International Journal of Chemical, Materials and Biomolecular Sciences
ISSN: 2415-6620
Vol:10, No:1, 2016

Control of Biofilm Formation and Inorganic Particle Accumulation on Reverse Osmosis Membrane by Hypochlorite Washing

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***Abstract*—**Reverse osmosis (RO) membranes have been widely used for desalination to purify water for drinking and other purposes. Although at present most RO membranes have no resistance to chlorine, chlorine-resistant membranes are being developed. Therefore, direct chlorine treatment or chlorine washing will be an option in preventing biofouling on chlorine-resistant membranes. Furthermore, if particle accumulation control is possible by using chlorine washing, expensive pretreatment for particle removal can be removed or simplified. The objective of this study was to determine the effective hypochlorite washing condition required for controlling biofilm formation and inorganic particle accumulation on RO membrane in a continuous flow channel with RO membrane and spacer. In this study, direct chlorine washing was done by soaking fouled RO membranes in hypochlorite solution and fluorescence intensity was used to quantify biofilm on the membrane surface. After 48 h of soaking the membranes in high fouling potential waters, the fluorescence intensity decreased to 0 from 470 using the following washing conditions: 10 mg/L chlorine concentration, 2 times/d washing interval, and 30 min washing time. The chlorine concentration required to control biofilm formation decreased as the chlorine concentration (0.5–10 mg/L), the washing interval (1–4 times/d), or the washing time (1–30 min) increased. For the sample solutions used in the study, 10 mg/L chlorine concentration with 2 times/d interval, and 5 min washing time was required for biofilm control. The optimum chlorine washing conditions obtained from soaking experiments proved to be applicable also in controlling biofilm formation in continuous flow experiments. Moreover, chlorine washing employed in controlling biofilm with suspended particles resulted in lower amounts of organic (0.03 mg/cm^2) and inorganic (0.14 mg/cm^2) deposits on the membrane than that for sample water without chlorine washing (0.14 mg/cm^2 and 0.33 mg/cm^2, respectively). The amount of biofilm formed was 79% controlled by continuous washing with 10 mg/L of free chlorine concentration, and the inorganic accumulation amount decreased by 58% to levels similar to that of pure water with kaolin (0.17 mg/cm^2) as feed water. These results confirmed the acceleration of particle accumulation due to biofilm formation, and that the inhibition of biofilm growth can almost completely reduce further particle accumulation. In addition, effective hypochlorite washing condition which can control both biofilm formation and particle accumulation could be achieved.

***Keywords*—**Biofouling control, hypochlorite, reverse osmosis, washing condition optimization.

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I. Introduction

WATER demand has increased due to growth caused by increased population, industrial expansion, tourism, and agriculture development in many water-stressed or arid regions or countries [1]. Moreover, the global needs for water are expected to reach 6900 billion m^3 by the year 2030 which is about 150% increase from the demand in 2009 (4500 billion m^3) [2], [3]. Desalination is an increasingly common solution to supply fresh water in many regions of the world where this resource is scarce [4]. RO is the most widely used desalination technology globally for desalination to purify water for drinking and other purposes [4], [5], with RO technology accounting for 59.9% of the desalination amount in the world [6]. In 2012, the sources for desalination are split into about 58.9% from seawater, 21.2% from brackish groundwater sources, and the remaining percentage from surface water and saline wastewater [6]. Recently, secondary effluent water has been also used as feed water for RO membrane technology because of worldwide water shortage [7]–[9].

In membrane technology, membrane fouling is categorized into crystalline fouling including mineral scaling, organic fouling, particle and colloid fouling, and microbiological fouling or biofouling [10]. The first three types of fouling can generally be controlled by pretreatment for foulant removal from feed water [10]; however, despite silt density index (SDI), biological, and chemical parameters of feed water being within limits prescribed by RO membrane suppliers, severe membrane fouling can still occur, with fouling layers mostly organic and of biological origin with minor inorganic amounts [11]. An inevitable problem of RO membrane technology is biofouling, which is caused by adhesion and accumulation of microorganisms, followed by growth and formation of biofilms [10], [12]. Biofouling is characterized by the presence of a biofilm on the membrane leading to increase in resistance and decline in membrane performance, such as water permeation and rejection of solutes [13]. Microorganisms are present in nearly all water systems and are capable of colonizing almost any surface [14]. Moreover, spiral wound elements, which contain feed spacers to keep membrane sheets apart and create the flow channel, were used for RO in current industrial practice [15]. Deposition of biofilms or particles had been analyzed for different feed spacer orientations (diamond and ladder) [16], [17] and it has been reported that the position of initial particle deposition is qualitatively similar to biofouling [17]. If some particles contaminate the feed water or are carried over from pretreatment of the raw water to the RO feed,

biofilms may form around the spacer along with inorganic particles in the membrane elements [18].

