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APPLICATION OF PHYCOREMEDIATION TECHNOLOGY IN THE TREATMENT OF FOOD PROCESSING WASTEWATER BY FRESHWATER MICROALGAE *Botryococcus* SP.

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ABSTRACT

The cultivating of freshwater microalgae namely *Botryococcus* sp. in outdoor conditions for phycoremediation of food processing wastewater was investigated. Dilution of wastewater and distilled water as pure sample (100%) and diluted sample (50%) of wastewater were examined. The highest growth of *Botryococcus* sp. in pure sample of food processing wastewater occurs at Day 12 at approximately 3.72×10^6 cell/mL with the highest removal of nitrate, phosphate, and total organic carbon are 86.62%, 78.23% and 76.66%, respectively. Diluted (50%) food processing wastewater, the *Botryococcus* sp. was able to reach maximum growth at Day 13 at cell concentration approximately at 9.7×10^5 cell/mL. Significantly reduction for some nutrients such as nitrate (78.78%), biochemical oxygen demand (69.03%) and total organic carbon (67.93%) were observed. This finding proved good indication of *Botryococcus* sp. grows in food processing wastewater. The information from these findings is potentially useful for biotechnology industries for further development of bio-based product from microalgae biomass.

Keywords: food processing wastewater, phycoremediation, *botryococcus* sp, microalgae, biomass.

1. INTRODUCTION

Food processing industry in Malaysia is one of the important industries that play a role in term of economic development [1, 2]. The interest in the food production industry is related to the production, consumption, and export-import products to boost country revenues. In food processing factory, there is a huge amount of water to be used and directly produced the volume of wastewater resulted from washing and processing activities [3-7]. Consequently, at the worldwide global market or scale, the food industry be able to generate a wastewater which is significantly effect to the environment if uncontrolled discharged to the water bodies [8]. By that, creation of innovative and sustainable idea to treat the food wastewater is highly required so that any food industry wastewater must meet the lowest requirement quality of the effluent standard before released to environment. The food processing wastewater such as food and milk processing industries consumed large volume of water then characterized by high BOD (442 – 523.5 mg/L) and COD (8960 – 11900 mg/L) with fats, oil and grease and other nutrient such as nitrogen, phosphorus, and potassium [9]. All these nutrients are suitable for the growth of microorganism that can cooperate to absorb the pollutant loads like algae. Ji et al. [5] also reported that food processing wastewater was rich in nutrient including nitrogen (1385 mg/L), phosphorus (108 mg/L), calcium (ND), iron (24.7 mg/L), aluminum (316.4 mg/L) and total organic carbon (14898 mg/L).

Sustainable biological wastewater treatment such as phycoremediation system establishes to overcome the problem as described above. This system was chosen to solve the constraints of the conventional method in industrial wastewater treatment system such as during tertiary treatment to eliminate nitrogen and phosphorus [10, 11]. Phycoremediation is the used of microalgae and macroalgae in wastewater bio-transform the pollutant loads into their cell and at once clean the wastewater before released to the environment [12-14]. However, the efficiency of removal of the pollutant load by microalgae shows variable changes among microalgae species [15]. Several advantages achieved by applying phycoremediation in wastewater [16, 33] and some of them are: 1) Phycoremediation is effective in costing, environmental friendly and non-hazardous process; 2) The algae used are non-pathogenic photosynthetic microorganism and they do not release any toxic emission; 3) Phycoremediation keeps bacterial population under control 4) Algae growth in the wastewater also removes waste CO_2 from air there by contributing to the reduction of greenhouse gases effect; 5) The algal biomass has potential nutrient value and can be used as a live feed for agro-aquaculture activity.

