Effects of C/N ratio on the performance of a hybrid sponge-assisted aerobic moving bed-anaerobic granular membrane bioreactor for municipal wastewater treatment

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Abstract

This study aimed to evaluate the impact of C/N ratio on the performance of a hybrid sponge-assisted aerobic moving bed-anaerobic granular membrane bioreactor (SAAMB-AnGMBR) in municipal wastewater treatment. The results showed that organic removal efficiencies were above 94% at all C/N conditions. Nutrient removal was over 91% at C/N ratio of 100/5 but was negatively affected when decreasing C/N ratio to 100/10. At lower C/N ratio (100/10), more noticeable membrane fouling was caused by aggravated cake formation and pore clogging, and accumulation of extracellular polymeric substances (EPS) in the mixed liquor and sludge cake as a result of deteriorated granular quality. Foulant analysis suggested significant difference existed in the foulant organic compositions under different C/N ratios, and humic substances were dominant when the fastest fouling rate was observed. The performance of the hybrid system was found to recover when gradually increasing C/N ratio from 100/10 to 100/5.

Keywords: C/N ratio; Sponge; Nutrient removal; Membrane fouling; Granular anaerobic membrane bioreactor

1. Introduction

Granular anaerobic membrane bioreactors (G-AnMBRs) offer a promising opportunity to transform conventional municipal wastewater plants into net producers of renewable energy with significantly reduced sludge handling costs and energy demand while occupying a small footprint (Chen et al., 2017a). Owing to the competitive advantages of granular biomass, G-AnMBRs have gained particular interest for fouling mitigation since membrane fouling has remained as one of the most critical challenges, hindering the progress of conventional AnMBRs (C-AnMBRs), predominantly in the form of continuous stirred tank reactor configuration (Chen et al., 2017b). Martin-Garcia et al. (2011) successfully applied a G-AnMBR, and reported that the G-AnMBR had much slower fouling than the C-AnMBR because the G-AnMBR sludge had a lower mixed liquor suspended solids (MLSS) and 50% less of soluble microbial products (SMP) than those of the C-AnMBR. More fouling reduction in G-AnMBR due to the significantly reduced solid and colloidal loading (by a factor of 10 and 3, respectively) on the membrane was also reported in another study of Martin-Garcia et al. (2013).

Recent research has found that the incorporation of membrane into the granular systems could negatively affect the integrity of anaerobic granules and lead to severe membrane fouling, thus exacerbating the long-term performance of G-AnMBRs (Ozgun et al., 2015; Chen et al., 2017a). The low-cost polyutrethane sponge, an ideal attached growth mobile carrier, has been successfully applied in many aerobic membrane bioreactors (AMBRs) studies to enhance the overall performance of AMBRs due to its high internal porosity and specific surface area, high stability to hydrolyse and light weight (Guo et al., 2010). Chen et al. (2017a) worked on a sponge-assisted G-AnMBR (SG-AnMBR), and indicated that sponge addition into G-AnMBR could enhance organic and nutrient removal, and maintain superior granular quality. Additionally, sponge media could not only positively affect the concentration and properties of microbial products (e.g. SMP and extracellular polymeric substances (EPS)) in granular sludge, cake layer as well as settling zone mixed liquor, but also reduce fouling resistance by 50.7%, thereby alleviating membrane fouling.

Although studies have proved that the sponge addition could improve nutrient removal (Nguyen et al., 2011), nutrient removal efficiencies were still considered quite low in the SG-AnMBR (Chen et al., 2017a), limiting its universal appeal for municipal wastewater treatment (Smith et al., 2012). Additionally, adopting conventional biological nutrient removal technologies at the downstream of SG-AnMBRs was also not feasible since low C/N ratio in SG-AnMBR effluents inhibited denitrification and phosphorus removal processes due to insufficient organic electron donor presented. Thus, C/N ratio is one of the most influential parameters affecting nutrient removal process as it affects the population and biodiversity of functional microorganisms (Lin et al., 2016). Moreover, membrane fouling can be significantly influenced by C/N ratio because C/N ratio profoundly affects the physiological property of microorganisms and chemical composition of biomass, and influences the concentrations of EPS and SMP and their protein and polysaccharides contents (Hao et al., 2016).

In this study, a new hybrid sponge-assisted aerobic moving bed-anaerobic granular membrane bioreactor (SAAMB-AnGMBR) was developed to overcome the two major issues (i.e. fouling and low nutrient removal) impeding the progress of G-AnMBRs. Based on the literature, it is the first development of the hybrid configuration for enhancing nutrient removal and fouling control of G-AnMBR during municipal wastewater treatment. The main aim of this study was to evaluate the effects of C/N ratio on the performance of such a hybrid system in terms of pollutants removal (particularly for nutrient removal) and membrane fouling. The system recovery after the overloaded nitrogen event was also evaluated in the study.

2. Methods

2.1. Wastewater and sponge

The synthetic wastewater was prepared with glucose, ammonium sulphate, potassium dihydrogen orthophosphate together with trace metals to simulate municipal wastewater just after primary treatment, providing dissolved organic carbon (DOC) of 105 - 128 mg/L, chemical oxygen demand (COD) of 330 - 370 mg/L, orthophosphate (PO_4-P) of 3.0 - 3.5 mg/L, ammonia nitrogen (NH_4-N) of 12 - 15 mg/L, nitrite nitrogen (NO_2-N) of 0 - 0.02 mg/L and nitrate nitrogen (NO_3-N) of 0.2 - 0.8 mg/L. $NaHCO_3$ (powder, analytical grade) or 2 M H_2SO_4 was used to adjust pH to 7. Porous polyester-urethane sponge (PUS) cubes (dimensions: 2.5 mm × 2.5 mm × 2.5 mm), named S28-

30/90R (density of 28 - 30 kg/m^3 with 90 cells per 25 mm) from Joyce Foam Products were used in the study.

2.2. Experimental set-up and operation conditions

The hybrid SAAMB-AnGMBR, consisting of a sponge-assisted aerobic moving bed reactor (SAAMBR) and a submerged sponge-assisted anaerobic granular membrane bioreactor (SS-AnGMBR), was continuously operated for 282 days in a temperature-controlled room (20 ± 0.5 ºC). Each of the SAAMBR and the SS-AnGMBR had effective working volume of 3 L, and sponge fraction was 20% of working volume. At the bottom of the SAAMBR, fine bubble diffuser was set to supply air in order to provide complete liquid-solid mixing and moderate sponge up/down motion, and maintain dissolved oxygen (DO) concentration of 3.5 - 4.8 mg/L. Prior to continuous operation, the SAAMBR with fresh sponge was acclimatized to synthetic wastewater for 30 days at HRT of 12 h until the system reached relatively stable treatment performance. The attached growth on the sponge also reached steady state at 1.02 ± 0.04 g MLVSS/g sponge. The sponges and anaerobic granular sludge were acclimatised to synthetic wastewater for 30 days until a stable treatment performance was reached. The SS-AnGMBR was seeded with anaerobic granular sludge with initial MLSS concentration of 20.12 ± 1.21 g/L, and biomass grown on sponge cubes after acclimatization was 1.78 ± 0.09 g MLVSS/g sponge. A polyvinylidence (PVDF) hollow fiber membrane module with a pore size of 0.22 μm and surface area of 0.06 m^2 was immersed in the settling zone of the SS-AnGMBR.

