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Research Article

Removal of Toxic Metal Presence in the Wastewater and Production of the Biomass from Microalgae *Chlorella* sp.

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Removal of high toxic metals from the wastewater was one of the mandate options to avoid the environmental pollution caused by the wastewater plant. Microalgae cultivation on the wastewater was one of the hopeful methods to convert the waste into useful by-product. In this study, the *Chlorella* sp. was used to remove the presence of the total nitrogen (TN) and total phosphorous (TP) in the wastewater. Added to the above, the biomass and lipids of the *Chlorella* sp. were examined with respect to the incubation time. *Chlorella* sp. was cultivated using BG11 medium. Here, two different types of wastewater had been used, one from leather industry and other priggery waste. The respective ratio of leather and piggery wastewater used in the current study was 0 : 100, 25 : 75, 50 : 50, 75 : 25, and 100 : 0. The total percentage of the nitrogen and phosphorous removal by the microalgae within 14 days was determined. Based on the findings, it is clear that *Chlorella* sp. L33 was highly efficient to absorb the nitrate and phosphorous content in the wastewater. With regard to the biomass production, the priggery wastewater treated reported the maximum biomass for $100\,\mu$mol/L with 0.55 g/L. However, the $100\,\mu$mol/L has higher pH content than other test samples. By varying the ratio of the wastewater, the removals rates can be improved.

1. Introduction

The environmental pollution is one of the talking points for decades. Hazardous metals in the wastewater damage the earth ecosystem massively [1, 2]. Over the years, many notable works had been carried out to reduce the toxic metals' presence in the wastewater (WW) [3, 4]. Microalgae are one of the promising methods to remove these toxic metals from the wastewater. Typically, microalgae absorb the toxic metals in the wastewater as nutrient for its growth. Microalgae are also used as the substitute candidate for the fossil fuel and other vital applications [5, 6]. Biodiesel from the microalgae is believed to be the next-generation fuel, since they are easy to grow at faster rate in cost-effective methods. However, the algae oil produced very minimum due to the less lipid content [7, 8]. Microalgae can also be cultivated in both indoor and outdoor areas. For instance, microalgae grow in both photo autotrophically and heterotrophically in wastewater [9–11]. The presence of heavy metals in the water poses a great threat to the human health due to its toxicity. Extreme concentrations of the heavy metals in wastewater result in lung insufficiency, bone damage, cancer, and other worse effects. Hence, application of the suitable method to remove these toxic metals is highly indispensable [12–14]. Algae are one of the biosorbents of the toxic metals owing to its ubiquitous nature. Microalgae growth on the wastewater neutralizes the carbon and environmental sustainability [15, 16]. Many notable works related to the microalgae were published recently. Leong et al. reviewed the removal of the heavy metals from the microalgae. The review has been performed with removal rates of the arsenic, cadmium, chromium, lead, and mercury. In addition to the removal rates, the potential of the value-added products was also examined [17]. Ahmed et al. reviewed the challenges in removing the toxic contents using microalgae strains. From the review, it is understood that growing the microalgae in the wastewater removes the toxic presence, it is due to the reason that microalgae absorbs nutrients present in the wastewater.

Introduction of these systems in real-time scenario was challenging due to the costing factors and filtration techniques [18]. In addition to the above, complexity in operations also occurred owing to HRT, pH, light, and temperature limitations. Singh et al. reported the sustainable approaches for remediation of the toxic metals in the contaminated water. Based on the study, they identified the removal rates of the metals which were nitrogen (90–98.4%), phosphorous (66%–98%), Pb (75%–100%), zinc (15.6–99.7%), chromium (52.54%–96%), mercury (77%–97%), copper (45%–98%), and cadmium (2–93.06%). In addition to the toxic metals, the microalgae remove the presence of complex pesticides also in the wastewater. On the other hand, the value-added products produced from the microalgae are biofuel, lipids, and proteins. Based on the above literature, it is evident that the use of microalgae for the removal of toxic metals in wastewater is possible and it is sustainable [19, 20]. Hence, this study is mainly engrossed to observe the removal rates of both phosphorous and nitrogen from the wastewater. In addition to the heavy metals, the biomass production for biofuel productions is also examined.