Nowadays, a lot of researchers had applied this sustainable method in many type of wastewater with various species of microalgae and macroalgae. Example of wastewater had been investigated such as dairy wastewater, greywater, municipal and domestic wastewater, palm oil mill effluent and leather industry using microalgae variable species such as *Botryococcus* sp., Oscillatoria limosa, Nostoc sp., Rhizoclonium hieroglyphicum, Spirulina sp. and Chlorella vulgaris [17-

21]. However, to the best of authors knowledge, no report has been found so far using food processing wastewater for phycoremediation especially by using *Botryococcus* sp. The study conducted by Ji et al. [5] used synthetic medium of food wastewater however, they cultivated Scenedesmus obliquus to produce biomass. This paper critically examines at different perspective of phycoremediation of food processing wastewater, specifically on *Botryococcus* sp. The aims of the present work are the following: (1) to quantify the removal of pollutant in different strength of food processing wastewater for phycoremediation by *Botryococcus* sp. (2) to investigate the growth of *Botryococcus* sp. during phycoremediation process.

2. MATERIALS AND METHODS

2.1. Sampling of wastewater

The wastewater sampling was from the effluent of a food processing factory located in Parit Raja, Batu Pahat, Johor, Malaysia. Samples were collected and transferred into a bottle according to the standard method [22]. Once they reached the laboratory the samples were kept at 4˚C prior to use for laboratory analysis.

2.2. The preparation of *Botryococcus* sp.

The *Botryococcus* sp. colonies were provided by the Microbiology Laboratory, Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia, and were ready for inoculation for preparation of the treatment medium. Prior to inoculation in the treatment medium, the inoculum was counted using a haemocytometer under a compound microscope (BS 748: 1982). An initial inoculum of 1.0 x 10^3 cell/ml was transferred from *Botryococcus* sp. stock based on the concentration calculation [22]. Series of flasks containing the inoculated treatment medium were placed at room temperature. The original culture of *Botryococcus* sp. was grown differently for six samples for 15 days. The sample of wastewater was categorized as pure wastewater (100%) and 50% concentration of wastewater (dilution of wastewater and sterile distilled water at ratio 1:1). Flasks were placed then in sunlight and shaken every day to ensure the *Botryococcus* sp. were homogenised with the wastewater sample during the cultivation process. The total volume in the flasks was 170 ml for each type of samples prepared in triplicates.

2.3. Laboratory test

The characteristics, such as COD, BOD, TOC, TN, phosphate and nitrate, were measured based on the Standard Method and Examination of Water and Wastewater [22]. The food wastewater parameters were examined at a sampling interval of 0 days, 7 days and 15 days of phycoremediation.

2.4. Microalgae growth measurement

The growth of *Botryococcus* sp. was measured using a haemocytometer to count the number of microalgae cells. The haemocytometer used are clean and sample was pipette at approximately 10µl at the edge of the cover slip and allow running under the cover slip. The haemocytometer grid under the microscope was observed at low magnification (10-20X). The focus of the lens was adjusted until individual counting grids are visible for enumeration of microalgae.

3. RESULTS AND DISCUSSION

3.1. Phycoremediation effectiveness

The total reduction of pollutants within 7 days and 15 days of phycoremediation treatment are analysed. The use of *Botryococcus* sp. for inoculation in food processing wastewater for phycoremediation has shown significant ($p<0.05$) nutrients removal results. The total reduction of pure wastewater for COD and BOD was 70.68% and 61.11%, respectively, on the 15^{th} day. The reduction of COD by microalgae was due to the absorption of carbon dioxide because, according to Hadiyanto et al. [24], microscopic microalgae are capable of converting COD into carbon sources for building their cells during the phycoremediation process. The same goes for BOD since employing microalgae is one of the biological treatments of wastewater in secondary treatment, then, indirectly, the BOD concentration experiences a decrease due to the degradation of the inorganic and organic nutrients by the microalgae [25]. These findings were similar to those obtained in a study by Sahu [26], who found that the maximum removal for COD and BOD was 66% and 74%, respectively, however, they used Chlorella vulgaris to treat domestic wastewater. Meanwhile, another researcher, Kshirsagar [27], proved that Scenedesmus provided better removal of COD, 70.97%, and BOD, 89.21%, when applied in the same type of wastewater as Sahu [26]. This study found that the *Botryococcus* sp. treated the domestic wastewater as good as Chlorella vulagaris and Scenedesmus since theybelong to the same division of green microalgae, which is Chlorophyta [28].

