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Utilization of Dairy Industry Wastewater for Nutrition of Microalgae *Chlorella vulgaris*

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Abstract. This experiment utilized dairy industry wastewater, which is located in Sukabumi, West Java, Indonesia. The aim of this experiment was to evaluate the biomass production and dairy wastewater treatment using *Chlorella vulgaris*. The experiment lasted for 6 months (July – December 2019). Monthly water analyses for a variety of physical-chemical factors were also investigated. Microalgae were cultivated in a 10 m³ raceway bioreactor type. The results indicated that a decrease in the concentration chemical substances of the wastewater, namely 74% N-amonia, 77% total nitrogen (TN), 78% total phosphate (TP), 92% Chemical Oxygen Demand (COD), and 77% Biological Oxygen Demand (BOD) within 10 days of the process. A maximum of 1.2 g/L dry biomass was obtained in 18 days. The percentage lipid content was determined by soxhlet extraction and was shown to be 25%. To sustain the growth of microalgae, it was necessary giving 3.5 mg NPK/L on the tenth day and then continuously added.

Keywords: biomass, dairy industry, lipid content, microalgae, wastewater, raceway pond.

1. Introduction

Waste from the milk industry is the result of the transformation of raw materials through the process of pasteurization into ice cream, milk and whey, powders, lactose, and condensed milk. In a report of industry practice in the PT Indolakto located in Sukabumi city in Indonesia the amount of dairy waste that must be processed per day is 2000 m³/day [1]. Based on a study dairy waste water has the physical characteristics with + 4000 m/L of BOD, + 2000 mg/L of COD and levels of suspended solids (TSS) dairy waste water is + 800 mg/L.

Along with the increase in milk production in Indonesia, the waste generated from the production of milk increased. The volume of wastewater generated from the dairy base is 3.9 L/kg of milk products coagulation processes such as milk and whey powder [2]. The threshold criteria for milk industry waste are 50 mg / L of BOD value, 100 mg / L of COD value and TSS of 40 mg / L coagulation processes such as milk and whey powder and TSS of 40 mg/L with discharge of waste production is 3.5 liters per kg of milk solids [2].

Bioremediation is often used to reduce pollutants from industrial waste. Low pollutant waste can function as a nutrient substitution for microorganisms [3]. Microalgae including microorganisms that can reduce pollutants and this remediation process is called phycoremediation. Many researchers report the results of their research on microalgae for the treatment of wastewater that can produce biomass [4]. Because of the ability of microalgae to reduce pollutants contained in wastewater, the nitrogen and phosphorus content in wastewater is used to grow microalgae [5,6].



Using dairy effluent for microalgal cultures is beneficial for minimizing the use of freshwater, reducing the cost of nutrient addition, removing the remaining nitrogen and phosphorus, and producing microalgal biomass as bioresources for biofuel or high-value by-products [4-7].

The purpose of this research is to determine the potential of the dairy industry wastewater as a nutrient substitution for microalgae so as to produce beneficial microalgal biomass. In addition to knowing the potential of microalgae living process to remediate the waste water as a phycoremediation treatment.

2. Methodology

2.1. Dairy Wastewater Effluent

The milk industry wastewater used comes from PT Indolaksto Sukabumi, West Java, Indonesia. This company produces large amounts (approximately 1,000 m³/day) of waste water. The water quality characteristics of the dairy waste water are summarized in Table 1. The characteristics of water quality was analysed by APHA (2012) methods [8]. The ratio of COD to BOD is 1.93 in the influent. The average pH was relatively low, determined to be 4.6 ± 0.4 for the influent tank and 6.1 ± 0.6 for the effluent tank. However, the values for the dairy effluent remained at 45.4 mg/L of COD, 37.6 mg/L of BOD, 0.43 of total nitrogen (TN), and 22.8 of total phosphate (TP).

The mass ratio of C:N:P for algal growth recommended a 46.1:7.7:1, whereas the dairy effluent wastewater we used in this study had a 45.9:0.43:22.8 mass ratio of C:N:P. C and N ratio values can positively influence the production of microalga biomass content [4]. The results of studies on N deficiency can increase fat content in microalgal cells [9].

