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Phycoremediation of Dairy Wastewater by Using Green Microlgae: *Botryococcus* sp.

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Abstract. Dairy wastewater are usually much polluted due to the high level of nutrient content such as phosphate, nitrate, sulfate etc. The pollution level are indicated by few characteristics such as BOD (243 mg/L), COD (324 mg/L), pH (9.08), Total Nitrogen (65.06 mg/L), Total Organic Carbon (143.3 mg/L), Total Carbon (312.9 mg/L) and Inorganic Carbon (169.6 mg/L). In this study, *Botryococcus sp.,* a species of green microalgae are used to determine whether it can purify dairy wastewater. In spite of that, the microalgae growth over phycoremediation process also determined daily based on hemocytometer counting. From 100% concentration of dairy wastewater, *Botryococcus* sp. was capable to reduce the parameters such as BOD of 73.3%, TOC of 65.1%, TC of 61.4%, IC of 58.3% and COD of 48.8% on the 15 days of phycoremediation. Meanwhile, for the 50% of dairy wastewater, the same microalgae be able to remove the parameter such as phosphate of 78.7%, BOD of 73.8%, TOC of 70%, TC of 68,8%, IC of 59.4% and COD of 50%. The overall result from this study shown that the *Botryococcus* sp. is useful to reduce the inorganic and organic pollutant in dairy wastewater and could be potential to be used for any different wastewater.

1. Introduction

Dairy industry is one of the major industry have economic importance in various industries of agricultural sector. Among the various dairy products producing countries, India has attained the first rank in milk production. For example, India is sharing about 13.1% of the total milk produced in the world [1]. There are large and small scale farm industries for example in India responsible for large number of waste production (solid and liquid). Specifically farm industry is noted as one of the significant contributor to water pollution. Farm waste is basically biodegradable, but produces undesirable color and odor. It is estimated that about 110 million tonnes of milk and about 275 million tonnes of wastewater are being generated annually from the Indian Dairy Industries by the year 2010 [2]. Farm wastewater is characterized by strong color, offensive odor, high BOD (40–48,000 mg/l), high COD (80–95,000 mg/l) [2] and variable pH [1]. It also contains sufficient nutrient like N (14– 830 mg/l) and P (9–280 mg/l) required for biological growth. Normally for

treatment of farm wastewater, physical and chemical treatment methods are involves, which are often very costly. There has been an increasing interest in the treatment of dairy waste water [3]. Most of the studies have concentrated on the use of fungi and bacteria for reducing the organic load of dairy waste water [4, 10, 11]. In recent years the use of microalgae in treatment and recycling of waste water has attracted great interest due to their central role in carbon dioxide fixation.

This study aimed focusing on one type of algae strain which is *Botryococcus* sp. to reduce the contamination in dairy wastewater. Therefore, the objective of this study is to determine the effectiveness of *Botryococcus* sp. to remove the pollutant load in diary wastewater and to measure the microalgae growth during phycoremediation process.

2. Materials and Methods

2.1. Dairy Wastewater sampling

Samples of wastewater were taken at the nearest discharge point, where the wastewater are disposed into the sewerage system after being used in the goat breeding farm at Yong Peng, Johor, Malaysia. Dairy wastewater had to filtered before undergo the phycoremediation process to remove suspended solid and also other microorganism. All sampling and preservation was done accordingly to the APHA, 2012.

2.1. Microalgae stock culturing

The *Botryococcus* sp. was isolated and kept in the Microbiology Laboratory of Faculty of Engineering Technology, UTHM. The inculum were counted using haemocytometer and initial inculums of 1.75×10^4 cell/ml was transferred to the dairy wastewater for phycoremediation process.

2.2. Parameters of wastewater

The characteristics such as Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), pH, Total Nitrogen (TN), Total Organic Carbon (TOC), Total Carbon (TC) and Inorganic Carbon (IC) was carried out based on the standard method and Examination of Water and Wastewater (2012). Dairy wastewater parameter was examined on the 0^{th} day, 7^{th} day and 15^{th} day of phycoremediation.

