Effect of biopolymer clusters on the fouling property of sludge from a membrane bioreactor (MBR) and its control by ozonation

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Running Head: Ozonation of BPC for MBR fouling mitigation
Abstract

Organic substances in the liquid phase of the sludge in a membrane bioreactor (MBR) have a profound impact on membrane fouling. In this study, a single-fibre microfiltration apparatus was developed to investigate the fouling propensity of MBR sludge and the effectiveness of ozonation in membrane fouling mitigation. The results show that biopolymer clusters (BPC) in the MBR suspension had a significant influence on the fouling potential of the sludge. An increase in BPC concentration by 20% and 60% from around 3.5 mg/l in the mixed sludge liquor drastically increased the fouling rate by 120% and 300%, respectively. Ozonation of the BPC solution greatly reduced the detrimental role of BPC in membrane fouling. An ozone dose of 0.03 mg/mg TOC of BPC could reduce the mean BPC size from 38 to 27 μm, which was further reduced to 12 μm at 0.3 mg O₃/mg TOC of BPC. In addition to BPC destruction, ozonation apparently also modified the surface properties of BPC, resulting in an increase in the filterable fraction and a decrease in the liquid viscosity. Based on the experimental findings, an approach for MBR membrane fouling control is proposed that applies ozonation to the supernatant containing BPC in a side-stream application.

Keywords: Biopolymer clusters (BPC); membrane bioreactor (MBR); membrane fouling; microfiltration; ozonation; soluble microbial products (SMP).
1. Introduction

Membrane bioreactors (MBRs) are increasingly being used as an advanced technology for biological wastewater treatment and reuse. With the use of a membrane for sludge filtration, the MBR ensures complete solid–liquid separation [1,2]. In MBRs, the sludge age and concentration can be effectively manipulated, affording this type of bioreactors several advantages over the conventional activated sludge (CAS) process [3]. At the same time, however, because of the retention by the membrane, some of the soluble microbial products (SMP) and other colloidal substances are unable to escape from the system with the effluent [4,5]. The organic interception by membrane filtration results in the formation and accumulation of organic foulants in the MBR sludge suspension, which in turn worsens the membrane fouling problem.

The effect on membrane fouling of liquid–phase organic substances in the MBR sludge mixture has long been recognised [6–10]. Recent research reveals the presence of a group of large-sized organic solutes, termed biopolymer clusters (BPC), in MBR systems [11–13]. BPC are neither biomass flocs nor SMP or extracellular polymeric substances (EPS). They can be larger than 10 µm in size, and are formed by the affinity clustering of SMP and loose EPS on the membrane surface [14]. It has been suggested that BPC may facilitate sludge deposition and the fouling layer formation on the membrane surface, and the detrimental role of BPC in membrane fouling has been demonstrated qualitatively during the operation of MBR systems [12,14]. However, more systematic studies remain to be conducted to determine the correlation between the membrane fouling rate and the BPC content of the sludge mixture. In addition, the effect of changes in BPC properties on the fouling potential
of the MBR sludge also merits investigation.

The reduction or modification of BPC in MBR sludge mixture is expected to be beneficial for the control of membrane fouling. Removal of BPC and their precursors, such as SMP and loose EPS, is an option, and indeed the use of adsorbents or coagulants in the MBR mixed liquor has been found effective in decelerating membrane fouling [15-19]. However, continuous addition of these chemicals may either be harmful to the membrane due to physical abrasion, as is the case for granular activated carbon, or affect the MBR treatment performance, as is the case with some coagulant metal ions (e.g., Fe(II) and Fe(III) that are reportedly toxic to the nitrifying bacteria [20]). More recently, the ozonation of bulk sludge has been tested as a means of membrane fouling control during continuous MBR operation [21-24]. The results show that at appropriate doses the membrane fouling rate can be effectively reduced, meanwhile, ozonation coupled with MBR appears to be an effective method for sludge reduction and toxic organic wastewater treatment [23,24]. However, a possible overdose of ozone and its impact on the biomass activity is a concern with direct sludge ozonation. Moreover, the underlying mechanisms of sludge ozonation for membrane fouling mitigation are not well understood.