Table 1. Characteristics of the Dairy Wastewater

Parameters	Influent (mg/L)	Effluent (mg/L)
pH	4.6 ± 0.4	6.1 ± 0.6
SS	587 ± 81.4	128 ± 21.9
COD	408 ± 378	45.4 ± 2.1
BOD	728 ± 64	37.6 ± 6.4
Total Nitrogen	1.91 ± 0.7	0.43 ± 10.7
Total Phosphor	49.1 ± 3.4	22.8 ± 55.4
Heavy metal	Very low	Very low

2.2. Experimental Design

The study used 10 m³ raceway pond for microalgae production model (Figure 1). Media liquid volume is 800 L. Media liquid from PT Indolakto's milk industry waste is inoculated with microalgae. Microalgae inoculum concentrations commonly used are at least 0.5 kg m⁻³ [10]. Microalgae *C. vulgaris* is a good source of ethanol due to its high starch content (~37% dry wt.) and up to 65% ethanol conversion efficiency [11]. For the seed culture, the *C. vulgaris* cells were cultivated in Basal Medium (BM) and prepared using deionized water under Light Emitting Diode (LED) lamps at ambient temperature. The initial concentration of the inoculated microalgae *C. vulgaris* was 0.05 ± 0.4 g/L. The experiments were conducted at a neutral pH (7.2 ± 0.3). In a sunny locale with a stable diurnal temperature of 25-32°C. CO₂ with a flow rate of 0.74 L/min was introduced into the reactor. To sustain the growth of microalgae, it was necessary giving 3.5 mg NPK/L on the tenth day and then continuously added.

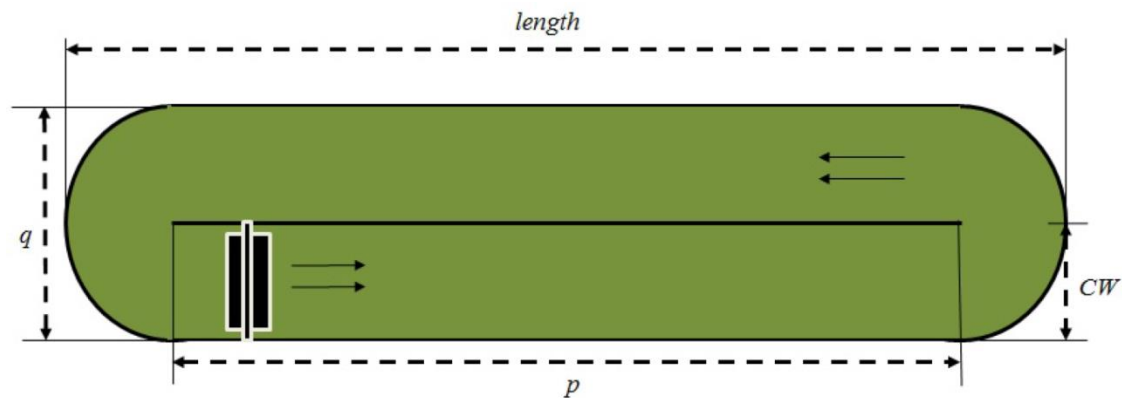


Figure 1. The schematic of an open channel raceway pond (view from the top).

2.3. Analytical Methods

The criterion for success in the use of milk industry wastewater for microalgae nutrition is to know the production of dry biomass. Calculation of dry biomass production with the following procedure. Biomass samples produced from ponds are taken as much as 50 mL every day. Then centrifuged for 15 minutes. The centrifuged sample was washed twice with distilled water and was dried at 105°C for 16 h. The biomass productivity, B_p , was defined using F . (1) as follows:

$$B_p = (B_p - B_{p0}) / (t - t_0) \quad (1)$$

where B_p and B_{p0} were the biomass concentrations at time t and at starting time t_0 , respectively.

