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Characterization of Organic Fraction Produced by *Spirulina platensis* in Oxidation Ditch Reactor

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ABSTRACT

The biggest source of river water pollution is domestic wastewater, about 60-70% with 6.1% treatment and 40% of the pollution comes from industry or others and is exacerbated by the uncontrolled algae growth. The diversity of algal organic matter (AOM) in domestic wastewater causes several problems in water treatment. Fluorescence Excitation Emission Matrix (FEEM) has been widely used to determine the structural and compositional characteristics of molecules. The purpose of this study was to determine the characteristics of the AOM fraction produced by the microalgae *Spirulina platensis* using FEEM. The peak percentage of EEM and the Fluorescence Regional Index (FRI) was used to represent the substance contained in the sample. The results showed the microalgae *Spirulina platensis* identified as containing fraction *aromatic protein-like*, *fulvic acid-like, soluble microbial product-like*, and *humic acid-like* where the percentage of FRI of each fraction fluctuates due to internal factors such as microbial carbon sources and external factors such as temperature and oxygen supply.

Keywords: Spirulina platensis, oxidation ditch, fluorescence excitation emission matrix, fluorescence regional index

Introduction

One of the sources of surface water pollutants derived from domestic waste, besides that algal blooms, is also a contributor to polluters of surface water. The presence of microalgae produces side effects such as discoloration, odor, causing the water environment in anoxic conditions, and producing algae organic matters (AOM) that are released through extracellular active metabolism and intracellular by autolysis (Khan et al., 2019). In Pivokonsky et al.'s (2016) observation, AOM is a contaminant produced from algae metabolism, released in extracellular organic matter (EOM) in the exponential phase and intracellular organic matter (IOM) in the death phase of microalgae. And the composition of AOM is influenced by differences in types, growing stage, condition, age of microalga cultivation, and water conditions (including a concentration of nutrients and toxins). According to Villacorte et al., 2015, AOM constituents consist of several organic compounds such as polysaccharides, proteins, amino acids, nucleic acids, lipids, and other organic substances. However, polysaccharides and proteins harm wastewater treatment, which causes membrane fouling and interferes with the coagulation process (Rehman et al., 2017).

Fluorescence Excitation Emission Matrix (FEEM) is one of the techniques that can determine the characterization of AOM fractions because it can inform about the structure and composition of molecules quickly and sensitively, supported by quantitative techniques Fluorescence Regional

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Index (FRI) makes FEEM technique able to read data points from 50 to >10000, by measuring the volume of EEM region by relating the area under the selected EEM spectrum (Chen, 2003). Based on a previous study by Khan et al. (2019) using *Microcystis aeruginosa* and *Chlorella Vulgaris* stated that the more towards the decline phase, AOM is more widely produced related to the substance *humic acid-like* for *Microcystis aeruginosa* and *like-tryptophan* for *Chlorella Vulgaris*, changes in EEM fluorescence in each phase of microalgae are highly dependent on microalgae conditions and the environment including solution composition, temperature, pH, nutrients, and oxygen supply. In this study, we characterize the fraction of organic matter produced by *Spirulina platensis* in an oxic and oxic anoxic condition in an oxidation ditch reactor with FEEM analysis.

Research Method

The samples were collected from the sewer to the holding tank of Rusunawa Pejaringan Sari 2 Pandugo, Surabaya and using microalga *Spirulina platensis,* but before the main research, *Spirulina platensis* were cultured first by adding *Spirulina platensis* seeds in water that had been added with baking soda and then added with F2 Guillard nutrients, during the cultivation process to consider the intake nutrients, aeration, and sufficient lighting. After the seedlings have been successfully cultured, acclimatization is carried out to determine the survival ability of microalgae to domestic waste in a 1:1 ratio by adding 5 liters of microalgae seeds in a container then adding 5 liters of wastewater in the container periodically for 5 days, the result of acclimatization showed that the microalgae changed color on day 5 which indicated that the microalgae could not survive more than 5 days.