The SS-AnGMBR was continuously fed with synthetic wastewater at a flow rate of 4.17 mL/min while wastewater from the SS-AnGMBR was continually transferred into the SAAMBR at the same flow rate. The SAAMBR effluent was recirculated back to the SS-AnGMBR through a nitrogen gas sparged buffer tank. The permeate pump in the SS-AnGMBR was operated in an intermittent mode with relaxation (8 min on and 2 min off) to acquire permeate from the membrane module with a constant filtration flux of 5.21 LMH. Both SAAMBR and SS-AnGMBR had HRT of 12 h, and upflow velocity in the SS-AnGMBR was maintained at 3.2 m/h using internal recirculation. The membrane fouling propensity was indicated by normalized trans-membrane pressure (TMP), which was recorded by a pressure transmitter. Operation was terminated when TMP exceeded 30 kPa, and fouled membrane was taken out for ex situ cleaning (Deng et al., 2016a).

The entire study period was divided in 2 phases according to the research objectives. In phase 1, the hybrid SAAMB-AnGMBR was fed with wastewater having C/N/P ratio = 100/5/1 (0 - 75 day), 100/6/1 (76 - 126 day), 100/8/1 (126 - 151 day) and 100/10/1 (151 - 166 day), respectively, with the aim to investigate the impact of C/N ratios on the performance of the hybrid system. In phase 2, after overloaded nitrogen events, the hybrid system was operated with C/N/P ratios of 100/6/1 (167 - 210 day) and then 100/5/1 (211 - 282 days) to investigate the extent of system recovery after the overloading nitrogen event.

2.3. Analytical methods

DOC analysis was performed using the Analytikjena Multi N/C 2000. Spectrophotometric method named spectroquant Cell Test (NOVA 60, Merck) was used to measure NH_4-N, NO_2-N, NO_3-N and PO_4-P. Volatile fatty acids (VFAs) were extracted using methods reported by Banel and Zygmunt (2011) and measured using GC-MS according to Chen et al. (2017a). Biogas produced was collected using a biogas sample bag and determined using a liquor displacement device. Methane composition was determined using potable biogas analyzer (Biogas 5000, Geotech, UK).

The granular sludge was collected at 3 sampling port at different heights of the SS-AnGMBR (Port 1: 20 cm, Port 2: 40 cm and Port 3: 60 cm height from the bottom) and mixed for analysis, in order to represent the overall characteristics of granular sludge. The analyses of MLSS, mixed liquor volatile suspended solids (MLVSS), sludge volume index (SVI), settling velocity, zeta-potential were carried out using Standard Methods (APHA, 1999). Attached biomass on sponge was determined by the method suggested by Nguyen et al. (2011). Batch tests were conducted according to methods proposed by Gong et al. (2012), to determine the specific nitrification rate (SNR) and specific denitrification rate (SDR) of sludge and sponge-attached sludge. Allylthiourea (ATU) of 1 mg/L was added as the nitrification inhibitor into the synthetic substrate to eliminate the influence of nitrifiers for the determination of SDR. SNR and SDR were calculated by the decreasing slope of NH_4-N and NO_3-N concentrations with time and divided by the initial MLSS concentration. Particle size distribution (PSD) was determined using Mastersizer Series 2000 (Malvern Instruments Ltd. UK) with a detection range of 0.02 - 2000 mm.

The detailed measurement protocol of membrane fouling resistances including total resistance (R_T), intrinsic membrane resistance (R_M), cake layer resistance (R_C) and pore blocking resistance (R_P), respectively, was followed by methods reported in Deng et al. (2015). Membrane fouling resistances were determined using the resistance-in-series model (Choo and Lee, 1996). The extraction and analysis of EPS and SMP in the granular sludge, mixed liquor and cake layer were performed based on the protocol of Deng et al. (2014). Modified Lowry method (Sigma, Australia) and Anthrone-sulfuric acid method were adopted for further determination of protein (EPS_P and SMP_P) and polysaccharide (EPS_C and SMP_C) concentrations of the extracted samples. Foulants attached on the membrane surface were extracted using the method suggested by Chen et al. (2017b). The extracted samples were further analysed using size exclusion liquid chromatograph with organic carbon detector (LC-OCD), a TSK HW 50-(S) column and a 0.028 mol/L phosphate buffer for the qualitative examination of foulant organics. Fluorescence measurements of membrane foulant (excitation emission matrix, (EEM)) were obtained using a Varian Cary Eclipse Fluorescence Spectrophotometer, USA, according to methods suggested by Hong et al. (2012). EEM spectra were plotted as the elliptical shape of contours using software OriginPro 9.1. All the liquid, gas and sludge samples were tested in triplicate, with an average value and standard deviation for discussion.

3. Results and discussion

3.1. Overall treatment performance

The hybrid SAAMB-AnGMBR system showed sound organic removal even though influent C/N ratio varied. COD and DOC removal efficiencies were kept over 94.8% and 94.5% regardless of C/N ratios (Table 1). The results suggested that decreasing C/N ratio from 100/5 to 100/10 did not adversely influence the organic removal since complete retention of all particulate COD and macromolecular COD components was achieved by the membrane and most of the readily biodegradable COD was removed by aerobic and anaerobic biodegradation (Martinez-Sosa et al., 2011).

Table 1

Similarly, stable NH_4-N removal (Table 1) over 92.5% was observed in spite of varying C/N ratios in influent, because the population of autotrophic nitrifying bacteria was not impacted by the change of C/N ratios (Lin et al., 2016). The removal efficiencies were higher than those reported by Khan et al. (2013), who obtained NH_4-N removal efficiencies of 89.3 - 90.5% at C/N ratios of 20 - 10. The higher NH_4-N removal in this study was mainly ascribed to sufficient aeration for nitrifying process and the enhanced population of nitrifying bacteria retained by sponge cubes (Deng et al., 2016a). The attached biomass on sponge carriers in the SAAMBR showed minor change, with 1.07 ± 0.03, 1.05 ± 0.05, 1.01 ± 0.08 and 1.03 ± 0.09 g MLVSS/g sponge at varying C/N ratios from 100/5 to 100/10, respectively. Furthermore, with the decreased C/N ratio, SNR of attached biomass was enhanced (Table 2), which contributed to stable NH_4-N removal.

