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Effect of bamboo charcoal amendment on an AnMBR in the aspect of anaerobic habitat and membrane fouling†

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The effect of bamboo charcoal (BC) amendment on the anaerobic habitat such as alkalinity and membrane fouling in an anaerobic membrane bioreactor (AnMBR) was investigated in this study. Results showed that the addition of BC into an anaerobic EGSB reactor modified the effluent wastewater properties, and 0.19–0.27 g of alkalinity was produced with the consumption of 1 g of COD which favored the performance of the anaerobic system. Analyzed by energy diffusive X-ray analysis (EDX) and confocal laser scanning microscopy (CLSM), the content of dissolved microbial products (SMPs) as well as that of the elements Ca, Al, Si, and Fe decreased significantly with the addition of BC. At the same time, BC inhibited the enrichment of microbes such as *Bacteroidetes* contributing to the formation of dense cake layers on the membrane surface. This work described an effective strategy for the treatment of high-concentration refractory organic wastewater. The addition of BC not only improved the anaerobic habitat and effluent quality, but also alleviated the issue of membrane fouling effectively, which could provide a foundation for future scale-up studies.

Water impact

In recent years, anaerobic membrane bioreactors have played an important role in industrial wastewater treatment because of their high permeate quality and low energy and nutrient requirements. According to the key limitation of membrane fouling, an effective and energy-saving strategy is developed to improve the effluent quality and alleviate the membrane fouling.

1. Introduction

An anaerobic membrane bioreactor (AnMBR) is an efficient treatment system for most industrial wastewaters, due to its high permeate quality, low energy and nutrient requirements, and energy recovery.[1–4] Lots of studies indicated that the COD removal efficiency in AnMBRs was over 90% for the treatment of high-concentration starch wastewater, landfill leachate, *etc.*[5–7]

As we know, membrane fouling is the most prominent obstacle limiting the application of AnMBRs, as it decreases the permeate flux, increases the pressure, deteriorates the permeate quality, and finally, shortens the membrane life.[8] Therefore, a compositional analysis of membrane foulants is essential. Organic and inorganic materials play an important role in membrane fouling in AnMBRs.[9,10] Organic materials mainly include soluble microbial products (SMPs) and extracellular polymeric substances (EPSs), which are considered to be the most important pollutants. Indeed, EPSs contribute to the formation of cake layers, and pore blocking is caused by SMPs. Typically, as a result of the roles played by SMPs and EPSs, a dense cake layer develops on the surface of the membrane, causing an increase in trans-membrane pressure (TMP) and a decrease in flux. Both materials are produced through cell death or lysis and consist mainly of proteins and polysaccharides. Higher protein and polysaccharide concentrations result in higher membrane fouling rates.[11] Colloidal materials are also responsible for membrane fouling by forming a cake layer on the membrane surface. Inorganic

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substances also influence membrane fouling due to their precipitation in the membrane pores or on the surface.[12]

Alkalinity is an important parameter of anaerobic habitat for the stable operation of anaerobic reactors, because its buffer effect can maintain the suitable pH and prevent acidification of anaerobic reactors. If the acid concentrations including H_2CO_3 and VFA exceed the available alkalinity, the microbial activity will be severely inhibited, especially that of methanogens.[13] At the same time, alkalinity dosing is one of the major costs in many anaerobic processes. López-López *et al.*[14] found that in an UASB reactor treating tequila vinasse, the COD removal efficiency dropped below 50% when the alkalinity and pH decreased. It was due to insufficient alkalinity, which could not neutralize the acidity present at the inlet of the reactor. In membrane bioreactors, alkalinity is associated with the contaminant removal efficiency and membrane fouling. Hu *et al.*[15] reported that sodium bicarbonate (SB) addition into an MBR for reject water treatment enhanced the average COD and NH_4-N removal efficiencies by 14.6% and 38.3%, respectively. In addition, membrane fouling was mitigated because the deprotonation mechanism reduced EPS adsorption on the negatively charged membrane surface.

In recent studies, parameter optimization, membrane modification and cleaning methods were considered to get rid of the issue of membrane fouling.[16,17] Shin *et al.*[18] used granular activated carbon (GAC) in an anaerobic fluidized bed bioreactor (AFMBR) for the post-treatment of effluent. The TMP stabilized at 0.03 bar with GAC, whereas the TMP rapidly increased to 0.32 bar within 0.5 d without GAC. Bamboo charcoal, as a low-cost biocarrier, has been reported as an alternative for reducing the membrane fouling in recent years.[19,20] Herrera-Robledo *et al.*[21] reported that the TMP increased rapidly and the fouling lasted for 140 h when the SRT was 60 d, and the fouling time continued to 175 h when the SRT was extended to 100 d. An *et al.*[22] found that decreasing the HRT caused the accumulation of suspended solids and a decrease in membrane flux. Lu *et al.*[23] indicated that the water contact angle of the membranes decreased from 83° to 57° after adding nano-Al_2O_3 to modify ultra-filtration membranes, and the hydrophilicity and anti-fouling of the membranes were significantly improved. Cleaning methods are necessary to control the membrane fouling, which mainly include physical and chemical cleaning. Martinez-Sosa *et al.*[24] showed that physical cleaning could recover the flux of membranes to almost 100%, indicating that the membrane fouling was reversible. Wen *et al.*[25] also found that ultrasonic cleaning could inhibit the formation of cake layers and the decrease of membrane flux. However, chemical cleaning is demanded when physical cleaning is not suitable. Lin *et al.*[26] used 500 mg L^{-1} NaOCl and 2000 mg L^{-1} citric acid for *in situ* chemical cleaning, and 1000 mg L^{-1} NaOCl and 2000 mg L^{-1} citric acid for heterotopic cleaning. Although membrane fouling could be mitigated effectively, the operational costs increased and the cleaning agents had certain corrosiveness towards the membrane. Meanwhile, the fouling mechanism and microbial community on the anaerobic membrane surface are still unclear.