The experiment was conducted on an oxidation ditch reactor with batch flow type and brush aerator set at a rotation speed of 60 rpm, using a ratio of wastewater samples to microalgae 1:1 in 250 liters, as well as variations in oxic (24 hours) and oxic-anoxic conditions (7 hours in oxic condition and 3 hours in anoxic condition), samples were treated for 5 days and samples were taken on first, third, and fifth days. Before measurement, the samples were filtered through a microfiltration membrane (0.45 μm), then using fluorescence spectrophotometer F2000 Hitachi, Japan with 1-cm-path quartz cuvettes at 24 ^{0}C. Excitation and emission were scanned together at a wavelength ranging from 200-400 nm at 10 nm intervals and 250-547.5 nm at 0.5 nm intervals. Calculation of fluorescence regional index (FRI) was used to integrate the area under the selected EEM spectrum, the FRI analysis procedure described in Chen's study in 2003 (Chen, 2003).

Result and Discussion

The initial concentration test was used to determine the characterization of the initial fluorophore organic matter in domestic wastewater before conducting the research, which is shown in table 1.

Table 1. Domestic wastewater characteristics

Parameter	Test Result
pH	7.4
Temperature	27 ^{0}C
Fluorescence Excitation Emission Matrix (FEEM) (%)	
Aromatic protein-like	12.96
Fulvic protein-like	18.75
Soluble microbial product-like	38.16
Humic acid-like	30.11

Four types of fractions were identified in domestic wastewater with the wavelength of each fraction mentioned in Hidayah et al. (2020) namely area I as the *aromatic protein-like* area excitation/emission wavelength <250 / <330 nm, area II as the *fulvic acid-like* area excitation/emission wavelength 200-250 / >380 nm, area III at excitation/emission wavelength 250-280 / >380 nm is known as *soluble microbial product-like* area, and the IV at excitation/emission wavelength >280 / >380 nm is known as *humic acid-like* area.

Aromatic Protein-like Fraction

One of the by-products produced by microalgae during biological processing is a protein-like substance, with one of the main constituents of which is an amino acid containing an aromatic ring, there are four types of amino acids that contain an aromatic ring, namely histidine, phenylalanine, tyrosine, and tryptophan (Novitasari et al., 2021).

Table 2. FRI percentages of *aromatic protein-like* fraction

Sampling Time (d)	Oxic Condition (%)	Percent Increase (%)	Oxic-Anoxic Condition (%)	Percent Increase (%)
0	12.96	0.00	12.96	0.00
1	12.07	-6.87	8.40	-35.19
3	14.10	8.80	6.99	-46.06
5	7.75	-40.20	6.61	-49.00

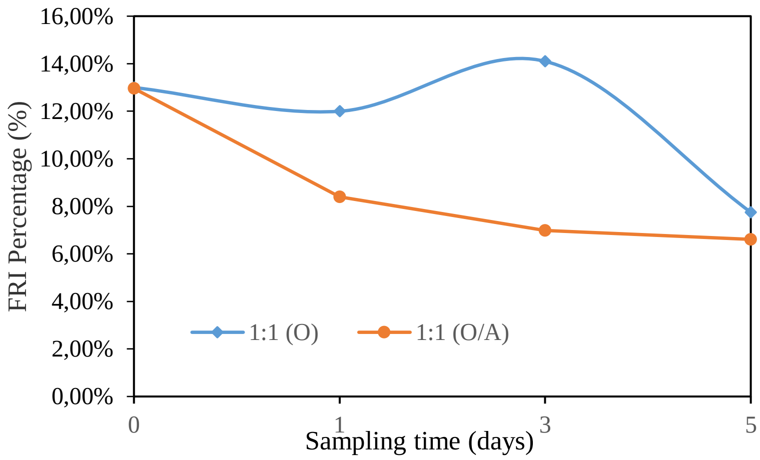

Figure 1. FRI percentages of the *Aromatic Protein-like* Fraction in Oxic (O) and Oxic-Anoxic (O/A Conditions

Figure 1 shows the decrease in FRI percentages of the *aromatic protein-like* fraction in oxic and oxic-anoxic conditions varying from day 0 to day 5. Under oxic conditions, the percentage of FRI of the *aromatic protein-like* fraction identified decreased and increased. The increase was shown on day three from 12.07% to 14.10%. This was probably due to the presence of non-biodegradable substances (Xue et al., 2017). According to Xu et al., 2010 the non-biodegradable substance related to the *aromatic protein-like* fraction is the tryptophan-like substance. While in oxic-anoxic conditions, a decrease in the percentage of FRI was identified on all sampling days. The lowest decrease of both conditions was lowest on day 5, and the lowest percentage of FRI was identified in oxic-anoxic conditions, it indicated the interaction of *aromatic protein-like* substances with bacteria in wastewater by utilizing them as an energy source during the biological treatment process (Li et al., 2020).

