ESPR_Using molecular weight–based fluorescent detector to characterize dissolved effluent organic matter

by Euis Nurul Hidayah

SHORT RESEARCH AND DISCUSSION ARTICLE

Using molecular weight–based fluorescent detector to characterize dissolved effluent organic matter in oxidation ditch with algae

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Abstract

Implementation of microalgae has been considered for enhancing effluent wastewater quality. However, it can cause environmental issues due to the release of extracellular and algal organic matter in the biological process. This study aimed to investigate the characteristics of dissolved effluent as algae- and bacteria-derived organic matter during the oxidation ditch process. Furthermore, experiments were conducted under three combinations filled by *Spirulina platensis*, *Chlorella vulgaris*, and without microalgae. The results showed that dissolved effluent organic matter was more aromatic and hydrophobic than before treatment. Fluorescence spectroscopy identified two components—aromatic protein–like and soluble microbial product–like components—at excitation/emission of 230/345 nm and 320/345 nm after treatment, instead of fulvic acid–like at 230/420 nm and humic acid–like at 320/420 nm in raw wastewater. These components were fractionated based on the average of molecular weight cut-offs (MWCOs), and high (MWCOs > 50,000 Da), medium (MWCOs 50,000–1650 Da), and low molecular weights (MWCOs < 1650 Da) were reported. Biological oxidation ditch under symbiosis algal bacteria generated humic and fulvic acid with a higher MWCOs than the process without algal. The quality and quantity of dissolved effluent organic matter in an oxidation ditch reactor were significantly affected by algal-bacteria symbiotic.

Introduction

Microalgae are considered an alternative solution to enhance wastewater treatment due to their ability to use inorganic nitrogen and phosphorous. Microalgae can remove pathogens and heavy metals as well as furnish O_2 to heterotrophic aerobic bacteria for the degradation of organic pollutants (Munoz and Guieysse 2006). The algae-bacteria biomass has been observed, and can be used as a source of food for

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humans (Wells et al. 2017), animal feed (Madeira et al. 2017), and organic fertilizer for sustainable agriculture (Baweja et al. 2019). It may also contain a recalcitrant compound in treated wastewater effluent (Leloup et al. 2013). Algae-bacteria biomass could be used for energy production, such as biogas (Montingelli et al. 2015) and biofuel (Benemann 2013). Algae can cause undesirable odor and taste as well as release toxins in the water bodies (Caruana and Amzil 2018; Leloup et al. 2013). Many studies observed that microorganisms can release extracellular polymeric substances (EPS) and soluble microbial products (SMP), while microalgae could release extracellular organic matter (EOM) (Qu et al. 2012). Furthermore, organic matter produced by algal organic matter (AOM) has been identified recently, including its production, evolution, and characteristics, which depend on the species and growth phases (Rehman et al. 2017). Those microbial products are essential because the constituents found in wastewater lead to membrane fouling, sludge bulking in activated sludge (biological treatment), disinfection by-products (DBPs) during chemical

disinfection, and floating matter and films in waterways (Shon et al. 2012).

Some characterizations of effluent organic matters and AOM have been developed to identify qualitative and quantitative properties. Regarding the effluent organic matter, EPS and SMP are generated from biological wastewater treatment. EPS is a complex high molecular weight compound produced by microorganisms in bioreactors when organic materials exist in wastewater (Ni et al. 2009). Meanwhile, SMP is composed of molecular weight (MW) < 1 kDa, and it is released during biomass metabolism but decays in biological processes (Liu et al. 2014). According to the organic fractions, the effluent from the biological process consists mainly of biopolymers, humics, building blocks, and low molecular weight acid, characterized using a liquid chromatography-organic carbon detector (LC-OCD) (Gonzales et al. 2013). Fluorescence excitation-emission matrices (FEEM) spectrometry has identified three main components of intracellular and extracellular substances of activated sludge for proteins, humic and fulvic-like substances at excitation (Ex)/emission (Em) of 280/350, 340/400, and 390/450 nm, respectively (Li et al. 2008; Ni et al. 2009). Previous studies observed that both EOM and intracellular organic matter (IOM) of *Chlorella* sp. presented less than 0.05 A.U. of UV_{254} and UV_{280} indicating a low absorbance (Hua et al. 2019a). Characteristics of AOM were mainly hydrophilic as detected by resin fractional, while EOM is mainly composed of fulvic acid–like, SMP-like, and humic acid–like substances. Furthermore, IOM, comprising aromatic protein and SMP-like substances, was identified using FEEM spectroscopy (Hua et al. 2017; Zhu et al. 2015; Dong et al. 2019). EOM and IOM were distributed in low (< 1 kD and high MW (> 100 kDa) fractions, and characterized by high-performance size exclusion chromatography (HPSEC) with ultraviolet (UVD) and organic carbon detectors (OCD) (Zhou et al. 2015). According to Fourier transform infrared (FTIR), algae and AOM show many absorption peaks at 3400–3200 cm^{-1} (hydrogen bonds O-H), 2950–2850 cm^{-1} (CH_2), and 1650–1580 cm^{-1} (amide group C-N/carboxylate group COOH) (Her et al. 2003).

