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Effect of Intermittent Aeration on Characteristic of Effluent Organic Matter in Oxidation Ditch Microalgae *Chlorella sp.*

Okik Hendriyanto Cahyonugroho✉ | Euis Nurul Hidayah | Emira Aulia Rahma

Department of Environmental Engineering, Universitas Pembangunan Nasional Veteran Jawa Timur, Post Code 60296, Surabaya, Indonesia

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ABSTRACT

Wastewater treatment with microalgae such as oxidation ditch algae reactor (ODAR) could reduce organic matter, however, the process might produce by-products that are toxic when dissolved in water. Effluent organic matter and algae organic matter are by-products of the microalgae process, that are released as well in ODAR system. The presence of these compounds in water can be a precursor for the formation of disinfection by-products (DBPs). The aim of this study is to determine the characteristics of effluent organic matter contained in domestic ODAR using the microalgae *Chlorella sp*. under variations of oxic and oxic-anoxic conditions. Novelty of this study is oxidation ditch combined with microalgae and the effect of aeration and intermittent aeration in the quality of wastewater effluent. Microalgae were applied in ODAR under oxic for 24 hours of aeration and oxic-anoxic through a brush aerator on for 7 hours and off for 3 hours with a ratio of waste volume to microalgae 1:1 with sampling time up to 5 hours. The results showed that BOD concentration tends to decrease up to 45% and 67% for oxic-anoxic and oxic, respectively. The UV254 value increased up to 110% and 147% for oxic-anoxic and oxic, respectively. Further, fluorescence excitation-emission matrix (FEEM) analysis identified the changing of four organic fractions as measured by the fluorescence regional index (FRI). The results indicate a decrease of aromatic protein-like significantly up to 62% and a decrease in soluble microbial products up to 30%. While humic acid-like and fulvic acid-like tends to increase by about 25-29% and 44-46%.

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INTRODUCTION

Culture of Chlorella pyrenoidosa by utilizing the wastewater has been done previously by other researchers which suggests that 20% of tofu wastewater media obtained the most significant results, particularly in the cell density (Arsad et al., 2020). The utilization of wastewater enhanced the growth and biomass of microalgae *Trichoderma* sp (Asiandu et al., 2023). Besides the effect on microalgae itself, the utilization of wastewater in microalgae culture was known to be the best way to reduce the organic matter effluent (Hidayah et al., 2021). The presence of microalgae in waters can be an alternative to wastewater treatment that is environmentally friendly and has economic value in degrading excessive nutrients such as nitrogen, phosphorus, and CO_2 (Ly et al., 2019). Oxidation Ditch Algae Reactor (ODAR) is a biological treatment by utilizes the role of microalgae and bacteria combination for nutrient and pathogen removal in grey water, which needed oxygen supply for aeration (Farahdiba et al., 2020; Hidayah et al., 2022). The usage of algae in wastewater treatment is beneficial

*Corresponding Author Email: *okikhc@upnjatim.ac.id*

because the process has a high growth rate, photosynthetic efficiency, and biomass productivity (Gururani et al., 2022). However, the usage of algae in water treatment process generates by-products of various organic substances that cause the changing in water characteristics such as watercolor, odor, anoxic conditions, and toxicity problems due to proliferation of microscopic algae which is significantly presented in certain seasons (Villacorte et al., 2015). Therefore, this issue is necessary to be explored since biological process is mostly used in wastewater treatment.

It has been well known that biological processes produce organic material, which is called effluent organic matter, which is generated from microbial metabolism. Effluent organic matter (EfOM) consists of various compounds such as refractory compounds, biodegradable residual substrates, intermediate substrates, organic compounds, and soluble microbial products (Ni et al., 2010). Biological processing using microalgae may contain of algae organic matter which is a by-product of biological activities such as symbiotic processes with bacteria. Algae organic matter is composed of protein (30-50% dry algal biomass), polysaccharides, amino acids, and other organic acids such as fatty acids. In addition, algae organic matter consists of more than 50% biopolymer as the main ingredient, such as proteins, and polysaccharides, while the remaining fraction consists of refractory organic substances (such as humic-like substances), low molecules of heavy acids, and neutral compounds (Villacorte et al., 2015; Hidayah et al., 2022; Cahyonugroho et al., 2023). Briefly, biological process is very useful to remove organic compound, however the existence of biotic component will release metabolic compound, which is categorize as organics. Those metabolite compound will contribute the quantity and quality of organic matter in water.

