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RESEARCH NOTE

Phytoremediation of Palm Oil Mill Effluent Using *Pistia Stratiotes* Plant and Algae *Spirulina sp* for Biomass Production

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ABSTRACT

Producing crude palm oil (CPO) generates significant amount of palm oil mill effluent (POME). Besides high COD /BOD contents, POME contains high amount of nutrients (nitrogen, phosphor and mineral). Traditional treatment of POME using facultative anaerobic method do not reduce COD and BOD into allowable limit. The objective of this research was to utilize two stage phytoremediation (water lettuce and algae) to reduce COD, and nutrients in POME with variables of retention times and activated sludge concentration. The residence time was varied from 1-5 days and activated sludge was in the range of 35-60% vol. The result showed that water lettuce as aquatic plant in the first remediation process could reduce the COD content up to 39.1-59.66%, absorb nitrogen and phosphorous contents up to 17.73 -30.78%, and 6.14 -18.46%, respectively. At the second remediation process using algae *Spirulina*, about 90% of nutrients could be absorbed.

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1. INTRODUCTION

Wastewater is still considered as a problematic issue for industries and therefore innovative treatments of industrial wastewater is still a primary concern for some countries. Most of industries use chemical processes in order to avoid environmental degradation and subsequent threats to human health. However, utilization of such facilities has disadvantages, particularly when considering economical aspects [1].

Consequently, there has been a great deal of interest in the development of alternative methods of treatment that adopt natural processes occurring in the ecosystem. One of these alternatives is the use of remediation method. Bioremediation is process to degrade a contamination in solid or aquatic medium. Polluted soil and ground waters may be degraded by environmental constrains known as dissolved oxygen, pH, temperature, hazardous compounds, oxidation and reduction potential and availability of inorganic nutrients[2]

Use of aquatic or semi-aquatic plants in the actions of technology-based wastewater treatment plants by removing unwanted nutrient such as nitrogen and phosphor as well as chemical oxygen demand (COD) and biological oxygen demand (BOD) is still promising. This method, which is called phytoremediation, is still efficient for removing nutrients in the wastewater in spite of its longer processing time and difficulty to find source of aquatic plants [3, 4]. Another possibility to reduce COD is by converting the POME into biogas which result in 85% COD removal efficiencies [5].

Phytoremediation is an energy efficient and an emerging technology that is facilitated by aquatic plants and their associated micro biota such as microalgae to remove environmental contaminants with low to moderate level of contaminations [6]. Although the natural remediation is practically slow, however from economical point of view this method is still highly

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feasible [7]. The main principle of phytoremediation is the ability of aquatic plants to transform molecules and make them available to the biota, thus contributing to the self-purification of the polluted environment [8]. Removal of nutrients like N and P using aquatic plant has been demonstrated by several reserachers to remediate euthrophic water [9, 10].

The plants are selected for their toleranc to soils, climatic conditions, and seasonal cycles of wet and drought. Floating aquatic plants, in particular, have been reported to achieve very high nutrient removal efficiencies due to their high nutrient uptake capacity, fast growth rates and high biomass productivity [11].

Aquatic plants such as *Eichhornia crassipes* also known as water hyacinth and apu-apu (*Pistia stratiotes*) are common native plant in Indonesia that naturally grow in the lake, have fast growth rate and colonize the surrounding marshy land areas (Figure 1).

Apu apu (*Pistia stratiotes*) plant size is smaller than *Eichhornia crassipes* and its leaves are wide so can cover a larger absorption area of wastes. This leads to advantages that apu-apu plant can be utilized for waste remediation. However, most of phytoremediation studies use single stage process where the remediation is only performed using aquatic plant and the remediated effluent is directly discharged to environment.

Nevertheless, the remediated effluent eventually still consists of high amount of nutrients such as nitrogen or phosphorous which may also be important for photosynthetic reaction of algae. Therefore, extended remediation is required in order to utilize the remediated waste for cultivation of algae. Phytoremediation using microalgae has been demonstrated in the work of Promya et al. [12] to treat kitchen waste, where COD/BOD successfully reduced and nitrogen contents reduced up to 90%. The advantages of using microalgae are generation of various products that can be extracted from their biomass.

Palm oil is one of industrial corps in Indonesia and contributes to the economical growth due to its high production (about 28.7 million ton). About 1 ton of fresh fruit bunch (FFB) in palm oil industry contains only 20-23% crude palm oil (CPO), and the rest is considered as waste which may be potential source of pollution for human and environment, due to its high COD and BOD contents of 50,000 ppm and 25,000 ppm, respectively [12].