Oxidation ditches are widely used in wastewater treatment to remove organic pollutants in industrial waste worldwide (Zhang et al. 2016). Oxidation ditches are a modified activated sludge process with advantages, such as long hydraulic retention time, less sludge, high capability of nitrification, and denitrification. Diffused air is provided through horizontally or vertically mounted aerators to increase oxygen transfer, create enough mixing, alternate aerobic and anoxic zones within a channel, and achieve simultaneous nitrification-denitrification (Jin et al. 2015; Zhou et al. 2015). In the oxidation ditches, the role of bacterial population in nutrients and organics under diverse conditions has been effectively carried out (Terashima et al. 2016; Xu et al. 2017; Luo
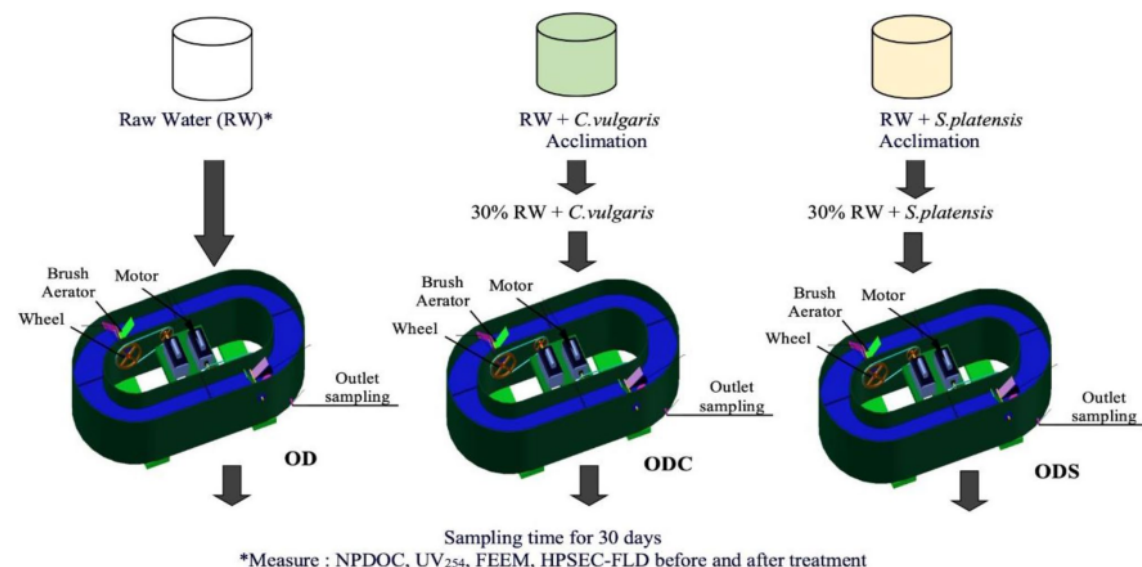
et al. 2020). Recently, oxidation ditches in conjunction with filled algae have been identified as promising processes for removing wastewater nutrients. These processes are analogous to the activated sludge that utilizes a symbiotic relationship between algae and bacteria in a controlled system (Maiti et al. 1988; Noüe et al. 1992). The capability of the biological algae reactor has shown a significant development for nutrients (Maiti et al. 1988; Farahdiba et al. 2020), organic (Munoz et al. 2004; Hidayah et al. 2020), and even heavy metals (Munoz and Guieysse 2006) in high percentage under the different operating system.

According to the identification of algae properties, the algae may contribute to the characteristic of organic matter in water quantitatively and qualitatively. As mentioned previously, microbial in wastewater treatment can release organic in terms of SMP. Therefore, algae-bacteria's symbiosis in the wastewater treatment process contributes to the concentration and characteristics of organic matters in effluent water. Symbiotics will release algae-derived and microorganism-derived organic matter with different properties. Wastewater reclamation has been developed as an alternative method for producing water resources; therefore, derived organic matter in treated effluents should be minimized to prevent membrane fouling and clogged pore-activated carbon (Tran et al. 2015). Another issue is that wastewater effluents have been discharged into water bodies, contributing to the organic matter properties. Organic matters may contribute as precursors to DBPs, which are generally essential for forming C-DBPs and N-DBPs in chlorination or chloramination (Zhou et al. 2015).

Characterization and compositional differences of dissolved effluent organic matter (dEfOM) in oxidation ditches have not been fully explored, specifically in the absence of studies regarding dEfOM from *Spirulina platensis*, and *Chlorella vulgaris*. This study conducted the oxidation ditches under three combinations filled with *S. platensis*, *C. vulgaris*, and without microalgae. During the processes, the characteristic of dEfOM was monitored by fluorescent spectrometry and the molecular weight–based fluorescent detector. Finally, the different species' derived organic composition in the oxidation ditches can be elucidated.

Materials and methods

The raw sample was collected from tofu wastewater, and three sets of oxidation ditches were prepared. The oxidation ditch system consists of a single channel within a ring. Oval and horizontally mounted airbrushes have been installed at the edge of the reactor for aeration and oxygen transfer. The airbrushes rate of 60 rpm was set up during operation. The reactor has a capacity of 300 L in a batch system with a size of 208 cm long, 25 cm inside wide, and 30 cm deep (Fig. 1).

