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Using Molecular Weight-Based Fluorescent Detector to Characterize Dissolved EffluentOrganic Matter in Oxidation Ditch with Algae

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Abstract

Implementation microalgae has been considered for enhancing effluent wastewater quality. However, algae can cause environmental issues due to algae released extracellular organic matter, algal organic matter, instead of bacteria-derived organic matter in the biological process. The objectives of this study are to investigate the characteristics of dissolved effluent organic matter as algal-derived organic and bacteria-derived organic during the oxidation ditch process. Experiments were conducted in the oxidation ditch without algae, with *Spirulina platensis* and *Chlorella vulgaris*. The results showed dissolved effluent organic matter increased into higher dissolved organic carbon, more aromatic and hydrophobic than that before treatment. Fluorescence spectroscopy identified two component, namely aromatic protein-like at excitation/emission 230/345 nm and soluble microbial products-like at 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. Fractionation of dissolved organic fluorescence based on average molecular weight cut-offs (MWCOs) has obtained that fractions aromatic protein-like, fulvic acid-like, humic acid-like, and soluble microbial products-like has respectively a high MWCOs 50,000 Da, a high to low MWCOs <1650 Da, medium MWCOs 1650 Da to low MWCOs. Biological oxidation ditch under symbiosis algal-bacteria generated humic acid-like and fulvic acid-like with a higher MWCOs than oxidation without algal. The quality and quantity of dissolved effluent organic matter in oxidation ditch algal reactor has been significant affected by algal-bacteria symbiotic.

Introduction

Microalgae have been considering as an alternative solution to enhance wastewater treatment due to their ability to use inorganic nitrogen and phosphorous for their growth. Microalgae could also remove pathogens, heavy metals, and furnish O2 to heterotrophic aerobic bacteria to degrade organic pollutants (Munoz and Guieysse 2006). The potential uses of algae-bacteria biomass have been well observed, which can be used for a human food source (Wells et al. 2017), animal feed (Madeira et al. 2017), organic fertilizer for sustainable agriculture (Baweja et al. 2019). However, it may contain a recalcitrant compound that could be found in treated wastewater effluent (Leloup et al. 2013). Also, algae-bacteria biomass could be used for energy production, such as biogas production (Montingelli et al. 2015) and biofuel (Benemann 2013). Instead of its advantages, algae can cause undesirable odor and taste, release toxins and organics in the water bodies (Caruana and Amzil 2018; Leloup et al. 2013). Many studies have observed that microorganisms released extracellular polymeric substances (EPS) and soluble microbial products (SMPs), while microalgae released extracellular organic matter (EOM) (Qu et al. 2012). Also, organic matter produced by algal or algal organic matter (AOM) has been well identified recently, including its production, evolution, and characteristics, which depended on the species and growth phases (Rehman et al. 2017). Those microbial products are essential because the constituents are found in wastewater lead to membrane fouling, sludge bulking in activated sludge (biological treatment), disinfection by-products upon chemical disinfection, and floating matter and films in waterways (Shon et al. 2006).

Some characterization of effluent organic matters and AOM have been developed to identify organics properties quantitatively and qualitatively. Regarding the effluent organic matter, extracellular polymeric substances (EPS) and soluble microbial products (SMP) are generated from biological wastewater treatment. EPS, a complex high molecular weight compound, is produced by microorganisms in bioreactors when organic materials exist in wastewater (Ni et al. 2009). SMP is composed of molecular weight < 1 kDa, and it is released during biomass metabolism and decay in biological processes (Liu et al. 2014). According to molecular weight organic fractions, the effluent from the biological process consists mainly of biopolymers and humics, in addition to building blocks, low molecular weight neutral, and low molecular weight acid as characterized using 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, namely: proteins, humic and fulvic-like substances at excitation (Ex)/emission (Em) at 280/350, 340/400, and 390/450 nm, respectively (Li et al. 2008; Ni et al. 2009). Regarding the characteristic of AOM, previous studies observed that both EOM and IOM of *Chlorella sp*. presented about less than 0.05 a.u. of UV254 and UV280, which indicated a low absorbance (Hua et al. 2019a). Characteristic of AOM was mainly hydrophilic as detected by resin fractionation, EOM is mainly composed of fulvic acid-like, soluble microbial products (SMP)-like, and humic acid-like substances, and IOM is dominantly comprised of aromatic protein substances and SMP-like as identified using FEEM spectroscopy (Hua et al. 2017; Zhu et al.2015; Dong et al. 2019). EOM and IOM were mainly distributed in low- molecular-weight (MW) (< 1 kDa) and high MW (> 100 kDa) fractions as characterized by high-performance size exclusion chromatography (HPSEC) with an ultraviolet detector (UVD) and organic carbon detector (OCD) (Zhou et al. 2015). According to Fourier transform infrared (FTIR), algae and AOM shows many absorption peaks at 3400 – 3200 cm^{-1} (hydrogen bonds O-H), at 2950 – 2850 cm^{-1} (CH_2), 1650 – 1580 cm^{-1} (amide group C-N/carboxylate group COOH) (Her 2003).

