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Evaluation of eluted organic matter characteristics and biofilm formation potential from polymeric material in contact with treated water

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ABSTRACT

Polymeric materials are widely used in contact with water at home (e.g., shower hose, plumbing, etc.). Organic matter eluted from polymeric materials may release carbon sources for microorganisms and cause biofilm formation in our household water systems. Therefore, further understanding of the organic matter derived from polymeric materials in water distribution systems is needed. In this study, BioMig was used to investigate the elution of carbonaceous matter, through migration potential (MP) experiments, and the possibility of microbial growth, through biomass formation potential (BFP). Additional analysis was also conducted on the eluted organic matter to better understand the relationship between the MP and BFP. Four different piping materials were used in the experiments each composed of polyethylene (PE), chlorinated polyvinyl chloride (CPVC), ethylene propylene diene monomer (EPDM) rubber and natural latex. Results showed that the total amount of elution decreased with selected materials with flexible EPDM, and natural latex having higher elution than PE and CPVC. The assimilable organic carbon (AOC) decreased with time for PE, and CPVC, where EPDM, and natural latex increased. The BFP agreed with the MP, where polymer materials with higher AOC amounts also showed higher biomass formation.

Keywords: Biofilm formation potential; BioMig; Migration potential; Polymeric materials

1. Introduction

For safe drinking water consumption, water treatment plants undergo rigorous processes to manage the quality and quantity of the treated water. However, the quality control of drinking water is only monitored at the final release point of the drinking water treatment plant. During the distribution process, the water will be in constant contact with polymeric piping that may influence its quality through secondary pollution. The secondary pollution refers to the physical, chemical, and biological changes in water quality during the distribution. Secondary pollution process occurs through the diffusion of chemical substances from polymeric materials that are used for water pipes. This has been well known to occur with polymeric food packaging, where plasticizers, antioxidants, thermal stabilizers, slip compounds, and monomers are known to be capable of migrating from the polymers [1]. Piping materials have also been known to migrate organic compounds into the water resulting in the alteration of the taste and odor of the water [2–4]. The migrated organic matter is also known to influence the growth of biofilm in the water [4–7]. Previous studies have shown that with residual chlorine concentrations below 0.3 mg/L resulted in the growth of biofilms on polyethylene (PE) and polyvinyl chloride (PVC) exceeded that of glass and concrete [8,9]. Biofilm formation in the distribution systems are reported to induce corrosion of the pipe materials and reduce the effectiveness of chemical disinfectants [8,10]. The growth of microorganisms does not only occur on the surface of the piping but at the final contact points of the consumer as well. Due to the low chorine concentration and stagnation,

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building plumbing to final contact points such as faucets and appliances also show a high degree of microbial growth [11–13].

In many European countries, water-related materials are regulated for their microbial growth potential, where standard tests are conducted in the certification process. The United Kingdom uses the mean dissolved oxygen difference (MDOD) to assess the oxygen consumption of growing microorganisms, where the Netherlands uses biomass production potential (BPP) test of NVN 1225 to measure the adenosine triphosphate (ATP) of the existing microbial species. Additionally, Germany uses the code of practice W270 as a regulatory measure. Although these methods are known to be reliable, they are also time consuming and only measure the microbial growth potential during the process. To overcome the mentioned limitations, Wen et al. [14] developed BioMig [14], which is an improved version of European methods (MDOD, BPP, etc.). The method is designed to measure the biomass formation potential (BFP) in shorter periods, with additionally measuring the migration of dissolved organic matter from the polymer substances. A summary of the characteristics of the different biomass growth measuring methods are summarized in Table 1.

In this study, the BioMig was used to verify the elution of organic matter (migration potential [MP]), and BFP for four selected piping materials. The materials selected for the research were commonly used polymer materials used for the piping and spacing of the drinking water distribution systems, being PE, chlorinated polyvinylchloride (CPVC), ethylene propylene diene monomer (EPDM) rubber and natural latex. As mentioned in the development process, the results of the MP and BFP do not necessarily correlate. So to further understand the connection between the two variables, additional analysis was conducted on the eluted organic matter. In the process, excitation emission matrix (EEM), liquid chromatography-organic carbon detector (LC-OCD), and dynamic light scattering (DLS) analysis were conducted.

2. Materials and methods

2.1. Materials tested

For the experiment, PE, CPVC, EPDM rubber and natural latex (cis-1,4-polyisoprene) were used, and each material was manufactured by different companies. The piping materials were cut directly from the purchased polymer piping, and cut into four 2.5 × 5 cm^2 pieces to make a total surface area of 100 cm^2.

