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Role of spent coffee ground biochar in an anaerobic membrane bioreactor for treating synthetic swine wastewater

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Abstract: Using anaerobic membrane bioreactors (AnMBRs) to treat swine wastewater is an effective method to recover bioenergy. However, due to the inhibitory effect of high concentrations of organic matter and ammonia nitrogen on microbial activities in swine wastewater, some problems are evident such as low recovery efficiency and serious membrane fouling. In this study, biochar prepared from spent coffee grounds (SCG-BC) was added to AnMBR to investigate its effect on the operation process. Results reported that methane yield rose from 0.227 LCH_4/g-$COD_{removed}$ to 0.267 LCH_4/g-$COD_{removed}$ along with a reduction in CO_2 being produced at 35.25% after adding SCG-BC. It confirmed that in-situ biogas upgrading was achieved. As well, the total volatile fatty acids declined to a low concentration of 194.87 ± 51.82mg/L while pH remained steadily at 7.70 ± 0.31. Adding SCG-BC reduced irreversible membrane fouling by 34.69%. Microbial community analysis showed that SCG-BC increased the relative abundance of methanogenic archaea, especially *Methanosarcina* (from 1.47% to 8.03%). Also, *Anaerolinea* and *Methanosaeta* participating in direct interspecies electron transfer were enriched onto biochar. They acted together to enhance the biogas production. It can be concluded that AnMBR with SCG-BC addition has good application prospects in recovering bioenergy from wastewater.

Keywords: Anaerobic membrane bioreactor, Spent coffee grounds biochar, Methane production, Membrane fouling, Microbial community

1. Introduction

The swine industry has become one of the fastest growing sectors in China, with 47.3% of the world's swine production in 2020, mainly due to the rising demand by people for meat [1]. At present, large-scale breeding is a crucial change in how the swine industry in China functions, and single farms can now produce much more. However, the large-scale swine factory has the problems of large wastewater discharge, which comprises mixed solid and liquid, many insect eggs and microorganisms, and large amounts of heavy metals, antibiotics and hormones [2, 3]. Besides, the swine wastewater produced contains a high level of organics, nitrogen, phosphorus, potassium and other chemical pollutants [4, 5]. In general, the traditional processes treating swine wastewater, such as lagoon, anaerobic digestion tank or three-stage A/O process, can only remove less than 90% of chemical oxygen demand (COD) and the removal capacity of nitrogen and phosphorus is extremely limited. Subsequently, this might cause a large amount of recyclable energy loss and long-term environmental problems [6]. In recent decades, as environmental regulations become increasingly strict, it is of importance to guarantee effluent quality of swine wastewater for a society much more concerned about what is happening to the environment. Moreover, simultaneous wastewater treatment and energy recovery has become a hotly debated topic. The purpose is to drive the technical efficiency of the wastewater treatment plant as well as its profitability [7].

Anaerobic digestion (AD) consists of hydrolysis, acidogenesis and methanogenesis, which is widely used to degrade swine wastewater and recover bioenergy presently, including biogases such as H_2S, CO_2, H_2 and CH_4 and digestive fluid containing residual

COD and other nutrients [8-11]. However, its poor efficiency in producing methane is a serious problem in practical application, which is due to the slow growth of anaerobic microorganisms, especially methanogens [12, 13]. Microbial activity in the reactor is an important factor for the efficient operation of the anaerobic digestion process [14, 15]. Anaerobic membrane bioreactor (AnMBR) can effectively separate solid retention time (SRT) and hydraulic retention time (HRT) through membrane modules, and be applied to wastewater treatment in recent years. In some cases, AnMBR achieved a high organic matter removal rate (81%-95%) and methane recovery rate (60%-80%) with a low sludge yield (0.05-0.22 gVSS/g-$COD_{removed}$) [16- 18].

AnMBRs can effectively improve the digestion performance but there are still some difficulties and challenges in them when treating swine wastewater. On one hand, the organic matter in swine wastewater is very high amount-wise. Tang et al. [18] found that the methanogenic capacity of anaerobic digestion becomes weak (lower than 0.24 L/g-$COD_{removed}$) in swine wastewater with a high organic concentration (13.5-27.2 gCOD/L). This is because the larger amount of organic matter in the substrate resulted in producing more volatile fatty acid, which inhibited the growth of methanogens. With the increase of organic load, the contents of soluble microbial products (SMP) and extracellular polymers (EPS) in the reactor also rose. SMP and EPS are considered to be the main pollutants that trigger membrane fouling [20]. On the other hand, swine wastewater also contains a lot of ammonia nitrogen. According to the research by Yan et al. [21], with the ammonia nitrogen shock of 4.5 gNH_4^+-N/L, the methane production diminished rapidly, which was 48.7-58.2% lower than before. This was because methanogens are very sensitive to ammonia toxicity during methane production from intermediate products, and their methanogenic performance decreases sharply with the impact of high concentration ammonia nitrogen in wastewater [22, 23].

Some corresponding strategies were put forward, such as controlling the anaerobic digestion temperature and prolonging HRT, to overcome these problems. The measures are widely used to enhance the performance of the reactor to a certain extent; however, they have the disadvantages of reducing the volume load and increasing operational costs [24]. Some researchers have focused on the use of various enhancers in AnMBR, including waste yeast, iron, calcium, polyaluminum chloride, zeolite and beans [25-30]. Among these materials, iron and calcium can improve biogas production in the digestion process. However, they also introduce serious inorganic pollution problems. Beans damage the membrane module to some degree during fluidization [31]. Moreover, these materials greatly increase the cost of reactors' operations.

Carbon-based materials as AnMBR performance enhancers play an important role in improving COD removal rate, improving membrane flux and delaying the formation of filter cake layer. These materials are also environmentally friendly as they are produced from agricultural wastes [32]. Sohn et al. [20] found that the removal rates of COD and total organic carbon (TOC) in AnMBR significantly increased by 15.7% and 15.6%, respectively, after the addition of 5g/L powdered activated carbon (PAC). A higher methane yield of about 3483 nmol/L was achieved in the degradation of rapeseed oil, while the methane yield of the control group without GAC was lower than 3000 nmol/L [33]. By adding bamboo charcoal in AnMBR to treat bamboo industrial wastewater, the removal rate of COD increased by 5%, as well as the content of SMP and the resistance of filter cake layer abated [34]. Chen et al. [35] found that the AnMBR with the addition of biochar exhibited an enhancement of 4.6% in the COD removal rate when treating pharmaceutical wastewater. It also improved the methane content in biogas production and effectively curtailed membrane fouling. Moreover, some research showed that carbon-based materials can strengthen the direct interspecies electron transfer (DIET) process by enriching microbes that can directly produce or receive electrons, such as *Geobacter metallireducens*, *Geobacter sulfurreducens* and *Methanosaeta* [36]. Thus, the electron transfer of microorganisms does not need to pass through the intermediate media that easily cause energy loss, such as conductive pili and hydrogen, and greatly increases the conversion rate from organic matter to methane [24]. This is also an important reason why carbon-based materials can effectively promote anaerobic digestion and control membrane fouling.

