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Impact of isolated dissolved organic fractions from seawater on biofouling in reverse osmosis (RO) desalination process

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ABSTRACT

The biofouling potential of three isolated dissolved organic fractions from seawater according to their molecular weights (MWs), namely, fractions of biopolymers (F.BP, MW > 1000 Da), humic substances and building blocks (F.HS&BB, MW 350–1000 Da), and low molecular weight compounds (F.LMW, MW < 350 Da) were characterized by assimilable organic carbon (AOC) content. The AOC/DOC ratio was in the order of F.LMW (~35%) > F.BP (~19%) > F.HS&BB (~8%); AOC/DOC of seawater was ~20%; organic compositions of seawater were BP ~6%, HS&BB ~52% and LMW ~42%; LMW accounted for >70% of AOC in seawater. Their impact on SWRO biofouling in term of flux decline rate was in the order of F. LMW (~30%) > F.BP (~20%) > F.HS&BB (<10%). Despite being the major organic compound in seawater, HS&BB showed marginal effect on biofouling. The role of indigenous BP was less critical owing to its relatively low concentration. LMW, which was the major AOC contributor, played a significant role in biofouling by promoting microbial growth that contributed to the build-up of soluble microbial products and exopolymeric substances (i.e., in particular BP). Therefore, seawater pretreatment shall focus on the removal of AOC (i.e., LMW) rather than the removal of biopolymer.

1. Introduction

Seawater reverse osmosis (SWRO) desalination technology has been widely employed for the production of fresh water for domestic, agriculture and industrial use (Lattemann and Höpner, 2008). However, the bottleneck of SWRO technology is RO membrane fouling, in particular biofouling, which eventually leads to significant flux decline or increase in applied pressure, increase in energy consumption, higher chemical usage, and shortens the membrane lifetime. In general, the mechanisms of biofilm formation can be categorized into four successive stages: (i) the attachment of organic matter onto the membrane surface, leading to the formation of a conditioning film that facilitates the subsequent bacteria attachment; (ii) the deposition of microorganisms onto the membrane surface; (iii) proliferation and production of exopolymeric substances (EPS) and soluble microbial products (SMP) to form a mature biofilm; and (iv) dispersal of biofilm, where cells and organic matter are released from the biofilm into the environment (Barker and Stuckey, 1999; Flemming, 2011).

Undoubtedly, the biofilm formation on RO membrane is closely associated with the organic matter in RO feed water. For instance, bacteria consume organic matter in RO feed water to proliferate; EPS are secreted by bacteria cells; SMP are released from metabolism of feed organic matter (i.e., utilization associated products, UAP) as well as during cell lysis and hydrolysis of EPS (i.e., biomass associated products, BAP); a portion of the SMP could be easily utilized by bacteria (Kunacheva and Stuckey, 2014). As raw seawater will undergo pretreatment processes to remove the particulate matter prior RO process, particulate matter are not considered in this study, instead, the focus is only on the dissolved matter in seawater. In general, dissolved organic matter (DOM) is defined as the organic matter that can pass through or is not retained by a 0.45 μm filter. Dissolved organic carbon (DOC), a common parameter used to indicate DOM, is measured by the total organic carbon (TOC) method after the sample is filtered through a

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0.45 μm filter. It can be further categorized into biopolymer (BP, molecular weight, MW > 1000 Da), humic substances and building blocks (HS&BB, MW 350−1000 Da), and low molecular weight compounds (LMW, MW < 350 Da) by liquid chromatography-organic carbon detection (LC-OCD) analysis (Huber et al., 2011; Yin et al., 2019). Biodegradable organic matter or biodegradable dissolved organic carbon (BOM or BDOC) is defined as the DOM or DOC that can be mineralized by heterotrophic microorganisms; while assimilable organic carbon (AOC) is that portion of BDOC that can be readily utilized to support microbial growth (Huck, 1990; Wang et al., 2014). Over the years, AOC measurement has been widely applied as a surrogate to predict the biofouling potential of various types of water in various water treatment processes (Terry and Summers, 2018; Water and Solutions 2005; Weinrich et al., 2016) including seawater applications (Dow Water and Process Solutions, 2005; Jeong et al., 2013; Weinrich et al., 2016). The AOC measurement method based on colony forming units was first proposed by Van der Kooij (van der Kooij et al., 1982), and later improved by other researchers to enhance its accuracy and efficiency (Kaplan et al., 1993; LeChevallier et al., 1993; van der Kooij et al., 1982). In addition, flow cytometry instrument has been used to measure the AOC rapidly by counting the true volumetric cells in the solution (Elhadidy et al., 2016; Hammes and Egli, 2005). Assessing the AOC concentration in SWRO process is critical as it has been reported that after pretreatment processes of raw seawater, the RO feed water still have high biofouling potential as surviving cells can proliferate by consuming the residual biodegradable substances (Matin et al., 2011). For example, organic molecules (MW < 1000 Da) in water that has undergone (or been pretreated) using coagulation or ultrafiltration showed a strong relationship with AOC content (Hem and Efraimsen, 2001). In addition, it was reported that an increase in AOC level was associated with excessive chemical dosing (i.e., chlorination and then dechlorination with sodium bisulfite), fluctuation of water quality (i.e., algal blooms) and organic oxidation (Weinrich et al., 2016). Furthermore, study also showed that NF permeate with LMW compounds (MW < 300 Da) such as acetate and with very low DOC amount could still be preferentially consumed by the bacteria (Meylan et al., 2007).

Despite large amount of research work on organic fouling and biofouling in RO process, the role of organic compositions in seawater on SWRO biofouling remains unclear, more importantly the interplay between them. Most of previous studies used nutrient broth or acetate as the nutrient source to simulate biofouling (Chong et al., 2008; Siddiqui et al., 2015), which might not represent the organic matter in seawater and failed to capture the actual mechanism in SWRO biofouling. Our previous study based on isolated dissolved organic fractions from seawater had identified a strong interaction of organic-membrane, hence organic fouling in SWRO, in the sequence of BP ≫ LMW > HS&BB. However, the effect of these isolated organic fractions on SWRO biofouling behavior has not been systematically investigated. For instance, bacteria responded differently, i.e., attachment, to clean and preconditioned membrane surfaces with model foulants of BSA and alginate (i.e., common foulants used to represent biopolymer) and membrane bioreactor (MBR) permeate (Suwarno et al., 2016). Since biopolymers and humic substances were identified as the major organic foulant on RO membrane, it was recommended to reduce the high molecular weight organic content in RO feed to reduce the flux decline (Jeong et al., 2013). On the other hand, it was established that the removal of AOC, i.e., reduction in biofouling potential, was accompanied by the removal of LMW in seawater pretreated with biofilter and MBR (Naidu et al., 2013; Jeong et al., 2014), nevertheless the studies did not perform SWRO biofouling test to confirm its actual impact. Therefore, this warrants further investigation on the effect of different dissolved organic compounds on biofouling in SWRO process in order to formulate an effective pretreatment that target at the culprit for fouling mitigation.