Use of free chlorine has been considered as a promising and effective method for biofilm formation control. However, at present, most polyamide RO membranes, which have been primarily used for water recycling and desalination applications [19], [20], have no resistance to chlorine, since it has been pointed out that if free chlorine is not completely removed before the RO membrane filtration unit, residual free chlorine in the feed solution can cause the degradation of polyamide [21]–[25]. The pretreatment of feed solution with free chlorine has been widely used as a standard practice in current RO systems [14], [26]. For years, continuous chlorine treatment has been the industrial standard [27], [28], but intermittent chlorine treatment is also considered in recent years. In [29], when simulations of the required minimum chlorine concentration and injecting time of intermittent chlorine treatment obtained from experimental studies of the growth and sterilization rates of microorganisms in seawater of Middle East were done, intermittent chlorine treatment was found to be the most effective chlorine injection mode to achieve the best RO performance for desalination. The reduction of biofilm by continuous or intermittent shock chlorination of seawater for the pretreatment of RO system was also evaluated in other studies [30], [31]. In [30], the intermittent shock chlorination done every 15 days with 1 mg/L of residual free chlorine for 2 h of exposure time could not reduce biofilm formation, and improvement of the application method (higher frequency or different dosage) was recommended to prevent the development of the biofilms on the RO membranes [30]. It was suggested that the microorganisms, which could not be reduced completely during pretreatment, most likely formed biofilms on the RO membrane. In another study, it was reported that the biofilm formed by *Pseudomonas aeruginosa* PAO1 GFP on the RO membrane surface was inactivated by continuous chlorine treatment with 10 mg/L of initial chlorine concentration for 30 minutes [32]. The intermittent chlorine mode of treatment, which reduces biofilms with an exposure amount smaller than the continuous chlorine mode treatment is an effective mode of disinfection from the point of view of membrane degradation since it will be able to reduce biofilm formation with minimum membrane damage due to chlorine. However, despite many reports about the degradation of polyamide RO membrane by chlorine treatment, the reduction of biofilm on the RO membrane has not been examined in various conditions of chlorine treatment, and has not also been quantitatively evaluated.

Chlorine-resistant polyamide membranes are being developed to allow direct treatment with free chlorine and chloramines to avoid biofilm formation on the membrane [20], [23], [33]–[37], and are expected to be used in the near future. Therefore, direct chlorine treatment/washing will be an option to avoid biofouling on the chlorine-resistant membrane. Chlorine washing will have the capability of reducing biofilm formation thoroughly and will allow simplification of the conventional pretreatment, making it similar to disinfection methods of microorganisms in feed water. Furthermore, if particle accumulation control is possible by using chlorine washing, expensive pretreatment for particle removal will be removed or simplified. Thus, quantitatively understanding the control of biofilm formed and inorganic particle accumulated on the membrane surface by chlorine washing will be very important in designing and developing chlorine-resistant membranes.

In this study, the reduction of the biofilm formed on the RO membrane by hypochlorite was examined by optimization of various chlorine washing conditions: free chlorine concentration, washing frequency, and washing time, using a conventional polyamide RO membrane. Moreover, the reduction of inorganic particle accumulated with biofilm formation in a continuous flow channel with membrane and spacer was also examined.

II. Materials and Methods

A. Materials

Commercially available polyamide RO membrane NTR-759HR, provided by Nitto Denko Co. (Osaka, Japan) was used in the study. All virgin membranes were thoroughly washed with pure water first before use. A diamond type polypropylene spacer (3 × 3 mm mesh size) was also used in the study. *Bacillus subtilis* (JCM 2499, Riken, Japan) was used for enhanced microbial growth. Tryptic Soy Broth (TSB) was purchased from Merck (Darmstadt, Germany). McFarland Turbidity Standard was purchased from bioMérieux, Inc. (Marcy l'Etoile, France). Glucose ($C_6H_{12}O_6$), kaolin (5-10 μm), and sodium chloride (NaCl) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Sodium hypochlorite (NaOCl) (approx. 10% available chlorine) and Chicago Sky Blue 6B were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Other reagents were purchased as analytical grade from Kanto Chemical Co. Inc. (Tokyo, Japan). DPD (N,N-diethyl-p-phenylenediamine) was purchased for free chlorine analysis from Hanna Instruments Japan, Inc. (Chiba, Japan). Pure water was prepared using a Milli-Q Reference Ultrapure Water Purification System (Merck Millipore Corp., Darmstadt, Germany). A 0.85% NaCl solution was prepared from NaCl. Secondary effluent water discharged after activated sludge treatment and settling but before disinfection was taken from Higashi-Hiroshima Wastewater Treatment Plant, and then was filtered through a glass fiber filter (1.0 μm, GF/B, Whatman, UK). The filtered secondary effluent water was used for all experiments for biofilm formation.