One quantitative and qualitative method to characterize organic matter's fraction is fluorescence spectroscopy. The usage of fluorescence excitation-emission matrix (FEEM) in the decomposition of organic matter can show good characterization of dissolved organic compounds. In addition, fluorescent method could be applied for detecting toxic diazinon in water under a specific procedure (Jamalipour et al., 2022). FEEM analysis test aims to determine the EfOM fraction so that it can quickly and sensitively inform the structure and composition of the molecule by using the Fluorescence Regional Integration (FRI) technique (Chen et al., 2003; Hidayah et al., 2017). The FRI technique is used to measure the volume of the excitation-emission matrix region by linking the area under the selected excitation-emission matrix spectrum and being able to read 50 to >10,000 data points. Two compounds that can be identified by FEEM in wastewater are biogenic substances or derived from microbes and humic-like substances. The most abundant biogenic substances found in water are protein-like substances and peptides containing aromatics, which are mainly tryptophan and tyrosine, the main peak λexcitation/λemission (λex/λem): a peak such as tryptophan T1, a peak such as tryptophan T2, a peak such as tyrosine B1 and a peak such as tyrosine B2 (Chen et al., 2003). However, it turned out that neither carbohydrates nor lipids, which are common constituents of waste, could be detected by FEEM (Khan et al., 2019). This study aims to determine the characteristics of effluent organic matter contained in domestic wastewater treatment and to know the effect of oxic and oxic-anoxic conditions, which was applied in an oxidation ditch with microalgae *Chlorella sp*., to the changing of characteristic effluent organic matter. Novelty of this study is oxidation ditch combined with microalgae and the effect of aeration and intermittent aeration in the quality of wastewater effluent. The results of this study could be used as references for wastewater treatment plants, especially for biological process, that combination of aeration with micro biotic could be effective or less effective in removing organic compound.

MATERIALS & METHODS

Microalgae *Chlorella sp*. was cultured and acclimatized in the media for 5 days until it met the required amount for further experiment. Acclimatization was carried out for 5 days after the density was suitable for the experiment by using domestic wastewater to determine the ability of *Chlorella sp*. to adapt to the new environment (Farahdiba et al., 2020). Domestic wastewater was taken from the urban living area which was derived from human activities. Collected domestic wastewater as raw samples were kept in the 500 L storage tank.

Samples were analyzed before treatment in order to know the initial concentration, and the treated samples were analyzed as well to know the parameter concentration after treatment. The parameters used in this study were the results of the functional group, the effluent organic matter fraction, organic concentration as Biological Oxygen Demand (BOD) parameter, and ultraviolet at 254 nm wavelength (UV_{254}). Further, organic the fraction was obtained by filtering the sample using a fluorescence spectrophotometer F2000 Hitachi, Japan with a 1-cm-path cuvette at 24°C. The excitation and emission were scanned simultaneously at wavelengths ranging from 200-400 nm at 10 nm intervals and a wavelength of 250-547.5 nm at 0.5 nm intervals (Hidayah et al., 2017; Hidayah et al., 2022). The study was conducted for 5 days under various conditions of oxic with brush aerator on for 24 hours and oxic-anoxic with brush aerator on for 7 hours and off for 3 hours, with a ratio of waste volume to microalgae 1:1 (Farahdiba et al., 2020).

FEEM spectra is presented into a contour plot, which shows the fluorescence intensity of various peak, through Sigma Plot. One sample will present in one figure, therefore the output of FEEM contour plot resulted amount of number sample. Fluorescence Regional Index (FRI) was applied in order to represent qualitative information of contour plot (Chen et al., 2003), further percentage decreasing or increasing of FRI value could be calculated. Percentage removal of BOD and UV_{254} were calculated according to initial sample concentration to treated sample concentration. All the data presents in the figure in order to discuss trending data and its changing concentration during treatment process.