The aims of this study are to investigate the biofouling potential of the isolated organic fractions from seawater and to evaluate their impacts on membrane biofouling in SWRO desalination process. First, three major dissolved organic fractions were isolated from seawater by a membrane-based fractionation and concentration process using a combination of ultrafiltration (UF) and nanofiltration (NF) membranes. Second, the biofouling potential of the isolated dissolved organic fractions was characterized by the AOC measurement. Third, the organic transformation that occurred during the bacteria growth (i.e., model bacteria of *Vibrio*) in the solution with isolated dissolved organic fractions was examined. Fourth, the bacteria-clean/fouled membrane interactions were characterized by atomic force microscopy (AFM) analysis. Last, the impact of isolated dissolved organic fractions on SWRO biofouling was investigated using a laboratory cross-flow RO setup.

2. Materials and methods

2.1. Fractionation and concentration of dissolved organic fractions from seawater

We have previously developed a membrane-based discontinuous-diafiltration technique to fractionate and concentrate the dissolved organic fractions from seawater according to their molecular weights into three main fractions: F.BP (MW > 1000 Da), F.HS&BB (MW 350−1000 Da), and F.LMW (MW < 350 Da) (Yin et al., 2019). Overall, >80% of the organic matter could be recovered using this protocol. Similar procedure was employed in this study; the seawater samples used in this study were collected from a R&D site next to a SWRO desalination plant in Singapore. The details of seawater fractionation and concentration protocol, as well as adjustment of salt concentrations in the isolated dissolved organic fractions to similar level as original seawater can be found in the Supporting Information.

2.2. Characterization of organic compounds in seawater

2.2.1. LC-OCD and FEEM analysis

Liquid chromatography organic carbon detector (LC-OCD Model 8, DOC-LABOR, Germany) was employed to characterize the organic compositions in water samples (Huber et al., 2011). Each water sample was pre-filtered through a 0.45 μm syringe filter prior to analysis. Quantification of the LC-OCD results were done using a customized software program (ChromCALC, DOC-LABOR, Karlsruhe, Germany). Fluorescence-excitation emission matrix (F-EEM) (Cary Eclipse Fluorescence Spectrometer, Agilent) was employed to characterize the fluorescent organic matter in water samples (refer to Supporting Information). Five regions were displayed in FEEM spectrum to represent tyrosine protein-like organic matter (Region I), aromatic protein-like organic matter (Region II), fulvic-like organic matter (Region III), microbial by-product-like organic matter (Region IV), and humic-like organic matter (Region V) (Chen et al., 2003; Yin et al., 2019).

2.2.2. Assimilable organic carbon (AOC) measurement

AOC analysis was used to quantify the biofouling potential of isolated dissolved organic fractions based on the method developed by Hammes et al., (2008). All glassware and plastic caps were cleaned to remove organic residues (i.e., AOC-free) following the protocol developed by Elhadidy et al., (2016). Briefly, the glassware and caps were acid- and alkaline-washed by an automated washer

(Miele Professional) followed by rinsing with DI water. Subsequently, the glassware was soaked overnight in 0.2 M HCl solution (Sigma, USA), followed by flushing with DI water for at least 5 times to ensure that they were acid-free. Then, the glassware was covered with aluminium foil and dried in an oven at 100 °C for 2 h. Subsequently, the dried glassware was baked in a muffle furnace at 450 °C for 6 h before cooled down to room temperature prior use. The plastic caps were soaked in a foil-covered beaker with 10% sodium persulfate solution (Sigma, USA) and then placed in a 60 °C water bath for at least 1 h. Lastly, the plastic caps were washed with DI water and dried in an oven at 100 °C for 30 min prior use.

Fig. 1 illustrates the AOC measurement: (i) To obtain the inoculum, the raw seawater (5L) was first vacuum-filtered through a 11 μm filter paper (Whatman, Grade 1, England) to remove the large particles. Subsequently, the filtrate was sent to a second vacuum filtration unit fitted with a 0.2 μm membrane (Whatman, Nuclepore Track-Etched polycarbonate, UK) to obtain 500 mL of concentrated bacteria solution. This solution was then incubated at 30 °C and 40 rpm for 21 days until the stationary phase of cell growth was reached, as determined from the flow cytometer (BD Accuri C6, USA) measurement. (ii) To obtain the growth medium, each isolated dissolved organic fraction (10 mL, ~0.5 mg-C/L) was first filtered through a 0.22 μm PES syringe filter (Millipore, USA) into a 40 mL AOC-free vial. The syringe filter was pre-washed by filtering with 100 mL of DI water prior use. Subsequently, the sample (10 mL) was heated in a 70 °C water bath for 30 min to inactivate the residual bacteria before left to cool down to room temperature. (iii) 0.5 mL of inoculum was injected into each vial to achieve an approximate cell count of 1×10^4 cells/mL, which was identified as the initial cell count ($N_{initial}$). Then, the test samples were incubated in a temperature-controlled shaker-incubator (Model: ZQZY-70AF, Shanghai Zhichu Instrument Co., Ltd) at 30 °C and 40 rpm. The cell count was measured using a flow cytometer

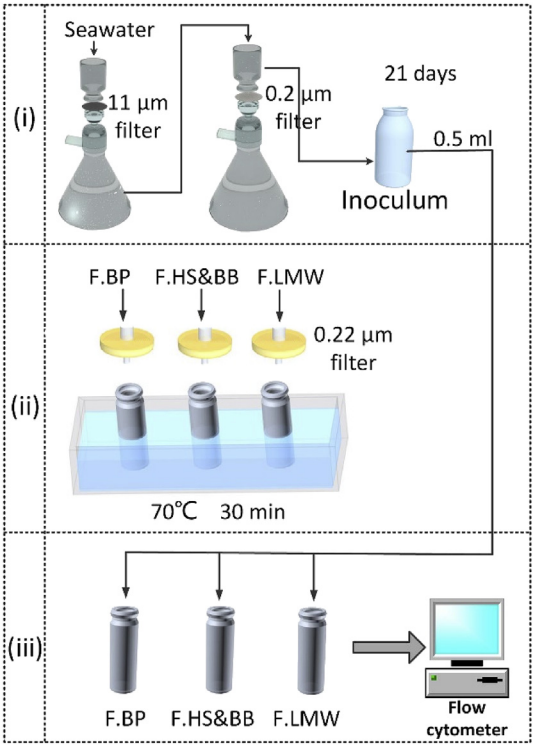

Fig. 1. The illustrations of (i) inoculum preparation, (ii) sample preparation and (iii) cell count measurement in AOC analysis.