Of carbon-based materials, the specific surface area and conductivity of biochar are much lower than those of activated carbon, yet it has been reported that biochar has the same ability to promote anaerobic digestion and enhance DIET as activated carbon [37]. Although powdered activated carbon can promote anaerobic digestion, it is a potential pollution source. Because the small particle PAC increases the turbidity of the mixture, it results in more membrane hole blockage and membrane surface abrasion [31]. The specific gravity of granular activated carbon (GAC) is higher than that of biochar, which means that it takes more energy to mix GAC with sludge into fluidization [38]. Furthermore, terms of preparation cost, biochar does not need an activation process, which can undoubtedly reduce overheads [39]. These are also the reasons why biochar has better prospects compared with activated carbon in the application of anaerobic digestion.

In conclusion, biochar has good application potential as an enhancer in AnMBR for treating swine wastewater. It may affect methanogenic performance and membrane fouling by changing the microbial community structure and microbial interspecific cooperation. These need to be further studied. In this work, spent coffee grounds biochar (SCG-BC) was introduced in AnMBR acting as a performance enhancer. The objectives were to: (i) evaluate the digestion performance of AnMBR system after the addition of spent coffee grounds biochar; (ii) investigate membrane fouling behaviors; and (iii) explore the impact of biochar on microbial community.

2. Materials and methods

2.1 Preparation of biochar

The spent coffee grounds were sourced from a local Starbucks Cafe in Tianjin, China. First, the collected waste coffee grounds were dried to constant weight in an oven at 105 ℃. Then the waste coffee residue was put into a muffle furnace to prepare biochar via pyrolyzation. The spent coffee grounds were heated to 900 ℃ at the rate of 5 ℃/min and maintained for 2 hours under oxygen limitation conditions [40]. Finally, the produced biochar with a particle size of 0.15-0.178 mm was passed through an 80-mesh sieve to remove large particles and retained by a 100-mesh sieve to remove small particles. The characteristics of SCG-BC are shown in supplementary material.

2.2 AnMBR set-up and operation

The AnMBR system consists of anaerobic digestion reactor, gas circulation equipment, gas-liquid replacement gas gathering device and membrane module (shown in Figure 1). The effective working volume of AnMBR is 2.5 L and the total volume is 4 L. The membrane information is shown in supplementary material.

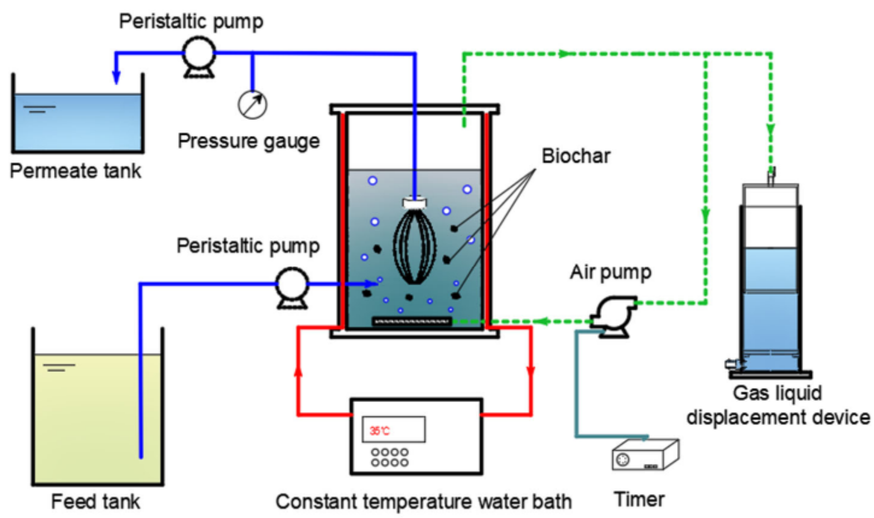

Fig. 1. Schematic of the AnMBR system

The inoculated sludge of the reactor was taken from the SBR sludge of a sewage treatment plant in Tianjin, China. The mlvss amount was 8 g/L. Following the inoculated sludge was domesticated for two months to adapt to synthetic swine wastewater. The experiments were divided into two stages. The first was phase 1(P1), running for 50 days (day 1-50) after acclimatization. The second phase commenced on the 51st day, when spent coffee grounds biochar was added into the reactor at the dose of 4 g/L, and the effect of biochar on AnMBR was investigated. This period (P2) lasted from day 51 to 100.

During the whole operation, HRT was set at 8.3d, the temperature was maintained at 35 ± 1 ℃, sludge was discharged regularly, and the sludge residence time was 120d. The biogas inside the reactor was extracted from the top gas space through the pump and transported to the aeration plate at the bottom of the reactor to activate the gas cycle. The aeration process was intermittent for 1 min every 30 min with the flow of 1 L/min.

The swine wastewater treated by the reactor was synthesized in the laboratory. Glucose, NH_4Cl and KH_2PO_4 were carbon, nitrogen and phosphorus sources, respectively. The ratio of COD: N: P is 350: 27: 1. The swine wastewater quality indicators at different operation periods are shown in supplementary material. To promote microbial growth and granular sludge formation, 10 mL of trace element solution was added to each liter of synthetic swine wastewater. The trace element solution is shown in supplementary material.

2.3 Analytical methods

The COD concentration was analyzed using the rapid digestion-spectrophotometric method (Shimadzu, UV-2600). The concentrations of protein (PN) and polysaccharide (PS) were determined utilizing a modified Folin Ciocalteu colorimetry method [41] and phenol-vitriolic acid colorimetry system [42], respectively. Volatile fatty acids (VFA) and biogas composition were measured by gas chromatograph (Perkin Element GC590, USA) and gas chromatograph (Perkin Element GC500, USA), respectively. TMP was recorded by vacuum pressure gauge and paperless recorder. The surface functional groups of biochar were detected by Fourier transform infrared spectroscopy (FTIR, Nicolet iS10, US). The specific surface area, pore volume and pore diameter of carbon-based materials were measured by Brunauer Emmett Teller (BET). The morphology of the original and used biochar was analyzed by scanning electron microscope (SEM, ESCAN MIRA4, Czech Republic).