2. Materials and Methods

2.1. Cultivation Method. The microalgae used in the study are *Chlorella* sp. which is the freshwater algae which can effectively grow in BG11 medium [21]. BG11 has a major composition of $NaNO_3$ 1.5 g/L and K_2HPO_4 0.04 g/L. Here, two types of the wastewater had been tested, leather wastewater and piggery wastewater at various concentrations as follows: L0:P100 (0% leather industry and 100% piggery), L25:P75 (25% leather industry and 75% piggery), L50:P50 (50% leather industry and 50% piggery), L75:P25 (75% leather industry and 25% piggery), and L100:P0 (100% leather industry and 0% piggery). The 500 mL of the wastewater was collected and treated with 100 mL of the BG11 at 25°C and allowed to shake continuously in the presence of the 6000 Lux white fluorescent light illumination for 28 days. The flasks were shaken manually two times in a day to eliminate the uncertainty. The measured uncertainty is within the acceptable limit.

2.2. Parameter's Measurement. The collected biomass was filtered through 0.45 μm Millipore filter and dried under the constant temperature of 25°C for 24 hours. Biomass was pre-weighted before the drying process. Lipid content in the microalgae has been determined using colorimetric method [22]. The pH level was determined by PHS-25. The total phosphorous was determined by ammonium molybdate spectrophotometry. The heavy metals are determined according to Changlei Xia et al. [8].

Basic formulas used to derive are the following [8, 9],

$$\text{Dry biomass weight (g/L)} = 0.1836 * OD_{600}, \quad (1)$$

$$\text{Biomass productivity (g/L/d)} = \text{Biomass concentration} \left(gL^{-1}\right)/\text{Time (days)}, \quad (2)$$

$$\text{Lipid productivity (g/L)} = \text{mass of lipid (g)}/\text{Volume (L)}, \quad (3)$$

$$\text{Lipid content (\%)} = \text{Mass of lipid (g)}/\text{Mass of culture (g)} * 100. \quad (4)$$

3. Results and Discussion

The series of the test was conducted using different combinations of the leather industry and piggery. Both leather and piggery wastewater controlled at the portions of 0%, 25%, 50%, and 100% with respect to each other. Herewith, the test combinations are L0:P100 (0% leather industry and 100% piggery), L25:P75 (25% leather industry and 75% piggery), L50:P50 (50% leather industry and 50% piggery), L75:P25 (75% leather industry and 25% piggery), and L100:P0 (100% leather industry and 0% piggery).

3.1. Removal of Total Nitrogen. The contents of the total nitrogen removal in the different cultures are depicted in Figure 1. Based on the procured results it is identified, the total nitrogen removal rates vary based on the incubation time and the wastewater combination. The maximum concentration of nitrogen in the leather and piggery wastewater was 1160 mg/L and 810 mg/L, respectively. These rates were dropped massively when the incubation period increased. Initially at day 3, the nitrogen rates of the samples L0:P100, L25:P75, L50:P50, L75:P25, and L100:P0 were 1150 mg/L, 1055 mg/L, 965 mg/L, 872 mg/L, and 790 mg/L, respectively. Due to the increase of the incubation period, the removal rate after 3 days witnessed the reduction of 0.8%, 1.5%, 1.5%, 2%, and 2.5% of the total nitrogen content in the wastewater compared to the initial day. As the incubation time increases, it is typical to witness all the samples reported reduction of the nitrogen content. At the 15th day, the total nitrogen present in the wastewater was 1005 mg/L, 905 mg/L, 801 mg/L, 701 mg/L, and 610 mg/L. The lowest levels of the nitrogen were reported for the neat leather wastewater. Furthermore, the total nitrogen removal in the span of 15 days incubation time 14%, 16%, 18%, 20%, and 23% nitrogen has been removed from the wastewater. Incubation time needs to be optimized for the better cultivation and superior removal rates. When the incubation time increases, more nutrient uptake leads to reduced metal contents in the wastewater. In the perspective of the better removal rate, leather industry wastewater grown microalgae absorb more nitrogen content compared to the piggery wastewater. With regard to the combination, the second-best removal efficiency was witnessed for L25:P25 combination [23, 24]. Although the removal rates were different, addition of the leather wastewater and piggery wastewater exhibits better total removal efficiency than neat 100% piggery wastewater.

