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**Euis Nurul Hidayah <euisnh@gmail.com>**

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**WATE** <em@editorialmanager.com> Mon, Oct 14, 2019 at 4:53 PM
Reply-To: WATE <aenna.masangkay@springernature.com>
To: euis nurul hidayah <euisnh@gmail.com>

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# WATE-D-19-01779 - Submission Confirmation

1 message

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To: euis nurul hidayah <euisnh@gmail.com>

Dear Dr. hidayah,

Thank you for submitting your manuscript,
"Characterization of Molecular Weight-Based Fluorescent Organic Matter and Its Removal in Combination of Constructed Wetland with Activated Sludge Process", to Water, Air, & Soil Pollution

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# Confirmation mail for Article 10.1007/s11270-020-4405-5

1 message

**CorrAdmin4@spi-global.com** <CorrAdmin4@spi-global.com> Mon, Jan 13, 2020 at 9:47 PM
Reply-To: CorrAdmin4@spi-global.com
To: euisnh.tl@upnjatim.ac.id

Journal: Water, Air, & Soil Pollution
DOI: 10.1007/s11270-020-4405-5
Title: Characterization of Molecular Weight–Based Fluorescent Organic Matter and Its Removal in Combination of Constructed Wetland with Activated Sludge Process

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# Your Submission WATE-D-19-01779

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**Jack T. Trevors** <em@editorialmanager.com> Thu, Dec 12, 2019 at 9:14 AM
Reply-To: "Jack T. Trevors" <lesliebarker@execulink.com>
To: euis nurul hidayah <euisnh@gmail.com>

Dear Dr. hidayah,

We have received the reports from our advisors on your manuscript, "Characterization of Molecular Weight-Based Fluorescent Organic Matter and Its Removal in Combination of Constructed Wetland with Activated Sludge Process", submitted to
Water, Air, & Soil Pollution

Based on the advice received, I have decided that your manuscript can be accepted for publication after you have carried out the corrections as suggested by the reviewer(s).

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Totally agree.
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it points out combination of constructed wetland and activated sludge treatment for removal of fluorescence organic matter, technically sound
5) Does the paper contain up-to-date references?
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Reply-To: WATE <aenna.masangkay@springernature.com>
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Dear Dr. hidayah,

We acknowledge, with thanks, receipt of the revised version of your manuscript, "Characterization of Molecular Weight-Based Fluorescent Organic Matter and Its Removal in Combination of Constructed Wetland with Activated Sludge Process", submitted to Water, Air, & Soil Pollution

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**Jack T. Trevors** <em@editorialmanager.com> Sat, Jan 4, 2020 at 4:34 AM
Reply-To: "Jack T. Trevors" <lesliebarker@execulink.com>
To: euis nurul hidayah <euisnh@gmail.com>

Dear Dr. hidayah,

We are pleased to inform you that your manuscript, "Characterization of Molecular Weight-Based Fluorescent Organic Matter and Its Removal in Combination of Constructed Wetland with Activated Sludge Process", has been accepted for publication in
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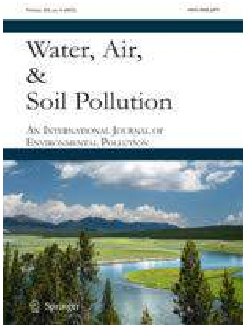


*Water, Air, & Soil Pollution*

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

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**Euis Nurul Hidayah** <euisnh.tl@upnjatim.ac.id> Thu, Jan 23, 2020 at 5:31 AM
To: Dr RAM BABU Pachwarya <pachwarya@gmail.com>, alrjnu@gmail.com

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**A.L. Ramanathan Ramanathan** <alrjnu@gmail.com> Thu, Jan 23, 2020 at 4:00 PM
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**Dr RAM BABU Pachwarya** <pachwarya@gmail.com> Thu, Jan 23, 2020 at 4:10 PM
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Water, Air, & Soil Pollution

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

--Manuscript Draft--

| | |
|---|---|
| **Manuscript Number:** | WATE-D-19-01779R1 |
| **Full Title:** | Characterization of Molecular Weight-Based Fluorescent Organic Matter and Its Removal in Combination of Constructed Wetland with Activated Sludge Process |
| **Article Type:** | Full research paper |
| **Keywords:** | constructed wetland; soluble microbial byproducts; fluorescence; HPSEC |
| **Corresponding Author:** | euis nurul hidayah<br>University of Pembangunan Nasional Veteran Jawa Timur<br>Surabaya, INDONESIA |
| **Corresponding Author's Institution:** | University of Pembangunan Nasional Veteran Jawa Timur |
| **First Author:** | euis nurul hidayah |
| **Order of Authors:** | euis nurul hidayah |
| | Ram Babu Pachwarya |
| | Okik Hendriyanto Cahyonugroho |
| | A.L. Ramanathan |
| **Funding Information:** | |
| **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 byproducts from substrate metabolism and cell lysis. The presence of those compounds in effluent of wastewater treatment causes problems in source water. This study combine 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 shows that three components of fluorescence organic: fulvic acid-like (Ex/Em 245/440 nm), SMPs-like (Ex/Em 280/350 nm), humic acid-like (Ex/Em 340/420 nm) have been identified in all samples by FEEM. Further, 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 substances-like (3000-650 Da). Peak-fitting determines that area of MMW is higher than area of HMW of all fluorescence organic components, and area of HMW of fluorescence fulvic acid-like is comparable with area of SMPs-like, and no HMW of humic acid-like detected. Humic acid-like and fulvic acid-like removed during treatment, while metabolite byproduct released as shown by increasing fluorescence SMPs-like and TOC concentration. This method gives new sight to characterize organic matter for assessing effluent of wastewater quality and determining the appropriate water treatment. |
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| | 4. EPs, is it the same with EPS? if yes, please shows a consistency word. if not, please give an abbreviation prior to acronym<br>Response: EPs is the same with EPS. It has been revised in the manuscript<br><br>5. PARAFAC, Please give an abbreviation prior to acronym<br>Response: abbreviation has been added into the revised version. Please check page 2 paragraph 3 of the revised manuscript<br><br>6. Ex; Em, Please give an abbreviation prior to acronym<br>Response: abbreviation has been added into the revised version. Please check page 2 paragraph 2 of the revised manuscript<br><br>7. Please explain about the activated sludge system, including: size of aeration tank and clarifier, air flow rate, etc.<br>Response: The activated sludge system has been added in the manuscript. Please check page 2 paragraph 4 of the revised manuscript<br><br>8.Please describe the meaning of 2 pieces Canna indica, including: number of leaves, age of plant, height, etc.<br>Response: the meaning of 2 pieces Canna indica has been added in the revised manuscript, please see page 2 paragraph 4 of the revised manuscript.<br><br>9. Please give a short information in discussion, regarding refractory organic matter could be degraded through phytoextraction, rhizofiltration, phytostabilization, phytodegradation.<br>Response: this information has been added in the revised manuscript Page 3, paragraph 2.<br><br>10. Please shows that fluorescence peak has been shifted less than 1%.<br>Response: Please see the attached file, standard error of the shifted fluorescence peak.<br><br>11. According to this study, the data shows that domestic wastewater treatment with activated sludge had generated soluble microbial-byproducts (RW-AS), further those SMPs was eliminated through constructed wetland (RW-AS-CW2).<br>Does it mean that activated sludge is not recommended for domestic wastewater treatment because the process will generate SMPs which is one of the DBPs precursors? Please give briefly explanation.<br>Response: Activated sludge is most widely used biological treatment process, although it is found that SMPs-like will be generated during the process. SMPs as one of type of organic matter could be degraded, treated, and removed by next processes, as shown in this study.<br>It has been added in the revised manuscript, page 8, paragraph 1. |
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| If yes, how is this research significant to furthering worldwide knowledge on this topic | This research is significant to characterize organic matter for controlling DBPs formation in source water through water treatment processes |
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| Please provide the name, affiliation and address, and e-mail address of three | 1. Prof. Lai Wen Liang (laisiff@gmail.com)<br>Graduate School of Environmental Management, Tajen University, Taiwan |

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

Euis Nurul Hidayah[(a)*], Ram Babu Pachwarya[(b)], Okik Hendriyanto Cahyonugroho[(a)], A.L. Ramanathan[(c)]

(a) Department of Environmental Engineering, University of Pembangunan Nasional Veteran Jawa Timur, Indonesia
(b) Department of Chemistry, Motillal Nehru College, University of Delhi, India
(c) School of Environmental Sciences, Jawaharlal Nehru University, India
*e-mail: euisnh.tl@upnjatim.ac.id

**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 byproducts from substrate metabolism and cell lysis. The presence of those compounds in effluent of wastewater treatment causes problems in source water. This study combine 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 shows that three components of fluorescence organic: fulvic acid-like (Ex/Em 250/440 nm), SMPs-like (Ex/Em 280/350 nm), humic acid-like (Ex/Em 340/420 nm) have been identified in all samples by FEEM. Further, 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 substances-like (3000-650 Da). Peak-fitting determines that area of MMW is higher than area of HMW of all fluorescence organic components, and area of HMW of fluorescence fulvic acid-like is comparable with area of SMPs-like, and no HMW of humic acid-like detected. Humic acid-like and fulvic acid-like removed during treatment, while metabolite byproduct released as shown by increasing fluorescence SMPs-like and TOC concentration. This method gives new sight to characterize organic matter for assessing effluent of wastewater quality and determining the appropriate water treatment.