2.4 Membrane fouling resistance analysis

In order to investigate the filtration characteristics of the membrane, the membrane fouling resistance was measured by resistance-in-series model [20].

$$J = \Delta P/\mu R_t \quad (1)$$

Where J is the permeate flux, ΔP stands for the TMP, and μ denotes the viscosity of the permeate. The total resistance R_T consists of four parts, which are R_c, R_m, $R_{p\text{-}org}$ and $R_{p\text{-}inorg}$. It can be described as follows:

$$R_T = R_m + R_{p\text{-}org} + R_{p\text{-}inorg} + R_c \quad (2)$$

Where R_m is the inherent resistance of the membrane, R_c, $R_{p\text{-}org}$ and $R_{p\text{-}inorg}$ represent the resistance of the cake layer, organic matter blockage, and inorganic matter blockage, respectively.

After the operation was completed, the membrane module was taken out and R_T with different flow in distilled water was measured. Next the membrane was rinsed with distilled water and wiped gently with a sponge to remove the cake layer. The measured value at this stage was $R_m + R_{p\text{-}org} + R_{p\text{-}inorg}$. Then the membrane was immersed in alkaline reagent (0.1% NaClO solution) for 24 hours to remove any organic matter remaining in the membrane pore. The measured value at this stage was $R_m + R_{p\text{-}inorg}$. Finally, the membrane was soaked in an acidic reagent citric acid solution (10 g/L) for 4 hours to remove inorganic pollutants from the membrane pores. The measured value at his stage was R_m.

2.5 Microbial analyses

The four samples (SS, SP1, SP2 and BC) for microbial analysis were the seed sludge, the mixed sludge taken from the AnMBR on day 45 and day 96, and the biomass taken from SCG-BC carriers on day 101, respectively. The universal primer set 515F (5’-GTGCCAGCMGCCGCGG-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’) served to target both bacterial and archaeal 16S rRNA V4 regions. The AxyPrepDNA Gel Extraction Kit (AXYGEN, USA) helped to purify each sample. The samples of this experiment were entrusted to Shanghai Majorbio (Shanghai, China) for high-throughput sequencing. Data analysis was conducted on the Majorbio bioinformatics date cloud platform.

3. Results and discussion

3.1 COD removal and biogas production

Fig. 2 shows the COD removal efficiencies of AnMBR system before and after the SCG-BC addition. The COD removal rate during phase 1 without SCG-BC addition was 96.88 ± 0.27% with the effluent concentration of 326.61 ± 42.58 mg/L. In phase 2, after adding SCG-BC, the COD removal rate was 98.85 ± 0.13% when the effluent concentration was 109.73 ± 35.43 mg/L. It can be seen that the effluent COD concentration diminished significantly after adding SCG-BC. This be due to the abundant pore structure and large pore volume of SCG-BC (see supplementary material), which facilitated: firstly, the adsorption of undegraded organic matter; and secondly, the reduction of effluent COD concentration [43, 44]. Meanwhile, according to SEM analysis of pure and used SCG-BC (see supplementary material), it can be observed that a large number of microorganisms grew and colonized onto the surface and pores of SCG-BC. Biochar provided a good environment for microorganisms in which to grow and raised microbial metabolic activity and its adaptability, so this contributed to the COD removal rate [24]. This also explained why the effluent from the AnMBR in P2 maintained a low COD concentration.

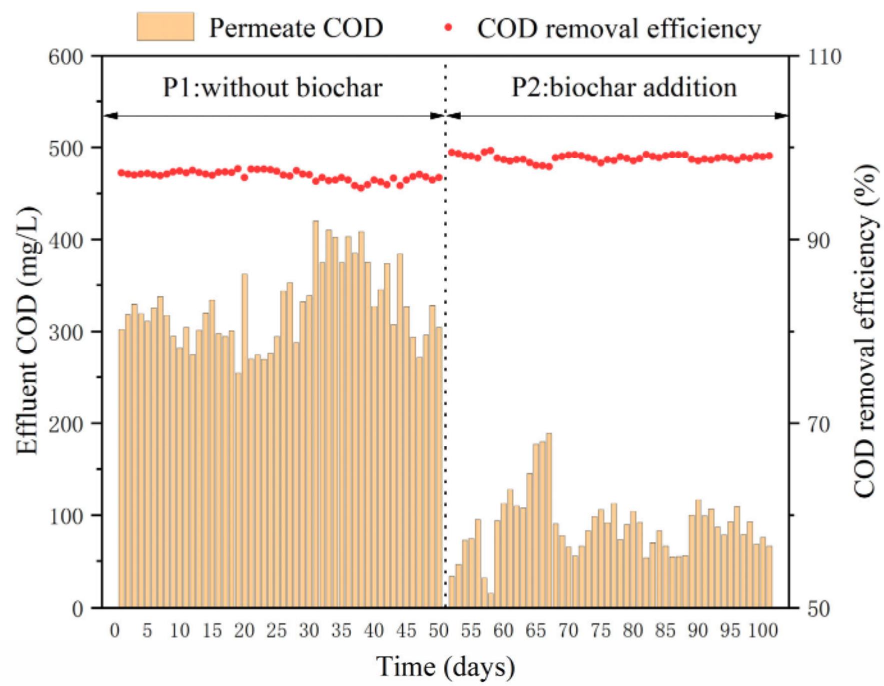

Fig. 2. COD concentration and removal efficiency before (P1) and after (P2) adding SCG-BC

The biogas production performance and CH_4 yield of the AnMBR system in P1 and P2 are illustrated in Fig. 3. Before and after adding SCG-BC, the CH_4 production of AnMBR was 593.10 ± 33.75 mL/d and 692.92 ± 56.54 mL/d, while the CO_2 production was 89.26 ± 8.96 mL/d and 58.57 ± 5.96 mL/d, respectively. Compared with P1, the CH_4 production in P2 increased significantly by 16.83%, while CO_2 production clearly decreased by 34.37%. The methane yield reached 0.256 LCH_4/gCOD in P2, which was

12.7% higher than 0.227 LCH_4/gCOD in P1. The elevated CH_4 production was due to the rapid decomposition of organic matter and more efficient microbial metabolism in the anaerobic digestion process enhanced by SCG-BC [21]. As well, the reduction of CO_2 production confirmed the in-situ purification and upgrading of biogas due to the addition of SCG-BC. The phenomenon can be explained in the following two ways. On one hand, biochar has a good adsorption effect on CO_2 and can reduce the loss of CO_2 [45]. On the other hand, the pH value of AnMBR modified by SCG-BC was slightly alkaline. It may be because more inorganics were retained in the SCG-BC prepared at the higher pyrolysis temperature. As a result, base cation and carbonate in the SCG-BC increased, while acidic functional groups decomposed [40]. In this state, CO_2 was transformed into bicarbonate/carbonate and stored in the liquid phase, which helped convert it into methane by hydrogenotrophic methanogens [46].

