example, Chen et al. (2012) found that the removal efficiencies of SMX and SDZ in a conventional anaerobic treatment process were only 31% and 8.3%, respectively. Han et al. (2020) observed negative removal efficiencies of sulfonamide antibiotics in the anaerobic digestion process. Although the authors explained that this phenomenon may be attributed to the reverse conversion of the antibiotic metabolites to their parent antibiotics, it also reflected their low removal rate. The studies by Zhao et al. (2018) also found no elimination of SMZ and SDZ in the anaerobic digestion process. As observed in Fig. 1 (b), the membrane module of AnMBR has a limited effect on the total removal of SMs, as only small portions of SMs (1.14 - 2.22%) were eliminated by the membrane rejection. This outcome was consistent with previous reports on the removal of sulfonamides in membrane bioreactors (Xiao et al., 2017; Zhao et al., 2018). Considering the negligible adsorption removal of SMs onto anaerobic sludge as reported previously (Zhao et al., 2018), the increase in SMs removal is mainly attributed to the enhanced biodegradation in the AnMBR.

From day 73, biochar (0.5 g/L) was added into the AnMBR to investigate the removal efficiency of SMs in the AnMBR with biochar (BC- AnMBR). Fig. 1 showed that (a) a significant increase in the removal of all three SMs was observed immediately after the addition of biochar. The removal efficiency of SMX in BC-AnMBR was still high and constant, which stabilized between 89.37 ± 1.24% and 97.29 ± 0.07%, as high as its removal in Staged Anaerobic Fluidized Membrane Bioreactor (SAF-MBR) (88-100%) and anaerobic membrane bioreactor with nanofiltration (AnMBR-NF) (>98%) (McCurry et al., 2014; Wei et al., 2016). Comparatively, AnMBR with the addition of PAC is more efficient in removing SMX (>99%) compared to this study, which may be because the addition of higher PAC (1 g/L) and lower initial concentration SMX (< 4 μg/L) (Xiao et al., 2017). Conversely, great improvements were obtained for the removal of SMZ and SDZ in BC-AnMBR, which ranged from 74.12 ± 0.52% to 47.66 ± 1.59% and from 80.1 ± 1.22% to 54.33 ± 0.61%, respectively, during day 73 and day 86. These results indicated that adding biochar played a positive role in the removal of SMs in the AnMBR. The enhanced removal of SMX and other antibiotics was also observed by adding powder and granular activated carbon in the AnMBRs (Dutta et al., 2014; Xiao et al., 2017). The author demonstrated that improved removal of antibiotics was attributed to the combined effects of activated carbon's adsorption and further enhanced degradation. Due to favourable adsorption of SMs onto the biochar as observed in one previous study (Cheng et al., 2020a), adsorption removal of SMs might account for a significant proportion. Similar to the activated carbon, biochar could act as a biocarrier of microorganisms, and further form a biofilm on the biochar surface, thereby enhancing the activity of microorganisms, and then improving the biodegradation and removal of SM (Mumme et al., 2014). However, further research is required to investigate the mechanisms of biochar's contribution to enhanced SMs removal.

3.2. COD removal and methane production in the AnMBR and BC-AnMBR

The performance of the AnMBR in terms of COD removal and methane production are displayed in Fig. 2. The reactor operated in a stable fashion for 50 days before the addition of SMs into the reactor. When the influent OLR was 3.27 kg COD/($m^3 \cdot d$), the average concentration of COD in permeate was 39.11 ± 1.96 mg/L, which corresponded to a COD removal efficiency of 98.7 ± 0.91%. This performance is comparable with previous studies about the treatment of swine wastewater in AnMBR systems (Jiang et al., 2020; Padmasiri et al., 2007). As observed from Fig. 2, a sharp increase of the permeate COD concentration (240.36 ± 2.41 mg/L) was observed after the addition of SMs (100 μg/L for each) into the AnMBR. During the first two weeks of SMs addition, the removal efficiency of COD fluctuated between 91.99 ± 0.08% and 95.61 ± 0.33%, which coincided with 3.2- to 6.2-fold increase in the permeate COD concentration. This result reflected inhibition effects of SMs on COD removal. In contrast the COD removal efficiency recovered gradually and remained stable at 95.99 ± 0.08% - 96.55 ± 0.05% during the third week of SMs addition (Fig. 2). Like this study, Wijekoon et al. (2015) observed an immediate decline in COD removal in the AnMBR right after the addition of trace organic contaminants into the influent, but the average value fully recovered after two weeks. An inhibition effect of SMX on the COD removal efficiency in the AnMBR system was also found by Wei et al. (2019), with the initial SMX concentration of 10 mg/L. However, there are reports where the findings vary. They indicated that antibiotics did not inhibit COD removal and biogas production in the AnMBR (Xiao et al., 2017). The inconsistency of the above results is possibly due to the different types and initial concentrations of antibiotics in the system. The review paper by Cheng et al. (2018a) stated that the influence of antibiotics on the COD removal of anaerobic treatment processes significantly related to the concentrations of antibiotics.