B. Bacteria Stock Preparation

Bacillus subtilis has emerged as an alternative model organism for studying the molecular basis of biofilm formation [38]. It has also been found to be one of the many bacterial species that participate in biofouling on RO membranes [14], [39], and thus has been used in studies that involve seawater RO membrane biofouling [40] and in the development of antibacterial polyamide RO membranes [41], and thus the use of *B. subtilis* is acceptable for enhancing biofilm development

on the membrane surface. The bacterial stock was prepared by growing overnight cultures in 3% Tryptic Soy Broth with shaking at 45 rpm at 37 °C for 24 h. The bacterial cells were recovered by centrifugation at 2,000 rpm for 5 min and washed for at least three times with 0.85% NaCl. The recovered bacterial suspension was resuspended in 0.85% NaCl to achieve an optical density of 0.4–0.5 at 550 nm using spectrophotometer (UV-1800, Shimadzu Co., Kyoto, Japan), resulting to a bacterial concentration in the range of 3–6 × 10^8 cfu/mL in the stock mixture, indicated by the McFarland Turbidity Standard. This stock of *B. subtilis* suspension was then added to the sample waters with the necessary dilutions required by the experiment.

C. Biofilm Formation Reduction by Soaking Test

Reduction of biofilm formation on the RO membrane surface was conducted by soaking test with continuous or intermittent chlorine washing of the membrane. The membrane was cut using sterile scissors into 20 × 20 mm pieces, and was washed with pure water. To enhance biofilm formation potential on the membrane surface, the filtered secondary effluent water was added with 1 mM of glucose and/or 3–6 × 10^7 cfu/mL of *B. subtilis*, and was used as soaking solution for all experiments. The membranes were soaked in the soaking solution, and then the biofilm was allowed to form at 37 °C in the dark with shaking at 45 rpm for 48 h. The chlorine washing for reduction of biofilm formation was continuously or intermittently conducted using aqueous sodium hypochlorite solution. In continuous washing, the soaking solution used was added with hypochlorite with free chlorine concentration maintained at 1.1 ± 0.2 mg/L. The soaking solution was replaced with fresh every 12 h. In intermittent washing, different conditions for chlorine washing were devised: the residual free chlorine concentration in the soaking solution, the washing frequency, and the washing time. For concentration studies, the residual free chlorine concentrations of the secondary effluent water were within 0.5–10 mg/L, and the free chlorine concentration under study was maintained during the washing. For washing frequency and washing time, the soaking solution was replaced with secondary effluent water added with hypochlorite every 6, 12, and 24 h, and then the membranes were exposed to the water with free chlorine for 1–30 minutes, respectively. After the membrane was chlorine washed, the secondary effluent water with free chlorine was replaced with fresh soaking solution. As control washing experiment, the membrane was exposed to the secondary effluent water without free chlorine, and then was replaced with fresh soaking solution. Biofilm formed on the membrane, before and after the chlorine washing, was determined by fluorescence intensity analysis.

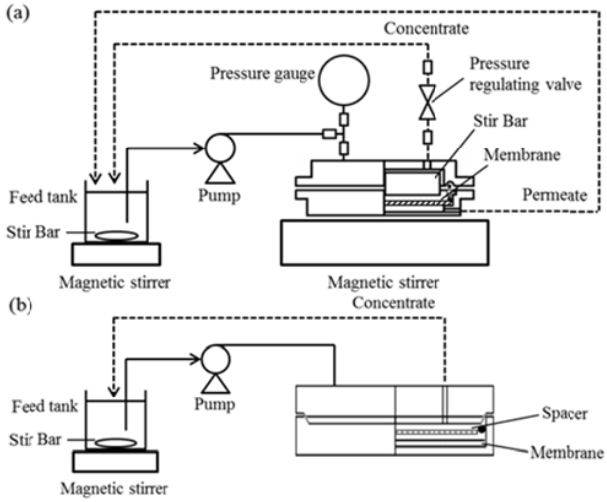

Fig. 1 Schematics of test cells for continuous test: (a) Cross-flow test cell with permeate, (b) Cross-flow test cell without permeate