RESULTS AND DISCUSSIONS

An initial parameter test was conducted to determine the concentration of organic matter content in the wastewater. The results of the initial parameter tests are shown in Table 1. FEEM is used to determine the characteristics of the organic matter fraction based on the selected excitation-emission matrix spectrum. In the initial test, the FEEM spectrum test in domestic wastewater is shown in Figure 1. The results of the FEEM analysis detected 4 regions such as organic matter in region I is an aromatic protein-like fraction with excitation (Ex)/Emission (Em) <250/<380 nm), region II is fulvic acid-like with Ex/Em 200-250/ >380 nm, region III is soluble microbial product-like with Ex/Em 250-280/>380 nm, and region IV is humic acid-like with Ex/Em>280/>380 nm (Chen et al., 2003; Hidayah et al., 2017). By using the fluorescence regional index (FRI), the percentage area of fluorescence EEM spectrum is 12.96% in the

Table 1. Initial characteristics of domestic waste

Parameter	Value
pH	7.4
Temperature (°C)	27
Fluorescence Excitation Emission Matrix (FEEM) (%)	
- aAromatic protein-like	12.96
- Fulvic acid-like	12.05
- Soluble microbial product-like	38.16
- Humic acid-like	36.83
Biochemical Oxygen Demand (BOD) (mg/L)	64.00
Ultraviolet 254 (UV_{254}) (cm^{-1})	0.4355

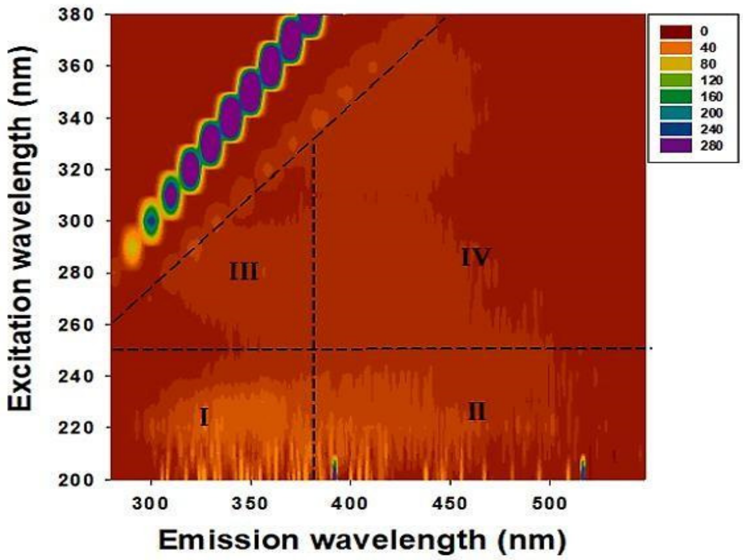

Fig. 1. Preliminary test of Fluorescence EEM spectrum organic matter fraction of domestic wastewater