Insert Fig. 2

Before introducing SMs into the feed wastewater, the average methane content in the produced biogas was 56.25%, and the average methane yield was 0.2 L/g $COD_{removed}$ in the AnMBR. In the first two weeks of SMs addition, the average methane content and yield fell to 41.12% and 0.13 L/g $COD_{removed}$, respectively, which showed the inhibition of SMs at the observed concentrations of methane production. Similar to the effect of SMs on the COD removal, the inhibition of SMs to methane production gradually waned as microorganisms in the AnMBR slowly adapted to SMs, since a recovery trend for methane production was observed in the third week. The methane content and yield produced in this study were consistent with values reported in typical AnMBRs (Wijekoon et al., 2015). For example, Wijekoon et al. (2015) demonstrated that the methane composition in biogas and the methane yield in an AnMBR for the treatment of wastewater containing micropollutants was around 61% and 0.2 L/g COD, respectively. Ng et al. (2015) found a similar average methane yield (0.22 L/g $COD_{removed}$) during the stable period of a bioaugmented anaerobic membrane bioreactor when treating pharmaceutical wastewater. The impact of antibiotics on the COD removal and methane production could be explained by the microbial community data as follows.

Samples collected from before and after the addition of SMs into the AnMBR were analysed. As displayed in Fig. 3, SMs addition did not alter the identity of dominant orders in the reactor microbial community. In total, 17 dominant orders were detected in all samples and they belong to different functional groups of the anaerobic process. *Clostridiales* consist of members with highly versatile functions, and this was the most abundant bacterial orders found in all samples (30.8 - 43.9%). Members of this order include hydrolytic bacteria, fermenters and acetogens (all steps prior to methanogenesis) (Nguyen et al., 2018). Hydrolytic and fermentative bacteria include: *Anaerolineales*, *Bacteroidales*, *Coriobacteriales, Lactobacillales*, and *Selenomonadales* (Asato et al., 2019; Cetecioglu et al.,

2016; Nguyen et al., 2019). Meanwhile, *Synergistales* and *Spirochaetales* participate in acetogenesis/syntrophic acetate oxidation to provide precursors for methanogenesis, which is performed by *Methanosarcinales*, *Methanobacteriales* and *Methanomicrobiales* (Asato et al., 2019; Nguyen et al., 2018).

Insert Fig. 3

A decrease in relative abundance of most hydrolytic-fermentative bacterial orders and an increase in relative abundance of methanogens were observed after 2 weeks of continuous SMs addition (Fig. 4(a)). Prior to the addition of SMs, the ratio between total relative abundance of methanogenic archaeal orders and bacterial orders (A/B) was 0.37. Conversely, the addition of antibiotics resulted in a two-fold increase of A/B ratio to 0.73, indicating an imbalance between different functional groups. In anaerobic treatment processes, a balance among functional groups must be maintained in order to achieve stable and efficient process performance (Nguyen et al., 2019). Specifically, *Clostridiales* belongs to the *Firmicutes* phylum, and plays an important role in the reactor's performance and biogas production (Cetecioglu et al., 2016). Hence, the observed decrease for the relative abundance of *Clostridiales* possibly explained the reduction in COD removal efficiency and methane production in the AnMBR. The negative effect of antibiotics on *Firmicutes* phylum in the anaerobic reactor was also observed in previous studies (Deng et al., 2012). Cetecioglu et al. (2016) noted that *Clostridium* sp. in the order of *Clostridiales* decreased after introducing SMX into the reactor.

