Performance of a recirculated biogas-sparging anaerobic membrane bioreactor system for treating synthetic swine wastewater containing sulfadiazine antibiotic

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Abstract

Anaerobic Membrane Bioreactor (AnMBR) is an efficient system for treating synthetic swine wastewater (SW). However, the presence of antibiotics in SW discourages the activity of microorganisms, resulting in less pollutants removal and biogas production. In this paper, a recirculated biogas-sparging anaerobic membrane bioreactor system was used to treat swine wastewater containing sulfadiazine (SDZ). The effects of different concentrations of SDZ on the AnMBR system's performance were explored, in terms of pollutant removal, biogas production, membrane fouling and microbial community. Results indicated that the larger concentration of SDZ triggered a strong suppression in the system's performance. When treating 1.0 mg/L SDZ, the biogas-sparging AnMBR system achieved about 77% COD removal and 0.23 L/g $COD_{removed}$ biogas production, which without SDZ fell to 21% COD being removed and dropped biogas production by 30%. As well, the presence of SDZ (1.0 mg L) increased by about half the amount of soluble microbial product (SMP) and extracellular polymeric substances (EPS) with lower protein/polysaccharide ratio and reduced sludge particle size by 49%. Meanwhile, microbial community analysis revealed that the abundance of *Firmicutes* increased while *Chloroflexi* diminished. These jointly contributed to a shorter membrane fouling cycle declining from the initial 23 d to 7 d. Furthermore, the shift from acetoclastic methanogens to hydrogenotrophic methanogens resulted in less methane production due to the presence of SDZ, while the hydrogenotrophic methanogen *Methanobacterium* promoted the degradation of SDZ. The work showed AnMBR can effectively treat swine wastewater containing antibiotics and provides basis for practical application.

Keywords: Anaerobic membrane bioreactor, Antibiotic, Swine wastewater, Biogas production, Membrane fouling,

Abbreviations : Anaerobic membrane bioreactor (AnMBR), anaerobic sequential batch reactor (ASBR), upflow anaerobic sludge bed filter (UBF), upflow solid reactor (USR), upflow anaerobic sludge blanket (UASB), swine wastewater (SW), sulfonamides (SAs), sulfadiazine (SDZ), chemical oxygen demand (COD), organic loading rates (OLR), hydraulic retention time (HRT), sludge retention time (SRT), polyvinylidene fluoride (PVDF), transmembrane pressure (TMP), mixed liquid suspended solids concentration (MLSS), mixed liquid volatile suspended solids concentration (MLVSS), soluble microbial product (SMP), extracellular polymeric substances (EPS), Volatile fatty acids (VFAs), methane (CH_4), hydrogen (H_2), nitrogen (N_2), carbon dioxide (CO_2), electron donating functional groups (EDG), operational taxonomic units (OTU).

1. Introduction

In recent years, with the huge expansion of the livestock and poultry breeding industry, the scale of intensive breeding continues unabated, resulting in the increasing amount of swine wastewater discharged from farms and subsequently harms and pollutes the environment [1]. Swine wastewater generally originates from livestock and poultry feces and cleaning wastewater and these substances contain a lot of organic matter. Due to the needs of disease resistance, epidemic prevention and production requirements, various antibiotics have been deployed as feed additives in the livestock industry and animal husbandry to promote animal growth and reproduction, and to prevent and cure animal disease symptoms, etc. [2, 3]. However, most antibiotics cannot be fully inhaled and metabolized when they are used for

animals, and about 30%-90% of antibiotics are excreted with feces and urine. They are only partially metabolized by animals in the form of compounds, conjugates, oxidation or hydrolysis products [4, 5]. According to research, the total antibiotics residues detected in swine wastewater were up to 3780 μg/L [6]. The most frequently detected classes of antibiotics in swine wastewater are sulfonamides, tetracyclines and macrolides, with the concentration of 324.4, 388.7 and 72.0 μg/L, respectively [7]. Consumption of antibiotics continue rising as the global population increases and the demand for pig products also increases. In order to reduce the impact of antibiotics on the environment and human health, antibiotic residues in swine wastewater have triggered a lot of concern about how to solve this problem [8].

At present, anaerobic process treatment is an effective method to treat swine wastewater because of its low energy consumption, high removal rate, methane-rich biogas production, and less sludge volume [9]. For example, the anaerobic sequential batch reactor (ASBR), upflow anaerobic sludge bed filter (UBF) and upflow solid reactor (USR) can remove 75%-80% chemical oxygen demand (COD) from swine wastewater and produce a maximum volume methane yield of 1.234-1.679 L/L·d [10]. However, these anaerobic treatments have some disadvantages such as long hydraulic retention time and poor stability [11]. Of the anaerobic processes, anaerobic membrane bioreactors (AnMBRs) combine the characteristics of anaerobic technology and membrane filtration, and perform remarkably well in the treatment of high COD wastewater. Therefore, AnMBR can be applied to effectively treat swine wastewater. Pu et al. investigated the functioning of AnMBR for treating swine wastewater at different organic loading rates (OLR). Their results showed that the AnMBR could achieve high COD removal (71.9%-83.6%) and CH_4 energy recovery (0.18-0.23 L/g $COD_{removed}$) when the OLR ranged from 0.25 to 0.5 g COD/g VSS·d [12].

Compared with a conventional upflow anaerobic sludge blanket (UASB), AnMBR achieved superior COD removal and methane production than the UASB (increased by 30% and 0.04 L/g $COD_{removed}$, respectively) [13]. Tang et al. investigated the effects of temperature and hydraulic retention time (HRT) on an anaerobic membrane bioreactor (AnMBR). These researchers' results confirmed that HRT of 15 days and 35 ℃ were the ideal experimental conditions for enhanced anaerobic digestion, achieving high methane production (0.24 L/g $COD_{removed}$) and microbial activity (6.65 mg COD/g VSS·h) [14]. Bu et al. treated swine wastewater with AnMBR, which could achieve an average methane yield of 0.28 L/g VSS·d and remove 96% COD [15]. However, most current studies have ignored the impact of antibiotics in swine wastewater [16]. As is well known, the presence of antibiotics may lead to microbial activity reduction or microbial populations' variation in anaerobic processes, subsequently affecting pollutants' removal and biogas production [17]. Some studies have confirmed that antibiotics in wastewater interrupt the digestion performance of anaerobic systems, and destroy the stability of the system, essentially causing the accumulation of VFAs and other metabolic intermediates [17-19]. In this way the efficiency of anaerobic treatment, etc., is seriously compromised. When treating swine wastewater, the effect of antibiotics on the operations of the AnMBR system must be explored to generate better energy production and water resource reuse.

