Treatment Performance of Municipal Sewage in a Submerged Membrane Bioreactor (SMBR) and Mechanism of Biochar to Reduce Membrane Fouling

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Abstract: Submerged membrane bioreactors (SMBRs) are a promising technology for municipal sewage treatment, but membrane fouling has limited their development. In this study, biochar (BC), which has a certain adsorption capacity, was added to an SMBR to investigate its potential in treating municipal sewage and alleviating membrane fouling. The results showed that the average removal rates of ammonia nitrogen (NH$_4^+$-N), total nitrogen (TN), total phosphorus (TP) and chemical oxygen demand (COD) were 94.38%, 59.01%, 44.15% and 83.70%, respectively. After BC was added and operation was stable, the ratio of mixed liquor volatile suspended solids to mixed liquor suspended solids (MLVSS/MLSS) was maintained between 0.78 and 0.81. The concentrations of soluble microbial products (SMPs) and extracellular polymeric substances (EPSs) were stabilized between 63.05 ± 8.49 mg/L and 67.12 ± 1.54 mg/L. Trans-membrane pressure (TMP) and scanning electron microscopy (SEM) analyses showed that BC reduced the TMP by reducing the thickness and compactness of the cake layer on the membrane surface. The high-throughput sequencing results showed that the microorganisms associated with biofilm formation (proteobacteria, γ-proteobacteria and α-proteobacteria) were significantly reduced in the BC-enhanced SMBR system. BC promoted the enrichment of functional microorganisms such as Chloroflexi, Acidobacteriota, Anaerolineae and Planctomycetes. Compared with traditional anti-fouling methods, the results of this study may provide a low-cost membrane fouling mitigation method for industrial applications of SMBRs.

Keywords: SMBR; municipal sewage; biochar; membrane fouling; microbial population dynamics; microbial community

1. Introduction

In many countries, especially China, the discharge of inadequately treated municipal sewage is a growing concern [1]. In recent years, the rapid development of urbanization in China has led to the production of a large amount of municipal sewage and water pollution is becoming increasingly serious, which poses a threat to people’s daily life [2]. At present, the combination process of “conventional activated sludge (CAS) + coagulation/filtration + disinfection” is still widely used in sewage treatment plants, which has many shortcomings such as a low treatment efficiency, a high energy consumption, a large area and cumbersome management [3]. Under the condition of increasing pollution levels, the CAS process cannot meet the national wastewater treatment requirements. Membrane bioreactors (MBRs) are a potential alternative to the CAS process, which uses a membrane process instead of a traditional sedimentation tank for biomass separation [3]. This new wastewater treatment process combines activated sludge (AS) biological treatment with membrane filtration technology, which has the advantages of a high biomass concentration, flexible operation, less sludge production and a small footprint [4]. However, at present, the most important factor hindering the wide application of MBRs is the difficulty in
their operation and maintenance, which is related to the delay in solving the problem of membrane fouling [5].

In order to understand the mechanism of membrane fouling in the sewage treatment process, a large number of studies have been conducted [6–8]. The properties of mixed liquor, the operating conditions, the membrane properties and external environmental conditions are factors affecting membrane fouling [6,9]. Among them, membrane fouling is mainly driven by soluble microbial products (SMPs) and extracellular polymeric substances (EPSs) in the mixed liquor [10,11]. EPSs are a complex mixture of polysaccharides (PS), proteins (PN), lipids/phospholipids, humic substances and other intercellular polymers [12]. SMPs consist of the hydrolyzed products of EPSs and the decay products of active cells, which are related to the formation of autotrophic and heterotrophic aggregates of organic carbon and ammonia removal in wastewater [10]. They jointly affect the sludge settling performance and membrane filtration performance in MBR systems, including direct blocking of membrane pores, formation of a gel layer and deposition of a cake layer on the membrane surface [10,13]. Therefore, the potential mechanisms of membrane fouling can be further investigated by understanding the changes in the mixture composition.