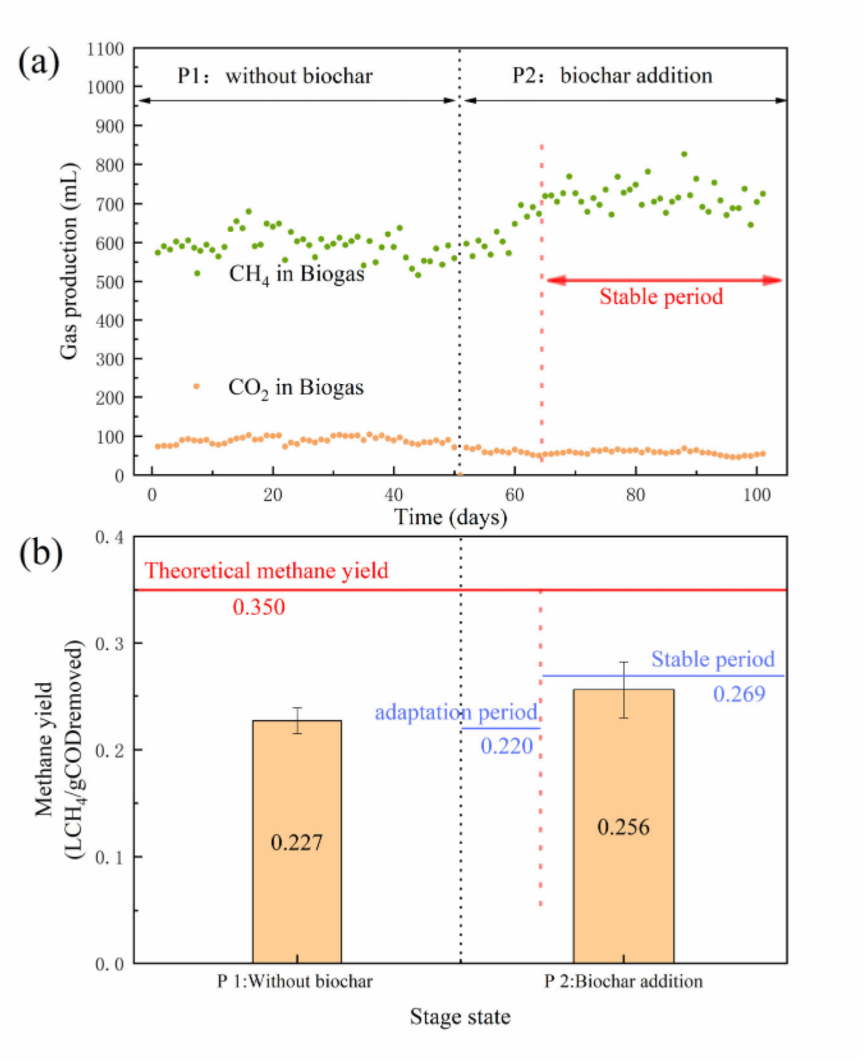

Fig. 3. Biogas production performance of AnMBR (a) and normalized methane yield (b)

Specifically, in the P2 phase it can be seen that CH_4 and CO_2 production showed a gradual upward and downward trend, respectively, at the initial stage of SCG-BC addition. After day 64, the methane production reached a relatively stable state with the CH_4 and CO_2 production of 717.37 ± 35.37 mL/d and 57.79 ± 5.67 mL/d, respectively. Compared to the scenario without SCG-BC, they increased by 20.95% and decreased by 35.25%, respectively. This is close to the 17.6% increase in methane production mentioned in one recent study [47]. The addition of SCG-BC led to more biogas production and enhanced biogas production structure. This was because a large number of microorganisms adhere to the outer and inner surfaces of the biochar, making better use of the adsorption capacity and conductivity [48].

Obviously, the addition of SCG-BC had a good effect on improving the methanogenic capacity of the AnMBR system. The CH_4 yield reached 0.269 $LCH_4/gCOD$ during the stable period of P2 which was increased by 18.50%, compared with the yield of 0.227 $LCH_4/gCOD$ in phase P1. Similarly, Kaur et al. [49] found that wheat straw pellet biochar increased the CH_4 yield in the digestion process by 24%. However, Giwa et al. [50] added biochar to the medium temperature continuous reactor, and discovered the methane yield only increased by 5%. These research studies revealed the different biogas production performance varied with the biochar properties and operation conditions of reactors. In general, when the yield of CH_4 increases by more than 15%, it means that biochar can significantly improve the energy recovery potential of AnMBR [16].

3.2 Variations of pH and VFAs

VFAs as important raw materials in methanogenic process, affect the performance of AD. The accumulation of VFAs reduces the alkalinity and pH value, thus inhibiting the methane production process [7]. The pH and VFAs of the AnMBR system in the two phases are shown in Fig. 4. As can be seen from Fig. 4, the pH values before and after SCG-BC addition were 6.76 ± 0.28 and 7.70 ± 0.31, respectively. After SCG-BC addition, the pH in phase 2 increased significantly and then stayed above 7.22. Therefore, SCG-BC helped restrict any decline in the pH level and improved the operational stability of the AnMBR. This was because SCG-BC possessed a large number of functional groups (see supplementary material), such as -OH, C=O, carboxyl C-O and aromatic C-H, which could provide alkalinity [51, 52]. The total VFAs (TVFA) concentrations before and after SCG-BC addition were 497.73 ± 129.48 mg/L and 194.87 ± 51.82 mg/L, respectively.