Table 2

When C/N ratio was maintained at 100/6 or higher, more than 84% of TN removal was achieved (Table 1). Due to effective simultaneous nitrification and denitrification (SND) process in the hybrid system, effluent NO_3-N concentrations were maintained at low levels with 0.39 ± 0.15 and 1.63 ± 0.54 mg/L at C/N ratios of 100/5 and 100/6. Table 2 showed the SDR values for each type of functioning biomass in the SAAMB-AnGMBR system. Anaerobic granular biomass exhibited the highest SDR, hence contributing the most to the denitrification process. SDR of aerobic sponge-attached biomass were found comparable to that of anaerobic sponge-attached biomass, although DO in the aerobic compartment was maintained at 3.5 - 4.8 mg/L. The main reason was that nitrification occurred on sponge surfaces while declining DO levels along the sponge inward depth favored the formation of anoxic/anaerobic zones, contributing to efficient denitrification process (Khan et al., 2011; Deng et al., 2016b).

However, lower C/N ratios of 100/8 and 100/10 showed significant reduction on TN removal efficiencies to 68.9% and 44.1%, respectively. Biological nitrogen removal is achieved by nitrification (an autotrophic bioprocess), followed by denitrification (a heterotrophic bioprocess) (Kim et al., 2016). Since the NH_4-N removal efficiencies were kept more than 92.5% at all designated C/N ratios, the low TN reduction was mainly attributed to the poor denitrification when insufficient carbon was present for heterotrophic denitrifier for cell growth and nitrate reduction (Yang et al., 2012). Insufficient carbon source restrained the denitrification process, caused relatively higher NO_3-N concentrations in the effluent (5.33 ± 1.08 and 12.46 ± 1.19 mg/L for C/N ratios of 100/8 and 100/10, respectively) and broke the balance between nitrification and denitrification processes (Lin et al., 2016). Low carbon source not only negatively affected the abundance of heterotrophic population but also significantly influenced vital parameters of denitrification process such as SDR. SDR of granular biomass was significantly reduced when decreasing C/N ratio to 100/8 and 100/10 (Table 2). This finding might be related to the deteriorated granule quality (details discussed in Section 3.2.3) as a result of decreasing C/N ratio. However, the attached growth in the SS-AnGMBR kept relatively stable at 1.74 - 1.84 g MLVSS/g sponge over the entire operation period. The impact of C/N ratio on the SDR of aerobic and anaerobic attached biomass could be neglected since their values did not vary significantly. Nitrite production, known to strongly inhibit denitrification process (Yang et al., 2012), was found not the reason for the low TN removal under low C/N ratio since very low nitrite concentrations (<1.52 mg/L) were detected in both SAAMBR and SS-AnGMBR.

Monclús et al. (2010) suggested that phosphate could be occupied by phosphate accumulating microorganisms (PAOs) in the oxic/anoxic zones or consumed for biomass growth during the operation. Since the SND process effectively reduced NO_3-N content at higher C/N ratios of 100/5 and 100/6, low amount of NO_3-N in the anoxic zones of the biofilm contributed to effective PO_4-P release, hence promoting 93.3% and 88.6% of PO_4-P removal, respectively (Deng et al., 2016b). However, when further reducing C/N ratio to 100/8 and 100/10, PO_4-P removal declined significantly to 63.5% and 39.3%, as higher concentration of NO_3-N could inhibit phosphorus release and result in sharp reduction of PO_4-P removal (Ng et al., 2016). Lower C/N ratio may also cause excessive growth of heterotrophic bacteria, hence limiting the growth of PAOs and slow growing denitrifiers (Lin et al., 2016; Khan et al., 2013). VFAs were deemed to be the most preferred carbon source for denitrifier and PAOs (Singhania et al., 2013). It is interesting to observe that more VFAs were accumulated with increased nitrogen content in the feed, and total VFAs concentration for each C/N ratio was 2.77 ± 0.56 (100/5), 5.72 ± 1.12 (100/6), 9.38 ± 1.02 (100/8) and 11.51 ± 1.28 (100/8) mg/L. Acetic acid was found the most sensitive to the change of C/N ratio, whose concentration at C/N ratio of 100/10 (8.92 ± 0.95) was nearly 4 times higher than that at C/N ratio of 100/5 (1.82 ± 0.35 mg/L). Higher levels of residual VFAs, especially for acetic acid at lower C/N ratio, might indicate the inhibition of denitrification and phosphate removal processes. In addition, decreasing methane yields were found at 137.40 ± 3.83, 126.58 ± 3.07, 115.25 ± 2.29 and 102.12 ± 1.92 mL $CH_{4\ (STP)}$/g $COD_{removed}$ (STP: volume of methane produced at and 0 °C Standard Temperature and 1 atm Pressure) at C/N ratios of 100/5, 100/6, 100/8 and 100/10, respectively.

3.2. Membrane fouling behaviour

3.2.1 Membrane filtration performance

The TMP of the hybrid system increased from 0.90 to 30.80 kPa in 75 days (C/N ratio = 100/5); 1.20 to 30.80 kPa (100/6) in 50 days; 1.10 to 30.20 kPa in 25 days (100/8); 1.20 to 30.90 kPa in 15 days (100/10), respectively. Accordingly, the SAAMB-AnGMBR had the fastest fouling rate of 1.98 kPa/d at C/N ratio of 100/10, which was almost 4 times higher than the corresponding value (0.40 kPa/d) obtained at C/N ratio = 100/5. While for C/N ratios of 100/6 and 100/8, the fouling rates were 0.59 and 1.16 kPa/d, respectively. These results implied that lowering influent C/N ratio could deteriorate membrane filterability and shorten the operation period before membrane cleaning. The cause is discussed in Section 3.2.2.

3.2.2 EPS and SMP of the mixed liquor

In the SS-AnGMBR, the membrane module was only challenged by the mixed liquor in the settling zone. Hence, the EPS and SMP concentrations of the mixed liquor and their polysaccharide (EPS_C, SMP_C) and protein contents (EPS_P, SMP_P) were analysed in order to explain the relationship between the mixed liquor properties and fouling propensity, and the results were shown in Table 3. EPS at C/N ratio of 100/10 (48.41 mg/L) were found more than 4.27, 2.04, 1.29 times to the corresponding values obtained at C/N ratios of 100/5, 100/6, 100/8 (11.33, 23.66, 37.40 mg/L), respectively. At the lowest C/N ratio (100/10), the SS-AnGMBR contained the highest EPS content, which was mainly responsible for the highest fouling rate. Studies have reported that EPS were biopolymers attached on flocs or cells surface, and were known to significantly influence membrane fouling (Hao et al., 2016), because EPS were closely associated with cake layer formation. Any variation in EPS concentration and composition would alter the interactions between floc surface and membrane surface or between fouling layer surface and floc surface, thus affecting cake layer formation rate and structure (Hao et al., 2016). Lower EPS_c/EPS_p ratio in sludge flocs was reported to increase floc hydrophobicity, thus deteriorating membrane fouling by their deposition on the membrane (Deng et al., 2016c). In this study, EPS_C/EPS_P ratio remained stable, mainly fluctuating from 0.46 to 0.61, suggesting that influent C/N ratio exerted a limited impact on EPS composition. Hence, EPS composition was not an influential factor affecting fouling.