(BD Accuri C6, USA) at 24h intervals, and the highest cell count (N_{final}) was recorded. The test sample without inoculum was prepared as the negative control ($N_{negative}$). Prior to measurement, all samples were stained with SYTO9 (Molecular Probes, USA) and left in the dark for 20 min. Each AOC measurement was done in triplicates. The calculation of AOC concentration is (Elhadidy et al., 2016):

$$\text{AOC } (\mu g - C/L) = \frac{\left(N_{final} - N_{initial} - N_{negative}\right)\left(\frac{Cells}{L}\right)}{\text{Inoculum yield } \left(\frac{Cells}{\mu g\ C}\right)} \quad (1)$$

The method to determine the inoculum yield from a calibration curve constructed using sodium acetate as carbon source is outlined in the Supporting Information. In this study, the inoculum yield was determined to be 1.1623×10^5 cells/μg-C (Fig. S2).

2.3. Organic transformation during bacteria growth in isolated dissolved organic fractions

Vibrio sp. B2, which was isolated from a fouled SWRO membrane in previous work (Kim and Chong, 2017), was selected as the model bacterium to study the organic transformation during bacteria growth in isolated dissolved organic fractions. The observations in this test would be useful to explain the observations in SWRO biofouling test. The inoculum was prepared as following: the bacteria was first cultured in two Erlenmeyer flasks with 200 mL marine broth solution (37.4 g/L, BD) and incubated with shaking at 180 rpm and 37 °C for 24 h. The bacteria free from marine broth was harvested by (i) centrifugation at 4000 rpm for 20 min at room temperature, (ii) the supernatant was discarded, (iii) the pellets were washed with 0.85% NaCl solution; the above procedure was repeated 3 times, followed by centrifugation at 4000 rpm for 10 min at room temperature. Subsequently, the bacteria was re-suspended into NaCl solution (35 g/L, Merck) to achieve an optical density OD_{600nm} of 0.1 (Shimadzu, model UV 1800). The preparation of growth medium of isolated dissolved organic fraction was same as described in Fig. 1 (ii). The *Vibrio* sp. B2 inoculum was injected into each vial to achieve an approximate cell count of 6×10^5 cells/mL, then was incubated at 30 °C and 40 rpm for 3 days. A blank test with only the salts solution, i.e., carbon-free synthetic seawater, was conducted to analyze the background from the inoculum alone (Supporting Information). The organic compounds in each test solution were characterized by the LC-OCD and F-EEM analyses.

2.4. Atomic force microscopy (AFM) measurement

The adhesion and cohesion force between bacteria-clean RO membrane, bacteria-F.BP, bacteria-F.HS&BB, bacteria-F.LMW, and bacteria-bacteria were examined by atomic force microscopy (AFM, model XE-100, Park systems, Korea). The observations in this test would be useful to explain the bacteria-membrane/fouled membrane interactions in the SWRO biofouling test. Commercial AFM cantilever with SiO_2 particles (5.0 μm in diameter) from Nanoscan (USA) was applied in this study. The spring constant of the cantilever was 0.06 N/m. The bacteria-coated cantilever tip was prepared by soaking the tip into the *Vibrio* sp. B2 stock solution with concentration of 6×10^6 cells/mL at 4 °C for 48 h prior to use (Villacorte et al., 2015). The fouled membrane coupons by bacteria, F.BP, F.HS&BB and F.LMW was obtained by using dead-end filtration setup according to the method in our previous study (Yin et al., 2019). A liquid cell was used in this study, and the test solution applied was synthetic seawater. In each measurement, average

results were obtained from at least five different locations and measured at least 6 times.

2.5. Impact of isolated dissolved organic fractions on SWRO biofouling

2.5.1. RO system and biofouling experiment

The impact of isolated dissolved organic fractions on SWRO biofouling was investigated using a laboratory crossflow RO setup. The detailed description of the RO setup can be found in previous publication (Yin et al., 2019). The commercial RO membrane (SW30-HR, DOW FilmTec, USA) with an effective area of 0.0045 m^2 was used. The RO membrane was soaked in DI water for 24 h prior use and was compacted at 6.1 MPa for at least 12 h with DI water to achieve a stabilized permeate flux. Then, the DI water in feed tank was replaced to the test solution with isolated dissolved organic fraction (DOC = 0.5 mg-C/L). The initial flux was set at 30 L/m^2h and crossflow velocity of 0.17 m/s. The RO system was operated in a fully recycled mode, where the retentate and permeate were returned to the feed tank to maintain constant volume and concentration. To initiate biofouling, the *Vibrio* sp. B2 stock solution (the preparation method is similar to inoculum preparation as described in Section 2.3) was injected at a flowrate of 0.5 mL/min by an injection pump (ELDEX, Model 5979 OptosPump 2HM) into the feed line to achieve an average cell concentration of 1.5×10^4 cells/mL. The bacteria stock solution was replaced every 2 days. To prevent the feed tank from turning into a bioreactor, 2 units of 0.2 μm cartridge filters were installed at the retentate line before returning to the feed tank and the feed solution was replenished daily. The biofouling experiments lasted for 6 days.

2.5.2. Membrane autopsy

At the end of SWRO biofouling experiment, the fouled membrane was taken out from the RO crossflow cell for autopsy analysis. Subsequently, the fouled membrane coupon (3 cm × 4 cm) was soaked in 25 mL of DI water, followed by sonicating for 30 min, and vortexing for 1 min. The foulant solution was then characterized by LC-OCD, F-EEM and EPS analyses.

The EPS in foulant solution, which was made up of polysaccharide and protein, was quantified using the method described in previous study (Suwarno et al., 2012). In brief, for the measurement of polysaccharide content, 2 mL of sample solution was added to a mixture of 1 mL of 5% (w/v) phenol solution and 5 mL of H_2SO_4, and the solution was left to cool to room temperature. The absorbance at 490 nm was measured using a UV spectrometer (UV-1800, SHIMADZU, Japan) and glucose solution (Merck, USA) was used as the calibration standard. The protein concentration was measured using the Bicinchoninic Acid (BCA) assay kit (Pierce, product #23225). One mL of sample solution was mixed with the working reagent (2 mL) and incubated in dark for 2 h at room temperature. The absorbance at 562 nm was measured and bovine serum albumin (BSA) solution was used as the calibration standard.

The biofilm on the fouled membrane surface was characterized by confocal laser scanning microscopy (CLSM) analysis. The membrane coupon (3 cm × 4 cm) was stained with the LIVE/DEAD BacLight bacterial viability kit (Molecular Probes, L7012) according to the procedure provided by manufacturer. In brief, the staining reagent was prepared by combining 1.5 μL of SYTO 9 and 1.5 μL of PI in 0.5 mL of DI water. Subsequently, the membrane coupon was soaked in the staining reagent and incubated in dark for 30 min at room temperature. Then, the membrane coupon was rinsed with 1 mL of DI water and placed on a glass slide. The microscopic observation and image acquisition were obtained by CLSM (Zeiss, model LSM710), and the biovolume ($\mu m^3/\mu m^2$) of biofilm was calculated by IMARIS software (Bitplane, version 7.3.1).