The addition of SCG-BC greatly reduced the content of VFAs in the AnMBR. At the beginning of P1 the main volatile acid was propionic acid. As the operation continued, pentanoic acid, isobutyric acid and isovaleric acid also appeared. In phase P2, with the addition of SCG-BC, the content of TVFA on days 51-54 was lower than that in the phase P1. This may be due to the adsorption of volatile acids by SCG-BC. On days 68-101, TVFA in the reactor reached a good concentration status. This phenomenon can be interpreted in two possible ways. First, the microbial community varied duo the introduction of SCG-BC, and the methanogenic microorganisms which could degrade VFAs enriched. Second, SCG-BC acted as an electronic catheter to strengthen DIET. Because biochar could skip the electron carriers such as hydrogen to transfer electrons, in this way microbial degradation of VFA was accelerated [53]. In general, adding the SCG-BC not only buffered pH but also reduced the concentration of VFA in the reactor., So these contributed to keeping the stable operation and enhancing the AnMBR system' production of biogas.

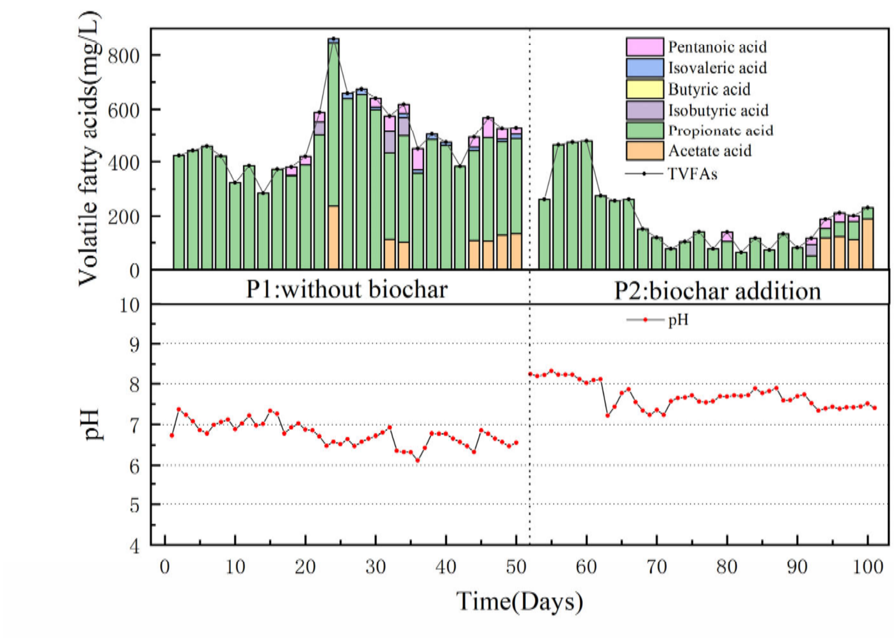

Fig. 4. Variations of pH and VFA before and after SCG-BC addition

3.3 Membrane fouling

Variations of transmembrane pressure (TMP) during the operation processes are shown in supplementary material. It can be seen that the growth rate of TMP decreased after adding SCG-BC. To further explore the effect of SCG-BC on membrane fouling, the membrane resistance was analyzed (Fig. 5). The proportion of each form of membrane resistance in the total resistance was very similar in the two operation stages, however, their values clearly varied. After adding SCG-BC, excluding the inherent resistance of the membrane (R_m), the total value of other membrane resistance fell by 12.33%. The resistance of the cake layer, organic and inorganic fouling (R_c, $R_{p\text{-}org}$ and $R_{p\text{-}inorg}$) dropped by 6.50%, 28.41% and 58.61%, respectively.

Clearly, the resistance of the cake layer was dominant in these two phases. Judging by the R_c value shown in Fig. 5, the addition of SCG-BC reduced the formation of a cake layer. Compared with the cake layer that can be removed by physical cleaning, irreversible fouling caused by membrane pores blockage, including organic matter and inorganic matter blockage, was more difficult to be removed by physical cleaning. However, the addition of SCG-BC reduced irreversible fouling by 34.69%. The reason for the decline in irreversible fouling was that the simultaneous adsorption and biodegradation of biochar greatly reduced the organic and inorganic pollutants in the reactor such as EPS and SMP, and diminished the blocking of membrane pores [54]. The conclusion can be further confirmed by the SEM results of the membrane surface (see supplementary material). There were many pollutants on the surface of both two membranes, however, the membrane module used in P2 was smoother than the membrane module used in P1, and the membrane pores were purer. It strongly suggested that SCG-BC had a positive effect on reducing membrane fouling, especially the irreversible fouling in membrane pores.

3.4 SMP and EPS in mixed sludge

Fig. 6 illustrated the SMP and EPS contents in the AnMBR system in two phases. It was well known that SMP and EPS were the main factors guiding membrane fouling. SMP was the main contaminant forming the gel layer due to adhering to the membrane surface, thus reducing the permeation flux of the membrane [55]. Additionally, the secreted EPS was conducive to the condensation between microorganisms and as a result promoted the formation of cake layer [19, 20]. The concentrations of SMP and EPS decreased by 28.58% and 49.36%, respectively. The main reason for the synchronous decline of SMP and EPS may be due to simultaneous adsorption and biodegradation of SCG-BC. In addition, SCG-BC improved pH and alkalinity of AnMBR, suppling a suitable environment for microorganisms to function well. It reduced the secretion of EPS as a protective secretion and meanwhile, less cell lysis led to less SMP accumulation [56, 57]. The decline in SMP and EPS helped to increase membrane flux and reduce membrane pollution.

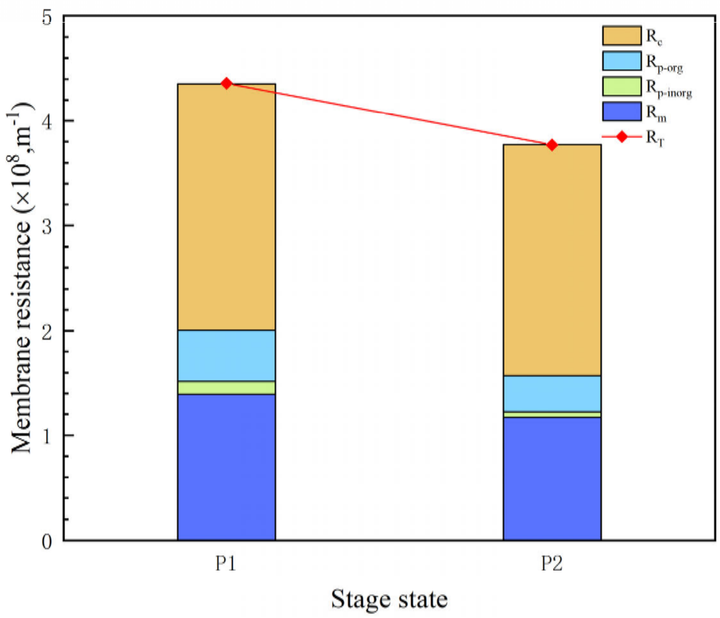

Fig. 5. The variations of membrane fouling resistance of two phases (R_T, total resistance; R_m, inherent resistance of the membrane; $R_{p\text{-}org}$, resistance of organic matter blockage; $R_{p\text{-}inorg}$, resistance of inorganic matter blockage; Rc, resistance of the cake layer)

