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Characterization of Molecular Weight–Based Fluorescent Organic Matter and Its Removal in Combination of Constructed Wetland with Activated Sludge Process

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Abstract Integrated constructed wetland into activated sludge process has a potential in improving treated wastewater with high organic loading. However, biological activities on those processes will generate microbial by-products from substrate metabolism and cell lysis. The presence of those compounds in effluent of wastewater treatment causes problems in source water. This study combines fluorescence excitation emission matrices (FEEM) with high-performance size exclusion chromatography with fluorescence detector (HPSEC-FLD) to characterize molecular weight–based fluorescent of organic matter and its removal in combination of constructed wetland with activated sludge process. The results show that three components of fluorescence organic: fulvic acid–like (Ex/Em 250/440 nm), SMP-like (Ex/Em 280/350 nm), humic acid–like (Ex/Em 340/420 nm), have been identified in all samples by the FEEM. Further, the HPSEC-FLD, which was set up based on chosen fluorescence wavelength, revealed two different apparent molecular weight (AMW) fractions: high molecular weight (HMW)/biopolymer (50,000 Da) and medium molecular weight (MMW)/humic substance–like (3000–650 Da). Peak-fitting determines that the area of MMW is higher than the area of HMW of all fluorescence organic components, and the area of HMW of fluorescence fulvic acid–like is comparable with the area of SMP-like, and no HMW of humic acid–like detected. Humic acid–like and fulvic acid–like were removed during treatment, while metabolite by-product were released as shown by increasing fluorescence SMP-like and TOC concentration. This method gives new insight to characterize organic matter for assessing effluent of wastewater quality and determining the appropriate water treatment.

Keywords Constructed wetland · Soluble microbial byproducts · Fluorescence · HPSEC

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1 Introduction

Constructed wetlands, a green treatment technology by simulating natural wetlands, has been widely used to treat various kinds of wastewater such as agricultural wastewater, industrial effluent, mine drainage, landfill leachate, storm water, polluted river water, and urban runoff in the last few decades (Yalcuk and Ugurlu 2009; Akizuki et al. 2018). Constructed wetlands represent a technology which may address more rigorous nutrient removal while resulting in relatively low maintenance

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requirements and operating costs. In some cases, a standalone constructed wetland system is unable to achieve high removal of pollutant and meet the standard water quality effluent. Therefore, constructed wetlands with pretreatment systems could enhance the treatment process (Liu et al. 2015; Su et al. 2018). Integrated constructed wetlands into biological process, such as activated sludge, have a potential for removing high organic loading; thus, they could improve treated water quality (Liu et al. 2015). It has been published that biological processes released soluble microbial products (SMPs), which are derived from metabolite activities during biomass growth, and cell lysis from biomass decay. Another metabolite product during biological process is extracellular polymeric substances (EPS), which support the aerobic granule formation and protect bacterium from harsh environmental conditions (Ni et al. 2010; Xie et al. 2012). Both of SMPs and EPS are made up from different kinds of macromolecules such as carbohydrates, proteins, fulvic, humic, and nucleic acids and contain large quantities of aromatic structures and unsaturated fatty chains with various types of functional groups (Shon et al. 2012; Xie et al. 2016). The presence of complex and heterogeneous soluble organic compounds in effluent of wastewater treatment may adversely affect the effluent quality, further causing serious impact to the quality of receiving waters as drinking water sources. Soluble organic had disinfection by-product (DBP) formation potential (DBPFP) around 5.6 μmol/mmol DOC (dissolved organic carbon). It is found that proteins, and humic-like substances in SMPs, can act as reactive DBP precursors (Liu et al. 2014).

In practice, natural organic matter (NOM) is usually represented by total organic carbon (TOC), dissolved organic carbon (DOC), or absorption of UV-light (UV_{254}). However, it mostly provides the quantity information of NOM, while offering limited information about its characteristics (Matilainen et al. 2011). A number of characterization methods have been conducted to provide a rapid qualitative and quantitative indications of organic matter in wastewater treatment systems (i.e., SMPs and EPS), such as fluorescence excitation emission matrices (FEEM), high-performance size exclusion chromatography (HPSEC) under different detectors, and its combination (Sillanpää et al. 2015). The FEEM is a simple, fast detection, and very sensitive tool that requires small sample volume and little or no sample pre-treatment; however, only fluorophores containing dissolved organic matter (DOM) constituents will respond to this method, and indicating the lack of characterization of non-fluorophores fractions. In contrast, the HPSEC, which fractioned organic matter based on its molecular weight (MW), is consuming time and materials (Huber et al. 2011; Lai et al. 2015; Hidayah et al. 2017). Peak-fitting is considered a tool for distinguishing quantitative information of HPSEC chromatogram area (Chow et al. 2008; Lai et al. 2015; Hidayah et al. 2017).