Among all the antibiotics currently present in the environment, sulfonamides (SAs) are the most widely used class in the farming industry and the main antibiotic content detected in swine wastewater [20, 21]. SAs are usually negatively charged and repelled from the sludge surface by electrostatic repulsion, thus resulting in negligible removal by adsorption on anaerobic sludge [6]. SAs possess many aromatic rings and double-bonded functional groups that can limit microbial growth by interfering with microbial protein production, DNA replication, or other aspects of cellular metabolism. Thus, they are hardly removed by

conventional anaerobic processes [7]. Prolonging sludge retention time (SRT) may improve the degradation of antibiotics. Therefore, anaerobic membrane bioreactors (AnMBRs) are a promising alternative to conventional anaerobic processes. As previously reported, the increase in SM removal was primarily attributed to enhanced biodegradation in the AnMBR [22]. However, there still needs to be more information about AnMBRs specifically for treating swine wastewater containing SAs, including the effect of SAs on the anaerobic system and the removal mechanism. In addition, previous studies noted that membrane fouling can significantly affect the progress and rigour of the experiments, as severe membrane fouling requires membrane cleaning or membrane replacement [7, 23]. Therefore, in this study, the simple and efficient way of recirculating biogas sparging was used to extend the life cycle of membrane fouling further to ensure the rigour and smoothness of the experiment operation [24]. In this paper, the operational performance of AnMBR when treating swine wastewater in the presence and absence of sulfonamides antibiotic was compared and analyzed, including organic matter removal, gas production, membrane fouling and microbial community characteristics. The results help to devise appropriate strategies that will: firstly, improve the stability and efficiency of anaerobic treatment of actual swine wastewater; and secondly, reduce the discharge of antibiotics into the environment. It can also provide theoretical reference and guidance for the design and operation of swine wastewater treatment methods.

2. Materials and methods

2.1 The AnMBR system device and operation

The biogas-sparging AnMBR system used in the experiment is shown in Fig. 1. The whole reactor is constructed of Plexiglas, with an inner diameter of 130 cm, a height of 315 cm, and an effective volume of 3 L. The membrane module selected the hollow fiber membrane made of polyvinylidene fluoride (PVDF) (Guangzhou Haike Membrane Technology Co., Ltd.), with a pore diameter of 0.1 μm and a specific surface area of 0.042 m^2. During the operation, the membrane module is completely immersed in the reactor. The water inlet and outlet flow rate of the reactor were changed by adjusting the pump speed through a peristaltic pump to the AnMBR. A pressure sensor (MBS1900, Danfoss, Denmark) and a paperless recorder (BRW500-5100, Fürst) were connected to the effluent section of the membrane module to monitor the differential of the transmembrane pressure (TMP). The reactor is operated at a constant temperature of 35 ± 1°C which is maintained by a water bath circulation device (BILON-CX-05, Wuxi Bilang Experimental Instrument Manufacturing Co., Ltd.). The self-circulation biogas is recycled to the bottom section by the gas pump (APN-085LV-1, Iwaki, Japan) to sparge the reactor. The biogas produced by anaerobic digestion is collected by aluminum foil biogas collection bags.

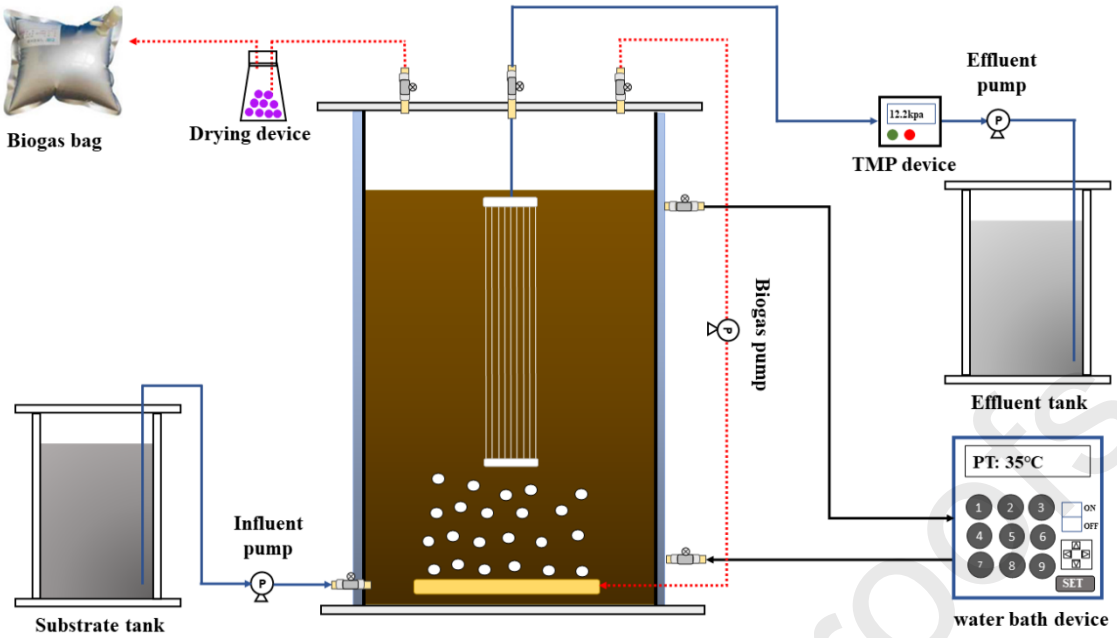


Fig. 1. Schematic diagram of the biogas-sparging AnMBR system

The anaerobic sludge used in the experiment was taken from the anaerobic digester of a sewage treatment plant in Tianjin, and the concentration of the seed sludge in the reactor amounted to 2.73 gVSS/L. The AnMBR operation is divided into three phases (shown in Tab. 1) and carried out in an uninterrupted manner. The wastewater used in this experiment was synthetic swine wastewater consisting of glucose as the main carbon source, and different concentrations of sulfadiazine (SDZ) were added in different phases of the experiment. The main component of synthetic swine wastewater was glucose (9000 mg/L), NH_4Cl (1800 mg/L), KH_2PO_4 (150 mg/L), $MgSO_4 \cdot 7H_2O$ (30 mg/L) and small amounts of essential trace elements. Essential trace elements include $FeCl_3 \cdot 6H_2O$ (13.5 mg/L), $MnCl_2 \cdot 4H_2O$ (1 mg/L), $ZnCl_2$ (1 mg/L), $NiCl_2 \cdot 6H_2O$ (4.1 mg/L), $CoCl_2 \cdot 6H_2O$ (1.4 mg/L), $CuCl_2 \cdot 2H_2O$ (0.25 mg/L), H_3BO_3 (0.1 mg/L) and $Na_2MoO_4 \cdot 2H_2O$ (0.24 mg/L). The stock solutions of SDZ were prepared by dissolving 10 mg of antibiotics in 100 mL of sodium hydroxide solution, and stored in Brown bottles at - 4°C.