There is thus a need to determine the effect of BPC in MBR sludge mixture on membrane fouling, and to investigate the effectiveness of the ozonation of BPC in reducing the fouling propensity of sludge. In this study, a lab-scale MBR was operated to supply both biomass sludge and BPC dispersion. A newly designed single-fibre microfiltration (MF) system was fabricated for the membrane filtration-fouling tests on different sludge–BPC mixture samples under well-controlled hydrodynamic conditions. Ozonation was applied to
the BPC solution only, rather than the entire sludge mixture, before mixing into the sludge suspension. The objectives of the experimental study were (1) to determine the fouling propensity of MBR sludge with different BPC contents, and (2) to investigate the effectiveness of the ozonation of BPC in minimising membrane fouling during sludge filtration. The mechanism of sludge ozonation to mitigate fouling was also identified, and on this basis a more reliable ozonation approach for MBR fouling control is proposed.

2. Materials and Methods

2.1. Filtration setup and operation

A single-fibre filtration apparatus was fabricated for the sludge filtration and fouling tests (Fig. 1). The apparatus was made of a plexiglass tube 1.5 cm in internal diameter and 50 cm in height. A polyethylene (PE) hollow-fibre MF membrane (pore size = 0.4 μm, diameter = 0.14 cm, working length = 40 cm, surface area = 16 cm², Mitsubishi Rayon, Japan) was installed along the centreline of the filtration tube. The sludge suspension in a feed tank was pumped through the MF test tube by a helical pump (SELTZ-L40 II, Hydor, USA). A constant cross-flow rate of 2 l/min (0.19 m/s) was applied by the recirculation of the sludge suspension for continuous membrane surface cleaning. The permeate was drawn out through the MF membrane by a suction pump (MasterFLEX, Cole-Parmer, USA) at a constant flux of 37.5 l/m² h. An electronic balance (Arrw 60, OHAUS, USA) was used to record the permeate production during the filtration-fouling tests. Unless sampled for analysis, the collected permeate was returned manually to the feed tank at regular intervals to maintain the same
sludge concentration. A pressure sensor (PTX Ex-0129, Druck, USA) was installed before
the suction pump to record the trans-membrane pressure (TMP) during sludge filtration. Both
the permeate production and TMP data were transferred to a PC for continuous data
recording (Fig. 1). The membrane fouling rate was measured by the increase in TMP with the
amount of permeate produced (filtrate depth, L), or $\Delta$TMP/$\Delta$L. After each filtration-fouling
test, the membrane fibre was taken off the filtration tube and washed with 100 ml of DI water
at 40°C to recover all of the sludge and foulants deposited on the membrane surface. The
sludge and foulant dispersion was then settled for 2 h at 4°C and the supernatant was analysed
for total organic carbon (TOC) and chemical composition, including proteins (PN),
polysaccharides (PS) and humic-like substances (HS). The sludge in the dispersion was
collected on a filter, dried for 2 h at 105°C and then weighed to obtain the suspended solids
(SS) content.

2.2. MBR activated sludge and biopolymer clusters

The sample activated sludge (AS) and biopolymer substances for the filtration tests were
collected from a submerged MBR (SMBR). The laboratory SMBR had a working volume of
5 l and contained a submerged 0.4 $\mu$m polyethylene MF module (surface area = 0.2 m$^2$,
Mitsubishi Rayon, Japan). The SMBR system had been in stable operation for more than four
years before the present experiment [12,25]. The influent (feeding wastewater) to the SMBR
was a mixture of a glucose-based synthetic wastewater prepared according to the basic recipe
given in the Environmental Engineering Process Laboratory Manual of the AEESP [26] and
domestic sewage collected from the Stanley Sewage Treatment Works in Hong Kong. The
sewage fraction supplied around 10% of the total organic load in the influent. The wastewater influent had a chemical oxygen demand (COD) of around 500 mg/l and a COD:N:P ratio of 100:9:3. NaHCO₃ was added to the influent at 50 mg/l or higher to maintain the pH of the MBR suspension between 6.5 and 7.5. The biomass concentration, food-to-microorganism (F/M) ratio, solid retention time (SRT) and hydraulic retention time (HRT) of the SMBR system were 10 g/l, 0.2 g COD/g SS d, 25 d and 8 h, respectively.