Insert Fig. 4

The discrimination in antibiotics impacts bacteria and methanogens which can be attributed to the working mechanism of antibiotics used in this study. Sulfamethoxazole (SMX), sulfamethazine (SMZ) and sulfadiazine (SDZ) all belong to the antibiotic class "sulfonamides" with a similar structure to p-aminobenzoic acid (PABA), a precursor for folic

acid biosynthesis (Khelaifia and Drancourt, 2012). Sulphonamides can compete with PABA for the bacterial enzyme dihydropteroate synthase and disrupt folic acid synthesis, blocking bacterial growth (Khelaifia and Drancourt, 2012). In methanogens, the role of folic acid as C1 carrier is fulfilled by methanopterin, which can be synthesized from PABA through a different pathway (Allen et al., 2014). It has been shown that methanopterin biosynthesis is not impacted by sulfonamides (Rasche and White, 1998).

The impact of adding antibiotics to a microbial composition seems to be partly compensated after 3 weeks of continuous addition (Fig. 4 (b)). Particularly, order *Clostridiales* demonstrated a significant increase in relative abundance from 31.05 ± 0.29 to 43.23 ± 0.65% (Student t-test, p-value < 0.05). The order *Coriobacteriales* also illustrated increased abundance although it was not significant. This contributed to a reduction in the A/B ratio from 0.73 (after 2 weeks) to 0.56 (after 3 weeks), represent a more "balanced" community. The increase in bacterial abundance was probably due to the developed resistance of bacteria to added sulphonamides via antibiotic resistance genes transfer and/or antibiotics degradation (Vila-Costa et al., 2017).

Results from microbial community analysis was in agreement with the performance data observed. Since SMs addition blocks the growth of bacterial orders that perform the first stage of digestion, the ability of the reactor to convert influent COD into precursors for acetogens and methanogens declined (indicated by increased effluent COD), resulting in less methane being produced (indicated by lower methane content and yield). Nevertheless, when the balance between different functional groups recovered, the reactor's performance also recovered as shown by a decrease in effluent COD and much better COD removal efficiency.

Some previous reports have indicated that biochar supplementation could enhance the performance of anaerobic digestion processes in terms of methane production (Wang et al., 2020; Zhang et al., 2019). Specifically, Wang et al. (2020) concluded that biochar addition

could enhance the methane production rate, and its final yield by increasing buffering capacity and the diversity of methanogens of the anaerobic process. Shanmugam et al. (2018) discovered that higher methane yields could be obtained in the system with biochar in comparison to the one with activated carbon. During the operation time from day 73 and day 86, the average COD removal efficiency was 95.66 ± 0.84%, and the average methane content and yield were 55.18% and 0.19 L/g $COD_{removed,}$ respectively. Little change of COD removal and methane production was observed in the BC-AnMBR, and this agrees with the conclusion of Mumme et al. (2014). The possible reason for the different result might be due to the type and dosage of biochar used in various studies. In this research, the dosage of biochar in the AnMBR was only 0.5 g/L, which is much smaller than previous studies, such as 10 g/L in Wang et al. (2020), 6.2 - 26.1 g/L in Zhang et al. (2019), and 8.3 - 25.5 g/L in Sunyoto et al. (2016). It seems that the methane yield and biochar concentration have a positive correlation when increasing the biochar dosage to a certain amount. Zhang et al. (2019) confirmed that the cumulative methane yield was 17.80%, 46.99% and 57.47% over the blank group when the amount of biochar added was 6.2, 15.9 and 26.1 g/L, respectively.