D. Biofilm Formation Reduction by Continuous Flow Test

To confirm the validity of the chlorine washing condition suggested by the soaking test, the reduction of biofilm formation on the RO membrane surface was conducted by continuous flow test with intermittent chlorine washing. A schematic diagram of a cross-flow test cell unit with permeation is presented in Fig. 1 (a). The stainless steel continuous cross-flow RO test cell (spin flow cell; Tritec Co., Ltd., Tokyo, Japan) with internal diameter of 75 mm and equipped with a pump (KP-22, Flom, Tokyo, Japan) was used. The filtered secondary effluent water was used as feed water, and was adjusted to pH 6.0 using HCl and NaOH during the operation. The membrane was then placed inside the RO test cell, and the test cell was operated by running feed water with stirring at a flow rate of 50 mL/min (initial flux 19.3 $m^3/m/^2d$), operating pressure of 1.5 MPa, and at 25°C for 72 h. The effective filtration area of the operating membrane was 37.4 cm^2. Concentrate and permeate were disposed during the initial 4 minutes (200 mL), and then were circulated into the feed tank. The secondary effluent water with free chlorine was intermittently flowed for 30 minutes of washing time every 12 h to reduce biofilm formation on the membrane. The residual free chlorine concentrations in the secondary effluent water were within 3–10 mg/L, and the free chlorine concentration under study was maintained by adding hypochlorite solution during the washing.

E. Reduction of Inorganic Particle Accumulation with Biofilm Formation

To evaluate the reduction of inorganic particle accumulation attributed by the biofilm formation on the membrane surface and spacer, during continuous flow experiments, continuous chlorine washing was employed to a membrane with biofilm and particle deposited. A schematic diagram of a cross-flow test cell without permeation is presented in Fig. 1 (b). The acrylic test flow cell with internal space to put the polyamide RO membrane (45 × 90 mm) and the spacer and equipped with

a pump (DSP-100SA, AS ONE Co., Osaka, Japan) was used. To determine the accumulation of an inorganic particle quantitatively with and without hypochlorite, filtered secondary effluent water added with 1 mM of glucose and 3–6 × 10^7 cfu/mL *B. subtilis* to enhance biofilm formation and added with 300 mg/L of kaolin, was used as feed water. The membrane and then a spacer on top of the membrane were then placed inside the test flow cell, and the test flow cell was operated by running feed water at a flow rate of 30 mL/min and at 25 °C for 24 h without pressure. The biofilm and the inorganic particle were allowed to form and deposit/accumulate on the membrane and spacer. The effective filtration area of the operating membrane was 40.5 cm^2. The chlorine washing was continuously conducted, and during the washing, the residual chlorine concentration in the secondary effluent water was maintained at 10.1 ± 1.6 mg/L.

To detect the presence of the kaolin particles, the membrane and spacer with biofilm and inorganic particles were stained by running Chicago Sky Blue 6B solution through the test flow cell, and then images of the membrane and spacer surface were taken using a digital microscope (Dino-Lite Basic DINOAM2001, Thanko Co., Tokyo, Japan) to analyze the site for biofilm formation and inorganic particles deposition. Light microscopic images were taken at 20 × magnifications at 0 h and 24 h. To detect biofilm formation, the membrane and spacer with biofilm and inorganic particles were taken out of the test flow cell, carefully cut into 20 × 20 mm pieces, and then were stained with SYTO 9 using the method described in Section II *F*. The green fluorescence from the microorganisms present on the stained membrane and spacer was viewed using a fluorescence microscope which is an upright microscope (CX-41, Olympus Co., Tokyo, Japan) equipped with a power supply unit (U-RFLT50, Olympus Co., Tokyo, Japan). Images were obtained to detect biofilm formation using a camera (EOS Kiss X50, Canon Inc., Tokyo, Japan) which is attached to the fluorescence microscope. Accumulated particle deposits on the membrane were determined through loss on ignition (LOI) tests. Pure water was filtered through a glass fiber filter (1.0 μm, GF/B) as rinsing, and then the filter was dried for 1 h at 110°C. The dried filter is then burned to 550°C for 30 minutes to get the mass of dried and ignited filter (m_1). Membrane and spacer were carefully removed from the test flow cell after each experiment, and the spacer was also carefully removed from the membrane top. The accumulated mass on the membrane surface was thoroughly removed from the membrane surface by rinsing and brushing using nylon toothbrush (Lion Co., Japan) in pure water. The resulting water was then filtered in a pre-weighed GF/B filter. The collected mass and filter was then dried for 1 h at 110°C, and then weighed (m_2). Total mass was calculated as m_1 mass subtracted from m_2 mass. The dried mass and filter was further ignited to 550°C and then weighed (m_3) to obtain inorganic mass. Inorganic mass was calculated as m_1 mass subtracted from m_3 mass, and organic mass was calculated as inorganic mass subtracted from total mass.