There was a significant reduction of TOC for both concentrations of wastewater. The results indicated a slightly higher removal of TOC at 76.66% for the pure concentration of wastewater compared to the 50% concentration at 67.93% after 15 days of treatment. The pure wastewater contained much higher total organic carbon compared to the 50% diluted wastewater, since the TOC is the result of the total between the TC and IC. This result is relatively high compared to the previous research by Mata et al. [29] indicated that the removal of TC in brewery effluent wastewater using a green microalgae species with maximum removal at 56.9% after 13 days cultivation. Other parameters, such as TN and phosphate, also showed the removal after 7 days and 15 days of treatment, in which the pure wastewater concentration was reduced by *Botryococcus* sp. by approximately 72.49% and 78.23%, respectively. While for the 50 % diluted wastewater the removal of TN and phosphate were at 65.44% and 67.15% for, respectively. The last parameter tested was for the presence of nitrate reduction in food wastewater within 7 days and 15 days of

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phycoremediation. The results showed that for the pure wastewater concentration, the nitrate was reduced to 86.62%, and 78.78% for the 50% concentration. Both of them occurred over the 15 days of treatment. The reduction of nitrate occurred because the microalgae need these nutrients to grow their cells, as a result of the photosynthesis process. The nitrate provides food to the microalgae so that they can grow optimally; clearly the question has been answered concerning why the nitrate declined after the treatment was completed. In wastewater, growing microalgae has the capacity to consume nutrients and absorb CO_2 and release oxygen through photosynthesis. Overall, the removal results based on the day and per cent concentration can be observed in Figure-1. The pure wastewater for phycoremediation by *Botryococcus* sp. was seen to be more effective in the removal of the nutrients in the wastewater due to the sample containing more amounts of nutrients for the growth of the microalgae.

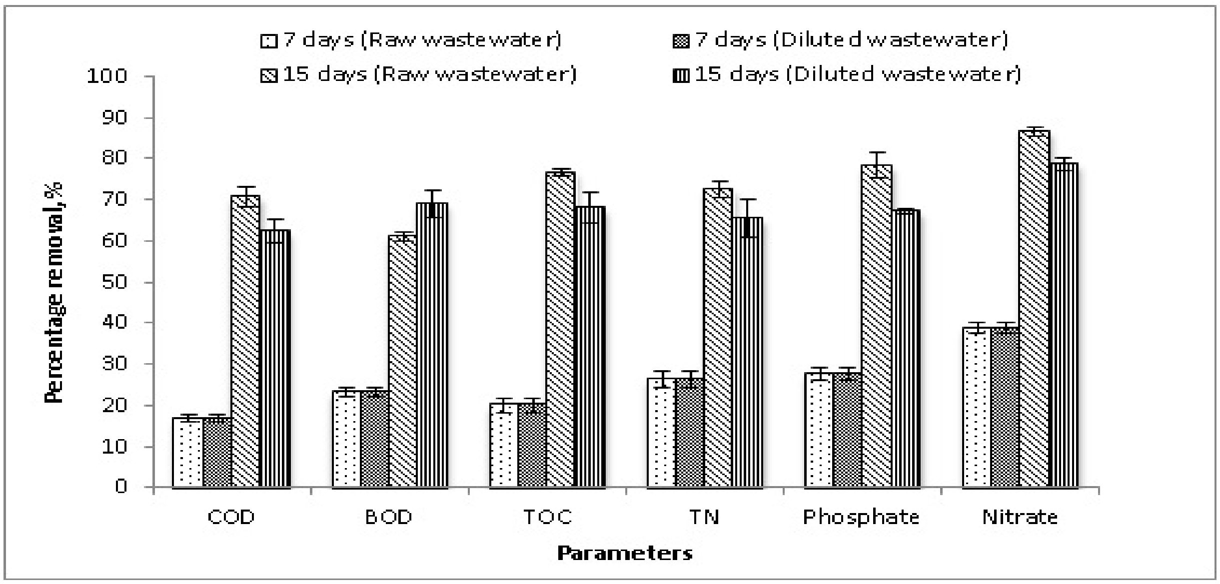

Figure-1. Percentage removal of food processing wastewater parameters (*n*= 3).