In recent years, various methods have been used to alleviate membrane fouling, including optimizing the operating parameters (hydraulic retention time (HRT), sludge retention time (SRT), etc. [14,15]), improving the shear force [16], modifying membrane cleaning methods [17], adding additives, etc. [5,13,18–20]. Ultimately, in order to reduce the cost of operation and maintenance (O&M), researchers have been working on using granular materials to control membrane fouling in MBRs. Many common materials have been used to mitigate membrane fouling [21], including adsorbents [19], granular media with air scouring, granular biomass [22] and media entrapped with quorum-quenching enzymes/microorganisms [23]. Carbon-based materials, particularly activated carbon and biochar (BC), stand out among adsorbents, and are produced from agricultural waste, environmentally friendly and easy to recycle when produced in granular form. A large number of studies have been conducted on the mitigation of membrane fouling by activated carbon, indicating that activated carbon can indeed mitigate membrane fouling to a large extent [24]. Li et al. [25] reported that the effect of activated carbon was similar to that of BC, but the cost of BC was 1/16 of that of activated carbon [26]. Studies on the removal of pollutants from wastewater by BC are very extensive at home and abroad, and mainly focus on the adsorption characteristics of BC, but there is a lack of studies related to membrane fouling and microbial response.

In this study, the removal of ammonia nitrogen (NH\textsubscript{4}+–N), total nitrogen (TN), total phosphorus (TP) and chemical oxygen demand (COD) in a submerged membrane bioreactor (SMBR) was investigated. Subsequently, BC was added to improve the degradation of organic matter and mitigate membrane fouling. The feasibility of adding BC to the SMBR was verified by studying and evaluating the effects of the COD removal rate, mixed liquor characteristics, membrane fouling control and the microbial community. This detailed study on the performance of an SMBR in municipal sewage treatment and the mechanism of membrane fouling can provide a theoretical basis for the control of SMBR membrane fouling and lay a foundation for the practical application of SMBRs.

2. Materials and Methods

2.1. SMBR Set-Up

A schematic diagram of the SMBR is shown in Figure 1. The ceramic membrane was supplied by Ya’an Weclean Environmental Technology Co. (Ya’an, China). The ceramic membrane is made from α-Al\textsubscript{2}O\textsubscript{3}, it has a pore size of 0.1 μm and the membrane area is 0.125 m\textsuperscript{2}/piece. The ceramic membrane has the advantages of a long service life, strong anti-fouling abilities, excellent hydrophilicity and a good oxidation resistance.
The SMBR with a work volume of 50 L (total volume of 100 L) is made of stainless steel and operates at ambient temperature. The membrane module is composed of four pieces of ceramic membrane (L 24.5 cm × W 0.6 cm × H 24.5 cm). The influent and effluent of the SMBR are controlled by diaphragm booster pumps. The wastewater enters the device and is mixed with AS for biological treatment under the action of an aeration disc, with a maximum aeration rate of 60 L/min. The treated wastewater is then immediately discharged through the hollow ceramic membrane. There are three rotor flow meters to control the aeration (maintained at around 40 L/min) and inlet and outlet flow rates throughout the operation. The device is also equipped with a vacuum pressure meter for real-time monitoring of the trans-membrane pressure (TMP) in the system. An aeration pump is placed at the top of the SMBR and an aeration disc is equipped at the bottom of the upstream area to supply fine air bubbles. The HRT was set at 2 days without sludge discharge. The dissolved oxygen (DO) concentration was maintained at 0.5–1.2 mg/L and the pH was adjusted to 6–9.

2.2. Experimental Design

AS was collected from a hydrolysis acidification tank at Futian slaughterhouse (Chengdu, China), which contains a large number of functional bacteria with high activities. Municipal sewage was obtained from the Keya Wastewater Treatment Plant (Chengdu, China). This sewage treatment plant is surrounded by both homes and factories; thus, it treats a mix of industrial wastewater, domestic wastewater and piped rainwater.

