Effect on composition and quantity of extracellular polymeric substances in an innovative hybrid membrane bioreactor under different sludge retention times

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ABSTRACT
This study investigated the influence of sludge retention time (SRT) in three hybrid membrane bioreactors to treat domestic wastewater at different SRTs (10, 20, and 40 d) simultaneously, based on extracellular polymeric substances (EPS) analysis. The results showed that significant positive correlation existed between SRT and humic acids quantity in TB-EPS, humic acids quantity in S-EPS, DNA quantity in S-EPS, and total quantity in TB-EPS, with the correlation coefficient 0.9992, 0.9980, 0.9973, 0.9821, respectively. Significant negative correlation existed in SRT and proteins in TB-EPS, proteins quantity in S-EPS, and polysaccharide in S-EPS, with the correlation coefficient 0.9993, 0.9107, 0.8552, respectively. A high logarithmic relationship existed between supernatant turbidity and SVI. The optimal SRT was 20 d and the corresponding operational cycle of the membrane module was 109 d.

1. Introduction
In recent years, membrane bioreactors have been widely employed for sewage and wastewater treatment. These reactors can also help to alleviate water shortages, which have become a global concern. Hybrid submerged membrane bioreactors (HMBRs) have many advantages such as bio-stability, a lower land footprint, and stability effluent quality despite high shock [1–5]. However, problems associated with membrane fouling pose obstacles to the large-scale application of HMBRs in wastewater treatment plants [6–8]. Further, the behavior of HMBR fouling is quite complex compared to that of most other membrane bioreactors because of the unique characteristics of activated sludge [9,10]. Several studies have focused on the membrane fouling process of HMBR. However, there is no clarity regarding its mechanism. Previous studies recognized extracellular polymeric substances (EPS) as the main material leading to membrane fouling, and HMBR, which is composed of a conventional MBR (CMBR) and biofilm technology, exhibits good membrane fouling resistance [7,10–12]. EPS are typically formed by the metabolism and cellular digestion of microorganisms commonly found in the internal and on the external surfaces of activated sludge flocs. The term EPS is typically used as a generic terminology for different types of high molecular substances. The relative molecular mass of EPS is often in excess of 10,000 Dalton. The main components of EPS are polysaccharides, proteins, glucuronic acid, humic acids, nucleic acids, lipids, and so on. Further, the formation and degradation of EPS are closely related to the external environment and the survival state of the microbes. EPS are generally categorized as soluble EPS (S-EPS) and bound EPS (B-EPS), with the latter being subdivided into loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS).

Some scholars have studied the components of EPS using three-dimensional fluorescence excitation-emission spectrometry (EEM) [13–16]. Most studies have investigated its proteins and carbohydrates, but few have analyzed the remaining components of EPS. Chabalina and Pastor [17] suggested that the major membrane fouling components in B-EPS are proteins. Janus and Paul [18] stated that the major membrane fouling components in S-EPS are proteins. Several researchers have analyzed the components of EPS under different working conditions, and they have also compared different EPS extraction methods [19–27]. Operating parameters, membrane characteristics, activated sludge, and dissolved organic matter (DOM) characteristics have been implicated in the occurrence of membrane fouling [20,21,25,28,29]. Among the factors that influence membrane fouling, solid retention time (SRT) is the most influential operating parameter [10,30] as it can change DOM characteristics inbound EPS, soluble microbial products (S-EPS), sludge supernatants, and the characteristics of effluent discharged by membrane bioreactors (MBRs). Note that the above research results are based on artificial wastewater rather than real wastewater. Thus, reports focusing on real municipal and synthetic sewage...
are lacking. Therefore, the theoretical understanding for optimum operations of wastewater treatment plants (WWTPs) appears to be lacking with regard to some factors such as seasonal changes and fluctuations in raw wastewater quality [10,31,32].

In this research, the effect of SRT in HMBRs is studied from the perspective of EPS, as this material is known to cause membrane fouling. Section 2 provides information required to repeat the experiments and validate their results, including how the raw feed wastewater was obtained and details on the equipment setup and sample analysis. Section 3 discusses the influence of SRT in three hybrid membrane bioreactors based on the analysis of the EPS. The paper also discusses the development of supernatant turbidity and sludge volume index (SVI).

2. Materials and methods

2.1 Raw feed wastewater

Wastewater samples obtained from the primary settling tank at the municipal WWTP in Xuzhou, Jiangsu Province (China) were used in the investigation. The wastewater samples are representative of typical urban mixed sewage. The characteristics of the raw feed wastewater appear in Table 1.