SMP, known as biopolymers released from microorganisms into solution, has the strongest relationship with fouling rate (Zhang et al., 2015). In this study, despite the increase in N concentration in the feed, SMP showed the relatively low concentrations (5.58 - 8.08 mg/L) at all four C/N ratios. This might be due to the fact that sponge addition into hybrid system could significantly reduce the mixed liquor SMP values

(Deng et al., 2014). The highest SMP value was observed at C/N ratio of 100/5 (8.08 mg/L), followed by those at C/N ratios of 100/6 (7.53 mg/L), 100/8 (6.85 mg/L) and 100/10 (5.58 mg/L). The higher SMP levels at higher C/N ratios in the hybrid system might be due to the SMP accumulation with evolution of time in the mixed liquor since prolonged operational time was recorded at higher C/N ratios (i.e. the time slots were 75, 50, 25, 15 days for C/N ratios of 100/5, 100/6, 100/8, 100/10, respectively). In this case, the total SMP concentration was not responsible for faster fouling at lower C/N ratios. Nevertheless, an increase in SMP_C/SMP_P was positively correlated with faster fouling rate at lower C/N ratio. The averaged values of SMP_C/SMP_P increased from 0.54 to 0.79, 1.14, and 1.44 when lowering C/N ratios from 100/5 to 100/6, 100/8 and 100/10, respectively, and it suggested that higher amount of hydrophilic SMP_C than SMP_P were presented in the mixed liquor at lower C/N ratios. SMP_C were more susceptible to membrane fouling since they could penetrate into the cake layer and membrane pore and lodge insides, thus inducing severe pore clogging and gel layer formation, and causing significant membrane filterability loss (Deng et al., 2016a).

Table 3

3.2.3 EPS and SMP of the anaerobic granular sludge

According to Table 3, both EPS_P and EPS_C concentrations of the granular sludge were noticeably reduced when influent N was increased. The lowest EPS concentration (EPS_P and EPS_C of 4.92 and 2.18 mg/L, respectively) was observed at C/N ratio of 100/10. Since EPS played an vital role on integrating cells into granules, the dramatic decrease in EPS content at lower C/N ratio might indicate weaker, scattered and looser structures of flocs and granules, contributing to granules breakage (reduced sludge particle size) and to the increase in EPS of the mixed liquor in settling zone (Chen et al., 2017a). The granular growth rates (ΔMLSS/Δt) were found at 0.052, 0.045, 0.038 and 0.035 g/L·d, at C/N ratios of 100/5, 100/6, 100/8 and 100/10, respectively, suggesting that lower C/N ratio discouraged the growth of retained sludge agglomerates in the granular sludge bed. PSD analysis confirmed that smaller anaerobic granular sludge particles existed when operating at lower C/N ratios. Granular sludge with the biggest median particle size of 355 μm was observed at C/N ratio of 100/5, while lower values of 308 μm, 223 μm and 165 μm were found at C/N ratios of 100/6, 100/8 and 100/10, respectively. As a result, the disintegration of granules decreased settling capacity of the granular sludge.

Additionally, the deterioration of the granular sludge settling capacity also promoted inefficient solid entrapment of the granular sludge bed. Hence, this encouraged the accumulation of fine sludge particles such as non-settling particles and small colloidal substances in the mixed liquor of the settling zone due to the membrane complete retention of particulate and colloidal matter and biomass. The elevated EPS levels in the mixed liquor (Section 3.2.2) at lower C/N ratios were mainly attributed to the higher MLSS concentrations in the mixed liquor of SS-AnGMBR settling zone as a result of granule breakage. At the end of experiment, the MLSS concentration in the SS-AnGMBR reached up to 293.8 mg/L at C/N ratio of 100/10, which was nearly 1.5, 2.7 and 3.9 times higher than those at C/N ratios of 100/8, 100/6 and 100/5, respectively. Moreover, the highest SMP level in the granular sludge (SMP_P and SMP_C of 14.21 and 2.12 mg/g VSS) was observed at C/N ratio of 100/10 (Table 3), which confirmed that the major fraction of the proteins and polysaccharides existed in the soluble form rather than being the part of the anaerobic granules in the form of EPS. These results highlighted that severe fouling at lower C/N ratios was not only associated with larger amounts of EPS, higher SMP_C/SMP_P ratio of mixed liquor but also due to the deteriorated granule quality.

3.2.4 Fouling resistance and cake layer analysis

Membrane fouling resistances were obtained for each of C/N regimes when cleaning of membrane was performed (Fig. 1). With increasing N dose in the feed, R_T increased from 2.61×10^{12} to 3.65×10^{12} m^{-1} at C/N ratios of 100/5 and 100/6, which further rose to 5.62×10^{12} and 6.56×10^{12} m^{-1} at C/N ratios of 100/8 and 100/10, respectively. R_C and R_P values followed the similar trends as shown in Fig. 1 in which R_C (2.03×10^{12} to 5.19×10^{12} m^{-1}) increased with a greater magnitude than R_P (3.9×10^{11} to 1.18×10^{12} m^{-1}), while R_M (1.89×10^{11} to 1.92×10^{11} m^{-1}) remained stable for all C/N ratios. These results suggested that higher N dose in the feed deteriorated membrane permeability due to more cake formation and pore clogging. Although R_p contributed to declined permeability, the values of R_C/R_T (over 77% at all C/N ratios) indicated that cake formation was the predominant fouling mechanism of the hybrid system, contributing more significantly to membrane fouling. As discussed in Section 3.2.2, the mixed liquor containing SMP with higher SMP_C/SMP_P ratio and higher amounts of EPS was responsible for the elevated R_C and R_P at lower C/N ratios. More EPS resulted in cake formation whist SMP with higher SMP_C/SMP_P ratio could modify the surface properties of membrane and outer cake layer to promote the self-accelerating fouling phenomena (accelerate cake formation rate) (Hao et al., 2016).

Fig. 1.

