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Effects of micro-organism growth phase on the accumulation and characteristics of soluble microbial products in MBR

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ABSTRACT

Soluble microbial products (SMP) have been demonstrated as a primary obstacle for the widespread application of membrane bioreactor (MBR). This study has investigated the effects of micro-organism growth phase (MGPs) on the accumulation and characteristics of SMP in MBR. The results showed that there were three MGPs in the experiment, including exponential growth phase (EGP), deceleration growth phase (DGP), and stationary growth phase (SGP), respectively. Polysaccharides and proteins in SMP were steadily increased in the EGP. While in the DGP, the SMP were increased firstly and then decreased sharply. Finally, the concentrations of SMP maintained at a low steady level in the SGP. Furthermore, the biomass associated products (BAP) batch experiment showed that the production potential of SMP was the largest in the DGP. The percentage of SMP (>30 kDa) was remarkably increased, then dropped due to abruptly decreasing production potential of BAP and degradation by acclimated micro-organisms. The results analyzed by excitation-emission matrix fluorescence spectroscopy indicated that the variation of protein-like substances showed a similar trend with the total SMP, while the humic acid-like substances were related with micro-organism slightly. Our results demonstrated that different MGPs played a significant role in the quantity and quality of SMP in MBR.

Keywords: Soluble microbial products; Membrane bioreactor; Micro-organism growth phase; Excitation-emission matrix fluorescence

1. Introduction

Soluble microbial products (SMP) are mainly composed of a complex mixture such as proteins, polysaccharides, and humic substances [1]. Based on biomass phase, they can be further classified into utilization associated products (UAP) and biomass

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associated products (BAP) [2]. It has been reported that SMP, as major organic foulants to the commonly used microfiltration or ultrafiltration membranes in membrane bioreactors (MBRs), contribute 17–81% of membrane fouling depending on the experimental conditions [3,4].

Considering the significance of SMP in MBR, numerous research efforts have been made recently. Process parameters, such as hydraulic retention

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time (HRT), sludge retention time (SRT), and organic loading rate (OLR), are identified to affect the production and elimination of SMP in MBR. Johir et al. [5] reported that the hydrophobic and hydrophilic compounds in SMP were increased with higher OLR. Shan et al. reported that longer HRT could result in higher SMP production [6]. Chen et al. found that the concentration of SMP in the supernatant firstly decreased when the SRT was prolonged from 10 to 30 d, and then showed a little increase as the SRT was further extended to 50 d [7]. In fact, the changes of operating conditions would disturb and alter the metabolism and physiological stages of micro-organism, which play an important role in the production and degradation of SMP. However, the mechanisms underlying production and elimination of SMP in the sludge supernatant of MBR have not been understood well, and especially little attention has been paid to the role of different micro-organism growth phases (MGPs) in this process. Thus, the relationship between the MGPs and the production of SMP should be studied thoroughly.

Previous studies regarding SMP formation by micro-organism in different MGPs were based on short-term batch tests and pure bacteria cultures. For example, Kevin et al. [8] reported that the protein and carbohydrate content in SMP increased as the cells progressed from the exponential to stationary phase. Nevertheless, most of the MBRs are usually operated with continuous feeding mode and mixed bacteria populations. Gao et al. [9] pointed out that the growth regulation of mixed bacteria populations as a whole had similarities with that in pure bacteria culture, and the control of different MGPs of mixed bacteria populations played a crucial role in the operation of sewage treatment system. However, there is little investigation that elucidates how SMP are produced during different MGPs in open systems such as MBRs, and that is of high significance for understanding the mechanisms of SMP formation in such continuously feed processes.

Therefore, the main objective of this study was to investigate the influence of MGPs on the accumulation and characteristics of SMP during the long-term operation of an MBR. The concentration and characteristic of molecular weight distribution of SMP in the sludge supernatant were analyzed. The chemical characteristic of SMP was also analyzed using excitation-emission matrix (EEM) fluorescence spectroscopy. The production potential of BAP was investigated with a series batch experiments. The results of this study will reveal the formation mechanism of SMP and supply a support to control membrane fouling in the startup of MBRs.