2.2. Glassware and water preparation

Glass bottles used for both MP and BFP tests were of 250 mL jars with Teflon linings. All glass bottles were immersed in hydrochloric acid (0.2 M) for 24 h, dried in air, and inserted into a muffle furnace for 3 h at 500°C for carbon-free conditions. For the parts not composed of glass, they were immersed in sodium persulfate solution (100 g/L) for at least 1 h, washed with distilled water and dried. Mineral waters used in the experiments were conducted with Evian bottled water. Evian was selected, due to its higher similarity with tap water, and higher release of organic matter while conducting MP experiments [14]. The Evian water was filtered through a 0.1 μm filter (Sartorius, Germany) before experiments.

2.3. Migration potential test

Prior to the experiments, the glassware was filled with filtered mineral water and sterilized for 24 h at 60°C. The water was then removed and filled with 100 mL of filtered mineral water. Test materials were added to the glass jars, where the migration experiments were conducted at 60°C for 24 h. Seven migration steps were conducted for a total of 7 d, each using filtered mineral water. The samples were each named M1–M7 based on the day the experiment was performed. After each step, the migrated water was tested for total organic carbon (TOC) and assimilable organic carbon (AOC).

Table 1
Comparison of test methods currently used in Europe for evaluating the potential of polymeric materials to promote microbial growth [14]

	MDOD (United Kingdom)	W270 (Germany)	BPP (Netherlands)	BioMig (Eawag)	
Temperature (°C)	30±1	Ambient (>6°C)	30±1	MP	BFP
				60±1	30±1
Surface (cm^2) of sample pieces	150 (or 75)	800	50 (or 25)	100	100
Volume of water (cm^3)	1,000	100,000	900	100	100
S/V (cm^{-1})	0.15	Not applicable	0.166	1	1
Duration (weeks)	7.5	12	16	2	
Replacement	Twice a week	Continuously	Weekly	Once a day (7 d)	Once in 2 weeks
Test water	Tap water (dechlorinated)	Tap water (dechlorinated)	Tap water (dechlorinated)	Mineral water (Evian)	
Parameter	Oxygen consumption	Slime volume	ATP	Organic carbon, TCC, ATP	
Pass–fail criteria	2.4 mg-O_2/L	0.05 mL-slime/800 cm^2	2 × 10^3~10^4 pg ATP/cm^2	–	–

Total organic carbon was measured with a TOC analyzer (TOC-VCPH, Shimadzu, Japan). A schematic of the experimental procedure is shown in Fig. 1.

2.4. Biomass formation potential assay

For BFP experiments, the sterilization process was conducted in the same manner as the MP test. After adding the piping materials, 100 mL of filtered mineral water with 3.4 mL of mineral medium stock solution was added. The mineral medium was composed of buffer, trace element, and EDTA solutions. The buffer stock solution was composed of ultrapure water with 1.28 g/L $Na_2HPO_4{\cdot}2H_2O$, 0.30 g/L KH_2PO_4, and 1.77g/L $(NH_4)2SO_4$ (Showa Chemical Co., Tokyo, Japan). Trace element stock solution was composed of ultrapure water with various salts in a concentration of 8 g $CaCO_3$, 1.15 g $MnCl_2{\cdot}4H_2O$, 0.15 g $CuSO_4{\cdot}5H_2O$, 0.13 g $CoCl_2{\cdot}6H_2O$, 0.40 g ZnO, 0.13 g H_3BO_3, 13.42 g $MgCl_2{\cdot}6H_2O$, and 1.04 g $Na_2MoO_4{\cdot}2H_2O$ (Showa Chemical Co., Tokyo, Japan). EDTA solutions were composed of ultrapure water and 3 g/L of $EDTA{\cdot}Na_4{\cdot}2H_2O$. The glass jars were placed on a temperature controlled shaking incubator at 30°C and 90 rpm for 14 d.

After the incubation period, the samples were tested for the sessile total cell count (sTCC), planktonic total cell count (pTCC), and residual organic carbon (rDOC). For the sTCC, the cells were sonicated at 10 kHz, 100 W, for 30 s. The detached cells were stained and counted by a flow cytometry (FCM). The FCM (Partec, Cube 6, Germany) was composed with a blue 50 mW laser emitting light at a fixed wavelength of 488 nm, and a red laser of 25 mW emitting a 638 nm wavelength. For the staining process, 20 μL of SYBR Green I (Sigma-Aldrich, USA) was added to 1 mL of the microbial sample and incubated in the dark for 10 min at 36°C. The electronic gating and cell count calculation were performed via FCS Express 4 software (De Novo Software, CA, USA). A schematic of the experimental procedure is shown in Fig. 1.