3. Results and discussion

3.1. Isolated dissolved organic fractions from seawater

As shown in Fig. 2, the seawater contained 6% BP, 52% HS&BB and 42% LMW. These dissolved organic compounds were successfully fractionated and concentrated into three major fractions, i.e., F.BP (MW > 1000 Da), F.HS&BB (MW 350–1000 Da) and F.LMW (MW < 350 Da). For each fraction, the final organic concentration was adjusted to DOC = 0.5 mg-C/L while the ions concentrations were adjusted to level similar to the original seawater (as verified by the ICP-OES measurements, Supporting Information) as summarized in Table 1. These solutions will be used in the subsequent tests.

3.2. Assimilable organic carbon (AOC) analysis

The biofouling potential of each isolated dissolved organic fraction was characterized by the AOC content, which was based on the growth curve of indigenous bacteria at DOC = 0.5 mg-C/L (Fig. 3), are summarized in Table 2. The contribution of AOC and DOC from each organic compound in seawater is summarized in Table 3. The F.LMW showed the highest growth rate of 0.60 d^{-1} and AOC value was 172 ± 15 μg-C/L; F.BP displayed much slower cell growth of 0.20 d^{-1} and AOC value was 101 ± 32 μg-C/L; F.HS&BB showed the lowest cell growth of 0.16 d^{-1} and AOC value was 43 ± 13 μg-C/L. Thus, the AOC/DOC ratio was in the sequence of F.LMW (~35%) > F.BP (~19%) > F.HS&BB (~8%). Typically, humic substances showed high resistance to biodegradation (van der Kooij et al., 1989), thus there was poor correlation between AOC and HS&BB (Jeong and Vigneswaran, 2015). Unlike HS&BB, LMW which composed of high proportion of protein-like organic matter (Yin et al., 2019) as well as BP which mainly composed of protein and polysaccharides (Villacorte et al., 2017), could serve as the nutrient source in supporting microbial growth, thus both gave higher AOC/DOC readings. The time for the highest cell growth varies for each isolated dissolved organic fraction, i.e., F.LMW was more readily consumed by bacteria as compared to F.BP, in which the bacteria growth took longer time (~× 2) to reach the maximum cell count. In addition, when comparing F.LWM with F·BP, the cell growth rate was ~ × 3 higher while the AOC concentration was only

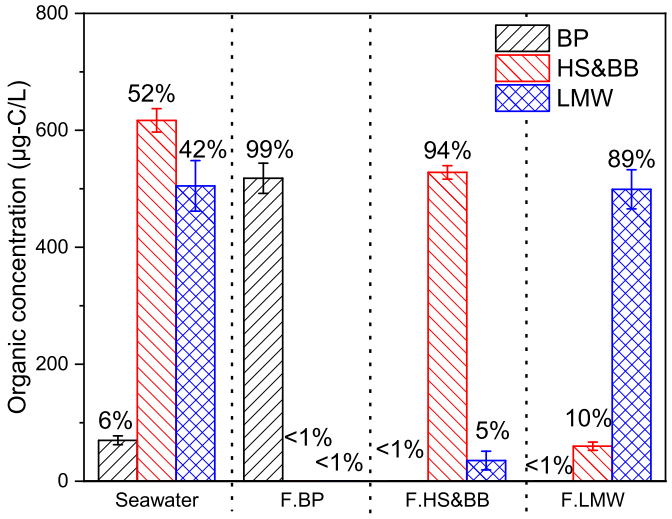

Fig. 2. LC-OCD analysis of organic compounds in original seawater and isolated dissolved organic fractions. The percentage value is the % ratio of DOC of BP, HS&BB, or LMW to total DOC.

Table 1
Concentration of dissolved ions in isolated dissolved organic fractions before and after ionic adjustment.

	Original (mg/L)			Conductivity	Adjusted (mg/L)			Conductivity
	Ca^{2+}	Mg^{2+}	Na^{+}	mS/cm	Ca^{2+}	Mg^{2+}	Na^{+}	mS/cm
Seawater	371.1 ± 0.9	1189 ± 9	10589 ± 101	47.2 ± 0.2	No adjustment of ions concentration			
F·BP	<1.4	<3.2	<0.9	<0.3	361.5 ± 1.8	1213 ± 16	10670 ± 105	47.3 ± 0.2
F·HS&BB	34.5 ± 3.2	153.1 ± 2.8	21.6 ± 3.6	4.4 ± 0.1	400.3 ± 0.9	1198 ± 18	10790 ± 97	47.9 ± 0.2
F.LMW	313.9 ± 2	1109 ± 9	10310 ± 30	46.2 ± 2.1	391.9 ± 3.3	1122 ± 15	10770 ± 121	47.8 ± 0.1

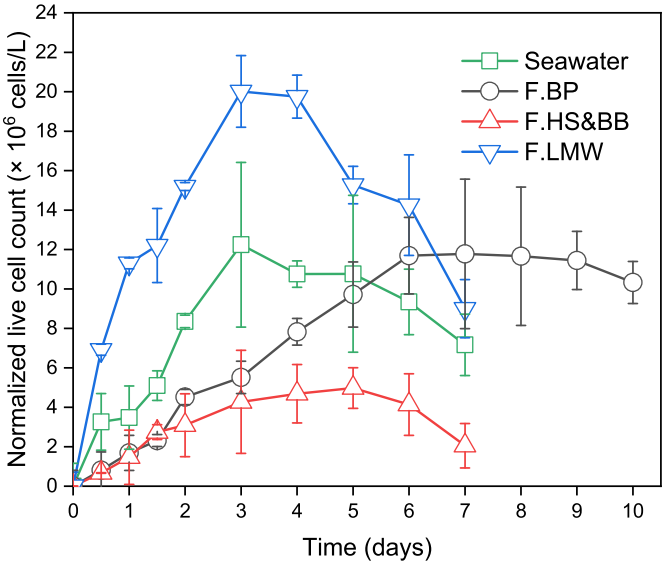

Fig. 3. Growth curve of indigenous inoculum in different isolated dissolved organic fractions from seawater. Initial concentration of organic in each fraction = 0.5 mg-C/L.

Table 2
AOC/DOC of isolated dissolved organic fractions.

