The model binary/ternary mixtures for actual EPS solution extracted from the activated sludge in MBR using dead-end membrane filtration cell

Shuang Zhao, Longyue Shi, Yu Ma and Zhan Wang

ABSTRACT

In order to find a model solution to simulate actual extracellular polymeric substances (EPS) solution in terms of filterability behavior, a series of experiments were conducted in a dead-end unstirred cell with 0.1 μm polyvinylidene fluoride membranes using binary/ternary mixtures consisting of sodium alginate (SA), bovine serum albumin (BSA) and humic acid (HA). Three target parameters (cumulative filtrate volume (CFV), specific cake resistance ($\alpha_c$) and rejection (R)) were compared and the roles of mixture components were investigated. The order of degree of influence on CFV, $\alpha_c$ and R in ternary mixture was SA (94.5%, 85.6% and 88.2%, respectively) > BSA (5.2%, 10.3% and 8.0%) > HA (0.3%, 4.1% and 3.8%). Meanwhile, when the composition of ternary mixture was SA/BSA/HA = 285.1/150.1/10.2 mg·L$^{-1}$, the deviation for CFV, $\alpha_c$ and R was 7.65%, 19.6% and 7.27%, respectively, while the corresponding values for the most suitable binary solution (SA/BSA = 140.4/50.35 mg·L$^{-1}$) were 12%, 1% and 16% respectively. This indicated that the ternary solution demonstrated a more accurate estimation than the binary solution for imitating the filterability of actual EPS solution. Therefore, the ternary mixture could be employed efficiently to replace the actual EPS solution in terms of three target parameters in practice applications.

Key words | bovine serum albumin, filterability, humic acid, microfiltration membrane, model solution, sodium alginate

INTRODUCTION

In recent years, the membrane bioreactor (MBR) has gained wide attention as an efficient separation approach in biotechnology development, food industry and industrial wastewater treatment (Peiris et al. 2010; Buetehorn et al. 2012; Huang et al. 2016). However, an intractable problem related to MBR is the significant decrease of the membrane permeability mainly due to the membrane fouling. Therefore, the alleviation of membrane fouling still remains as a formidable challenge. Consequently, the determination of the main foulant in MBR and the investigation of its filterability behavior are of great significance.

The extracellular polymeric substances (EPS), which mainly consist of polysaccharide, protein, humic substances, uronic acid and DNA (Frølund et al. 1995; Dignac & Urbain 1998; Zhang et al. 1999; Tsuneda & Park 2001; Houghton & Stephenson 2002), is considered as a key component affecting membrane fouling in MBR (Arabi & Nakhla 2010; Santos et al. 2011; Lin et al. 2014; Campo et al. 2017). The main foulant in MBR has been investigated extensively (Basuvaraj et al. 2013). For example, Yao et al. found that the membrane fouling was mainly controlled by cake resistance rather than pore absorption and the lower protein/polysaccharide ratio demonstrated a less severe membrane fouling circumstance than the higher ratio (Yao et al. 2010). Kimura et al. reported that irreversible fouling was mainly caused by polysaccharides and presumably iron and manganese to some extent (Kimura et al. 2004). Also, the filtration characteristics and membrane fouling in cross-flow microfiltration mode using bovine serum albumin (BSA)/dextran binary suspension was studied (Wang et al. 2010). An increase in cross-flow velocity/transmembrane (TMP) pressure led to a relatively higher pseudo-steady filtration flux, which resulted in an obvious decrease of membrane fouling. In addition, the fouling behavior of mixed liquor suspended solids (Kenji et al. 2011), supernatant
of activated sludge (Benoît et al. 2011; Zhu et al. 2016) and EPS extracted from MBR have been well studied (Meng et al. 2006; Wang et al. 2009b; Lin et al. 2014).