The total BFP is categorized into the planktonic biomass formation potential (pBFP), and the sessile biomass formation potential (sBFP). Planktonic biomass formation is the cell count of the suspended microbial species by the area of the pipe material tested. The equation is as follows:

$$\text{pBFP} = (\text{TCC} \times V_A)/A_{\text{tot}} \quad (1)$$

where the TCC is the total cell concentration (cell count/mL), measured from the FCM. V_A is the volume of the water tested (mL), and A_{tot} is the total surface area of the tested piping material.

The sBFP is the cell count of attached cells per surface area of the tested piping material. The equation is as follows:

$$\text{sBFP} = ((\text{TCC}_{\text{US1}} + \text{TCC}_{\text{US2}}) \times V_{\text{US}})/A_{\text{US}} \quad (2)$$

TCC_{US1} and TCC_{US2} were the total cell concentration (cell count/mL) of the first and second sonication procedures, respectively. U_{VS} is the volume of water during the test, where A_{US} is the total surface area of the tested piping material. The total BFP is the combination of planktonic BFP and sessile BFP.

2.5. Assimilable organic carbon

To evaluate the AOC of the organic matter eluted from each material, the analysis was carried out according to the AOC measurement method using FCM [15]. Before the analysis, glass vials were sterilized in an autoclave under 105°C for 1 h. After the sterilization process, the eluted samples were placed in each glass vial and pasteurized at 60°C–70°C for 30 min. After pasteurization, the samples were filtered with a 0.1 μm PES filter and injected into a carbon-free glass vial to 20 mL. In addition, 680 μL of the mineral stock solution and 10 μL of $FeCl_3$ (10 mM) were injected to form carbon restriction conditions. As a microorganism strain, each sample was amended with a 1 mL aliquot of unfiltered mineral bottled mineral water (Evian bottle water). The initial number of microorganisms was determined by the FCM after inoculation (about 105 cells/mL), and the number of final microorganisms was determined after incubation for 6 d in a dark room at 36°C. AOC conversion was calculated with a commonly used conversion factor [16], as proceeded as follows. This experiment was expressed as AOC (μg/cm²) per unit area of the material.

$$\text{AOC (μg/L)} = \text{net grown cells (cells/L)}/\text{conversion factor } (1 \times 10^7 \text{ cells/μg}) \quad (3)$$

2.6. Fluorescence excitation emission matrix

Fluorescence EEM is an analysis capable of qualitative analysis of organic matter by detecting fluorescence of organic substances. The analytical method is as follows. After immersing the analytical sample in a quartz

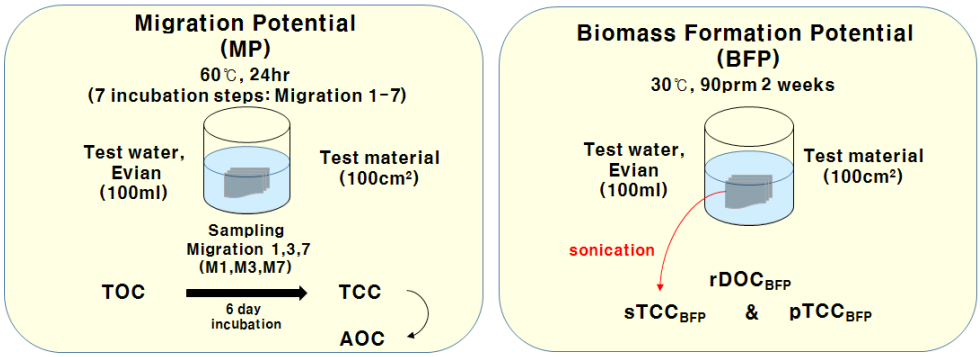

Fig. 1. Schematic overview of BioMig test method migration potential (MP) test and biomass formation potential (BFP) test.

cuvette (Hellma, USA), fluorescence is emitted by using RF-5301 spectrofluorometer (Shimadzu, Japan) excitation wavelengths (220–400 nm) and emission wavelengths (280–600 nm) were scanned and measured and analyzed by arbitrary unit (AU) diluted to a maximum intensity of not more than 1,000 AU. To analyze the fluorescence characteristics of the material, blank mineral water was measured for its fluorescence and used as a baseline to remove any noise from the measured eluted samples.

2.7. Size exclusion chromatography-dissolved organic carbon (SEC-DOC)

Quantitative analysis was performed using LC-OCD Model 8 (DOC Labor, Germany) to characterize the molecular weight of the eluted organics. UV absorbance was measured with a spectrophotometer at wavelengths of 254 nm, with additional organic carbon measurements. Samples were injected into a size exclusion chromatography (SEC) column (Toyopearl HW-50S) filled with resins to separate the organic matter according to their molecular size. Samples were eluted with phosphate buffer (28 mM of phosphate buffer, pH 6.58) at a flow rate of 1.1 mL min^{-1}.