Fig. 1 The oxidation ditch system diagram

After the seventh day, the algal acclimation in tofu wastewater concentration of 30% has shown a decreasing number of cells measured by a hemocytometer. Therefore, the experiment applied 30% tofu wastewater as a raw sample. The ratio of 1:1 of wastewater to algal volume in oxidation ditches was filled with *S. platensis*, *C. vulgaris*, and without algae to control the experiment. Samples were collected before or raw water (RW) and after treatment, which is effluent from the oxidation ditch (OD) with *S. platensis* (ODS) and *C. vulgaris* (ODC) once per day for a month of observation. Operation conditions were maintained under DO, pH, and temperature values of 5–6 mg/L, 7–8, and 27–30 °C. The lux level of sunlight was about 10,000–25,000 for full daylight because the experiment was conducted in an open space (Fields et al. 2016), and the samples were filtered through a 0.45-μm filter paper (cellulose acetate, Toyo Roshi, Japan) to make it particle-free. Filtered samples were analyzed for dissolved organic matter parameters, including non-purgeable dissolved organic carbon (NPDOC). The filtered samples were analyzed using TOC Analyzer 5000A Shimadzu; ultraviolet absorbance at 254 nm (UV_{254}), Carry 100 Bio UV-Visible Spectrophotometer (APHA, AWWA, and WEF 2012), and specific ultraviolet absorbance (SUVA) through dividing UV_{254} value to NPDOC concentration (Edzwald and Tobiason 2011).

Besides, fluorescence spectroscopy (PerkinElmer LS-55) and high-performance liquid chromatography with fluorescence spectroscopy as a detector were used to validate the dEfOM characterization (HPLC-FLD type LC-20 ATV Shimadzu, Japan). First, it was set up at excitation wavelengths

(Ex) between 230 and 400 nm at 10 nm and emission wavelengths (Em) between 300 and 550 nm at 0.5 nm. The Raman scattering peak in the fluorescence analysis was corrected through the blank sample of deionized water at Ex 350 nm and Em 375–420 nm (Chen et al. 2003). This method was applied for selecting wavelengths to set up a detector by determining the average of selected peak maxima location of Ex/Em wavelength. Second, chromatography was used to fractionate dEfOM based on its apparent molecular weight (AMW) through fluorescence detection, according to the previously selected peak of excitation-emission wavelength (Hidayah et al. 2020). The peak-fitting technique, PeakFit version 4.12, Systat Software Inc., CA, USA, was applied to resolve the chromatograph described in the previous study (Lai et al. 2015).

Results and discussion

Characteristic of dEfOM in raw tofu wastewater and during oxidation ditch processes

Table 1 shows that tofu wastewater contains high organic matter regarding biological oxygen demand (BOD) and chemical oxygen demand (COD) instead of bulk parameters of dEfOM. The concentrations of dEfOM in natural organic matter surrogate parameters, including NPDOC, UV_{254}, and SUVA, are presented in Fig. 2a, b, and c, respectively. The average concentrations of NPDOC in OD, ODS, and ODC

Table 1 Characteristics of tofu wastewater

Parameters (unit)								
pH	NPDOC (mg/L)	UV$_{254}$ (cm^{-1})	SUVA (L/mg-m)	Turbidity (NTU)	BOD (mg/L)	COD (mg/L)	NH$_3$-N (mg/L)	PO$_4$-P (mg/L)
5.5 ±0.4	15.45 ±0.25	0.080 ±0.015	0.52 ±0.04	385 ±24	3100 ±122	7585 ±334	36.1 ±2.4	1.85 ±0.03

Fig. 2 The concentration of dEfOM during oxidation ditch processes in terms of (**a**) NPDOC, (**b**) UV$_{254}$, and (**c**) SUVA value
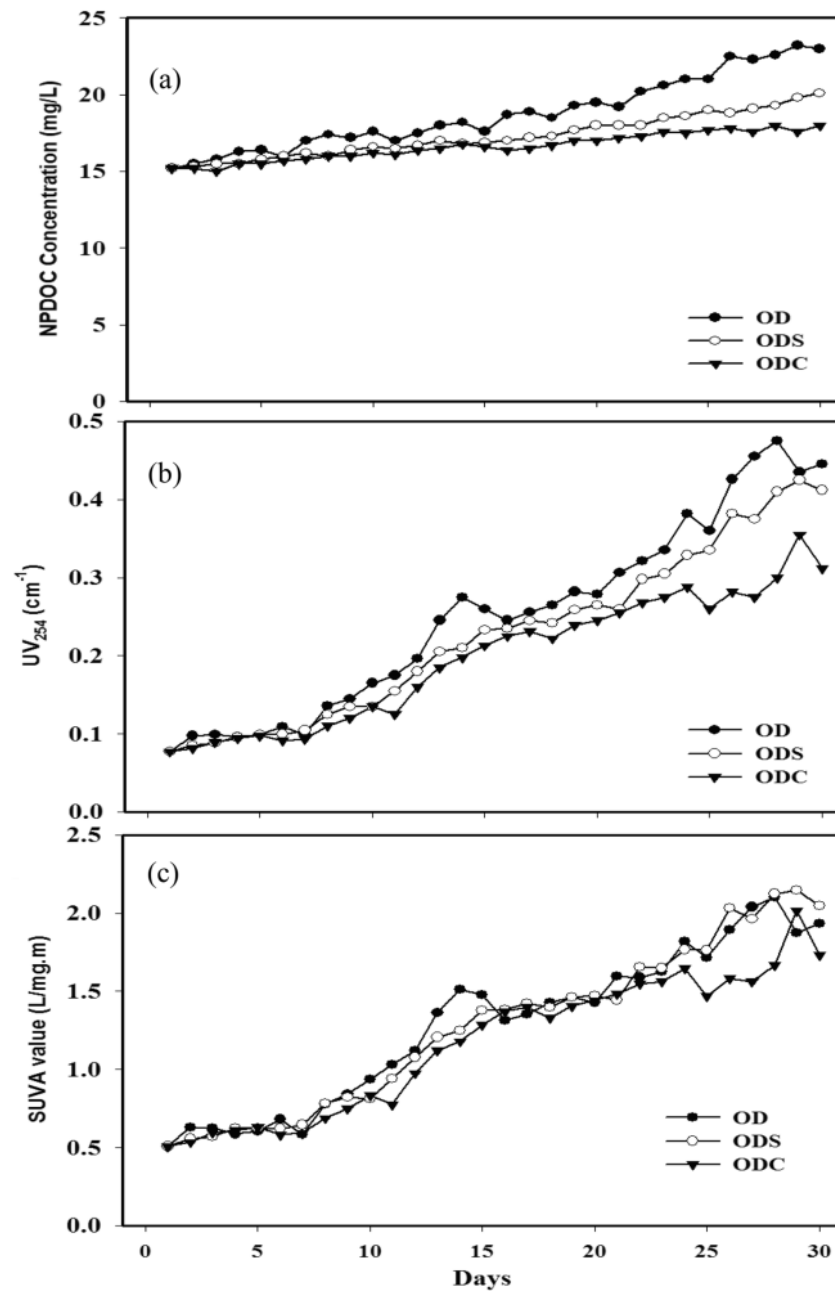
systems are 18.77 ± 2.33 mg/L, 17.29 ± 1.36 mg/L, and 16.61 ± 0.88 mg/L, respectively.