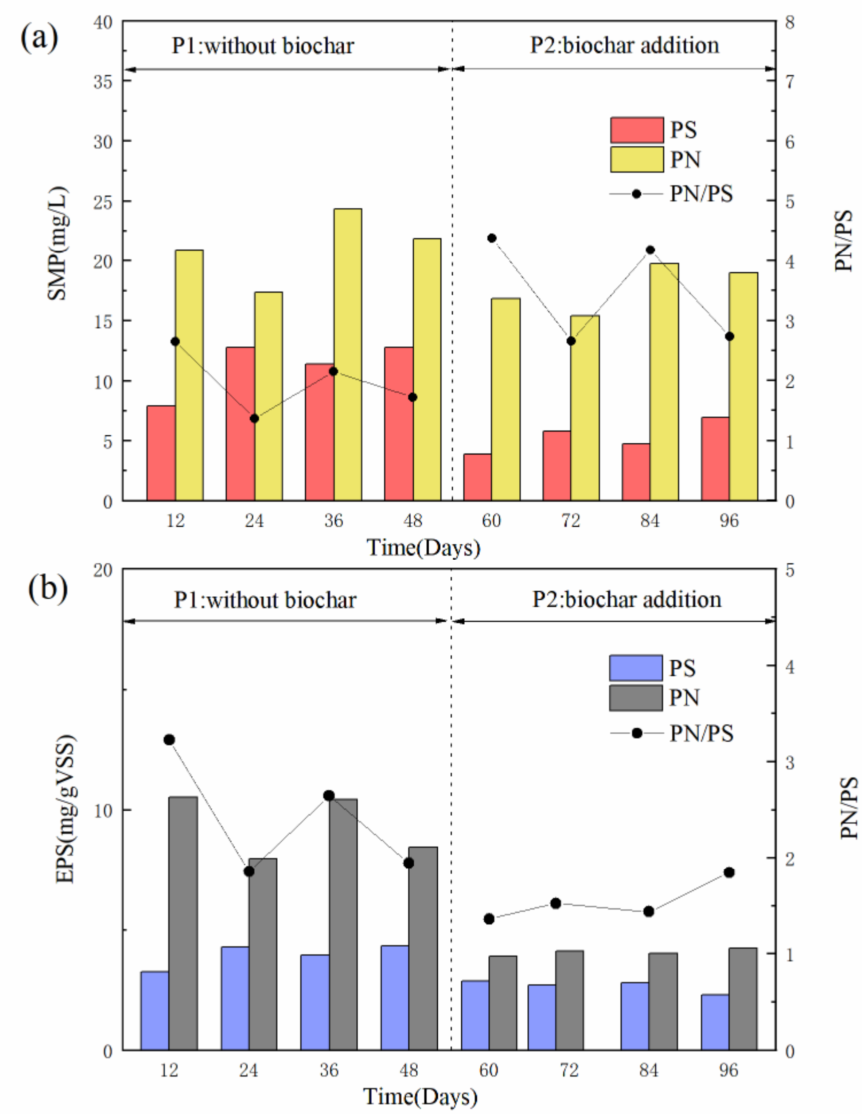

Fig. 6. SMP(a) and EPS(b) contents in the AnMBR

The SMP_{PS}, SMP_{PN}, EPS_{PS} and EPS_{PN} decreased by 52.33%, 15.99%, 31.05% and 56.31%, respectively. Furthermore, the ratio of protein to polysaccharide (PN/PS) of SMP and EPS was also an important factor affecting membrane fouling. As shown in Fig. 6, the PN/PS ratio of SMP increased significantly after the addition of SCG-BC. The SMP with high protein ratio would produce better settleability through better hydrophobicity, which helped to control membrane fouling, especially irreversible fouling in membrane pores. The PN/PS ratio of EPS in AnMBR with SCG-BC was lower than AnMBR without SCG-BC. The smaller protein content resulted in the weaker hydrophobicity and the smaller viscosity of the sludge, thus delaying the formation of the cake layer [58]. Combined with the membrane fouling analysis in section 3.3, it can be observed that the membrane fouling degree was positively correlated with the variety of SMP and EPS content. With the decrease of SMP and EPS after SCG-BC addition, both cake layer and the pore blockage were indeed inhibited. Therefore, adding SCG-BC to AnMBR proved to be an effective method for curtailing membrane fouling and increasing membrane flux.

3.5 Microbial community diversity analysis

The alpha diversity index is shown in the supplementary material. Compared with the seed sludge， sample of SP1 showed lower community richness by Chao1 and ACE value, which meant some microorganisms were eliminated during the AD process. After adding SCG-BC, poorer community richness can be found from diversity indices. Meanwhile, the lower Shannon index and higher Simpson index revealed a lower evenness. It was evident that SCG-BC affected the environment of microorganisms and led to some microorganisms becoming enriched as the dominant species in AnMBR. It was worth noting that microorganisms attached to SCG-BC highlighted less community richness and evenness compared with other samples. The percentages of archaea increased from 4.47% (seed sludge) to 17.79% (SP1), and 23.64% (SP2). At the same time, methanogens accounted for more than 99.90% of the total number of archaea, which was 2.91% higher than that of seed sludge (see supplementary material). The proportion of methanogens increased during the AD process, indicating that functional methanogens were enhanced. After adding SCG-BC the methanogens further increased, and thus improved methane production (shown in Fig. 3).