F. SYTO 9 Staining Procedure and Fluorescence Analysis

After soaking or filtration experiments, the membranes were retrieved, and then subjected for staining. The green dye, SYTO 9 from the BacLightTM Bacterial Viability Kit L13152 (Invitrogen/Molecular Probes, USA) stains both live and dead cells with a fluorescent green color. The SYTO 9 solution was prepared according to manufacturer specifications, and was kept in the dark and inside the refrigerator until analysis. A 20 × 20 mm piece of the membrane was stained with 100 μL of SYTO 9 for 30 minutes in the dark, and then the excess dye was carefully removed from the membrane by pipetting.

Amount of biofilm formed on the dyed membranes is quantified by fluorescence intensity (FI). The FI was analyzed using a microplate reader (Gemini EM, Molocular Devices Japan Co., Tokyo, Japan) with SoftMax®Pro Microplate Microplate Data Acquisition & Analysis Software with Excitation scan set at 485 nm and Emission scan set at 545 nm. The FI values for 144 points per membrane were analyzed. Averages of FI values for each membrane were then calculated. The FI values are then reported as the averages of 3 replicate membranes for soaking tests or the averages of 6 cut pieces from the membrane used in continuous filtration tests, and the precision reported as standard deviations for n = 3 or 6 membranes. The ΔFI, which indicates the amount of biofilm remaining, was calculated as the FI of the virgin membrane subtracted from the FI of the membrane with biofilm formed.

III. Results and Discussion

A. Biofilm Formation Control by Continuous Washing and Intermittent Washing

Behavior of biofilm formation (ΔFI) by continuous washing with 1 mg/L of residual free chlorine and intermittent washing every 12 h with 1 and 10 mg/L of residual free chlorine are shown in Fig. 2. The biofilm formation on the RO membrane without chlorine washing increased with soaking time, and ΔFI after 48 h was 440. The ΔFI by continuous washing with 1.1 ± 0.2 mg/L of free chlorine decreased every sampling, and was 12 (97% reduction) after 48 h. According to [42], a residual free chlorine concentration of 0.5–1.0 mg/L should be maintained throughout the whole pretreatment for disinfection of microorganisms. This observation could explain such result during continuous chlorine washing. On the other hand, the ΔFI by intermittent washing every 12 h was 454, and was the same for the control experiment, showing very low biofilm control, which is in agreement with another study where continuous chlorination enables better reduction of total aerobic bacteria than intermittent chlorination [30]. The intermittent chlorine washing reduces the biofilm by removing the biofilm that has formed on the membrane. The microorganisms and biofilm fragments, which had detached from the biofilm during intermittent chlorine washing might have resulted in an increased biofilm formation on RO membrane [30] due to the biofilm fragments as nutrient source to support biofilm growth [12]. The intermittent washing with 1 mg/L of the free chlorine, in this case, seemed to be an extremely insufficient condition, which aids instead of reduce biofilm formation. However, in the intermittent washing with 10 mg/L of the free chlorine, the ΔFI was 0 (100% controlled), and reduced the biofilm

formation completely. The CT value in the continuous washing with 1 mg/L of the free chlorine for 1 day, which is calculated as the product of free chlorine concentration and the washing time, was 24 mg·h/L. On the other hand, CT values in the intermittent washing for 1 day were 1 mg·h/L for 1 mg/L of free chlorine and 10 mg·h/L for 10 mg/L of free chlorine, respectively. These values suggest that intermittent washing conditions which can reduce the biofilm formation with small CT value is more beneficial than the continuous washing, since it reduces the risk of membrane degradation by chlorine due to the low CT value.

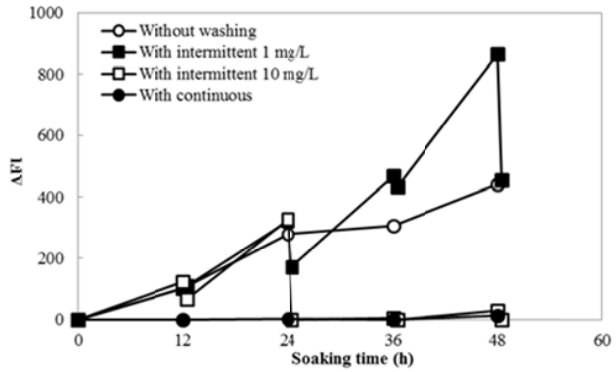

Fig. 2 Behaviors of biofilm formation during continuous washing with 1 mg/L of residual free chlorine concentration and intermittent washing every 12 h