Tab. 1. Operating parameters of the biogas-sparging AnMBR system

Experimental Phase	SDZ Dosage (mg/L)	Operation time (d)	Hydraulic retention time (h)	Influent COD concentration (mg/L)
Phase 1	0	45		
Phase 2	0.5 ± 0.1	30	96	9600 ± 200
Phase 3	1.0 ± 0.1	20		

2.2 Analysis methods

During the operation of the AnMBR system, the pH value of the influent and effluent was measured by a pH portable tester (Hach HQ11D, USA), while COD was detected by potassium dichromate rapid digestion spectrophotometry [25]. The mixed liquid suspended solids concentration (MLSS) and the mixed liquid volatile suspended solids concentration (MLVSS) were analyzed using the gravimetric method [26]. Soluble microbial product (SMP) was extracted by centrifugal filtration, and extracellular polymeric substances (EPS) were further separated from the sludge mixture by pyrolysis according to Hao et al. [25]. Carbohydrates and proteins in SMP and EPS were determined by the phenol-sulfuric acid method and the Folin-Ciocalteu method, respectively [27]. The sludge particle size was measured by Malvern laser particle size analyzer (Malvern Masters Sizer 2000, Malvern Instruments, UK). Volatile fatty acids (VFAs) were determined by gas chromatography (PerkinElmerClarus, USA). The autosampling volume was 20 μL, the mobile phase was 0.05% dilute phosphoric acid at a flow rate of 0.7 mL/min, the analytical column model was CosmosilPacked Column 5C18-PAQ (5 μm, 4.6×250 mm) and the column oven setting was maintained at 45 ℃. A UV detector was used with a measurement wavelength of 210 nm. Biogas components were investigated by gas chromatography (GC-2014, Shimadzu, Japan) for methane (CH_4), hydrogen (H_2) and nitrogen (N_2). The inlet temperature was set at 150℃; the column model was a 5A molecular sieve, and nitrogen and hydrogen acted as the carrier gas with shunt mode when the column flow rate was 1.81mL/min, and the column temperature was 50℃. GC equilibrium time of 3min, reference flow rate of 30mL/min, blowing flow rate of 3mL/min, and the detector heating temperature of 180℃ were employed. Carbon dioxide (CO_2) was detected through the absorption method [28]. The concentration of sulfadiazine was tested by high performance liquid chromatography-triple quadrupole mass spectrometry (LC-MS8050, Shimadzu, Japan). Solid phase extraction (SPE) was used as pre-treatment for SDZ analysis, and the extraction cartridge was Oasis (HLB) (500 mg, 6 cc, Waters, USA), according to Zhang et al. [29]. The column type was Shimadzu-packGISTC18 (size 2.1 mm, length 2 μm), and the column temperature was set at 40 °C. The interface temperature was set at 300 ℃, the interface voltage was 4 kV, and the interface current was 1.7 μA. The flow rate of the mass spectrometer dryer was 10 L/min, and the temperature of the heating block was set at 400℃. The mobile phase components were 0.1% formic acid solution and acetonitrile solution in a volume ratio of 20:80, the flow rate of the mobile phase was 0.4 mL/min, the autosampling volume was set to 5 μL, and the program run time was set to 3 minutes. The linear calibration curve is y = 2.50760·107 x + 16502, the correlation coefficients R2 were > 0.9990, the recovery was 77.12%-126.37%, the detection limit was 0.001-0.260 ng/L, and the relative standard deviation was < 9.34%.

2.3 Microbial community analysis

2.3.1 DNA extraction and testing

The initial inoculation sludge sample was select as S0. The sludge samples were taken from the reactors during the operation periods of Phase 1, Phase 2 and Phase 3, and subsequently named S1, S2 and S3, respectively. All the samples were analyzed by high-throughput sequencing. DNA was extracted by using E.Z.N.A.® soil kit (Omega Bio TEK, Norcross, GA, USA), the concentration and purity of DNA were detected by nanodrop 2000, and the quality of DNA extraction was detected by 1% agarose gel electrophoresis.

2.3.2 Amplicon sequencing and bioinformatics analysis

PCR amplification of the variable region of colony V3-V4 was done by primers 515F (5'-GTGCCAGCMGCCGCGG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'),

detected and quantified using QuantiFluor™-ST (Promega, USA) and then sequenced on Illumina's Miseq PE300 platform from Illumina (commissioned by Shanghai Meiji Biomedical Technology Co., Ltd.). The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered by fast version 0.20.0 and merged by FLASH version 1.2.7 with the following criteria: (i) the 300 bp reads were truncated at any site receiving an average quality score of < 20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp were discarded, reads containing ambiguous characters were also discarded; (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of the overlap region is 0.2. Reads that could not be assembled were discarded; (iii) samples were distinguished according to the barcode and primers, and the sequence direction was adjusted, exact barcode matching, 2 nucleotide mismatches in primer matching. Operational taxonomic units (OTUs) with 97% similarity cutoff were clustered using UPARSE version 7.1, and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 against the 16S rRNA database (e.g. Silva v138) using a confidence threshold of 0.7.

3. Results and Discussion

3.1 Operational performance of the biogas-sparging AnMBR system

3.1.1 COD removal

In Phase 1, the amount of COD removed by AnMBR is very high and stable at about 97.5 ± 0.7%, indicating that the system was very efficient in treating high organic load wastewater. This was consistent with the conclusion of Liu et al. regarding the removal of multi-antibiotic swine wastewater by AnMBR [6]. In Phase 2, at the presence of SDZ (0.5 mg/L), the COD removal rate still remained above 96% in the first 10 days and 95.7 ± 1.6% in the following 20 days, confirming that a small concentration of SDZ had only a slight impact on the AnMBR system. Therefore, the system enabled high COD removal perfomance for the treatment of wastewater containing low concentrations of antibiotics. The slight decline in COD removal may be due to the accumulation of refractory and toxic intermediates such as aniline and pyrimidin-2-amine in the degradation process of SDZ [30-32]. In Phase 3, with the addition of SDZ (1.0 mg/L), the COD removal began to falter and decreased to 86.8% after 5 days, and this process continued in the following 15 days, finally dropping to 77.1%. This result was also consistent with a previous report by Cheng et al. [33]. They sequentially injected a mixture of SAs with total concentrations of 0, 0.3, 0.6, and 0.9 mg/L into the AnMBR, and the COD removal was reduced from the initial 94.21% to 58.72%, 51.65%, and 18.82%, respectively. This reflected those higher concentrations of antibiotics more strongly inhibited anaerobic microorganisms. The higher concentration antibiotic led to a stronger inhibition on the organic matter biodegradation [34]. Secondly, it may be that the antibiotics of SDZ were decomposed by the anaerobic microorganisms to produce more toxic intermediate products, including aniline, pyrimidin-2-amine and 3-(methylimino) prop-1-en-1-yl) hydroxylamine. These were difficult to degrade and gradually accumulated as the operating time continued [32]. They in fact affected the normal physiological metabolic behavior of microorganisms, and then caused a decrease in the removal of COD [30].