3.3. Membrane fouling of the AnMBR and BC-AnMBR

As shown in Fig. 5(a), an increase in TMP and shortening of membrane lifecycle were observed when SMs were introduced to the AnMBR, possibly due to the enhanced production of SMP and EPS in the mixed liquor. Fig. 5 (b) indicates that SMP and EPS increased from 4.22 ± 0.22 mg/L to 6.63 ± 0.21 mg/L and from 18.28 ± 0.76 mg/L to 37.67 ± 1.05 mg/L, respectively, during SMs addition period during this study. SMP and EPS have been considered as great contributors to biofouling through the formation of cake layer and pore blockage (Lin et al., 2012). Actually, the enhancement of SMP and EPS production by the presence of antibiotics in anaerobic reactors has been proposed elsewhere (Aquino and Stuckey, 2004; Du et al., 2018). Affected by the toxicity of antibiotics, more EPS were

secreted by microorganisms which acted as a protective "cocoon" to delay the penetration of toxic compounds into the cell body (Du et al., 2018). Meanwhile, more cell lysis caused by antibiotics induced the accumulation of SMP in the reactor (Aquino and Stuckey, 2004).

Insert Fig. 5

Polysaccharides and proteins are the major components of SMP and EPS, which play important roles in membrane fouling (Guo et al., 2012). As observed in Fig. 5 (b), the protein/polysaccharide ratio in SMP (SMP_P/SMP_C) and EPS (EPS_P/EPS_C) increased 1.64 and 2.34 times after the presence of SMs in the AnMBR. The research by Lay et al. (2012) also detected an increase in the protein/polysaccharide ratio in EPS after adding toxic micropollutants in an MBR. Such an increase might be caused by cell death and hydrolysis (Ma et al., 2018). Proteins with amino groups could enhance the hydrophobicity and surface charge of sludge flocs, thereby having a higher affinity for sludge flocs than polysaccharides (Massé et al., 2006). Hence, higher protein/polysaccharide ratio in the mixed liquor should have greater stickiness and thus promote the formation of cake layer (Lin et al., 2011).

The addition of biocarriers in membrane bioreactors have been considered as an effective method to solve membrane fouling. In this study, the TMP increase slowed down when introducing biochar into the AnMBR (Fig. 5(a)). Moreover, the total concentrations of SMP and EPS in the mixed liquor decreased, which contributed to the slowdown of the TMP increase. The research by Deng et al. (2014) also found lower concentrations of SMP and EPS in the AnMBR with biocarriers compared to the control reactor, where this might due to the adsorption of SMP and EPS onto the biocarriers. In addition, the enhanced removal of SMs by the biochar could weaken the inhibition of antibiotics in the production of EPS and SMP. Thus, adding biochar into the AnMBR is a promising strategy to mitigate membrane fouling. Nevertheless, the problem of membrane fouling still exists, so more investigations are

necessary to understand the effects of biochar on membrane fouling development in AnMBRs treating swine wastewater that contain antibiotics.

4. Conclusion

This study demonstrated that the BC-AnMBR is effective to treat swine wastewater containing SMs, when compared with other traditional anaerobic treatment processes. Although SMs showed inhibitive effects on COD removal and methane production, these effects gradually decreased as the microorganisms in AnMBR slowly adapted to the antibiotics. SMs in this study demonstrated a negative effect on membrane fouling of the AnMBR, due to the stimulated production of total SMP and EPS, as well as the enhancement of the protein/polysaccharide ratio in them. In short, the BC-AnMBR is a promising solution technology towards high SMs removal efficiency and membrane fouling control.

Acknowledgement

This research was supported by University of Technology Sydney, Australia (UTS, RIA NGO) and the Korea Institute of Energy Technology Evaluation and Planning (KETEP) and the Ministry of Trade, Industry & Energy (MOTIE), Republic of Korea (No. 20183020141270 and No. 20194110300040). The supports from China Scholarship Council (CSC) and UTS PhD scholarship are very grateful.