3. Results and Discussion

3.1. Production of Biomass

The growth of microalgae is to show the functioning of the nutrients provided. Adding dairy industry used for microalgae media *Chlorella vulgaris* showed the growth of microalgae biomass that was successfully separated and dried. Research on treating the milk industry wastewater with microalgae shows that wastewater is an effective nutrient for the production of microalgae biomass [4,12].

Figure 2 shows the biomass growth curves for the dairy effluent. The maximum biomass reached 1.2 g/L dry weights in 18 days from an initial concentration of 0.05 g/L. The biomass in the dairy wastewater was slightly decreased after 18 days. However, the biomass in the dairy effluent yielded 24-fold more than that of the initial concentration.

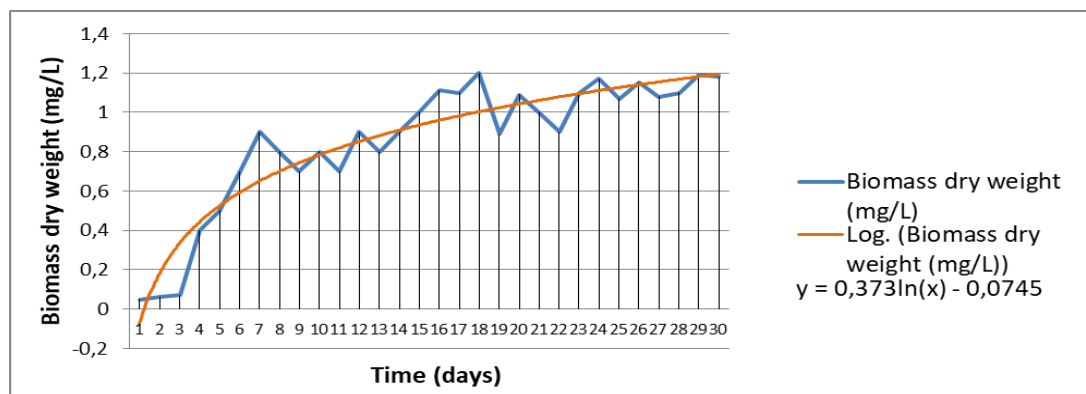


Figure 2. Growth curve of *C. vulgaris* in the dairy effluent.

The growth of the microalgae varied according to the species of microalgae in raw dairy wastewater [5]. Biomass productivity of 0.201 g/L/day for *Chlorella saccharophila* and 0.211 g/L/day for *Scenedesmus* sp. in untreated dairy farm wastewater [5]. In this study, dry *C. vulgaris* biomass was produced as much as 0.18 g / L within 13 days of cultivation. The production of *C. vulgaris* biomass is relatively higher than other types of microalgae [6].

3.2. Nutrient Removal

The calculated BOD and COD reduction values using *C. vulgaris* were 77% and 92% (Figure 3), respectively, in the dairy effluent at 10 days. The BOD and COD concentrations remained at 6.2 and 7.1 mg/L from the initial concentrations of 37.6 and 45.4 mg/L, respectively, in the dairy effluent after 10 days. In addition, the reduction process of the BOD and COD in the dairy effluent increased during the first 5 days and then the reduction rate was not changed. The reduction in the SS obtained in the dairy wastewater using microalgae had significantly less reduction, which reached only 19%, in comparison to that of the BOD and COD. This result indicated that the microalgae were ineffective due to the small solid particles that remained in suspension in the dairy effluent as a colloid or due to the motion of the dairy effluent.