Dissolved organic matters are very heterogeneous and composed of complex mixture of different molecular weights and fluorescence organics; thus, identification and interpretation of organic fractions are being difficult. Combination of the FEEM with HPSEC fluorescence detector (HPSEC-FLD) will give new insight to the characteristic of organic matter. The FEEM presented different spectral regions, which are interpreted as different fluorescence organics, and its combination with HPSEC can resolve different fluorophores within different molecular weights. A number of combination of the FEEM with HPSEC-FLD studies have been conducted for environmental application. Her et al. (2003) used the HPSEC with sequential detector, including organic carbon (OC), ultraviolet (UV), and FLD to provide qualitative information of organic matter based on chosen peak maxima of FEEM wavelength. Nagao et al. (2003) assessed the monitoring wavelength of excitation/emission (Ex/Em, 320/430 nm) and revealed four peaks with apparent MW of 2580–10,700 in river water. Chabaliná et al. (2013) conducted quantification and characterization of EPS by using 3D FEEM and HPSEC separately in a membrane bioreactor. Li et al. (2013) demonstrate that FEEM scan and HPSEC with fluorescence detector under multi-excitation scan or multi-emission scan could relate the polarity of fluorescent organic matter species in textile effluents. Even Xiao et al. (2018) found a correlation between lower wavelength (Ex/Em, $< 300/< 280$ nm) and the smaller MW, and Em > 400 nm correlated to the higher MW.

According to the previous research described above, a few number of combination FEEM and HPSEC-FLD studies had been applied to provide qualitative and quantitative information of fluorophores within different molecular weights in wastewater treatment, especially related to the metabolite by-products. To our knowledge, the FEEM with parallel factor (PARAFAC) analysis is most widely used to characterize and track organic matter in constructed wetland and biological process, without considering MW distribution of fluorescent organic (Yao et al. 2016; Sgroi et al. 2018; Moradi et al. 2018; Hidayah and Cahyonugroho 2019). Therefore, the objective of this study was to characterize molecular weight–based fluorescent organic matter and its removal in combination of constructed wetland with activated sludge process. Qualitatively, FEEM was used to identify the fluorescent peak of organic, and the chosen peak was selected to perform the HPSEC-FLD analysis for characterizing organic matter in domestic wastewater. Quantitatively, peak-fitting was used to determine the peak area for further removal analysis.

2 Material and Methods

2.1 Experimental Setup

Domestic wastewater was collected from Sewage Treatment Plant-Jawaharlal Nehru University (STP-JNU), New Delhi, India. The constructed wetland system consists of equalization tank to collect domestic wastewater; activated sludge process, including aeration tank, and clarifier; and two subsurface horizontal flow constructed wetlands (0.6 m long, 0.6 m wide, and 0.6 m deep) filled with 0.3 m gravel. Aeration tank has a capacity of 60 L; flow rate of 120 L/day; and size of 0.6 m long, 0.6 m wide, and 0.25 deep. Air flow rate of 3 L/min was injected during treatment. Subsurface-constructed wetlands were planted with 2 pieces of *Canna indica* per bed with consideration of density 4 plant/m^2 (Wu et al. 2015). Initial *Canna indica* has about 9–14 number of leaves and height of 43–105 cm. Range finding test was conducted in 7 days by observing the growth daily, and followed by 3-weeks plant acclimation for adapting *Canna indica* to the environmental conditions (Vymazal 2018). Domestic wastewater was fed continuously from equalization tank to the activated sludge 20 L/day and 50% sludge recirculation from clarifier tank; the system was maintained for F/M = 0.05–0.1 kg BOD/kg and SVI = 50–100 mL/g (Metcalf and Eddy 2002). Further, treated effluent from clarifier was discharged into constructed wetland 2 (CW2) at the same flow rate. For controlling experiment, domestic wastewater was discharged directly (without activated sludge) from equalization tank to constructed wetland 1 (CW1) system with flow rate of 20 L/day. Sample was collected before treatment (RW), effluent CW1, effluent of activated sludge (AS), and effluent CW2 within twice per week for a month of observation.