3.2. Removal of Phosphorous. In general, the presence of P in the wastewater will be in different forms like phosphate, poly-phosphate, meta-phosphate, and some other organic complex. These phosphates are very influential to the acid and alkaline environment. Some notable work predicted that
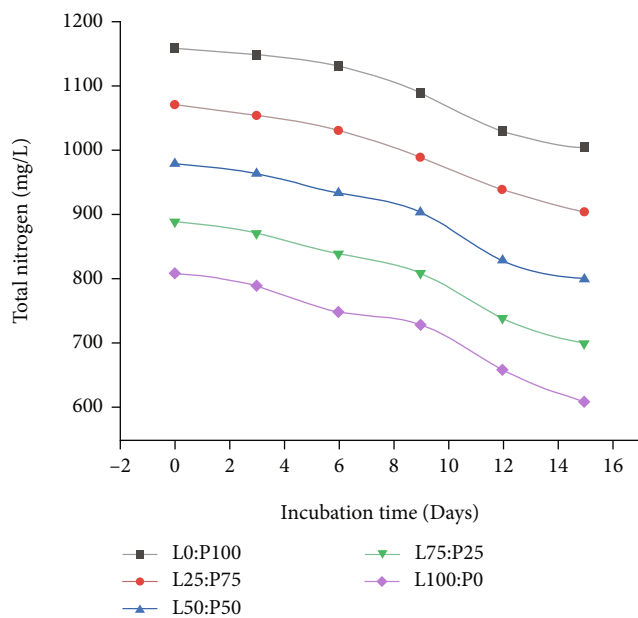
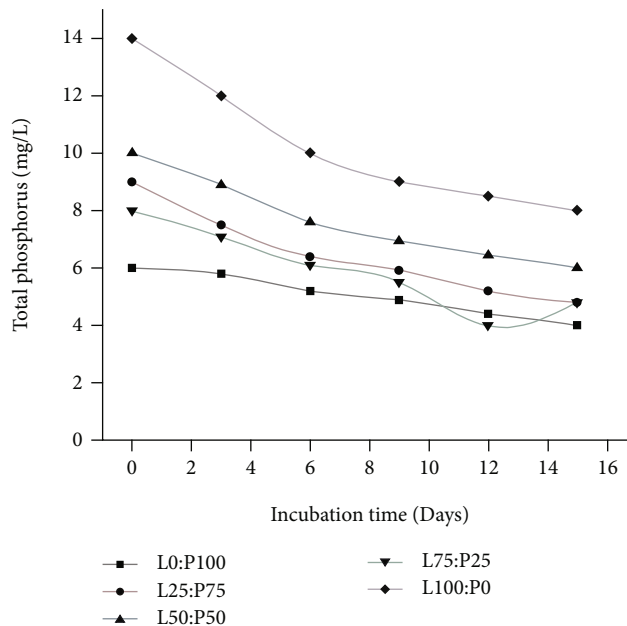FIGURE 1: Removal of total nitrogen from the wastewater.

FIGURE 2: Removal of total phosphorous from the wastewater.