3.1.2 Biogas production and composition analysis

Compared to Phase 1, biogas production in Phase 2 fell from 0.43 ± 0.04 L/g $COD_{removed}$ to 0.36 ± 0.03 L/g $COD_{removed}$. However, the methane content in biogas appeared to obvious

decrease from 56.7%-77.2% to 47.6%-59.7%. Despite the consequent reduction in methanogenic performance, the reactor continued to operate in a stable way. When in Phase 3, the biogas production declined sharply to 0.23 ± 0.03 L/g $COD_{removed}$, which was nearly 0.20 L/g $COD_{removed}$ lower than that in Phase 1. It was clear that the biogas production performance of the AnMBR system declined significantly when a larger SDZ concentration was present in the feed water. In addition, an interesting phenomenon was observed at the beginning of SDZ addition in Phases 2 and 3: the proportion of methane showed a consistent trend of decreasing and then increasing to a stable level. Similar to Cheng et al. [33], the methane content and production rate decreased from the initial 56.3% and 0.2 L/g CODremoved to 41.1% and 0.13 CODremoved, respectively, within two weeks after the addition of SMs, indicating the inhibitory effect of SMs at the observed methane production concentrations. While the inhibitory effect of SMs on methane production gradually weakened as the microorganisms in the AnMBR slowly adapted to the SMs, a recovery trend of methane production was observed in the third week. This revealed that the presence of SDZ affected the microflora related to biogas production in the bioreactor. A study by Xu et al. found that antibiotics increased lactate dehydrogenase release levels, a cytoplasmic substance released from damaged cells, indicating a disruption of cellular integrity [35]. These findings suggest that the presence of SDZ limited the growth of anaerobic bacteria and led to cell lysis of anaerobic bacteria, influencing anaerobic digestion and ultimately causing reduced methane production. Furthermore, the methane content droped to 44.1%-53.3%, meaning that methanogenic bacteria were very sensitive to SDZ, even at low SDZ concentrations. Although some methanogenic bacteria could attribute to degrading SDZ [32], the high concentration of antibiotics and the accumulation of intermediate products inhibited the activity of methanogens, thereby reducing the production of methane [30, 34].

3.1.3 pH and VFAs

pH is an important indicator of the normal operation of the AnMBR system. As shown in Fig. 2(c), pH did not change significantly and remained stable at 7.4-7.7 in Phases 1 and 2. However, in Phase 3, pH revealed a downward trend and floated in the 6.6-7.7 range, strongly suggesting the appearance of obvious acid accumulation. Further, the changes of VFAs in the reactor at each phase were analyzed. In Phase 1, the VFAs concentration varied between 418 mg/L and 814 mg/L. After the addition of SDZ (Phase 2), the VFAs rose to 533-1120 mg/L. Therein acetic acid and propionic acid concentration ascended from 95-212 mg/L to 102-341 mg/L and from 98-298 mg/L to 100-501 mg/L, respectively. Though the VFAs demonstrated a certain increase in Phase 2, in view of gas production and COD removal, the bioreactor's internal environment was relatively stable. Nevertheless, when in Phase 3 the VFAs appeared to virtually bolt from 649 to 4707 mg/L.

Correspondingly, the concentrations of acetic acid and propionic acid increased to 2587 mg/L and 1939 mg/L, respectively. The results showed that with the concentration increase of SDZ, a large accumulation of VFAs, especially acetic acid, occurred in the AnMBR system. The accumulation of VFAs revealed that the activity of both hydrolytic acidifying bacteria and methanogenic bacteria was inhibited due to the presence of SDZ (1.0 mg/L). Especially, the obvious accumulation of acetic acid indicated that the activity of acidophilic methanogenic bacteria was most severely inhibited. As a result, it caused the very evident decrease in the organic matter removal and gas production in Phase 3. Due to the inhibition of the activity of methanogenic bacteria in the bioreactor, the produced acetic acid could not be effectively transformed, leading to a decrease of pH and acidification in the reactor [36]. Cheng et al. proposed that sulfonamides prevented the addition of p-aminobenzoic acid into the folate molecule by competing for dihydropteroate synthase, thereby inhibiting the synthesis of folate required for the

synthesis of purines and nucleic acids, and thus limiting bacterial growth [7].

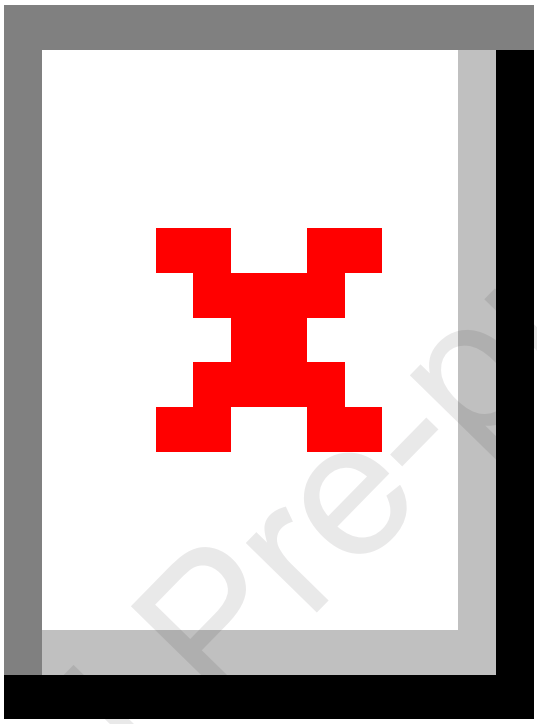


Fig. 2. (a) Removal of COD, (b) Biogas production, (c) pH and VFAs in AnMBR ($P < 0.05$, according to the test of One-Way ANOVA)

3.2 Sulfadiazine removal

In Phase 2, the SDZ concentration in the AnMBR effluent was stable below 0.05 mg/L with the removal rate of 92.6 ± 3.3%, indicating that AnMBR performed excellently in removing the SDZ antibiotic. When in Phase 3, the removal rate of SDZ fell slightly to 86.6 ± 4.7%, yet AnMBR still exhibited a high removal rate of SDZ. The results demonstrated the effectiveness and feasibility of AnMBR when treating swine wastewater containing SDZ. Biological degradation was the primary mechanism for SDZ removal. As reported, SDZ

(pKa2: 6.5) becomes negatively charged when the pH > pKa2, leading to electrostatic repulsion between sulfadiazine and biofilms, and a low LogKow value of SDZ (LogKow < 2) leads to the poorer adsorption capacity of biofilms [29]. A 112-day mass balance test by Wang et al. demonstrated that biodegradation with negligible adsorption is the primary pathway for removing SDZ [37]. Some research had proved that antibiotics containing electron donating functional groups (EDG), such as sulfonamides, showed high biodegradability in AnMBR [38]. In addition, the interception of AnMBR created a longer retention time for SDZ in the system [39], and this contributed to the SDZ removal. However, in Phase 3, the removal rate of SDZ showed only a slight decrease. This was due to the excessive toxic effect of SDZ on microorganisms and the accumulation of intermediate products in the SDZ degradation process. Some studies have reported that sulfa antibiotics can form a variety of transformation products during the biodegradation process, including aniline, pyrimidin-2-amine and 3-(methylimino) prop-1-en-1-yl) hydroxylamine. These degradation by-products are more toxic and more stable than the parent [30]. Furthermore they inhibited the activity of microorganisms and resulted in undermining the efficiency of SDZ degradation.