When the reactor was started, low concentration synthetic wastewater was supplied initially [27], and the contents of COD, NH$_4^+$-N and TP were 500, 10 and 2 mg/L, respectively [28]. When the pollutant removal rate was stable above 90%, the SMBR ran stably. The formal test was then started and the SMBR ran for 79 d. The operation process was divided into two stages: stage I (1–51 d)—operational performance and stage II (52–79 d)—BC addition (BC = 5 g/L). When the membrane was seriously fouled or the setpoint/threshold value was reached (TMP = 30 kPa), BC was added to investigate the mitigation mechanism of membrane fouling. BC was obtained by pyrolysis of bamboo sawdust in a Muffle furnace at 500 °C for 4 h in a nitrogen atmosphere. The specific surface area of the BC was 120.69 m$^2$/g and the pore volume was 0.037 cm$^3$/g.

2.3. Sampling Methods

The influent and effluent were filtered through a 0.45 µm aqueous polyethersulfone (PES) needle membrane filter (JINTENG, Zhoushan, China). Water samples were collected every 2 days in stage I and every day during stage II. The mixed liquor was collected.
weekly and pre-treated. The main components of the mixed liquor were SMP and EPS, and the extraction method was as follows: After the mixed liquor sample was centrifuged at 6000 × g for 10 min, the supernatant was filtered by a 0.45 µm membrane filter and the filtrate was the SMP. The residual biomass was re-suspended in ultra-pure water after topping up to the original volume, repeated three times, and then heated in a constant temperature water bath at 70 °C for 30 min. Then, the mixed liquor sample was centrifuged at 8000 × g for 10 min and the supernatant was filtered by a 0.45 µm membrane filter. The filtrate was used to determine the EPS content.

2.4. Analytical Methods

The COD concentration was determined by a rapid analyzer (5B-3C, Lianhua, Linxia, China). The main components of SMP and EPS are PN and PS, and their concentrations were determined by the Folin–Ciocalteu phenol reagent method and the anthrone method, respectively [29]. Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were determined by the weight method according to the Urban Construction Industry Standard of the People’s Republic of China (2005) [30]. The concentrations of NH₄⁺-N, TN, nitrate nitrogen (NO₃⁻-N) and nitrite nitrogen (NO₂⁻-N) were determined by the China Standard Method for Monitoring and Analysis of Water and Wastewater (2002) [31]. Three parallel experiments were conducted for each index.

The morphology of the polluted ceramic membrane was observed by scanning electron microscopy (SEM). A trace of the dried sample was glued directly to the conductive adhesive and sprayed at 10 mA for 90 s using an Oxford Quorum SC7620 sputtering coater, then scanned using a Zeiss Sigma 300 at 20 kV acceleration voltage. A contact angle measuring instrument (SL200B) was used to measure the contact angle of the membrane sample surface.

2.5. Microbiome Analysis

Microbial samples were collected at the end of the two stages and stored at −80 °C. The samples from the two stages in different regions of the system were suspended sludge, fixed sludge (located in the membrane module but not attached to the membrane) and cake layer sludge, named J_1_1, J_1_2, J_1_3, J_2_1, J_2_2 and J_2_3 in turn. After DNA extraction from the environmental samples, genomic DNA was evaluated by 1% agarose gel electrophoresis. Bacterial primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used to construct the PCR amplification library, and MiSeq sequencing was performed on the V4 region of the bacterial 16S rRNA gene. Sequencing was performed using Illumina’s Miseq PE300/NovaSeq PE250 platform (Shanghai Majorbio Biomedical Technology Co., Ltd., Shanghai, China). The sequencing data were analyzed using Meiji cloud platform. Sequence clustering with similarity ≥97% was in operational classification units (OTU). The community diversity index based on the OTU was obtained in the MOTHUR program [13].