The AS mixture collected from the SMBR was settled for 1 h, and the settled sludge was then diluted with a 0.05% NaCl solution to a mixed liquor suspended solids (MLSS) concentration of 3 g/l. Large organic substances, or biopolymer clusters, were obtained from the cake sludge (CS) deposited on the surface of the membrane in the SMBR. When the membrane was seriously fouled, the CS layer was scraped off the membrane using a spatula. The CS was then re-suspended and dispersed by stirring it in a 0.05% NaCl solution. The CS suspension was then separated by sedimentation at 4°C for 12 h and the supernatant was collected. The organic substances in the CS supernatant were regarded as biopolymer clusters [12,14]. The CS supernatant, or BPC solution, was analysed for TOC and PN, PS and HS content.

The BPC solution was added into the AS suspension (3 g/l) at different doses. Each sludge suspension was then tested for its fouling propensity using the single-fibre MF filtration apparatus. In this way, the effect of the BPC content in the sludge mixture on the fouling potential of the sludge during membrane filtration was determined.

2.3. Ozonation of the BPC solution
Ozonation was also applied to the BPC solution with an intention to modify the BPC properties before their addition to the sludge. Ozonation was performed quantitatively by adding ozone-containing water into the BPC solution. Ozone was generated in the gaseous phase by an ozone generator (5000 BF, Enaly, China) that was supplied with pure oxygen. To dissolve the ozone in water and prepare an ozone solution, 500 ml ultra-pure water (Milli-Q-Advantage, Water Purification, Millipore, USA) was bubbled with the ozone gas at 4°C for 10 min or longer. The ozone concentration achieved in the ozone solution was about 8 mg/l. A pre-determined amount of the ozone solution was then added to 30 ml of the BPC solution. The mixed solution was placed in the dark and stirred for 5 min at 60 rpm to ensure complete ozonation. Similar to the previous sludge filtration tests, the ozonated BPC solution was added into the AS suspension at different doses, and the sludge mixtures were then tested for their fouling potential using the single-fibre filtration apparatus.

2.4. BPC characterisation

In the characterisation of the organic substances in the BPC solution, the fraction that could not pass through a 0.4 μm membrane filter (polycarbonate, Osmonics, USA) was defined as non-filterable BPC. The proportion of non-filterable BPC to the total organic content in the BPC solution was termed as the BPC cut-off ratio [11]. The BPC solution before and after ozonation and its filtrate were analysed to determine the TOC concentration and the PN, PS and HS content.

The BPC size distribution was determined by using a laser diffraction particle analyser (LS 13 320, Beckman Coulter, USA). Before the particle sizing and counting, the BPC in the
solution were stained with NanoOrange (Molecular Probes, Eugene, USA), which is a fluorescent probe that targets proteins in organic polymers. Five millilitres of NanoOrange dye solution was added to 30 ml of BPC solution for a final dye concentration of 20 mg/l, and the mixture was kept in the dark for 30 min. After staining, the transparent BPC became detectable by a laser particle analyser [14]. Moreover, both before and after ozonation, the BPC were filtered on a membrane filter and examined directly under a confocal laser scanning microscope (CLSM) (LSM Pascal, Zeiss, Thornwood, USA), following the procedures described previously [5,27]. For the CLSM observations, BPC (actually non-filterable BPC) and other solids collected on a 0.4 μm black polycarbonate membrane (25 mm, Osmonics, USA) were stained using a combination of two probes: SYTO9 to target the bacterial cells and ConA-TRITC to target the polysaccharides with D-glucose or D-mannose [12].