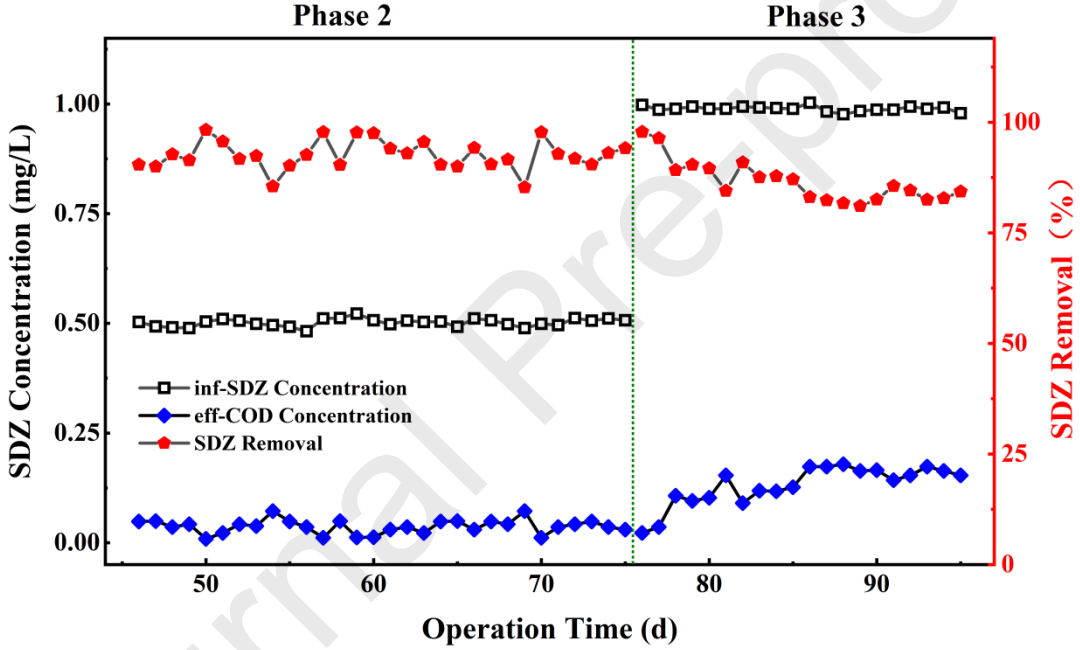


Fig. 3. SDZ removal by AnMBR

3.3 Membrane fouling

3.3.1 TMP

During the operation that lasted 45 days in Phase 1, membrane cleaning was conducted twice (on days 23 and 43). The fouling process was slow during the period of 0-20 d and 24-40 d, respectively, with TMP increasing slowly from 0 to 10 kPa. The fast membrane fouling rate suddenly increased during the operation time of 21-23 d and 40-43 d, with TMP jumping from 10 kPa to 33 kPa, respectively. The reason for the slow fouling rate was that the organic and inorganic particles penetrated and deposited in the membrane pores, which promoted the formation of the filter cake layer at a later stage. Meanwhile the fast fouling was mainly caused by the compression of the filter cake layer [40, 41]. After adding SDZ to the reactor, the membrane fouling cycle was shortened, and the membrane fouling rate was accelerated. In Phases 2 and 3, the membrane cleaning cycle decreased to 12 d and 7 d, respectively.

Membrane fouling was accelerated by 47.8% and 69.6%, respectively, compared to that without the addition of SDZ. This was restrictive for the long-term operation of AnMBR. As can be seen from Fig. 4(a), the higher SDZ concentration caused the shorter cleaning cycle and faster TMP growth. The main reason for this was due to the presence of SDZ in the bioreactor stimulating microorganisms to secrete more SMP and EPS in response to toxicity and inhibition [42]. As a result, they can adhere to the membrane surface and then accumulate gel layers and contribute to the occurrence of membrane fouling [43].

3.3.2 MLSS, MLVSS and Sludge particle size

The characteristics of the sludge in the bioreactor, such as MLSS, MLVSS and sludge particle size, are closely related to membrane fouling. The changes of MLSS and MLVSS and sludge particle size are depicted in Fig. 4(b). In Phase 1, the concentrations of MLSS and MLVSS remained stable at around 21.96 ± 1.26 g/L and 17.37 ± 0.84 g/L, respectively. Meanwhile, the sludge particle size showed a steady upward trend, rising from an initial 47.11 μm to 82.72 μm, which was conducive to alleviating membrane fouling. Given the larger difference between the suspended matter and membrane pore size, it is less likely that the membrane will be blocked. On the contrary, when the particle size is closer to the membrane pores, more particles are attached to the membrane, thereby causing more serious membrane fouling [44, 45]. In addition, sludge flocs with larger particle size exhibit greater interaction forces and only with some difficulty are deposited on the membrane. While particles with smaller size have poor hydraulic effect and continuously accumulate on the membrane surface to form a tight filter cake layer, this accelerates membrane fouling [46].

In Phases 2 and 3, the concentrations of MLSS and MLVSS remained largely stable, which ensured the proper functioning of the AnMBR systems. However, the sludge particle size first increased from 82.72 μm to 98.31 μm (Phase 2) and then decreased to 50.46 μm (Phase 3). In Phase 2, the growth of sludge particle size was probably because the small concentrations of sulfadiazine did not yet affect the sludge too much, but nonetheless stimulated the secretion of EPS and SMP. This enhanced the adsorption of suspended particulate matter by the sludge particles, and led to sludge particles increasing in size. However, in Phase 3 the high concentration of SDZ and its toxic intermediates had a significant toxic effect on the microorganisms, resulting in the destruction of the sludge flocs and a further reduction in sludge particle size [47-49]. As the particles shrunk in size, they were easily accumulated and blocked near the membrane pores, which further caused serious membrane fouling of the AnMBR.