Despite all this, the filterability behavior and fouling mechanism of EPS were not well understood due to the variability in extracting mass, composition and concentration information for real MBR systems (Zhang et al. 2015). On this account, a series of model EPS solutions (sodium alginate (SA), (Chang et al. 2016), glucon (Nataraj et al. 2008), dextran, BSA and myoglobin (Susanto et al. 2008), β-lactoglobulin (Steinhauer et al. 2003), humic acid (HA) (Chang et al. 2015), polyphenol (Zator et al. 2009) and their compounds) were employed to replace the actual EPS solutions to investigate their filterability and fouling behaviors. For example, polysaccharides were used as a model solution for EPS solution (Frank & Belfort 2005). SA was employed as a model polysaccharide to study fouling mechanism in the presence of calcium and alum (Listiarini et al. 2009). Recently, the EPS model solution has also been shifted from a single substance to binary mixture. For example, a binary mixture (BSA and SA) was used to model the EPS solution from MBR (Ye et al. 2005) and the protein/polysaccharide mixture from a fermentation tank was used to study the membrane fouling mechanism (Susanto et al. 2008). However, to the best of our knowledge, the model solution still requires further development to imitate the actual EPS solution. Therefore, it is crucial to find a model solution to study an actual EPS solution from MBR and describe its filterability.

The purpose of this study is to find a suitable model solution to simulate the actual EPS solution extracted from MBR in terms of filterability behavior. For the first time, the study of an EPS model solution is shifted from single or binary mixture to ternary mixture, and the orthogonal methodology is used to select the appropriate ternary mixture to simulate the actual EPS in terms of filterability behavior. Furthermore, another novelty in this paper is that the role of each component (BSA, HA and SA) in the binary/ternary mixtures is quantified by the influence degree and average variation rates (multivariate linear regression methodology) to deeply understand the membrane fouling behavior. Meanwhile, the comparison of the deviations between simulated solution and the actual EPS solution on the three target parameters (cumulative filtrate volume (CFV), specific cake resistance (a) and rejection (R)) is a novel and intuitive method to select a suitable model EPS solution. In this study, the model EPS solution is compared with actual EPS solutions in terms of filterability behavior in a dead-end microfiltration unstirred cell using 0.1 μm polyvinylidene fluoride (PVDF) membranes. Because the batch tests could not capture the dynamics of the continuous flow MBR, there are still limitations compared with the industrial application.

### EXPERIMENTAL

#### Materials and preparation process

A flat sheet PVDF membrane with pore size of 0.1 μm (Ande (China)) was used as filtration membrane. Before each experiment, the PVDF membrane was soaked in deionized (DI) water for 6–10 h to remove protective agent glycerin. SA was supplied by Sinopharm (China). BSA (molecular weight = 67 kDa) was supplied by Fuchen (China). HA was purchased from Beijing Chemical Reagent Co. (China). All chemicals were used without further purification. The preparation of model substances was as follows. The polysaccharide solution was prepared by mixing SA with sodium bicarbonate buffer solution. The protein solution was prepared by dissolving BSA in a potassium dihydrogen phosphate/sodium hydroxide buffer solution at pH = 7.0. The humic substance solution was prepared by mixing HA with sodium bicarbonate buffer solution at pH = 8.0. Arbitrary pairs of SA, BSA and HA solutions were selected to form the binary mixture (the model EPS solution), and then the prepared solution was stored in a refrigerator.

#### Extraction of the actual EPS

The formaldehyde–NaOH extraction method was chosen to extract the EPS in this study. The detailed steps were as follows: the activated sludge sample was left to settle for 1.5 h and then the supernatant was carefully decanted away. The thickened sludge was centrifuged at 2,000 g for 15 min at 4 °C. The sludge pellets were re-suspended to their original volume using a buffer (pH = 7.0) consisting of 2 mmol·L⁻¹ Na₃PO₄, 4 mmol·L⁻¹ NaH₂PO₄, 9 mmol·L⁻¹ NaCl and 1 mmol·L⁻¹ KCl. Meanwhile, the EPS solution was extracted as follows: formaldehyde (36.5%) (0.06 mL per 10 mL sludge) was added to the above suspension at 4 °C, which was maintained for 1 h, and then 1 g·L⁻¹ NaOH (4 mL per 10 mL sludge) was added at 4 °C, which was maintained for 3 h. The extracted EPS was harvested by centrifuging the formaldehyde/ NaOH /sludge suspension sample at 20,000 g for 20 min, followed by 0.2 μm membrane filtration at 25 °C. Extractant residues in the solution were removed by the dialysis membrane filtration (3,500 Da; Pierce, USA) in the subsequent treatment (Meng et al. 2006). The compositions
of the actual EPS solution extracted from the activated sludge of MBR are shown in Table 1.