In this study, AnMBRs were fed with real bamboo product wastewater to investigate the effect of bamboo charcoal (BC) amendment on the anaerobic habitat such as alkalinity, dissolved organic matter (DOM) and inorganic substances in an anaerobic membrane bioreactor (AnMBR). High-throughput pyrosequencing was used to investigate the microbial communities in the cake layers of fouled membranes. Through advanced characterization of the cake layers, a better understanding of the fouling mechanisms in terms of microbiology can be achieved.

2. Experimental

2.1 AnMBR reactor operation

AnMBR systems, which consisted of an expanded granular sludge blanket reactor (EGSB) and a polyvinylidene fluoride (PVDF) hollow membrane module set externally with a surface area of 0.07 m^2 and a pore size of 0.02 μm, were operated to treat bamboo industry wastewater (BIWW), which was collected from a treatment plant located in Zhejiang Province, China and contained a high concentration of organic matter and NH_4^+-N. The effective working volume of the EGSBs was 5.5 L, and the recirculation flow ratio was 10. The gas discharged from the top of the reactors passed through a water-sealed bottle and its rate was measured using a wet gas flow meter.

Both reactors were inoculated with 16 g VSS L^{-1}, originating from an upflow anaerobic sludge blanket from an urban sewage treatment plant in Hangzhou, China. The particle size of the BC was 0.5 mm, which was purchased from Watson Bamboo Charcoal Products Company in Zhejiang. The BC particles were sieved, washed with deionized water and mixed with the inoculated sludge. They were added into the reactor only at the start-up point and the dosage was 100 g (1 : 1 for the MLSS of the inoculated sludge). This system is defined as a B-AnMBR. The reactor without added BC was the control reactor (AnMBR). The HRT was set to 3 days during the whole operation, and the temperature was maintained at 32 ± 2 °C. At the initial stage, the two reactors were operated with 9-fold diluted BIWW. During the start-up period, the dilution factor was reduced to that of real BIWW, and the OLR reached approximately 6 kg COD m^{-3} d^{-1}. Both membrane systems were set to 20 min with 5 min of relaxation. The permeate was withdrawn using a suction pump. At 50 and 108 days, the membranes were removed for physical and chemical cleaning.

2.2 Analytical methods

Alkalinity measurement was based on an acid titration method described in the Method of Water and Wastewater (4th edition) from the State Environmental Protection Administration of China.[27] SMPs were determined using the analysis described by Huang *et al.*[11] The total carbohydrate and protein concentrations of SMPs were determined using the Dubois phenol–sulfuric acid method with glucose as the

carbohydrate standard and a modified Lowry method with bovine serum albumin as the protein standard, respectively.[28,29]

The three-dimensional fluorescence spectra (3D-EEM) of dissolved organic matter in effluent from different BC doses were recorded on an F-5500 fluorescence spectrophotometer (Hitachi, Japan). The three-dimensional spectra were obtained by repeatedly measuring the emission spectra ranging from 200 to 600 nm while varying the excitation wavelength from 350 to 650 nm in 50 nm increments. The excitation and emission slits were maintained at 5 nm. The Origin 9.0 program was used to process the EEM data. The membrane samples were stored in a 2.5% solution of glutaraldehyde at 4 °C for 12 h and then sputter coated with 20 nm of gold using an Emitech K550 sputter coater. A Hitachi TM-1000 SEM (Tokyo, Japan) was used to capture micrographs. All images were acquired digitally using the Quartz PCI software (Vancouver, BC, Canada), which was also used for image analysis.

The elements C, O, F, Al, Si, P, S, K, Ca, and Fe were detected using an SEM coupled with an EDX spectrometer (JEOL JSM 5900 LV). The membrane samples were excised, and 100 μL of 0.1 M $NaHCO_3$ buffer was added to the samples until pH 7.0 was reached. Then, 10 μL of FITC (10 g L^{-1}) was dropped onto the membrane. The mixture was shaken at 25 °C for 1 h. Then, 100 μL of 250 mg L^{-1} Con A was added, and the membrane was stained for 30 min. Next, the cells were stained with CW (300 mg L^{-1}, 100 μL) for 30 min. Finally, the samples were washed two times with phosphate buffer (pH 7.2) to remove excess dye. The stained samples were stored at 4 °C until using confocal laser scanning microscopy (CLSM, LSM 710) to obtain CLSM images. The quantitative analysis of fluorescence intensity of the digital CLSM images was performed with ImageJ software (http://rsbweb.nih.gov/ij/).

2.3 Microbial community analysis

To analyze the microbial communities on the membranes, the membrane samples were collected during the steady period. Community structures of the two membranes were characterized by 454 high-throughput sequencing. To determine the DNA sequence of the M1 and M2 samples, the membrane samples were cut into pieces and extracted using a Power Soil DNA extraction kit (MO BIO Laboratories Inc.). In this experiment, the V4 region was used for sequencing, which had a bacterial length of approximately 280 bp. Polymerase chain reaction (PCR) amplicon libraries were constructed with the Illumina MiSeq platform, using the primers AYTGGGYDTAAA GNG and TACNVGGGTATCTAATCC. PCRs were conducted using the following procedure: 98 °C for 30 s; 25–27 cycles at 98 °C for 15 s, 50 °C for 30 s, and 72 °C for 30 s; and finally, 72 °C for 5 min. A Quant-iT PicoGreen dsDNA assay kit was used to quantify the PCR products with a microplate reader (BioTek, FLx800), as well as the mixed sample according to the amount of required data. The libraries were normalized and quantified using an Agilent High Sensitivity DNA kit, and the results were used in the Quant-iT PicoGreen dsDNA assay kit. The final sequencing was conducted using a MiSeq Reagent Kit V3 (600 cycles). The library (index non-repeatable) was diluted to 2 nM and was then mixed into the required amount of data. The library was normalized and quantified using the Agilent High Sensitivity DNA kit, and the results were used in the Quant-iT PicoGreen dsDNA assay kit. The final sequencing was conducted using the MiSeq Reagent Kit V3 (600 cycles).