P will vary based on the pH levels of the wastewater. Basically, the microalgae absorb the phosphorous content in the wastewater and utilize them as one of the nutrients. Here, the study has been conducted on two different wastewaters [21, 23]. Compared to the piggery wastewater, the leather industry wastewater has more phosphorous content in them. Both the samples are combined with the configuration of L0:P100, L25:P75, L50:P50, L75:P25, and L100:P0. The maximum phosphorous reported for piggery and leather wastewater was 6 mg/L and 14 mg/L. Compared to the several combinations, L75:P25 recorded lowest levels of phosphorous at the end of day 15 according to Figure 2. As the incubation time increases, the concentration of the phosphorous has been dropped significantly for leather compared to piggery wastewater. Based on the findings, it is more evident that mixing both the wastewater can improvise the removal rates. At day 3, 3.5%, 18%, 11.5%, 11.9%, and 15.3% reduction in the phosphorous has been noticed for the samples L0:P100, L25:P75, L50:P50, L75:P25, and L100:P0. The total removal rate on day 9 was 1.1 mg/L, 3 mg/L, 3 mg/L, 2.5 mg/L, and 5 mg/L; from these stats, it is very evident there is massive reduction in the phosphorous presence in the wastewater. On the incubation period of day 15, the total difference in the phosphorous was 40%, 60%, 50%, 51%, and 54% for the respective samples L0:P100, L25:P75, L50:P50, L75:P25, and L100:P0. The major reduction in the phosphorous has been witnessed for L25:P75 test sample and the second best was reported by L75:P25.

3.3. Effects of Wastewater Concentration on Microalgae Growth.

Figure 3 presents the dry biomass productive of the microalgae at different incubation time in different wastewater combinations. The maximum dry biomass produced was 1.75 g/L. As the incubation time increases, the dry biomass increased massively. At day 3, 0.45 g/L, 0.6 g/L,
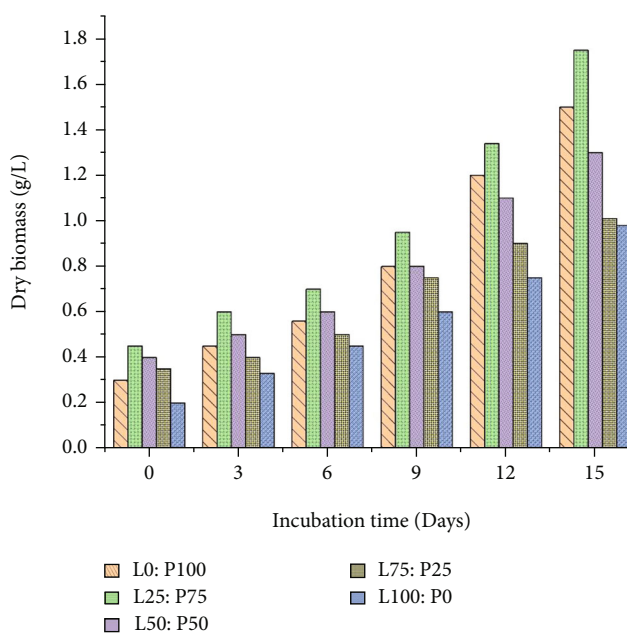

FIGURE 3: Dry biomass production from the microalgae.