2. Materials and methods

2.1. MBR setup

A bench-scale MBR with a working volume of 13.5 L was used in this study, in which a hollow-fiber membrane module (PVDF, $0.03 \mu m$, Tengxiang Corp., Hangzhou, China) with an effective area of 0.2 m^2 was installed. A balance box with a float-ball valve was used to control water level in the reactor. The transmembrane pressure was recorded by a paperless recorder to measure the membrane fouling rate (MFR).

The membrane module was constantly sucked with a dump pump. As the transmembrane pressure reached 30 kPa, the membrane module was extracted and washed with high-pressure water repeatedly, then soaked in 0.06% (w/v) NaClO solution for about 8 h. Clean water flux was measured to confirm the extent of membrane cleaning. Dissolved oxygen was maintained at 2.5-3.0 mg/L. HRT and SRT were set at 5.4 h and 26 d. The sludge corrected from Xianlin Wastewater Treatment Plant (Nanjing, China) was acclimatized for one month before taken to the reactor and initial volatile suspended solid (VSS) concentration was 2,500 mg/L. A complex synthetic wastewater simulating municipal wastewater contained glucose (300 mg/L), peptone (50 mg/L), NH₄Cl (114.6 mg/L), and KH₂PO₄ (12 mg/L) as primary nutrients and required trace metals. Na2CO3 was added to adjust the pH between 7.0 and 7.5.

2.2. Analytical methods

EPS and SMP were extracted as the following heat extraction method [10]. Protein (Pn) was analyzed using the Lowry method [11]. Polysaccharide (Ps) analysis was conducted using the modified Anthrone method [12]. The VSS in sludge pellets were analyzed according to standard methods [13] to calculate the concentration of EPS (mg/g VSS). The molecular weight distribution of SMP were determined by ultrafiltration fractionation method with a series ultrafiltration membranes of different molecular size (PVDF, 100, 30, 10, and 3 kDa) reported by Liang et al. [14]. A fluorescence spectrophotometer equipped with a xenon lamp (F-7000, Hitachi, Japan) was used for the measurement of EEM spectra of SMP. The fluorescence regional integration (FRI) method was employed to analyze the EEM spectra semi-quantitatively [15].

2.3. BAP batch experiment

The sludge in different MGPs may have different production potential of SMP. In this study, the

production potential of SMP was investigated by BAP generation. The UAP were not considered because the production potential of UAP was always stable in a reactor performed in a continuous flow mode and the UAP were small molecular substances that discharged easily in MBR [16]. As a result, a series batch experiments were conducted to investigate the BAP production potential of the sludge on1, 15, 23, 29, 35, 40, and 46 d. For each batch experiment, a total of 750 mL mixed suspension sludge was extracted from the reactor and washed with deionized water for three times. Then the sludge was diluted to 1.5 L with deionized water and cultivated without any substrate added. The concentrations of proteins and polysaccharides were detected at 0 h, 12 h, 24 h, 36 h, 48 h, 60 h, and 72 h, respectively. Meanwhile, the corresponding VSS was also measured. The experiments were conducted in triplicate to ensure reproducibility. The production potential of BAP was calculated as Eq. (1) and Eq. (2):

$$BAP Pn_t = (Pn_t - Pn_0)/VSS$$
(1)

$$BAPPs_t = (Ps_t - Ps_0)/VSS$$
(2)

where $BAPPn_t/Ps_t$ represent the Pn/Ps content of BAP produced by per gram sludge at time of *t* (mg/g VSS). Pn_t/Ps_t and Pn_0/Ps_0 are the Pn/Ps concentrations in the supernatant at *t* and 0 h, respectively (mg/L). VSS represents the volatile mixed liquid suspended solids (g/L).