3. Results and discussion

3.1 Effect of alkalinity on the performance of AnMBRs

The addition of BC improved the performance of AnMBRs in the treatment of BIWW, showing a higher COD removal efficiency and methane yield,[30] but the influent wastewater required the addition of $NaHCO_3$ to maintain the pH at 6.8–7.2 to promote methanogen activity. Therefore, an analysis of the effect of BC addition was necessary to minimize dosing agents and save process costs. Fig. 1 shows that with the decrease in the influent alkalinity, the COD removal efficiency also decreased, indicating that the fluctuation of the pH of the system destabilized the self-regulation of the alkalinity in the reactor. Table 1 shows that the effluent alkalinity in the AnMBRs was higher than that of the influent, implying that the reactor itself produced a certain amount of alkalinity. González *et al.*[31] reported that when the Alk_0 (influent alkalinity)/COD_0 ratio was approximately 4.0, the pH was maintained at 7.0 for the treatment of sugar wastewater in an UASB.

In this study, Alk_0/COD_0 was maintained at 0.03–0.25 as the influent alkalinity decreased, but when Alk_0/COD_0 was 0.03, the COD removal efficiency of the B-AnMBR was higher than 89.2%, and the reactor operated well. The ratio of Alk_P (alkalinity production) and COD_R (COD removal) showed that the AnMBR would produce 0.18–0.24 g of alkalinity with the consumption of 1 g of COD, and 0.19–0.27 g of alkalinity was produced in the B-AnMBR. In our previous study, analysis of microbial communities demonstrated that BC could increase the microbial diversity and promote the activity of *Methanosaeta*, *Methanospirillum*, and *Methanobacterium*.[30] And in this study, a higher methane yield was achieved with the addition of BC. These results are consistent with previous studies.[32,33] Therefore, BC could promote methanogen activity to consume the acids in the process of methanogenesis, leading to the enhancement of the reactor alkali capacity. Higher alkalinity is also conducive to the precipitation of toxic metals in wastewater and beneficial for improving sludge settling. In Table 1, 31 g, 28 g, 24 g, 20 g, 17 g, 14 g and 0 g $NaHCO_3$ were required to maintain 4328 mg L^{-1}, 3831 mg L^{-1}, 3299 mg L^{-1}, 2840 mg L^{-1}, 2205 mg L^{-1}, 1685 mg L^{-1} and 549 mg L^{-1} alkalinity, respectively. 3461.4 mg L^{-1} alkalinity could be produced in the B-AnMBR in the absence of $NaHCO_3$ and the COD removal efficiency was 89.2%, which were both higher than those of the AnMBR (3056.9 mg

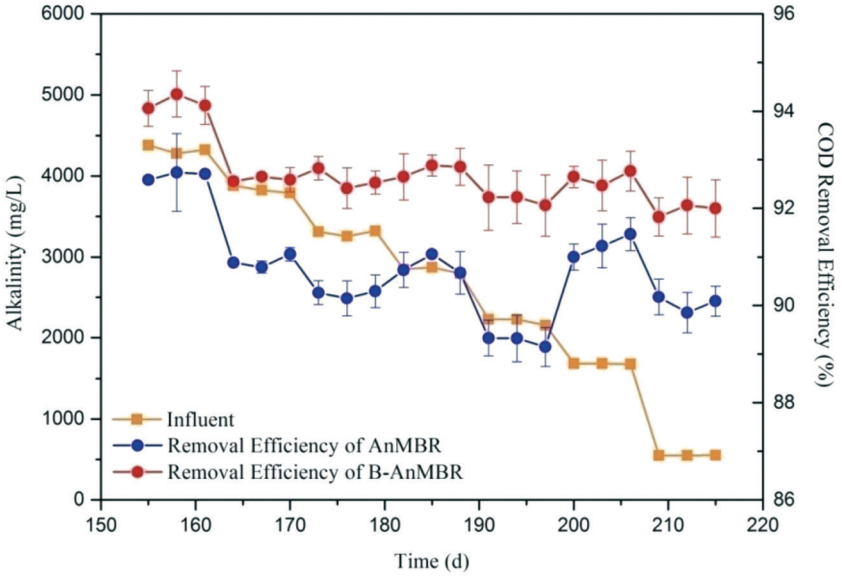

Fig. 1 The COD removal efficiency at different influent alkalinity values.

Table 1 Parameter values at different influent alkalinity values

Reactors	Parameters	Index value						
AnMBR	$NaHCO_3$ (g)	31	28	24	20	17	14	0
	Alk_0 (mg L^{-1})	4328	3831	3299	2840	2205	1685	549
	Alk_0/COD_0	0.25	0.22	0.19	0.17	0.13	0.10	0.03
	Effluent alkalinity (mg L^{-1})	7015.2	6698.6	6485.6	5588.9	5545.7	5301.9	3605.9
	Alk_P (mg L^{-1})	2687.2	2867.6	3186.6	2748.9	3340.7	3616.9	3056.9
	Effluent COD_1 (mg L^{-1})	1923.3	2056.7	2461.7	2151.7	2081.3	2086.7	2306.3
	COD_R (mg L^{-1})	15236.7	15103.3	14698.3	15008.3	15078.7	15073.3	14853.7
	Alk_P/COD_R	0.18	0.19	0.22	0.18	0.22	0.24	0.21
	COD removal efficiency (%)	88.79	88.01	85.65	87.46	87.87	87.84	86.56
B-AnMBR	$NaHCO_3$ (g)	31	28	24	20	17	14	0
	Alk_0 (mg L^{-1})	4328	3831	3299	2840	2205	1685	549
	Alk_0/COD_0	0.25	0.22	0.19	0.17	0.13	0.10	0.03
	Effluent alkalinity (mg L^{-1})	8116.8	6814.7	6632.5	6229.4	6034	5800.6	4010.4
	Alk_P (mg L^{-1})	3788.8	2983.7	3333.5	3389.4	3829	4115.6	3461.4
	Effluent COD_1 (mg L^{-1})	1643.3	1781.7	1806.7	1690	1732.7	1770	1860.7
	COD_R (mg L^{-1})	15516.7	15378.3	15353.3	15470	15427.3	15390	15299.3
	Alk_P/COD_R	0.24	0.19	0.22	0.22	0.25	0.27	0.23
	COD removal efficiency (%)	90.42	89.62	89.47	90.15	89.90	89.69	89.16

L^{-1} and 86.6%, respectively). Therefore, the consumption of $NaHCO_3$ could be greatly reduced by adding BC, achieving a cost-effective and stable anaerobic system.