**Analytical methods**

The actual EPS solution was extracted from activated sludge samples produced by the intermittent-aeration MBR (Figure 1(a)), and the chemical oxygen demand, NH$_3$-N, and pH of EPS solution were 350–500 mg·L$^{-1}$, 65–80 mg·L$^{-1}$ and 7.0, respectively. Also, all of the actual EPS was extracted in the same reactor, and the concentration of mixed liquor suspended solids was constant. In addition, the contents of protein and humic substance in actual EPS solution were measured by the modified Lowry method (Lowry et al. 1951; Frølund et al. 1993), where BSA and sodium humate were used as respective standards. The content of polysaccharide was measured by the anthrone method (Dubois et al. 1956; Raunkjær et al. 1994), where glucose was used as a standard. The viscosity was measured using a viscometer (Visco Tester 6L, Thermo-Haake, Germany) and the total organic carbon (TOC) was measured using a TOC analyzer (TOC-VCPH, Shimadzu, Japan). The pH was measured using a pH meter (pHS-3C, Leici, China).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The composition of the actual EPS solution extracted from the activated sludge of MBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$<em>{</em>{\text{polysaccharide}}}$  (mg·L$^{-1}$)</td>
<td>100.7 ± 3.5</td>
</tr>
<tr>
<td>C$<em>{</em>{\text{protein}}}$  (mg·L$^{-1}$)</td>
<td>140.4 ± 18</td>
</tr>
<tr>
<td>C$<em>{</em>{\text{humic substance}}}$  (mg·L$^{-1}$)</td>
<td>11.4 ± 1.1</td>
</tr>
<tr>
<td>C$<em>{</em>{\text{EPS}}}$  (mg·L$^{-1}$)</td>
<td>252.5 ± 22</td>
</tr>
<tr>
<td>TOC  (mg·L$^{-1}$)</td>
<td>257.6 ± 24</td>
</tr>
</tbody>
</table>

Figure 1 | (a) Schematic diagram of integrated intermittent-aeration MBR, (b) schematic diagram of dead-end filtration.
Experimental procedure

All the experiments were conducted in a batch mode. The microfiltration experiment was performed at a constant TMP of 0.1 MPa and temperature of 25 °C with a laboratory scale dead-end filtration system consisting of a filtration cell with effective membrane area of 24.19 cm² (Figure 1(b)) (Wang et al. 2009a). Firstly, the pure water flux of the membrane was measured with DI water under a constant TMP of 0.1 MPa, and then the EPS solution used as feed solution was replaced in the filtration cell and filtrated under the same TMP at 25 ± 2 °C. Secondly, the permeate was collected into a filtrate receiver and the permeate weight was recorded with an electronic balance at intervals of 30 s. Finally, the permeate obtained after 780 s was collected to measure total EPS concentration using a TOC analyzer (Katsoufidou et al. 2010; Liang et al. 2017).

Fouling mechanism

The fouling mechanism of the membrane can be determined by the blocking law (Sabia et al. 2015; Bourcier et al. 2016; Iritani & Katagiri 2016; Trzaskus et al. 2016) and the empirical equations in Table 2 were used (Charfi et al. 2012, 2017).

The calculations of selected target factors

Based on cake filtration mechanism, the specific cake resistance ($\alpha_c$) can be determined by plotting $t/V$ vs $V$ (Wang et al. 2009b):

$$\frac{t}{V} = \frac{R_m \mu}{\Delta P A_m} + \alpha_c \left( \frac{\mu \rho_s C}{2 \Delta P A_m^2} \right) V$$

(1)

where $V$ is the CFV at time $t$ ($m^3$); $A_m$ is effective filtration area ($m^2$); $\mu$ is the viscosity (Pa·s); $\rho_s$ is the sludge density (kg·m$^{-3}$); $R_m$ is the intrinsic membrane resistance ($m^{-1}$); $\Delta P$ is the TMP (Pa) and $C$ is the concentration of the solution model (mg·L$^{-1}$).

The rejection ($R$) of the membrane was determined by a TOC analyzer and it can be calculated using the following equation:

$$R = \left( 1 - \frac{C_p}{C_f} \right) \times 100\%$$

(2)

where $C_p$ and $C_f$ are the concentration of the filtrate and feed solution, respectively (mg·L$^{-1}$). The concentrations of the filtrate and feed solution were measured using a TOC analyzer.

The average variation rate ($r_v$) of unit concentration variation of one substance in the binary/ternary mixture on target factor can be calculated by the following equation:

$$r_v = \frac{OF_{ave} \times I}{C_{ave}}$$

(3)

where $OF_{ave}$ is the average value of the target factor; $I$ is the influence degree of substance in the binary/ternary mixture on target factor (%) and $C_{ave}$ is the average concentration (mg·L$^{-1}$).