First, NPDOC concentration results indicated an increase in NPDOC concentration after-treatment processes. Even effluent organic matter from OD exhibited the highest NPDOC concentration. Increasing NPDOC during the biological process may be due to microorganisms and released algal by-products during growth activities and decay (Ni et al. 2010; Qu et al. 2012; Hua et al. 2017). Second, NPDOC concentrations in OD are higher than those in ODS and ODC. The algal-bacteria symbiotic transforms the quality and diminishes dEfOM in wastewater, which depends on the characteristic of algae, bacteria, and their interaction. According to Ji et al. (2017), the symbiosis of algae and bacteria can eliminate dissolved nutrients and organic in wastewater because of the chlorophyll metabolism–related genes. Third, average UV_{254} values, representing aromatic compounds of organic in water, at OD, ODS, and ODC systems, are 0.251 ± 0.12 cm^{-1}, 0.227 ± 0.11 cm^{-1}, and 0.197 ± 0.08 cm^{-1}, respectively. The highest UV_{254} in OD had been confirmed, and the increasing value may be attributed to biological processes. Previous studies proved that dEfOM from the biological process could be produced from substrate utilization, microbial growth, and endogenous phase. Several molecules derived from bacteria with an aromatic structure, such as lipopolysaccharide and amino acid, are detectable in recalcitrant dissolved organic matter (Jiao et al. 2010). Fourth, the symbiotic *C. vulgaris*-bacteria in the ODC system resulted in lower aromatic concentrations detected by UV_{254}. This symbiotic system's interaction achieved lower aromatic properties than *S. platensis*-bacteria in ODS and OD systems. *C. vulgaris* contains a higher component of polyunsaturated fatty acid than *S. platensis*, because total level of unsaturated fatty acid, alpha linolenic, oleic, and hexadecatrienoic acids in *C. vulgaris*, was found in high amounts (Otles and Pire 2001). The presence of those component indicated that *C. vulgaris* contains less aromatic compounds because the unsaturated fatty acid is a straight chain of an even number of carbon atoms, with hydrogen atoms along the length of chain with the carbon-to-carbon bonds is triple. In addition, the interaction between microalgae and bacteria was mutually beneficial, which could be important for each metabolism. For example, bacteria have been found to secrete the phytohormone indole-acetic acid, an algal growth promoter, and some bacteria release algaecides. On the other hand, microalgae excrete chlorellin, an antibacterial substance, and provide organic matter for bacterial growth (Dellagreca et al. 2010).

Fifth, tofu raw wastewater has the lowest SUVA value, and the data shows an insignificant divergence in SUVA value distribution. The average SUVA values at OD, ODS, and ODC systems are 1.28 ± 0.49 L/mg-m, 1.27 ± 0.52 L/mg-m, and 1.16 ± 0.44 L/mg-m, respectively. In other words, the SUVA values in OD with or without algae were close, and UV_{254} was affected by the variations of ion concentrations, specifically for the utilization of nitrogen or phosphorus by algae grown in the OD system. Edwards et al. (2001) proved that UV at 205 nm and 300 nm is suitable for detecting nitrate with dissolved organic carbon concentration up to 20 mg/L. SUVA value slightly increased during treatment to describe the variation of hydrophilicity and hydrophobicity of organic properties. SUVA value may indicate organic, humic, hydrophobic, and hydrophilic composition (Edzwald and Tobiason 2011). Generally, organic matter surrogate parameters show that raw tofu wastewater's organic concentration has lower NPDOC and aromatic compounds and is more hydrophilic than treated tofu raw wastewater. These organic or dEfOM concentrations increased after the biological oxidation ditch process, indicating that the properties may be attributed to organic-derived bacteria (Bhatia et al. 2013; Hua et al. 2019a; Hidayah et al. 2020).

Furthermore, AOM is mainly composed of polysaccharides, lipids, nucleic acids, proteins, amino acids, and other organic acids. The proportions of those components may vary depending on species, age of culture, and environmental conditions. High proportions of polysaccharides and proteins increase hydrophilic organic matter properties in water (Rehman et al. 2017). AOM is more hydrophilic than natural organic matter in the growth phase. The decline phase indicates a decrease in the cell number of algae and then the release of intracellular compounds from autolysis mainly composed of amino acids, peptides, and other organic acids such as fatty acids (Leloup et al. 2013).