**Keywords**: constructed wetland, soluble microbial byproducts, fluorescence, HPSEC

## 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 relatively low maintenance requirements and operating costs. In some cases, a standalone constructed wetlands systems is unable to achieve high removal of pollutant and meet the standard water quality effluent. Therefore, constructed wetlands with a pretreatment systems could enhanced the treatment process (Liu et al. 2015; Su et al. 2018). Integrated constructed wetlands into biological process, such as activated sludge, has a potential for removing high organic loading, thus could improve treated water quality (Liu et al. 2015). It has been published that biological processes released soluble microbial products (SMPs), which is 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 is supported the aerobic granules formation and protect bacterium from harsh environmental conditions (Ni et al. 2010; Xie et al. 2012). Both of SMPs and EPS are made up of from different kind of macromolecules such as carbohydrates, proteins, fulvic, humic, nucleic acids, etc 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 receiving waters as drinking water sources. Soluble organic had disinfection by-products (DBPs) 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 is mostly provide the quantity information of NOM, while offering limited about its characteristics (Matilainen et al. 2011). A number of characterization method have been conducted to provide a rapid qualitative and quantitative indication 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). 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, HPSEC, which fractioned organic matter based on its molecular molecular weight (MW), is consuming time and materials (Huber et al. 2011; Lai et al. 2015; Hidayah et al. 2017). Peak-fitting is considered as 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 is very heterogeneous and composed with complex mixture of different molecular weight and fluorescence organic, thus, identification and interpretation of organic fractions is being difficult. Combination FEEM with HPSEC fluorescence detector (HPSEC-FLD) will give new insight to the characteristic of organic matter. FEEM presented different spectral regions, which is interpreted as different fluorescence organic, and its combination with HPSEC can resolve different fluorophores within different molecular weight. A number of combination FEEM with HPSEC-FLD studies have been conducted for environmental application. Her et al. (2003) used 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. Chabalina et al. (2013) conducted quantification and characterization of EPs by using 3-dimensional FEEM and HPSEC separately in 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 researches 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 weight in wastewater treatment, especially related to the metabolite byproducts. To our knowledge, 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. 2017; Moradi et al. 2018; Hidayah et al. 2019). Therefore, the objectives of this study were 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 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 Set Up

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, 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 capacity 60 L, flow rate 120 L/day, and size 0.6 m long, 0.6 m wide, and 0.25 deep. Air flow rate 3 L/min was injected during treatment. Subsurface constructed wetlands were planted with 2 pieces *Canna indica* per-bed with considering density 4 plant/$m^2$ (Wu et al. 2015). Initial Canna indica has about 9-14 number of leaves and height 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/gr (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 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 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 focus on dissolved organic matter (APHA 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 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 wavelength (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) has concluded that SUVA values higher than 4 indicate that organic matter is composed mainly of humic or hydrophobic matter, while SUVA values less than 2 exhibits 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 parameters concentration 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.

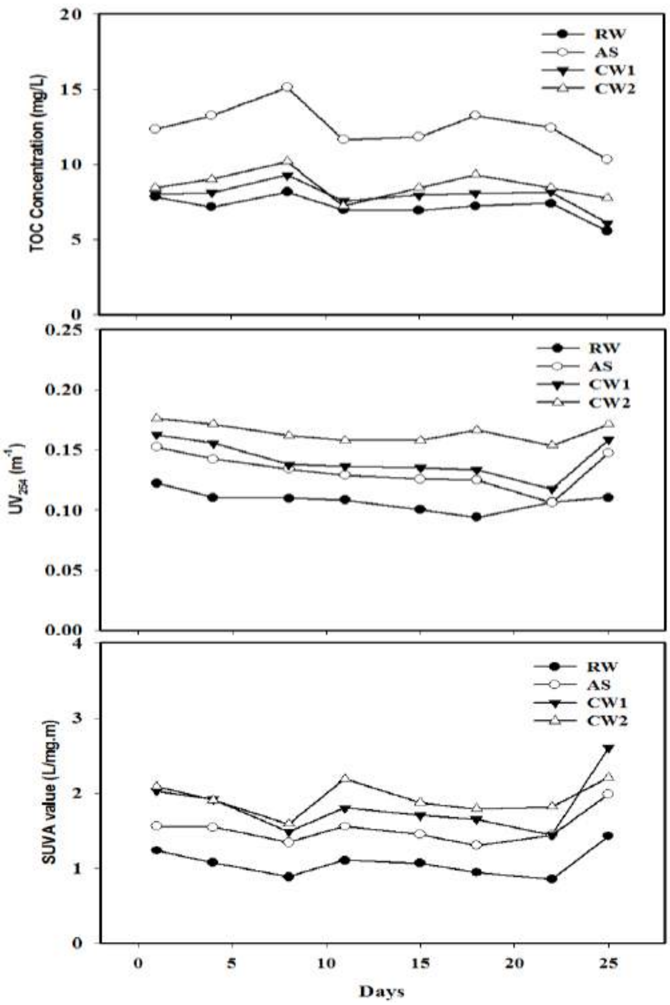


**Fig.1** Concentration of organic matter: (a) TOC, (b) $UV_{254}$, (c) SUVA value of raw domestic wastewater and treated wastewater

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, 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 plants roots to absorb and uptake those refractory organic matter. Those organic matter are immobilized through adsorption onto root surface and precipitation on it.

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 sample, and its contour plot describes the intensity of FEEM. The results explains that domestic wastewater consisted of mainly three component 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 byproducts-like (SMPs-like), and the last component is humic acid-like (HA-like), which have shown the highest intensity peak at Ex/Em 340/420 nm. Fluorescence components in this study show a consistency 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 natural dissolved organic matter are mostly existed in term 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 compound have been identified in effluent from biological wastewater treatment plants, in term 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, SMPs formation was contributed by heterotrophs in higher percentage than autotrophs (Ni et al. 2010; Xie et al. 2012; Xie et al. 2016).

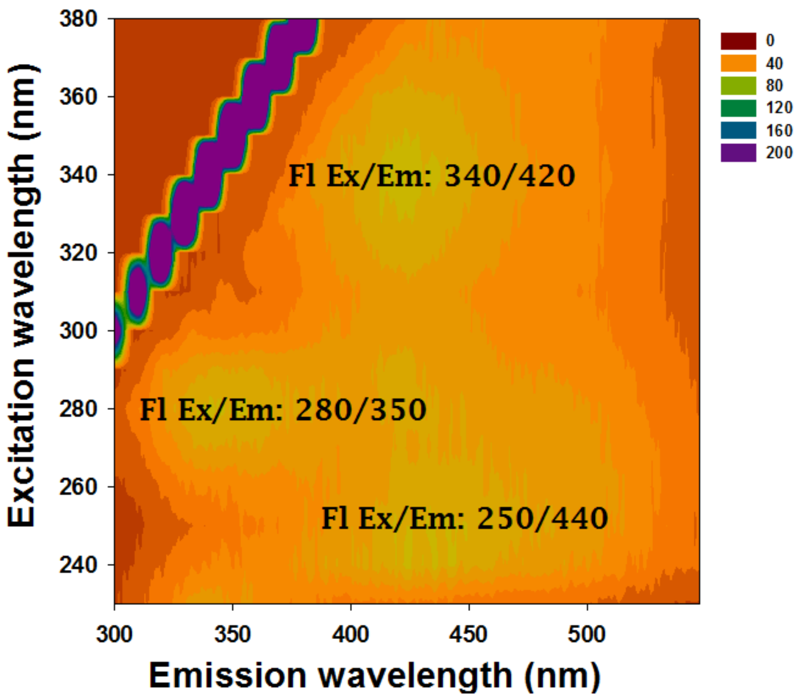


**Fig. 2** Spectra of fluorescence excitation emission matrices of raw domestic wastewater

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 <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 HPSEC-FLD was determined according average fluorescence peak and 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 FEEM. The tables describes that all samples have the same components, that is FA-like, SMPs-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 a consistency results with characteristic of organic matter surrogates, as mentioned earlier. The changing of intensity of fluorescence spectra indicated that the increasing or decreasing fluorescing components due to 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).