The gas production and methane yield at different influent alkalinity levels are shown in Fig. 2. With the addition of BC, the B-AnMBR had a higher biogas output than the AnMBR, achieving an average biogas output of 14.29 L d^{-1} in 60 days, while the corresponding value for the AnMBR was 11.03 L d^{-1}. The methane yield of the B-AnMBR (0.31 L CH_4 per g COD) was higher than that of the AnMBR with an average value of 0.17 L CH_4 per g COD. Two reasons were probably responsible for the enhancement of the biogas production and methane yield. The addition of BC led to greater gas production due to the large amounts of microorganisms attached to the carriers. The other possible reason was that BC might retain more biomass which contributed to the methane production.[30]

As the influent alkalinity decreased, the gas production of both reactors increased slightly. When the influent alkalinity decreased to 549 mg L^{-1}, the gas yields of the AnMBR and B-AnMBR were 11.06 L d^{-1} and 14.03 L d^{-1}, respectively. The methane yield of the B-AnMBR was 0.32 L CH_4 per g COD, higher than 0.17 L CH_4 per g COD in the control group. According to the three-stage theory of anaerobic digestion by M. P. Bryant: the first stage is hydrolysis, and the macromolecular organic substances (such as protein, starch, polysaccharide, *etc.*) in the wastewater are used to produce simple organic substances such as amino acids and glucose by fermentation bacteria. The second stage is acidification and hydrogen production. The intermediate products produced in the previous stage were decomposed into CH_3COOH, H_2 and CO_2 by hydrogen-producing acetogenic bacteria. The third stage is methanogenesis. H_2, CO_2 and organic acids are utilized by methanogens to produce methane. Studies suggested

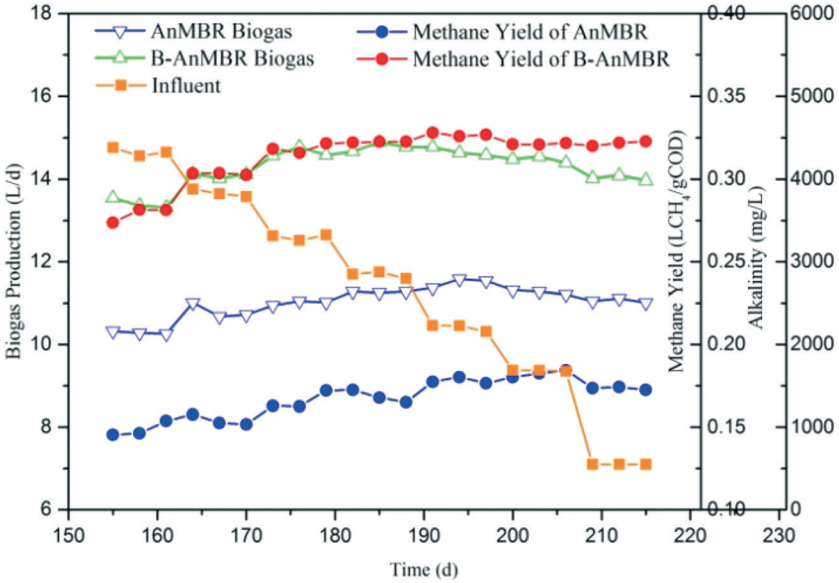

Fig. 2 Gas production and methane yield at different influent alkalinity levels.

Table 2 Different effluents with different doses of bamboo charcoal

Sample	S1	S2	S3	S4	S5	S6	S7
Anaerobic effluent	R1	R1	R1	R1	R1	R1	R2
BC	0 g	25 g	50 g	75 g	100 g	125 g	0 g

that the optimum pH values for methanogens were 6.8–7.2.[34] Hence, proper reduction of influent alkalinity to control the pH was beneficial to enhancement of the methanogen activity.

3.2 Effect of BC addition on effluent quality in an EGSB system

Addition of BC maintained the self-regulation of alkalinity in the anaerobic reactor in the steady state. However, the influence of BC addition on the EGSB effluent properties that directly affect membrane fouling should be determined. Therefore, different doses were added to the EGSB effluent to prove the enhanced performance with the addition of BC, which was collected from the R1 EGSB effluent and used R2 EGSB effluent, denoted as S7 (Table 2).

Table 3 shows that the COD concentrations were basically unchanged after adding different doses of BC to the R1 effluent; however, the COD removal efficiency of S7 was 41.5% higher than that of S1. The pH, turbidity, and chroma slightly increased, as the BC contained a certain amount of impurities and alkaline substances. The concentrations of the SMP_{PN} and SMP_{PS} decreased with the increase in BC, although they were still higher than those in the R2 effluent. In addition, when the dose of BC was 125 g, the SMP_{PN} and SMP_{PS} reached 303.73 ± 0.91 mg L^{-1} and 38.24 ± 1.71 mg L^{-1}, respectively, indicating that excess addition of BC had a negative effect on the reduction in SMPs. Compared to S5, the SMP_{PN} and SMP_{PS} in S7 decreased by 13.4% and 27.1%, respectively, revealing that the addition of BC into R2 can modify the properties of the mixed solution and improve the removal performance, whereas no improvement occurred when adding the same dose of BC to the R1 effluent.