2.2 Analytical Methods

Samples were made particle-free by passing through a 0.45-μm filter (cellulose acetate, Toyo Roshi, Japan) because this study focused on dissolved organic matter (APHA, AWWA,, and WEF 2012). Filtered samples were measured for quantitative analysis, such as total organic carbon (TOC), using TOC Analyzer 5000A Shimadzu; ultraviolet absorbance at 254 nm (UV_{254}), using Carry 100 Bio UV-Visible Spectrophotometer (APHA, AWWA,, and WEF 2012); and specific ultraviolet absorbance (SUVA) through dividing UV_{254} value to TOC concentration (Edzwald and Tobiason 2011). Qualitatively, filtered samples were measured by using fluorescence spectroscopy and chromatography. Fluorescence peak spectra were generated for each sample by scanning over excitation wavelengths (Ex) between 230 and 400 nm at interval of 10 nm and emission wavelengths (Em) between 300 and 550 nm at intervals of 0.5 nm through Perkin Elmer LS-55. Region of fluorescence spectra was determined according to Chen et al. (2003). Average of the chosen peak maxima location of excitation-emission wavelength was used to select wavelengths for chromatography analysis. High-performance exclusion chromatography type, HPLC, LC-20 ATV, Shimadzu, Japan, with online fluorescence detector under different selected Ex/Em wavelengths (HPSEC-FLD), was conducted to fractionate organic matter based on its apparent molecular weight (AMW). HPSEC-FLD instrumental setup and peak-fitting technique for resolving chromatograph are described in previous study (Hidayah et al. 2017).

3 Results and Discussion

3.1 Characteristics of Raw Domestic Wastewater and Treated Wastewater

Figure 1 presents the concentrations of organic matter, including TOC, UV_{254} to indicate aromatic level of organic matter, and SUVA as indicator of phobicity organic compound. Edzwald and Tobiason (2011) have concluded that SUVA values higher than 4 indicate that organic matter is composed mainly of humic or

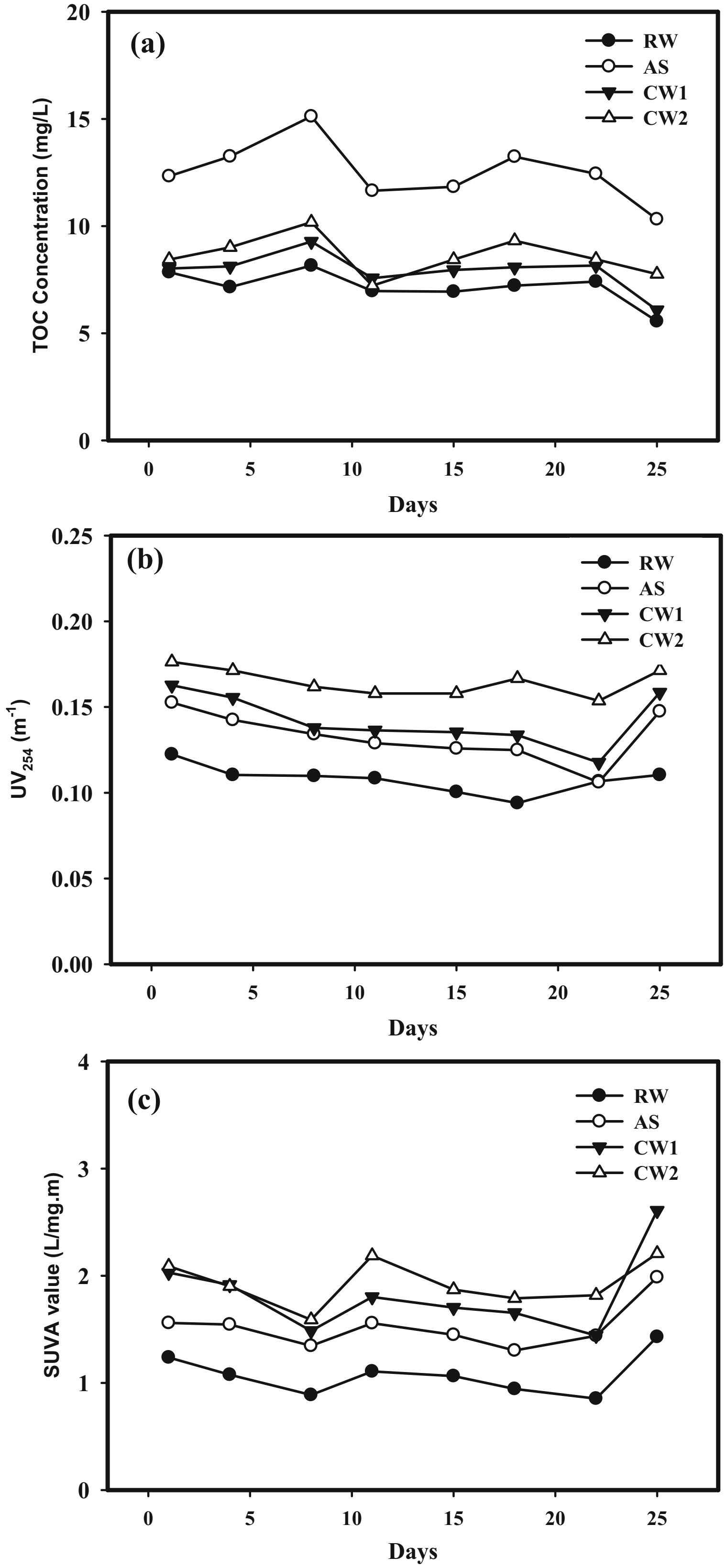

Fig. 1 Concentration of organic matter. **a** TOC. **b** UV_{254}. **c** SUVA value of raw domestic wastewater and treated wastewater

hydrophobic matter, while SUVA values less than 2 exhibit a non-humic or hydrophilic matter. The results show that raw water has lower TOC, lower aromatic compound, and more hydrophilic. Organic matter in domestic wastewater can be conjectured to be composed mainly of non-humic, less aromatic, and hydrophilic matter. These organic parameter concentrations increased significantly after activated sludge process, then slightly decreased at higher concentration than raw water, after constructed wetland processes. It means that characteristic of organic matter was changing during activated sludge process and constructed wetland treatment.