In addition, the waste called palm oil mill effluent (POME), also has high nutrients (i.e. N, P, K, Mg, and Ca) which may be utilized for corps and medium for microalgae growth although it has been treated using facultative anaerobic [13-15]. The objective of this research was to use aquatic plants and microalgae for phytoremediation of POME. Two stages of phytoremediation were proposed to treat palm oil waste as well as to gain high algae biomass.

Figure 1. Natural aquatic plants grown in Indonesia: (a) Apu-Apu and (b) Enceng Gondok

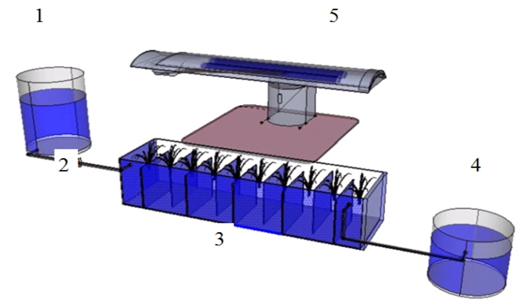

Figure 2. Phytoremediation unit: (1) POME tank, (2) valve, (3) pond/baffle reactor, (4) effluent tank for *Spirulina* remediation and (5) light source.

2. MATERIALS AND METHOD

2. 1. Preparation of POME Palm oil mill effluents (POME) was collected from PTPN VII Unit Bekri Lampung, Indonesia as effluent of 4^{th} anaerobic pond. POME was analyzed to determine COD, BOD, phosphorus and nitrogen content before being used as medium for phytoremediation. The POME has 1400 mg/L of COD, 760 mg/L of BOD, 420 mg/L of total nitrogen and 89 mg/L of phosphorous.

2. 2. First Stage of Phytoremediation The first stage of POME phytoremediation used an aquatic plant, water lettuce (*Pistia stratiotes*), at various retention times of 1-5 days combined with use of facultative anaerobes in the ratio (activated sludge:POME) of 35, 50, and 65%. Phytoremediation pond was designed for 21 liters (dimension 75cm x 20cm x 14cm) equipped with baffles and the plant was exposed to incident light intensity of 2 x 15 Watt. Water lettuce was planted at water surface with area of 75 x 20 cm^2. The treated effluent of POME from the first stage was analyzed daily to determine the COD, nitrogen, and phosphorus contents before being transferred to second stage of algae *Spirulina* remediation (Figure 2).

2. 3. Second Stage of Phytoremediation The 2^{nd} stage of the phytoremediation used microalgae *Spirulina sp.* Cultivation of *Spirulina* was carried out for 15 days and every two days synthetic nutrients were added to the culture with concentration of 50 ppm $NaHCO_3$, 40 ppm urea, and 0.5 ppm $FeCl_3$. Light intensity was maintained constant using 2 x 15 Watt lamp and the culture was continuously aerated at flowrate of 1 cm^3/h. The POME was measured for its nitrogen contents by using macro Kjedahl method, and a close reflux was used to measure COD and BOD content [16, 17]. The algae biomass was measured using spectrophotometer for its optical density (OD) at 680 nm.

2. 4. Nutrients Analysis For nutrient analysis, samples were filtered using a glass microfiber filter (Whatman, USA). Then the filtrates were appropriately diluted and analyzed for COD and BOD according to the Hach DR 2700 spectrophotometer manual (Hach Company, USA). Total nitrogen (TN) and total phosphorus (TP) were determined colorimetrically as nitrate and phosphate after the samples had been oxidized.

3. RESULT AND DISCUSSION

3. 1. Influences of Activated Sludge Concentration on COD Reduction in POME The COD content decreased by increasing active sludge concentration (Figure 3). The activated sludge with 35% volume could obtain 39-60% reduction of COD within 2-6 days of retention time. At retention time of 5 days, increasing volume of activated sludge up to 65% could obtain 68% COD reduction.

Figure 3 also concluded that the reduction of COD content is a function of concentration of active sludge and residence time. Anaerobic bacteria was a paramount parameter in activated sludge to digest organic content in the POME. Activated sludge also contains two environmental zones, aerobic and anaerobic zone. Anaerobic microorganisms grow through anaerobic respiration, without oxygen and nitrate, and are able to digest complex organic source into simple organic matter. This simple organic matter is then used as source of nutrient for metabolism.

Aerobic organism grows in the presence of oxygen where the oxygen is provided by water lettuce, as an electron acceptor, to oxidize organic matter into stable molecules (i.e. CO_2, nitrite, and phosphate). Water lettuce also stabilizes cycle of aerobic and anaerobic zones by absorbing organic molecules produced by aerobic microorganism as nutrient source in photosynthesis reaction. Oxygen produced from photosynthetic reaction is absorbed for aerobic microorganism. A cycle between anaerobic microorganism, aerobic microorganism, and water lettuce influences COD content and it tends to decrease [19].

