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Mechanism of biofouling mitigation on nanofiltration membrane by non-oxidizing biocide

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ABSTRACT

In the present study, the impact of non-oxidizing biocide (NOB) on the structure of biofilm formed on a nanofiltration (NF) membrane was investigated during an accelerated biofouling test. The results showed that although minimal toxic effect of NOB on suspended microbial growth was observed, NOB hindered attached biofilm growth on the membrane surface, thereby, retarded flux decline. This result was correlated with the decreased amount of extracellular polymeric substances (EPSs) in biofilm on the NF membrane surface in the presence of 5 mg/L of NOB. In addition, images taken by confocal laser scanning microscopy clearly showed that the number and density of microorganisms and the biofilm thickness significantly decreased on the fouled NF membrane with 5 mg/L of NOB compared with the control NF membrane. Therefore, it was concluded that the addition of NOB was effective at retarding attached biofilm growth on the NF membrane surfaces by suppressing the microbial activity as well as the secretion of EPS.

Keywords: Biofilm formation; Biofouling; Extracellular polymeric substances; Nanofiltration membrane; Non-oxidizing biocide

1. Introduction

With the increasing stringency of water quality criteria, nanofiltration (NF) membranes, one of the most recently developed pressure-driven membrane systems, are becoming a promising technology in advanced water treatment [1]. Since NF membranes typically possess pore size of 1 nm and negatively charged surface in aqueous solution [2], organic matters (e.g., natural organic matters), viruses, and divalent ions (e.g., Ca^{2+}, Mg^{2+}, and SO_4^{2-}) can be easily rejected [3]. Consequently, NF membrane processes have been frequently applied for groundwater softening, removal of potential disinfection by-products, and advanced water treatment for removal of contaminants in emerging concern [4]. However, membrane fouling, resulting in the rapid decline of the permeate flux or the increase of operational pressure, has been found to be the biggest limiting factor in the wide-spread application of NF membrane systems for water treatment [5].

Among classified four fouling mechanisms — colloidal fouling, biofouling, inorganic fouling, and organic fouling [6], biofouling caused by the formation of biofilm has long been considered a critical concern in fouling control [7]. Four steps are generally considered to be involved in sequential biofilm formation, including: (i) conditioning membrane surface by formation of a conditioning film; (ii) attachment of pioneer microorganism cells on surfaces; (iii) accumulation of microorganisms by irreversible adhesion via secretion of extracellular polymeric substances (EPSs); and (iv) subsequent development of mature biofilms [8]. During the step (iii), secreted EPSs are mostly composed with polysaccharides and proteins, and provide to the biofilm that allows increased resistance to dispersant and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the biofilm [9]. Hence, after the formation of thick EPS layer, extensive membrane cleaning methods such as dosing of chemical cleaning agents are often required to deteriorate biofilm on membrane

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surfaces. Thus, physical methods including modifications of membrane surfaces and optimization of pretreatment and operational parameters have been widely studied as they can prevent the conditioning and attachment of microbial cells on membrane surfaces [10,11].

As an alternative to above methods, inactivation of microorganism using various oxidizing chemicals are tested as pretreatment step of NF membrane processes. However, oxidizing chemicals such as HOCl, Cl_2, and H_2O_2 can cause significant oxidative damage in polyamide membranes, leading to decreased membrane life time [12]. In addition, these oxidizing chemicals can react with organic matter and produce carcinogenic agents as by-products.

Recently, stabilized halogens such as chlorosulfate and bromosulfate have been introduced as non-oxidizing biocides (NOBs) to reduce biofouling not only with no adverse effect on polyamide membrane structure but also with greater persistence of bactericidal effect. Moreover, stabilized halogens were not classified as toxic pollutants [13,14]. Accordingly, several studies reported the addition of NOBs to membrane systems to mitigate biofouling in water treatment [15–17]. Previous work also reported that NOBs can rapidly induce microbial growth inhibition by penetrating into biofilms about eight times faster than oxidizing chemicals [15,18]. However, there have been only a few studies on the changed morphology, composition, and binding structure of biofilms. In addition, the effects of NOBs on controlling fouling of NF membranes for water treatment are yet unclear.