2.4. Membrane fouling rate

The MFR is defined as mean value of transmembrane pressure (TMP) during the operation time and calculated as Eq. (3) [17]:

$$MFR = (TMP_f - TMP_i)/T$$
(3)

where TMP_{f} is the final transmembrane pressure and TMP_{i} is the initial transmembrane pressure. *T* is the period from TMP_{i} to TMP_{f} .

3. Results and discussion

3.1. Effects of MGPs on the accumulation of SMP

The removal rate of COD was 92% and NH_3-N was 85% after the ninth. As showed in Fig. 1, the variation of VSS displayed a clear curve of microorganism growth related to three typical MGPs: (1) exponential growth phase (EGP, 0–15 d), (2) deceleration growth phase (DGP, 15–40 d), and (3) stationary



Fig. 1. Evolution of SMP and VSS over operating time (HRT = 5.4 h, SRT = 26 d, volume of the reactor = 13.5 L).

growth phase (SGP, 40-60 d). As the sludge had been acclimated for over one month, no lag growth phase was found in the curve. At the beginning of the MBR operation, the bioreactor had a lower VSS concentration of 2,500 mg/L. As such, the micro-organisms involved in the bioreactor were exposed to a higher OLR, leading to a higher micro-organism growth rate in the initial operating time. However, the microorganisms were gradually subject to an increase of substrate limitation because of the increasing sludge concentration, which subsequently resulted in the slowdown of micro-organism growth rate (i.e. DGP) and an SGP was finally reached (i.e. the micro-organism growth was balanced with sludge decay and sludge discharge). Interestingly, it was found that the concentrations of polysaccharides and proteins were increased steadily in the EGP, however, which were increased sharply (from 15 to 21 d), and then decreased continuously (22-40 d) in the DGP. Furthermore, the concentrations of proteins were higher than polysaccharides, especially during 15-30 d. Finally, the concentrations of polysaccharides and proteins were both maintained at a low level in the SGP. Similarly, Gómez et al. [18] found that the evolution of several SMP components showed a same trend during 200 days long operation in a full-scale membrane reactor plant except that the carbohydrates concentration was higher than the proteins due to different influent and operation conditions. In addition, it is well known that the BAP are hydrolyzed by EPS, and the rate of BAP formation is proportional to EPS concentration [19,20]. The EPS concentration in DGP reached the highest level (see Fig. 2), thus it could make contribution to the high concentration of SMP. These results demonstrated that different MGPs played a significant influence on the evolution of proteins and polysaccharides in SMP.



Fig. 2. Evolution of EPS over operating time (HRT = 5.4 h, SRT = 26 d, volume of the reactor = 13.5 L).

Moreover, the average MFR in different MGPs (EGP, DGP, and SGP) was 0.74 ± 0.08 , 1.25 ± 0.12 , and 0.86 ± 0.06 k Pa/h, respectively. As a result, the highest level of MFR was observed in DGP, in which the concentration of SMP was also at the highest level. However, it should be noted that the MFR in EGP was lower than that in SGP even the concentration of SMP was higher. The possible reason is that the concentration of SMP is not the only factor affecting membrane fouling. The characteristics of SMP might be also a potential fouling factor. For example, the SMP with lower concentration but higher size could present a higher fouling potential [21]. Therefore, the size nature of SMP in supernatant in different stages will be further discussed.