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Figure Captions

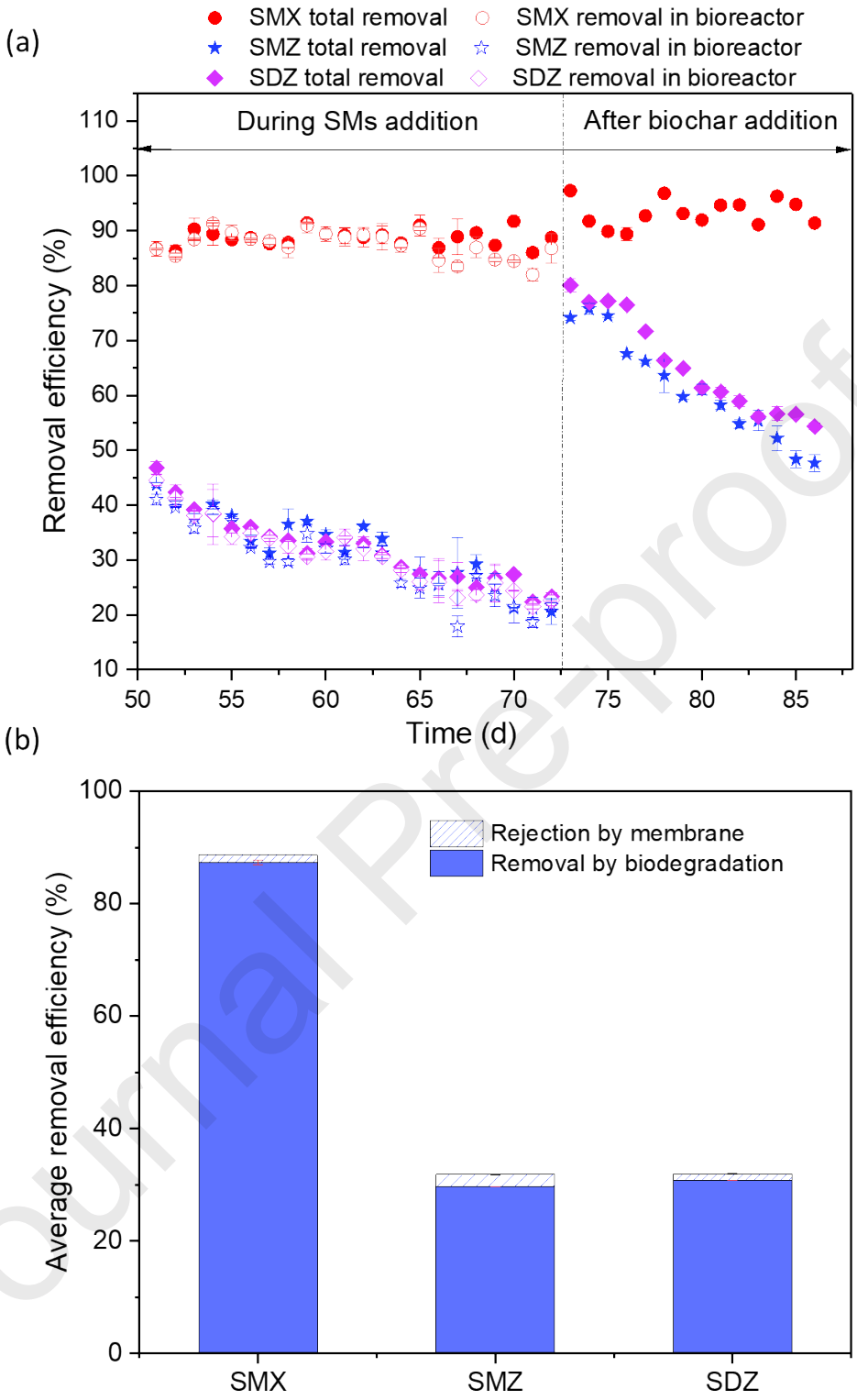
Fig. 1 SMs removal efficiencies during the operation of the AnMBR system (a); and their average removal by biodegradation and membrane rejection (b).

Fig. 2 The COD permeate concentration and removal efficiency during the operational period of the AnMBR.

Fig. 3 Microbial composition in the reactor before and after 2 and 3 weeks of continuous SMs addition.

Fig. 4 Log2-fold change in the relative abundance of dominant orders in the reactor (a) before and after 2 weeks of continuous antibiotics addition, and (b) after 2 and 3 weeks of continuous antibiotics addition. Higher enrichment means increased relative abundance while depletion means lower abundance. Orders use colors to highlight functional groups and orders with significant changes are also marked with an asterisk (Student t-test, p-value < 0.05).