The changing of organic matter dramatically in activated sludge is probably due to microbial activity during microbial growth and microbial decay (Xie et al. 2012; Chabalina et al. 2013). Decreasing of organic matter after constructed wetland indicated that existed refractory organic matter could be degraded through mechanism process in constructed wetland, such as phytoextraction, rhizofiltration, phytostabilization, and phytodegradation (Wu et al. 2015; Vymazal et al. 2018). Refractory organic matter is poorly biodegraded; therefore, using plants to transfer those refractory organic matter from source of wastewater to shoots, using plant roots to absorb and uptake those refractory organic matter, is necessary. Those organic matter are immobilized through adsorption onto root surface and precipitation on it.

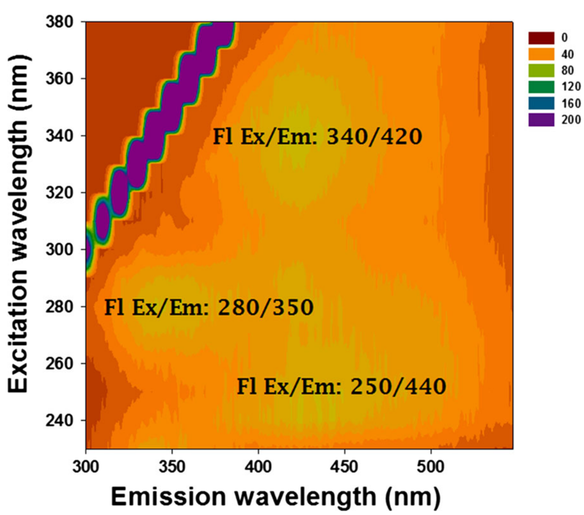

Fig. 2 Spectra of fluorescence excitation emission matrices of raw domestic wastewater

3.2 Characteristic of Molecular Weight–Based Fluorescent Organic Matter in Raw and Treated Domestic Wastewater

Figure 2 represents the FEEM spectra from one of the reference samples, and its contour plot describes the intensity of the FEEM. The results explain that domestic wastewater consisted of mainly three components of fluorescence-dissolved organic. The first component is fulvic acid–like (FA-like) with peak at Ex/Em 250/440 nm; the second component is found at Ex/Em 280/350 nm which is identified as soluble microbial by-product-like (SMPs-like); and the last component is humic acid–like (HA-like), which has shown the highest intensity peak at Ex/Em 340/420 nm. Fluorescence components in this study show consistent results with the previous studies (Her et al. 2003; Yao et al. 2016; Hidayah et al. 2017; Moradi et al. 2018). HA-like and FA-like in naturally dissolved organic matter mostly existed in terms of carboxylic and phenolic functional groups. These fluorescence structures are commonly present as a significant percentage of humic substances, which typically represent over 50% of NOM (Shon et al. 2012). HA-like and FA-like compounds have been identified in effluent from biological wastewater treatment plants, in terms of aromatic double bond, due to microbial activities during their metabolism and their decay (Ni et al. 2010). SMPs in domestic wastewater are composed mainly of carbohydrates, proteins, and humic substances; are produced from microbial metabolism; and constitute a major component of residual organic material. In activated sludge, SMP formation was contributed by heterotrophs in higher percentage than autotrophs (Ni et al. 2010; Xie et al. 2012; Xie et al. 2016).

This study results in a shift in the location of peaks due to impact of different treatment processes. The error in the shifted fluorescence peak is < 1% (Supporting Information); the resultant shifted fluorescence peak could not be ignored if the error is higher than 5% (Baghoth et al. 2008). Therefore, the chosen fluorescence peak for setting the HPSEC-FLD was determined according to average fluorescence peak and the number should be rounded off. Table 1 shows the variation intensity of fluorescence spectra in the dissolved organic component at Ex/Em peak of the raw water and treated water, obtained by the FEEM. The table describes that all samples have the same components, that is, FA-like, SMP-like, and HA-like, of dissolved organic at similar Ex/Em peak. This means that component distribution of dissolved organic in all samples did not change after treatment; however, the peak of fluorescence intensity spectra changed substantially. The changing of fluorescence intensity spectra shows consistent results with characteristic of organic matter surrogates, as mentioned earlier. The changing of intensity of fluorescence spectra indicated that the increasing or decreasing of fluorescent components was due to the effect of treatment process. However, only organic matter with molecules that contain fluorophores, which emit fluorescence at specific wavelengths, can be detected by fluorescence spectroscopy (Wünsch et al. 2015; Hidayah et al. 2017).