Oxidation ditches are widely used in wastewater treatment for removing organic pollutants in industrial waste all over the world (Zhang et al. 2016). Oxidation ditches are a modified activated sludge process, which has advantages in long hydraulic retention time, produces 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). The bacteria community's potential key role in removing nutrients and organics under various operational in the oxidation ditches has been well implemented (Terashima et al. 2016; Xu et al. 2017; Luo et al. 2020). Recently, oxidation ditches combined with filled-algae have been referred to as promising processes in removing wastewater nutrients. This process is analogous to the activated sludge that utilizes a symbiotic relationship between algae and bacteria in a controlled system (Maiti et al. 1988; Noue et al. 1992). The capability of biological algae reactor has shown a significant development, which removal nutrient (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, it conjectures that algae-derived organic matter might be contributed the characteristic of organic matter in water quantitatively and qualitatively. As mentioned previously, microbial in wastewater treatment might release organic in terms of soluble microbial products. Therefore, the symbiosis of algae-bacterial into the wastewater treatment process is inferred to contribute organic matter's concentration and characteristic in effluent water. Symbiotic between algae and microorganisms will release algae-derived organic matter and microorganism-derived organic, comprised of different organic properties. Wastewater reclamation has been developed as an alternative method for producing water resources; therefore, the existence of derived organic matter in treated wastewater effluents should be minimized because organic matters can cause membrane fouling, 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 in water bodies. Organic matters may contribute as precursors of the disinfection by-products (DBPs) formation, generally took an essential part in forming C-DBPs and N-DBPs, either in chlorination or in chloramination (Zhou et al. 2015).

To the best of our knowledge, characterization and compositional differences of dissolved effluent organic matter (dEfOM) in oxidation ditches filled with algae and without algae have not been fully explored, especially for absence of studies regarding to dEfOM from *Spirulina platensis*, and *Chlorella vulgaris*. In this study, the oxidation ditches process were conducted under three different combination, that is filled by *Spirulina platensis*, *Chlorella vulgaris*, and without microalgae. During the processes, the characteristic of dEFOM was monitored by fluorescent spectrometry and using the molecular weight-based fluorescent detector. Finally, the composition of derived organic released by different species in the oxidation ditches could be clearly elucidated.