Fig. 5 Variation of TMP (a), total SMP and EPS concentration in the mixed liquor, as well as protein/polysaccharide ratio in SMP and EPS during the operation of the AnMBR (TMP: average daily value; Time: the time of membrane inside the AnMBR).



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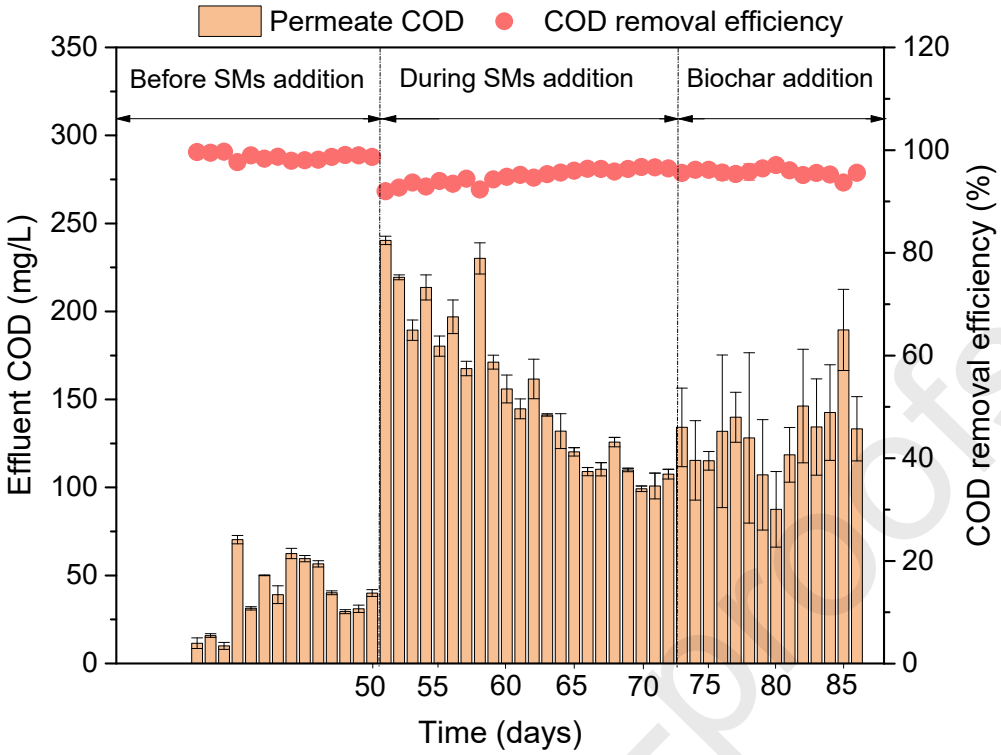

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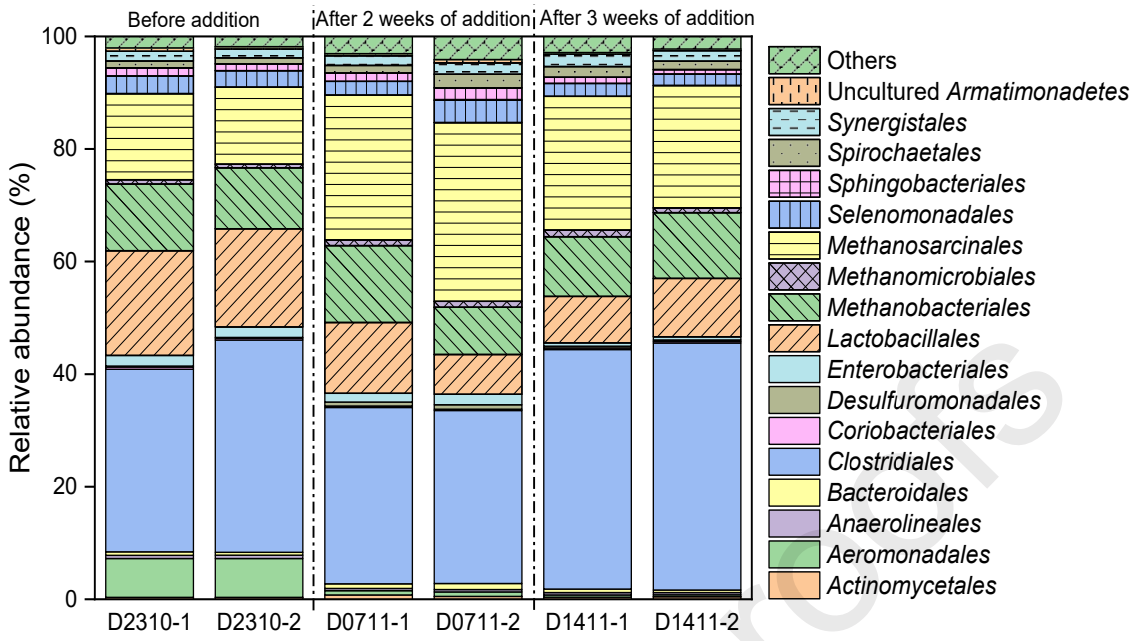