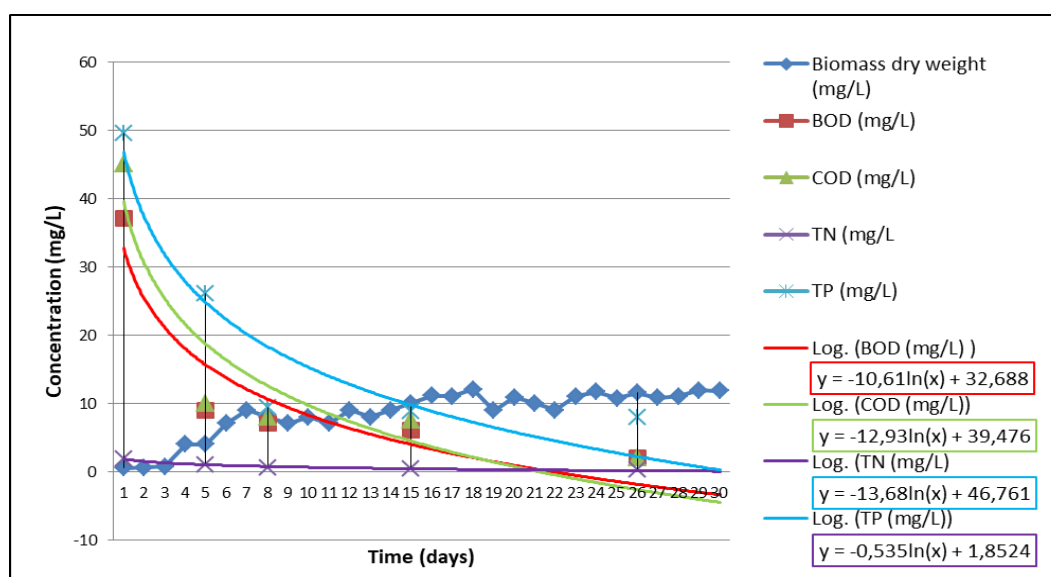


Figure 3. Relationship graph reduction of chemical characteristics of milk industry wastewater that is used as a nutrient for microalgae *C. vulgaris*.

The maximum TN reduction in the dairy effluent by *C. vulgaris* was 77% in 10 days. Nitrogen could be reduced through assimilation by microalgae. The microalgae, *C. vulgaris*, were able to consume high concentrations of nitrate ions and, therefore, could help in the purification of dairy wastewater [13]. The results obtained for the TP removal showed that 78% was removed at an initial TP concentration of 49.17mg/L. The microalgae consumed 22% more TN than TP. Phosphorus uptake by algae is not always stoichiometric and can be affected by the algal physiology as well as the phosphorus concentration and its chemical forms, light intensity, pH, and temperature. There have been observations that showed that phosphorus uptake is inversely related to the internal phosphorus concentrations of the cell [14].

The correlation coefficient (R^2) for the logarithmic relationships of the biomass productivity, and the BOD, COD, TN, and TP in the dairy effluent were determined to be 0.880, 0.868, 0.983, 0.924, respectively (Figure 4).

Depending on the source, raw waste water has sufficient nutrients for algal growth [15]. However, when algal biofilms are utilized for tertiary wastewater treatment (effluent polishing) nutrient addition

may be required to optimize the molar stoichiometric ratios of carbon, nitrogen, and phosphorus (C:N:P) necessary for growth [16].