RESULTS AND DISCUSSION

Membrane fouling mechanism

A series of experiments with different substance concentrations in binary/ternary mixture were conducted. The fouling mechanism was investigated by empirical equations given in Figure 2 and Table 2. Good agreement between the model predictions and the experimental data (Table 3) indicated that the whole filtration process was predominated by cake filtration except for some points at the early stage of filtration (pore plugging).

The selection of suitable binary mixtures

As presented in Table 4, arbitrary pairs of SA, BSA and HA were selected to form the binary mixture (the model EPS solution), where $C_{SA}$, $C_{BSA}$ and $C_{HA}$ respectively were 0.1,
Comparison of the filtration behavior

The values of three key parameters (CFV, $\alpha_c$ and R) of binary mixture were compared with those of the actual EPS solution. As shown in Figure 3(a), a big deviation was observed between the CFV of binary mixture BSA/HA (Group 2) and the actual EPS solution, while CFV of binary mixtures BSA/SA and SA/HA (Group 1 and Group 3) was significantly closer to that of actual EPS solution. This revealed that polysaccharide was a key component in the actual solution and this provided the possibility to match the actual EPS solution by altering the ratios of BSA/SA or SA/HA in binary mixture. For BSA/SA mixture, when CSA was 2.8% (Group 1-4), 12.5% (Group 1-5) and 22.3% (Group 1-6), the corresponding CFV was $5.7 \times 10^{-5}$, $3.53 \times 10^{-5}$ and $2.86 \times 10^{-5}$ m$^3$, respectively. Meanwhile, CFV was nearly constant ($3.49 \times 10^{-5}$, $3.53 \times 10^{-5}$ and $3.01 \times 10^{-5}$ m$^3$) with the increase of CBSA from 73.6% (Group 1-2), to 87.7% (Group 1-5) and 93.3% (Group 1-8). This revealed that SA molecules played a dominant role in cake formation while BSA molecules only played a minor role despite

0.5, 1.0, 2.5 and 5.0 times the value of $C_{\text{polysaccharide}}$, $C_{\text{protein}}$ and $C_{\text{humic substance}}$ ($C_{\text{humic}}$) in actual EPS solution (in parentheses in Table 4).

Table 3 | The value of parameters for different solutions

<table>
<thead>
<tr>
<th>Solution</th>
<th>$K_c$</th>
<th>$J_0$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual EPS</td>
<td>1.6072 ± 1.77×10$^{-2}$</td>
<td>0.0572 ± 9.41×10$^{-4}$</td>
<td>0.99454</td>
</tr>
<tr>
<td>BSA/SA = 702/100.7</td>
<td>1.7601 ± 5.29×10$^{-3}$</td>
<td>0.0491 ± 1.75×10$^{-4}$</td>
<td>0.99966</td>
</tr>
<tr>
<td>BSA/HA = 702/57</td>
<td>0.0729 ± 3.89×10$^{-3}$</td>
<td>0.1364 ± 2.59×10$^{-3}$</td>
<td>0.94341</td>
</tr>
<tr>
<td>SA/HA = 100.7/57</td>
<td>1.3768 ± 2.24×10$^{-2}$</td>
<td>0.0446 ± 6.08×10$^{-4}$</td>
<td>0.99077</td>
</tr>
<tr>
<td>BSA/SA/HA = 702/100.7/57</td>
<td>1.4064 ± 7.64×10$^{-3}$</td>
<td>0.0985 ± 1.07×10$^{-3}$</td>
<td>0.99917</td>
</tr>
</tbody>
</table>