3. Results and Discussion

3.1. Reactor Performance

3.1.1. Nitrogen and TP Monitoring

Nitrogen monitoring results at stage I are shown in Figure 2. After acclimating AS with the low-load synthetic wastewater, the SMBR ran stably when pollutant removal rates were stable above 90%. After stable operation, the synthetic wastewater was replaced with municipal sewage, and the removal rates of NH₄⁺-N and TN were both high within the first week, with the highest removal rates reaching 98.85% and 94.21%, respectively. This was because the slow water intake under constant flux conditions enables the microorganisms to adapt to environmental changes slowly without activity stagnation [32,33]. Under continuous influent conditions, the NH₄⁺-N removal rate was stable and efficient, with an average removal rate of 91.57%. The removal rate of NO₂⁻-N decreased first and then increased, while the removal rate of TN decreased. At this time, the effluent NO₂⁻-N
increased to the highest level at 8.09 mg/L. Thereafter, the effluent NO$_2^-$-N continued to decline, and the NH$_4^+$-N removal rate was maintained at a high level, with an average of 94.38%. This might be due to the emergence of the Planctomycetota phylum in the SMBR after domestication (see Section 3.3.1), which contains all currently known ammonia-oxidizing bacteria (AOB) [34]. Wang et al. reported similar conclusions and speculated that AOB enrichment might be caused by a suitable feedwater substrate and the superior properties of MBRs [35]. From 27 to 51 d, the effluent TN concentration continued to decrease and tended to be stable, with an average removal rate of 59.01%, indicating that the SMBR could adapt to the change in water quality. The SMBR showed good decontamination performance, mainly due to the good mixing of municipal sewage and AS by aeration and the maximum retention of microorganisms by the membrane [4]. At the same time, the NO$_3^-$-N concentration in the effluent increased gradually and was maintained at 20.20 ± 1.15 mg/L. It might be that the concentration of organic matter in urban sewage was too low, which led to the inactivation of denitrifying bacteria and the loss of electron donors, resulting in the inhibition of denitrifying bacteria [36]. In the whole process, NH$_4^+$-N was efficiently and stably removed, indicating that the SMBR was in an efficient denitrification state since its successful start-up.

![Figure 2](image_url)

**Figure 2.** Performance of the SMBR for municipal sewage treatment in stage I. (a) NH$_4^+$–N, (b) NO$_3^-$–N and NO$_2^-$–N, (c) TN.

After a period of operation, the treatment effect of the SMBR on municipal sewage tended to be stable, and the TP removal rate was monitored (Figure 3). Initially, the removal rate of TP by the SMBR was more than 50%, which might be attributed to phosphate accumulating organisms (PAOs) and inorganic matter precipitation ingestion by (PAOs) and inorganic matter precipitation ingestion by phosphate accumulating organisms (PAOs) and inorganic matter precipitation ingestion by (PAOs) and inorganic matter precipitation ingestion by phosphate accumulating organisms (PAOs) and inorganic matter precipitation ingestion by (PAOs) and inorganic matter precipitation ingestion by phosphate accumulating organisms (PAOs) and inorganic matter precipitation ingestion by (PAOs) and inorganic matter precipitation ingestion by phosphate accumulating organisms (PAOs) and inorganic matter precipitation ingestion by (PAOs) and inorganic matter precipitation ingestion by phosphate accumulating organisms (PAOs) and inorganic matter precipitation ingestion by (PAOs) and inorganic matter precipitation ingestion by phosphate accumulating organisms (PAOs) and inorganic matter precipitation ingestion by (PAOs) and inorganic matter precipitation ingestion by phosphate accumulating organisms (PAOs) and inorganic matter precipitation ingestion by (PAOs) and inorganic matter precipitation ingestion by phosphate accumulating organisms (PAOs) and inorganic matter precipitation ingestion by (PAOs) and inorganic matter precipitation ingestion by phosphate accumulating organisms (PAOs) and inorganic matter precipitation ingestion by (PAOs) and inorganic matter precipitation ingestion by phosphate accumulating organisms (PAOs) and inorganic matter precipitation ingestion by (PAOs) and inorganic matter precipitation ingestion by phosphate accumulating organisms (PAOs) and inorganic matter precipitation ingestion by...
3.1.2. Comparative Analysis of the Removal of COD

The performance of the SMBR was investigated with and without BC for the removal of COD (Figure 4). In stage I, COD was successfully removed in phase I with an average removal rate of 92.72% [41], which may be due to the breaking of the nutrient-poor environment in the start-up period. Nutrients in the actual wastewater promoted the growth of microorganisms in the system, thus improving the utilization rate of substrates [20]. During phase II (11~51 d), COD removal rates were above 70%, slightly lower than the results of Aslam et al. [42] and Sathya et al. [43], mainly because the municipal sewage used in this study contained industrial wastewater with substances that are difficult to degrade and have low biochemical properties. The average COD removal rate was 83.70% in stage I, indicating that the SMBR has a good effect on the treatment of actual municipal sewage.