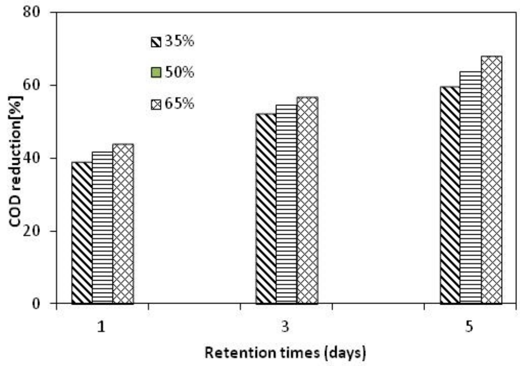

Figure 3. COD reduction efficiencies at various activated sludge volume and residence time

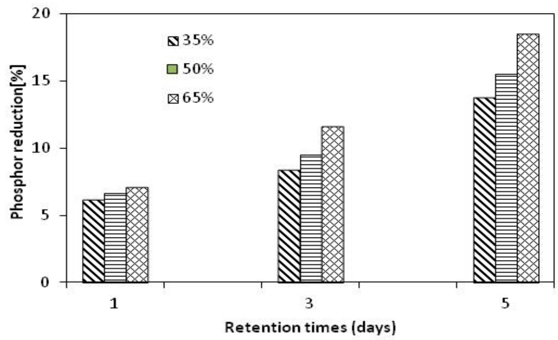

Figure 4. Phosphorus reduction efficiencies as function of resident time and amount of activated sludge in reactor

Retention time is playing important role in reducing COD contents. Digesting process of complex organic matter by microorganism in longer retention time produces more organic molecules. Longer retention time also gives a chance for aerobic bacteria to perform degradation. A cycle between anaerobic and aerobic microorganism in the activated sludge leads to increase of adsorption by water lettuce; then it will lower COD content in the POME [18]. Dipu et al. [16] showed that retention time had influences in lowering COD content in dairy wastewater treatment using different kinds of water plant within 5 to 15 days.

3. 2. Phosphorus Reduction Phosphorus is also the component available in POME waste which is important nutrient for plant and microorganism for their photosynthesis. Phosphorous helps for saving, transfer energy, and biochemistry reaction in photosynthetic reaction and its role cannot be replaced by other nutrients. Phosphorus also plays important role in ATP formation, nucleic acid, and coenzyme production [19].

Figure 4 shows that longer retention time lead to decrease the phosphorous content in the waste up to 18% within 6 days for various activated sludge contents. This was also supported by Dipu et al. [16] who showed that water lettuce plant reduced phosphorus up to 69.3%

in tofu wastewater within 20 days of retention time. Longer retention time and increasing sludge ratio give better cycle contact for water lettuce and organic bacteria in sludge, which leads to high absorption of phosphorus in POME. Phosphorus is then degraded to phosphate molecule (PO^{4-}) by bacteria, and is absorbed by water lettuce plant for photosynthetic reaction.

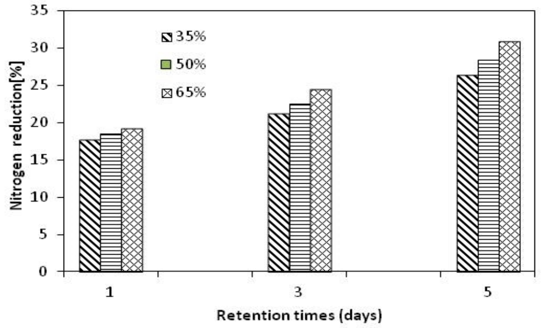

Figure 5. Phosphorus reduction efficiencies as function of resident time and amount of activated sludge in reactor

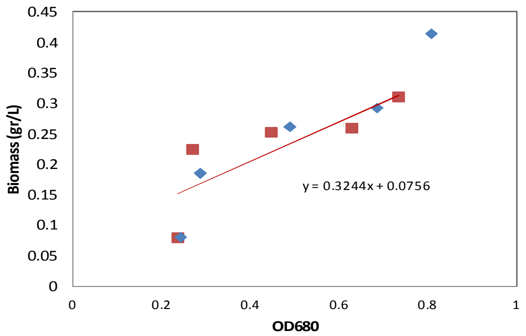

Figure 6. The correlation between optical density (OD) at 680 nm and biomass (protein) of *Spirulina* cultivated in POME medium