Material And Methods

The raw sample was collected from tofu wastewater, and three sets of oxidation ditches were prepared. The oxidation ditches 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. The algal acclimation in tofu wastewater concentration 30% has shown a decreasing number of algal cells, which was measured by haemocytometer, after the seventh day. Therefore the experiment applied 30% tofu wastewater as a raw sample. The ratio 1:1 of wastewater to algal volume in oxidation ditches was filled with *Spirulina platensis*, with *Chlorella vulgaris*, and without algae for controlling experiment. Samples were collected before treatment (RW) and after treatment, which is effluent from oxidation ditch (OD), from oxidation ditch with *Spirulina platensis* (ODS), and from oxidation ditch with *Chlorella vulgaris* (ODC) once per day for a month of observation. Operation conditions was maintained under DO value of 5–6 mg/L, pH value of 7–8, temperature value of 27-30^{o}C, and got the same natural light intensity from morning to afternoon. Samples were filtered through a 0.45 um 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), 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 NPDOC concentration (Edzwald and Tobiason 2011). Besides, dEfOM characterization was qualified by using fluorescence spectroscopy (Perkin Elmer LS-55) and high-performance liquid chromatography combined with fluorescence spectroscopy as a detector (HPLC-FLD, type LC-20 ATV Shimadzu, Japan). First, fluorescence spectroscopy was set up at excitation wavelengths (Ex) between 230 and 400 nm at interval 10 nm and emission wavelengths (Em) between 300 and 550 nm at an interval of 0.5 nm (Chen et al. 2003). This method is applied for selecting wavelengths to set up a fluorescence detector by determining the average of chosen peak maxima location of excitation-emission wavelength. Second, chromatography was used to fractionate dEfOM based on its apparent molecular weight (AMW) through fluorescence detection, according to the chosen peak of excitation-emission wavelength previously (Hidayah et al. 2020). The peak-fitting technique, PeakFit Version 4.12, Systat Software Inc., USA, CA, was applied to resolve chromatograph as describes in the previous study (Lai et al. 2015).

Results And Discussion

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

The concentration of dEfOM in term of natural organic matter surrogates parameters, including NPDOC, UV_{254}, SUVA, is presented in Fig. 1a, 1b, 1c, respectively. The average concentrations of NPDOC at OD, ODS, and ODC system are 18.77 ± 2.33 mg/L, 17.29 ± 1.36 mg/L, 16.61 ± 0.88 mg/L, respectively. First, the results of NPDOC concentration indicated an increasing of NPDOC concentration after treatment processes, even effluent organic matter from ODS exhibited the highest NPDOC concentration. Increasing NPDOC during the biological process may be due to microorganisms and released algal by-product 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. It seems that algal-bacteria symbiotic transforms the quality and diminishes of dEfOM in wastewater, which depends on the characteristic of algae, bacteria, and their interaction. According to the report of Ji et al. (2017), the symbiosis of algae and bacteria had the ability to eliminate dissolved nutrients and organic in wastewater because of the chlorophyll metabolism-related genes to bacterial rRNA genes, which might support to remove of nutrients in wastewater.

Third, average UV 254 values, representing aromatic compound of organic in water, at OD, ODS, and ODC system, are 0.251 ± 0.12 cm^{-1}, 0.227 ± 0.11 cm^{-1}, 0.197 ± 0.08 cm^{-1}, respectively. The highest UV254 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, and several molecular derived from bacteria detectable in recalcitrant dissolved organic matter with an aromatic structure, such as lipopolysaccharide, an amino acid (Jiao et al. 2010). Fourth, the symbiotic *Chlorella vulgaris*-bacteria in the ODC system resulted in lower aromatic concentration detected by UV254. This symbiotic system's interaction could achieve lower aromatic properties than those of symbiotic *Spirulina platensis*-bacteria in ODS and bacteria only in the OD system. Fifth, tofu raw wastewater has a lowest SUVA value, and the data shows insignificant divergence SUVA value distribution.

The average SUVA values at OD, ODS, and ODC system are 1.28 ± 0.49 L/mg-m, 1.27 ± 0.52 L/mg-m, 1.16 ± 0.44 L/mg-m, respectively. In other words, the SUVA values in OD with or without algae were close, probably UV_{254} was affected by the variations of ion concentrations, especially for the utilization of nitrogen or phosphorus by algae grown in OD system. Edwards et al. (2001) had proved that UV at 205 nm and 300 nm is suitable to detect nitrate with dissolved organic carbon concentration up to 20 mg/L. SUVA value slightly increased during treatment; it could describe variation of organic hydrophilicity and hydrophobicity of organic properties. SUVA value could indicate the composition of organic, whether humic, hydrophobic matter or non-humic, hydrophilic matter (Edzwald and Tobiason, 2011). Overall, NOM surrogates parameters reveals that the organic concentration of raw tofu wastewater has lower NPDOC, lower aromatic compounds, and more hydrophilic than those tofu raw wastewater treated. These organic or dEfOM concentrations increased after the biological oxidation ditch process, indicating that the properties of organic matter may be attributed by organic-derived bacteria or organic-derived from algal-bacteria symbiotic (Bhatia et al. 2013; Hua et al. 2019a; Hidayah et al. 2020).