Fulvic acid-like fraction

The fulvic acid substance is the main component of humic substance which consists of hydrophilic molecules and has a relatively low molecular weight (Wang et al., 2020).

Table 3. FRI percentages of *fulvic acid-like* fraction

Sampling Time (d)	Oxic Condition (%)	Percent Increase (%)	Oxic-Anoxic Condition (%)	Percent Increase (%)
0	12.05	0.00	12.05	0.00
1	14.07	16.76	12.37	2.66
3	14.39	19.42	13.54	12.37
5	15.46	28.30	13.98	16.02

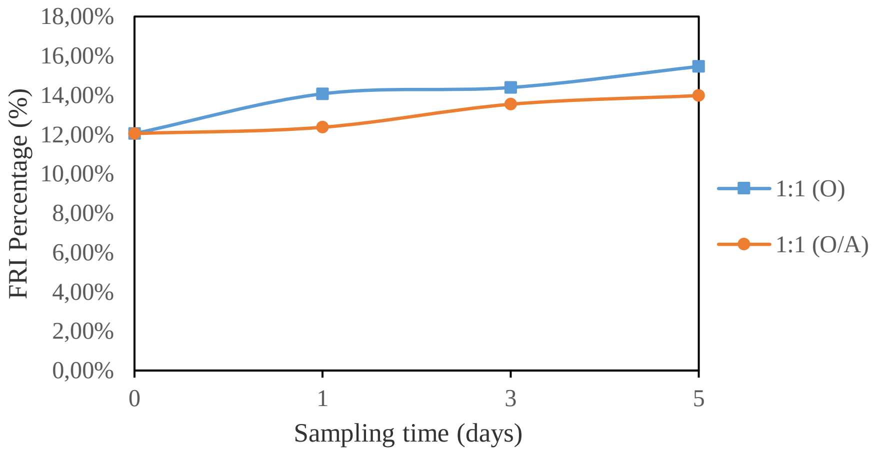

Figure 2. FRI Percentages of the *Fulvic Acid-like* Fraction in Oxic (O) and Oxic-Anoxic (O/A) Conditions

Figure 2 shows an increase in FRI percentages of the fulvic acid-like fraction in oxic and oxic-anoxic conditions, which varied from day 0 to day 5. In oxic conditions, the highest increase in FRI percentage was shown on day 5, reaching 15.46% from day 0, which was 12.05%. The increase in the percentage of FRI of the *fulvic acid-like* fraction also occurred in oxic-anoxic conditions, from 12.05% on day 0 to 13.98% on day 5. Between the two conditions, the oxic condition had the highest increase in the percentage of FRI. This indicates the highest accumulation of *fulvic acid-like* organic matter most in this condition. The increase in the percentage of FRI of the substance *fulvic acid-like* is because the substance is the result of non-biodegradable microbial biodegradation (Meng et al., 2016), which is not degraded during the biological treatment process and makes it a high contributor of organic matter in wastewater (Wang & Chen, 2018).

Soluble microbial product-like fraction

Soluble microbial product-like substances are produced from microbial metabolism, so it can be said that soluble microbial product-like is residual organic matter in wastewater. The presence of soluble microbial product-like substances can reduce the efficiency of wastewater treatment processes such as membrane fouling (Wang & Zhang, 2010).