2.2 Experimental setup

Three identical HMBRs (Figure 1) with an effective volume of 100 L were operated for different SRTs (10, 20, and 40 d) for a period of 12 months under the same hydraulic retention time (HRT) of 10 h. The HMBRs used in this study consisted of a 1-m³ reactor membrane bio-reactor tank with a submerged enhanced polyvinylidene fluoride (PVDF) membrane module. The area was 10 m² and the effective pore size was 0.1 μm. With an attached feed reservoir, up to 100 L of water could be filtered per hour for the ultrafiltration experiments. Air bubbles were injected from the bottom of the membrane tank at a predetermined flow rate of 2 m³·m⁻²·h⁻¹ to scour the membranes and mix the contents of the reactor, and to provide oxygen for biomass suspension. A peristaltic pump at a constant flux of 2.5 L·m⁻²·h⁻¹ was used to remove the permeate. Pressure transducers with online data acquisition were used to monitor the transmembrane pressure (TMP) every 5 min. Other operational conditions of the HMBRs are listed in Table 2.

2.3 Sample analysis

Mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), chemical oxygen demand (COD₅), total nitrogen (T-N), and total phosphorus (T-P) were measured according to standard methods [33,34]. The methods suggested by previous studies [35–37] for extracting and analyzing S-EPS, LB-EPS, and TB-EPS from the mixed liquor and biofilm were also used in this study. These methods are described below.

2.3.1 Extraction of EPS from the mixed liquor

The S-EPS are obtained by directly filtering 5 mL of mixed liquor through a 0.22-mm-pore size filter. High-speed centrifugation was used to extract LB-EPS from the mixed liquor. First, 5 mL of the mixture was taken in
Table 2. Operational conditions of HMBR.

| Item                        | Unit  | Working volume 1.0 m³ |
|-----------------------------|-------|-----------------------|
| Biological padding volume fraction | 50.0% |
| Hydraulic retention time (HRT) | 10 h  |
| Solid retention time (SRT)   | 10, 20, 40 d |
| Initial SRT                 | 10    |
| Membrane area               | 11.0 m² |
| Membrane flux               | 1.00 L m⁻¹ h⁻¹ |
| pH                          | 7.5   |
| Dissolved oxygen            | 1.0 mg·L⁻¹ |
| Temperature                 | 20.0°C|

The general performance of the HMBRs over 12 months of operation under different SRTs (R1: 10 d, R2: 20 d, and R3: 40 d) is shown in Table 3, in terms of MLSS, MLVSS, COD, T-N, and T-P.

Table 3 indicates that the HMBRs could effectively remove organic substances, with an average COD removal efficiency of up to 91%. Further, the COD removal efficiency increased as SRT increased. The HMBRs were operated under oxic conditions, resulting in nitrification. They removed nutrients effectively, with average T-N and T-P removals at 62.0% and 96%, respectively, which did not increase with an increase in SRT. Higher T-P removals were achieved for R2 (96.4%), followed by R1 (96%) and R3 (95.8%), and the T-P removal was correlated with the amount of excess sludge under different SRTs. Biological denitrification and biological phosphorus removal require two processes: nitrification under aerobic conditions and denitrification under anoxic conditions, and the release of phosphorus under anaerobic conditions and the phosphorus uptake reaction under aerobic conditions, respectively. The traditional theory of biological nitrogen and phosphorus removal considers that different reactions must occur in different structures owing to the different conditions required for the reactions [38,39]. The results showed that both the anoxic and anaerobic zones may exist in the interior of the activated sludge, with a certain biofilm thickness, under low dissolved oxygen (DO) concentration, and this can be attributed to influence of the oxygen mass transfer resistance [40]. Thus, the anoxic condition (to drive the denitrification reaction) as well as the anaerobic condition (to drive the phosphorus release reaction) co-exist. DO was controlled at about 1 mg·L⁻¹ in this experiment, and the reactors clearly influence both nitrogen and phosphorus removals.

MLSS and MLVSS concentrations increased with the extension of the SRT from 10 d (R1) to 40 d (R3). The ratio of MLVSS/MLSS decreased with increasing SRTs, ranging from 0.7 to 0.5 as the SRT increased from 10 d to 40 d.

3. Results and discussion

3.1. MBR performance

The concentration of EPS is usually expressed as milligram per unit mass VSS, and are characterized by total organic carbon (TOC) content. The components of EPS include polysaccharides, proteins, humic acids, DNA, and so on. Sugar was detected by anthrone colorimetry, using glucose as the standard reference. Protein and humic acid concentration were determined by the improved Lowry method, using bovine serum albumin as the standard reference. The detection method for the S-EPS is the same as that for the mixed liquid.