Table 4 | The substance concentration (mg·L$^{-1}$) in the binary mixture

<table>
<thead>
<tr>
<th>No.</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$c_{\text{CSA}}$</td>
<td>$c_{\text{CBSA}}$</td>
<td>$c_{\text{CSA}}$</td>
</tr>
<tr>
<td>1</td>
<td>10.07(0.1)</td>
<td>140.4(1.0)</td>
<td>140.4(1.0)</td>
</tr>
<tr>
<td>2</td>
<td>50.35(0.5)</td>
<td>140.4(1.0)</td>
<td>140.4(1.0)</td>
</tr>
<tr>
<td>3</td>
<td>100.70(1.0)</td>
<td>140.4(1.0)</td>
<td>140.4(1.0)</td>
</tr>
<tr>
<td>4</td>
<td>10.07(0.1)</td>
<td>351.0(2.5)</td>
<td>351.0(2.5)</td>
</tr>
<tr>
<td>5</td>
<td>50.35(0.5)</td>
<td>351.0(2.5)</td>
<td>351.0(2.5)</td>
</tr>
<tr>
<td>6</td>
<td>100.70(1.0)</td>
<td>351.0(2.5)</td>
<td>351.0(2.5)</td>
</tr>
<tr>
<td>7</td>
<td>10.07(0.1)</td>
<td>702.0(5.0)</td>
<td>702.0(5.0)</td>
</tr>
<tr>
<td>8</td>
<td>50.35(0.5)</td>
<td>702.0(5.0)</td>
<td>702.0(5.0)</td>
</tr>
<tr>
<td>9</td>
<td>100.70(1.0)</td>
<td>702.0(5.0)</td>
<td>702.0(5.0)</td>
</tr>
</tbody>
</table>
altering the BSA/SA ratio in binary mixture (Hashino et al. 2014). This could be explained by BSA molecules having a lower molecular weight (67 kDa) in comparison with SA molecules. Therefore BSA can easily pass through the membrane without forming a cake layer on the membrane surface. Also, for SA/HA mixture, the similar conclusion was obtained due to molecular weight reasons. When the concentrations of HA increased from 10.2% (Group 3-4), to 36.1% (Group 3-5) and 53.1% (Group 3-6), the corresponding variation of CFV was unobvious. The relatively small amounts of SA substance can form a cake, screening the molecules of HA substance, and this prevented HA substance further contacting with the membrane surface (Katsoufidou et al. 2010). In summary, the CFV of actual EPS solution could be matched by altering the concentration of SA substance in the binary mixture due to the dominant role of SA in cake formation.

The sequences of $\alpha_c$ for different binary mixtures were: Group 3 (mixture SA/HA) > Group 1 (mixture BSA/SA) > Group 2 (mixture BSA/HA) (Figure 3(b)). Firstly,
the $\alpha_c$ value of Group 2 was comparatively small. Therefore, BSA/HA mixture cannot reach the identical value as for actual EPS solution. This can be explained by the fact that a loose cake was formed by mono-dispersed suspensions with larger particles while small particles (BSA) mostly permeated through the membrane. The most possible structure of the cake layer is shown in Figure 3(d-3). Moreover, although CFV for binary mixture SA/HA (Group 3) was similar to that of the actual EPS solution (Figure 3(a)), there was a large deviation between binary mixture with different ratios of SA/HA and actual EPS solution in terms of $\alpha_c$ value (Figure 3(b)). The cake layer on the membrane surface consisting of particles with various sizes (Figure 3(e)) would normally have a smaller porosity than that with single size particles and this resulted in an enhancement of $\alpha_c$ value compared with other combinations (Figure 3(d-2)). Therefore, both binary mixtures BSA/HA and SA/HA cannot be used to model actual EPS solution in terms of $\alpha_c$.

R depends mainly on the substance type in the binary mixture (Figure 3(c)). It increased with the rise of $C_{SA}$, while it decreased with the rise of $C_{BSA}$ or $C_{HA}$. The interaction between BSA and SA substances (Group 1) was larger than that between BSA and HA substances (Group 2) or SA and HA substances (Group 3). For example, R increased nearly 17 times (from 3.9% (Group 1-7) to 67.9% (Group 1-3)) for Group 1 series, while it increased 5 times (from 1.4% (Group 2-7) to 6.7% (Group 2-2)) and 4 times (from 24.2% (Group 3-2) to 97.9% (Group 3-4)) for Group 2 series and Group 3 series, respectively. These results indicated that the variation of the ratio of BSA and SA can induce an apparently big change in R value, and also the SA ratio in mixture was directly proportional to R.

The deviation between model and actual EPS solution on the target factors (CFV, $\alpha_c$ and R) was studied by orthogonal method (Wang et al. (2010)). As shown in Figure 3(f), the deviations were obvious for CFV, $\alpha_c$ and R. For instance, the deviation between Group 2 and actual EPS solution on all three target factors was obvious and the maximum deviation was almost 500%. However, the deviation between Group 1-2 (SA/BSA) and actual EPS solution for CFV, $\alpha_c$ and R was $-12\%$, $1\%$ and $164\%$ respectively. Although the deviation for R (164%) was relatively significant, the binary mixture solution demonstrated the potential to imitate the actual EPS solution in terms of filterability.