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

| **Day** | **Sample** | **Intensity (A.U.)** | | |
|---|---|---|---|---|
| | | **Humic Acid-Like Ex/Em: 340/420** | **Fulvic Acid-Like Ex/Em: 280/350** | **SMPs-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 |

Figure 3, one of representative sample, shows the distribution molecular weight of organic fractions as detected by different wavelength of 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 medium molecular weight (MMW) with AMW of about 1650 Da and it is indicated as humic substances-like, building block, low molecular weight acid (Huber et al. 2011; Lai et al. 2015; Hidayah et al., 2017). The chromatograms explains that fluorescence organic of FA-like (Ex/Em 250/440 nm) and SMPs-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 is mentioned 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 shows the similar shape of the chromatograms HPSEC-FLD, 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 that a decreasing or increasing concentration of dissolved organic compound during treatment processes.

Figure 4a and 4b 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 substances-like, which is existed about more than 50% of the dissolved organic carbon (Shon et al. 2012). In addition, the major organic fractions of the MMW was composed of three different fractions with wide AMW range, namely humic substances-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 SMPs-like in MMW. Second, area of HMW of fluorescence FA-like is comparable with area of fluorescence SMPs-like, and no HA-like detected. Organic matter in wastewater is combination of natural organic matter, SMP, and trace chemicals.

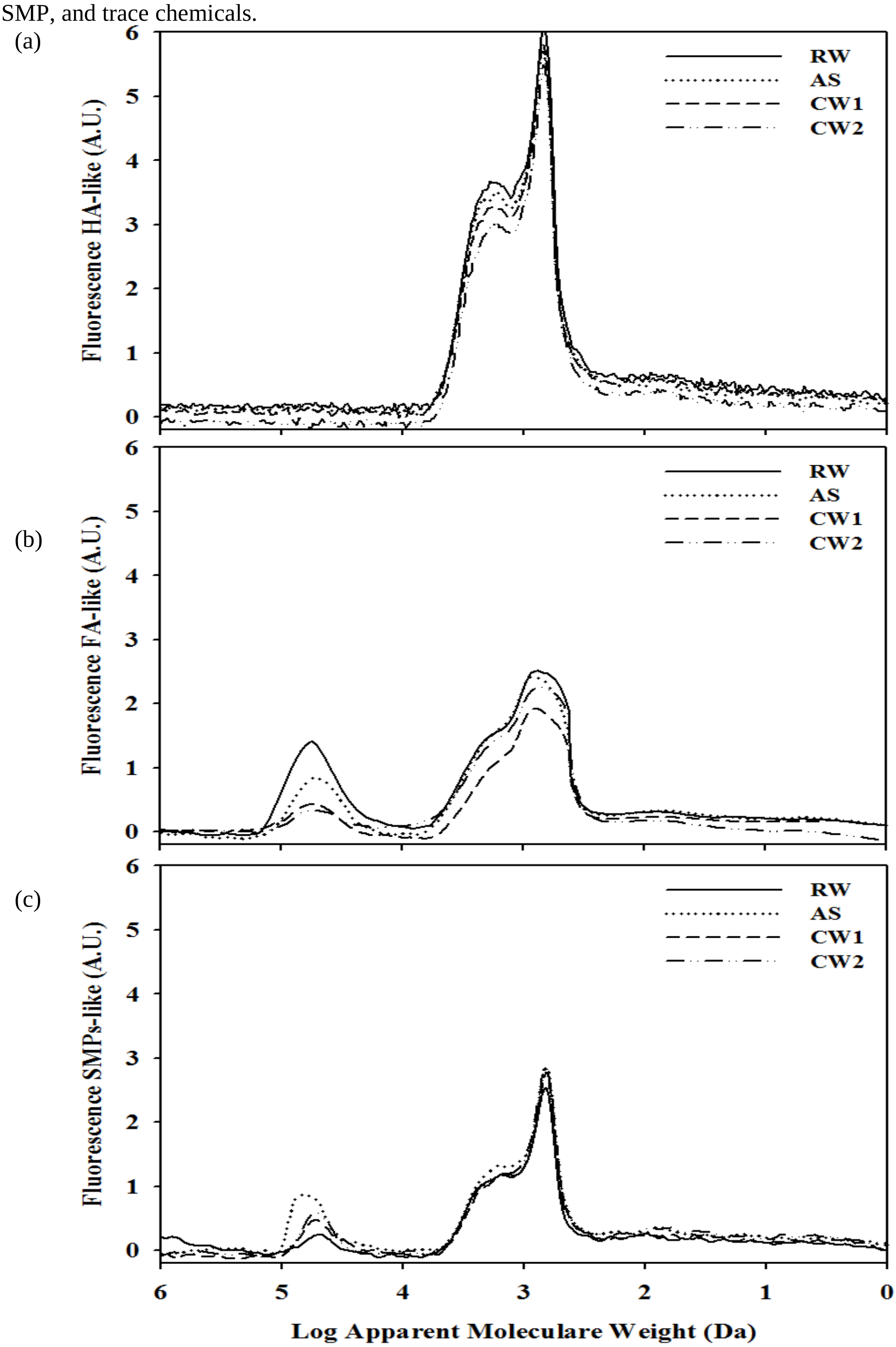


**Fig.3** Distribution molecular weight of fluorescence organic (a) humic acid-like; (b) fulvic acid-like; (c) soluble microbial byproducts-like into different fraction as detected by HPSEC-FLD.

The organic composition of wastewater is approximately 50% protein, 40% carbohydrates, and the rest is trace contaminants (Shon et al. 2012; Xie et al. 2016). Previous studies found that SMPs and FA-like in wastewater and in effluent organic matter has a greater amount of HMW compounds, which is generated from substrate utilization, microbial growth, endogenous phase (Ni et al. 2010; Xie et al. 2016; Zhiji et al. 2017). Third, fluorescence organic SMPs-like in both high and medium molecular weight indicates a higher area of treated water than that of raw water, and vice verca 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 SMPs-like peak area decreased in both MW, it probably due to different kinetic production or formation rates of SMPs-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 process.

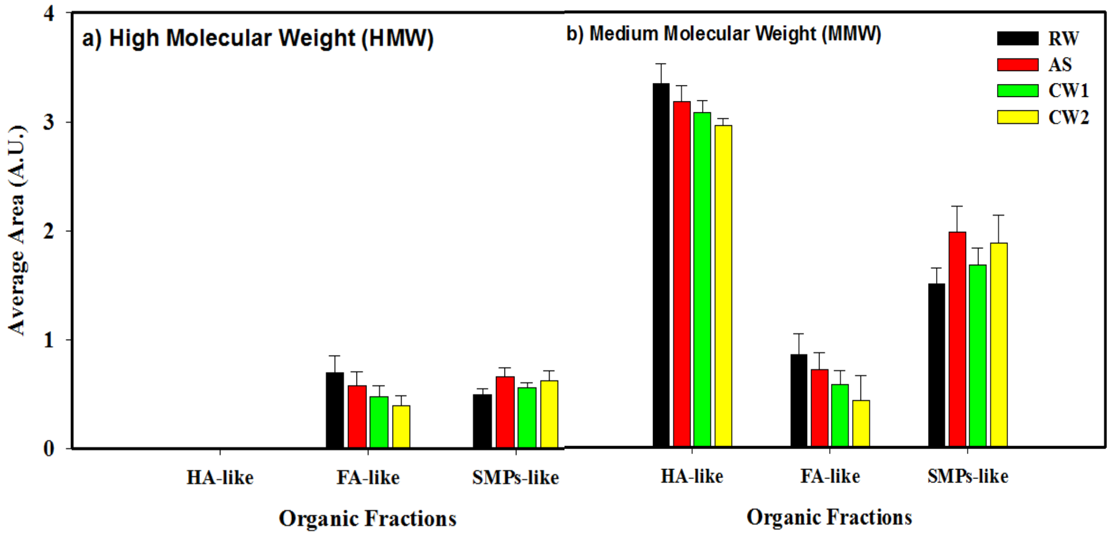


**Fig. 4** Average of peak area of (a) HMW and (b) MMW of the HPSEC-FLD chromatograms in all samples by peak-fittings

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

Figure 5 describes the average percentage reducing and increasing in the area of each peak of chromatogram HPSEC-FLD 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 total removal of HA-like (11.62%). It is probably due to peak of MMW 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 a large proportion fractions, including humic substances fraction with humic acid and fulvic acid species, building block fraction that reflects breakdown products of humic substances or low molecular weight of humic substances-like material, low molecular-weight acids fraction and low molecular-weight neutrals that are highly complex composition and its peaks may appears overlap in surface water and shows as an asymptotic steady line in ground waters sample. Fractionation of organic in the activated sludge has been clarified that decrease in humic-like substances 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 substances 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 polysaccharides and biopolymers 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 compound and being removed through those mechanism (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 SMPs-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 SMPs-like shows a consistency results with increasing TOC as shown in Figure 1. Activated sludge process and constructed wetlands contributed to the highest percentage increasing of SMP production. Microorganism plays an important roles 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 most widely used biological treatment process, although it is found that SMPs-like will be generated during the process. SMPs as one of type of organic matter could be degraded, treated, and removed by next processes, as shown in this study.