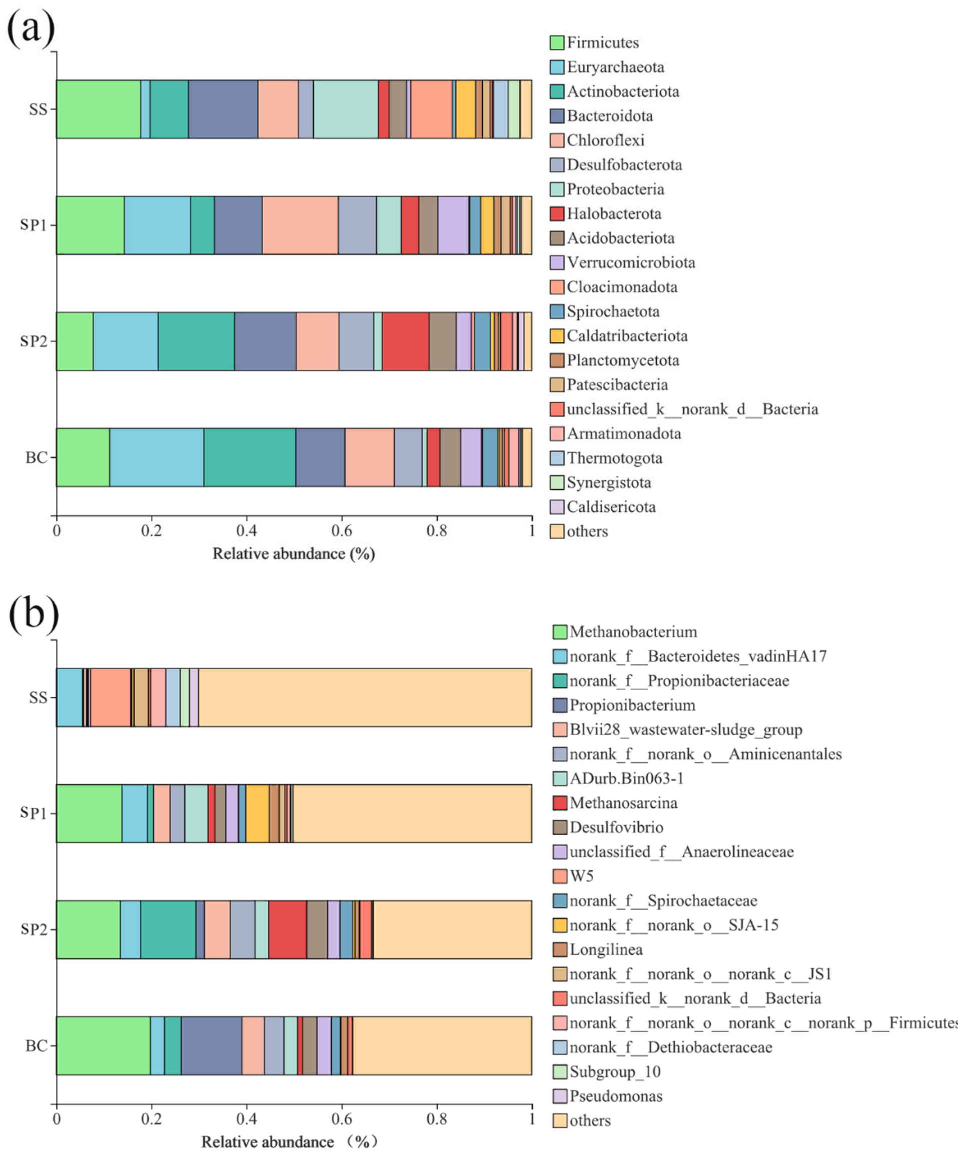

Fig. 6. Relative abundance of microbial communities at phylum (a) and genus (b) level

The relative abundance of bacterial and archaeal communities at phylum is illustrated in Fig. 6a. The relative abundance of microorganisms varied significantly with the operation of AnMBR. Among them, phylum *Thermotogota*, the dominant strain in high-temperature anaerobic digestion, decreased from 3.10% to 0.11%. Except some special cases were temporarily classified as phyla *Bathyarchaeota* and *Verstrateearchaeota*, most of the known methanogens belonged to phyla *Euryarchaeota* and *Halobacterota* [59, 60]. The phylum *Euryarchaeota* increased significantly from 1.97% in the seed sludge to 13.91% in SP1. Meanwhile the phylum *Halobacterota* increased 1.63-fold. The growth of these two phyla means that methanogenic microorganisms adapted to the operating conditions of the reactor and possessed good methanogenic capacity.

The predominant phyla in SP1 were *Chloroflexi* (15.99%), *Firmicutes* (14.32%), *Euryarchaeota* (13.91%), *Bacteroidota* (10.09%) and *Desulfobacterota* (8.04%). After the addition of SCG-BC, the predominant phyla were *Actinobacteriota* (16.10%), *Euryarchaeota* (13.62%), *Bacteroidota* (12.95%), *Halobacterota* (9.85%) and *Chloroflexi* (9.01%). Therefore, it can be stated that the relative abundance of fermentation microorganisms changed significantly. *Firmicutes, Bacteroidota*, *Proteobacteria* and *Actinobacteriota* phyla were the main fermentation microorganisms in the hydrolysis and acidogensis stage which were responsible for converting organic matter into volatile fatty acids, H_2 and CO_2 [61-63]. The total relative abundances of these microbial phyla increased by 11.65%. This meant that the efficiency of the hydrolysis acidification stage was greatly improved, and the substrate was degraded more efficiently. Among them, the relative abundance of *Proteobacteria* decreased from 5.17% in SP1 to

1.78% in SP2. This was because *Proteobacteria* was the dominant strain under acidic conditions, but adding SCG-BC led to the increase of pH. This subsequently limited the growth of *Proteobacteria*.

Additionally, *Proteobacteria* was the main bacteria causing membrane fouling. The decline in its relative abundance played a positive role in controlling membrane fouling [57]. The relative abundance of *Firmicutes* was 45.67% lower than that before adding SCG-BC, which indicated that the addition of SCG-BC had certain restrictions on its growth. This may be because the existence of SCG-BC greatly improved the growth environment of microorganisms in the bioreactor, making *Firmicutes* lose its competitive advantage of strong tolerance [64]. In contrast, the relative abundance of *Actinobacteriota* increased by 3.22 times after adding SCG-BC, and replaced *Firmicutes* as the main phylum in the hydrolysis and acidification stage. This was effective in alleviating membrane fouling. Further, *Actinobacteriota* can easily decompose polysaccharide and protein matrix in anaerobic environment, which contributed to the reduction of SMP and EPS, helping to improve the reactor's stability [65, 66].