Influence degree and average variation rates

The influence degree of different substances in binary mixtures on the three target factors (CFV, $\alpha_c$ and R) were studied by multivariate linear regression method (Wang et al. (2010)) and the results are presented in Figure 4(a). For Group 1 and Group 2, the influence degree of SA or HA was much greater than that of BSA. Meanwhile, the influence degree of SA was much greater than that of HA in Group 3. Therefore, only one substance played a major role in each binary mixture. If $C_{SA}$ was changed in Group 1, the values of CFV would be changed markedly.

Figure 4 | The influence degree of different substances in binary solution on the three target factors (CFV, $\alpha_c$, and R) (a) and the average variation rates of the three target factors (CFV, $\alpha_c$ and R) for different binary mixtures (b). Experimental conditions: 0.1 $\mu$m PVDF membrane, TMP = 0.1 MPa, $T = 25 \pm 2 ^\circ C$ and pH = 7.5.
In order to further evaluate the influence of different substances in binary mixture on the three target factors (CFV, $\alpha_c$ and $R$), the average variation rates were calculated according to Equation (3). As presented in Figure 4(b), $r_V$ of SA was the largest among all values. However, impact of $r_V$ for HA on the three target factors was larger than that for BSA in binary mixture BSA/HA. After the comparison of the absolute value of $r_V$, it was suggested that the interaction between SA and BSA or between SA and HA substances was comparatively small. It indicated that only one substance played a decisive role on the three target factors in the binary mixtures. However, the composition of the actual EPS solution was complex, and the role of different components in actual EPS solution was also inextricably linked. Therefore, there was an obvious gap between binary model solutions and actual EPS solution in terms of filterability behavior, due to the simple composition of the binary solution and the weak interaction between each component. Hence the ternary mixtures were employed, described in the following section.

### Selection of suitable ternary mixtures

A factor-level orthogonal table for the ternary mixture consisting of SA, BSA and HA substances is shown in Table 5, where the $C_{SA}$ is 0.1, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 times the value of $C_{polysaccharide}$, and $C_{BSA}$ and $C_{HA}$ are 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 times the value of $C_{protein}$ and $C_{humic}$ in actual EPS solution (in parentheses in Table 5), respectively. Forty-nine parallel experiments were conducted and the results are shown in Figures 5–8.

The deviations between actual EPS solution and the ternary mixture in terms of three target parameters

<table>
<thead>
<tr>
<th>No.</th>
<th>$C_{SA}$</th>
<th>$C_{BSA}$</th>
<th>$C_{HA}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.07(0.1)</td>
<td>70.2(0.5)</td>
<td>5.7(0.5)</td>
</tr>
<tr>
<td>2</td>
<td>50.35(0.5)</td>
<td>140.4(1.0)</td>
<td>11.4(1.0)</td>
</tr>
<tr>
<td>3</td>
<td>100.7(1.0)</td>
<td>280.8(2.0)</td>
<td>22.8(2.0)</td>
</tr>
<tr>
<td>4</td>
<td>151.1(1.5)</td>
<td>421.2(3.0)</td>
<td>34.2(3.0)</td>
</tr>
<tr>
<td>5</td>
<td>201.4(2.0)</td>
<td>561.6(4.0)</td>
<td>45.6(4.0)</td>
</tr>
<tr>
<td>6</td>
<td>251.8(2.5)</td>
<td>702.0(5.0)</td>
<td>57.0(5.0)</td>
</tr>
<tr>
<td>7</td>
<td>302.1(3.0)</td>
<td>842.4(6.0)</td>
<td>68.4(6.0)</td>
</tr>
</tbody>
</table>

Figure 5 | The comparisons of different substances ratios in the ternary solution in terms of (a) CFV, (b) $\alpha_c$ and (c) $R$.  

A factor-level orthogonal table for the ternary mixture consisting of SA, BSA and HA substances is shown in Table 5, where the $C_{SA}$ is 0.1, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 times the value of $C_{polysaccharide}$, and $C_{BSA}$ and $C_{HA}$ are 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 times the value of $C_{protein}$ and $C_{humic}$ in actual EPS solution (in parentheses in Table 5), respectively. Forty-nine parallel experiments were conducted and the results are shown in Figures 5–8.