Cake layer as the dominant mechanism of R_T was further investigated and characterized by the composition of EPS and SMP. As shown in Fig. 2, the cake layer at C/N ratio of 100/5 contained the lowest concentration of EPS_P and EPS_C. The reduction of C/N ratio to 100/10 induced a noticeable rise of EPS_P and EPS_C to 10.95 mg/g MLSS and 25.59 mg/g MLSS, respectively, which were 6.0 and 7.8 times to the corresponding values obtained at C/N ratio of 100/5. The results revealed that higher nitrogen dose increased R_C by the accumulation of EPS within the sludge cake on the membrane surface. On the other hand, in spite of varying C/N ratios, SMP presented significantly low concentrations and minor variations, with SMP_P of 0.47 - 0.52 mg/g MLSS and SMP_C of 0.87 - 1.08 mg/g MLSS, which further confirmed that SMP were not the primary fouling factor for this study as discussed in Section 3.2.2.

Fig. 2.

3.2.5 Foulant characterization

LC-OCD provides critical information regarding the different components of the foulant organics by dividing the total organics into two groups: hydrophobic organics (HPO) and hydrophilic organics (HPI). The hydrophilic group can be further characterized into subgroups such as biopolymers, humic substances, building blocks, and lower molecular weight (LMW) neutrals and acids (Johir et al., 2012). Fig. 3 showed that larger HPO occurred at lower C/N ratios, but they might not be the main foulants due to their much lower concentrations as compared to those of HPI. Within HPI, lower nitrogen dose favoured the accumulation of biopolymers in the foulant while humic acids were dominant at higher N dosage, contributing to the faster fouling rate. Building blocks, as the breakup of the humics, were found decreasing in response to the increase in influent nitrogen content. Though building blocks and LMW neutrals and acids were found in relatively lower concentrations, Aryal et al. (2009) suggested that they were the significant factors affecting fouling as their assemblage could promote biopolymers formation on the membrane surface, exacerbating fouling propensity.

Fig. 3.

The EEM spectra of membrane foulant organics were obtained at different feed C/N ratios. The observation indicated that the hybrid system at different C/N ratios yielded different organics structures and components in the membrane foulants. Peaks at Exitation/Emission (Ex/Em) of 270/300 and 220/320 nm under C/N ratio of 100/5 were associated with biopolymers and their precursors (LMW small amino acid type organics) in the foulant. In comparison, the foulant at C/N ratio of 100/6 demonstrated less intense spectra at the similar peaks' locations, indicating the foulant had lower content of biopolymers along with their precursors. In contrast, C/N ratios of 100/8 and 100/10 showed humic and fulvic acid types peaks at Ex/Em of 310/390 and 230/390 nm, respectively, which suggested that foulant formed at such C/N ratios contained higher content of humics but less biopolymers. The EEM results were found in agreement with the LC-OCD results in the sense that the higher N dosage at lower C/N ratio favoured the relative dominance of the humic-like substances in the extracted foulant, resulting in faster fouling. Similar results were reported by Hong et al. (2012) that humics were prevalent when the fastest fouling was observed.

3.3 System recovery from high nitrogen loading

As mentioned above, the performance of hybrid system including nutrient removal and membrane filtration was deteriorated in phase 1 as a result of the decrease in C/N ratios from 100/5 to 100/10. Therefore, the hybrid system was further investigated in phase 2 by gradually increasing C/N ratio back to 100/5 to observe the extent of system recovery after the overloaded nitrogen event.

3.3.1 Treatment performance of the hybrid system

The system performed reasonably well in term of organic and NH_4-N removal in phase 2, with the comparable removal efficiencies of DOC and COD over 95.2% and NH_4-N over 93.5%. On the other hand, TN and PO_4-P removal efficiencies (82.3 ± 8.8% and 85.7 ± 6.7%, respectively) were found significantly improved after C/N ratio was increased from 100/10 to 100/6. When further increasing C/N ratio to 100/5, the hybrid system could achieve TN and PO_4-P removal efficiencies of 90.1 ± 6.8% and 92.1 ± 8.2%, respectively, indicating that the inhibitory effects on denitrification and phosphate removal processes imposed by overloaded nitrogen were not permanent. The system recovery in terms of nutrient removal was mainly attributed to efficient SND process and effective elimination of residual NO_3-N in the system (Deng et al., 2016b).

3.3.2 Membrane fouling behaviour analysis

Table 4 showed that the fouling rate was significantly reduced when increasing C/N ratio from 100/10 to 100/6 (0.69 kPa/d) and then to 100/5 (0.47 kPa/d) in phase 2. As compared to fouling resistance at C/N ratio of 100/10, the hybrid system in phase 2 exhibited much lower R_T values at C/N ratios of 100/6 and 100/5. During the recovery stage, EPS contents in the mixed liquor were maintained at 28.38 and 13.21 mg/L at C/N ratios of 100/6 and 100/5, respectively, which were about only 59% and 27% of the corresponding values obtained at C/N ratio of 100/10. The analysis of the cake layer also revealed that the hybrid system in phase 2 contained less EPS_P and EPS_C at C/N ratios of 100/6 and 100/5. Thus, increasing C/N ratio helped reduce membrane fouling mainly through reducing EPS in the mixed liquor and cake layer, thereby alleviating fouling (Chen et al., 2017a; Hao et al., 2016). Furthermore, lower SMP_C/SMP_P (0.88 and 0.59, respectively) under C/N ratios of 100/6 and 100/5 revealed less severe fouling in the hybrid system, compared with that at C/N ratio of 100/10. The major foulant organics in phase 2 were found to be biopolymers. The results also indicated that the impact of overloaded nitrogen on fast fouling propensity was temporary.

Table 4

4. Conclusion

This study examined the overall performance of the SAAMB-AnGMBR operated at different C/N ratios. Decreased C/N ratio deteriorated TN and PO_4-P removal, worsened the granular quality, and exacerbated cake layer formation and pore blocking, thereby aggravating membrane fouling. R_C and R_P increased as a result of increased EPS and SMP_C/SMP_P ratio in the mixed liquor. This study also revealed humics were the dominant organic foulant at lower C/N ratios. Additionally, the hybrid system could be recovered by increasing C/N ratio from 100/10 to 100/5 through improving SND process and the properties of mixed liquor and cake layer.

Supplementary information

Summary of sludge properties of seed sludge and granular sludge in the SS-AnGMBR at different C/N ratios, and EEM fluorescence spectra of membrane foulants at different C/N ratios can be found in the supplementary information.