Table 4. FRI Percentages of *Soluble Microbial Product-like* Fraction

Sampling Time (d)	Oxic Condition (%)	Percent Increase (%)	Oxic-Anoxic Condition (%)	Percent Increase (%)
0	38.16	0.00	38.16	0.00
1	29.79	-21.93	32.35	-15.23
3	36.53	-4.27	33.28	-12.79
5	29.58	-22.48	33.43	-12.40

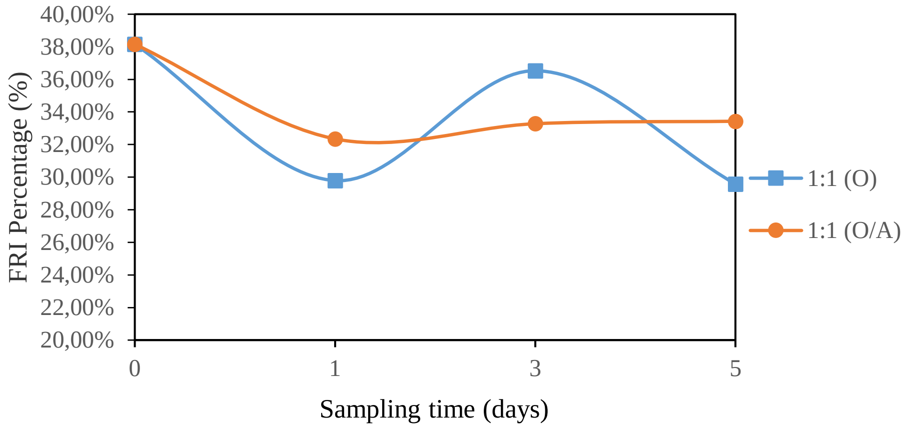

Figure 3. FRI Percentages of the *Soluble Microbial Product-like* Fraction in Oxic (O) and Oxic-Anoxic (A/O) Conditions

Figure 3 shows the decrease in the percentage of FRI of the *soluble microbial product-like* fraction in oxic and oxic-anoxic conditions. It can be seen that the *soluble microbial product-like* fraction tends to decrease as indicated by a decrease in the percentage of FRI which varies from day 0 to day 5 for both conditions. From table 4, it is known that there was a significant decrease and increase in the percentage of FRI in oxic-anoxic conditions, on day 1 reaching 29.79% to 36.53% on day 3, and decreased on day 5 to reach 29.58%. And the same thing was identified in the oxic-anoxic conditions, but the change was not too significant, 32.35% on day 1, decreased on day 3, and increased on day 5. According to Hidayah et al., 2020, the *soluble microbial product-like* fraction produced during biological processing is released by microbes as residual organic matter from the metabolic process. A decrease in the percentage of FRI of the *soluble microbial product-like* fraction indicates the presence of cell decomposition and/or lysis (Ni et al., 2010). And the increase of the *soluble microbial product-like* fraction described by Wang & Zhang (2010) was caused by microbes under stress conditions due to the unfulfilled carbon source from the *aromatic protein-like* fraction that produced directly by microalgae, and an increase in temperature also affected, which was indicated by an increase in temperature in the range of 28.0 to 29.5 at both conditions.

Humic acid-like fraction

Humic acid is one part of humic substances which is dominated by hydrophobic compounds, humic acid substances are known to be soluble in alkaline solutions (Wang et al., 2020). The humic acid-like fraction is one of the non-biodegradable fractions that are not produced by microalgae directly but produced by microbial activity in degrading AOM (Stedmon & Cory, 2014).

Table 5. FRI Percentage of *Humic Acid-like* Fraction

Sampling Time (d)	Oxic Condition (%)	Percent Increase (%)	Oxic-Anoxic Condition (%)	Percent Increase (%)
0	30.11	0.00	30.11	0.00
1	32.63	8.37	44.29	47.09
3	40.20	33.51	45.93	52.54
5	46.25	53.60	47.36	57.29