In stage II, the COD removal rate further increased to more than 90%, with the maximum removal rate reaching 95.43% in phase I. During phase II (64~79 d), the SMBR gradually adapted to the BC environment and ran stably, and the average removal of COD was 86.47%, which was slightly higher than in stage I (83.70%). Chen et al. [20] mentioned...
that after BC was added, the COD removal rate increased from 89.2 ± 2.2% to 93.8 ± 1.7%. Mian et al. [44] also reported that BC could promote COD removal depending on the adsorption properties of BC itself (promoting the absorption of organic matter) and some of the carbon-containing groups (providing a good carbon source and stimulating microbial activity). Obviously, BC may promote the growth and activity of sludge [19] and thus the degradation of organic compounds. It has also been speculated that the ability of BC to accept and provide electrons provides a better REDOX pathway for microorganisms [45].

3.2. Impacts of BC Addition on the Properties of the Mixed Liquor

3.2.1. Sludge Characterization

The changes in the sludge can reflect the operating efficiency and stability of SMBRs [46]. The initial MLSS and MLVSS amounts were 12.18 and 8.97 g/L, respectively. As shown in Figure 5, in stage I, MLSS and MLVSS showed an upward trend, gradually increasing from 0.52 to 0.88, and the value of MLVSS/MLSS was superior to conventional wastewater treatment processes [46]. These results indicated that SMBRs have better stability, which is consistent with the excellent pollutant removal performance shown in Figures 2 and 4. Previous studies have shown that SMBRs operated for long SRTs will produce slow-growing bacteria, which can degrade inert substances and avoid their significant accumulation in sludge [47]. In stage II, after BC was initially added, the MLVSS/MLSS decreased from 0.88 to 0.21. It is speculated that this may be due to the inherent adsorption of BC; the substrates in the mixture are rapidly adsorbed onto BC, resulting in a rapid reduction in biomass [44]. After internal circulation aeration, AS re-entered the mixture and the MLSS value increased and was maintained in a suitable range of 10.63–13.41 g/L. From 69 to 78 d, MLVSS/MLSS and MLVSS levels increased stably, and were higher than that at the beginning of BC addition. This was because the porous structure of BC provided more attachment sites for microorganisms, which ultimately led to the increase in sludge. Lei et al. [19] and Zhao et al. [48] have reported that BC can help attached microorganisms transfer electrons, thereby reducing the energy loss in the traditional microbial electron transfer process, which is conducive to the activity and growth of AS.

![Figure 5. Analysis of MLSS, MLVSS and MLVSS/MLSS levels during continuous operation.](image)

3.2.2. SMPs and EPSs in the Mixed Liquor

The contents of SMPs and EPSs at different stages of continuous operation of the SMBR were determined, as shown in Figure 6. In stage I, both SMPs and EPSs showed an upward trend, from 31.12 mg/L and 107.41 mg/L on the 7th d to 87.40 mg/L and 244.23 mg/L on the 51st d, respectively. Correspondingly, the PS concentrations of SMPs gradually increased from 2.01 to 17.05 mg/L (Figure 6a). This demonstrates that membrane fouling slowly becomes more severe in the later stages of stage I. The effect of SMPs on membrane fouling is believed to be a mixed process of direct plugging and adsorption on
membrane pores, especially for PSs of SMPs [49]. These results show that SMPs are one of the main factors influencing membrane filtration resistance at the beginning of SMBR operation, and Banti et al. have also reported a similar finding [50].