References

1. APHA, 1999. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington.
2. Aryal, R., Legegue, J., Vigneswaran, S., Kandasamy, J., Heran, M., Grasmick, A., 2009. Identification and characterisation of biofilm formed in membrane bioreactor. Sep. Purif. Technol. 67, 86 - 94.
3. Banel, A., Zygmunt, B., 2011. Application of gas chromatography-mass spectrometry preceded by solvent extraction to determine volatile fatty acids in wastewater of municipal, animal farm and landfill origin. Water Sci. Technol. 63, 590 - 597.
4. Chen, C., Guo, W.S., Ngo, H.H., Liu, Y., Du, B, Wei, Q., Wei, D., Nguyen, D.D., Chang, S.W., 2017a. Evaluation of a sponge assisted-granular anaerobic membrane bioreactor (SG-AnMBR) for municipal wastewater treatment. Renew. Energy 111, 620 - 627.
5. Chen, C., Guo, W.S., Ngo, H.H., Chang, S.W., Nguyen, D.D., Nguyen, P.D., Bui, X.T., Wu, Y., 2017b. Impact of reactor configurations on the performance of a granular anaerobic membrane bioreactor for municipal wastewater treatment. Int. Biodeterior. Biodegrada. 121, 131 - 138.
6. Choo, K.H., Lee, C.H., 1996. Membrane fouling mechanisms in the membrane coupled anaerobic bioreactor. Water Res. 30, 1771 - 1780.

7. Deng, L.J., Guo, W.S., Ngo, H.H., Zhang, J., Liang, S., Xia, S., Zhang, Z., Li, J., 2014. A comparison study on membrane fouling in a sponge-submerged membrane bioreactor and a conventional membrane bioreactor. Bioresour. Technol. 165, 69 - 74.
8. Deng, L.J., Guo, W.S., Ngo, H.H., Zuthi, M.F.R., Zhang, J., Liang, S., Li, J.X., Wang, J., Zhang, X.B., 2015. Membrane fouling reduction and improvement of sludge characteristics by bioflocculant addition in submerged membrane bioreactor. Sep. Purif. Technol. 156, 450 - 458.
9. Deng, L.J., Guo, W.S., Ngo, H.H., Du, B., Wei, Q., Tran, N.H., Nguyen, N.C., Chen, S.S., Li, J.X., 2016a. Effects of hydraulic retention time and bioflocculant addition on membrane fouling in a sponge-submerged membrane bioreactor. Bioresour. Technol. 210, 11 - 17.
10. Deng, L.J. Guo, W.S., Ngo, H.H., Zhang, X.B., Wang, X.C. Zhang, Q.H., Chen, R., 2016b. New functional biocarriers for enhancing the performance of a hybrid moving bed biofilm reactor–membrane bioreactor system. Bioresour. Technol. 208, 87 - 93.
11. Deng L.J., Guo, W.S., Ngo, H.H., Zhang, H.W., Wang, J., Li, J.X., Xia, S.Q., Wu, Y., 2016c. Biofouling and control approaches in membrane bioreactors. Bioresour. Technol. 221, 656 - 665.
12. Gong, L.X., Jun, L., Yang, Q., Wang, S.Y., Ma, B., Peng, Y.Z., 2012. Biomass characteristics and simultaneous nitrification–denitrification under long sludge retention time in an integrated reactor treating rural domestic sewage. Bioresour. Technol. 119, 277 - 284.
13. Guo, W.S., Ngo, H.H., Dharmawan, F., Palmer, C.G., 2010. Roles of polyurethane foam in aerobic moving and fixed bed bioreactors. Bioresour. Technol. 101, 1435 - 1439.
14. Hao, L., Liss, S.N., Liao, B.Q., 2016. Influence of COD: N ratio on sludge properties and their role in membrane fouling of a submerged membrane bioreactor. Water Res. 89, 132 - 141.
15. Hong, S., Aryal, R., Vigneswaran, S., Johir, M.A.H., Kandasamy, J., 2012. Influence of hydraulic retention time on the nature of foulant organics in a high rate membrane bioreactor. Desalination 287, 116 - 122.

16. Johir, M.A.H., Vigneswaran, S., Sathasivan, A., Kandasamy, J., Chang, C.Y., 2012. Effects of organic loading rate on organic matter and foulant characteristics in membrane bio-reactor. Bioresour. Technol. 113, 154 - 160.
17. Khan, S.J., Ilyas, S., Javid, S., Visvanathan, C., Jegatheesan, V., 2011. Performance of suspended and attached growth MBR systems in treating high strength synthetic wastewater. Bioresour. Technol. 102, 5331 - 5336.
18. Khan, S.J., Ilyas, S., Rehman, Z.U., 2013. Impact of nitrogen loading rates on treatment performance of domestic wastewater and fouling propensity in submerged membrane bioreactor (MBR). Bioresour. Technol. 141, 46 - 49.
19. Kim, H., Kim, J., Shin, S.G., Hwang, S., Lee, C., 2016. Continuous fermentation of food waste leachate for the production of volatile fatty acids and potential as a denitrification carbon source. Bioresour. Technol. 207, 440 - 445.
20. Lin, J.Y., Zhang, P.Y., Li, G.P., Yin, J., Li, J., Zhao, X.H., 2016. Effect of COD/N ratio on nitrogen removal in a membrane-aerated biofilm reactor. Int. Biodeterior. Biodegrada. 113, 74 - 79.
21. Martinez-Sosa, D., Helmreich, B., Netter, T., Paris, S., Bischof, F., 2011. Anaerobic submerged membrane bioreactor (AnMBR) for municipal wastewater treatment under mesophilic and psychrophilic temperature conditions. Bioresour. Technol. 102, 10377 - 10385.
22. Martin-Garcia, I., Mokosch, M., Soares, A., Pidou, M., Jefferson, B., 2013. Impact of reactor configuration on the performance of anaerobic MBRs: Treatment of settled sewage in temperature climates. Water Res. 47, 4853 - 4860.
23. Martin-Garcia, I., Mokosch, M., Soares, A., Pidou, M., Le-Clech, P., Judd, S.J., McAdam, E.J., Jefferson, B., 2011. Impact of membrane configuration on fouling in anaerobic membrane bioreactors. J. Membr. Sci. 382, 41 - 49.
24. Monclús, H., Sipma, J., Ferrero, G., Comas, J., Rodriguez-Roda, I., 2010. Optimization of biological nutrient removal in a pilot plant UCT-MBR treating municipal wastewater during start-up. Desalination 250, 592 - 597.
25. Ng, K.K., Shi, X., Yao, Y., Ng, H.Y., 2014. Bio-entrapped membrane reactor and salt marsh sediment membrane bioreactor for the treatment of pharmaceutical wastewater: treatment performance and microbial communities. Bioresour. Technol. 171, 265 - 273.