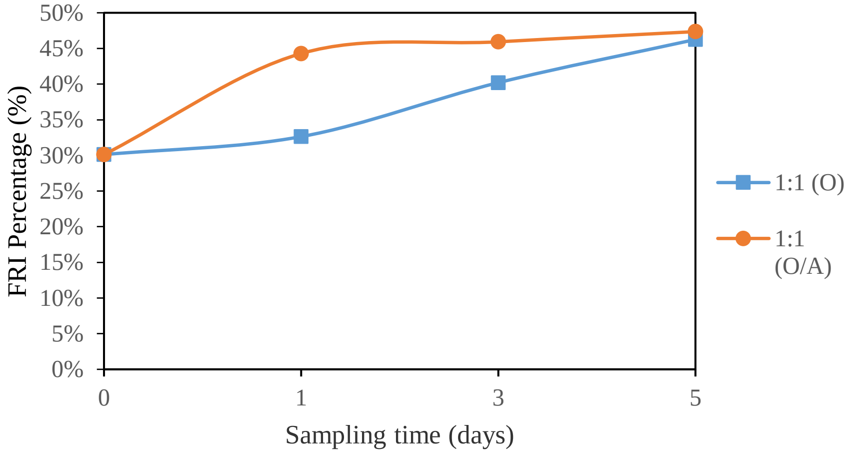

Figure 4. FRI Percentage of the *Humic acid-like* Fraction in Oxic (O) and Oxic-Anoxic (O/A) Conditions

Figure 4 shows the increase in FRI percentage of the humic acid-like fraction under oxic and oxic-anoxic conditions, which varied from day 0 to day 5. From table 5, it is known that in oxic conditions, the *humic acid-like* fraction increased, and the largest identified on day 5 is 46.25%. The same thing was also identified in the oxic-anoxic condition, and the largest was on day 5, reaching 47.36%. The highest increase in the percentage of FRI between the two conditions was identified in oxic-anoxic conditions, this indicates that the microbe as a producer of the *humic acid-like* fraction lacks nutrients (carbon sources) and or in unfavorable conditions such as lack of oxygen intake (Villacorte et al., 2015), and the highest increase was identified on day 5, according to Luo et al. (2019) the longer contact time between microalgae and bacteria in wastewater, causing the accumulation and making them a contributor of organic matter to the *humic acid-like* fraction in wastewater. And an increase in the *humic acid-like* fraction can contribute to a much higher membrane fouling compared to the *fulvic acid-like* fraction (Peiris et al., 2011).

Conclusion

Domestic wastewater of Rusunawa Penjaringan Sari 2 Pandugo, Surabaya, which was treated in an oxidation ditch reactors with the addition of microalgae *Spirulina platensis* in a 1:1 ratio, identified 4 types of fraction, that is *aromatic acid-like*, *fulvic acid-like*, *soluble microbial product-like*, and *humic acid-like*. Where each fraction increased and decreased in the percentage of FRI, this was influenced by internal factors such as the use of carbon sources by bacteria, and external factors such as temperature and supply of oxygen.

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References

Chen; B. (2003). *Fluorescence Excitation - Emission Matrix Regional Integration to Quantify Spectra for Dissolved Organic Matter*. 5701–5710.

Hidayah, E. N., Lai, W. L., Cahyonugroho, O. H., & Rizqa, F. (2020). Organic matter from biofilter nitrification by high performance size exclusion chromatography and fluorescence excitation-emission matrix. *Global Journal of Environmental Science and Management*, *6*(2), 133–144. https://doi.org/10.22034/gjesm.2020.02.01

Khan, S., Zamyadi, A., Rao, N. R. H., Li, X., Stuetz, R. M., & Henderson, R. K. (2019). Fluorescence spectroscopic characterisation of algal organic matter: Towards improved: In situ fluorometer development. *Environmental Science: Water Research and Technology*, *5*(2), 417–432. https://doi.org/10.1039/c8ew00731d

Li, G., Li, L., Song, K., Yuan, Z., Zhu, S., Zhang, J., & Xie, F. (2020). Photochemical degradation characteristics of algasourced dissolved organic matter in Chaohu Lake, China. *Water Science and Technology: Water Supply*, *20*(8), 3083–3095. https://doi.org/10.2166/ws.2020.203

Luo, Y., Henderson, R. K., & Le-Clech, P. (2019). Characterisation of organic matter in membrane photobioreactors (MPBRs) and its impact on membrane performance. *Algal Research*, *44*(September), 101682. https://doi.org/10.1016/j.algal.2019.101682