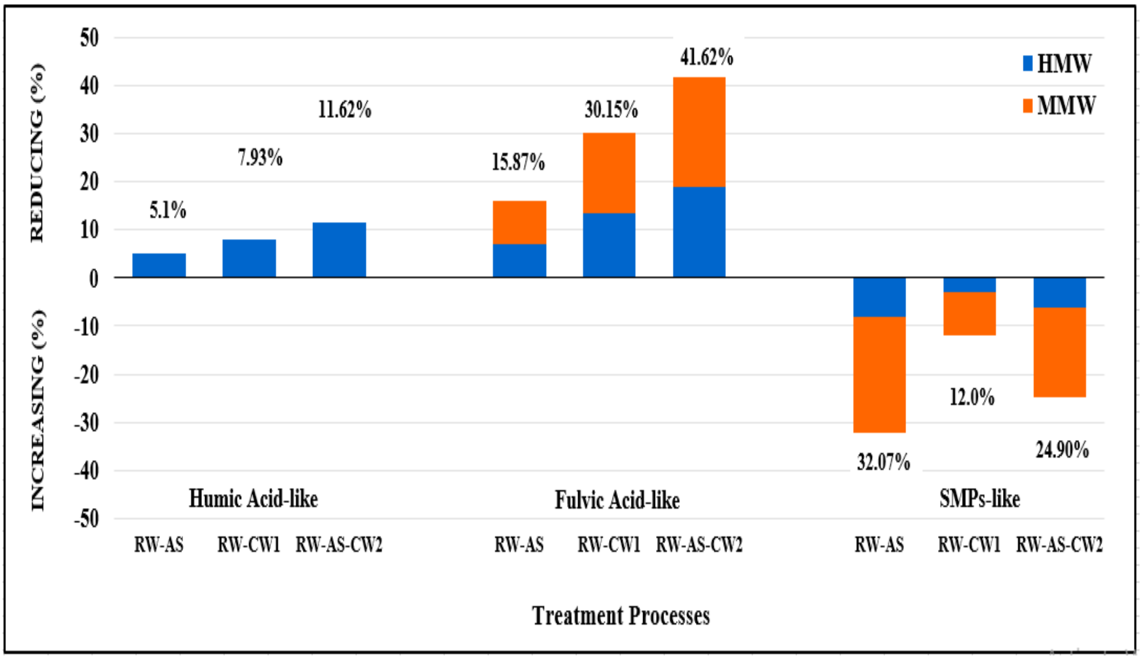


**Fig. 5** Average percentage reducing and increasing in the area of each peak of chromatogram HPSEC-FLD after various treatment

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 it contains a 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 2018). In activated sludge, microorganism are mixed with wastewater, come in contact with the biodegradable materials and consume it as food (Metcalf and Eddy 2012). In both processes, microorganism activities, including growth and decay phase, generated metabolite byproducts or SMPs, that is utilization-associated products, which is 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. SMPs-like have been found to have a wide range of molecular weight distribution and different structures. SMPs form utilization products were found to be more carbonaceous compounds with MW more than 10,000 Da, while biomass products has MW range 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 caused lowering of increased SMPs-like in CW1 and CW2. Using combination of FEEM with HPSEC-FLD could reveal the main compound of fluorescence organics and identify its 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 used polluted source water.

## 4 Conclusion

Combination 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. 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 SMPs-like (Ex/Em 280/350 nm), and those fluorescence organics is fractionated into HMW with AMW 50,000 Da and MMW with AMW 3000 – 650 Da. FEEM shows the changing of fluorescence intensity spectra peak due to effect of treatment process. 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 SMPs-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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**Supporting Information.**

Standard error of the shifted fluorescence peak

| Day | Sample | Humic Acid-Like | | Fulvic Acid-Like | | Soluble Microbial Products (SMPs) | |
|---|---|---|---|---|---|---|---|
| | | Ex (nm) | Em (nm) | Ex (nm) | Em (nm) | Ex (nm) | Em (nm) |
| 1 | RW | 340 | 420 | 280 | 350 | 250 | 440 |
| | AS | 340 | 420.5 | 280 | 350 | 250 | 440 |
| | CW1 | 340 | 420.5 | 280 | 350 | 250 | 440 |
| | CW2 | 340 | 420 | 280 | 350 | 250 | 440 |
| 2 | RW | 340 | 420.5 | 280 | 350 | 250 | 440 |
| | AS | 340 | 420 | 280 | 350.5 | 250 | 440 |
| | CW1 | 340 | 420.5 | 280 | 350 | 250 | 440 |
| | CW2 | 340 | 420 | 280 | 350 | 250 | 440.5 |
| 3 | RW | 340 | 420 | 280 | 350 | 250 | 440 |
| | AS | 350 | 420.5 | 280 | 350 | 250 | 440 |
| | CW1 | 340 | 420.5 | 270 | 350 | 250 | 440 |
| | CW2 | 340 | 420 | 280 | 350 | 250 | 440 |
| 4 | RW | 340 | 420 | 280 | 350 | 250 | 440 |
| | AS | 340 | 420.5 | 280 | 350 | 250 | 440 |
| | CW1 | 340 | 420.5 | 280 | 350 | 250 | 440 |
| | CW2 | 340 | 420 | 280 | 350 | 250 | 440 |
| 5 | RW | 340 | 420 | 280 | 350 | 250 | 440 |
| | AS | 340 | 420 | 280 | 350 | 250 | 440 |
| | CW1 | 340 | 420.5 | 280 | 350.5 | 250 | 440 |
| | CW2 | 340 | 420 | 280 | 350 | 250 | 440 |
| 6 | RW | 340 | 420 | 280 | 350 | 250 | 440 |
| | AS | 340 | 420 | 280 | 350 | 250 | 440 |
| | CW1 | 340 | 420.5 | 280 | 350 | 240 | 440 |
| | CW2 | 340 | 420 | 280 | 350 | 250 | 440 |
| 7 | RW | 340 | 420 | 280 | 350 | 250 | 440 |
| | AS | 340 | 420 | 280 | 350.5 | 250 | 440 |
| | CW1 | 340 | 420 | 280 | 350 | 250 | 440 |
| | CW2 | 340 | 420 | 280 | 350 | 250 | 440 |
| 8 | RW | 340 | 420 | 280 | 350 | 250 | 440 |
| | AS | 340 | 420 | 280 | 350 | 250 | 440 |
| | CW1 | 340 | 420 | 280 | 350 | 250 | 440 |
| | CW2 | 340 | 420.5 | 280 | 350 | 250 | 440 |
| Standard Error | | 1.739926 | 0.237479 | 1.739926 | 0.14574 | 1.739926 | 0.086996318 |
| Average Standard Error | | 0.948333=0.95% < 1% | | | | | |

Note:

RW=raw water; AS= activated sludge; CW1=constructed wetland 1; CW2=constructed wetland 2

# Author's Response To Reviewer Comments

Close

Respond to Reviewer:
1. DBPs, Please give an abbreviation prior to acronym.
Response: abbreviation has been added into the revised version. Please check page 1 paragraph 1 of the revised manuscript

2. NOM, Please give an abbreviation prior to acronym
Response: abbreviation has been added into the revised version. Please check page 1 paragraph 2 of the revised manuscript

3. DOM, Please give an abbreviation prior to acronym
Response: abbreviation has been added into the revised version. Please check page 2 paragraph 1 of the revised manuscript

4. EPs, is it the same with EPS? if yes, please shows a consistency word. if not, please give an abbreviation prior to acronym
Response: EPs is the same with EPS. It has been revised in the manuscript

5. PARAFAC, Please give an abbreviation prior to acronym
Response: abbreviation has been added into the revised version. Please check page 2 paragraph 3 of the revised manuscript

6. Ex; Em, Please give an abbreviation prior to acronym
Response: abbreviation has been added into the revised version. Please check page 2 paragraph 2 of the revised manuscript

7. Please explain about the activated sludge system, including: size of aeration tank and clarifier, air flow rate, etc.
Response: The activated sludge system has been added in the manuscript. Please check page 2 paragraph 4 of the revised manuscript

8.Please describe the meaning of 2 pieces Canna indica, including: number of leaves, age of plant, height, etc.
Response: the meaning of 2 pieces Canna indica has been added in the revised manuscript, please see page 2 paragraph 4 of the revised manuscript.