3.2. Effects of MGPs on the molecular weight distribution of SMP

As reported in the literature, the molecular weight distribution of SMP is directly related to membrane fouling and is wide of concern to reveal the mechanism of the SMP production and degradation in MBR [1,14]. As showed in Fig. 3, the SMP had a broad spectrum of molecular weight and a bimodal pattern which is consistent with previous studies [14,16]. On first day, the majority of SMP had molecular weight smaller than 3 kDa accounting for around 57.2%, whereas the components with high molecular weight (>30 kDa) only constituted 19% of SMP. Interestingly, the percentage of SMP with small molecular weight decreased slightly and the large SMP (>30 kDa) increased steadily in the EGP. In the DGP, the percentage of large SMP firstly increased, subsequently reached the highest level on 35th day, and finally



Fig. 3. The molecular weight distribution of SMP in MBR (HRT = 5.4 h, SRT = 26 d, volume of the reactor = 13.5 L).

declined to 38% on 40th day. It has been reported that the UAP were small molecular weight materials and easily to be reutilized by micro-organisms [22]. While, the BAP generated in the microbial growth phase and endogenous phase were mainly composed with high molecular weight and slowly degradable materials [23]. Furthermore, it was reported that the proportion of BAP production increased with ascending sludge concentrations when the MBR operated at fixed HRT [24]. Thus, the refractory compounds with high molecular weight increased continuously in the EGP. Especially during 15–29 d in the DGP that presented higher SMP production rate, the large molecular weight components composed the majority of SMP.

However, the percentage of large molecular weight compounds decreased in the SGP. Correspondingly, the small molecular weight compounds progressively became the majority of SMP. In a relatively long SRT, the accumulated SMP that were mainly composed of BAP could be degraded into low molecular weight compounds by acclimated micro-organisms [25] and the readily degradable compounds could be quickly consumed [23]. This might be one reason to the decrease of total SMP in the supernatant of MBR after a long-term operation. It is obvious that the BAP played a significant role in the evolution of molecular weight of SMP in the MBR process. Next, we will analyze the BAP production rate in different MGPs to further understand the influence of MGPs on the size of SMP.

3.3. Effect of MGPs on the chemical characteristics of SMP

Fig. 4 illustrates four typical EEM spectra of the SMP samples on 1, 8, 27 and 51 d, respectively. A total



Fig. 4. The EEM results of SMP on 1, 8, 27, and 51 d (HRT = 5.4 h, SRT = 26 d, volume of the reactor = 13.5 L).

of four distinct peaks could be identified from the spectra. The first main peak was located at the excitation/emission wavelengths (Ex/Em) of 220–240/330–355 nm (peak A), while the second distinct peak was observed at 265–280/320–340 nm (peak B). These two peaks were associated with the aromatic protein-like substances and tryptophan-like substances, respectively [10]. The other two typical peaks were located at 350–370/425–440 nm (peak C) and 240–265/430–445 nm (peak D), separately. Similar peak locations were reported for natural organic matter determination and regarded as humic acid-like substances and fulvic acid-like materials [15,26].

The fluorescence peak locations did not change much during the whole process, but the fluorescence peak intensities changed substantially. The EEM results revealed that the intensity of protein-like peaks (peak A and peak B) showed a more prominent variation than peak C and peak D during the operating process which indicated that proteins could be the main content of SMP. In detail, the protein peaks of spectra on 8 d showed a stronger intensity compared with the spectra on 1 d. Particularly on 27 d, the protein-like peaks presented a significant summit. Subsequently, the intensity of protein-like peaks came back to a similar level as begin on 51 d. Clearly, the changes of protein-like peaks were consistent with the evolution of proteins concentration in the supernatant. On the contrary, the intensities of peak C and peak D changed slightly and it revealed that the relative concentrations of humic acid-like substances maintained at a steady level during the process. As a result, humic acid-like substances and fulvic acid-like materials showed a loose relationship with the MGPs. It was worth noting that humic acid and fulvic acid were components with small molecular weight, thus they were prone to pass the membrane and presented in the membrane permeate [22]. Moreover, The FRI distribution (see Fig. 5) of protein-like substances increased steadily from 30 to about 60% during EGP and reached the most of 88% in DGP. While in SGP, the FRI distribution of peak C and peak D constituted the majority of the spectra which indicated that the humic acid-like substances and fulvic acid-like substances became the major components of SMP. In general, the EEM results combined with the FRI distribution showed that MGPs played a remarkable role in the chemical characteristics of SMP during overall MBR operation.