(c)

AOM is mainly composed of polysaccharides, lipids, nucleic acids, proteins, amino acids, and other organic acids, and its proportions of those components may vary depending on species, age of culture, and environmental conditions. High proportions of polysaccharides and protein in AOM cause hydrophilic organic matter properties in water (Rehman et al. 2017). It has been mentioned that AOM is more hydrophilic than natural organic matter during the growth phases; even the decline phase resulted in more hydrophilic than previous phases. The decline phase indicates a decrease in the cell number of algae and then the release of intracellular compounds from cell autolysis, mainly composed of amino acids, peptides, and other organic acids like fatty acid (Leloup et al. 2013).

Figure 2 Fluorescence excitation-emission spectra of (a) raw wastewater (RW) and after treatment with (b) oxidation ditch only (OD), (c) oxidation ditch *Chlorella vulgaris* (ODC), and (d) oxidation ditch *Spirulina platensis* (ODS).

wastewater and 15th days treatment samples. After treatment, the treated sample from the oxidation ditch without algae, the oxidation ditch *Chlorella vulgaris* and *Spirulina platensis*. Spectra was divided into four regions, Region I is aromatic protein-like (AP-like) at Ex/Em < 250/<380 nm, Region II is fulvic acid-like (FA-like) at 200–250/>380 nm, Region III is soluble microbial products like (SMPs-like) at 250–280/<380 nm, and Region IV is humic acid-like (HA-like) at > 280/>380 nm, as described by Chen et al. (2003). The spectra of raw tofu wastewater indicated two main components of organic fluorescence: fulvic acid-like (FA-like) with the peak at Ex/Em 230/420 nm and humic acid-like (HA-like) with the peak at Ex/Em 320/420 nm. After oxidation ditch without algae and oxidation ditch with algae treatment, fluorescence organic fractions of samples indicated two additional components that are aromatic protein-like (AP-like) with the peak at Ex/Em 230/345 nm and soluble microbial products like (SMPs-like) with the peak at Ex/Em 320/345 nm. A 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, which was performed as HA-like and FA-like compounds, from microbial activities during their metabolism and decay (Ni et al. 2010). Protein components were generated from the metabolic products of algae and bacteria activities, as well. Proteins-like exhibited materials containing tryptophan-like, such as alpha-amino acid, are used in protein biosynthesis, and tyrosine-like 4-hydroxyphenylalanine, and further synthesize protein by cells (Rehman et al. 2017; Bhatia et al. 2013). According to the results, it can be conjectured that bacteria and microalgae had contributed to the quality and quantity of organic fractions in water during the biological process in the oxidation ditch. Comparing raw tofu wastewater and treated ones showed an increasing fluorescence intensity in each region. Further, each peak's Ex/Em peak was applied as a wavelength on the fluorescence detector to reveal fluorescence organic properties based on its molecular weight organic (Hidayah et al. 2020). The error in the shifted fluorescence peak should be examined before setup a fluorescence peak as a fluorescence wavelength if the error reached up to 5% (Baghoth et al. 2011).

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

Figure 3 shows molecular weight distribution of dissolved effluent organic fluorescence during