The deviations between actual EPS solution and the ternary mixture in terms of three target parameters
(ΔCFV, Δαc and ΔR) are shown in Figure 5. These deviations fluctuated around the horizontal axis, i.e. at the zero line. It implied that by altering the ratio of substances in the ternary mixture, a certain ternary mixture could be found to exactly describe the filterability of actual EPS solution. For example, experiment numbers 15, 22, 30 and 40 for CFV, 15, 23, 31, 38 and 45 for αc and 9, 17, 25, 32, 33 and 41 for R, were relatively close to the zero line. Therefore, these solutions could be used as potential ternary model solutions to model the actual EPS solution.

The contribution of different substances in the ternary mixture to the three target parameters was investigated using multivariate linear regression methodology. It can be seen from Figure 6(a) that SA was still the most important substance and it dominated the three target parameters in the ternary mixture. However, compared with the binary mixtures, the interaction among substances in the ternary mixture was strengthened. For example, impacts of BSA on CFV were changed from 0.4% (the binary mixtures of Group 1) to 5.2% (the ternary mixtures) owing to the presence of HA substance (despite the impact of HA on CFV being only 0.3%). Impacts of BSA on αc and R were also enhanced. This could be because the hydrophobic parts of HA bound...
onto the hydrophobic parts of the membrane while the hydrophilic parts of HA were directed towards the solution. As a result, the membrane became more hydrophilic and more negatively charged. Therefore, the hydrophilic BSA was absorbed on the membrane surface more easily (Mänttäri et al. 2000). As a consequence, the impacts of BSA on CFV $\alpha_c$ or R were strengthened, while the impact of HA substance on the three target factors were weakened. The order of impact in the ternary mixture on the three target parameters was as follows: SA $>$ BSA $>$ HA. The total influence degree of SA, BSA and HA on CFV was 94.5%, 5.2% and 0.3% while on $\alpha_c$, it was 85.6%, 10.3% and 4.1%, and on R it was 88.2%, 8.0% and 3.8%, respectively.

The average variation rates ($\Delta r$) of the three target parameters CFV, $\alpha_c$ and R as function of SA, BSA and HA were obtained by multivariate linear regression methodology and the results are shown in Figure 6(b). SA played a leading role in the variation of the filterability of the ternary solution while BSA and HA substances played a supporting role. This can be explained by the fact that SA contains plenty of hydroxyl groups while BSA and HA have substantial amino acid residue groups and carboxyl groups, respectively (pH = 8.0 in this study). The size of the BSA molecule is much smaller than the pore size of the membrane (0.1 $\mu$m). Therefore, it can be adsorbed on the surface of pores and block internal pores. Meanwhile, stronger intermolecular interaction results in the aggregation of SA molecules on the membrane surface to form a dense cake layer. Therefore, the presence of BSA results in a lower normalized flux (Hou et al. 2017). It is concluded that SA and BSA play an important role in the ternary mixture. The range analysis of the orthogonal table was carried out and the optimum formulations of $\text{SA}_6\text{BSA}_3\text{HA}_2$, $\text{SA}_7\text{BSA}_3\text{HA}_2$ and $\text{SA}_3\text{BSA}_4\text{HA}_4$ corresponding to the three target parameters were obtained, respectively. In addition, the optimum normalized formulation $\text{SA}_6\text{BSA}_3\text{HA}_2$. HA$_2$ can be obtained using normalized process for the three target parameters and the optimum compositions are shown in Table 6.

Verification experiments were also conducted to check the result of optimal formulations of $\text{SA}_6\text{BSA}_3\text{HA}_2$, $\text{SA}_7\text{BSA}_3\text{HA}_2$, $\text{SA}_3\text{BSA}_4\text{HA}_4$ and $\text{SA}_6\text{BSA}_3\text{HA}_2$ and the results are summarized in Figure 7. For the formulation of $\text{SA}_6\text{BSA}_3\text{HA}_2$, HA$_2$, the deviations for CFV, $\alpha_c$ and R were $-11.1\%$, 90.6% and 147.5%, respectively. Similarly, for the formulation of $\text{SA}_7\text{BSA}_3\text{HA}_2$, $\text{SA}_3\text{BSA}_4\text{HA}_4$ and $\text{SA}_6\text{BSA}_3\text{HA}_2$, these deviations were $-11.1\%$, 31.8% and 60.3%, 36.4%, $-46.7\%$ and $-27.6\%$, and $-13.2\%$, 2.48% and 22.2%, respectively. In such way, the formulation of $\text{SA}_6\text{BSA}_3\text{HA}_2$, i.e. $C_{\text{SA}}, C_{\text{BSA}}$ and $C_{\text{HA}}$ respectively equal to 2.5, 2 and 1 times the value of $C_{\text{polysaccharide}}, C_{\text{protein}}$ and $C_{\text{humic}}$ in actual EPS solutions ($\text{SA}/\text{BSA}/\text{HA} = 251.8/280.8/114\text{mg} \cdot \text{L}^{-1}$), can be applied as a suitable ternary solution to simulate actual EPS solution in terms of three target parameters simultaneously.