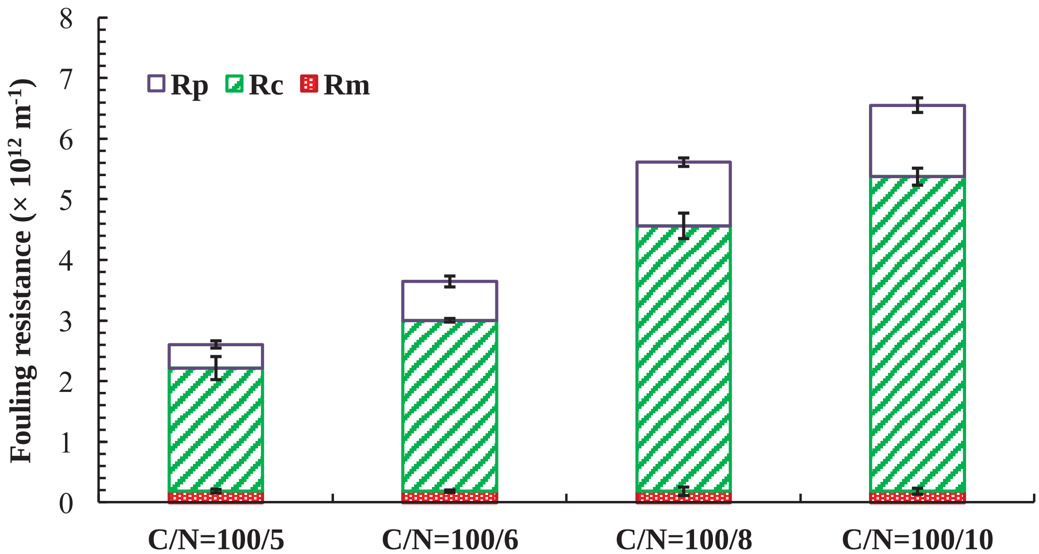
26. Nguyen, T.T., Ngo, H.H., Guo, W.S., Phuntsho, S., Li, J.X., 2011. A new sponge tray bioreactor in primary treated sewage effluent treatment. Bioresour. Technol. 102, 5444 - 5447.
27. Ozgun, H., Gimenez, J.B., Ersahin, M.E., Tao, Y., Spanjers, H., van Lier, J.B., 2015. Impact of membrane addition for effluent extraction on the performance and sludge characteristics of upflow anaerobic sludge blanket reactors treating municipal wastewater. J. Membr. Sci. 479, 95 - 104.
28. Smith, A.L., Stadler, L.B., Love, N.G., Skerlos, S.J., Raskin, L., 2012. Perspectives on anaerobic membrane bioreactor treatment of domestic wastewater: a critical review. Bioresour. Technol. 122, 149 - 159.
29. Yang, X.P., Wang, S.M., Zhou, L.X., 2012. Effect of carbon source, C/N ratio, nitrate and dissolved oxygen concentration on nitrite and ammonium production from denitrification process by *Pseudomonas stutzeri* D6. Bioresour. Technol. 104, 65 - 72.
30. Zhang, X., Yue, X., Liu, Z., Li, Q., Hua, X., 2015. Impacts of sludge retention time on sludge characteristics and membrane fouling in a submerged anaerobic–oxic membrane bioreactor. Appl. Microbiol. Biotechnol. 99, 4893 - 4903.

Figures captions

Fig. 1. Fouling resistance distribution under different C/N ratios.

Fig. 2. Compositions of EPS and SMP of cake layer in the hybrid system at different C/N ratios.

Fig. 3. Nature of foulant organics at different C/N ratios.



Fouling resistance (× 10^12 m^-1)
8
7
6
5
4
3
2
1
0
Rp
Rc
Rm
C/N=100/5
C/N=100/6
C/N=100/8
C/N=100/10

Fig. 1.

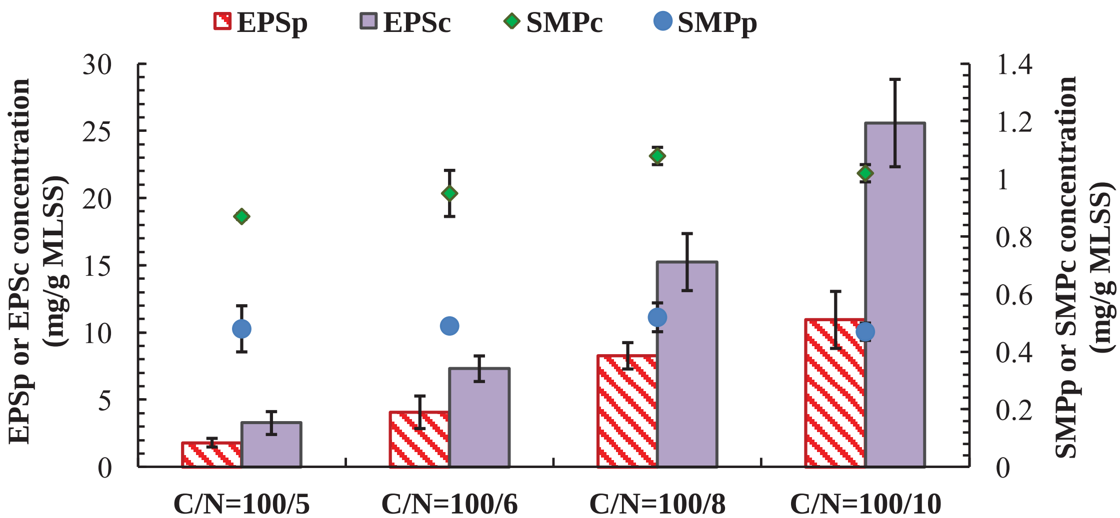

EPSp
EPSc
SMPc
SMPp
EPSp or EPSc concentration (mg/g MLSS)
SMPp or SMPc concentration (mg/g MLSS)
0
5
10
15
20
25
30
0
0.2
0.4
0.6
0.8
1
1.2
1.4
C/N=100/5
C/N=100/6
C/N=100/8
C/N=100/10

Fig. 2.

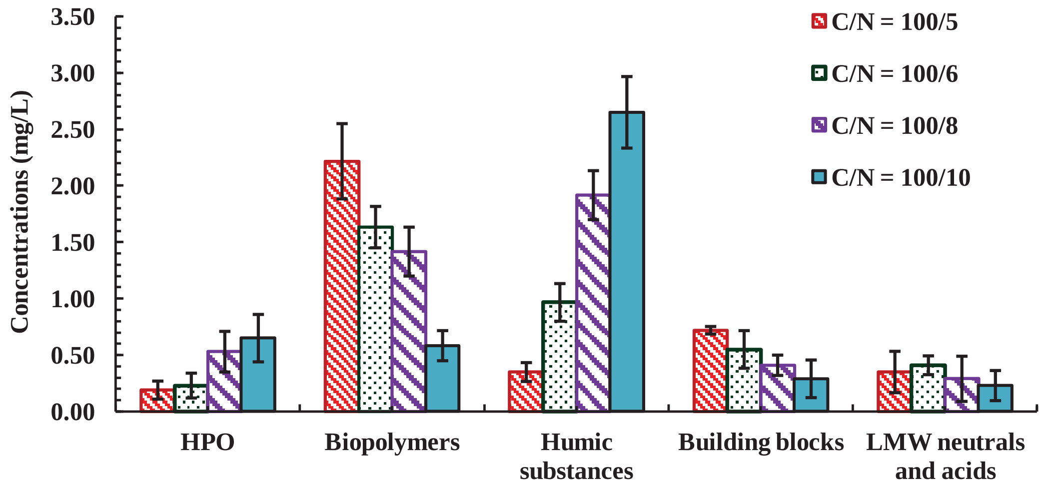

3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Concentrations (mg/L)
C/N = 100/5
C/N = 100/6
C/N = 100/8
C/N = 100/10
HPO
Biopolymers
Humic substances
Building blocks
LMW neutrals and acids

Fig. 3.