Figure 3 describes the fluorescence spectra of samples taken from the sources of raw tofu wastewater and 15th day treatment. Spectra were divided into regions I–IV of aromatic protein–like (AP-like) at Ex/Em < 250/< 380 nm, fulvic acid–like (FA-like) at 200–250/380 nm, soluble microbial product–like (SMP-like) at 250–280/< 380 nm, and humic acid–like (HA-like) at > 280/> 380 nm, as described by Chen et al. (2003). The raw tofu wastewater (RW) spectra showed FA-like and HA-like components with peaks at Ex/Em 230/420 nm and 320/420 nm. Furthermore, fluorescence organic fractions indicated two additional AP-like and SMP-like components with peaks at Ex/Em 230/345 nm and 320/345 nm during ODC and ODS treatment. At the same time, AP-like components appeared during OD treatment, and similar result under different biological processes was reported by previous studies (Moradi et al. 2018; Hidayah et al. 2020). Effluent from biological processes has identified an aromatic double bond performed as HA-like and FA-like compounds from microbial activities during their metabolism and decay (Ni et al. 2010). It is consistent with the emitted color spectra of the OD, ODS, and ODC systems. Protein
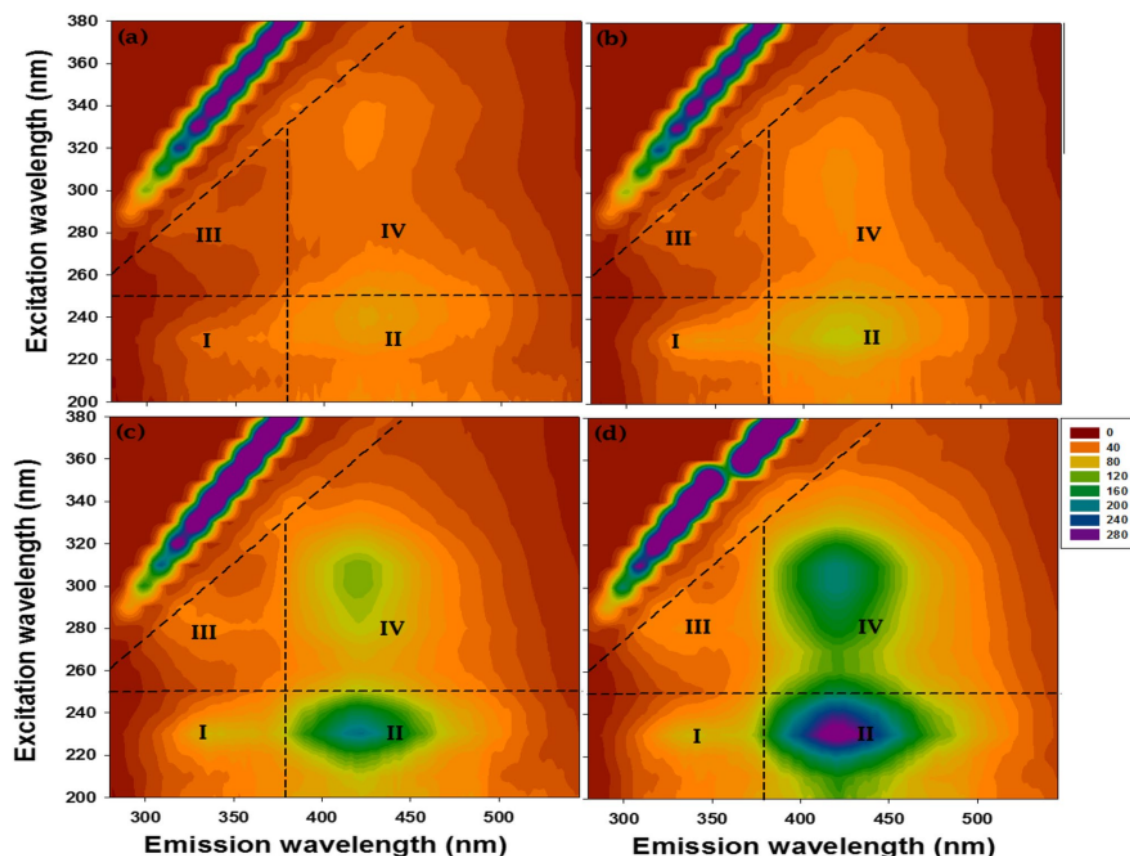
Fig. 3 Fluorescence excitation-emission spectra of (**a**) raw wastewater (RW) and after treatment with (**b**) oxidation ditch only (OD), (**c**) oxidation ditch *C. vulgaris* (ODC), and (**d**) oxidation ditch *S. platensis* (ODS)

components were generated from the metabolic products of algae and bacteria activities. It is relevant to the increasing fluorescence spectra in the system, which indicated the performance of bacteria in the OD and that of algae-bacteria symbiosis in the ODS and ODC systems. Protein-like components contain tryptophan and tyrosine, such as alpha-amino acid and 4-dihydroxyphenylalanine used in protein synthesis (Rehman et al. 2017; Bhatia et al. 2013). According to the results, bacteria and microalgae contributed to organic fractions' quality and quantity during the oxidation ditch's biological process. The comparison of raw and treated tofu wastewater showed an increasing fluorescence intensity in each region. Furthermore, each peak's Ex/Em was applied as a wavelength on the fluorescence detector to show organic properties based on its molecular weight (Hidayah et al. 2020). The shift error should be examined before setting up a fluorescence peak

as a wavelength when the error reaches up to 5% (Baghoth et al. 2011).