3.3.3 EPS and SMP

As is well known, SMP and EPS are the main factors causing membrane fouling. SMP is mainly produced from the endogenous respiration of microorganisms, and its main components include polysaccharides, proteins, nucleic acids, and humic acids. EPS refers to various types of macromolecular polymers secreted by bacteria and wrapped in vitro and between bacteria, which is the support structure of biofilm and activated sludge. EPS and SMP are regarded as the main substances causing membrane fouling [50, 51]. As shown in Fig. 4(c), EPS and SMP concentrations tended to increase during the whole operation. SMPc (polysaccharides in SMP) and SMPp (proteins in SMP) rose from 6.01 ± 0.61 mg/L and 31.30 ± 1.29 mg/L to 24.85 ± 2.52 mg/L and 35.62 ± 3.98 mg/L, respectively. The ratio of SMPp / SMPc were 5.25 ± 0.55、2.16 ± 0.35 and 1.43 ± 0.10 in Phases 1, 2 and 3. EPSc and EPSp increased from 10.10 ± 1.35 mg/gVSS and 24.31 ± 1.92 mg/gVSS to 20.51 ± 1.33 mg/gVSS and 35.62 ± 3.98 mg/gVSS, respectively. In the meantime, EPSp/EPSc reduced from 2.45 ± 0.44 to 1.45 ± 0.08. Based on this it was clear that after adding SDZ, the SMP and EPS on the cake layer of the membrane module increased.

The increase of SMP concentration caused by the SDZ in the AnMBR may be due to the large production of VFAs and cell lysis products [52]. The EPS production by microorganisms was a natural reaction to the toxic environment, and played an important role in protecting microorganisms against the presence of antibiotics. Microorganisms secreted more SMP and EPS to form a protective "cocoon" which delayed the entry of toxic compounds into the cell body [42]. SMP and EPS have complex properties including surface charge, hydrophobicity/hydrophilicity and adhesive characteristics, etc., and affect the flocculation, stability and adhesion behaviors of sludge flocs, thus their dramatic increase accelerated membrane fouling [53]. In addition, with the increase of SDZ concentration, the ratio of protein/polysaccharide decreased when the SMP and EPS concentration increased. The polysaccharides in EPS were preferentially used by microorganisms, therefore the decrease of protein/polysaccharide ratio may be due to the gradual inhibition of microbial activity by toxicity, resulting in the increase of residual polysaccharide concentration [54]. According to the reported research, the smaller ratio of protein/polysaccharide in SMP would increase irreversible fouling of membrane modules [25].

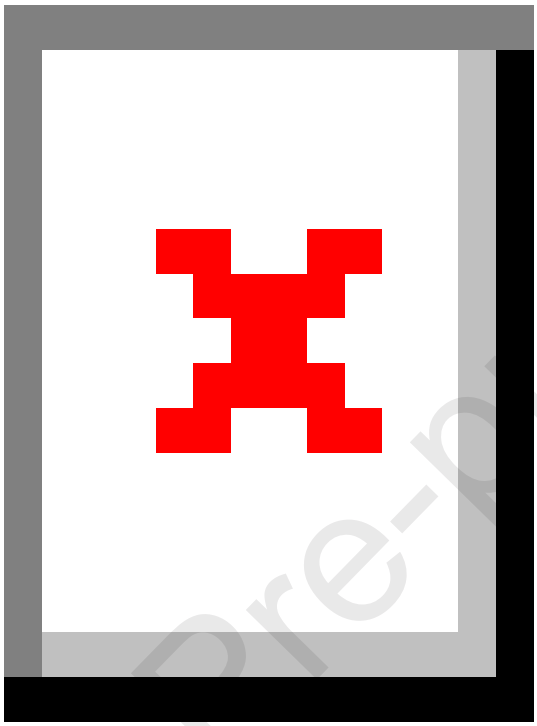


Fig. 4. (a) TMP, (b) MLSS, MLVSS and Sludge particle size and (c) SMP and EPS in AnMBR

3.4 Microbial community analysis

To explore the dynamic changes occurring in the microflora in the AnMBR system, microbiological samples were respectively analyzed in different operation phases. The operational taxonomic units (OTU) is the classification operation unit, which is obtained by clustering Reads at a similarity level of 97.0%. As shown in Tab. 2, the coverage index was greater than 0.998 in all phases, indicating that the sequencing data were sufficient to capture the actual diversity of the microflora in the samples. The Sobs index, ACE index and Chao1 index showed that 0.5 mg/L SDZ only slowed down the growth of microflora, while 1.0 mg/L SDZ greatly reduced the abundance of microflora. Simpson's and Shannon's indices indicated

that the diversity followed the same trend as the abundance of microflora.

Variations in the abundance of microorganisms at different phases in the AnMBR were obtained by high-throughput sequencing analysis of the mixed sludge. The abundance of the main phylum is shown in Fig. 5(a). During the entire operation, *Actinobacteria*, *Proteobacteria*, *Halobacterota, Synergistota*, *Firmicutes, Spirochaetota* and *Thermotogota* were the top seven dominant phyla. As is well known, *Actinobacteria, Proteobacteria, Firmicutes* and *Chloroflexi* play the key roles in anaerobic hydrolytic acidification [55, 56]. *Actinobacteria* have a strong ability to degrade complex carbohydrates and can generate acetic and propionic acid from glucose [57]. *Firmicutes* contain a variety of hydrolytic and acid-producing fermentative bacteria for the production of propionic and acetic acids that contribute to the removal of complex and refractory organic matter [58]. *Chloroflexi* and *Proteobacteria* are also recognized as hydrolytic bacteria [59]. Therefore, in Phase 1 the abundance of *Actinobacteria, Proteobacteria, Firmicutes* and *Chloroflexi* was dominant and greater than 55%, which ensured very thorough removal of organic matter and adequate supply of volatile fatty acids. Among the dominant phyla, Firmicutes can cause membrane fouling [60, 61]. The abundance of *Firmicutes* increased by 10% and 38% in Phases 2 and 3, respectively, due to the addition of SDZ, thus contributing to the accelerated membrane fouling. As reported, *Chloroflix* can use SMP and EPS as organic carbon sources for growth [62].

Compared with that in Phase 1, the abundance of *Chloroflexi* dropped by 33% and 56% in Phases 2 and 3, respectively, meaning that the presence of SDZ inhibited the growth of *Chloroflexi* and exacerbated membrane fouling. *Synergistetes* and *Thermotogota* can co-metabolize with methanogenic bacteria, and *Synergistetes* can degrade long-chain fatty acids to acetic acid [63] and *Thermotogota* can reduce the CO_2 and H_2 through acetate oxidation [64]. In Phase 2, the abundance of *Synergistetes* and *Thermotogota* increased by 86% and 369%, respectively, which promoted the metabolism of VFAs and caused a smaller methane ratio in biogas to emerge. In Phase 3, the abundance of *Synergistetes* and *Thermotogota* increased by 5% and 70%, respectively, which caused the accumulation of VFAs and lower biogas production and methane ratio. Additionally, as reported, *Thermotogota* enhanced biodegradation of sulfonamides in the biological treatment of amoxicillin wastewater [6]. In this study, the change in abundance of the *Thermotogota* phylum coincidentally corresponded to antibiotic concentration and degradation performance. *Spirochaeta* can produce acetic acid, ethanol, H_2, CO_2 and other intermediates through glucose fermentation [65]. After the addition of SDZ in Phases 2 and 3, the abundance of *Spirochaetota* decreased by 64% and 70%, respectively. This did not favor biogas production. Interestingly, after the addition of SDZ in Phases 2 and 3, the total abundance of *Halobacteria* and *Euryarchaeota*, which included various methanogenic bacteria, increased by 120% and 55%, respectively. The observed differences in the growth of bacteria and archaea may be attributed to the working mechanism of the sulfonamide antibiotics used in this study. The structural similarity of sulfonamide antibiotics to p-aminobenzoic acid (PABA), a precursor for folate biosynthesis, has been reported to cause sulfonamides to compete with PABA for dihydropteroate synthase, which is used by bacteria to synthesize folic acid, thereby reducing the amount of folic acid necessary for bacterial growth and inhibiting bacterial growth. In contrast, the role of folate as a C1 carrier in archaea is fulfilled by methotrexate, which can be synthesized from PABA via different pathways [66]. Therefore, the addition of sulfadiazine only affected most bacteria's growth but not archaea's growth.