2.8. Particle size distribution

The DLS (Zetasizer Nano, Malvern Instrument, UK) was used to measure the size distribution of eluted organic particulates. The DLS has a particle size measuring a range of 0.3 nm to 10 μm, equipped with a He-Ne laser source with a wavelength of 633 nm and 90° fixed angle detector. The eluted material was added to cuvettes under ambient temperatures and placed in the instrument for measurement. Measurements were conducted in triplets with a 30 s interval.

3. Results and discussions

3.1. Elution of organic matter from polymer materials

To evaluate the organic elution and its contribution to microbial growth, four selected polymer materials (PE, CPVC, EPDM, and natural latex) were cultured at 60°C for 24 h. The 24-h experiments were repeated for seven times separately for a week. Each of the trials was separately analyzed for the TOC and AOC, where each 24 h sample was labeled M1–M7. Fig. 2 shows the results of the eluted organic matter of M1, M3, and M7, where the TOC of all materials showed a decrease in the elution with time. The total amount of eluted organic matter was higher in the materials with amorphous structure such as EPDM and natural latex compared with PE and CPVC. This corresponded with previous migration experiments where the amorphous or flexible materials show higher tendencies to elute organic matter [17,18].

Aside from the TOC results, the AOC from the selected materials showed different elution tendencies, where the AOC elution of PE and CPVC decreased with time, EPDM showed an increase in elution with time. Additionally, natural latex also showed distinctive characteristics, with an increase in AOC on the third trial and decreased amounts on the seventh trial. This may be due to the surface coating of the EPDM and

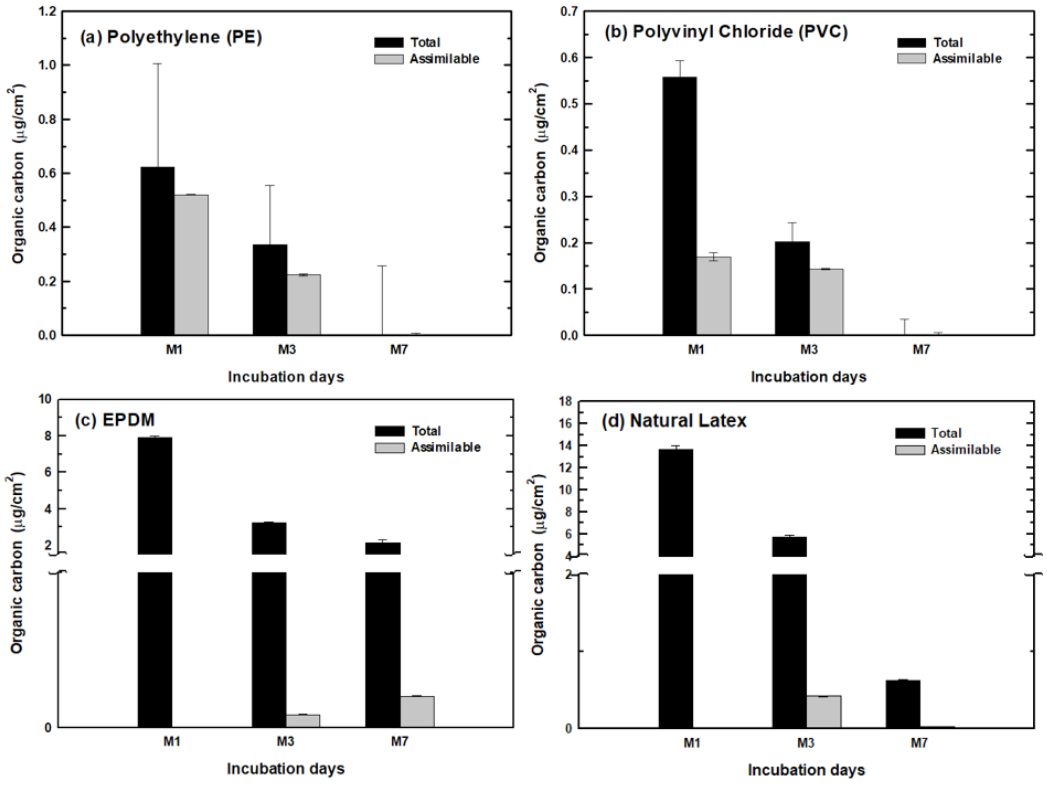

Fig. 2. MP test: total organic carbon (total) and assimilable organic carbon (assimilable) of quantified on result from incubation days (M1, M3, and M7).