2.3. Phycoremediation culture design

There were 3 type of diary wastewater design for the phycoremediation process namely 100% concentration of wastewater with *Botryococcus* sp., 50% concentration of wastewater with *Botryococcus* sp. and control sample. All those process conducted in triplicate of 250 ml flask and filled up with 170ml of wastewater for each flask. Each of treatment medium was inoculated with 1.75×10^4 cell/ml to perform the phycoremediation process. During te treatment, the medium was exposed to the natural sunlight and aerated manually by hand shaking twice a day then supplied with air CO_2 to maintain on the photosynthesis process. The phycoremediation is conducted for total 15 days.

2.4. Microalgae growth

Microalgae cell was determined by counting the number of cell using haemocytometer. The haemocytometer consists of a thick glass microscope slide with a rectangular indentation that creates a chamber. This chamber is engraved with a laser-etched grid of perpendicular lines. The device is carefully crafted so that the area bounded by the lines is known, and the depth of the

chamber is also known. It is therefore possible to count the number of cells or particles in a specific volume of fluid, and thereby calculate the concentration of cells in the fluid overall.

3. Result and discussion

3.1 Characteristics of Raw Dairy Wastewater

Eight parameters were selected to characterize the dairy wastewater samples. These parameters are important for the effluent characterization. These values also need to meet the Standard A and B established by the Malaysia Wastewater Effluent Discharge (2000) guidelines prior being released into watercourse. Table 1 below shows the characteristics for those eight parameters.

Table 1 Summarized characteristics for Raw Dairy Wastewater

Parameter	pH	COD, mg/L	BOD, mg/L	TOC	TC	IC	TN	Phosphate
Concentration (n=3)	9.08	342	243	143.3	312.9	169.6	65.06	17.5
Allowable limit (Standard A)	6 – 9	80	20	-	-	-	-	-
Allowable limit (Standard B)	5.5 - 9	200	50	-	-	-	-	-

3.2 Analysis of pH

Figure 1 shows the results of pH after treatment of wastewater by using *Botryococcus* sp. The patterns for both concentration are almost identical. The pH was decreased from 9.08 to 8.44 in 100% concentration of dairy wastewater while 8.35 to 7.97 in 50% concentration of wastewater. According to the research done by Tumsri, [5], during the treatment of dairy wastewater with microalgae, hydroxide ions are produced along with hydrogen bubbles; thus increasing the level of pH for that treated water. The elevation of pH is likely due to the depletion of inorganic carbon during photosynthesis process [6]. All those data obtained are higher than the allowable limits by the Standard guidelines A and B (2000). However, in distilled water sample, there is no significant changes in pH because there are no indication sign of microalgae growing in it.

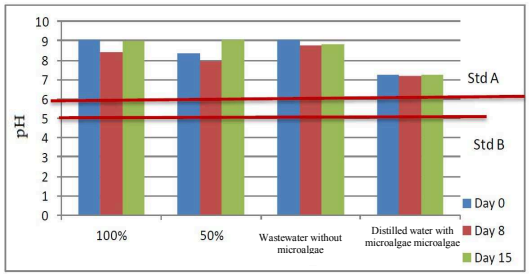

Figure 1 pH variation of dairy wastewater during phycoremediation

3.3. Analysis of COD and BOD

Figure 2 and 3 showed the results of COD and BOD after 15 days of treatment. For 100% concentration of dairy wastewater, the value of COD for initial day was 342 mg/L then drop to 246 mg/L on the 7th day then 175 mg/L on the last day of treatment. Meanwhile, 50% concentration of dairy wastewater, the concentration of COD for the 0 day was 190 mg/L and the last day of phycoremediation was 95 mg/L. From both concentrations, the significant different value of COD is

due to amount concentration of wastewater used. According to Figure 2, value of COD for both concentration was below than Standard B allowable limit. As in Figure 3, from the 0 day of treatment, both concentration showed a high value if compared to the Standard A and B. However, for 100% concentration of wastewater, the value of BOD was higher than the Standard A and B whereas 50% concentration also indicated the same situation as 100% concentration. By that, overall result could be concluded that the water quality of the treated wastewater was improved.