The changes in genus-level abundance at different phases are shown in Fig. 5(b). *Norank_f_Propionibacteriaceae* and *Brooklawnia* were overwhelmingly dominant. *Norank_f_Propionibacteriaceae* could transport complex nutrients for fermentative metabolism into substances such as propionic acid and butyric acid. *Brooklawnia* plays

important roles in hydrolysis and acid production during anaerobic degradation by taking up VFAs as the main fermentation products [67, 68]. Their stable presence ensures the proper hydrolytic acidification functioning of the system. Genus-level identifications indicated that *Thermotogota* phylum was composed entirely of *Mesotoga*, which was first described as a mesothermal genus [69]. *Mesotoga*, a genus of functional bacteria related to hydrolytic acidification, can co-oxidize with methanogenic bacteria to complete the removal of organic acids. They can use acetic acid to produce H_2 and CO_2 in anaerobic systems which helps to acetic acid accumulation and promote hydrogenotrophic methanogenesis [70, 71]. Furthermore, as mentioned above, the Thermotogota phylum was the bacteria associated with the degradation of sulfonamide antibiotics, and Mesotoga was the only component of Thermotogota species in this study. The abundance of Mesotoga at each phase was 1.83% (initial sludge), 1.4% (Phase 1), 11.9% (Phase 2), and 4.5% (Phase 3). The abundance increased rapidly after the addition of SDZ. Although there was a decrease in Phase 3, it was still 300% higher than in Phase 1. The abundance presented a positive correlation with the SDZ removal rate, indicating that Mesotoga may be beneficial in enhancing the SDZ removal. Similarly, norank_f_Synergistaceae abundance increased from 1.2% (Phase 1) to 5.6% (Phase 1) and 2.8% (Phase 3) after SDZ addition. A positive correlation between this genus and antibiotic removal was reported by Liu et al. [6]. *Treponema* (belonging to the *Spirochaetota* phylum) is a homoacetogenic bacteria that can transform organic matter while also reducing CO_2 to acetate by using H_2 as electron donors [72]. This can enhance the utilization of organic matter and methane. With the addition of SDZ, the abundance of *Treponema* decreased by 48% and 70% in Phases 2 and 3, respectively, which may be one explanation for the smaller amount of methane in the biogas.

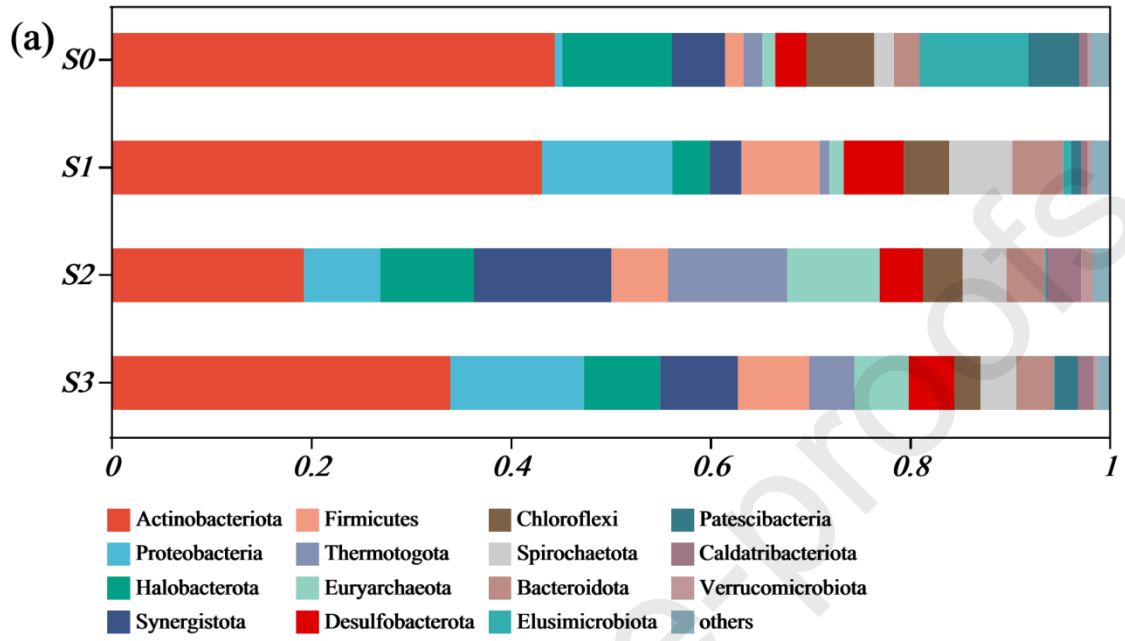
In order to analyze the methane production changes, methanogenic archaea were analyzed in detail as shown in Fig. 5(c). *Methanothrix*, *Methanosarcina* and *Methanobacterium* were the top three archaeal genera with the relative highest abundance, and their total abundance was greater than 85%. *Methanothrix*, *Methanosarcina* and *Methanobacterium* belong to acetoclastic methanogens, hybrid multipathway and hydrogenotrophic methanogens, respectively [72, 73]. With the addition of SDZ, the total abundance of *Methanothrix* and *Methanosarcina* decreased by 17% and 20%, while the abundance of *Methanobacterium* increased by 33% and 67% in Phases 2 and 3, respectively. This indicated that the hydrogenotrophic methanogens had higher substrate utilization, growth rate and cell yield when exposed to a high concentration of SDZ [74]. Combined with the changes in the VFAs and methane content, it is suggested here that the accumulation of VFAs (especially acetic acid and propionic acid) and reduction in methane was related to the inhibition to *Methanothrix* and *Methanosarcina* by SDZ in Phases 2 and 3. In addition, hydrogenotrophic methanogens *Methanobacterium* increased rather than decreased with SDZ concentration rise, further confirming the previous conclusion that anaerobic degradation of sulfonamide antibiotics is driven by a combination of hydrogenotrophic methanogens and homoacetylated methanogens [60]. In addition, *methanobacterium* were also noted to potentially contribute to the mineralization of some by-products of SDZ [32].