oxidation ditch without algae, oxidation ditch filled with *Spirulina platensis*, oxidation ditch with *Chlorella vulgaris*. The dEfOM was fractioned by HPLC-FLD and expressed in the molecular weight cut-offs (MWCOs). Observed from Fig. 3, three significant dissolved organic fractions in tofu wastewater are included, including high molecular weight (HMW), medium molecular weight (MMW), and low molecular weight (LMW) with AMW of about 50,000 Da, 1650 Da, less than 1650 Da, respectively (Huber et al. 2011; Lai et al. 2015; Hidayah et al. 2017). The typical compound of HMW is biopolymers, while MMW is presented as humic substance-like and building blocks, and LMW is indicated as low molecular weight acid and neutral. The fluorescence chromatograms show that the chromatograph shape of all samples is similar, and the height of peaks is different. It means that all samples produce similar organic fractions in different organic concentration quantities and quality of organic properties. The higher height of the peak indicates the 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. It has been found in the previous studies that aromatic protein is mainly composed of high molecular weight of natural organic matter with AMW around 50,000 Da (Chow et al. 2008). During the lag phase 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 three fractions: HMW, MMW, and LMW. For HMW, it shows that the peak increased dramatically; even ODS exhibited a higher height of peak than that one of ODC. For MMW, the chromatograph presented the height of all peaks increased significantly in the OD system, except in ODS and ODC. The height of peak LMW in ODS and ODC showed a lower height than the RW and OD. It seems that microorganisms solely in the oxidation ditch reactor released more FA-like components, mainly non-growth associated, which were generated in the endogenous phase. The FA-like component in the polymer matrix could be assigned to either NADH and pyridoxine or directly fulvic acids (Ni et al. 2010).

Third, SMPs-like fluorescence chromatograms of RW exhibited MMW and LMW organic fractions. The peak of MMW appeared at a lower height of the fluorescence chromatogram of OD than that of ODS and ODC. On the other hand, the LMW peak height is comparable among organic matter released from OD, ODS, and ODC systems. It has been found that SMP has been classified into two groups based on the bacteria phase from which they are derived. endogenous phase generates group of biomass-associated products (BAP), while the original substrate in microbial growth is categorized as utilization associated products (UAP) (Ni et al. 2010). The utilization-associated products (UAP) in SMP, produced in the substrate-utilization process, were carbonaceous compounds. The BAP was mainly cellular macromolecules classified into the growth-associated BAP (GBAP), which were produced in the microbial growth phase, and the endogeny-associated BAP (EBAP) were generated in the endogenous phase. It has been well assigned that 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; even the fractionated organic in OD exhibited the highest peaks. The effluent organic matter was mainly composed of humic-like materials associated with existing organic matter in water bodies, and SMP biomass-associated products (BAP) released during the endogenous phase. This study is in accordance with previous studies, which found significant peaks from effluent organic matter, include polysaccharide, protein-like, humic-like substances, and low molecular weight organic acids (Shon et al. 2006; Ni et al. 2010; Xiao et al. 2018; Hidayah et al. 2020). Nevertheless, this method has a main limitation to detect non-fluorescing component by fluorescence spectroscopy. Organic matter has different molecular weight, however only organic matter with molecules containing fluorophores, could emit fluorescence at specific wavelengths, and then detected by fluorescence spectroscopy (Hidayah et al., 2017).

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Figure 4 The peak area of fluorescence organic fractions based on its molecular weight in (a) raw wastewater and during biological processes with (b) OD, (c) ODS, and (d) ODC

Fractions of both FA-like and SMPS-like have a similar pattern on the peak area of fluorescence organic in OD system with algae. The peak area HMW of FA-like and SMPs-like increased significantly during oxidation ditch processes, although increasing HMW of FA-like is higher than SMPs-like. The highest peak area HMW of FA-like and SMPs-like was detected in the ODC system, while the OD system released the highest peak area of MMW and LMW. Interestingly, peak area MMW of FA-like and SMPs-like was undetected in the ODS and ODC, and peak area LMW of FA- like decreased in oxidation ditch with algal. The interpretation for these results is that increasing HMW significantly indicates the growth phases of algal organic matter. Biopolymers production, mainly composed of polysaccharides and has a much higher weight, increased throughout 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 to mainly material of extracellular polymeric substances (EPS), which means that EPS is a generic term of biopolymers (Huber et al. 2011). In biological process with ODC and ODS, algal organic matter can be classified based on their origin and organic properties, that is extracellular organic matter (EOM), intracellular organic matter (IOM), and cellular-bound organic matter (COM) (Hua et al. 2019). EOM mainly comprises polysaccharides, proteins, and humic-like substances, mainly distributed in the HMW and hydrophobic fractions during lag and exponential phases and high polysaccharide content during the stationary and declining phases (Qu et al. 2012). The limitation of nutrients for cells to support their growth led to cell mortality, and the death phase occurs and causes releasing of cellular organic matter, such as IOM and COM (Hua et al. 2019). Characterization of EOM and IOM by using fluorescence spectroscopy has shown that EOM contents of HA-like, FA-like, and SMPs-like compounds, while IOM is dominantly composed of SMPs-like and AP-like substances. Quantitative measurement of fluorescence using an average fluorescent intensity of each component indicated that FA-like has a higher average fluorescence intensity than SMPs-like (Li et al. 2012; Hua et al. 2019a). FA-like in biological processes is mostly composed of higher level of total and aromatic carbon than SMPs-like. SMPs-like containing carbon of amino acids or refers to tryptophan and carbohydrate with lower molecular weight (Chen et al. 2002; Ni et al. 2009). In addition, it seems that the contribution of symbiosis bacteria-algal in the oxidation ditch could manage in lowering the quantity of MMW and LMW of FA-like and SMPs-like. The results suggested that different molecular weight distribution of FA-like and SMPs-like have been released during the biological process growth phase.