![Figure 6.](image)

In stage II, due to the effect of BC, SMPs and EPSs decreased sharply, reaching the lowest values of 31.76 and 35.51 mg/L on the 53rd d, respectively, which was consistent with the decrease in MLVSS in Figure 5. Duan et al. [51] reported that in a reactor with BC added, the hydrolysis of PS and PN increased to 51.12% and 68.3%, respectively. This may be due to the increased activity of enzymes such as protease and dextranase cause by BC [52]. It is obvious that BC can significantly promote the hydrolysis of organic substrates significantly in SMBRs. After the reactor was stabilized, the contents of SMPs and EPSs stabilized at 63.05 ± 8.49 mg/L and 67.12 ± 1.54 mg/L, respectively. Overall, the trends in SMPs and EPSs are similar, and there may be a conversion relationship between the two, which is consistent with the unified theory of SMPs and EPSs [53,54]. In addition, the contact angle of the ceramic membrane is 87.3°, indicating that it has good hydrophilicity and tends to repel the adhesion of SMPs and EPSs to the membrane surface [55]. The above results demonstrate that the combination of hydrophilic ceramic membranes and BC in SMBRs is a good method to mitigate membrane fouling [19,20,56,57].

3.2.3. TMP Analysis

The SMBR ran at a constant flux for TMP monitoring, as shown in Figure 7. When the membrane was severely fouled or reached the setpoint/threshold (TMP = 30 kPa) [13,24,58], BC was added to investigate the mitigation mechanism of membrane fouling. In stage I, TMP increased slowly until day 14, during which the concentrations of SMPs and EPSs were relatively low (Figure 6) due to the strong resistance of the clean ceramic membrane to membrane fouling. In addition, the initial microbial activity was low and the metabolism was slow, so that less EPSs and gel matrix were produced [21]. Subsequently, a “TMP jump” occurs [10], and MLSS, MLVSS, SMPs and EPSs tend to increase, indicating that these factors jointly lead to membrane fouling [6,59]. Among these factors, PSs of SMPs were the most important. Other studies have also reported that PSs of SMPs accumulate on the membrane surface and in the membrane pore, thus accelerating membrane fouling [49,50]. In stage II, the TMPs decreased after the addition of BC, reaching a minimum of 23.8 kPa, and then increased slowly, but never reaching the threshold values, indicating the retarding effect of BC in preventing membrane fouling [19]. The EPS concentration decreased most significantly in stage II (from 244.23 to 35.51 mg/L), which reduced the flow of biopolymer to membrane components, indicating that membrane pollution was mainly driven by EPSs [12,20].
3.2.4. Membrane Morphology Analysis

The influence of BC on membrane fouling was studied through membrane surface characteristics, as shown in Figure 8. SEM of the contaminated membrane on the 51st day showed that the ceramic membrane was covered by a sludge layer and only a few membrane holes were visible (Figure 8a). At this point, TMP’s reached the set value (>30 kPa), and a stable membrane effluent could not be maintained even under the pressure of the discharge pump, indicating that the membrane fouling of the SMBR has reached a maximum value. A large number of studies have reported that when the cake layer on the surface of the ceramic membrane is thick enough, the filtration resistance of the membrane will increase, resulting in an increase in TMPs [24,49,59]. In order to mitigate membrane fouling, BC was added. After 25 d of operation, the sludge layer clearly started to peel off, exposing the gel layer (Figure 8b), which was mainly composed of EPSs and SMPs [60]. This is consistent with the conclusion of Teng et al. [61] and Meng et al. [62], namely that the biofilm (cake layer) is mainly composed of a sludge cake layer and a gel layer, which together lead to membrane fouling. The results showed that BC can not only reduce the membrane fouling by changing the properties of the mixed liquor, but also reduce the thickness and compactness of the cake layer effectively by inhibiting the formation of the biofilm, improve the filtration performance of the ceramic membrane and reduce the resistance of the cake layer.

Figure 7. TMP profile of the effects of BC on membrane fouling process in the SMBR.

Figure 8. SEM analysis of ceramic membranes in the SMBR. (a) Polluted membranes operating on the 51st d (b) Polluted membranes operating on the 79th d.
3.3. Effect of BC Addition on the Microbial Community

3.3.1. The α-Diversity Analysis

In addition to the properties of the mixed liquor, the microbial community also plays an important role in the membrane fouling [63]. Therefore, the α-diversity of the SMBR during operation was analyzed by high-throughput sequencing. The observed Good’s coverage rate of OTU was 99.62 ± 0.06%, and the rarefaction curves showed clear asymptotes (Figure 9), indicating that the sampling of this community was nearly complete [64].