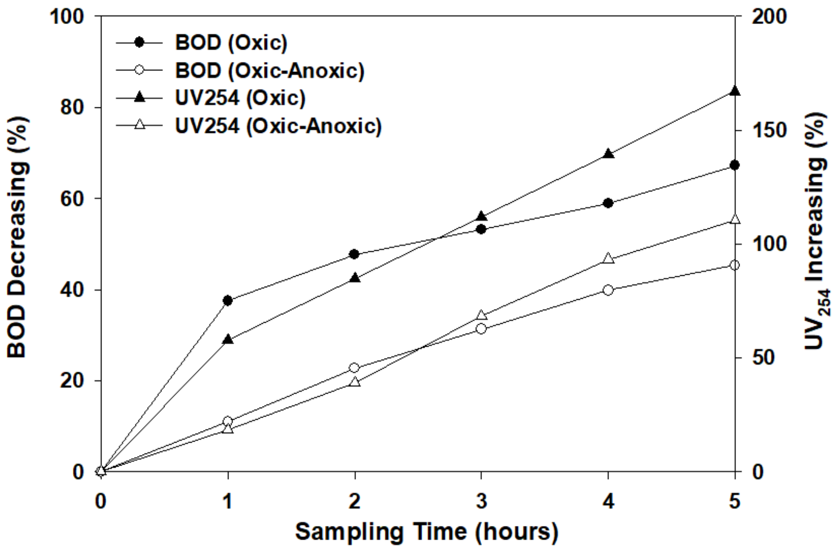

Fig. 2. The changing of BOD concentration and UV_{254} value under different system of oxidation ditch algae reactor in oxic and oxic-anoxic condition

aromatic protein-like fraction, 12.05% in the fulvic acid-like fraction, 38.16% in the soluble microbial product-like fraction, and 30.11% of humic acid-like fraction.

The BOD test was carried out to determine the amount of dissolved oxygen needed by microorganisms to decompose organic matter in the waters. The high BOD content indicates the minimal amount of dissolved oxygen in the water. The lack of oxygen affects the organisms that play a role in degrading organic matter.

The increase in organic matter in the water results in an increase in the concentration of BOD (Edzwald and Tobiason, 2011). The BOD concentration and UV_{254} values under various sampling times at oxic and oxic-anoxic conditions were obtained as shown in Figure 2. In this study, BOD concentration clearly shows that oxic condition performed a higher BOD reduction than oxic-anoxic. The difference value between those two aeration conditions was about 25%

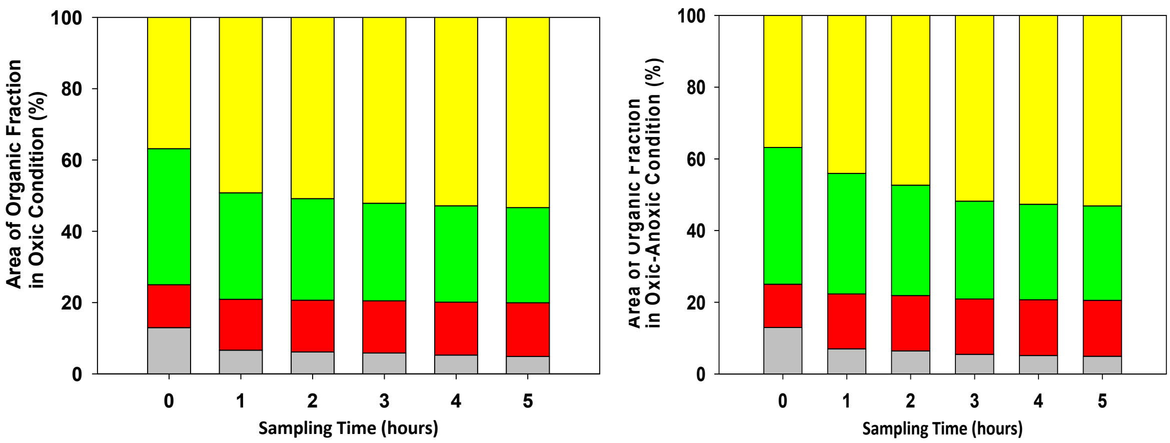

Fig. 3. Fluorescence spectrum in terms of percentage area of each region based on FRI analysis at oxic condition (a) and oxic-anoxic (b) condition of oxidation ditch algae reactor

due to 45% compared with 67% of BOD reduction. It was probably due to the oxic-anoxic condition having less dissolved oxygen because of the intermittent aeration processes (Hidayah et al., 2022). Previous studies have found that intermittent aeration will affect the characteristics of biomass, which tends to increase the sludge volume index and filamentous index value (Singh et al., 2018).

In addition, other supporting analytical data used is (UV_{254}) analysis, which can read the quantity of dissolved organic carbon that is aromatic or carbon that has C double bonds (C=C) (Xing, 2012). It shows the UV_{254} value under oxic and oxic-anoxic conditions over sampling time. The results indicate that both aeration processes caused increasing UV_{254} value, which means the oxic condition contributed to a higher UV_{254} value than the oxic-anoxic condition. In addition, according to Figure 2 and Figure 3, it is obviously shown that the optimum condition in the removal of oxidation ditch algae reactor is 5 hours. The increasing processing time could be occurred in the following time, although the efficiency removal might be slightly increased.