Table 3. Performance of the HMBRs under different SRTs.

| Item   | Unit     | Influent                  | R1 (SRT: 10 d)        | R2 (SRT: 20 d)        | R3 (SRT: 40 d)        |
|--------|----------|---------------------------|-----------------------|-----------------------|-----------------------|
| MLSS   | mg·L⁻¹   | –                         | 4100.0 ± 30.0         | 5300.0 ± 150.0        | 71000.0 ± 300.0       |
| MLVSS  | mg·L⁻¹   | –                         | 2950.0 ± 20.0         | 32000.0 ± 70.0        | 380000.0 ± 260.0      |
| MLVSS/MLSS | –     |                           | 0.7                   | 0.7                   | 0.5                   |
| COD    | mg·L⁻¹   | 120.0–180.0               | 180.0 ± 2.0           | 160.0 ± 1.0           | 150.0 ± 2.0           |
| TN     | mg·L⁻¹   | 25.0 ± 5                  | 100.0 ± 1.0           | 91.0 ± 1.0            | 90.0 ± 1.0            |
| TP     | mg·L⁻¹   | 5.0 ± 1.0                 | 0.2 ± 0.05            | 0.18 ± 0.05           | 0.21 ± 0.05           |
| LB EPS | mg·g⁻¹ VSS | –                        | 488.5 ± 6.3           | 223.3 ± 4.0           | 300 ± 1.0             |
| TB EPS | mg·g⁻¹ VSS | –                        | 65.9 ± 7.2            | 78.1 ± 5.2            | 87.8 ± 9.5            |
| S-EPS  | mg·L⁻¹   | –                         | 37.0 ± 0.5            | 20.1 ± 0.5            | 18.5 ± 0.5            |
from 0.72 (R1) to 0.54 (R3) in the HMBRs. The lowest MLVSS/MLSS ratio was observed in R3, indicating the low viability of biomass from bio-decay effects and/or the accumulation of inert substances in the HMBRs after a long SRT.

The variation of S-EPS with SRT was the same as that of COD. When SRT was 10 d, the average concentration of S-EPS was 37.0 mg·g$^{-1}$. The average concentration of S-EPS decreased gradually with an increase in SRT, and the average concentration of S-EPS decreased to 18.0 mg·g$^{-1}$ when the SRT was 40 d.

### 3.2. Effect of SRT on the total quantity and components of EPS

Ali [41] found that a low food-to-microorganisms (F/M) ratio (substrate-limiting conditions) and long SRT influence the microbial activity of biomass in MBRs; they suggested that the microbial activity of the biomass increased as SRT decreased. Figures 2–4 show the average concentrations of bound EPS (mg TOC·g$^{-1}$ VSS) obtained from R1, R2, and R3. It can be seen that LB-EPS concentrations decreased with the extension of SRT from 10 d (R1) to 20 d (R2). However, they remained stable as SRT was prolonged. On the other hand, TB-EPS concentrations increased with the extension of SRT. The average concentrations of S-EPS in the sludge supernatants were 37.1, 19.59, and 19.44 mg·L$^{-1}$ for R1, R2, and R3, respectively. Both S-EPS and LB-EPS increased with decreasing SRT, indicating that more utilization-associated products may be present in S-EPS under short SRTs (high F/M ratio).

In LB-EPS, proteins and polysaccharose decreased rapidly with an increase in SRT and stabilized thereafter; the concentrations of humic acid and DNA first reduced and then increased as SRT increased. With regard to TB-EPS, a continued decline was noticed for proteins. Otherwise, both polysaccharose and humic acids showed a continued rise as SRT increased. Humic acids and DNA ranged widely in S-EPS compared to LB-EPS and LB-EPS.

Figure 3 showed that no significant correlation was found between SRT and humic acids quantity in LB-EPS, DNA quantity in TB-EPS (Table 5). Significant positive correlation existed successively between humic acids quantity in TB-EPS (Table 5, 0.9992), SRT and humic..
significantly negative correlation existed, such as proteins quantity in TB-EPS (Table 5, 0.9993), proteins quantity in S-EPS (Table 6, 0.9107), and polysaccharose in S-EPS (Table 6, 0.8552). Among these components, humic acids affect the flocculation capacity of the sludge, which is not conducive to the granulation of the sludge. Because they are mainly aliphatic and aromatic high polymers and their hydrogen ions can be dissociated and exchanged with cations in solution as they contain functional groups such as carboxyl groups and phenolic groups. Therefore, the content of proteins and humic acids should be controlled by adjusting the SRT to improve sludge flocculation capacity. This implied that the proteins and humic acids could be employed as a universal indicator of membrane fouling potential for HMNRs at different SRTs. This is an important finding that has not been reported before.