2.5. Analytical methods

The TOC was measured by a TOC analyser (IL550 TOC-TN Analyzers, Lachat, USA) using the high-temperature combustion method. The protein and humic concentrations were determined via an UV/VIS spectrophotometer (Lambda 25, Perkin Elmer, USA) following the modified Lowry method using albumin bovine (Sigma, Germany) and humic acid (Fluka, Italy), respectively, as the standards [28]. The polysaccharide content was measured according to the phenol method using glucose as the standard [29]. The MLSS concentration of the sludge was measured in accordance with the Standard Methods [30]. The concentration of dissolved ozone in the ozonated water was determined based on the UV absorbance as
measured by an UV/VIS spectrophotometer (Lambda 25, Perkin Elmer, USA) following the conventional indigo method [31,32]. The liquid viscosity was measured by a vibration viscometer (SV-10, A&D, Japan).

3. Results and Discussion

3.1. Significance of BPC in membrane fouling

The membrane fouling rate for the sludge samples was well indicated by the increase in TMP during the filtration process (Fig. 2). For the filtration-fouling tests with the single-fibre MF apparatus, the sludge suspension collected from the SMBR was kept at a SS concentration of 3 g/l. At a constant filtration flux, the membrane fouling rate shown by the TMP increase was reflective of the fouling propensity of the sludge samples. As all of the conditions were identical except for the amount of BPC added to the sludge mixture, the comparative results directly demonstrate the effect of BPC on membrane fouling, and clearly show that an increase in BPC concentration in the MBR sludge mixture led to a significant acceleration in membrane fouling during the sludge filtration. However, it should be pointed out that a fixed signal fibre MF membrane was used in the present study to determine the fouling rate during sludge filtration. The actual MBR situation is more complication with aeration and membrane fibre movement. The membrane fibre movement caused by aeration turbulence is expected to reduce the membrane fouling rate; however, the contact between membrane fibres in a membrane module would reduce the fluid shear over the membrane surface, worsening the fouling situation.
The control sludge (without extra BPC) had a background liquid-phase organic concentration $C_0$ of around 3.5 mg/l. A small BPC addition of $0.2C_0$ resulted in a notable increase in the membrane fouling rate (Fig. 2a), and a further addition of BPC beyond $0.6C_0$ increased the fouling rate dramatically. The membrane fouling rate during sludge filtration increased almost linearly with the BPC content in the sludge suspension (Fig. 3). The results of the well-controlled sludge filtration experiments thus prove that BPC are a crucial foulant in MBR systems. BPC are a group of organic solutes formed by the affinity clustering of soluble and colloidal substances on the membrane surface during MBR operation [11]. It is believed that large-sized BPC in the MBR sludge mixture function as a “glue” that facilitates sludge attachment and the formation of a fouling layer on the membrane surface [12–14].

3.2. Reduction of the membrane fouling rate by BPC ozonation

Ozonation was applied to the BPC solution before its addition into the sludge suspension. No residual ozone was found in the ozonated BPC solutions. At the ozone dose employed, which was less than 1 mg O$_3$/mg TOC of BPC, the amount of BPC removed was minimal, as shown below (Fig. 5 in Section 3.3) by the insignificant TOC reduction, but BPC destruction by ozonation was expected. The fouling test results demonstrate that ozonation can greatly reduce the detrimental effect of BPC on membrane fouling during sludge filtration. Upon ozonation of the BPC solution, the membrane fouling rates of the sludge–BPC mixtures decreased significantly compared with the identical test cases without ozonation (Fig. 2b). The average fouling mitigation efficiency by BPC ozonation was over 70% (Fig. 3). Thus, the ozonation of BPC may be an effective fouling control measure in SMBR systems.
After each filtration test, the fouling sludge layer on the single-fibre membrane was collected and analysed for the solid and BPC content to determine the average deposition rates of the solid matter and BPC on the membrane surface during sludge filtration. The results show that the deposition rates of both the solids and BPC decreased as the ozone dose applied to the BPC solution increased (Fig. 4). It should be noted that the biomass solids were the predominant foulant material (over 95%) in the fouling (cake) layer on the membrane surface. The BPC content in the sludge cake layer averaged around 14.2 mg TOC/g SS. The proportional deposition of BPC and suspended solids suggests that BPC function as the “glue” in cake layer formation. In comparison, BPC after ozonation apparently lost their “gluing” capability to a great extent. However, the effectiveness of ozonation in reducing foulant attachment on the membrane surface did not continue to increase with an increasing amount of ozone. This implies that the improvement of the sludge filterability by BPC ozonation has a limit. Fortunately, a small ozone dose is effective in reducing the fouling potential of sludge.