Fraction HA-like comprised of MMW and LMW as well as SMPs-like, and the peak area of HA-like in OD system increased significantly. It seems that bacteria released more HA- like than microalgal, decreasing MMW and LMW in the ODS and ODC system. The HA-like area was rapidly increased during oxidation ditch processes; it seems that both bacteria and algal released protein during biomass growth and that are released from cell lysis during biomass decay. A higher increasing of HA-like at oxidation ditch process is in accordance with a higher NPDOC concentration at OD system than those of OD systems with algae. HA-like was mainly composed of apparent MWCOs of 3–10 kDa and LMW with apparent MWCOs of 1–3 kDa and less than 1 kDa (Hua et al. 2019). This study reveals that biological processes, whether involved bacteria or symbiosis algal-bacteria, will release four organic components, namely AP-like, FA-like, SMPs-like with mostly composed of HMW, and HA-like, which is composed of MMW and LMW. A biological process under symbiosis algal-bacteria generated a lower quantity of MMW and LMW than without algal. Nevertheless, the type of algal will affect the quantity and characteristic of generated organic matter in the biological process under symbiosis algal- bacteria, as respectively presented in the symbiosis *Chlorella vulgaris*-bacteria and *Spirulina platensis*-bacteria in oxidation ditch process.

Conclusion

Several important finding are summarized in the following statements. The organic concentration of original tofu wastewater has lower NPDOC and lower aromatic more hydrophilic than those treated. The concentration of dEfOM reveals increasing trend during the biological oxidation ditch process. The original tofu raw wastewater shows two main components of organic fluorescence, namely, fulvic acid-like (FA-like) at Ex/Em 230/420 nm and humic acid-like (HA-like) at Ex/Em 320/420 nm, which produced extra fluorescent positions located at Ex/Em 230/345 nm (aromatic protein-like or AP-like) and at Ex/Em 320/345 nm (soluble microbial products like or SMPs-like). Three significant dissolved organic fractions of the tofu wastewater and treated wastewater are appeared, including HMW, MMW, LMW with average MWCOs close 50,000 Da, 1650 Da, less than 1650 Da, respectively. The peak area of fluorescence organic fractions based on its molecular weight indicated that AP-like, FA-like, SMPs-like was mostly composed of HMW, while HA-like was composed of MMW and LMW. This study recommends that a molecular weight-based fluorescent detector could be applied to characterize and track the changing of dEfOM in term of algal-derived organic and bacteria-derived organic during the oxidation ditch process.

Declarations

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files

Author contribution

ENH: arranged experiment; analyzed data; write the manuscript; review manuscript; OHC: conducted experiment; arranged data; analyzed data; write the manuscript; ENF: conducted experiment; visualized data; editing manuscript; NK: review the manuscript.

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Ethics approval and consent to participate

This article does not contain any studies involving human participants or animals performed by any of the authors.