natural latex, which may elute during the initial stages of the experiment. The coating materials of the polymer materials are known to inhibit the growth of microorganisms or contain non-degradable substances [18] lowering the amount of AOC ratio of the results. The bioavailability of the eluted organic matter, by polymer materials, is summarized in Table 2. Results show the ratio between the 7-d accumulated TOC and AOC. Bioavailability of EPDM and natural latex was very low due to the high TOC elution. This may indicate that the bioavailability may not fully represent the potential growth of microorganisms. However, with knowing the amount of organic elution from a material, the bioavailability may aid in the prediction of BFP. Additionally, in the case of EPDM and natural latex, since AOC tends to increase with time after M3, it is expected that microbial re-growth after 7 d becomes active and bioavailability is also increased.

Table 2
Bioavailability (ΣAOC/ΣTOC) of organic carbon migrated from selected polymer materials

Materials	Bioavailability (%)
Polyethylene (PE)	77.7
Chlorinated polyvinyl chloride (CPVC)	41.0
EPDM	1.1
Natural latex	1.5

3.2. Characteristics of eluted organic matter

3.2.1. Characteristics of initially eluted organic matter

To verify the type of organic matter eluted from the polymer, fluorescent EEM was conducted on the M1 samples. Background organic matter eluted from the glassware or found initially in the mineral water was removed from the samples. This was done by initially measuring the EEM of filtered mineral water from a blank MP experiment. Results of the four selected polymer materials are shown in Fig. 3. It can be seen that PE, EPDM, and natural latex show emission peaks below 380 nm. Emission peaks found in this region are in general found in an organic matter related with protein-like or other microbial exudates [19,20]. Among the three materials, each shows a slight difference in the excitation and emission peaks. EPDM show peaks of $\lambda_{excitation}/\lambda_{emission}$ <220/300, and 270/300. These two peaks are, in general, found in substances such as lignin and aromatic amino acids, such as tyrosine [20–22]. EEM peaks for PE are similar as seen with the EPDM, however the range of the peak at excitations of 220–230 are a slightly wider range of emission wavelengths. This may be due to more diverse forms of lignin matter such as vanillic or syringic acids [23]. Natural latex also has an emission peak under 380 nm; however, the emission wavelengths are slightly higher than PE, and EPDM ranging at 330–360 nm. This can be associated with protein-related substances such as tryptophan [24]. Further investigation is required to determine the compounds