	AOC [a] (μg-C/L)	DOC [b] (μg-C/L)	AOC/DOC (%)
Seawater (diluted) [c]	102 ± 35	523 ± 20	19.5
F·BP	101 ± 32	534 ± 29	18.9
F·HS&BB	43 ± 13	518 ± 47	8.3
F.LMW	172 ± 15	494 ± 50	34.8

[a] Measured value based on growth test of indigenous bacteria.
[b] Measured value by LC-OCD analysis.
[c] Seawater was diluted so that DOC was ~0.5 mg/L and salts concentrations were adjusted to level equivalent to original seawater.

Table 3
Contribution of AOC and DOC by different organic compounds in seawater.

	DOC [a]		AOC [b]		AOC/Total DOC
	(μg-C/L)	(%)	(μg-C/L)	(%)	(%)
BP	70 ± 8	5.9	13	5.5	1.1
HS&BB	617 ± 12	51.8	51	21.2	4.3
LMW	505 ± 19	42.3	176	73.3	14.8
Total	1192	100	240	100	20.1

[a] Measured value by LC-OCD.
[b] Calculated value based on measured value of DOC in original seawater (Fig. 2) and AOC/DOC ratio (Table 2).

about × 1.7 higher, could be attributed to the smaller molecular weight of LMW (MW < 350 Da) compared to BP (MW > 1000 Da), which was more readily consumed by microorganisms (Passow, 2002; Villacorte et al., 2017). This finding was important as water samples with the same amount of AOC but different organic compositions, could potentially result in different SWRO biofouling rates.

Overall, the ratio of AOC/DOC for seawater was only ~20%. The cell growth curve of seawater (Fig. 3) followed similar trend as F.LMW since LMW contributed ~73% of AOC in seawater (Table 3). Although HS&BB was the largest portion of organic compounds in seawater (i.e., ~52% of DOC), it did not play a significant role in supporting the microbial growth, i.e., contributed to ~21% of AOC in seawater. Meanwhile, BP which accounted for only 6% of DOC in seawater, contributed only ~5% of AOC in seawater. The findings suggested that the contribution to biofouling potential of seawater was in the order of LMW ≫ HS&BB > BP; which corroborated the linear correlation between LMW and AOC in treated seawater by MBR (Jeong et al., 2014).

3.3. Organic transformation during bacteria growth in isolated dissolved organic fractions

The bio-transformation of organic matter during bacteria growth of *Vibrio* sp. B2 in isolated dissolved organic fractions and carbon-free synthetic seawater (blank) are shown in Fig. 4a and Fig. S3, respectively. In the blank test, maximum increase in BP, HS&BB and LMW were only ~10, ~10 and ~30 μg-C/L, respectively, after 3-day incubation. In F·BP as nutrient source, the concentration of BP decreased while the concentration of LMW increased to 142 ± 63 μg-C/L, due to biodegradation of larger molecules of BP into smaller molecules of LMW that would be more favourable for assimilation (Naidu et al. 2013, 2015); HS&BB was also released as the concentration increased (73 ± 22 μg-C/L) but the amount was much lesser than LMW. The bacteria could also generate BP through SMP and EPS production (Villacorte et al., 2017), but it was difficult to distinguish the BP produced as microbial product from the indigenous BP in F.BP. While in F.HS&BB as nutrient source, only small amount of BP and LMW (i.e., taking into account the LMW impurities in F·HS&BB (Fig. 2) as well as LMW release in blank test) were produced as humic substances were slowly or non-biodegradable and the microbial growth was not favoured. On the other hand, in F.LMW as nutrient source, a decrease in LMW was observed as LMW was easily consumed by bacteria for proliferation. An increase in BP (58 ± 21 μg-C/L) and HS&BB (74 ± 39 μg-C/L) was also noted, mainly due to the production of SMP and hydrolysis of EPS secreted by bacteria.

From the F-EEM analysis, F.BP and F.LMW mainly consisted of protein-like organic matter in region I and II, respectively, while F.HS&BB was a mixture of protein-like (region I, II), fulvic-like (region III) and humic-like (region IV) organic matter. After 3-day incubation, the results clearly showed the transformation of organic matter in all test solutions to protein-like (region II), microbial by-product-like (region IV), and humic-like (region V) organic matter. The organic transformation was critical as reported from previous study, microbial by-product-like organic matter were identified as an important foulant in membrane processes (Yu et al., 2015).