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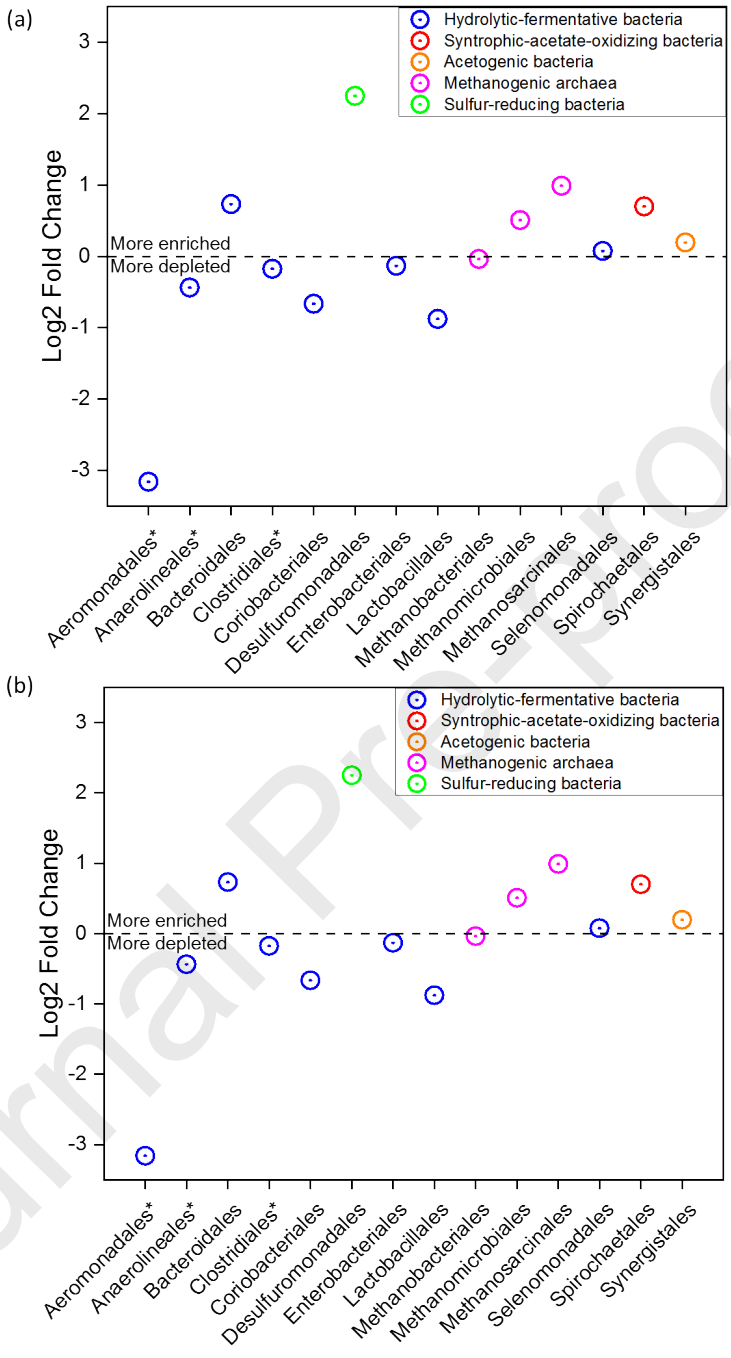