3.3. Development of trans-membrane pressure

The membrane hydraulic resistance was calculated via online data acquisition every 5 min. Changes in the TMP of R1, R2, and R3 are shown in Figure 5, and the increasing rate of TMP per unit time indicates a high fouling rate in HMBRs. Liu et al. [7] found that HMBRs show better membrane fouling control than CMBRs. When the transmembrane pressure reached 20 kPa, the HMBRs were operated for 143 d while the CMBRs were operated for only 57 d [38]. Figure 5 also shows that the relationship between the rate of increase and SRT is not linear; the point of inflection occurs when SRT is 40 d. Reactor dynamics under an SRT of 30 d should be studied to determine the inflection point accurately. This inflection point exists for LB-EPS and S-EPS too. Kornboonraksa and Lee [42] reported MLSS to be the dominant factor in influencing membrane filtration ability (with a negative correlation) for piggery wastewater treatment, while sludge viscosity, bound EPS, and S-EPS were considered as sub-factors for membrane fouling. However, researchers are still debating the correlation between MLSS concentration and membrane performance in MBR systems [43]. In this study, LB-EPS and S-EPS were considered as major factors in membrane fouling, and lower MLSS concentration leads to better MBR operation, resulting in fewer fouling problems. The operation time was prolonged as SRT changed from 10 d to 20 d, but thereafter, it decreased rapidly when SRT increased.
Figure 5. TMP variations over time for R1, R2, and R3.

Figure 6. Correlations of supernatant turbidity and SVI under different SRTs.

Figure 7. Correlations of (a) SRT and supernatant turbidity, (b) SRT and SVI.
### 3.4 Development of supernatant turbidity and SVI

The SVI (units: mL·g⁻¹) is defined as the ratio of the volume to dry weight of the sludge mixture after sedimentation for 30 min in a 1-L measuring cylinder. SVI is an important index as it serves as an indicator of various problems. A low SVI value indicates that the particle size is small and dense, but the proportion of inorganics is large. A high value indicates that the sludge settling performance is not good, and sludge bulking will occur or may have already occurred. The SVI of treated municipal sewage typically ranges from 50 to 150 mL·g⁻¹, and 150 mL·g⁻¹ is often regarded as the limit for sludge expansion.

Table 7 and Figure 6 showed that SVI presented the same change tendency as supernatant turbidity and a high logarithmic relationship existed between these two parameters. Otherwise, different correlations were found between these two parameters and SRTs, a linear relationship between SVI and SRT, and logarithmic relationship between supernatant turbidity and SRT (as shown in Figure 7, Table 8). These two aspects indicated that the best operating condition appeared under an SRT of 20 d, and the quality of supernatant deteriorated when SRT was prolonged, as SVI exceeded 150 mL·g⁻¹. This indicated that supernatant turbidity could be employed as a substitute indicator of SVI to investigate the sedimentation performance of sludge.

### 4. Conclusions

Three identical HMBRs treated with domestic wastewater under different SRTs (10, 20 and 40 d) were studied using EPS analysis. The results showed that HMBRs can remove organic substances effectively, with an average COD removal efficiency of up to 91%, TN removal of 62.0%, and TP removal of 96%. The COD removal efficiency increased as SRT increased, whereas TN and TP presented different trends. The HMBRs can provide anoxic and anaerobic conditions to improve the denitrification reaction and phosphorus release reaction, respectively. Based on observations of the TMP over 12 months, membrane fouling worsened as SRT was prolonged beyond 20 d, suggesting that an optimized SRT, of between 20 d and 40 d, likely exists. When the SRT was prolonged from 10 d to 20 d, the operational period of the membrane module increased by 22.1%, and thereafter, the operational period declined rapidly. Consequently, the optimal SRT is 20 d and the corresponding operational cycle of the membrane module is 109 d. The MLVSS/MLSS ratio decreased with increasing SRTs, indicating that the low viability of biomass from bio-decay effects and/or inert substances accumulated in the HMBRs after long SRTs. Analysis of EPS components showed that different trends appeared with the change of SRT. Significant positive correlation existed between humic acids quantity in TB-EPS, SRT and humic acids quantity in S-EPS, DNA quantity in S-EPS, and total quantity in TE-EPS. Significant negative correlation existed in TB-EPS, proteins quantity in S-EPS, and polysaccharose in S-EPS. The decrease in LB-EPS could improve sludge flocculation and sedimentation, resulting in an increase in operating time. A high logarithmic relationship existed between supernatant turbidity and SVI, indicating that supernatant turbidity could be employed as a substitute indicator of SVI to investigate the sedimentation performance of sludge.

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### Disclosure statement

No potential conflict of interest was reported by the authors.

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