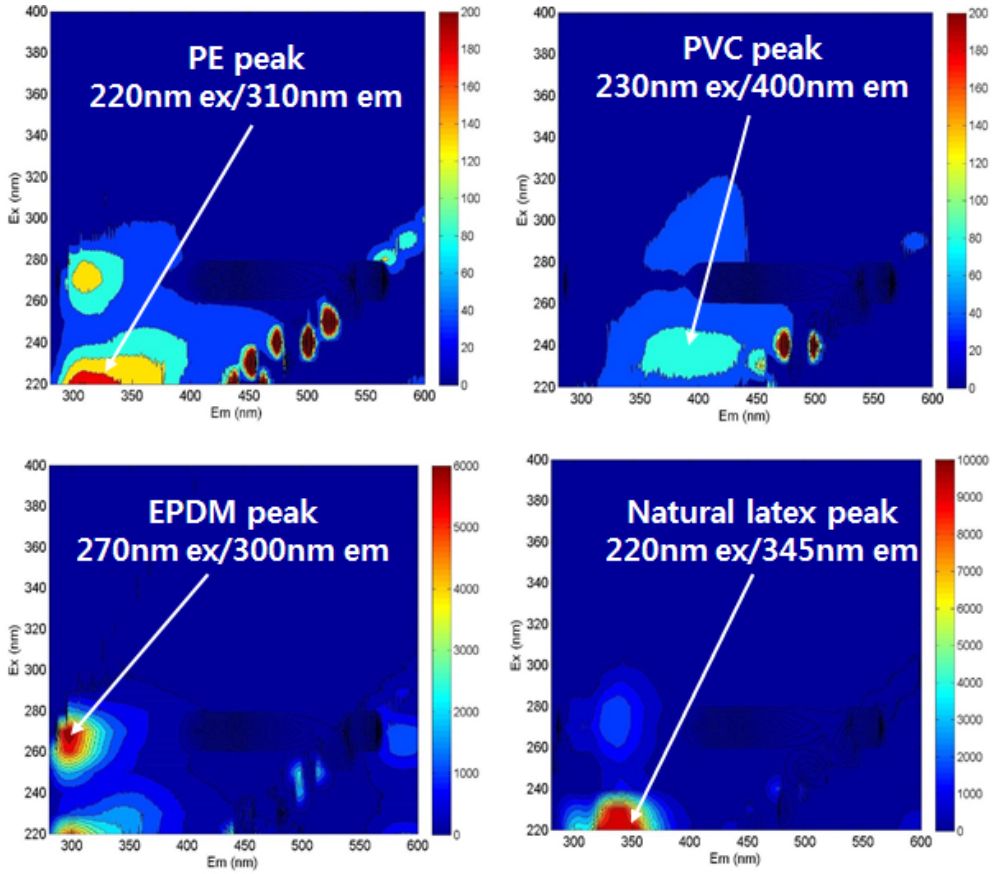

Fig. 3. EEM peak of the target material for water from M1 via migration potential.

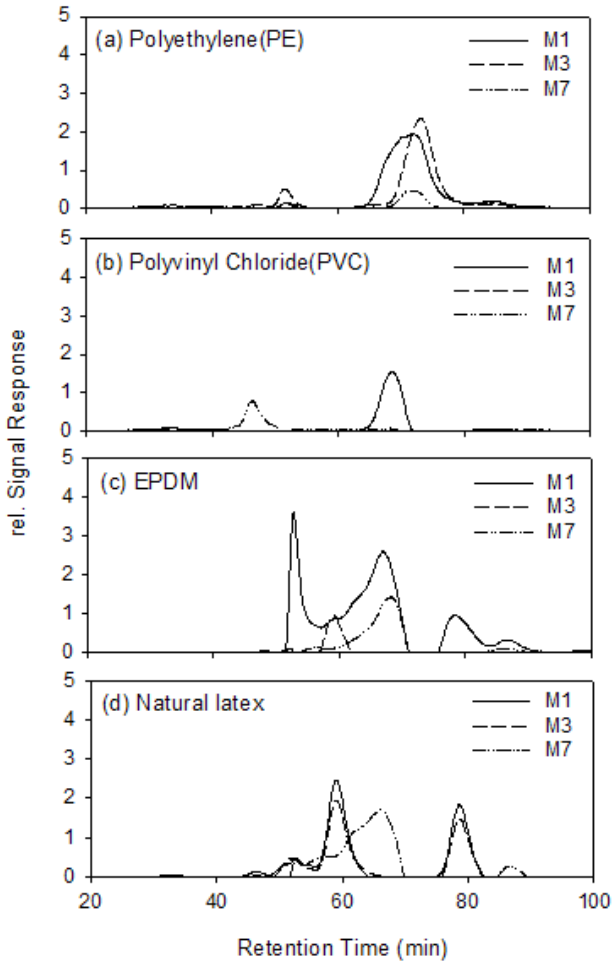

Fig. 4. SEC-DOC chromatograms of the target material for water from MP (migration potential) test.

that appeared in the region of protein-like substances. CPVC, however, show peaks in ranges different from the other materials, with $\lambda_{excitation}/\lambda_{emission}$ of 225–245/360–450. This excitation/emission range is known to be associated with polyaromatic hydrocarbons (PAHs) such as anthracene [25], which may act as toxins or inhibitory substances for microbial growth.

3.2.2. Elution of organic matter with time

As shown in Fig. 2, the amount and composition of eluted organic matter changed with time. Fluorescence EEM, LC-OCD, and particle size analysis were conducted. Fig. 4 shows the LC-OCD results of the M1, M3, and M7 samples from the MP tests. Results showed a general decrease in the size of organic matters eluted. In Fig. 4(a) PE peaks in the 60–80 min range show a decrease in size, where the M3, and M7 peaks show smaller organic matter compared with M1. This can also be seen with EPDM and natural latex, where the larger organic matter (shorter retention time) decreased or smaller organic matter increased. However, with CPVC it can be seen that the size of eluted organic matter increased from the M1 samples to the M7 samples.

DLS and fluorescent EEM were conducted to further analyze the size and quantity of trace organic matter. Fig. 5 shows the results of the DLS and fluorescence EEM of PE and CPVC samples. PE and CPVC samples were emerged in filtered mineral water and retained under MP experimental conditions for 72 h. The signal intensity of fluorescence EEM was used to characterize the trace amount of eluted organic matter, due to its high sensitivity. PE and CPVC were the only materials tested for the EEM intensity, due to the fact that the eluted organic matter concentration for the two materials was under 3 mg/L. It is known that the intensity of EEM signals and concentration has a good correlation up to 4 mg/L. However, with concentrations over 6 mg/L, the values will be under estimated, making it difficult to correlate the signal intensity with organic matter [26]. For comparison, filtered mineral water was also measured to verify the background organics in the