**Verification tests**

One of the verification experiments with the formulation of $\text{SA}_6\text{BSA}_3\text{HA}_2$ (285.05/150.12/10.2 mg·L$^{-1}$) was carried out (Table 7). The deviations in CFV, $\alpha_c$ and R were 7.65%, 19.6% and 7.27%, respectively. As shown in Figure 8, the membrane fouling caused by the actual EPS solution was consistent with that caused by the simulated solution. This means that this ternary mixture can be applied as a suitable model solution to replace actual EPS solutions in terms of the three target parameters.

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**Table 6** | The optimum composition of the ternary mixtures (mg·L$^{-1}$)

<table>
<thead>
<tr>
<th>Minimum of</th>
<th>$C_{\text{SA}}$</th>
<th>$C_{\text{BSA}}$</th>
<th>$C_{\text{HA}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta$CFV</td>
<td>251.8</td>
<td>70.2</td>
<td>22.8</td>
</tr>
<tr>
<td>$\Delta\alpha_c$</td>
<td>302.1</td>
<td>280.8</td>
<td>11.4</td>
</tr>
<tr>
<td>$\Delta$R</td>
<td>100.7</td>
<td>421.2</td>
<td>34.2</td>
</tr>
<tr>
<td>Normalization</td>
<td>251.8</td>
<td>280.8</td>
<td>11.4</td>
</tr>
</tbody>
</table>

**Table 7** | Verification tests between the ternary mixture and actual EPS solution

<table>
<thead>
<tr>
<th>Polysaccharide (mg·L$^{-1}$)</th>
<th>Protein (mg·L$^{-1}$)</th>
<th>Humic substance (mg·L$^{-1}$)</th>
<th>EPS (mg·L$^{-1}$)</th>
<th>Experimental results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracted EPS</td>
<td>114</td>
<td>75.05</td>
<td>10.2</td>
<td>199.25</td>
</tr>
<tr>
<td>Binary model EPS</td>
<td>285</td>
<td>150.1</td>
<td>10.2</td>
<td>435.1</td>
</tr>
<tr>
<td>Ternary model EPS</td>
<td>285</td>
<td>150.1</td>
<td>10.2</td>
<td>445.3</td>
</tr>
<tr>
<td>Deviation</td>
<td></td>
<td></td>
<td></td>
<td>1.96 $\times$ 10$^{-5}$</td>
</tr>
<tr>
<td>Deviation</td>
<td></td>
<td></td>
<td></td>
<td>5.72 $\times$ 10$^{-5}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$-191.8$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.81 $\times$ 10$^{-5}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.65</td>
</tr>
</tbody>
</table>
CONCLUSIONS

In this paper, the filterability of different binary/ternary mixtures consisting of SA, BSA and HA were studied using 0.1 μm PVDF membrane in a dead-end unstirred cell. The deviations of model EPS solutions and actual EPS solution in terms of the three target parameters (CFV, αc and R) were studied by orthogonal methodology and multivariate linear regression methodology. The results showed that the deviation between ternary mixture and actual EPS solution for the three target parameters was smaller than that for binary mixture, and a suitable ternary mixture (CSA/CBSA/CHA = 251.8/280.8/11.4 mg L⁻¹) was found (its deviations were 13.2%, 2.48% and 22.2% for the three target parameters respectively) to model actual EPS solution extracted from MBR in terms of filterability behavior. Since the extraction process for actual EPS is complex, this imitated ternary mixture can be employed to replace actual EPS solutions to investigate their filterability and fouling behaviors. Furthermore, the ternary mixture solution containing SA, BSA and HA functioned as a more robust mimic EPS solution combination compared with single or binary mixtures. Therefore, this study was of great importance and provides guidance for future EPS research.

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