The spectral characteristic of excitation-emission and its molecular weight distribution fluorescence of dEfOM

Figure 4 shows the MW distribution of dissolved effluent organic fluorescence during oxidation ditch without algae, filled with *S. platensis* and *C. vulgaris*. The dEfOM was fractioned by HPLC-FLD and expressed in the molecular weight cut-offs (MWCOs). Figure 5 shows three significant dissolved organic fractions in tofu wastewater, including high (HMW), medium (MMW), and low molecular weight (LMW) with AMW of about 50,000 Da, 1650 Da, and less than 1650 Da, respectively (Huber et al. 2011; Lai et al. 2015; Hidayah et al. 2017). The typical compound of HMW is biopolymers, and MMW is presented as humic
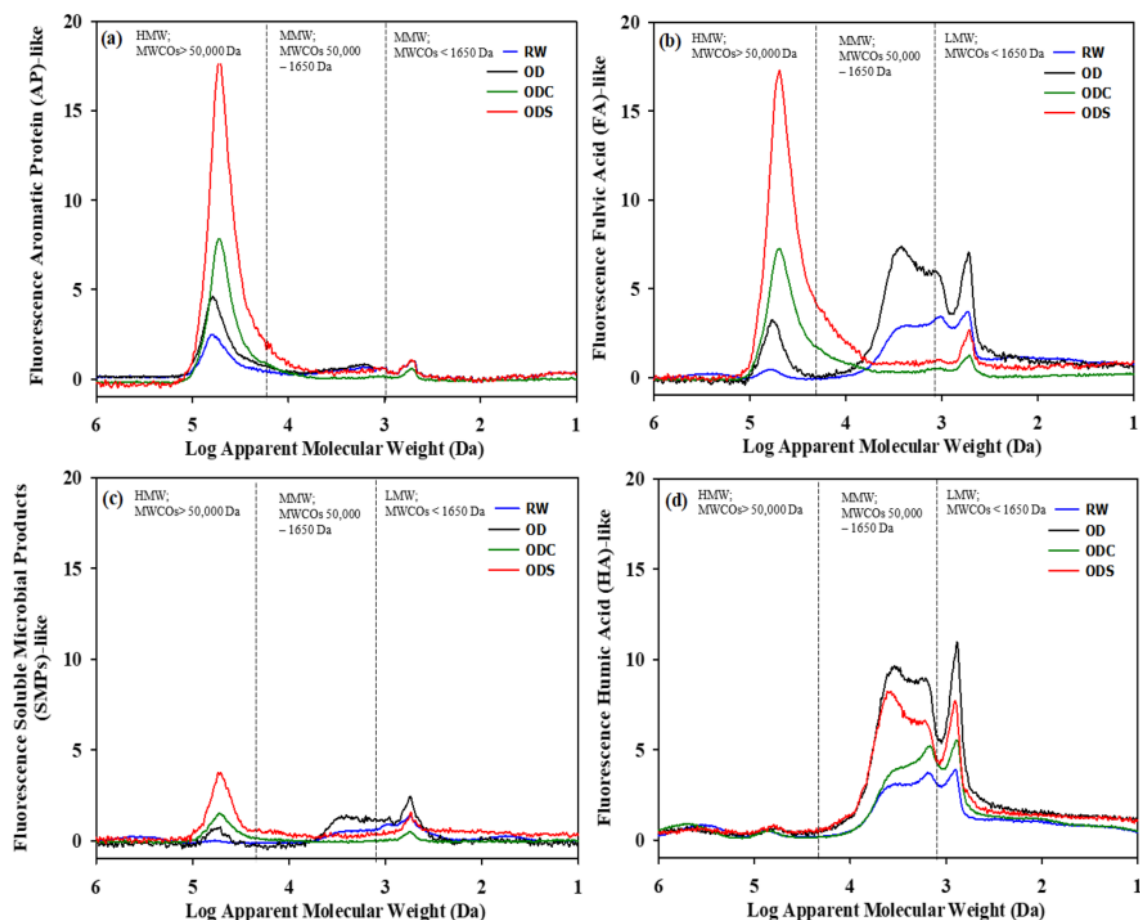
Fig. 4 Fractionation of dissolved effluent organic fluorescence as (**a**) AP-like, (**b**) FA-like, (**c**) SMP-like, and (**d**) HA-like, based on average molecular weight as obtained by HPLC-FLD

substance–like components and building blocks. Meanwhile, LMW stands for low molecular weight acid and neutral, and the chromatograph shape of all samples is similar, but the peak height varies. This indicates all samples produce similar organic fractions in different quantities and qualities. The higher height of the peak indicates a higher concentration of organic compounds.

First, AP-like fluorescence chromatograms identified a distribution HMW of organic fractions mainly. The heights of all the peaks increased during the oxidation ditch process with and without algae. According to previous studies, aromatic protein mainly consists of high molecular weight of natural organic matter with AMW of around 50,000 Da (Chow et al. 2008). During the lag and death phase, algae species may produce mainly biopolymers in polysaccharides and proteins containing fucose and sulfated functional groups (Villacorte et al. 2015). Second, FA-like

fluorescence chromatograms fractionated organic matter in raw wastewater into HMW, MMW, and LMW fractions. For HMW, the peak increased dramatically, and ODS exhibited a higher height than ODC. The chromatograph presented that the height of all peaks increased significantly in the OD system, except in ODS and ODC for MMW. The peak LMW in ODS and ODC showed a lower height than the RW and OD. Therefore, microorganisms in the oxidation ditch reactor released more FA-like components generated in the endogenous phase. The component in the polymer matrix could be assigned to NADH and pyridoxine or fulvic acids (Ni et al. 2010).