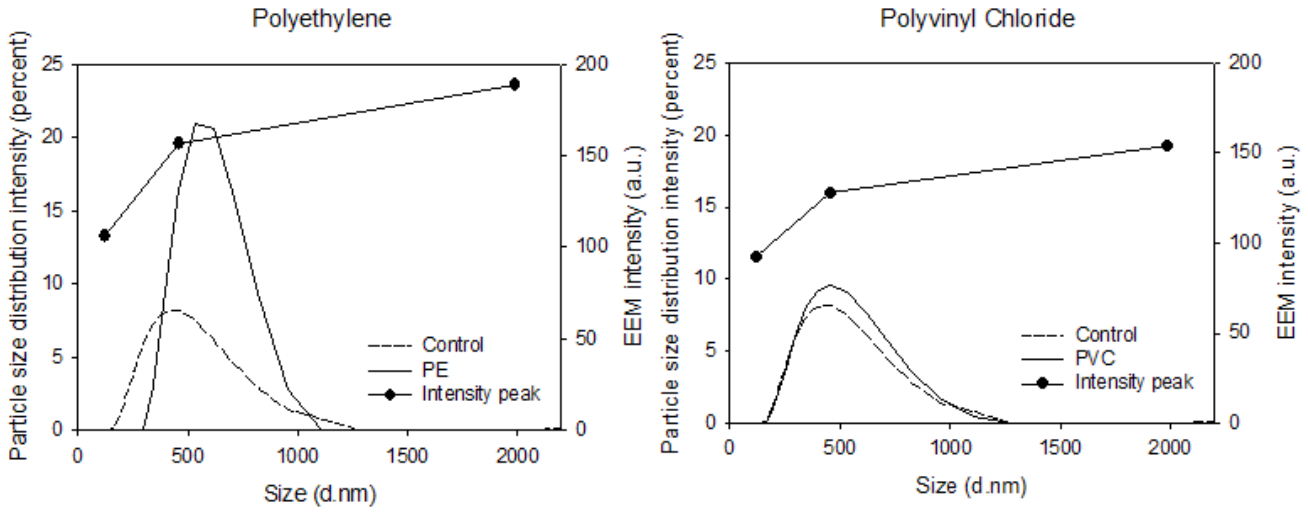

Fig. 5. Size distribution of retained water for 3 da (72 h) under MP (migration potential) test condition (60°C).

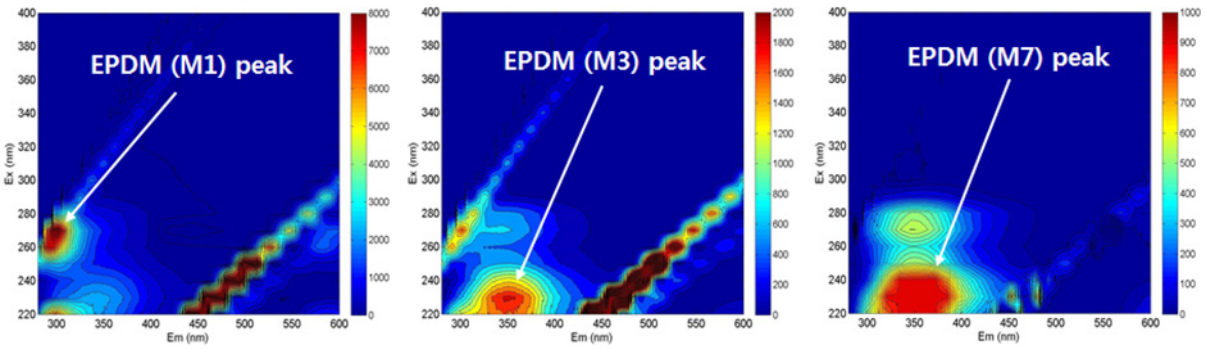

Fig. 6. EEM peak of the EPDM for water from MP (migration potential) test (M1, M3, M7).

samples. Prior to measuring the EEM intensity, three separate samples were prepared, each going through no filtration, 0.45 μm filtration, and 0.1 μm filtration to separate the organic matter by size. Results showed that the majority of eluted matter from both materials is composed of organic matter smaller than 0.1 μm. PE showed to have, approximately, half of the matter being below 0.1 μm, approximately, quarter of the amount between the sizes of 0.1 and 0.45 μm, and the remaining amount being larger than 0.45 μm. For CPVC, the majority of eluted materials were smaller than 0.1 μm. Comparing the results from Fig. 4(b), it can be speculated that the larger organic matter was eluted in the later stage of the elution. The DLS analysis results showed higher and larger amounts of organic matter with PE, where the majority of organic particulates were larger than 0.5 μm. However, CPVC showed the minimal difference with the background organic matter, again indicating that only small organic matter will elute from CPVC at initial stages.