SMPs have been confirmed to play an important role in membrane fouling.[35] Three-dimensional fluorescence spectroscopy (3D-EEM) was used to understand the change in SMPs after adding BC. From Fig. 3, we can see that two main peaks exist. One is a fulvic acid-like substance at excitation/emission wavelengths (Ex/Em) of 450–550/400–500 nm (peak A). The other peak is described as the fluorescence of a high color-like substance, which includes two categories, denoted as peak B and peak C.[10] As the amount of BC in the effluent increased, the peak position did not undergo significant

Table 3 Changes in the partial parameters at different doses of bamboo charcoal

Sample	COD (mg L^{-1})	pH	Turbidity	Chroma	SMP_{PN} (mg L^{-1})	SMP_{PS} (mg L^{-1})
S1	1880	8.36	260.4 ± 2.1	9521.66	130.46 ± 1.81	33.96 ± 0.45
S2	1250	8.6	218.0 ± 5.6	7997.12	124.79 ± 0.32	26.34 ± 1.34
S3	1260	8.57	126.4 ± 9.6	8784.8	116.70 ± 0.21	29.76 ± 0.63
S4	1185	8.8	295.4 ± 10.7	8859.26	122.23 ± 0.11	27.01 ± 0.64
S5	1165	8.68	220.3 ± 4.8	9384.36	128.37 ± 0.63	31.16 ± 1.23
S6	1230	8.88	345.9 ± 6.7	9594.47	303.73 ± 0.91	38.24 ± 1.71
S7	1100	8.52	239.9 ± 7.9	9456.08	122.92 ± 0.52	24.77 ± 1.74

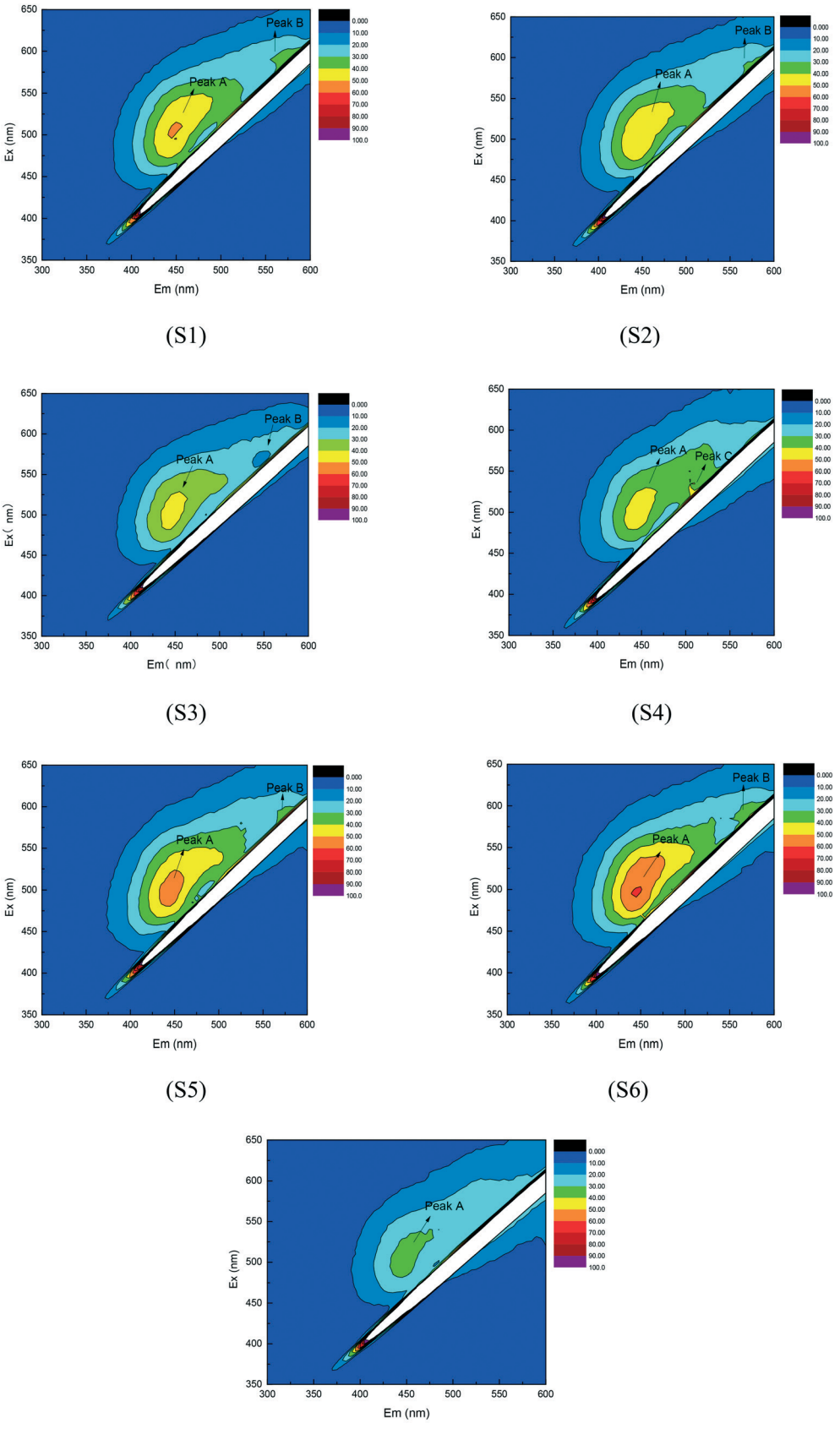

Fig. 3 3D-EEM images at different doses of bamboo charcoal: S1. R1 effluent with 0 g BC; S2. R1 effluent with 25 g BC; S3. R1 effluent with 50 g BC; S4. R1 effluent with 75 g BC; S5. R1 effluent with 100 g BC; S6. R1 effluent with 125 g BC; S7. R2 effluent with 0 g BC.