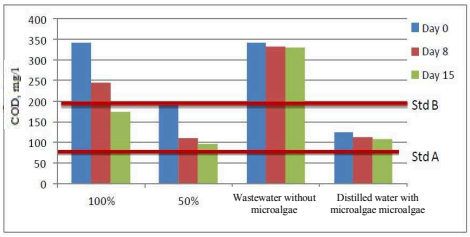

Figure 2 COD variation of dairy wastewater during phycoremediation

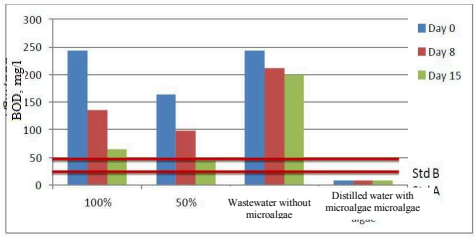

Figure 3 BOD variation of dairy wastewater during phycoremediation

3.4 TOC, TC and IC analysis

Based on Figure 4, the highest TOC removal is on 50% concentration from 81.05mg/l to 24.31mg/l) while for 100% concentration of 143.3mg/l to 50mg/l. Other than that, TC also show a good reduction for both concentration of wastewater. Percent removal for 100% and 50% concentration was 63.4% and 68.8% respectively. In addition, the IC seems to suffer from the same condition which is decrease from 169.6mg/l to 87.16mg/l for 100% concentration and decrease from 83.7mg/l to 43mg/l for 50% concentration. Carbon is also one of the important elements needed for the growth of microalgae because it constitutes over 5% in typical algal biomass [7,8]

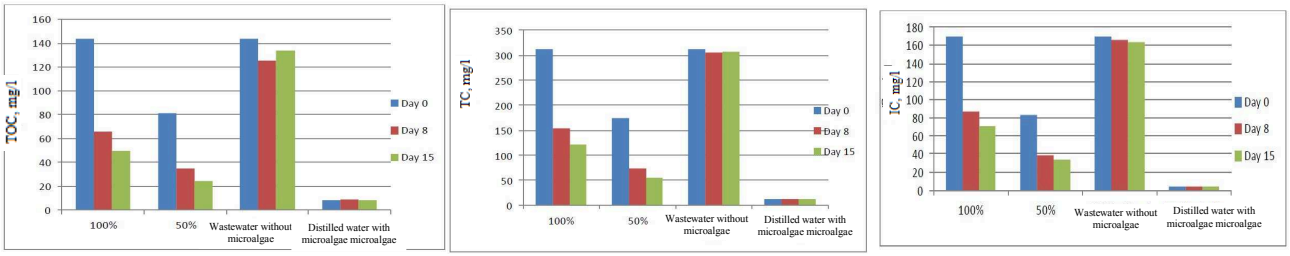

Figure 4 TOC, TC and IC variation of dairy wastewater during phycoremediation

3.5 Analysis of Total Nitrogen (TN) and Phosphate

Based on the Figure 5, total nitrogen for both samples were not showing any removal of this nutrient in fact increased during 15 days of phycoremediation. For 100% concentration, TN value increased about 8.1% on the last day of treatment while for 50% concentration also showed an increment much less 48.28% at the same day as 100% concentration. Meanwhile, Figure 6 is the summary of removals of phosphate. As we can see, 100% concentration of wastewater, phosphate content reduces by 21.4% at day 8 and then 62.71% by day 15. In 50% concentration of wastewater, phosphate content reduces by 45.4% by day 8 and 78.7% by day 15. Microalgae assimilate a significant amount of nutrients because they require high amounts of nitrogen and phosphorus for the synthesis of proteins (45-60% of microalgal dry weight), nucleic acids and phospholipids [9].