The main goal of this study was to investigate the impact of halogenated NOB on the build-up and structure of biofilm during an accelerated biofouling test on an NF membrane system. In order to elucidate the mechanism of NOB on the mitigation of biofilm formation, microbial growth and toxicity tests, changes in the morphology and properties of biofilm on the membrane surface, and properties of EPS in biofilm were analyzed.

2. Materials and methods

2.1. Membrane biofouling experiments

As an NOB, a mixture of two stabilized halogens (chlorosulfamate [CAS no. 17172-27-9, $NaHClNO_3S$] and bromosulfamate [CAS no. 134509-56-1, $NaHBrNO_3S$]) was used as diluted solution.

Commercial polyamide NF membranes (NF90, DOW FilmTec, USA) were used to evaluate the mitigation of biofouling. The molecular weight cut-off of the membrane was 200 Da and the pure water permeability was 9.43 LMH/bar, determined using a cross-flow cell at 23°C ± 1°C. The effective area of each membrane in the cross-flow cell was 100 cm^2 (20 cm × 5 cm) and the cross-flow velocity of NF membrane system was adjusted to 7 cm/s. Water flux was calculated by measuring the weight of the membrane permeate over time using a digital balance (GF-4000, AND Co., Japan).

For the accelerated biofouling test, trisodium citrate (CAS no. 6132-04-3, $Na_3C_6H_5O_7$) and *Pseudomonas aeruginosa* PAO1 were added to the feed tanks at concentrations of 0.2 mM and 2.5×10^5/mL, respectively, after 2 h of stabilization with synthetic water medium as reported [10]. Then, prepared NOB (5 mg/L) was added to the feed tank, while the other NF membrane system was operated without addition of NOB. During the operation, the permeate was recycled to the feed tank.

2.2. Microbial inhibition and toxicity tests

The effect of NOB on the growth of microorganism was evaluated by exposing *E. coli* to NOB in solutions containing different concentrations of NOB. LB broth (1/10) diluted by 0.9% NaCl were inoculated with *E. coli* (2.5×10^7/mL) in the presence of NOB with concentrations of 0, 0.1, 1, 5, 10, 50, and 100 mg/L. Then, all flasks were placed in a shaking incubator controlled at 37°C and agitated at 150 rpm, and cell concentrations over time were monitored using optical density at 600 nm.

Microbial toxicity test of NOBs was conducted by live/dead cell test. *E. coli* cells (2.5×10^7/mL) were contacted with 5 mg/L of NOB in 0.9% NaCl for 30 min at 150 rpm. The cells were then stained with 3.34 mM SYTO 9 and 20 mM propidium iodide (Invitrogen, USA) and stained cells were observed via fluorescence microscopy (BX43, Olympus, Japan) [19].

2.3. Characterization of biofilm on membrane surfaces

After operating the NF membrane systems, the fouled membranes were removed and gently rinsed with PBS to discard unattached and loosely bound microbial cells. To examine the morphology of biofilm on NF membrane surfaces, confocal laser scanning microscopy (CLSM, Carl Zeiss, Germany) was used. For CLSM test, membrane coupons (1 cm × 1 cm) were placed in staining solutions with 13.4 μM SYTO 9 and 80 μM propidium iodide then kept in the dark for 15 min.

To evaluate the biofilm properties, the biomass weight and EPS contents of the fouled membranes were analyzed. The fouled membranes were cut into squares with dimensions of 25 cm^2 and the biofilm was scraped with a silicone spatula. Residue was then filtered with a preweighed 0.2 μm paper filter (Whatman, USA). After complete drying at 105°C for 2 h, the biomass weight was calculated. To extract EPSs in biofilm, the heat extraction method was chosen [20]. Considering carbohydrates and proteins as the major compounds of EPS, phenol-sulfuric acid method with glucose as the standard and Coomassie (Bradford) Protein Assay kit (Thermo Scientific, USA) was used to analyze the total protein content in the EPS, respectively [21].