Figure 3, one the of representative samples, shows the molecular weight distribution of organic fractions as detected by different wavelengths of the HPSEC-FLD. The chromatograms fractioned two different apparent molecular weight (AMW) organic matter: first fraction is a high molecular weight (HMW) with AMW of about 50,000 Da and it is known as biopolymer; second is a

Table 1 Variation intensity dissolved organic component at Ex/Em peak of the source and treated water

Day	Sample	Intensity (AU)		
		Humic acid–like Ex/Em 340/420	Fulvic acid–like Ex/Em 280/350	SMP-like Ex/Em 250/440
1	RW	43.20	42.02	42.54
	AS	33.00	31.86	32.30
	CW1	22.98	21.67	22.17
	CW2	12.90	11.59	12.23
2	RW	45.62	41.96	42.11
	AS	33.37	31.85	32.70
	CW1	23.22	21.76	22.32
	CW2	13.04	11.64	12.58
3	RW	43.14	41.58	42.04
	AS	33.02	31.56	32.77
	CW1	22.95	21.52	22.30
	CW2	12.88	11.45	12.62
4	RW	43.48	42.03	42.40
	AS	33.31	31.85	32.41
	CW1	23.19	21.64	22.05
	CW2	13.04	11.51	12.15
5	RW	53.38	50.67	51.67
	AS	43.17	40.51	42.06
	CW1	33.08	30.46	31.79
	CW2	22.92	20.37	22.12
6	RW	53.26	50.86	51.25
	AS	43.18	40.65	41.64
	CW1	33.05	30.46	31.44
	CW2	22.94	23.97	21.56
7	RW	43.66	40.26	41.55
	AS	38.18	30.64	31.66
	CW1	25.02	20.76	21.23
	CW2	12.72	13.91	11.71
8	RW	50.24	45.86	47.25
	AS	41.68	37.65	40.24
	CW1	34.75	30.46	31.22
	CW2	20.64	23.97	21.51