3.4. BAP batch experiment

Fig. 6 shows the evolution of production rates of proteins and polysaccharides consisted of the major compositions in SMP by sludge during the MBR operating time. For the batch experiment performed on 1 d, the production potential of polysaccharides increased at the beginning, subsequently decreased from 48 h. Similarly, the proteins declined from 36 h. The results suggested that the micro-organisms possessed a higher degradation rate than the production rate for polysaccharides and proteins. It was also found that both polysaccharides and proteins had high production rates on 23 and 29 d. Therefore, it comes as no surprise that there were high level of SMP accumulated in the MBR in the DGP. A clear trend can also be observed that the production rates of proteins and polysaccharides in the batch study decreased rapidly as soon as the DGP was reached. Generally, the evolution trend of Fig. 6 is similar with



Fig. 5. The FRI distribution of SMP in the MBR (HRT = 5.4 h, SRT = 26 d, volume of the reactor = 13.5 L).



Fig. 6. BAP production of the sludge taken from different operating time in the MBR (HRT = 5.4 h, SRT = 26 d, volume of the reactor = 13.5 L, number of samples = 3).

that of SMP in Fig. 1. Thus, BAP production potential was one of the main reasons leading to SMP accumulation in MBR. In addition, the sufficient EPS in the DGP (see Fig. 2) could hydrolyze to more BAP due to micro-organism metabolism that has been reported to be a major source for BAP [19,20]. Moreover, some SMP could be utilized as substrate by micro-organism in starvation condition [27]. Possibly, due to the simultaneous but varied production and elimination rates of polysaccharides and proteins, the BAP concentrations of each batch experiment fluctuated. For instance, the production rate of proteins on 15th day increased steadily initially (i.e. production rate was larger than elimination rate) but declined quickly after 36 h of cultivation (i.e. elimination rate was larger than production rate). It deserved to pay a special attention that the production rate of polysaccharides decreased faster than the proteins which might supply an interpretation to the high level of proteins accumulated in the MBR. Generally, the BAP study could explain the importance of micro-organism growth on SMP accumulation in the MBR. In brief, the results showed that the sludge under the EGP and DGP would have a high potential to produce SMP.

4. Conclusions

This study investigated the influences of MGPs on the accumulation and characteristics of SMP during the long-term operation of MBR. The SMP concentrations were at the highest level in the DGP and then reached a low level in the SGP. Based on the BAP experiments, the production potential of SMP could reach the highest level in the DGP due to the abruptly limited substrate and higher microbial metabolism secondary activities. The components with large molecular weight in SMP increased in both EGP and DGP, then decreased in SGP on the contrary for the reason that micro-organisms might have acclimated for utilizing the accumulated SMP with high molecular weight after a long operating period. The protein-like peaks in SMP-EEM spectra showed a tighter relation with MGPs than the humic acid-like and fulvic acid-like peaks. In conclusion, the MGPs played a significant role in the quantity and quality of SMP in MBR. Additionally, more detailed characterization of the chemical and structural composition of the SMP, especially BAP, produced by micro-organisms under different MGPs is needed in the future.

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Nomenclature

BAP		biomass associated products
DGP	_	deceleration growth phase
EEM	_	excitation-emission matrix
EGP		exponential growth phase
EPS	_	extracellular polymeric substance
FRI	_	fluorescence regional integration
HRT	_	hydraulic retention time
MBR		membrane bioreactor
MFR	_	membrane fouling rate
		e e

MGP	_	micro-organism growth phase
OLR	_	organic loading rate
PVDF	_	polyvinylidene fluoride
SGP	_	stationary growth phase
SMP	_	soluble microbial products
SRT	_	sludge retention time
TMP	_	transmembrane pressure
UAP	_	utilization associated products
VSS	_	volatile suspended solid

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