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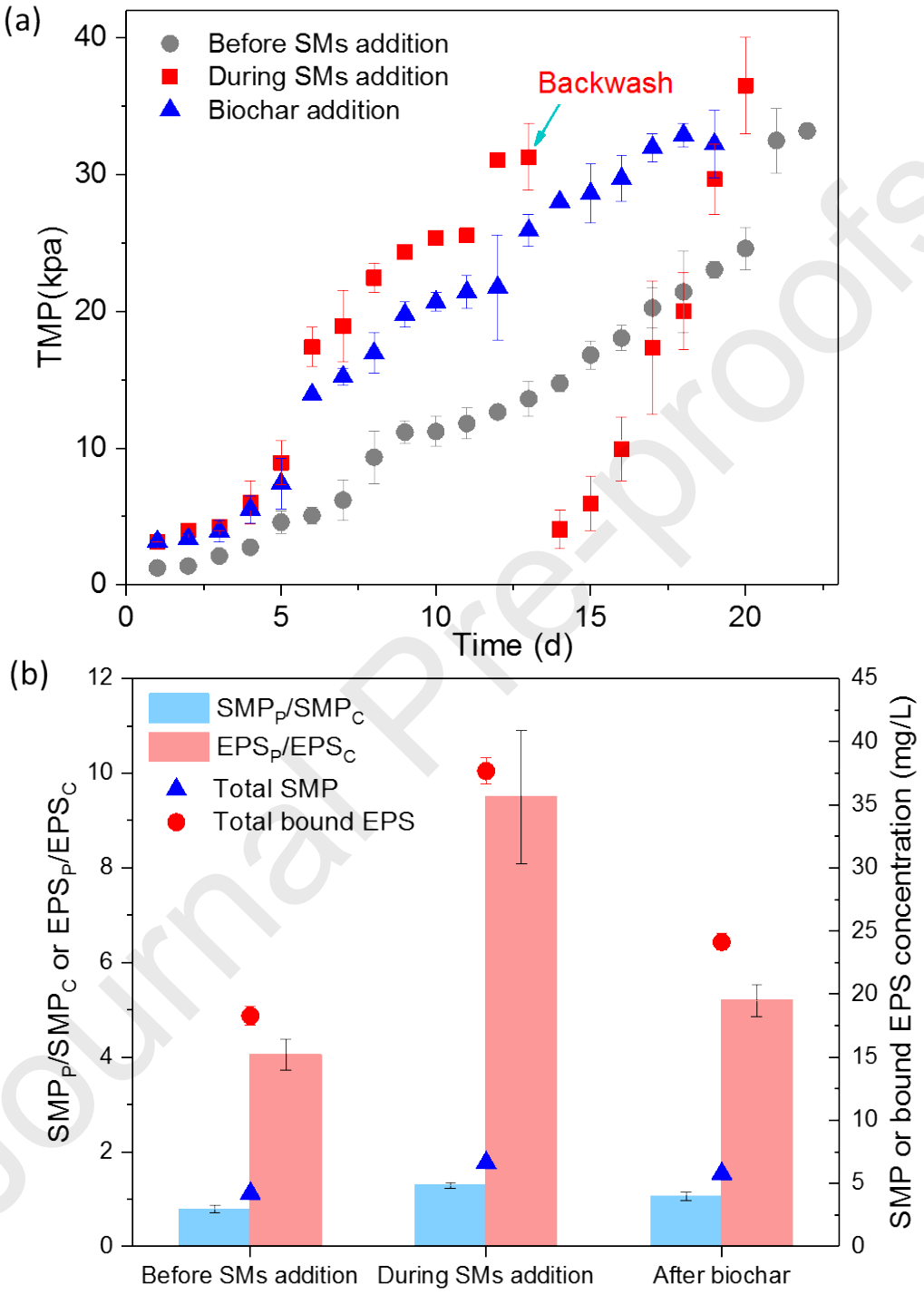

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