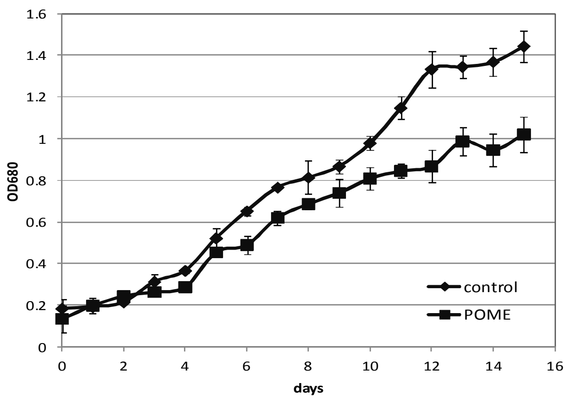

Figure 7. Algae *Spirulina* grown in control and remediated POME medium

3. 3. Nitrogen Reduction Nitrogen is an important macronutrient source, required for water lettuce and bacteria more than phosphorus. Nitrogen is able to be synthesized to amino acid, nucleic acid, fat, and other molecules. Nitrogen in form of N-organic and ammonia (NH_3) is converted to nitrite molecules by bacteria. The formed nitrite is utilized by water lettuce for photosynthetic reaction. Our findings indicated that reduction of nitrogen in 2 days retention time under 35 to 65% of sludge ratio were 17.73- 19.13% reduction (Figure 5).

3. 4. Utilization of POME Effluent as Microalgae Medium (2nd phytoremediation) Water lettuce plant has wide leaves, and can grow up to 14 cm. This plant also has a fiber root attached to leaves and sink in to water. This fiber root could inhibit aerobic organism in sludge and inhibit diffusion of oxygen in water. Bacterial organism in sludge tend to destroy water lettuce's root to absorb oxygen. However water lettuce could not absorb more nutrients in water, and slowly dies.

The COD and nutrients in the effluent of first stage (remediated by water lettuce plant) decreased to 50-70%, and this was fed to algae tank. Since the nutrient contents (nitrogen and phosphorus) were still high at 15 ppm and 1.1 ppm, respectively, then the waste was potential for microalgae medium. Based on photosynthetic reaction, algae can grow under carbon: nitrogen: and phosphorus (C: N: P) weight ratios of 56:8.6: 1.2 [20], and to supply the carbon source, we provided additional carbon using $NaHCO_3$.

The algae *Spirulina* sp was cultivated in control medium (fresh water with $NaHCO_3$: 50 ppm, urea : 40 ppm, $FeCl_3$: 0.5 ppm) and in remediated POME. The correlation between optical density at 680 nm and biomass is presented in Figure 6. The growth of algae in control and POME medium is shown in Figure 7. As compared to the control medium, the growth of algae in remediated POME is lower in achieving biomass. This is probably due to amount of excess substrate or nutrient derived from POME and synthetic nutrients can inhibit the growth of *Spirulina*. Bacteria from activated sludge were still present in the waste which may inhibit the growth of algae [21]. Figure 7 provides growth rate constant (μ) of 0.25/day for control variable and 0.19 /day for POME medium. The final COD, nitrogen and phosphor contents from second stage was 75, 1 and 0.4 mg/L, respectively.

4. CONCLUSIONS

The pretreatment of palm oil mill effluent using two sequential phytoremediation (water lettuce plant and algae *Spirulina* sp) was conducted. The content of COD, nitrogen and phosphorus in raw POME decreased by increasing the retention time (up to 6 days) and activated sludge ratio (35- 65 vol%) using water lettuce plant as the first and *Spirulina* as the second remediation. The first stage of remediation could reduce COD up to 70%, while nutrient decreased up to 30%. The second stage could reduce COD up to 50% and nutrient was lowered up to 40% by microalgae through photosynthetic reaction.

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چکیده

تولید روغن نخل خام (CPO) مقدار قابل توجهی پساب روغن نخل (POME) تولید می کند. علاوه بر COD / BOD بالا، POME حاوی مقدار زیادی مواد مغذی (نیتروژن، فسفر و مواد معدنی) است. روش سنتی تصفیه POME با استفاده از روش بی هوازی میزان COD و BOD را به حد مجاز کاهش نمی دهد. هدف از این پژوهش، استفاده از تکنولوژی phytoremediationدو مرحله ای (کاهوی آبی و جلبک) برای کاهش COD و مواد مغذی در POME در زمان های مختلف نگهداری و غلظت لجن فعال است. مدت زمان ماند از ۱–۵ روز و غلظت لجن فعال از ۳۵-۶۰٪. حجمی تغییر داده شد. نتایج نشان داد که کاهوی آبی به عنوان گیاه آبزی در اولین فرایند پاکسازی توانست محتوایCOD را تا ۳۹/۱– ۵۹/٦٦٪ کاهش دهد و جذب نیتروژن و جذب فسفر به ترتیب ۱۷/۷۳–۳۰/۷۸٪ و ٦/١٤–١٨/٤٦٪ بود. در فرایند پاکسازی دوم با استفاده از جلبک اسپیرولینا ۹۰٪ مواد مغذی جذب شد.

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