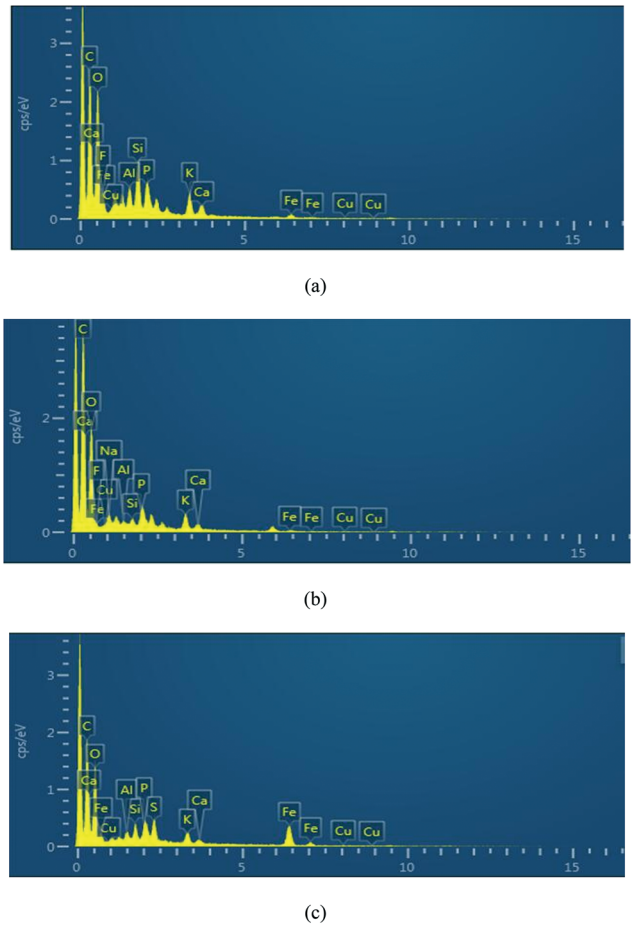

Fig. 4 EDX spectra of the AnMBR (a), B-AnMBR (b), and BC (c).

deviation. However, the intensities of peak A and peak B first disappeared and then increased. No peak B was detected in S4 or S7, which implied that the high color-like substance was absent; however, the peak A intensity was much higher in S4 than in S7. Therefore, it can be concluded that BC addition into the EGSB reactor results in a decrease in fulvic acid-like substances and high color-like substances. However, adding BC to the effluent of the EGSB did not reduce the SMPs, even when the intensity of the dissolved substances was increased due to excess addition. It can be assumed that BC improved the microbial activity and inhibited microbial metabolism and death to produce SMPs. However, amounts of SMPs and agglomerates remained in the effluent, and their fouling characteristics were unchanged, leading to membrane fouling similar to the case without BC addition.

3.3 Analysis of the TMP and cake layer

BC addition to the EGSB effluent did not alleviate membrane fouling because SMPs, colloids and other foulants still existed in the effluent. However, adding BC to the EGSB significantly reduced the TMP increasing rate, and the inorganic and organic substances, which were the main components of the cake layer.

The evolution of the TMP in the AnMBR and B-AnMBR is shown in Fig. S1.† In 60 days, the TMP of the AnMBR increased from 10 kPa to 30 kPa, whereas the TMP of the B-AnMBR increased from 10 kPa to 26 kPa. The TMP increasing rate was improved with the addition of BC in the B-AnMBR. The results indicated that the addition of BC mitigated the accumulation of foulants like EPSs, SMPs, and colloids on the membrane surface and then decreased the rate of formation of the cake layer.

Fig. 4 and Table 4 show that the surfaces of the membranes and BC contain similar elements, including C, O, F, Al, Si, P, K, Ca, and Fe. F was not present in BC, as the PVDF hollow fiber membrane contains the F element itself. Ca, Al, Si, and Fe have been reported to be the main inorganic components of membrane fouling. Microbial cells and macromolecules can gather on the membrane surface to form a dense and less porous cake layer by capturing metal elements *via* electrical neutralization.[36] BC is an environmentally friendly, low-cost and renewable bioresource with a porous structure, large specific surface area and characteristic surface functional groups.[37] Wang *et al.*[38] found that heavy metal ions (including Pb^{2+}, Cr^{3+}, Cd^{2+}, Ni^{2+}, As^{5+}, and Cu^{2+}) could be greatly adsorbed by Makino bamboo charcoal due to its large specific surface area and abundant micropores and mesopores. Besides the physical adsorption, Tan *et al.*[39] found that the surface oxygen functional groups especially carbonyl (C═O), carboxyl or ester groups (COOH) were proved to enhance the elemental mercury adsorption capacity of the BC sorbents. FTIR analysis revealed that –OH, C–H bending, C–O stretching vibration and carbonyl functional groups were mainly responsible for Pb(II) adsorption by activated bamboo charcoal (*Melocanna baccifera* Roxburgh).[40] In this study, BC can adsorb large amounts of metal elements *via* physical and chemical adsorption processes, to reduce its accumulation on the membrane and slow down the membrane fouling.

Proteins and polysaccharides were observed on both membrane surfaces (Fig. 5). However, without the addition of BC, proteins significantly accumulated on the membrane surface, forming a cake layer and blocking pores. A clear membrane

Table 4 Molar ratios of major elements in the membrane samples

Membrane sample	Molar ratio of the major elements (%)									
	C	O	F	Al	Si	P	S	K	Ca	Fe
AnMBR	52.75	30.45	5.39	1.34	2.93	2.04	—	2.36	1.27	1.47
B-AnMBR	62.63	30.41	1.97	0.2	0.45	1.35	—	1.86	0.71	0.42
BC	55.54	25.36	—	0.68	1.31	1.33	2.04	1.58	0.5	11.66