9. Please give a short information in discussion, regarding refractory organic matter could be degraded through phytoextraction, rhizofiltration, phytostabilization, phytodegradation.
Response: this information has been added in the revised manuscript Page 3, paragraph 2.

10. Please shows that fluorescence peak has been shifted less than 1%.
Response: Please see the attached file, standard error of the shifted fluorescence peak.

11. According to this study, the data shows that domestic wastewater treatment with activated sludge had generated soluble microbial-byproducts (RW-AS), further those SMPs was eliminated through constructed wetland (RW-AS-CW2).
Does it mean that activated sludge is not recommended for domestic wastewater treatment because the process will generate SMPs which is one of the DBPs precursors? Please give briefly explanation.
Response: Activated sludge is most widely used biological treatment process, although it is found that SMPs-like will be generated during the process. SMPs as one of type of organic matter could be degraded, treated, and removed by next processes, as shown in this study.
It has been added in the revised manuscript, page 8, paragraph 1.

Close

## Water, Air, & Soil Pollution

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

--Manuscript Draft--

| | |
|---|---|
| **Manuscript Number:** | WATE-D-19-01779 |
| **Full Title:** | Characterization of Molecular Weight-Based Fluorescent Organic Matter and Its Removal in Combination of Constructed Wetland with Activated Sludge Process |
| **Article Type:** | Full research paper |
| **Keywords:** | constructed wetland; soluble microbial byproducts; fluorescence; HPSEC |
| **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 byproducts from substrate metabolism and cell lysis. The presence of those compounds in effluent of wastewater treatment causes problems in source water. This study combine 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 shows that three components of fluorescence organic: fulvic acid-like (Ex/Em 245/440 nm), SMPs-like (Ex/Em 280/350 nm), humic acid-like (Ex/Em 340/420 nm) have been identified in all samples by FEEM. Further, 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 substances-like (3000-650 Da). Peak-fitting determines that area of MMW is higher than area of HMW of all fluorescence organic components, and area of HMW of fluorescence fulvic acid-like is comparable with area of SMPs-like, and no HMW of humic acid-like detected. Humic acid-like and fulvic acid-like removed during treatment, while metabolite byproduct released as shown by increasing fluorescence SMPs-like and TOC concentration. This method gives new sight to characterize organic matter for assessing effluent of wastewater quality and determining the appropriate water treatment. |
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| Once the article is submitted for review, no changes in authorship, the order of authors, or designation of the corresponding author will be permitted.<br><br>Have all authors been actively involved in making a substantial scholarly contribution to the design and completion of this research, interpretation of data and conclusions, assisted in drafting and revising the manuscript, and read and approved this submission? (YES/NO). If NO, please do not submit your manuscript. Please review the authorship guidelines on the journal's homepage for guidance. | yes |
| Does the article report existing science applied to a local situation? | No |
| If yes, how is this research significant to furthering worldwide knowledge on this topic | This research is significant to characterize organic matter for controlling DBPs formation in source water through water treatment processes |
| Is the article of local, national or international value? (Choose one.) | International |
| Please provide the name, affiliation and address, and e-mail address of **three** potential reviewers who do not pose a conflict of interest. Note that this information will be checked to ensure it is credible. | 1. Prof. Lai Wen Liang (laisiff@gmail.com)<br>Graduate School of Environmental Management, Tajen University, Taiwan<br>2. Dr. Yadanar Win Mynth (winmyint.yadanar@gmail.com)<br>Department of Research and Innovation, Ministry of Education, Myanmar<br>3. Prof. Munawar Ali (alimunawar960@gmail.com)<br>Department of Environmental Management ITS Surabaya, Indonesia |

# 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 byproducts from substrate metabolism and cell lysis. The presence of those compounds in effluent of wastewater treatment causes problems in source water. This study combine 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 shows that three components of fluorescence organic: fulvic acid-like (Ex/Em 245/440 nm), SMPs-like (Ex/Em 280/350 nm), humic acid-like (Ex/Em 340/420 nm) have been identified in all samples by FEEM. Further, 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 substances-like (3000-650 Da). Peak-fitting determines that area of MMW is higher than area of HMW of all fluorescence organic components, and area of HMW of fluorescence fulvic acid-like is comparable with area of SMPs-like, and no HMW of humic acid-like detected. Humic acid-like and fulvic acid-like removed during treatment, while metabolite byproduct released as shown by increasing fluorescence SMPs-like and TOC concentration. This method gives new sight to characterize organic matter for assessing effluent of wastewater quality and determining the appropriate water treatment.

**Keywords**: constructed wetland, soluble microbial byproducts, fluorescence, HPSEC

## 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 relatively low maintenance requirements and operating costs. In some cases, a standalone constructed wetlands systems is unable to achieve high removal of pollutant and meet the standard water quality effluent. Therefore, constructed wetlands with a pretreatment systems could enhanced the treatment process (Liu et al. 2015; Su et al. 2018). Integrated constructed wetlands into biological process, such as activated sludge, has a potential for removing high organic loading, thus could improve treated water quality (Liu et al. 2015). It has been published that biological processes released soluble microbial products (SMPs), which is derived from metabolite activities during biomass growth, and cell lysis from biomass decay. Another metabolite product d[illegible] biological process is extracellular polymeric substances (EPS), which is supported the aerobic granules forma[illegible] and protect bacterium from harsh environmental conditions (Ni et al. 2010; Xie et al. 2012). Both of SMPs and EPS are made up of from different kind of macromolecules such as carbohydrates, proteins, fulvic, humic, nucleic acids, etc 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 receiving waters as drinking water sources. Soluble organic had DBPs formation potential (DBPFP) around 5.6 µmol/mmol-DOC (dissolved organic carbon). It is found that pro[illegible], and humic-like substances in SMPs can act as reactive DBP precursors (Liu et al. 2014).

In practice, NOM is usually represented by total organic carbon (TOC), dissolved organic carbon (DOC), or absorption of UV [illegible] ($UV_{254}$). However, it is mostly provide the quantity information of NOM, while offering limited about its characteristics (Matilainen et al. 2011). A number of characterization method have been conducted to provide a rapid qualitative and quantitative indication 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). FEEM is a simple, fast detection, and very sensitive tool that requires small sample volume and little or no sample pretreatment, however only fluorophores containing DOM constituents will respond to this method, and indicating the lack of characterization of non-fluorophores fractions. In contrast, HPSEC, which fractioned organic matter based on its molecular molecular weight (MW), is consuming time and materials (Huber et al. 2011; Lai et al. 2015; Hidayah et al. 2017). Peak-fitting is considered as 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 is very heterogeneous and composed with complex mixture of different molecular weight and fluorescence organic, thus, identification and interpretation of organic fractions is being difficult. Combination FEEM with HPSEC fluorescence detector (HPSEC-FLD) will give new insight to the characteristic of organic matter. FEEM presented different spectral regions, which is interpreted as different fluorescence organic, and its combination with HPSEC can resolve different fluorophores within different molecular weight. A number of combination FEEM with HPSEC-FLD studies have been conducted for environmental application. Her et al. (2003) used 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 Ex/Em: 320/430 nm and revealed four peaks with apparent MW of 2580-10,700 in river water. Chabalina et al. (2[illegible]) conducted quantification and characterization of EPs by using 3-dimensional FEEM and HPSEC separately in 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 researches 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 weight in wastewater treatment, especially related to the metabolite byproducts. To our knowledge, FEEM with PARAFAC 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. 2017; Moradi et al. 2018; Hidayah et al. 2019). Therefore, the objectives of this study were 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 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 Set Up

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, two subsurface horizontal flow constructed wetlands (0.6 m long, 0.6 m wide, and 0.6 m [illegible]) filled with 0.3 m gravel. Subsurface constructed wetlands were planted with 2 pieces *Canna indica* per-bed with considering density 4 plant/m$^2$ (Wu et al. 2015). 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/gr (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 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 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 focus on dissolved organic matter (APHA 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 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 wavelength (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) has concluded that SUVA values higher than 4 indicate that organic matter is composed mainly of humic or hydrophobic matter, while SUVA values less than 2 exhibits 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 parameters concentration 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, phytodegradation (Wu et al. 2015; Vymazal et al. 2018).