The relative abundance of *Chloroflexi* phylum decreased from 15.99% to 9.01% after the addition of SCG-BC. As is well known, *Chloroflexi* was involved in the degradation of various macromolecular organics, including proteins and carbohydrates mainly in SMP and EPS produced by autotrophic microorganisms [67, 68]. It further proved that adding SCG-BC contributed to controlling the accumulation of SMP and EPS. As for methanogenic microorganisms, *Euryarchaeota* maintained a stable level before and after adding SCG-BC. The relative abundance of *Halobacterota* increased from 3.71%

to 9.85%. A large increase in the relative abundance of methanogenic microorganisms not only can accelerate the consumption of intermediate products, but also heighten the yield of methane. Interestingly, most of the phyla Euryarchaeota were hydrogentrophic methanogens, while phyla Halobacterota were mostly acetoclastic methanogens, such as genus *Methanosarcina* and genus *Methanosaeta*.

Thus, it can be seen that the addition of SCG-BC enhanced the acetoclastic methanogenic pathway. In summary, the community structure in hydrolysis stage was optimized by adding SCG-BC, which increased the organic matter degradation rate, reduced membrane fouling and enhanced the methanogenic efficiency. The relative abundance of bacterial and archaeal communities at genus was illustrated in Fig. 6b. As can be seen that *Methanobacterium* was a hydrogenotrophic methanogenic archaea, which was a dominant strain in the whole operation stage. Therefore, the existence of SCG-BC in the AnMBR wielded no significant effect on its relative abundance (13.49% - 13.80%). *Propionibacterium* can decompose polysaccharide into short chain fatty acids.

The lack of *Propionibacterium* would cause the accumulation of the matrix to a certain extent, resulting in a decline in reactor performance and the deterioration of effluent quality [57]. *Propionibacterium* was not detected in SP1, while its relative abundance rose to 1.79% in SP2. In the meantime, the relative abundance of *norank_f__Propionibacteriaceae*, a fermentation bacterium that could produce VFAs from polysaccharide, also increased from 1.25% to 11.64% after adding SCG-BC. This change may improve the degradation efficiency of substrate and the content of VFAs in AnMBR. However, a decrease in VFAs content was noted in phase P2 (see Fig. 4). This was attributed to *Methanosarcina*. *Methanosarcina* is a new dominant genus with a relative abundance of 8.03% after SCG-BC addition. Not only can it convert CO_2 into methane, but also carry out acetolactic methanogenesis [69]. The growth of *Methanosarcina* effectively consumed VFA in the reactor. It may be due to the relative abundance of *norank_f__Bacteroidetes_vadinHA17*, which can stimulate propionic acid production activity, decreasing by 19.70% [70].

Meanwhile, the relative abundance of *Blvii28_wastewater-sludge_group* and *Desulfovibrio* reported as acetogenic bacteria, increased from 3.50% to 5.43% and from 2.28% to 4.38%, respectively. This change indicated a higher proportion of acetic acid in VFA and a faster rate of methane production from acetic acid. Not only did propionic acid remain at a low level, but acetic acid was consumed rapidly, so TVFA showed a small concentration when an increase in methane production can be simultaneously realized. Interestingly, the increase total methanogens resulted in the decrease of the CO_2 output in Biogas. This is because almost all methanogens can produce methane through CO_2 reduction, thus resulting in the in-situ upgrading of biogas [46].

By analyzing the microbial community attached onto SCG-BC, it was found that several microbial genera were enriched, namely *Methanobacterium*, *Propionibacterium*, *Methanosaeta*, *Smithella* and *Anaerolinea*. Of these, *Methanosaeta* and *Methanosarcina* were indicative microorganisms of DIET [71]. However, *Methanosarcina* was not enriched on SCG-BC while in suspended sludge of SP2, and its relative abundance was 5.46 times that of SP1. This showed that the addition of SCG-BC enriched *Methanosarcina* in the reactor and strengthened the acetolactic methanogenic pathway.

The relative abundance of *Methanosaeta* on SCG-BC was 1.32%, which was 88.57% higher than that of SP1. Meanwhile, *Anaerolinea* was enriched on SCG-BC (relative abundance was 1.90%), which was 5.13 times more that of SP1 and 1.74 times more than that of SP2. Therefore, the addition of biochar promoted the syntrophic metabolism of *Anaerolineaceae* and *Methanosaeta* through DIET [72]. However, some studies documented those indicative microorganisms accounted for only a small part of the microbial community. These indicative microorganisms participate in DIET and other microorganisms were enriched on conductive carbon materials [71]. For example, *Desulfovibrio* and *Blvii28_wastewater-sludge_group* detected may also participate in DIET, because they exhibit a syntrophic growth relationship with hydrogenotrophic methanogenic microorganisms such as *Methanobacterium* [73, 74].

Interestingly, their relative abundance revealed a positive correlation, and they were enriched after adding SCG-BC. Similarly, Smithella, one of the main methanogenic symbionts, had a positive correlation with the abundance of methanogens. It was enriched on the surface of SCG-BC with the relative abundance increasing from 0.67% to 1.52%. These outcomes suggested that DIET may occur not only between *Anaerolinea* and *Methanosaeta*, but also between hydrolytic bacteria and hydrogenotrophic methanogens such as *Methanobacterium*, *Methanolinea*, *Methanospirillum* and *Methanoculleus*, especially *Methanobacterium* growing on SCG-BC [75]. As an electron conductor, SCG-BC transferred electrons for syntrophic microorganisms, which improved two things: the degradation efficiency and increased the yield of biomethane.

4. Conclusions

In this work, the effect of adding SCG-BC to assess the performance and membrane fouling of an AnMBR treating swine wastewater was systematically investigated. Results strongly suggested that SCG-BC played an active role in buffering pH and alleviating VFA accumulation in the AnMBR system. Further, the introduction of SCG-BC clearly enhanced COD removal and methane production in the AnMBR system. In the meantime, the in-situ upgrading of biogas was achieved due to the addition of SCG-BC. The analysis of the microbial community found that fermentation microorganisms such as *Propionibacteriaceae* were enriched, thereby accelerating the degradation of substrates. Meanwhile, *Methanosarcina* also increased in suspended sludge, and this in turn improved the utilization of VFAs and strengthened the methanogenic capacity of the AnMBR. The amounts of SMP and EPS significantly decreased after SCG-BC addition, while irreversible blocking of membrane pores was effectively alleviated. Finally, the AnMBR system with SCG-BC addition performed excellently as far as biogas production was concerned, and controlled membrane fouling when treating swine wastewater.

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