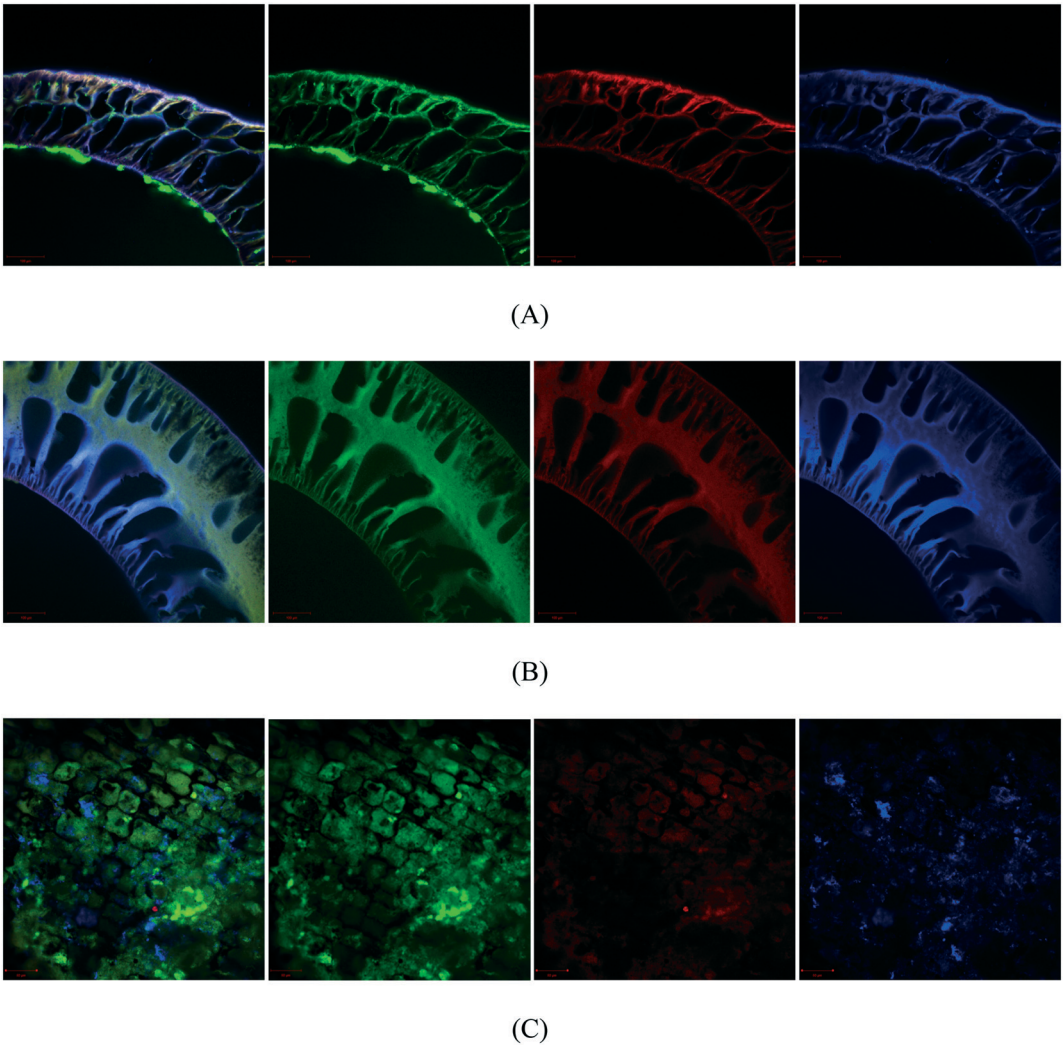

Fig. 5 CLSM images of the AnMBR membrane (A), B-AnMBR membrane (B), and bamboo charcoal (C) (protein, green; α-polysaccharide, red; and β-polysaccharide, blue).

structure and loose layer were observed when BC was added. Table S1† shows that the fluorescence intensities of protein, α-polysaccharide and β-polysaccharide on the AnMBR membrane were higher than those on the B-AnMBR, indicating more severe organic membrane fouling in the AnMBR. From the observation of the surface images of BC using CLSM and the fluorescence intensity of protein, α-polysaccharide and β-polysaccharide on BC, it was found that BC could remove dissolved substances in the solution *via* adsorption, hence reducing contamination of the subsequent membrane modules.

3.4 Microbial communities on the surface of carbon and the cake layer

The microbial communities in M1 and M2 were analyzed by 454 high-throughput sequencing in the steady state. The alpha diversity index is shown in Table 5 with the Chao1/ACE estimator and the Shannon and Simpson diversity indices. The Chao1/ACE was slightly higher in M1 than that in M2. Considering the richness and evenness of the community, the Shannon and Simpson diversity indices increased with the addition of BC. These results implied that adding BC could increase the microbial diversity. Kim *et al.*[41] also found that high microbial diversity favored the degradation of SMPs. The phylum level identification of the microbial communities is shown in Fig. 6. *Proteobacteria* (45.5%), *Firmicutes* (27.9%), *Bacteroidetes* (7.6%), and *OP11* (6%) were abundant in M1. *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *OP11* were abundant in M2 at 42.8%, 20.5%, 1.8%, and

Table 5 Diversity of microbial communities on the surface of the membranes and BC

Sample	Chao/ACE	Simpson	Shannon
M1	0.8026	0.77	5.25
M2	0.7898	0.98	6.88
BC	0.8103	0.88	5.27

2.4%, respectively. Gao *et al.*[42] reported that *OP11* and *Bacteroidetes* are the main microorganisms contributing to membrane fouling, resulting from the flow through and adsorption on the membrane pores. The proportion of *Chloroflexi* was higher in M2 than that in M1. Miura *et al.*[45] reported that the members of *Chloroflexi* were ecologically significant in the MBR treating municipal wastewater and were responsible for degradation of SMPs including carbohydrates and cellular materials, reducing the membrane fouling potential consequently. The content of *Gemmatimonadetes* was also significantly higher in M2 than in M1. Xie *et al.*[43] found that *Gemmatimonadetes* are a type of anaerobic ammonium oxidation bacteria, which can convert ammonia nitrogen to nitrogen, and thus, the enrichment of *Gemmatimonadetes* is conducive to ammonia removal. *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *OP11* were present at 18.6%, 60.1%, 5.3%, and 3.4%, respectively, in BC. Large numbers of *Firmicutes*, which are the dominant bacteria in pollutant degradation, were detected in BC. This was consistent with a previous finding that BC could enrich the dominant bacteria to improve the pollutant removal efficiency.[30] Moreover, BC could adsorb *OP11*, *Bacteroidetes* and other microorganisms to reduce enrichment on the membrane surface.