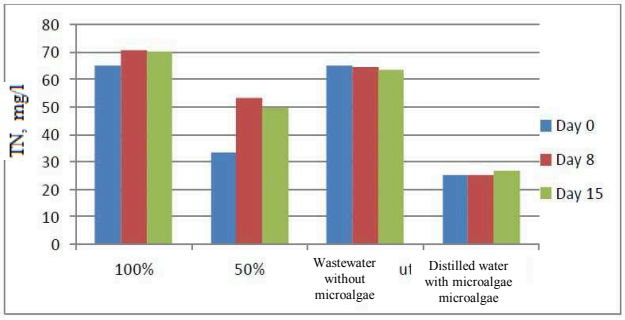

Figure 5 TN variation of dairy wastewater

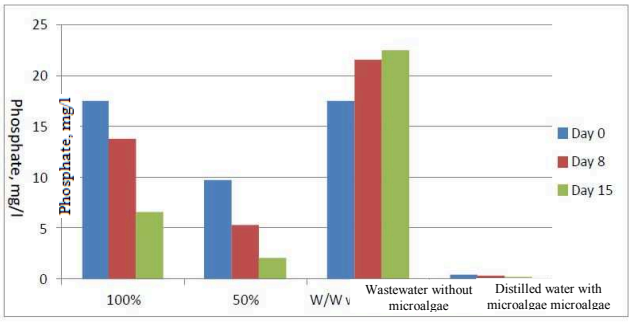

Figure 6 Phosphate variation of dairy wastewater

4. Conclusion

Based on the inclusive results, it is proved that *Botryococcus* sp. can be used to remove the pollutants load and nutrients except TN in dairy wastewater. The effectiveness of the microalgae was observed through the experiments carried out. The maximum removals of COD, BOD, TOC, TC, IC and phosphate in 100% concentration of dairy wastewater were 48.8%, 73.3%, 65.1%, 61.4%, 58.3% and 62.71% respectively. Those were quite a significant reduction for all the parameters except for TN. As for the 50% concentration of dairy wastewater, the changes of COD was 50%, BOD 73.8%, TOC 70%, TC 68.8%, IC 59.4% and phosphate 78.7%. Due to the positive results in both concentration of dairy wastewater, the optimisation of *Botryococcus* sp. for wastewater treatment is also worthy of the future research.

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References

[1] Kothari, R. et al., (2012). Experimental study for growth potential of unicellular alga Chlorella pyrenoidosa on dairy waste water: an integrated approach for treatment and biofuel production. *Bioresource technology*, 116, pp.466–70.

[2] Kushwaha, J.P., Srivastava, V.C. & Mall, I.D., (2011). An overview of various technologies for the treatment of dairy wastewaters. *Critical reviews in food science and nutrition*, 51(5), pp.442–52.

[3] Wang, L. et al., (2010). Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. *Bioresource technology*, 101(8), pp.2623–8.

[4] Maheswari, S. & Murugesan, A.G. (2009). Remediation of arsenic in soil by Aspergillus nidulans isolated from an arsenic-contamninated site. Environ Technol. 30: pp: 921-926.

[5] Tumsri, K., (2011). Optimizing Electrocoagulation-electroflotation Process for Algae Removal. , 6, pp.452–456.

[6] Grobbelaar J.U., (2004), Algal nutrition, A. Richmond (Ed.), Handbook of Microalgal Culture: Biotechnology and Applied Phycology, Blackwell Publishing, Oxford pp. 97–115.

[7] Lee, C., (2002). Nitrogen Removal from Wastewaters by Microalgae Without Consuming Organic Carbon Sources. 12, pp.979–985.

[8] Lee K. and Lee C.G. (2001), Effect of light/dark cycles on wastewater treatments by microalgae Biotechnol. Bioprocess Eng., pp. 194–199.

[9] Oswald W.J. (2003) My sixty years in applied algology. *J. Appl. Phycol.* 15.pp: 99-106.

[10] Kaur, A and Chaman, S. (2012). Bioaugmentation for Dairy wastewater. International Journal of Science and Research. 3(9). Pp. 316-320.

[11] Rajeshkumar, K and Jayachandran, K. (2004). Treatment of dairy wastewater using a selected bacterial isolate, *Alcaligenes* sp. Applied Biochemistry and Biotechnology. 18(1-3). Pp. 65-72.