Tab. 2. Sample alpha diversity index statistics

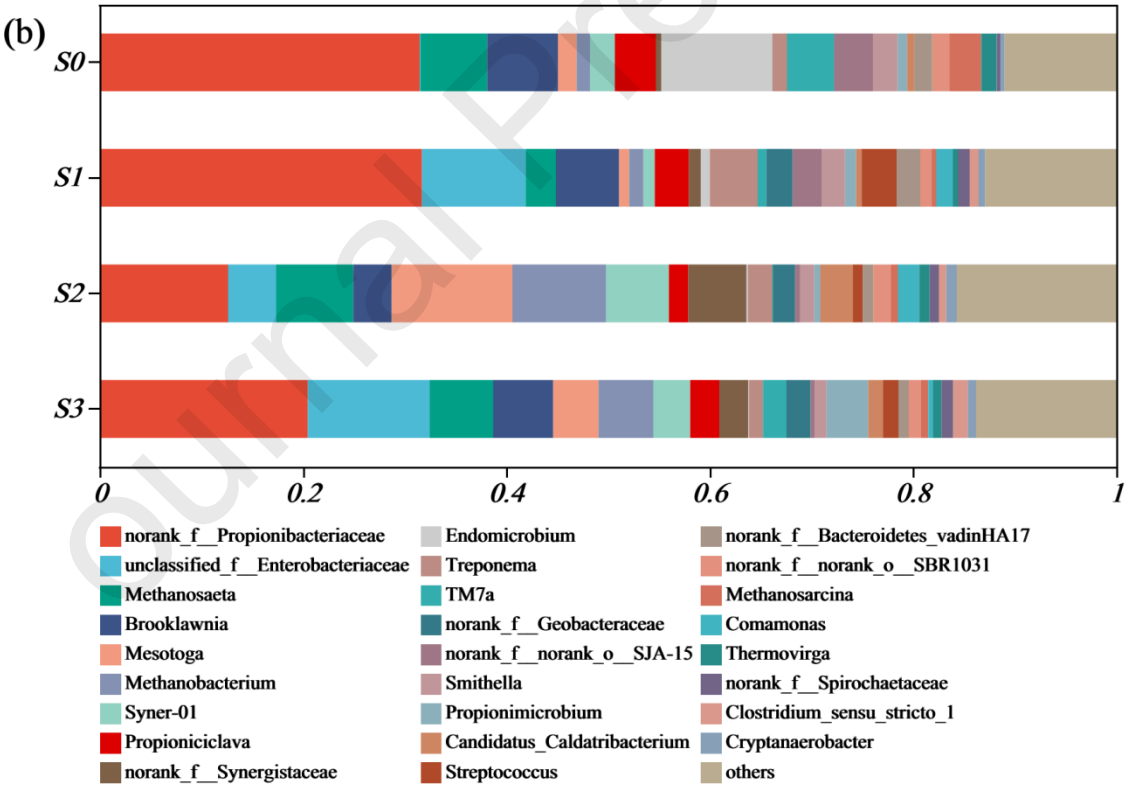
Sample	Sobs	Ace	chao	shannon	simpson	coverage
S0	478	579.935398	608.784314	3.073301	0.127575	0.998606
S1	553	642.722713	668.000000	3.278188	0.123076	0.998734

S2	599	704.748040	693.879518	3.715691	0.052295	0.998745
S3	564	652.736035	639.370370	3.536984	0.073227	0.998913

533



534



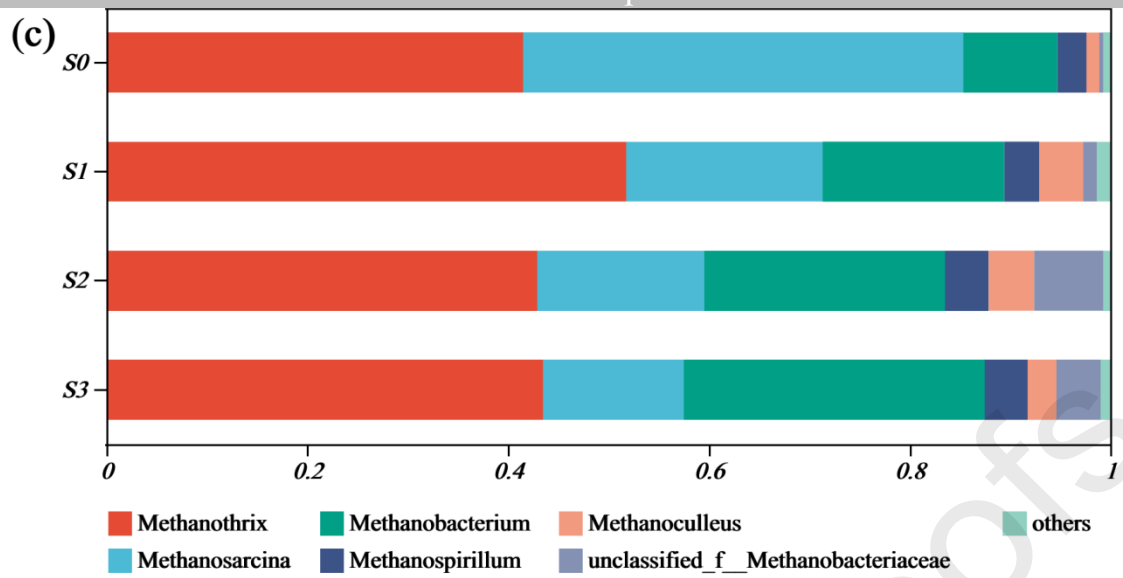


Fig. 5. Abundance at the level of (a) phylum microorganism, (b) genus bacterial and (c) genus archaea

4. Conclusions

The biogas-sparging AnMBR system was applied to treat swine wastewater containing the antibiotic SDZ. The system could achieve high COD and SDZ removal as well as methane production when exposed to SDZ, despite the accumulation of VFA and decrease in methane production occurring due to the presence of 1.0 mg/L SDZ. Moreover, SDZ stimulated the production of SMP and EPS, diminished the protein/polysaccharide ratio due to bacterial self-protection, and reduced sludge particle size. These ultimately exacerbated membrane fouling rate, which was unfavorable for the long-term operation of the AnMBR system. Meanwhile, the increase of *Firmicutes* and decrease of *Chloroflexi* contributed to a shorter membrane fouling cycle. Furthermore, the shift from acetoclastic methanogens to hydrogenotrophic methanogens in the system resulted in lower methane production due to the presence of SDZ. This work further demonstrated the promotion of SDZ degradation by hydrogenotrophic methanogen Methanobacterium, as did Mesotoga. This work can provide the basis for practical application and help to take effective strategies when the AnMBR system is applied to treat wastewater containing antibiotics. In addition, the specific antibiotics degradation pathways and membrane fouling mitigation still need further exploration.

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