3.3. Destruction of BPC by ozonation

As stated previously, ozonation did not result in significant BPC oxidation or organic mineralization. The TOC concentration remained largely unchanged in the BPC solutions after ozonation at different doses (Fig. 5). Moreover, according to the chemical analysis, ozonation did not lead to a clear trend of change in the chemical composition of BPC in terms of the polysaccharide, protein and humic content (Fig. 5). Apparently, oxidation of the organic polymers by ozone at the doses applied did not reach the level of their component
units, that is, simple sugars for polysaccharides, amino acids for proteins and aliphatic or phenolic acids for humic substances. The main change brought about by ozonation appeared to be the breaking up of large BPC. This is demonstrated by the significant reduction in BPC size after ozonation. According to the particle size analysis (Fig. 6), a small ozone dosage of 0.03 mg/mg TOC of BPC decreased the volume-based mean size of BPC from 38 μm to 27 μm. As the ozone dose increased to 0.30 mg/mg TOC of BPC, the mean BPC size decreased to about 12 μm. However, further increases in the ozone dose resulted in little decrease in BPC size. This indicates that larger BPC are more vulnerable than small BPC to break-up by ozonation.

The breaking up of large BPC by ozonation was further confirmed by CLSM examination (Fig. 7). BPC could be well observed by staining their polysaccharide components with fluorescent ConA-TRITC. The CLSM images show that large BPC, many of which were larger than 50 μm, disintegrated into smaller BPC after ozonation at small ozone doses of 0.03 to 0.18 mg/mg TOC of BPC.

In addition to BPC break-up, ozonation at a low dose also altered the chemical properties of the BPC. Ozone is a selective oxidant that reacts faster with some chemicals or functional groups than with others [33]. Polymeric substances and their clusters contain several sites that are reactive to ozone. For example, the glycosidic bonds inherent in chains of polysaccharides can be easily cleaved by ozone attack, resulting in their breakdown into short-chain polysaccharides or oligosaccharides [34,35]. Some reactive sites that are located at the branches of polymeric substances are readily cut by ozonation from the main chains [36]. This leads not only to the fragmentation of BPC, but also the modification of their
The viscosity of the BPC solution decreased as a result of ozonation (Fig. 8). A high viscosity generally suggests a high fouling potential of the feed liquid [37,38]. The significantly higher viscosity of the BPC solution than water is probably due to the abundance of large BPC and their interaction. The decrease in viscosity of the BPC solution after ozonation thus not only reflects the breaking up of the BPC, but also implies the modification of their surface properties.

3.4. Effect of BPC destruction on membrane fouling control

Due to the size reduction and possible modification of the surface properties of BPC after ozonation, the cut-off ratio of the BPC by filtration decreased (Fig. 8). In other words, the portion of filterable BPC increased considerably after ozonation, although the total amount of BPC hardly changed. The cut-off ratio provides an indication of the fouling propensity of BPC dispersion [11], as the fouling resistance greatly depends on the amount of foulant deposition. A reduction in size leads to a decrease in BPC retention due to the steric effect [39], whereas surface property modification affects the gelling propensity of the polymeric substances [40].

An SMBR is an almost completely enclosed system that does not allow the overflow of loose sludge flocs or organic foulants from the system. As a result, fouling materials, including SMP, loose EPS and colloidal organics, accumulate in the bioreactors. Sludge filtration through a large membrane surface provides a unique condition for BPC formation from polymeric organic substances [11]. BPC within the sludge cake deposited on the
membrane greatly increase the filtration resistance of the cake layer. The detachment of BPC from the membrane by aeration turbulence brings BPC back into the sludge suspension, which in turn worsens the fouling potential of the sludge [12,13]. It is therefore desirable to remove or destruct BPC regularly in an MBR system.