B. Optimization of Washing Condition for Biofilm on the Membrane

The optimization of washing condition for the intermittent chlorine washing was evaluated to reduce the biofilm formation completely and efficiently. The ΔFI after 48 h was evaluated to determine the effect of the different conditions during intermittent washing. The amounts of biofilm remaining using intermittent chlorine washing under different conditions are shown in Fig. 3. The washing conditions constitute 0.5–10 mg/L of free chlorine concentration, every 6, 12, and 24 h of washing frequency, and 1–30 min of washing time. ΔFI after 48 h for the control experiments (without free chlorine) with replacement of solutions at 6, 12, and 24 h were 701, 440, and 338, respectively. Fig. 3 (a) shows the effect of free chlorine concentration and washing frequency to the reduction of biofilm formation. Results show that the ΔFI with 30 min of washing time decreased as the free chlorine concentration increased in all washing frequencies employed, indicating that there is concentration dependence existing between reduction of biofilm formed on the membrane and the free chlorine amount. In particular, for every 12 h of washing frequency, ΔFI for 3 mg/L of free chlorine remarkably decreased to 146, compared to that for 2 mg/L of free chlorine (396). The intermittent chlorine washing using 1 and 2 mg/L of free chlorine are shown to be insufficient, resulting in an increase of biofilm formation after 48 h. In particular, at chlorine concentration of 2 mg/L, the ΔFI at 6, 12, and 24 h was 46.8, 396, and 423, respectively. The biofilm formation decreased as the washing frequency increased, and every 6 h of washing frequency indicated the highest reduction effect, which was 93%. After 6 and 12 h, ΔFI values of control experiment for every 6 h of washing frequency were 18 and 129, respectively (data not shown). Every 6 h of washing frequency could reduce the biofilm most effectively, because the free chlorine could attack microorganisms before or during the early stages of biofilm formation on the membrane. Based on these results, in order to reduce biofilm formation to more than 90% during intermittent washing, the necessary residual chorine concentrations were less than 2 mg/L for every 6 h of washing, and more than 10 mg/L for every 12 and 24 h washing. On the other hand, the minimum washing conditions, which could reduce the biofilm by 100% on RO membrane were 10 mg/L of free chlorine concentration and every 12 h of washing frequency.

Every 12 h of washing frequency was then selected and then CT value was used to evaluate the optimum washing condition of residual free chlorine concentration and washing time for reduction of biofilm formation (Fig. 3 (b)). ΔFI decreased as the CT value increased. For 48 h, the 90% reduction of biofilm formed on the membrane could be achieved using 5 mg/L of free chlorine and 5 min of washing time, with 1.7–20 mg·h/L CT values required to reduce the biofilm by 90%. These CT values were calculated from 5–20 mg/L of free chlorine and 3–30 min of washing time. Based on this, 10 mg/L of free chlorine concentration, washing frequency of every 12 h, and 5 min of washing time can be suggested as the optimum conditions for intermittent chlorine washing to sufficiently reduce the biofilm formed on the RO membrane, with a calculated CT value of 0.07 mg/L for 1 h. For general use, the polyamide membranes are designed to be exposed to maximum chlorine concentration of less than 0.1 mg/L [43], [44]. The free chlorine concentration required suggested by this study for intermittent washing for reduction of biofilm formation was within this recommended application conditions for the polyamide membrane, and thus the direct intermittent washing condition can be applied for conventional membranes. Membrane lifetime has been estimated as 3–5 years for continuous operation, depending on the feed stream characteristics and the operating conditions [45]–[47], and the general maximum chlorine resistance of the polyamide RO membrane is calculated to be 2,628 mg·h/L for 3 years of membrane lifetime. Using the suggested intermittent washing condition (CT = 1.7 mg·h/L for 1 day), the membrane lifetime allowed for direct chlorine washing was estimated to be 1,546 days. Furthermore, in order to achieve the general membrane lifetime of 3–5 years, CT values based on the conditions suggested by this study were 1,825–3,042 mg · h/L, which are within the allowed chlorine resistance values for the polyamide RO membrane.