In the archaeal class level identification of the microbial communities (Fig. S2†), *Methanomicrobia* (24.9%), *MCG* (3.1%) and *Methanococci* (2.2%) were present in M1, and *Methanomicrobia* (1.4%) and *MCG* (1.5%) were present in M2. From the analysis of the bacterial class level identification of the microbial communities, *Clostridia* (57.8%), *OP11-1* (1.7%), and *OP11-4* (1%) were abundant in M1, and *Clostridia* (18.1%), *OP11-1* (0.8%), and *OP11-4* (1.2%) were abundant in M2. The bacteria *Clostridia* (57.8%), *OP11-1* (1.7%), and *OP11-4* (1%) and the archaeal *Methanomicrobia* (24.9%), *MCG* (3.1%), and *Methanococci* (2.2%) were present in BC. These results revealed that the content of microflora on the membrane surface decreased after adding bamboo charcoal particles, and that bamboo charcoal could enrich *Methanomicrobia*, which is beneficial to methane production. This is consistent with a previous study.[33] Fonknechten *et al.*[44] found that *Clostridia* exist in granular sludge, which indicates that bamboo charcoal may act as a critical factor in promoting the formation of granular sludge. The results showed that the content of methanogenic bacteria in the membrane was low, which indicated that the dominant bacteria in the AnMBR process were well retained in the anaerobic reactor.

An analysis of the bacterial community in the anaerobic reactor revealed that the bacteria in the AnMBR included *Firmicutes* (6.0%), *Elusimicrobia* (7.0%), and *Acidobacteria* (17%), while *Firmicutes* (19.0%), *Elusimicrobia* (13.0%), *Acidobacteria* (13%) and *Chlorobi* (3%) were abundant in the B-AnMBR. After adding BC, the amount of *Firmicutes* and *Elusimicrobia* increased; these results are consistent with the results of Gao *et al.*,[42] who found that *Firmicutes* are the dominant COD degradation bacteria. Therefore, BC is beneficial for enhancing the pollutant removal performance of the system. Miura *et al.*[45] also found that *Chlorobi* was only present in the B-AnMBR, which inhibited SMP production.

Analysis of the archaeal community structure revealed that the microbial community of the AnMBR included *Methanosaeta* (60.7%), *Methanospirillum* (10.1%), *Methanobacterium* (4.4%), *Candidatus Methanoregula* (2.3%), *Methanosarcina* (2.1%), and *Methanomassiliicoccus* (1%). *Methanosaeta* (66.4%), *Methanospirillum* (13.3%), *Methanobacterium* (8.2%), *Candidatus Methanoregula* (2.2%), *Methanosarcina* (2%), and *Methanomassiliicoccus* (1.2%) were abundant in the B-AnMBR. The amounts of *Methanosaeta*, *Methanospirillum*, and *Methanobacterium* increased after adding BC. *Methanosaeta* played an important role in the decomposition of acetic acid into methane and carbon dioxide and could be used as a core to enhance the granulation process.[46–48] In summary, BC was able to increase the number of *Methanosaeta*, *Methanospirillum*, and *Methanobacterium* to promote methane production and anaerobic sludge granulation. This result, in combination with the results of the microbial communities, permitted speculation of the possible mechanism of BC in mitigating membrane fouling. The characteristics of BC include a large specific surface area and porosity that is able to adsorb colloidal substances and small particles, especially the proteins and polysaccharides of EPSs, to prevent them from adhering to

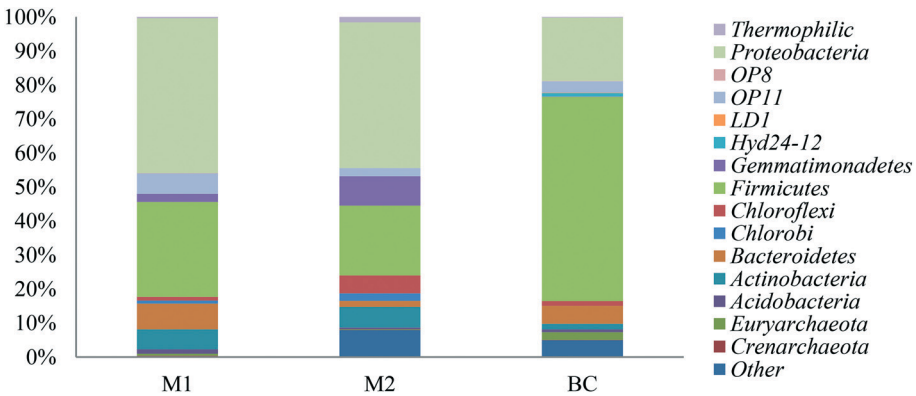

Fig. 6 Relative abundance of the microbial communities at the phylum level.

the membrane surface. Meanwhile, the use of BC as the "core" increased the speed of the sludge granulation process and decreased the ability of small granular sludge to block the pores. BC particles inhibited the increase of concentration of SMPs, which were produced from the lysis and death of cells, as well as slowed the membrane pore blocking and reduced the number of *Bacteroidetes* and *OP11* microbial communities enriched on the membrane surface.

4. Conclusion

The addition of BC in the EGSB reactor modified the properties of the anaerobic habitat, decreased the fulvic acid-like substances and high color-like substances, and produced a certain amount of alkalinity. At the same time, BC adsorbed large amounts of Ca, Al, Si, Fe, proteins and polysaccharides to alleviate membrane fouling. Analysis of the microbial community demonstrated that the enrichment of *Bacteroidetes*, *OP11* and other EPS-producing microbes was inhibited obviously to control the biofouling in the presence of BC. This work developed a promising BC assisted AnMBR for the treatment of high-concentration refractory organic wastewater.

Conflicts of interest

There are no conflicts to declare.

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