The change in the composition of the eluted EPDM material was verified through EEM analysis with the M1, M3, and M7 samples. Results showed in Fig. 6 indicate a shift in the composition, where the wavelength of the emissions became higher with time. The initial eluents from the M1 samples showed peaks associated with lignin, where the M7 samples show peaks associated with tryptophan-like substances. This is in agreement with Fig. 2, where the AOC increases with time, indicating that more biodegradable materials elute from the EPDM with time.

3.3. Biofilm formation potential

Fig. 7 shows the results of the BFP experiments of the four selected polymer materials, and PE, EPDM, and natural latex showed a high amount of biomass formation for both planktonic and sessile cell count. For PE, the planktonic cell count was the highest among the tested materials. This may be due to the high initial AOC migration, seen in Fig. 2. As shown in the EEM analysis (Fig. 3), the initial elution of organic matter was seen to be composed of biodegradable materials. The sessile cell count of PE has shown to be lower than EPDM, and natural latex which is also due to the decrease in AOC elution with time. EPDM, and natural latex show a higher amount of sessile biomass, due to the increase of AOC elution (Fig. 2). Additionally, the increase of smaller biodegradable organic matter may have enhanced the growth of the sessile biomass as well as the planktonic biomass. The

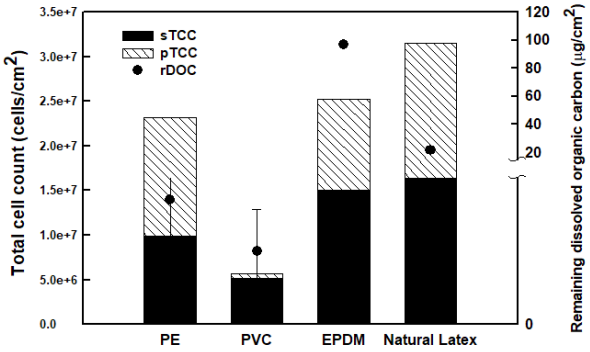

Fig. 7. BFP: the planktonic and sessile total cell count (pTCC and sTCC) and the remaining DOC (rDOC).

change in eluted materials from lignin type products to amino acids such as tryptophan also reinforces the growth of the total biomass (Fig. 6). The higher amount of sessile biomass in EPDM, and natural latex may not only be due to the higher amount of AOC elution but also due to the gradual increase of the process as well. With a general increase of AOC matter, the biomass attached to the surface of the polymer material may increase in numbers with the increasing AOC elution, matching the growth stages of the sessile biomass. For CPVC, however, the total biomass formation showed to be lower compared with the amount of this is due to the composition of the eluted organic matter, where the PAHs inhibit the growth of the biomass. Results of the BFP tests show that the PAH have a higher impact on the planktonic cells, compared with the sessile cell count. This may indicate that with longer elution periods, the amount of PAH decreases, resulting in the increase of sessile cells, while the residual PAH in the water will inhibit the suspended cell growth.

4. Conclusions

The current study was carried out to investigate characteristics of organic matter from the MP tests, and how it relates to the BFP using BioMig. EEM, LC-OCD, and DLS were conducted on the samples from the MP tests to assess the characteristics of the eluted organic matter from selected polymer materials. The BFP experiment was conducted to determine the potential biofilm formation on selected polymer materials. Based on the results of this study, we summarized as follows:

- In general, the more flexible materials showed a high amount of TOC elution with a low AOC to TOC ratio. However, the total amount of eluted organic matter was higher for the flexible EPDM, and natural latex.
- Based on the material characteristics, the amount and characteristics of the elution process may differ. The amount of total elution decreased in all selected materials. However, the elution characteristics differed by materials, where PE and CPVC showed a decrease in AOC elution with time, EPDM and natural latex showed a gradual increase. This may be due to coating materials used on the EPDM and natural latex. Additionally, EEM analysis indicated that eluted materials from PE, EPDM, and natural latex were composed of biodegradable matter, where CPVC eluted PAHs.
- The size and characteristics of the organic matter eluted from the polymer also changed with time. With longer testing periods, the organic matter eluted from the polymers was shown to be smaller in size. More biodegradable materials were proven to be eluted as well, where in the case of EPDM eluates changed from lignin-like substances to proteins such as tryptophan.
- With the combination of the MP tests and advanced organic matter characteristics tools, the migration analysis results showed a strong correlation with the BFP. CPVC, which eluted PAHs, showed the lowest amount of biomass formation. BFP results of PE, EPDM, and natural latex were proportional to the AOC amounts of the MP tests.

Acknowledgments

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