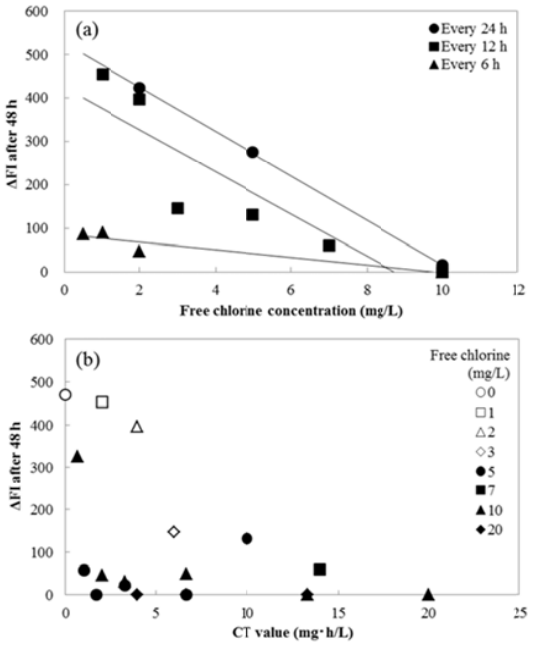

Fig. 3 Amount of biofilm remaining after 48 h during intermittent chlorine washing at different washing conditions; Washing conditions are 0.5–10 mg/L of free chlorine concentration, every 6 h, 12 h, and 24 h of washing frequency, and 1–30 minutes of washing time: (a) examination of free chlorine concentration and washing frequency with 30 minutes of constant washing time, (b) examination of free chlorine concentration and washing time with every 12 h of constant washing frequency

C. Confirmation of Validity of Optimized Washing Condition

To confirm the validity of the condition of intermittent chlorine washing based from the soaking test, the amount of biofilm remaining was evaluated by continuous filtration test with intermittent chlorine washing. The variations of amount of biofilm remaining after 72 h are shown in Fig. 4. Continuous filtration was conducted with 3–10 mg/L of chlorine concentration, 30 min of washing time, and every 12 h of washing frequency for 72h. ΔFI of control (without the free chlorine) in the continuous filtration test was 1,791, which was 3.8 times higher than that in the soaking test (470). This can be explained by easier biofilm formation in the continuous filtration test because the membrane surface was always subjected to pressure and velocity, enhancing contact between microorganisms in the feed water and the membrane surface. Unlike the results in the soaking test wherein there was significant biofilm control at 3 mg/L free chlorine concentration, in the continuous filtration experiments, the ΔFI hardly reduced at 3 mg/L of free chlorine concentration, and a marked decrease was observed at 4 mg/L of free chlorine concentration. However, ΔFI at 4 mg/L of free chlorine was still 142.8 after 72 h, and is expected to increase upon continued filtration since any remaining bacteria can feed on the dead cells [12]. Results showed that the biofilm formed on the membrane was completely reduced at greater than 5 mg/L of free chlorine concentration. The reduction of biofilm formation in the continuous filtration test required a free chlorine concentration that is lower than that in the soaking test because the membrane surface is continuously exposed to the chlorine washing due to the applied pressure and flow of the washing solution. However, biofilm that is remaining due to insufficient washing can still accumulate with the extension of operating time. Based on these results, for intermittent washing, 5 mg/L, although of lower concentration than what was suggested by the soaking test, is a sufficient free chlorine concentration that could reduce the biofilm formed on the RO membrane during continuous filtration operations. In this intermittent chlorine washing, the CT value for 30 min is 5 mg·h/L for 1 day, which was higher than the optimum washing condition suggested by the soaking test (1.7 mg·h/L). Thus, shorter washing time should be examined in future continuous filtration tests.

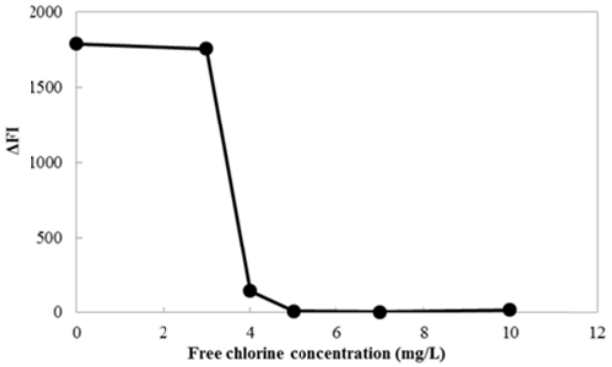

Fig. 4 The variation of the amount of biofilm remaining during intermittent chlorine washing in the continuous filtration test for 72 h

D. Reduction of Inorganic Particle Accumulation with Biofilm Formation

The continuous chlorine washing was conducted to evaluate the reduction of the accumulation of inorganic particles with biofilm formation on the membrane and spacer. The light images and fluorescence images of the membrane and spacer after 24 h with biofilm formed and accumulated kaolin with or without the chlorine washing are depicted in Fig. 5. The micrographs show that without free chlorine conditions, the accumulated kaolin, which was stained by a blue dye was observed mainly around the spacer, and the fluorescent green color from the biofilm formed was also observed mainly around the spacer. On the other hand, the presence of biofilm and kaolin on the membrane and spacer was not observed in the condition with 10.1 ± 1.6 mg/L of free chlorine. This suggests that the accumulation of inorganic particles on the membrane and spacer was reduced by the continuous chlorine washing along with the reduction of the biofilm formed.