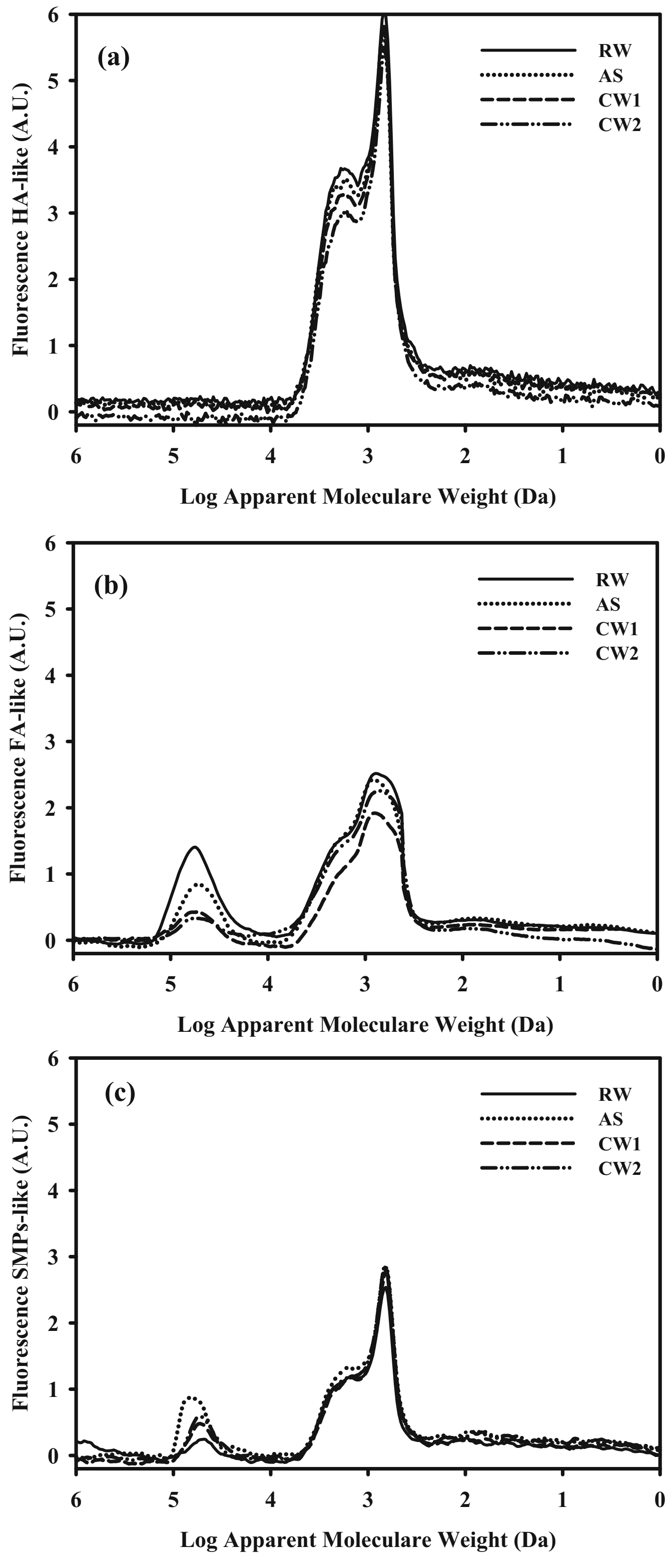

Fig. 3 Molecular weight distribution of fluorescence organic **a** humic acid–like; **b** fulvic acid–like; and **c** soluble microbial by-product-like into different fraction as detected by HPSEC-FLD

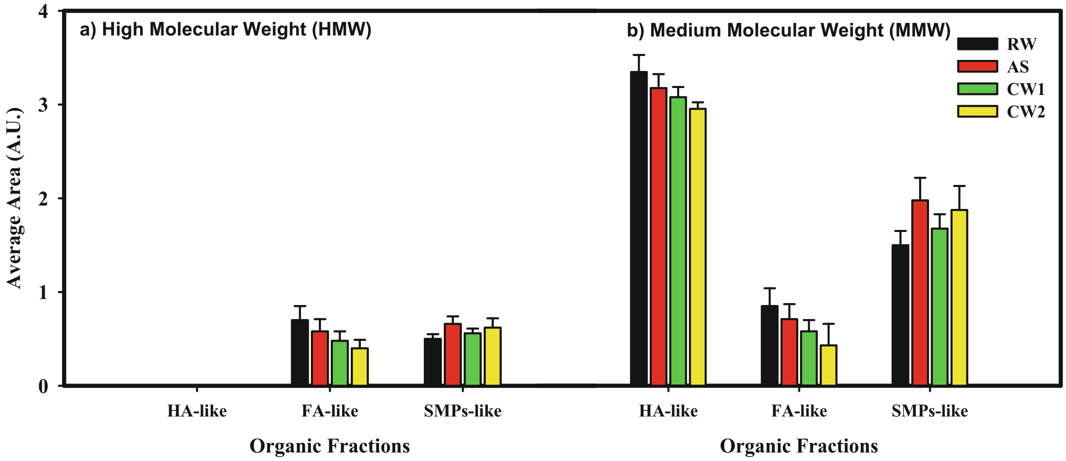

Fig. 4 Average of peak area of **a** HMW and **b** MMW of the HPSEC-FLD chromatograms in all samples by peak-fittings

medium molecular weight (MMW) with AMW of about 1650 Da and it is indicated as humic substance–like, building block, low molecular weight acid (Huber et al. 2011; Lai et al. 2015; Hidayah et al. 2017). The chromatograms explain that fluorescence organic of FA-like (Ex/Em 250/440 nm) and SMP-like (Ex/Em 280/350 nm) were composed of HMW and MMW, while HA-like (Ex/Em 340/420 nm) was contributed by MMW only. The result shows a consistency with previous study that mentions lower wavelength region Ex/Em < 300/280 nm) and higher wavelength region (Em > 400 nm) reflected the features of smaller and larger MW fractions, respectively (Xiao et al. 2018). All samples show the similar shape of the HPSEC-FLD chromatograms; it means that the AMW distribution of the dissolved organic content in all samples is similar. However, the heights of the peaks are different; it reflected a decreasing or increasing concentration of dissolved organic compound during treatment processes.

Figure 4 a and b summarize the average of peak area of HMW and MMW, respectively, of the HPSEC-FLD chromatograms in all samples by peak-fittings. First, area of MMW is much higher than area of HMW of all fluorescence organic component. MMW is mostly composed of humic substance-like, which existed about more than 50% of the dissolved organic carbon (Shon et al. 2012). In addition, the major organic fractions of the MMW were composed of three different fractions with wide AMW range, namely humic substance-like, building block, and low molecular weight acid, with AMW of about 1650, 1300, and 630 Da, respectively (Huber et al. 2011; Lai et al. 2015; Hidayah et al. 2017). It is consistent with the highest area of HA-like compared with FA-like and SMP-like in MMW. Second, area of HMW of fluorescence FA-like is comparable with area of fluorescence SMP-like, and no HA-like detected. Organic matter in wastewater is a combination of natural organic matter, SMP, and trace chemicals.