Third, SMP-like fluorescence chromatograms of RW exhibited MMW and LMW organic fractions. The peak of MMW appeared at a lower height of OD than ODS and ODC. On the other hand, the LMW peak height is comparable to organic matter released from the OD, ODS, and

Fig. 5 The peak area of fluorescence organic fractions based on their molecular weight in (**a**) raw wastewater and during biological processes with (**b**) OD, (**c**) ODC, and (**d**) ODS
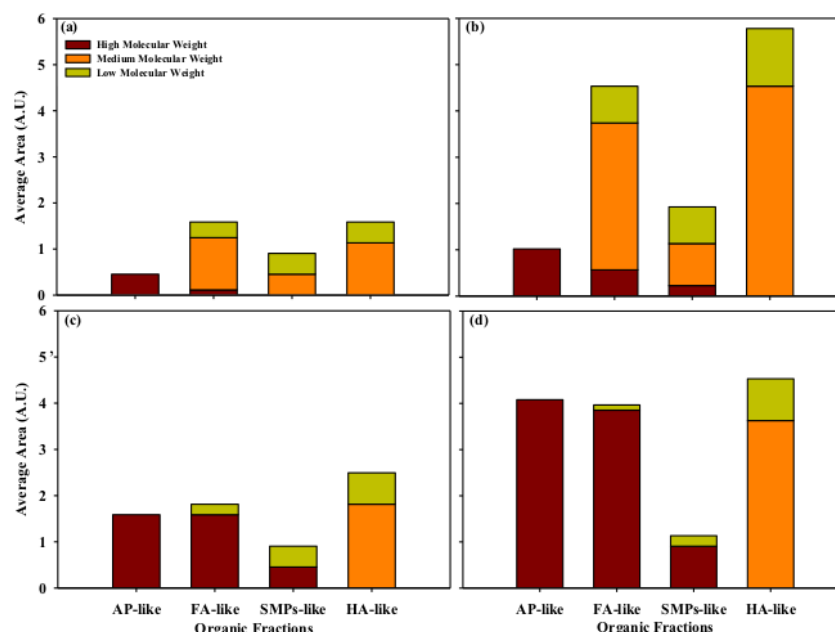
ODC systems. SMP has been classified into two groups based on the bacteria phase derived from them. The endogenous phase generates a group of biomass-associated products (BAP), while the original substrate in microbial growth is categorized as utilization-associated products (UAP) (Ni et al. 2010). UAP produced in the substrate-utilization process were carbonaceous compounds. Meanwhile, BAP was classified into growth-associated (GBAP) and endogeny-associated (EBAP) produced in the microbial growth and endogenous phases. SMP was a component of effluent organic matter associated with biomass decay during the biological process.

Fourth, HA-like fluorescence chromatograms of all samples fractionated organic matter into MMW and LMW. The height of all peaks increased significantly, and the fractionated organic component in OD exhibited the highest peaks. The effluent organic matter is mainly composed of humic-like materials and biomass SMP released during the endogenous phase. This is consistent with previous studies, which found significant peaks from effluent organic matter, including polysaccharides, protein-like and humic-like substances, and LMW organic acids (Shon et al. 2012; Ni et al. 2010; Xiao et al. 2018; Hidayah et al. 2020). However, this method is limited to detecting non-fluorescing components by fluorescence spectroscopy. This is because organic matter has a different molecular weight, and only molecules containing fluorophores emit fluorescence at specific wavelengths (Hidayah et al. 2017).

Figure 5 presents the peak area of fluorescence organic fractions based on molecular weight during biological processes with OD, ODS, and ODC, as shown using the peak-fitting technique (Chow et al. 2008; Lai et al. 2015). The aromatic protein area was dramatically increased during OD processes. The bacteria and algal released protein during biomass growth, cell lysis, or biomass decay. According to the results, aromatic protein is mainly composed of high molecular weight, biological processes can release HMW of AP-like (Qu et al. 2012; Hua et al. 2019a). The peak area HMW of AP-like in the ODS system is much higher than in the OD and ODC systems. Previous studies found that microalgae produced polysaccharides and protein biopolymers. The increasing peak area of aromatic protein seems probable due to releasing IOM during cell lysis or similar processes in the stationary or death phase (Qu et al. 2012; Rehman et al. 2017). More than 40% of the total mass of the IOM cells are aromatic proteins and amino acids (Tomlinson et al. 2016; Hong et al. 2008). These results conclude that bacteria-derived organic matter will release higher aromatic protein than algal-derived. The peak area of aromatic protein, derived in *S. platensis* and *C. vulgaris*, describes that algae may release a similar high molecular weight.

Fractions of FA-like and SMP-like have a similar pattern on the peak area of fluorescence organic in the OD system with algae. The peak area HMW of FA-like and SMP-like increased significantly during oxidation ditch processes. The

highest peak area HMW was detected in the ODC, while MMW and LMW were detected in the OD system. Interestingly, peak area MMW of FA-like and SMP-like was undetected in the ODS and ODC, and LMW of FA-like decreased in oxidation ditch with algal. The interpretation of these results is that increasing HMW significantly indicates the growth phases of algal organic matter. The production of biopolymers, primarily composed of polysaccharides and have a much higher weight, increased in the growth phases (Qu et al. 2012).