![Figure 9. The coverage curves in OTU levels.](image)

The α-diversity is the diversity within a particular region or ecosystem, which intuitively reflects the diversity and richness of bacteria in the SMBR. As shown in Table 1, the coverage of each sample was above 0.99, which meant that the obtained sequence libraries covered the microbial diversity of MBRs. After BC was added, the Shannon index increased from 4.70 to 5.76, the Simpson index decreased from 0.08 to 0.01 and the Ace index and Chao index showed increasing trends. These results indicated that BC addition and membrane filtration increased the diversity and richness of bacterial communities. It can also be seen from the composition of the microbial community (Figures 10 and 11) that BC affected the relative abundance of dominant bacteria in the SMBR (including Proteobacteria, Chloroflexi, Anaerolineae, etc.), thus resulting in a more balanced distribution of bacteria and an increased microbial diversity. Wang et al., Du et al. and Lou et al. also concluded that the addition of BC stimulated the growth of some dominant microorganisms, which was crucial for promoting the degradation of organisms [57,59,65]. Through the synergistic action of various microorganisms, the performance and anti-environmental effect of the BC-enhanced SMBR on municipal sewage treatment were improved.

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<th>No.</th>
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3.3.2. Microbial Community Analysis

As can be seen from Figure 10, the composition and percentage of microorganisms in different regions of the SMBR were relatively consistent due to the similar living environment at same stage. The types of microorganisms in different stages were highly similar, and the dominant bacteria at the phylum level were Proteobacteria, Chloroflexi, Actinobacteria, Acidobacteria, Planctomycetota, etc. However, there were significant differences in
the relative abundances of the microbial community. **Proteobacteria** and **Chloroflexi** were the two most abundant bacterial phyla [66] in all samples, accounting for 17.54~40.26% and 9.21~42.58% of the total effective bacterial sequences, respectively. **Proteobacteria** usually exists in aerobic systems as heterotrophic bacteria [64]. They are considered to be the quickest bacteria to attach to the membrane surface [64], and their relative abundance was more than 50% in stage I, much higher than that in stage II. This suggests that BC can effectively reduce membrane fouling mainly by mitigating microbial attachment to the membrane [40]. This was also consistent with the decreases in MLVSS (Figure 5) and TMP (Figure 7) in stage II. The relative abundance of **Chloroflexi** in AS (including the mixed liquor and cake layer) increased significantly in stage II, reaching 22.93~25.58%, which indicates that the addition of BC improves the degradation ability of complex organic matter [40].

![Figure 10](image-url)

**Figure 10.** Microbial community bar diagram of samples at different stages on the phylum level.

![Figure 11](image-url)

**Figure 11.** Microbial community bar diagram of samples at different stages on the class level.

In stage II, **Acidobacteriota** accounted for 7.75 ± 0.22%, which is different to the lower than 2% level reported by Nguyen et al. [40] in the sludge community. This demonstrates that the prolonged anoxic environment of the system promotes the accumulation of slow-growing microorganisms (i.e., **Acidobacteriota**, **Patescibacteria**, etc.) [67], so that MLSS can be maintained at an average of 12.01 g/L, reducing the production of residual sludge in the SMBR. Nguyen et al. [40] also mentioned in their review that **Proteobacteria**, **Chloroflexi**...
and Acidobacteriota commonly exist in MBR systems [68], and most of them can degrade nutrients, including a wide range of complex organic matter and polysaccharides [69]. An exponential increase in Planctomycetota, containing all known AOB, was also observed after the addition of BC [34]. These results showed that the nitrogen removal rate of the BC-enhanced SMBR increased, especially the utilization rate of NO$_2$-N (Figure 2).