3. Results and discussion

3.1. Effect of NOB on water flux decline

Changes in normalized water flux of NF membrane systems in the absence and presence of 5 mg/L NOB are shown in Fig. 1(a). As operation began, the water flux declined quickly for both membranes due to the accelerated operational condition. Until 6 h, the decrease in water flux of both membranes exhibited similar trend (89% ± 2% for the NF membrane with 5 mg/L of NOB and 87% ± 1% for the control NF membrane compared with initial water flux). With further operation, difference in flux declines due to the addition of NOB became significant. After 10 h of operation, the normalized water flux was reduced to 74% ± 4% with the absence of NOB, while the NF membrane with 5 mg/L of NOB maintained 81% ± 1% of initial flux. It was further found that 29.2 h were required to

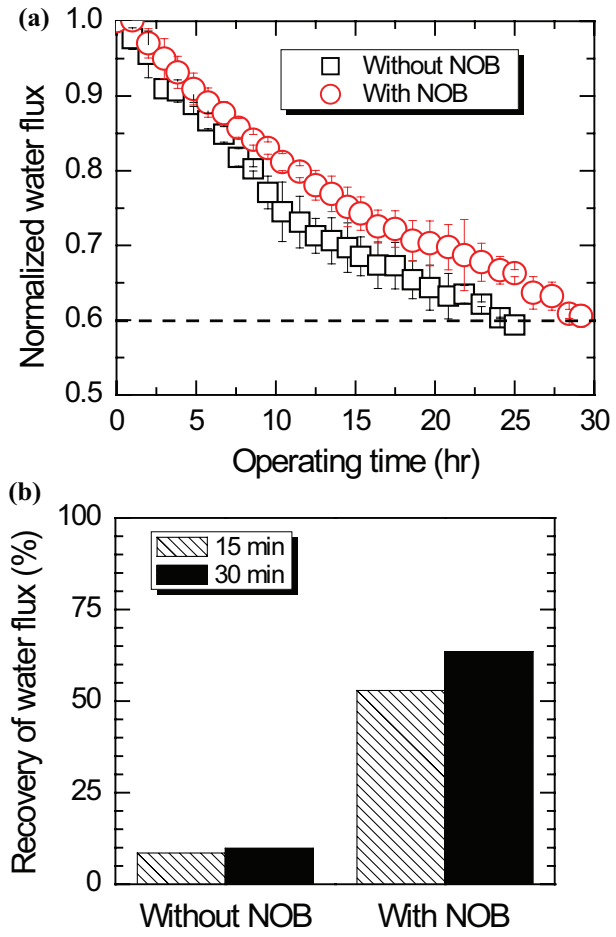

Fig. 1. (a) Change in normalized water flux of both NF membrane systems during accelerated biofouling test and (b) comparison of water flux recovery between two NF membranes after physical cleaning performed for 15 and 30 min.

reach the 60% of initial flux with the addition of NOB, while 25.0 h for the absence of NOB. These results correspond to those of previous studies, which have reported that NOB can retard biofouling [15].

To examine the integrity and strength of biofilm on each NF membrane surfaces, physical cleaning with cross-flow velocity of 10 cm/s was performed for 15 and 30 min (Fig. 1(b)). The recovery of NF membrane without NOB was only achieved 8.5% and 9.8% after 15 and 30 min of cleaning, respectively. Meanwhile, it was found that the water flux recovered in the NF membrane operated with 5 mg/L of NOB was reached 53.0% and 63.5% of initial water flux after 15 and 30 min of cleaning, respectively. These results indicate that the loose biofilm structure formed on the NF membrane surface in the presence of 5 mg/L of NOB. To further investigate the impact of NOB on the biofilm structure, characterizations of the biofilm properties were conducted.

3.2. Effect of NOB on microbial growth

To examine whether NOB could inhibit the growth of microorganism, and thus, lead to the retarded biofilm