In the biological process with OD, polysaccharides with some contribution from nitrogen-containing material such as proteins or amino sugars are considered as EPS (Huber et al. 2011). In biological processes with ODC and ODS, algae can be classified based on EOM, IOM, and cellular-bound organic matter (Hua et al. 2019b). EOM comprises polysaccharides, proteins, and humic-like substances, mainly distributed in the HMW and hydrophobic fractions during lag and exponential phases. It also comprises high polysaccharide content during the stationary and declining phases (Qu et al. 2012), and the limitation of nutrients to support the growth leads to cell mortality. During the death phase, cellular organic matters, such as IOM and cellular-bound organic matter, are released (Hua et al. 2019a). Using fluorescence spectroscopy, characterizing EOM and IOM showed the EOM contents of HA-like, FA-like, and SMP-like compounds. At the same time, IOM is composed of SMP-like and AP-like substances. Quantitative fluorescence measurement using an average fluorescent intensity of each component indicated that FA-like has a higher average intensity than SMP-like (Li et al. 2012; Hua et al. 2019a). FA-like in biological processes is mainly composed of higher total and aromatic carbon levels. On the other hand, SMP-like contains carbon of amino acids or refers to tryptophan and carbohydrate with lower molecular weight (Chen et al. 2003; Ni et al. 2009). The contribution of symbiosis bacteria-algal in the oxidation ditch can lower the quantity of MMW and LMW of FA-like and SMP-like. The results suggested that different molecular weight distributions of FA-like and SMP-like have been released during the growth phase of the biological process.

Fraction HA-like comprised MMW, LMW, and SMP-like, and the peak area in the OD system increased significantly. Bacteria released more HA-like than microalgal, decreasing MMW and LMW in the ODS and ODC systems. Bacteria and microalgae released similar organic matter, with different quantity and quality characteristics. Bacteria produce EPS through several mechanisms of excretion, secretion, cell lysis, sorption of constituents from wastewater, etc. EPS essentially consists of polysaccharides, identified as high molecular weight. It was the predominant component in the EPS fraction for the aerobic biological process (Laspidou and Rittmann 2002). Meanwhile, microalgae released

AOM, abundant in nitrogenous and protein substances such as AP- and SMP-like components with a very little aromatic structure (Hua et al. 2019b). The HA-like area was rapidly increased during oxidation ditch processes, and protein was released during biomass growth and decay. An increased HA-like at oxidation ditch process is consistent with a higher NPDOC concentration in OD than in the systems with algae. HA-like was mainly composed of apparent MWCOs of 3–10 kDa and LMW with MWCOs of 1–3 kDa and less than 1 kDa (Hua et al. 2019a). This study shows that biological processes can release AP-like, FA-like, SMP-like, and HA-like components. A biological process under algal-bacteria symbiosis generated a lower quantity of MMW and LMW than without algal. However, the type of algal will affect the quantity and characteristics of generated organic matter in the biological process presented in *C. vulgaris* and *S. platensis*.

Conclusion

Several significant findings are summarized in the following statements. First, the organic concentration of original tofu wastewater has lower NPDOC, and is more hydrophilic than those treated. The concentration of dEfOM shows an increasing trend during the biological oxidation ditch process. Second, the original tofu raw wastewater shows FA-like and HA-like components at Ex/Em 230/420 nm and Ex/Em 320/420 nm. This produced different fluorescent positions at Ex/Em 230/345 nm and Ex/Em 320/345 nm for AP-like and SMP-like components. Three significant dissolved organic fractions of the tofu and treated wastewater were reported, including HMW, MMW, and LMW, with average MWCOs close to 50,000 Da, 1650 Da, and less than 1650 Da. The peak area of fluorescence organic fractions based on the molecular weight indicated that AP-like, FA-like, and SMP-like components formed HMW. In contrast, HA-like was composed of MMW and LMW. This study recommends that a molecular weight–based fluorescent detector applied to characterize and track the changing of dEfOM in terms of algal-derived and bacteria-derived organic matter during the oxidation ditch process.

Abbreviations AMW: Apparent molecular weight; AOM: Algal organic matter; AP-like: Aromatic protein–like; BAP: Biomass-associated products; BOD: Biological oxygen demand; COD: Chemical oxygen demand; DBPs: Disinfection by-products; dEfOM: Dissolved effluent organic matter; EOM: Extracellular organic matter; EPS: Extracellular polymeric substances; Ex/Em: Excitation/emission; FA-like: Fulvic acid–like; FEEM: Fluorescence excitation-emission matrices; GBAP: Growth biomass-associated products; HA-like: Humic acid–like; HMW: High molecular weight; IOM: Intracellular organic matter; LC-OCD: Liquid chromatography-organic carbon detector; LMW: Low molecular weight; MMW: Medium molecular weight; MW: Molecular weight;

MWCOs: Molecular weight cut-offs; NPDOC: Non-purgeable dissolved organic carbon; OD: Oxidation ditch; ODC: Oxidation ditch with *C. vulgaris*; ODS: Oxidation ditch with *S. platensis*; RW: Raw water; SMP: Soluble microbial products; SUVA: Specific ultraviolet absorbance; UAP: Utilization-associated products; UV_{254}: Ultraviolet absorbance at 254 nm

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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