In Figure 11, the dominant species on the class level were γ-proteobacteria, Anaerolineae, α-proteobacteria, Planctomycetes, Bacteroidia, Actinobacteria and so on. Among them, the abundance of γ-proteobacteria and α-proteobacteria belonging to the phylum proteobacteria decreased by 37.04% after the addition of BC, which was associated with the formation of biofilms [13]. In the BC-enhanced system, Anaerolineae involved in carbohydrate degradation were enriched to 18.29% under anaerobic conditions. These results showed that BC may stimulate the growth of mycelia and act as an adhesive carrier for mycelia to aggregate with substrates [40,70,71]. The changes in the microbial community structure in the BC-enhanced SMBR system indicate that the adaptability of bacteria is important for effective treatment of municipal sewage.

4. Conclusions

The SMBR constructed in this paper showed an excellent pollutant removal performance and the BC-enhanced SMBR has a good mitigation effect on membrane fouling. When the SMBR was used to treat municipal sewage, the removal rate of NH$_4^+$-N, TN, TP and COD reached 94.38%, 59.01%, 44.15% and 83.70%, respectively. The large specific surface area, the surface functional groups and the scouring effect of BC promoted the growth of sludge, and the MLVSS/MLSS was maintained between 0.78 and 0.81, which was conducive to the degradation of organic matter. BC also reduced the thickness and compactness of the cake layer on the membrane surface, and both SMPs and EPSs decreased and stabilized at 63.05 ± 8.49 mg/L and 67.12 ± 1.54 mg/L, respectively. It can be seen that adding BC is an effective measure to mitigate membrane fouling.

The high-throughput sequencing results showed that the addition of BC increased the diversity and richness of microorganisms and promoted the enrichment of functional microorganisms. In conclusion, the treatment performance of the SMBR used in this study is stable and efficient. After BC strengthening, currently recognized SMBR membrane fouling can be controlled and alleviated to a certain extent, which provides theoretical support for the industrial application of SMBRs. At the same time, for comprehensive treatment equipment, if SMBRs can be combined with physical and chemical methods to form a mature process in practical applications, this may promote the overall development of sewage treatment. In addition, further studies on the effects of BC on microbial functional genes, the microbial community structure evolution and the dynamic migration of microorganisms between BC and sludge in SMBRs will be helpful to further explain how BC affects the microbial community structure and how the mechanism of BC alleviates membrane fouling.

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Abbreviations

AS  activated sludge
AOB  ammonia-oxidizing bacteria
BC  biochar
COD  chemical oxygen demand
DO  dissolved oxygen
EPS  extracellular polymeric substance
HRT  hydraulic retention time
MBR  membrane bioreactors
MLVSS  mixed liquor volatile suspended solids
MLSS  mixed liquor suspended solids
NH$_4^+$-N  ammonia nitrogen
NO$_3^-$-N  nitrate nitrogen
NO$_2^-$-N  nitrite nitrogen

References


16. Wang, C.; Ng, T.C.A.; Ng, H.Y. Comparison between novel vibrating ceramic MBR and conventional air-sparging MBR for domestic wastewater treatment: Performance, fouling control and energy consumption. *Water Res.* 2021, 203, 117521. [CrossRef]


36. Zhang, Y.; Fang, Y.; Wang, B.; Zhang, H.; Ding, J. Effects of stepwise adjustment of C/N during the start-up of submerged membrane bioreactors (SMBRs) on the aerobic denitrification of wastewater. Water 2021, 13, 3251. [CrossRef]


52. Yang, G.; Wang, J. Synergistic enhancement of biogas production from grass fermentation using biochar combined with zero-valent iron nanoparticles. Fuel 2019, 251, 420–427. [CrossRef]


61. Teng, J.; Wu, M.; Chen, J.; Lin, H.; He, Y. Different fouling propensities of loosely and tightly bound extracellular polymeric substances (EPSs) and the related fouling mechanisms in a membrane bioreactor. Chemosphere 2020, 255, 126953. [CrossRef]


63. Fu, C.; Yue, X.; Shi, X.; Ng, K.K.; Ng, H.Y. Membrane fouling between a membrane bioreactor and a moving bed membrane bioreactor: Effects of solids retention time. Chem. Eng. J. 2007, 309, 397–408. [CrossRef]


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