The organic composition of wastewater is approximately 50% protein and 40% carbohydrates, and the rest is trace contaminants (Shon et al. 2012; Xie et al. 2016). Previous studies found that SMP- and FA-like in wastewater and in effluent organic matter have a greater amount of HMW compounds, which are generated from substrate utilization, microbial growth, and endogenous phase (Ni et al. 2010; Xie et al. 2016; Zhiji et al. 2017). Third, fluorescence organic SMP-like in both high and medium molecular weight indicates a higher area of treated water than that of raw water, and vice versa for fluorescence FA-like in both molecular weight and humic-like. It seems that activated sludge process released more soluble dissolved organic during treatment processes that might be excreted by microorganism and be derived from microorganism decay (Xie et al. 2012; Chabaliná et al. 2013). After constructed wetland, average of SMP-like peak area decreased in both MW; it could probably be due to different kinetic productions or formation rates of SMP-like between activated sludge and constructed wetlands (Ni et al. 2010). Activated sludge process involves microorganism and organic matter as the main role components for biological process, while constructed wetlands associated with a number of mechanism processes, including physical, chemical, and biological processes.

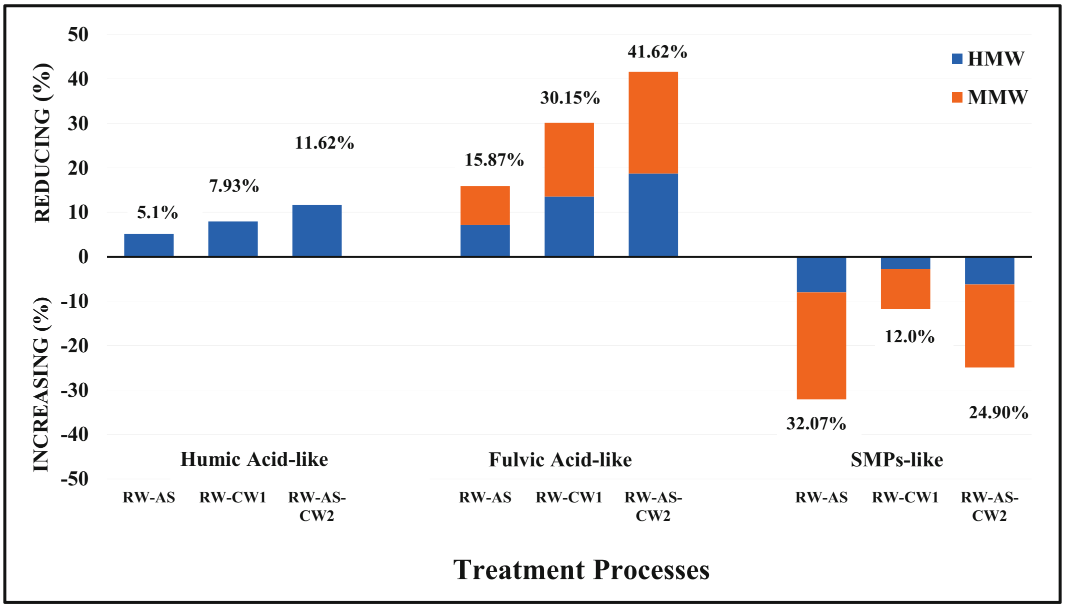

Fig. 5 Average percentage reducing and increasing in the area of each peak of HPSEC-FLD chromatogram after various treatments

3.3 Effect of Activated Sludge in Constructed Wetland to the Changing of Fluorescence Organic

Figure 5 describes the average percentage reducing and increasing in the area of each peak of HPSEC-FLD chromatogram after constructed wetland process without activated sludge (RW-CW1), with activated sludge process (RW-AS-CW2) and after activated sludge (RW-AS). Firstly, comparison between HMW and MMW removal of FA-like indicates that MMW removal is higher (22.85 ± 7.03%) than HMW removal (18.73 ± 5.76%). Thus, it can be seen that HMW of FA-like removal is higher (18.73 ± 5.76%) than HA-like removal (11.62 ± 2.94%); therefore, the total removal of FA-like is much higher (41.62%) than the total removal of HA-like (11.62%). It is probably due to peak of MMW that has wide range of AMW about 3000–650 Da, while peak of HMW has short range, about 50,000 Da. According to Huber et al. (2011), it has been classified that MMW has large proportion fractions, including humic substance fraction with humic acid and fulvic acid species, building block fraction that reflects breakdown products of humic substances or low molecular weight of humic substance–like material, low molecular weight acid fraction, and low molecular weight neutrals that are highly complex composition, and its peaks may appear overlap in surface water and show as an asymptotic steady line in ground waters sample. Fractionation of organic in the activated sludge has been clarified that decrease in humic-like substance concentration is attributed to hydrolysis and utilization by microorganism during growth, and fulvic acid–like were associated to non-growth phase (Ni et al. 2010; Zhiji et al. 2017). HA-like and FA-like of humic substance fraction in MMW seems to have high contribution in percentage removal of MMW (Chow et al. 2008; Hidayah et al. 2017). It is probably due to HMW pointed out to the presence of polysaccharide and biopolymer fractions with some contribution from nitrogen-containing material such as proteins or amino sugars (Huber et al. 2011; Hidayah et al. 2016).