The reduction of the inorganic particles accumulation and biofilm formation was also quantitatively evaluated. After 24 h, the amounts of organic and inorganic materials deposited on the membrane and spacer in the control experiment (pure water and 300 mg/L kaolin, without free chlorine) were 0 and 0.17 mg/cm^2, respectively. For secondary effluent water added with glucose, *B. subtilis*, and 300 mg/L kaolin, the amounts of

organic and inorganic materials deposited were 0.14 and 0.33 mg/cm^2, respectively; indicating an amount of 0.16 mg/cm^2 increase in inorganic material deposited due to biofilm formation on the membrane and spacer. It can be concluded that biofilm formation aided the accumulation of inorganic particle from the feed water on the membrane and spacer. The continuous chlorine washing with free chlorine concentration ranging from 10.1 ± 1.6 mg/L, resulted in 0.11 mg/cm^2 (79%) and 0.19 mg/cm^2 (58%) reduction in the amount of organic and inorganic material deposited. The organic and inorganic amounts deposited (0.03 and 0.14 mg/cm^2, respectively) after 24 h with chlorine washing were reduced to the same levels as those of the control experiment (without the free chlorine). These results confirmed the role of biofilm on the accumulation of inorganic particles. The results also indicate that chlorine washing can essentially reduce the accumulation of not only the biofilm but also the inorganic suspended particles.

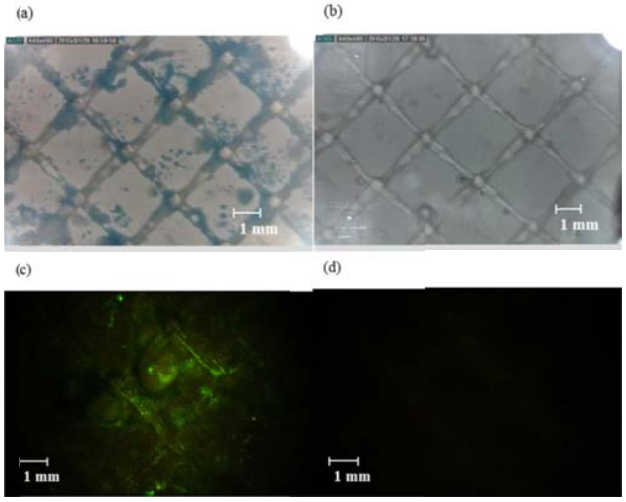

Fig. 5 Light images and fluorescence images of the membrane and spacer after 24 h which was formed the biofilm and accumulated the kaolin with or without the chlorine washing: (a) and (b) light images of the membrane and spacer, (c) and (d) fluorescence images of the membrane and spacer, (a) and (c) the membrane and spacer without the chlorine washing, (b) and (d) the membrane and spacer with the chlorine washing

IV. Conclusion

The reduction of biofilm formed on the RO membrane using hypochlorite was examined by optimization of the various chlorine washing condition using a conventional polyamide membrane. Intermittent washing which can reduce biofilm formation with a small CT value is more useful than continuous washing. The suggested optimum condition to sufficiently reduce the biofilm formed on the RO membrane for the intermittent chlorine washing is 10 mg/L of free chlorine concentration, washing frequency of every 12 h, and 5 min of washing time (CT = 0.07 mg/L for 1 h). The free chlorine concentration required for intermittent washing was found to be within the allowable application condition for conventional polyamide membrane. Moreover, based from the conditions suggested by this study, CT values that can be applied to achieve the usual membrane lifetime expected for RO membranes were also estimated, and were found to be within the chlorine resistance values allowed for polyamide RO membrane. Reduction of biofilm formation in the continuous filtration test required a free chlorine concentration lower than that from the soaking test. Furthermore, the reduction of inorganic particles, which accumulated with biofilm formation on the membrane and spacer in a continuous flow channel, was also examined. The accumulated inorganic particles and biofilm formed, which were observed mainly around the spacer, were reduced by continuous chlorine washing. The results indicate chlorine washing can essentially reduce the accumulation of not only the biofilm but also the inorganic suspended particles.

Acknowledgments

This study is supported by CREST (Core Research for Evolutionary Science and Technology) under the project Development of ROBUST reverse osmosis (RO)/nanofiltration (NF) membranes for various types of water resources.

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