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

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Figures

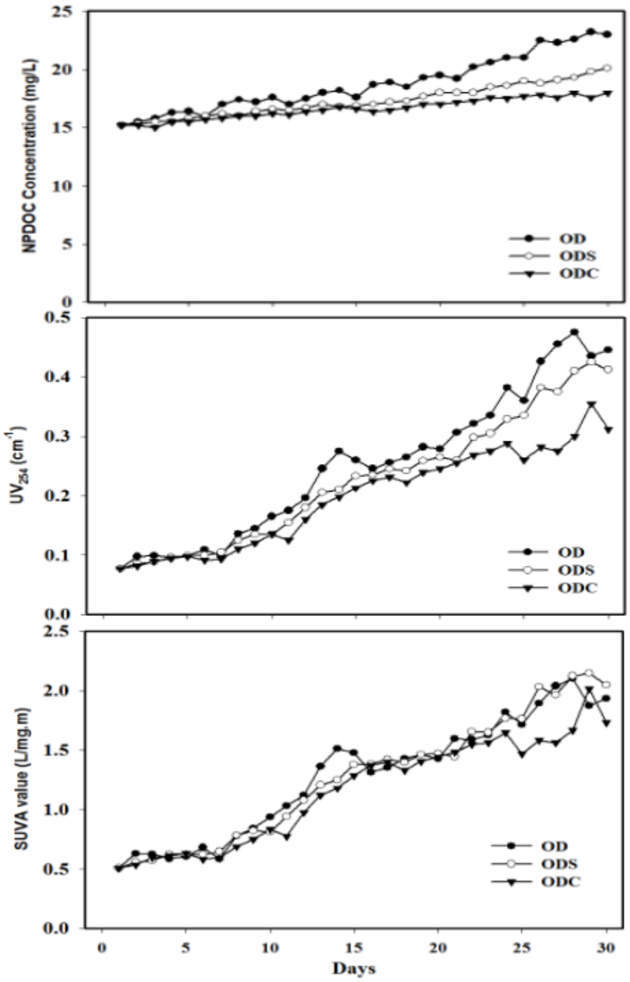

Figure 1

The concentration of dEfOM during oxidation ditch processes in term of (a) NPDOC, (b)UV254, and (c) SUVA value

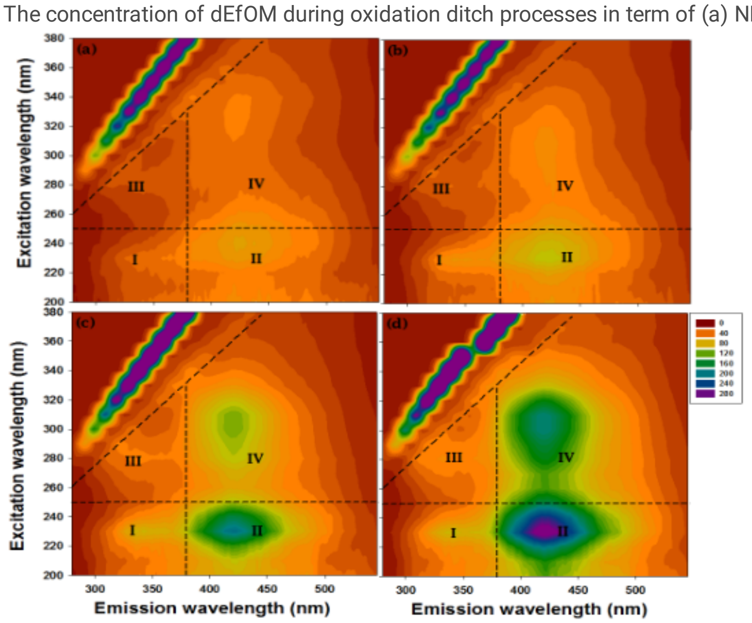

Figure 2

Fluorescence excitation-emission spectra of (a) raw wastewater (RW) and after treatment with (b) oxidation ditch only (OD), (c) oxidation ditch Chlorella vulgaris (ODC), and (d) oxidation ditch Spirulina platensis (ODS).