Secondly, percentage removal of FA-like and HA-like shows a sequence of degree treatment process RW-AS-CW2 > RW-CW1 > RW-AS. It is probably due to constructed wetland has various mechanism process in removing HA-like and FA-like organic matter, including biological process, physical process, and chemical process, in which organic matter could be associated with different compounds and being removed through those mechanisms (Wu et al. 2015; Vymazal et al. 2018). Activated sludge associated to biological process only; therefore, the percentage removal of HA-like and FA-like organic matter in activated sludge is lower than that of constructed wetlands. Third, it can be seen that the area of both HMW and MMW of fluorescence organic SMP-like increased after activated sludge process (32.07 ± 10.66%), after constructed wetland treatment CW1 (12 ± 3.96%), and after CW2 (24.9 ± 8.75%). Increasing of SMP-like shows consistent results with increasing TOC as shown in Fig. 1. Activated sludge process and constructed wetlands contributed to the highest percentage increasing of SMP production.

Microorganism plays an important role for organic degradation in constructed wetland (Wu et al. 2015; Liu et al. 2015) and in activated sludge process (Metcalf and Eddy 2012). Activated sludge is the most widely used biological treatment process, although it is found that SMP-like will be generated during the process. SMPs, as one of the types of organic matter, could be degraded, treated, and removed by next processes, as shown in this study.

In constructed wetland, microbial communities can be associated with all surfaces of the plant including leaves, stems, and roots. The root-associated bacteria are considered to be the most relevant composition, since they contain high abundance of microbial organisms. The high abundance of bacterial cells present in root-associated microbial communities is primarily a function of the organic deposits made by plant roots into the surrounding environment which act as a source of nutrients for the associated microbiota (Vymazal 2018; Clairmont and Slawson 2019). In activated sludge, microorganisms are mixed with wastewater, come in contact with the biodegradable materials, and consume them as food (Metcalf and Eddy 2012). In both processes, microorganism activities, including growth and decay phases, generated metabolite by-products or SMPs, that is, utilization-associated products, which are derived from the original substrate in microbial growth, and the biomass-associated products generated in the endogenous phase have been identified as soluble microbial byproducts. SMP-like has been found to have a wide range of molecular weight distribution and different structures. SMPs utilization products were found to be more carbonaceous compounds with MW more than 10,000 Da, while biomass products have MW range of 290–5000 Da (Ni et al. 2010; Xie et al. 2012; Zhiji et al. 2017). According to its MW, this study classified utilization-associated products as HMW, and biomass-associated products considered as MMW. Generated utilization-associated products of SMPs could be degraded by heterotroph or autotroph microorganisms, while generated biomass-associated products would accumulate in the system due to slower utilization than utilized products. Therefore, it might cause lowering of increased SMP-like in CW1 and CW2. Using combination of the FEEM and HPSEC-FLD could reveal the main compound of fluorescence organics and identify their molecular weight simultaneously; therefore, it could give new sight to characterize organic matter, especially for assessing effluent of wastewater treatment which is discharged into water bodies, and for determining the appropriate water treatment which will be used in polluted source water.

4 Conclusion

Combination of the FEEM with HPSEC-FLD in conjunction with peak-fitting could assess characteristic of fluorescence organic matter based on its molecular weight in domestic wastewater, and in treated wastewater through combination of constructed wetlands with and without activated sludge process. The FEEM characterized organic fractions in domestic wastewater into three fluorescence organics, namely humic acid–like (Ex/Em 340/420 nm), fulvic acid–like (Ex/Em 250/440 nm), and SMP-like (Ex/Em 280/350 nm), and those fluorescence organics are fractionated into HMW with AMW 50,000 Da and MMW with AMW 3000–650 Da. The FEEM shows the changing of fluorescence intensity spectra peak due to effect of treatment process. The HPSEC-FLD revealed that biological process and constructed wetland process released more soluble dissolved organic during treatment processes, as described by increasing fractionated AMW of SMP-like, after activated sludge process (32.07 ± 10.66%), after constructed wetland treatment CW1 (12 ± 3.96%), and after CW2 (24.9 ± 8.75%). Fractionated AMW of fluorescence shows a higher decreasing fulvic acid–like (41.62%) than humic acid–like (11.62%). Combination of activated sludge with constructed wetland could enhance efficiency removal of organic matter, and the appropriate water treatment could be determined after knowing the characteristic of organic matter.

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