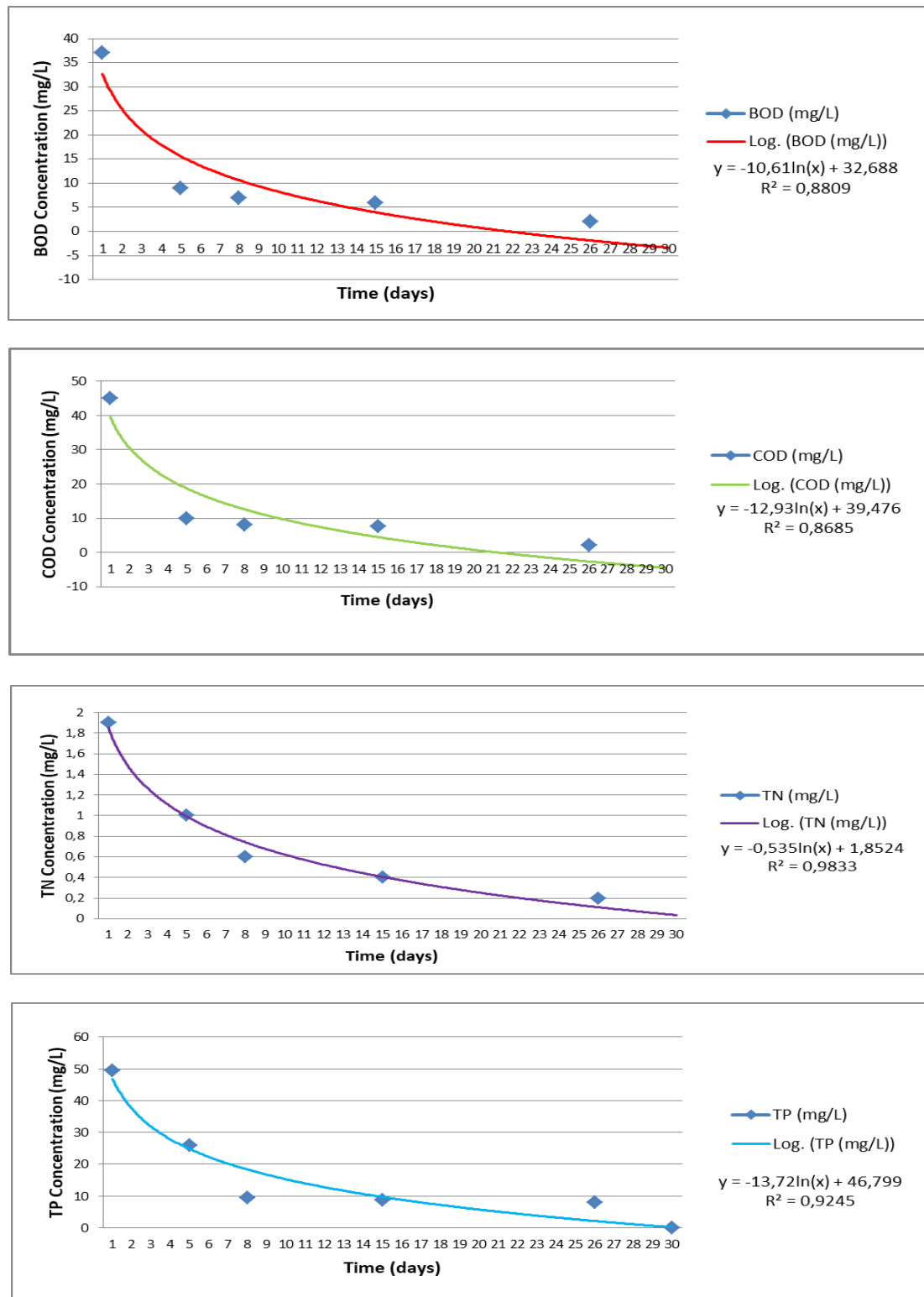


Figure 4. Graph of reduction in Biological Oxygen Demand, Chemical Oxygen Demand, Total Nitrogen and Total Phosphate content from milk industry wastewater treated by *C. vulgaris* microalgae.

Therefore, using dairy effluent for microalgal cultures could be useful and practical as an advanced environmentally friendly treatment process [17].

4. Conclusion

In summary, the objective of this study was to develop an efficient treatment for nutrients and the production of biomass in dairy wastewater effluent. The maximum biomass reached 1.2 dry weight g/L in 18 days from an initial concentration of 0.05 g/L. The calculated BOD, COD, TN, and TP reduction values using *C. vulgaris* were obtained as 77%, 92%, 77%, and 78% respectively, in the dairy effluent at 10 days. The correlation coefficient (R^2) for the logarithmic relationships of the biomass productivity, and the BOD, COD, TN, and TP in the dairy effluent were determined to be 0.880, 0.868, 0.983, 0.924, respectively. The biomass productivity was strongly influenced by the nutrient reduction in the dairy effluent. To sustain the growth of microalgae, it was necessary giving 3.5 mg NPK/L on the tenth day and then continuously added. The percentage lipid content was determined by soxhlet extraction and was shown to be 25%.

Acknowledgements

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