Meng, Y., Wang, Y., Han, Q., Xue, N., Sun, Y., Gao, B., & Li, Q. (2016). Trihalomethane (THM) formation from synergic disinfection of biologically treated municipal wastewater: Effect of ultraviolet (UV) irradiation and titanium dioxide photocatalysis on dissolve organic matter fractions. *Chemical Engineering Journal*, *303*, 252–260. https://doi.org/10.1016/j.cej.2016.05.141

Ni, B. J., Zeng, R. J., Fang, F., Xie, W. M., Sheng, G. P., & Yu, H. Q. (2010). Fractionating soluble microbial products in the activated sludge process. *Water Research*, *44*(7), 2292–2302. https://doi.org/10.1016/j.watres.2009.12.025

Novitasari, RTM; Anggo, AP; Agustini, T. (2021). Pengaruh kombinasi bahan pengisi maltodekstrin dan karagenan terhadap karakteristik bubuk flavor lemi dari rajungan. *Jurnal Ilmu Dan Teknologi Perikanan Volume 3 No 1*, *3*(1), 16–25.

Peiris, R. H., Budman, H., Moresoli, C., & Legge, R. L. (2011). Identification of humic acid-like and fulvic acid-like natural organic matter in river water using fluorescence spectroscopy. *Water Science and Technology*, *63*(10), 2427–2433. https://doi.org/10.2166/wst.2011.439

Pivokonsky, M., Naceradska, J., Kopecka, I., Baresova, M., Jefferson, B., Li, X., & Henderson, R. K. (2016). The impact of algogenic organic matter on water treatment plant operation and water quality: A review. *Critical Reviews in Environmental Science and Technology*, *46*(4), 291–335. https://doi.org/10.1080/10643389.2015.1087369

Rehman, Z. U., Jeong, S., Tabatabai, S., Emwas, A. H., & Leiknes, T. (2017). Advanced characterization of dissolved organic matter released by bloom-forming marine algae. *Desalination and Water Treatment*, *69*(May 2016), 1–11. https://doi.org/10.5004/dwt.2017.0444

Stedmon, C. A., & Cory, R. M. (2014). Biological Origins and Fate of Fluorescent Dissolved Organic Matter in Aquatic Environments. *Aquatic Organic Matter Fluorescence*, 278–300. https://doi.org/10.1017/cbo9781139045452.013

Villacorte, L. O., Ekowati, Y., Neu, T. R., Kleijn, J. M., Winters, H., Amy, G., Schippers, J. C., & Kennedy, M. D. (2015). Characterisation of algal organic matter produced by bloom-forming marine and freshwater algae. *Water Research*, *73*, 216–230. https://doi.org/10.1016/j.watres.2015.01.028

Wang, M., & Chen, Y. (2018). Generation and characterization of DOM in wastewater treatment processes. *Chemosphere*, *201*, 96–109. https://doi.org/10.1016/j.chemosphere.2018.02.124

Wang, Z. P., & Zhang, T. (2010). Characterization of soluble microbial products (SMP) under stressful conditions. *Water Research*, *44*(18), 5499–5509. https://doi.org/10.1016/j.watres.2010.06.067

Wang, Z., Shen, T., Yang, Y., Gao, B., Wan, Y., Li, Y. C., Yao, Y., Liu, L., Tang, Y., Xie, J., Ding, F., & Chen, J. (2020). Fulvic acid-like substance and its characteristics, an innovative waste recycling material from pulp black liquor. *Journal of Cleaner Production*, *243*(61), 118585. https://doi.org/10.1016/j.jclepro.2019.118585

Xu, H., He, P., Wang, G., & Shao, L. (2010). Three-dimensional excitation emission matrix fluorescence spectroscopy and gel-permeating chromatography to characterize extracellular polymeric substances in aerobic granulation. *Water Science and Technology*, *61*(11), 2931–2942. https://doi.org/10.2166/wst.2010.197

Xue, S., Jin, W., Zhang, Z., & Liu, H. (2017). Reductions of dissolved organic matter and disinfection by-product precursors in full-scale wastewater treatment plants in winter. *Chemosphere*, *179*, 395–404. https://doi.org/10.1016/j.chemosphere.2017.02.106