Aeration provides oxygen to bacteria for treating and stabilizing the wastewater, because bacteria need supplied oxygen, which is utilized by bacteria in wastewater. Therefore, bacteria growth and decay will release metabolite compounds which contain of soluble microbial products, extra polymeric substances. Those metabolite compounds consist of dissolved organic matter, which is presented in terms of UV_{254} as aromatic organic matter (Arsad et al., 2020; Hidayah et al., 2021; Cahyonugroho et al., 2022).

According to Figure 1, domestic wastewater contained of 4 regions with percentage area as represented at 0 as an initial sampling time. The FRI percentage area of each region has been calculated under various sampling times in the oxic and in the oxic-anoxic condition, as shown in Figure 3. In addition, according Figure 2 and Figure 3 then it can be resumed that Figure 2 describes a significant reduction of BOD and increasing aromatic organic as indicated by UV_{254} parameter in ODAR process. It has been reported well that microorganism in biological process will uptake organic, in term of BOD, which is existed in wastewater as their carbon sources (Xue et al., 2017). Those microorganism presented as suspended growth in the aeration tank (Hidayah et al., 2020). Microorganism experiences growing and decay phase, and some metabolite products will be released during their growth and death phase. Their metabolite products are known as soluble microbial products, which contains of protein, amino acids, biopolymers, and many more organic compounds (Ni et al., 2010; Li et al., 2020). These metabolite products is mostly identified as aromatic organic compounds, which could be detected by UV visible or known as UV_{254} parameter (Hidayah et al., 2017).

Further, Figure 3 described that both conditions performed a similar percentage of removal of dissolved organic matter as aromatic protein-like and soluble microbial product fractions. The aromatic protein-like decreased significantly by about 62%, a higher removal of organic matter than 30% removal of the soluble microbial products. Soluble microbial products in domestic wastewater are mainly produced from metabolite microorganism activities, which mostly contain different macromolecules such as carbohydrates, proteins, and nucleic acids and high amounts of aromatic structures, and unsaturated fatty chains with various kinds of functional groups of proteins (Ni et al., 2010; Li et al., 2020). Therefore, microalgae could utilize those kinds of organic matter as a substrate, which caused decreasing soluble microbial products and aromatic protein-like.

The main protein-like components consist of amino acids with fluorophores (such as tryptophan, and tyrosine), peptides, and proteins (Li et al., 2020). The study conducted by Meng et al. (2016) explained that an increase in the absorbance value of UV_{254} indicates a low value of the aromatic fraction. The decrease in FRI percentage indicated that there was a decrease in the concentration of the aromatic protein-like fraction and could indicate an interaction between the aromatic protein-like substance and the microbes present in the wastewater by utilizing protein-like components as source energy during the biological processing process (Li et al., 2020). Based on Xue et al. (2017), the increase in the substance also indicates the possibility of non-biodegradable substances, one of these substances is tryptophan-like. Soluble microbial products are produced from metabolic processes released by microbes so it can be considered that soluble microbial products are residual organic matter in wastewater. The main soluble microbial product components associated with disinfection byproduct and membrane fouling were identified as low molecular-weight hydrophobic acids and high molecular-weight hydrophilic polysaccharides (Shon et al., 2006; Ni et al., 2010). According to Ni et al. (2010), a decrease in the percentage of FRI of the soluble microbial product-like fraction indicated that there was cell decomposition or cells undergoing lysis. Meanwhile, the increase in the soluble microbial product-like fraction was caused by stressed microbial conditions because the carbon source from the aromatic protein-like fraction produced directly by microalgae was not met and the ambient temperature increased, thereby causing an increase in the production of the soluble microbial product-like fraction (Villacorte et al., 2015).