3.2. Growth of *Botryococcus* sp.

The growth of microalgae *Botryococcus* sp. over 15 days cultivation in the phycoremediation of food processing wastewater medium is illustrated in Figure-2. The growth of the *Botryococcus* sp. population was measured based on the increase in cell concentration over time. The lag phase of the microalgae in the medium occurred until Day 8 before it reached the exponential phase. Phycoremediation using pure wastewater showed the highest growth rate for *Botryococcus* sp. at day 12 with the number of cells equal to 3.72×10^6 cell/mL. The diluted wastewater sample indicated that the maximum growth occurred at day 13 with a cell concentration of approximately 9.7×10^5 cell/mL. Compared to the diluted wastewater, the *Botryococcus* sp. were able to grow better in the pure concentration of the medium. The peak point of both samples was achieved due to the amount of nutrients in the sample being very high. Although microalgae tend to grow very quickly under high nutrient availability, as *Botryococcus* sp. is short-lived this results in a high concentration of dead organic matter that starts to decay[30,32].

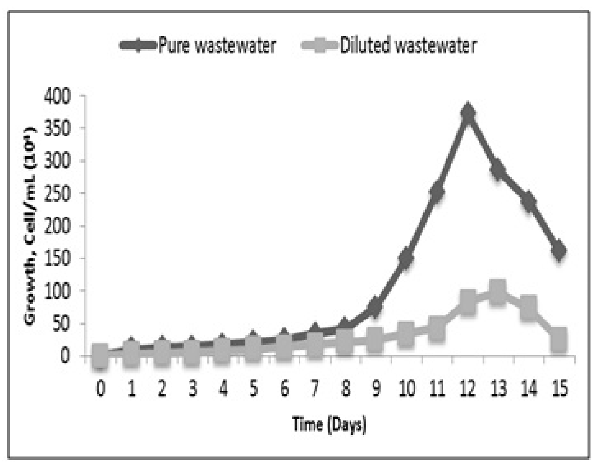

Figure-2. The growth of microalgae *Botryococcus* sp. in food processing wastewater.

The decaying process consumes dissolved oxygen in the water, resulting in hypoxic conditions and without sufficient dissolved oxygen in the water, the plants, especially microalgae, may die off in large numbers [31]. Significantly, this study shows that raw wastewater has high potential for alternative sustainable phycoremediation combined with *Botryococcus* sp. biomass growth. Considering the level of nutrient was slightly low in diluted wastewater, therefore the growth of *Botryococcus* sp. was also affected due to different pollutant strength. Nowadays, the constantly increasing interest in growing algae absolutely will lead to an increased demand for water and nutrients. It has been reported that in conventional wastewater treatment, nutrients have been added to ensure microorganisms growth for treating the wastewater [25]. This study showed that food processing wastewater can be used and treated by algae without the addition of artificial fertilizers.

4. CONCLUSIONS

The most obvious finding to emerge from this study is different strength of wastewaters pollutant for microalgae cultivation. These findings enhance our understanding that various purity of wastewater for phycoremediation operation gives different trend of growth. The current interest in microalgae as sources of biomass and bioenergy is constantly increasing therefore, the cultivation of microalgae industry demands large quantities of water and nutrients. The use of food processing wastewater to grow *Botryococcus* sp. is particularly interesting from the point of view of green sustainability. Further research might explore the potential of biomass from *Botryococcus* sp. as bio-based product such as hydrocarbons, biofuels, fish feeds and fertilizer.

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