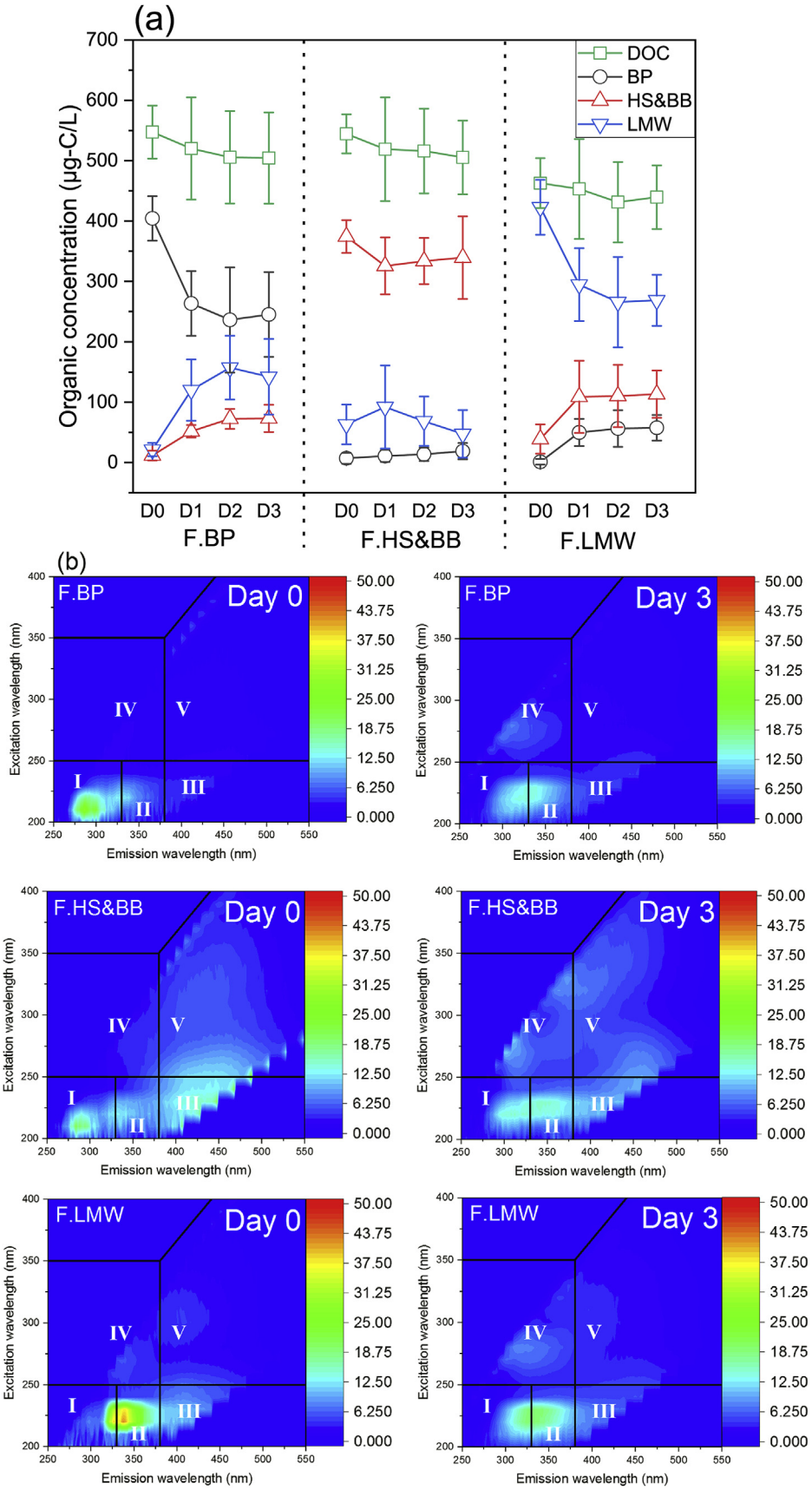

Fig. 4. Bio-transformation of organic matter by *Vibrio* sp. B2 (Kim and Chong, 2017) as inoculum in different isolated dissolved organic fractions from seawater (a) LC-OCD, and (b) F-EEM analysis.

3.4. Bacteria-clean/fouled membrane interactions

From the AFM analysis (Fig. 5) of the organic-fouled and bacteria-fouled membrane coupons obtained from the dead-end filtration, it can be seen that interaction of bacteria and clean membrane surface was not favoured. However, the presence of organic matter had modified the membrane surface, causing greater adhesion of bacteria to the organic-fouled membrane as compared to clean membrane, and the magnitude of interaction force was in the following order: bacteria-BP > bacteria-LMW > bacteria-HS&BB. First, the results suggested that organic conditioning film was an important precursor to biofouling as bacteria-membrane interaction was the weakest. Second, in previous study, BP was identified as the major contributor of organic fouling in RO due to its sticky nature that caused highest degree of BP-membrane interaction as compared to HS&BB and LMW (Yin et al., 2019). Similarly, the effect of bacteria-BP fouled membrane interaction was also more pronounced. In addition, once the bacteria colonized the membrane, more bacteria attachment was expected since bacteria-bacteria interaction was greater than bacteria-organic-fouled membrane interaction.

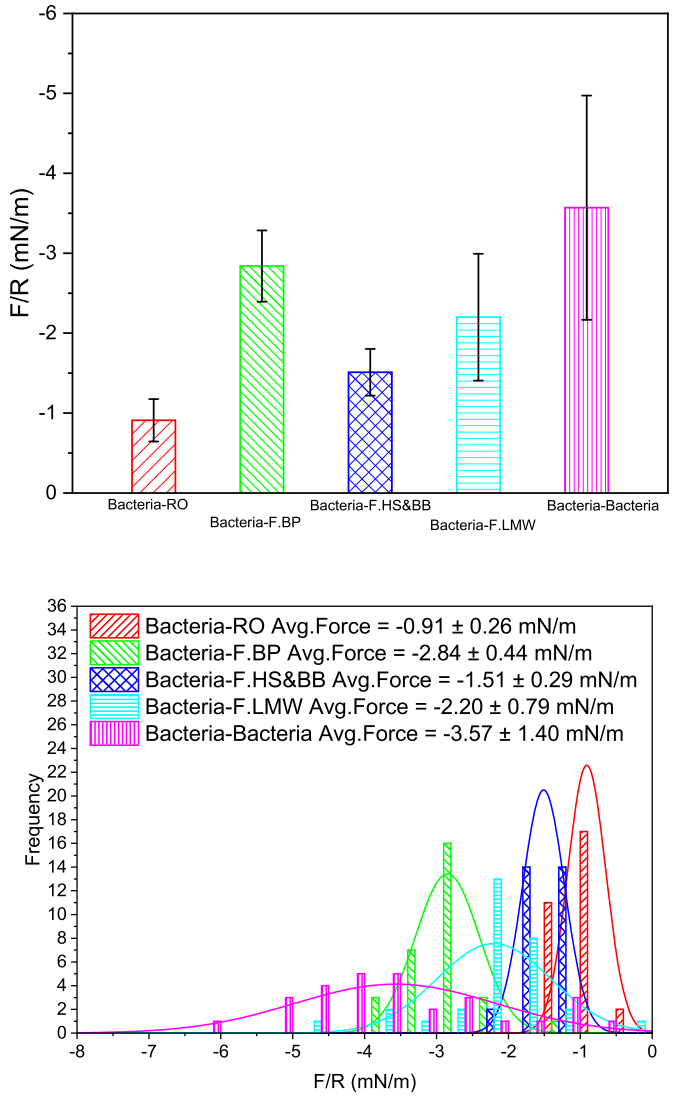

Fig. 5. AFM analysis of interaction of bacteria-clean RO membrane, bacteria-BP-fouled membrane, bacteria-HS&BB-fouled membrane, bacteria-LMW fouled membrane, and bacteria-bacteria.

3.5. Impact of isolated dissolved organic fractions on SWRO biofouling

In the crossflow RO experiments to simulate SWRO biofouling in different isolated dissolved organic fractions, the characteristics of test solution in the feed tank remained unchanged (i.e., concentration and organic compositions) as shown in Fig. S4. This was important so the feed tank and recirculating lines did not turn into a bioreactor that could impact the biofouling process. The flux decline rate during biofouling process was compared with the control without bacteria injection (i.e., organic fouling in our previous study (Yin et al., 2019)) as shown in Fig. 6. The impact of isolated dissolved organic fractions (at DOC = 0.5 mg-C/L) on biofouling based on the % flux decline rate after 140 h of operation was in the order of F.LMW > F.BP ≫ F.HS&BB. When compared to the control, significant difference in the flux decline rate was noted between biofouling and control (30% vs. < 5%) in F.LMW, while it was 20% vs. 12.5% in F.BP. Insignificant effect on flux decline rate was observed in both biofouling and control in F.HS&BB, i.e., <10%.

Further analysis of membrane autopsy also supported the flux decline profile. From the CLSM imaging (Fig. S5) and quantification of biovolume of live cells on RO membranes (Fig. 6a), higher value of $15.5 \pm 0.5\ \mu m^3/\mu m^2$ was noted for bio-fouled membrane in F.LMW than $12.5 \pm 1.3\ \mu m^3/\mu m^2$ in F.BP. Whereas lowest value of $4.9 \pm 1.1\ \mu m^3/\mu m^2$ was reported in F.HS&BB. It shall be noted that the biovolume of dead cells on RO membranes was negligible. The results agreed well with the AOC measurement, i.e., F.LMW > F.BP > F.HS&BB, where greatest microbial growth rate was observed in F.LMW as nutrient source.