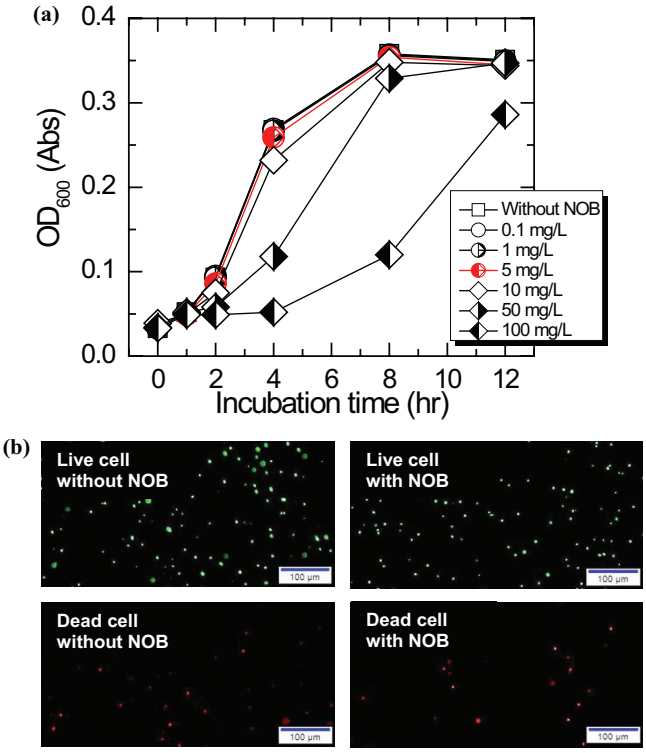

Fig. 2. (a) Effect of NOB on growth of microorganisms and (b) results of live/dead cell test analyzed by fluorescence microscopy.

growth, batch-type growth tests were conducted at various NOB concentrations (0–100 mg/L of NOB). As shown in Fig. 2(a), the inhibition of microbial growth by NOB was negligible up to 5 mg/L of NOB. In the case of the 10 mg/L NOB, there was a slight drop in microbial growth was observed and the inhibition became significant above 50 mg/L of NOB. Thus, considering the growth curves of *E. coli* in various NOB concentrations, the inhibition of microbial growth during the accelerated biofouling test might not induce the retarded flux decline with the addition of 5 mg/L of NOB.

Results of live/dead cell tests also confirmed that the addition of NOB had no toxic effect on the microorganisms as shown in Fig. 2(b). This result was again in agreement with results of previous studies, which reported that continuous low NOB dose rates can prevent toxic effects in microorganisms, while dosages of NOB about 200–300 mg/L can kill microorganisms [17].

3.3. Properties of biofilm

Fig. 3 shows the CLSM images for microbial density and biofilm thickness on the surface of NF membranes in the absence and presence of 5 mg/L NOB. Compared with the biofilm without NOB, the biofilm on the NF membrane with 5 mg/L of NOB had significantly lower microbial density and smaller thickness. Moreover, dense microbial aggregates were observed in the biofilm without NOB, but fewer microbial aggregates were found in mostly inactivated form (stained with red color) on the NF membrane with 5 mg/L of NOB. It implied that microorganisms which were continuously exposed to NOB exhibited suspended growth rather

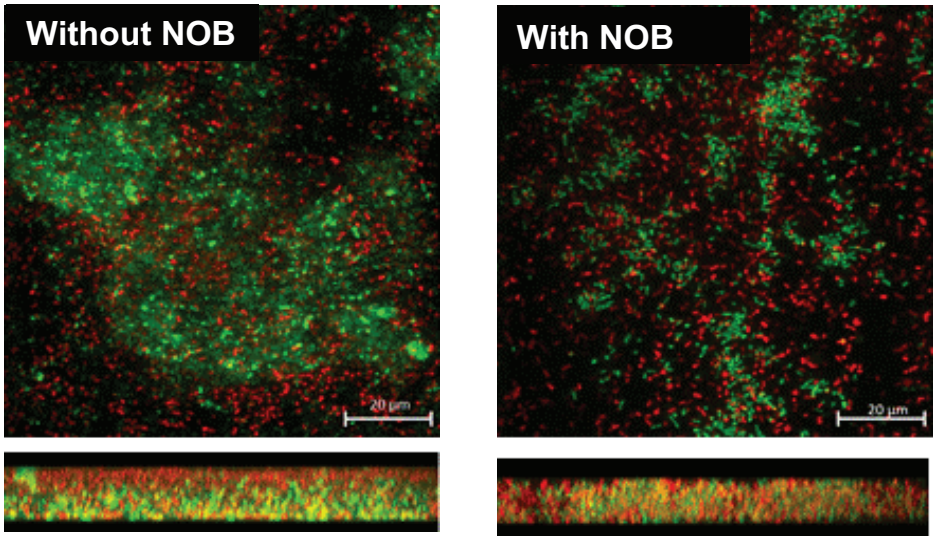

Fig. 3. Effect of NOB on microbial density and biofilm thickness on membrane surfaces analyzed by CLSM. Note that cells stained with green and red are intact and inactivated, respectively.