Secondly, humic substance-like compounds, such as fulvic acid-like and humic acid-like, shows increasing concentration of dissolved organic matter. Oxic-anoxic resulted in a slightly higher increasing concentration than oxic condition, as shown by about a 29% increase of fulvic acid-like and 46% increase of humic acid-like. Thirdly, increasing humic substance-like, which is presented as humic acid-like and fulvic acid-like, is comparable with increasing UV_{254} value. It means that biological oxidation ditch with algae generates more humic substances-like than microbial byproducts. Humic acid-like and fulvic acid-like compounds have been identified in effluent from biological wastewater treatment plants, in terms of aromatic double bonds, due to microbial activities during their metabolism and their decay (Ni e t al., 2010). Fulvic acid is the main component in humic substances, the amount can reach 81-95% of the total humic substances (Edzwald and Tobiason, 2011). According to Xue et al. (2017), this fulvic acid-like substance is included in the non-biodegradable fraction. The increase in the percentage of FRI humic acid-like and fulvic acid-like indicates that microalgae have sufficient oxygen intake that microalgae can produce a lot of fulvic acid-like fraction organic matter, which is one of the non-biodegradable organic materials resulting from microbial activity in degrading algogenic dissolved organic matter (AOM) (Xue et al., 2017; Khan et al., 2019). This is supported by a decrease in BOD concentration in both conditions, which indicates an increase in dissolved oxygen in the water. The increase that occurred on the 5th day showed that during the biological treatment process, the fulvic acid-like fraction was not degraded and accumulated so it became the cause of high organic matter in wastewater (Li et al., 2020). Humic-like materials are non-biodegradable during biological processing. Humic acid-like is one part of the humic substance in water. The amount of humic acid-like in the water is relatively smaller than fulvic acid-like because the low carboxyl content in humic acid-like reduces its solubility (Peiris et al., 2011). According to Luo et al. (2019), the high increase in the humic acid-like fraction could be caused by the contact time of microbes with microalgae. The longer the contact time between microbes and microalgae in wastewater, the more humic acid-like fraction produced and accumulated to be the highest contributor of humic acid-like organic matter. The increase in the humic acid-like fraction can contribute more to membrane fouling than the fulvic acid-like fraction (Shon et al., 2006). Nevertheless, humic acid could be removed by granular activated carbon through adsorption process, which is most widely used process in advanced treatment of water and wastewater treatment processes (Nazar et al., 2022).

CONCLUSIONS

According to This study concluded that domestic wastewater treatment using oxidation ditch reactor using microalgae *Chlorella* sp. with oxic and oxic-anoxic condition could remove organic pollutant in terms of BOD parameter up to 45% and 67% for oxic-anoxic and oxic, respectively. However, the usage of microalgae *Chlorella* sp. could increase aromatic dissolved organic matter up to 110% and 147% for oxic-anoxic and oxic, respectively. It can be concluded that organic matter could be reduced in less percentage removal by intermittent aeration in term of oxic-anoxic reactor than non-intermittent aeration in term of oxic reactor. The advantages of both systems were fit and appropriate to be applied with microalgae *Chlorella* sp. FEEM analysis had identified four fractions of dissolved effluent organic matter containing in the domestic wastewater, namely: aromatic protein-like, fulvic acid-like, soluble microbial products, and humic acid-like. FRI method could perform the changing of quantity of each fraction, that is a decreasing of aromatic protein-like significantly up to 62% and soluble microbial products up to 30%, while humic acid-like and fulvic acid-like tends to increase by about 25-29% and 44-46%. Future work of this study is using high performance size exclusion chromatography for characterizing dissolved organic carbon that could be released through metabolism of organism. In addition, another potential microalgae could be applied and be compared its effectiveness in removing organic pollutant.

ABBREVIATIONS

ODAR : oxidation ditch algae reactor
DBPs : disinfection by-products
FEEM : fluorescence excitation-emission matrixfor
FRI : fluorescence regional index
EfOM : Effluent organic matter
λex/λem : λexcitation/λemission
BOD : Biological Oxygen Demand
UV_{254} : ultraviolet at 254 nm wavelength
Ex/Em : excitation/emission
AOM : algogenic dissolved organic matter

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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