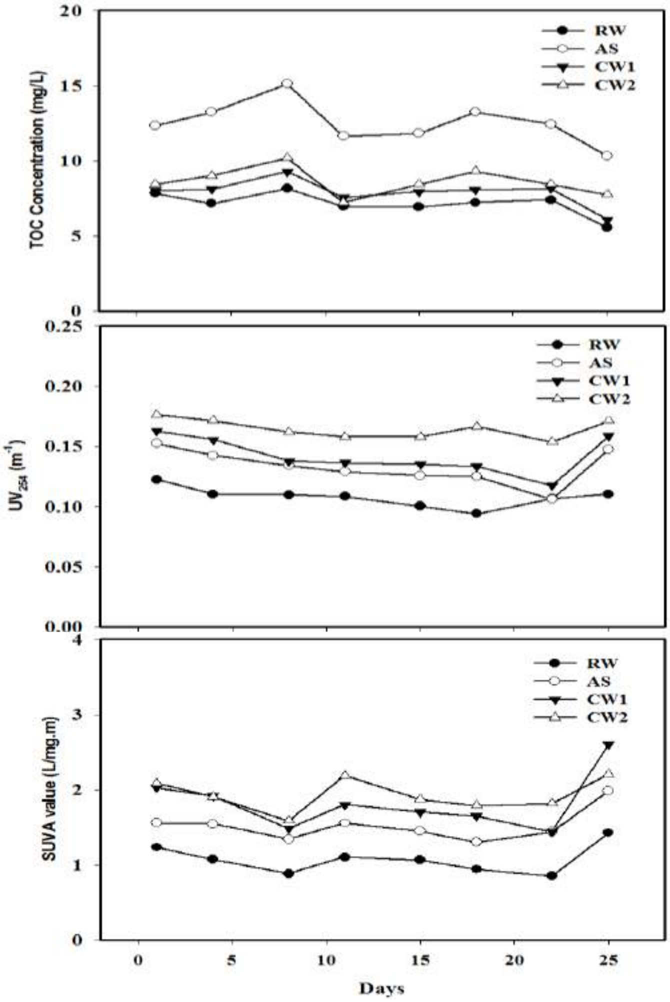


**Fig.1** Concentration of organic matter: (a) TOC, (b) $UV_{254}$, (c) SUVA value of raw domestic wastewater and treated wastewater

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 sample, and its contour plot describes the intensity of FEEM. The results explains that domestic wastewater consisted of mainly three component of fluorescence dissolved organic. The first component is fulvic acid-like (FA-like) with peak at Ex/Em: 245/440 nm, the second component is found at Ex/Em 280/350 nm which is identified as soluble microbial byproducts-like (SMPs-like), and the last component is humic acid-like (HA-like), which have shown the highest intensity peak at Ex/Em 340/420 nm. Fluorescence components in this study show a consistency 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 natural dissolved organic matter are mostly existed in term 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 compound have been identified in effluent from biological wastewater treatment plants, in term 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, SMPs formation was contributed by heterotrophs in higher percentage than autotrophs (Ni et al. 2010; Xie et al. 2012; Xie et al. 2016).

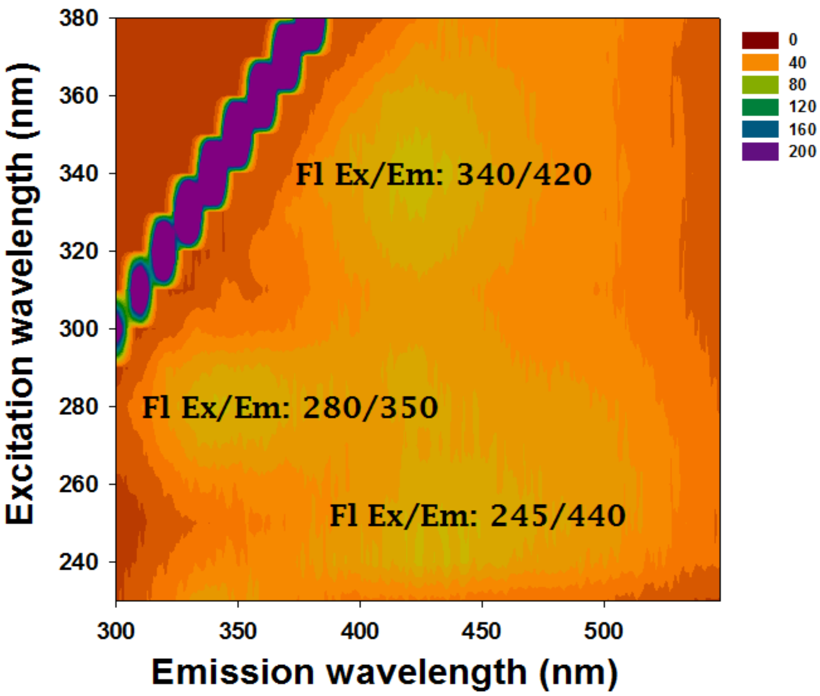


**Fig. 2** Spectra of fluorescence excitation emission matrices of raw domestic wastewater

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 <1%, 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 HPSEC-FLD was determined according average fluorescence peak and 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 FEEM. The tables describes that all samples have the same components, that is FA-like, SMPs-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 a consistency results with characteristic of organic matter surrogates, as mentioned earlier. The changing of intensity of fluorescence spectra indicated that the increasing or decreasing fluorescing components due to 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).

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

| Day | Sample | Intensity (A.U.) | | |
|---|---|---|---|---|
| | | **Humic Acid-Like Ex/Em: 340/420** | **Fulvic Acid-Like Ex/Em: 280/350** | **SMPs-Like Ex/Em: 245/440** |
| 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 |

Figure 3, one of representative sample, shows the distribution molecular weight of organic fractions as detected by different wavelength of 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 medium molecular weight (MMW) with AMW of about 1650 Da and it is indicated as humic substances-like, building block, low molecular weight acid (Huber et al. 2011; Lai et al. 2015; Hidayah et al., 2017). The chromatograms explains that fluorescence organic of FA-like (Ex/Em 245/440 nm) and SMPs-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 is mentioned 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 shows the similar shape of the chromatograms HPSEC-FLD, 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 that a decreasing or increasing concentration of dissolved organic compound during treatment processes.

Figure 4a and 4b 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 substances-like, which is existed about more than 50% of the dissolved organic carbon (Shon et al. 2012). In addition, the major organic fractions of the MMW was composed of three different fractions with wide AMW range, namely humic substances-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 SMPs-like in MMW. Second, area of HMW of fluorescence FA-like is comparable with area of fluorescence SMPs-like, and no HA-like detected. Organic matter in wastewater is combination of natural organic matter, SMP, and trace chemicals.