In addition, the LC-OCD analysis (Fig. 6b) of foulant extracted from the biofouled membrane also revealed that least biofouling occurred in F.HS&BB as RO feed. Several remarks could be made for biofouling in F.BP and F.LMW as RO feed:

i) In F.BP as RO feed, by comparing the feed and foulant compositions and amount, it was found that despite there was no LMW in the F.BP feed solution (Fig. 2 and Fig. S4), large amount of LMW was detected in the foulant (Fig. 7b). In addition, the amount of BP detected in biofouled membrane

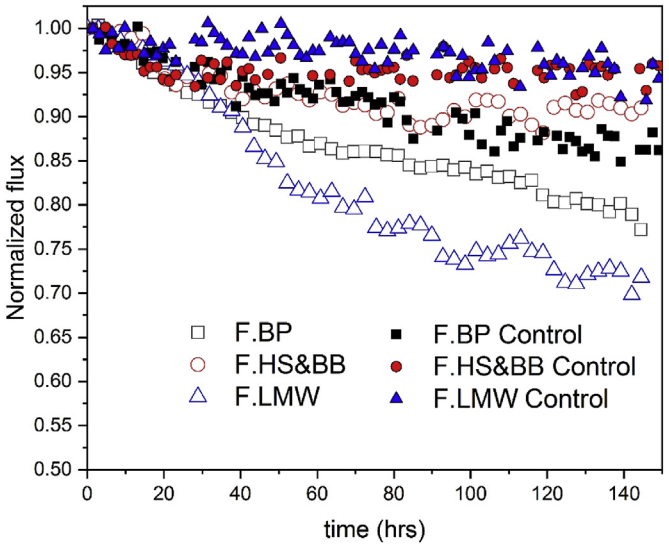

Fig. 6. Flux decline profile of RO biofouling by *Vibrio* sp. B2 (Kim and Chong, 2017) in different isolated dissolved organic fractions from seawater. The control experiments were tests without the presence of bacteria, taken from our previous work (Yin et al., 2019). Concentration of organic in each fraction = 0.5 mg-C/L. Initial flux = 30 L/m^{2}h.

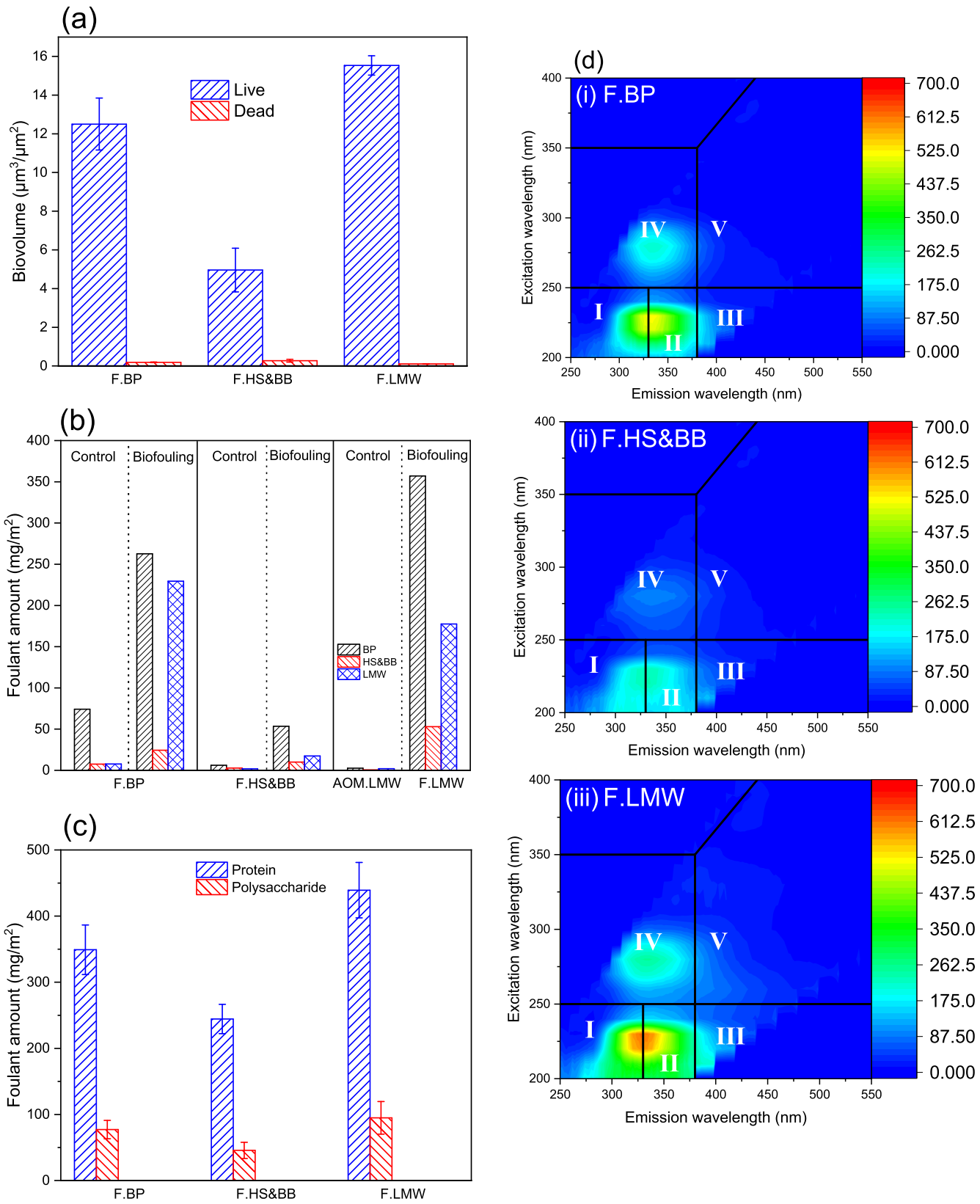

Fig. 7. (a) Biovolume ($\mu m^3/\mu m^2$) of live and dead cells, (b) LC-OCD, (c) EPS (protein and polysaccharide), (d) F-EEM of biofouled RO membranes by *Vibrio* sp. B2 (Kim and Chong, 2017) in different isolated dissolved organic fractions from seawater. The control experiments in (b) were tests without the presence of bacteria, taken from our previous work (Yin et al., 2019).