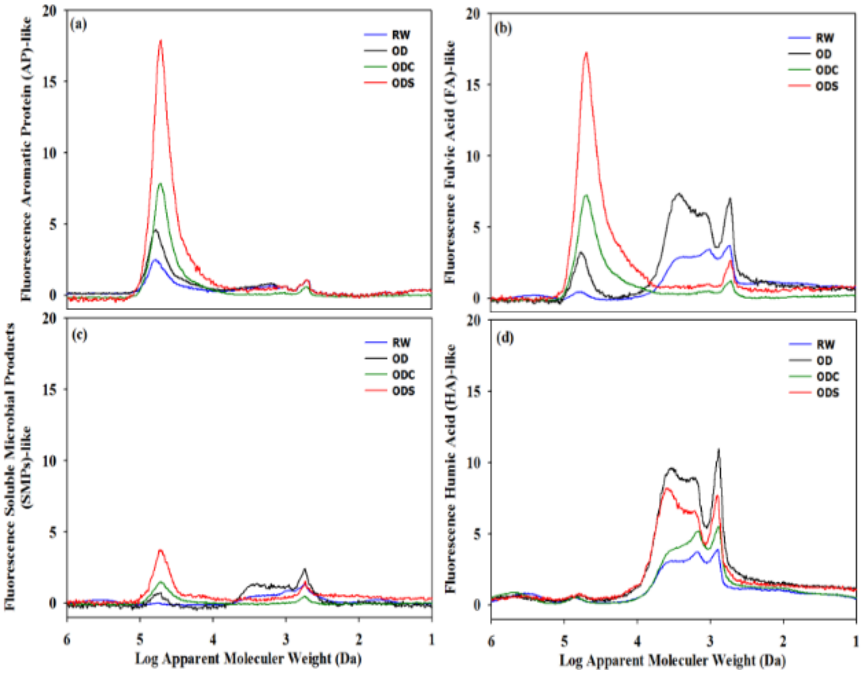

Figure 3

Fractionation of dissolved effluent organic fluorescence as (a) AP-like, (b) FA-like, (c) SMPs-like, (d) HA-like, based on average molecular weight as obtained by HPLC-FLD

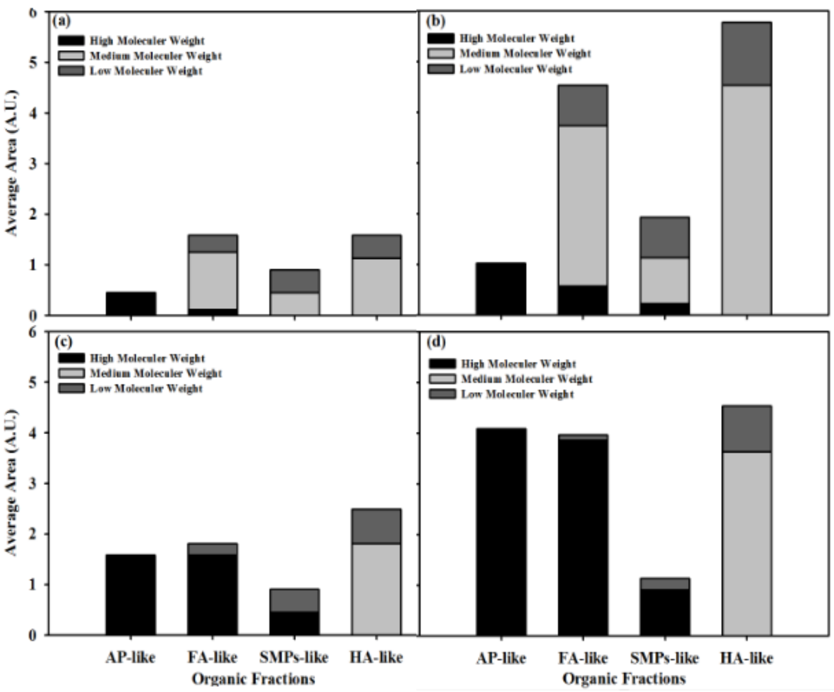

Figure 4

The peak area of fluorescence organic fractions based on its molecular weight in (a) raw wastewater and during biological processes with (b) OD, (c) ODS, and (d) ODC