Previous studies showed that direct sludge ozonation could practically reduce the membrane fouling rate in SMBR [19]. Huang and Wu [20] demonstrated that in continuous SMBR operation, ozonation of the bulk sludge at 0.25 mg O₃/g SS effectively controlled membrane fouling. The experimental findings of this study indicate that the underlying mechanism of ozonation for fouling minimisation reported in previous studies is probably the effective destruction of BPC by ozonation. In future MBR applications, a side-stream could be used with an intermediate sedimentation tank for simple liquid–solid separation to allow the ozonation of the supernatant alone to destroy BPC. The advantage of such an approach over direct ozonation of the entire bulk sludge mixture is that it maintains consistent membrane fouling alleviation whilst avoiding the damage of possible ozone overdoses on the biomass properties or MBR treatment performance.

4. Conclusions

- The single-fibre MF filtration system is a highly efficient testing device for the determination of the fouling propensity of MBR sludge samples with different BPC contents.
- Liquid-phase organic substances, particularly BPC, in MBR sludge suspension have a profound impact on the fouling potential of the sludge. A small increase in BPC
concentration by 20% and 60% from a background level of about 3.5 mg/l in the mixed sludge drastically increased the membrane fouling rate by 120% and 300%, respectively. 

- Ozonation of BPC is shown to be an effective means of controlling membrane fouling for MBR sludge. An ozone dose of only 0.18 mg/mg TOC of BPC can reduce the membrane fouling rate by up to 70%. Ozone is able to destruct large BPC and modify their surface properties, which increases the filterability of BPC and the sludge mixture. It appears that ozonation causes BPC to lose their “gluing” capability, thereby serving as an effective measure of membrane fouling mitigation.

- A side-stream approach may be developed that allows the ozonation of the BPC-containing supernatant before its return to the MBR. Such a technique can help control membrane fouling in MBRs whilst avoiding possible adverse effects on biomass properties and MBR treatment performance.

Acknowledgments

This research was supported by URC funding from the University of Hong Kong, Special Equipment Grant SEG_HKU10 from the University Grants Council (UGC) and Grant HKU7144/E07 from the Research Grants Council (RGC) of the Hong Kong SAR Government. The technical assistance of Mr Keith C. H. Wong is greatly appreciated.

References


Figure Captions:

**Fig. 1.** Schematic diagram of the single-fibre MF testing apparatus.

**Fig. 2.** Membrane fouling rate indicated by TMP increase during the filtration of the sludge (SS concentration = 3 g/l) with different BPC dose ratios: (a) BPC without ozonation; (b) BPC after ozonation at 0.18 mg O₃/mg TOC. C₀: background organic (TOC) concentration in the sludge suspension; C_{BPC}: TOC of the BPC added.

**Fig. 3.** Membrane fouling rate of the sludge during MF filtration as a function of the BPC content: comparison of the fouling effect between raw BPC and ozonated BPC.

**Fig. 4.** Changes in the sludge and BPC deposition rates in the fouling layer on the single-fibre membrane during sludge filtration as a function of the ozone dose applied to the BPC solution. The BPC dose ratio C_{BPC}/C₀ = 1.6.

**Fig. 5.** Comparison of the organic content and chemical composition of BPC before and after ozonation at different ozone doses.

**Fig. 6.** Change in (a) the size distribution and (b) the mean size of BPC after ozonation at different ozone doses.

**Fig. 7.** CLSM observation of BPC before and after ozonation: (a₁) and (a₂) before ozonation; (b₁) and (b₂) ozone dose ratio = 0.03 mg O₃/mg TOC; (c₁) and (c₂) ozone dose ratio = 0.18 mg O₃/mg TOC. (red: polysaccharides in the BPC; green: bacterial cells)

**Fig. 8.** Changes in the viscosity and cut-off ratio of the BPC solution after ozonation.
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