was much higher than the control, i.e., organic-fouled membrane. First, the large amount of LMW in foulant was due to the biodegradation of larger molecules of BP into smaller molecules of LMW for easy consumption by bacteria to proliferate. This hypothesis was supported by the biotransformation data in Fig. 4. Second, the results suggested that the amount of BP generated as SMP and EPS by bacteria far exceeded the deposition of indigenous BP from F.BP feed solution. Note that LMW could also be generated as part of the SMP and EPS.

ii) In F.LMW as RO feed, the deposition of LMW originated from F.LMW onto clean RO membrane was less favourable compared to BP in F.BP due to weaker LMW-membrane interaction (Yin et al., 2019). However, when LMW was consumed for microbial growth, BP was generated as SMP and EPS, i.e., biotransformation of LMW by bacteria as shown in Fig. 4. Hence, RO membrane surface could be modified as such it promoted subsequent bacteria deposition. Since the amount of BP produced via biotransformation was relatively lower than the availability of BP in F.BP, the initial biofouling rate in F.LMW was slower than F.BP (Fig. 6, from 0 to 30 h). However, once the BP conditioning layer was formed on the membrane, with the continuous supply of LMW in F.LMW, in which the amount was much higher than LMW obtained from the biodegradation of BP in F.BP, resulted in greater microbial growth and production of SMP and EPS (i.e., BP and LMW), thus the biofouling rate in F. LMW surpassed F.BP beyond 30 h (i.e., 30% vs. 20% in flux decline after 140 h).

iii) By comparing the flux decline profile and compositions of foulants extracted from fouled membranes and controls, it was noted the sequence of flux decline was F.LMW (30%) > F.BP (20%) > control F.BP (12.5%) ≫ control F.LMW (<5%), the amount of BP in foulant was in the order of F.LMW > F.BP > control F.BP ≫ control F.LMW, the amount of LMW in foulant was in the order of F.BP > F.LMW ≫ control F.BP > control F.LMW. There was a good correlation between flux decline and amount of BP in foulant. Note that majority of BP in foulant was accumulated through generation of SMP and EPS rather than from deposition of indigenous species. Based on these results and CLSM analysis, it was suggested that biofilm with high number of cells embedded in matrix of SMP and EPS (i.e., in particular BP) could result in high hydraulic resistance and biofilm enhanced osmotic pressure effect that lower the flux.

The EPS (Fig. 7c) and F-EEM analyses of foulants extracted from biofouled membranes also supported the remarks above. The amount of EPS in foulant was in the order of F.LMW > F.BP > F.HS&BB. It was noted that proteins occupied a large portion of EPS compared to polysaccharides, which agreed well with previous work (Jeong et al., 2013). Similarly, F-EEM analysis revealed that the foulant was mainly composed of tyrosine protein-like (Region I), protein-like organic matter (Region II), and microbial by-product-like organic matter (Region IV).

Overall, these findings are critical in providing the guidance for selection of efficient seawater pretreatment method to mitigate membrane biofouling. Most of the reported work focused on removing the biopolymer fraction by methods such as coagulation/flocculation and membrane filtration or combinations prior RO process (Jeong et al., 2013; Shutova et al., 2016). However, based on the AOC/DOC ratio of organic matter in seawater (Table 2) and SWRO biofouling test in this study, the role of indigenous BP in SWRO biofouling was less critical owing to its relatively low concentration (i.e., only accounted for 6% of DOC and 5% of AOC in seawater). On the other hand, LMW which accounted for >70% of AOC and 42% of DOC in seawater, played a significant role in SWRO biofouling by supporting microbial growth that contributed to the build-up of SMP and EPS (i.e., generation of BP). Even though HS&BB occupied about 52% of DOC in seawater, i.e., major organic compound, it had marginal role in SWRO biofouling. Therefore, seawater pretreatment prior RO process shall focus on the removal of AOC rather than the removal of biopolymer.

4. Conclusions

The biofouling potential of the isolated dissolved organic fractions from seawater, i.e., fractions of biopolymers (F.BP, MW > 1000 Da), humic substances and building blocks (F.HS&BB, MW 350−1000 Da), and low molecular weight compounds (F.LMW, MW < 350 Da), was characterized by assimilable organic carbon (AOC) content and their impact on SWRO biofouling was evaluated using *Vibrio* sp. B2 as model bacteria in a crossflow RO system. The findings are summarized below:

(i) The AOC/DOC ratio of the isolated dissolved organic fractions was in the order of F.LMW (~35%) > F.BP (~19%) > F.HS&BB (8%). The main contributor to AOC in seawater was LMW (>70%).

(ii) In SWRO biofouling, the flux decline was in the order of F.LMW (30%) > F.BP (20%) > F.HS&BB (<10%). The membrane autopsies data also supported these findings.

(iii) In F.BP as RO feed, the BP preferentially formed a conditioning layer on the RO membrane due to strong interaction of BP-membrane, followed by the deposition of bacteria due to greater interaction of bacteria-BP than bacteria-membrane. The BP was then biodegraded to LMW, which could be easily assimilated by bacteria for proliferation. BP and LMW was generated as SMP and EPS.

(iv) On the other hand, in F.LMW as RO feed, due to weaker interaction of LMW-membrane, the deposition of LMW onto membrane was not favourable. But bacteria consumed LMW and produced BP as a by-product, which could then form a conditioning layer on the membrane surface. This process was slower than the direct deposition of BP in F.BP, thus slower flux decline rate was observed in F.LMW as compared to F.BP during the initial stage of biofouling. However, due to continuous supply of LMW in F.LMW, in which the amount was much higher than the amount of LMW generated through biodegradation of BP in F.BP, rapid microbial growth and generation of SMP and EPS were observed, thus biofouling rate in F.LMW surpassed F.BP at the later stage of biofouling process.

(v) The large amount of BP and LMW presence (i.e., HS&BB was insignificant) in the foulants of biofouled membranes in F.BP and F.LMW as RO feed were not from the deposition of indigenous BP and LMW from the bulk feed solution, but rather from the generation of BP and LMW during microbial growth on the membrane surface through biodegradation of BP in F.BP and production of SMP and EPS in both F.BP and F.LMW. The accumulation of bacteria cells embedded in matrix of SMP and EPS (i.e., in particular BP) formed a biofilm that caused an increase in hydraulic resistance and biofilm-enhanced osmotic pressure effect, thus decline in flux.

In conclusion, even though HS&BB was the major organic compound in seawater (~52%), it had marginal role in SWRO biofouling. Meanwhile, the role of indigenous BP in SWRO biofouling was less critical owing to its relatively low concentration. On the other hand, LMW which accounted for >70% of AOC and ~42% of DOC in seawater, played a significant role in SWRO

biofouling by supporting microbial growth that contributed to the build-up of SMP and EPS (i.e., in particular BP). Therefore, seawater pretreatment prior RO process shall focus on the removal of AOC (i.e., LMW) rather than the removal of biopolymer.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2019.115198.

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