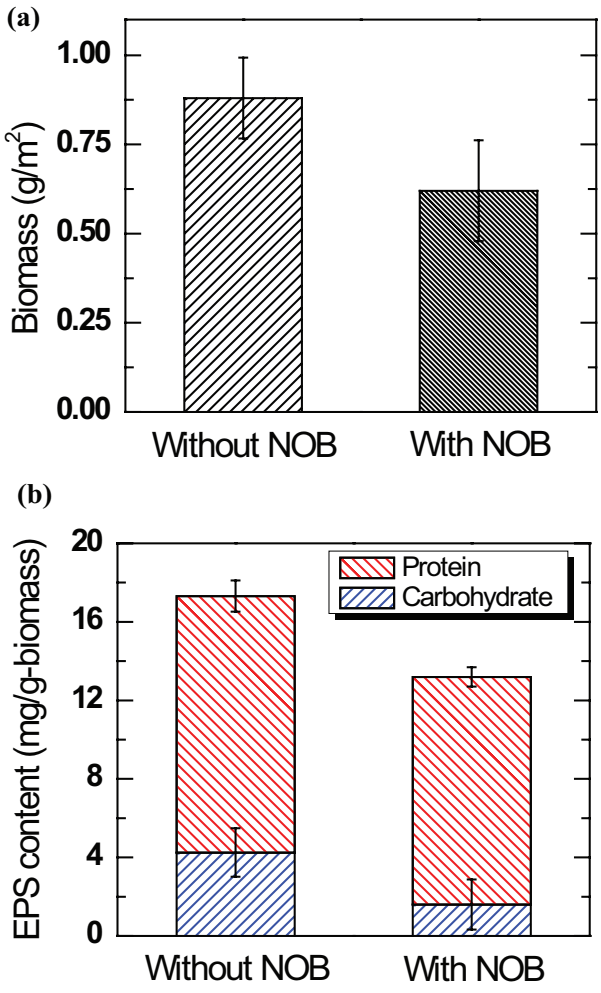

Fig. 4. (a) Mass of biomass and (b) EPS contents of biofilm taken from 25 cm² of fouled membranes with and without 5 mg/L of NOB.

than attached growth, and inactivated due to the long-term exposure to NOB as reported [15].

The measurement of the mass of biofilm on both NF membrane surface gave similar results as the microbial density and biofilm thickness shown in Fig. 3. The results showed that the total biomass were 0.88 ± 0.11 g/m² on NF membrane coupons in the absence of NOB, while the mass of biofilm was reduced to 0.62 ± 0.14 g/m² when 5 mg/L of NOB were added as shown in Fig. 4(a). It is in accordance with the previous observation that biofilm was less developed in the presence of 5 mg/L than that without NOB. In particular, the mass and composition of EPS provided important mechanistic explanations why the addition of NOB resulted the retarded biofilm growth and the weaker structural strength than those without NOB. As shown in Fig. 4(b), EPS contents were 13.20 and 17.31 mg/g-biomass in biofilm structures from NF membrane samples with and without 5 mg/L of NOB, respectively. Furthermore, it should be noted that the carbohydrate contents in the EPS were significantly lower in the case with NOB (1.60 ± 1.28 mg/g-biomass) than that without NOB (4.25 ± 1.24 mg/g-biomass), while the protein contents were similar (11.60 ± 0.49 and 13.06 ± 0.80 mg/g-biomass, respectively). It has been reported that EPS plays important roles in the biofilm structure [22], and carbohydrate fractions of EPS play critical roles in stabilizing the biofilm structure by forming a scaffold within the fruiting-body structure [23]. Consequently, these results corresponded to the results from Fig. 1(b), that the strength of biofilm was significantly weaker in the presence of NOB than without NOB due to the deficit of structural building block of biofilm such as carbohydrate-like EPS.

4. Conclusion

In this study, the impact of stabilized halogen as an NOB on the build-up and structure of biofilm was investigated by analyzing biofilm mass, morphology, and compositions using NF membrane system. Though NOB had no toxic effect

on microbial growth, the addition of NOB can effectively retard biofilm growth on NF membrane surfaces by suppressing both microbial activity and secretion of EPS. On the basis of the results obtained here, it is suggested that application of NOB such as stabilized halogens at low concentration can lead to reduced chemical cleaning frequency as well as increased membrane life time.

Acknowledgments

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