Table titles

Table 1. Treatment performance of the hybrid SAAMB-AnGMBR under various C/N ratios.

Table 2. Specific Nitrification rates and denitrification rates (SNR and SDR) of biomass under different C/N ratios.

Table 3. The concentration and properties of EPS and SMP in the mixed liquor of the SS-AnGMBR settling zone and anaerobic granular sludge.

Table 4. The summary of membrane fouling analysis results during system recovery after overloaded nitrogen event.

Table 1.

Treatment performance of the hybrid SAAMB-AnGMBR under various COD/N ratios.

Removal efficiency (%)	C/N = 100/5	C/N = 100/6	C/N = 100/8	C/N = 100/10
COD	95.5 ± 3.5%	95.2 ± 2.4%	95.0 ± 1.8%	94.8 ± 2.0%
DOC	95.3 ± 3.1%	95.1 ± 3.2%	94.8 ± 1.7%	94.5 ± 2.2%
NH_4-N	93.9 ± 4.5%	93.4 ± 2.0 %	93.2 ± 2.3%	92.5 ± 1.6%
TN	91.3 ± 4.7%	84.4 ± 3.1 %	68.9 ± 3.8%	44.1 ± 5.8%
PO_4-P	93.3± 5.5 %	88.6 ± 5.6 %	63.5 ± 5.7%	39.3 ± 7.2%

Table 2.

Specific Nitrification rates and denitrification rates (SNR and SDR) of biomass under different C/N ratios.

	SNR ($g\ NH_4$-N/g MLSS· d)	SDR ($g\ NO_3$-N/g MLSS· d)
C/N = 100/5	0.40 ± 0.23 [a]	0.44 ± 0.11 [b] 0.25 ± 0.02 [c] 0.22 ± 0.05 [d]
C/N = 100/6	0.45 ± 0.24 [a]	0.40 ± 0.10 [b] 0.23 ± 0.09 [c] 0.21 ± 0.03 [d]
C/N = 100/8	0.49 ± 0.16 [a]	0.31 ± 0.16 [b] 0.22 ± 0.13 [c] 0.18 ± 0.17 [d]
C/N = 100/10	0.52 ± 0.21 [a]	0.25 ± 0.13 [b] 0.21 ± 0.12 [c] 0.19 ± 0.09 [d]

[a] = SNR of aerobic attached biomass on sponge cube; [b] = SDR of anaerobic granular biomass; [c] = SDR of anaerobic attached biomass ; [d] = SDR of aerobic attached biomass on sponge cube.

Table 3.

The concentration and properties of EPS and SMP in the mixed liquor of the SS-AnGMBR settling zone and anaerobic granular sludge.

		C/N = 100/5	C/N = 100/6	C/N = 100/8	C/N = 100/10
Mixed liquor	EPS (mg/L)	11.33 ± 4.41	23.66 ± 6.04	37.40 ± 6.89	48.41 ± 7.13
	EPS_C/EPS_P	0.53 ± 0.03	0.46 ± 0.04	0.61 ± 0.04	0.54 ± 0.08
	SMP (mg/L)	8.08 ± 1.49	7.53 ± 1.78	6.85 ± 1.47	5.58 ± 1.07
	SMP_C/SMP_P	0.54 ± 0.04	0.79 ± 0.04	1.14 ± 0.28	1.44 ± 0.31
Granular sludge	EPS_P (mg/g VSS)	27.03 ± 5.13	13.30 ± 3.75	7.57 ± 2.12	4.92 ± 1.08
	EPS_C (mg/g VSS)	9.86 ± 2.36	5.15 ± 1.63	2.99 ± 1.02	2.18 ± 0.93
	SMP_P (mg/g VSS)	4.68 ± 1.23	9.58 ± 2.58	13.02 ± 2.88	14.21 ± 3.23
	SMP_C (mg/g VSS)	2.13 ± 0.85	1.95 ± 0.91	2.08 ± 0.95	2.12 ± 1.02

Table 4.

The summary of membrane fouling analysis results during system recovery after overloaded nitrogen event.

		C/N = 100/6	C/N = 100/5
Fouling rate	R (kPa/d)	0.69	0.47
Fouling resistance (m^{-1})	R_M (m^{-1})	$(1.93 \pm 0.08) \times 10^{11}$	$(1.88 \pm 0.06) \times 10^{11}$
	R_C (m^{-1})	$(2.97 \pm 0.11) \times 10^{12}$	$(2.18 \pm 0.20) \times 10^{12}$
	R_P (m^{-1})	$(7.31 \pm 0.22) \times 10^{11}$	$(4.32 \pm 0.35) \times 10^{11}$
	R_T (m^{-1})	$(3.89 \pm 0.21) \times 10^{12}$	$(2.80 \pm 0.30) \times 10^{12}$
SMP and EPS in the mixed liquor	EPS (mg/L)	28.38 ± 8.34	13.21 ± 6.83
	EPS_C/EPS_P	0.48 ± 0.05	0.51 ± 0.04
	SMP (mg/L)	6.24 ± 1.85	8.38 ± 1.08
	SMP_C/SMP_P	0.88 ± 0.11	0.59 ± 0.05
SMP and EPS in the cake layer	EPS_P (mg/g MLSS)	6.12 ± 0.89	2.35 ± 0.51
	EPS_C (mg/g MLSS)	9.83 ± 1.05	4.58 ± 0.91
	SMP_P (mg/g MLSS)	0.75 ± 0.12	0.49 ± 0.16
	SMP_C (mg/g MLSS)	0.98 ± 0.07	0.92 ± 0.11
Major foulant organics	Biopolymer (mg/L)	1.72 ± 0.34	2.33 ± 0.73

Highlights

- Effects of C/N ratio on SAAMB-AnGMBR performance were investigated.
- Lower C/N ratios negatively affected nutrient removal and granular properties.
- The SAAMB-AnGMBR exhibited lower fouling propensity at higher C/N ratios.
- EPS was the influencing factor to membrane fouling at varying C/N ratios.
- The SAAMB-AnGMBR performance could recover after increasing C/N ratio.

Graphical Abstract