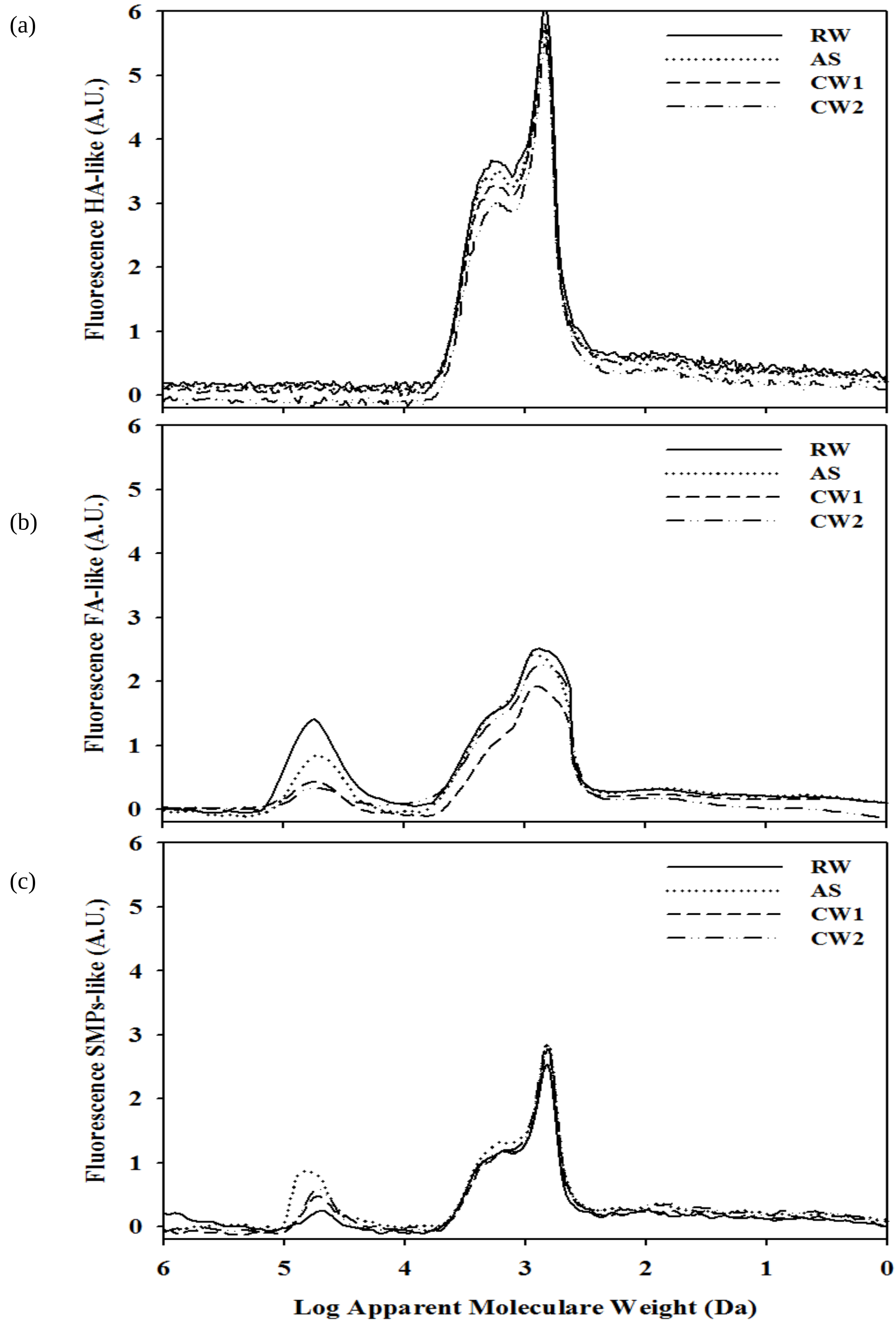


**Fig.3** Distribution molecular weight of fluorescence organic (a) humic acid-like; (b) fulvic acid-like; (c) soluble microbial byproducts-like into different fraction as detected by HPSEC-FLD.

The organic composition of wastewater is approximately 50% protein, 40% carbohydrates, and the rest is trace contaminants (Shon et al. 2012; Xie et al. 2016). Previous studies found that SMPs and FA-like in wastewater and in effluent organic matter has a greater amount of HMW compounds, which is generated from substrate utilization, microbial growth, endogenous phase (Ni et al. 2010; Xie et al. 2016; Zhiji et al. 2017). Third, fluorescence organic SMPs-like in both high and medium molecular weight indicates a higher area of treated water than that of raw water, and vice verca 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 SMPs-like peak area decreased in both MW, it probably due to different kinetic production or formation rates of SMPs-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 process.

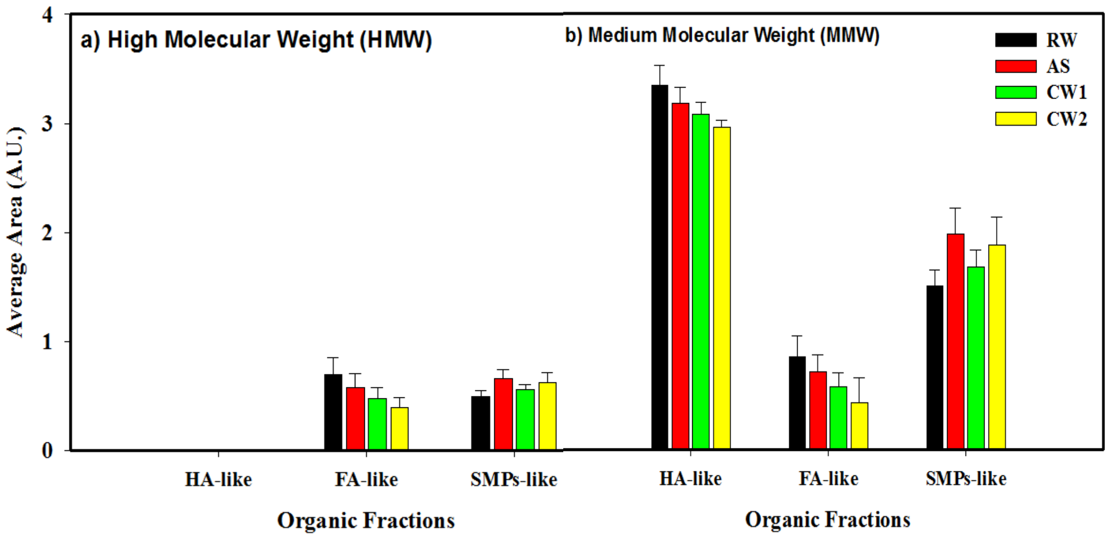


**Fig. 4** Average of peak area of (a) HMW and (b) MMW of the HPSEC-FLD chromatograms in all samples by peak-fittings

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

Figure 5 describes the average percentage reducing and increasing in the area of each peak of chromatogram HPSEC-FLD 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 total removal of HA-like (11.62%). It is probably due to peak of MMW 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 a large proportion fractions, including humic substances fraction with humic acid and fulvic acid species, building block fraction that reflects breakdown products of humic substances or low molecular weight of humic substances-like material, low molecular-weight acids fraction and low molecular-weight neutrals that are highly complex composition and its peaks may appears overlap in surface water and shows as an asymptotic steady line in ground waters sample. Fractionation of organic in the activated sludge has been clarified that decrease in humic-like substances 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 substances 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 polysaccharides and biopolymers 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 compound and being removed through those mechanism (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 SMPs-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 SMPs-like shows a consistency results with increasing TOC as shown in Figure 1. Activated sludge process and constructed wetlands contributed to the highest percentage increasing of SMP production. Microorganism plays an important roles for organic degradation in constructed wetland (Wu et al. 2015; Liu et al. 2015) and in activated sludge process (Metcalf and Eddy 2012).

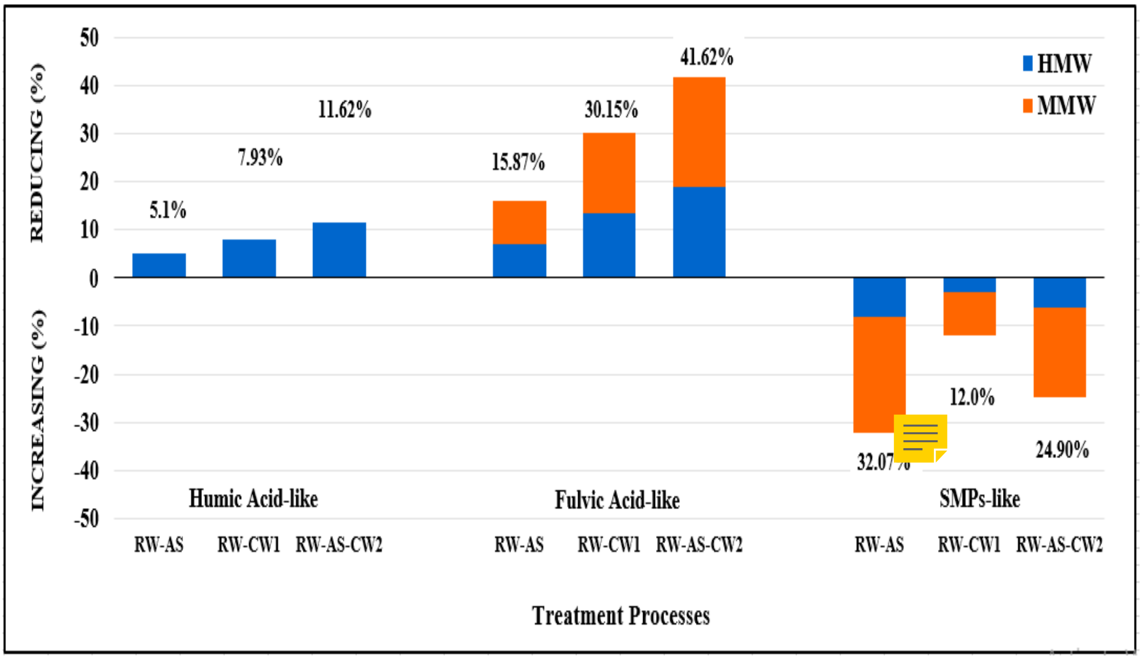


**Fig. 5** Average percentage reducing and increasing in the area of each peak of chromatogram HPSEC-FLD after various treatment

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 it contains a 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 2018). In activated sludge, microorganism are mixed with wastewater, come in contact with the biodegradable materials and consume it as food (Metcalf and Eddy 2012). In both processes, microorganism activities, including growth and decay phase, generated metabolite byproducts or SMPs, that is utilization-associated products, which is 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. SMPs-like have been found to have a wide range of molecular weight distribution and different structures. SMPs form utilization products were found to be more carbonaceous compounds with MW more than 10,000 Da, while biomass products has MW range 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 caused lowering of increased SMPs-like in CW1 and CW2. Using combination of FEEM with HPSEC-FLD could reveal the main compound of fluorescence organics and identify its 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 used polluted source water.

## 4 Conclusion

Combination 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. 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: 245/440 nm) and SMPs-like (Ex/Em 280/350 nm), and those fluorescence organics is fractionated into HMW with AMW 50,000 Da and MMW with AMW 3000 – 650 Da. FEEM shows the changing of fluorescence intensity spectra peak due to effect of treatment process. 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 SMPs-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%).

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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 shows that three components of fluorescence organic: fulvic acid–-like (Ex/Em 250/440 -nm), SMPs-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 substances-–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 SMPs-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 SMPs-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

Cconstructed wetland

Ssoluble microbial byproducts

Ffluorescence

HPSEC


## 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 requirements and operating costs. –In some cases, a standalone constructed wetlands systems is unable to achieve high removal of pollutant and meet the standard water quality effluent. Therefore, constructed wetlands with a pretreatment systems could enhanced the treatment process (Liu et al. 2015; Su et al. 2018). Integrated constructed wetlands into biological process, such as activated sludge, hasve 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 isare 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 is supported the aerobic granules formation and protect bacterium from harsh environmental conditions (Ni et al. 2010; Xie et al. 2012). Both of SMPs and EPS are made up ~~of~~ from different kinds of macromolecules such as carbohydrates, proteins, fulvic, humic, and nucleic acids~~, etc.~~ 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-products (DBPs) 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 ~~is~~ 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 ~~molecular~~ weight (MW), is consuming time and materials (Huber et al. 2011; Lai et al. 2015; Hidayah et al. 2017). Peak-fitting is considered ~~as~~ 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 ~~is~~are very heterogeneous and composed ~~with~~ of complex mixture of different molecular weights and fluorescence organics~~,~~; thus, identification and interpretation of organic fractions ~~is~~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 ~~is~~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 3~~-dimensional~~D 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~~s~~ 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~~s~~ of this study w~~ere~~as 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 Set~~-~~u~~U~~p

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~~ing~~ 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/~~gr~~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 D~~d~~omestic W~~w~~astewater and T~~t~~reated W~~w~~astewater

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) ha~~s~~ve concluded that SUVA values higher than 4 indicate that organic matter is composed mainly of humic or hydrophobic matter, while SUVA values less than 2 exhibit~~s~~ 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~~s~~ 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.

Fig. 1 Concentration of organic matter~~:~~. ~~(~~**a**~~)~~ TOC~~,~~. ~~(~~**b**~~)~~ UV$_{254}$~~,~~. ~~(~~**c**~~)~~ SUVA value of raw domestic wastewater and treated wastewater

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~~s~~ 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.

## 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~~s~~ 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~~s~~-like (SMPs-like)~~,~~; and the last component ~~–~~is humic acid~~-~~–like (HA-like), which ha~~ve~~s shown the highest intensity peak at Ex/Em 340/420 ~~-~~nm. Fluorescence components in this study show ~~a~~ consisten~~cy~~t 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 ~~are~~

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, SMPs formation was contributed by heterotrophs in higher percentage than autotrophs (Ni et al. 2010; Xie et al. 2012; Xie et al. 2016).

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

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 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 tables describes that all samples have the same components, that is, FA-like, SMPs-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 a consistentcy results with characteristic of organic matter surrogates, as mentioned earlier. The changing of intensity of fluorescence spectra indicated that the increasing or decreasing of fluorescenting 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).

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

| Day | Sample | Intensity (A~~.~~U~~.~~) | | |
|---|---|---|---|---|
| | | Humic ~~A~~acid~~–~~-~~L~~like Ex/Em~~:~~ 340/420 | Fulvic ~~A~~acid~~–~~-~~L~~like Ex/Em~~:~~ 280/350 | SMP~~s~~-~~L~~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 |

Figure ~~-~~3, one the of representative samples, shows the ~~distribution~~ 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 medium molecular weight (MMW) with AMW of about 1650 ~~-~~Da and it is indicated as humic substance~~s -~~-like, building block, low molecular weight acid (Huber et al. 2011; Lai et al. 2015; Hidayah et al.~~,~~ 2017). The chromatograms explain~~s~~ that fluorescence organic of FA-like (Ex/Em 250/440 ~~-~~nm) and SMP~~s~~-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 ~~is~~ mention~~ed~~s 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~~s~~ the similar shape of the ~~chromatograms~~ 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 ~~that~~ a decreasing or increasing concentration of dissolved organic compound during treatment processes.

Figure ~~-~~4~~a and 4b~~ 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~~s~~-like, which ~~is~~ existed about more than 50% of the dissolved organic carbon (Shon et al. 2012). In addition, the major organic fractions of the MMW w~~as~~ere composed of three different fractions with wide AMW range, namely humic substance~~s~~-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~~s~~-like in MMW. Second, area of HMW of fluorescence FA-like is comparable with area of fluorescence SMP~~s~~-like, and no HA-like detected. Organic matter in wastewater is a combination of natural organic matter, SMP, and trace chemicals.

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

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-~~s~~ and FA-like in wastewater and in effluent organic matter ha~~s~~ve a greater amount of HMW compounds, which ~~is~~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~~s~~-like in both high and medium molecular weight indicates a higher area of treated water than that of raw water, and vice ver~~c~~sa 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~~s~~-like peak area decreased in both MW~~,~~; it could probably be due to different kinetic productions or formation rates of SMP~~s~~-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. 4 Average of peak area of ~~(~~**a**~~)~~ HMW and ~~(~~**b**~~)~~ MMW of the HPSEC-FLD chromatograms in all samples by peak-fittings

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

Figure ~~-~~5 describes the average percentage reducing and increasing in the area of each peak of ~~chromatogram~~ 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 ~~a~~ large proportion fractions, including humic substance~~s~~ 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~~s-~~-like material, low molecular~~-~~ weight acid~~s~~ fraction, and low molecular~~-~~ weight neutrals that are highly complex composition, and its peaks may appear~~s~~ overlap in surface water and show~~s~~ 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~~s~~ 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~~s~~ 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~~s~~ and biopolymer~~s~~ 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~~s~~-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~~s~~-like shows ~~a~~ consisten~~cy~~t results with increasing TOC as shown in Fig.~~ure~~ 1. Activated sludge process and constructed wetlands contributed to the highest percentage increasing of SMP production. Microorganism plays an important role~~s~~ 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~~s~~-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.

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

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 ~~it~~they contain~~s~~ ~~a~~ 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 ~~it~~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 ~~is~~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~~s~~-like ha~~ve~~s been found to have a wide range of molecular weight distribution and different structures. SMPs ~~form~~ utilization products were found to be more carbonaceous compounds with MW more than 10,000 ~~-~~Da, while biomass products ha~~s~~ve 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~~d~~ lowering of increased SMP~~s~~-like in CW1 and CW2. ~~–~~Using combination of the FEEM ~~wi~~and~~th~~ HPSEC-FLD could reveal the main compound of fluorescence organics and identify ~~it~~their~~s~~ 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~~s~~-like (Ex/Em 280/350 ~~-~~nm), and those fluorescence organics ~~is~~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~~s~~-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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## A. Electronic supplementary material

ESM 1 (DOCX 15 ~~-~~kb)

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