0.5 g/L, 0.4 g/L, and 0.33 g/L dry biomass were produced for the wastewater samples L0:P100, L25:P75, L50:P50, L75:P25, and L100:P0. Compared to day 3, day 9 reported massive growth in the microalgae 21%, 15.3%, 18.1%, 22%, and 30.7% [8, 9]. From the findings, it is clear that industry leather wastewater produced higher dry biomass compared to the piggery wastewater. With regard to the combination, L75:P25 reported higher cultural growth. For instance, at day 12, the dry biomass procured from the culture was 1.2 g/L, 1.34 g/L, 1.1 g/L, 0.9 g/L, and 0.75 g/L, respectively. Incubation time of day 15 has witnessed the higher biomass productivity; the respective values are 1.5 g/L, 1.75 g/L, 1.3 g/
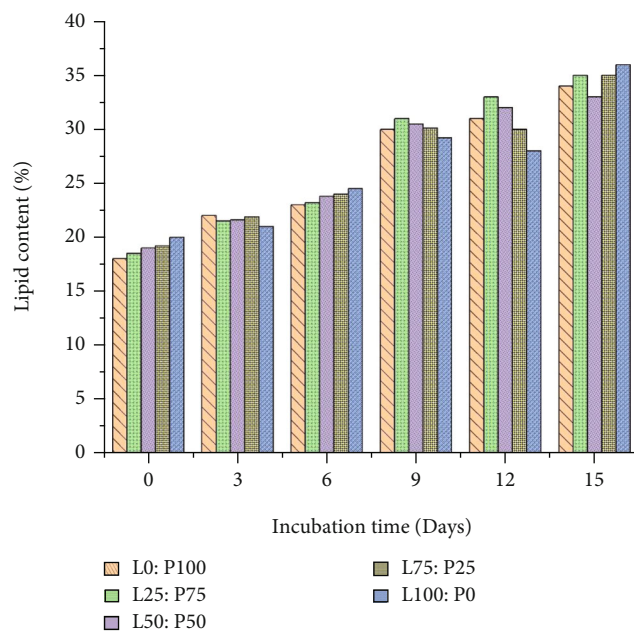
FIGURE 4: Lipid content presence in the cultivated microalgae.

L, 1.01 g/L, and 0.98 g/L. There is a rapid increase in the production of the dry biomass for the L25:P25 that has been observed irrespective of the incubation period. Unlike above, the specimen L50:P50 also showed some positive effects on the dry biomass accumulation. Compared to the leather wastewater, piggery wastewater microalgae growth is 42% higher due to the presence of high nitrogen and less phosphorous.

3.4. Lipid Content in the Cultivated Microalgae. Microalgae lipids are the value-added resource for the production of biodiesel, chemicals, and cosmetics. Figure 4 shows the lipid yield of the microalgae at different cultivated conditions. Based on the procured results, it is identified that the lipid content in the leather wastewater is higher. As the incubation time elevated, the lipid content was increased irrespective of the WW sample. Initially, on day 3, the sample L0:P100 reported 22% of the lipid content which is 1% higher than the piggery cultivated microalgae. Although the increase is minimal, as the incubation time increases, the difference between the lipid from leather WW and piggery WW intensely increased [25, 26]. The samples such as L0:P100, L25:P75, L50:P50, L75:P25, and L100:P0 reported the lipid content of 22%, 21.5%, 21.6%, 21.9%, and 21% on day 3. Samples of day 3 between every sample are marginally different. However, as the incubation time increases, there was a very notable change that had been observed. At the final moment of day 15, the respective lipid content of the samples is 1.5%, 1.75%, 1.3%, 1.01%, and 0.98%, respectively. Lipid content was dramatically improved when they are cultivated in the leather wastewater than piggery [9, 22]. The main reason for the better lipid productivity for the leather WW was absorbed nutrients. With regard to the type of the specimen, at day 9, L25:P25 reported the marginal increase in the total lipid content than the other

samples. After the 9th day, there is no massive improvement in the lipid content that has been noted.

4. Conclusion

The cultivation of microalgae with leather industry and piggery wastewater under various dilution ratios was conducted to remove the heavy metals in the wastewater and derive the value-added products. A set of experimental calibrations were done for the samples and to determine the removal rates of total nitrogen and total phosphorous. In addition to the removal rates of heavy metals, the microalgae growth on the biomass production and the lipid content was also measured. Based on the findings, it is clear that the total nitrogen content was higher for piggery WW compared to the leather WW. The respective concentrations were 1160 mg/L and 810 mg/L. As the incubation time increases, the total nitrogen absorbed by the *Chlorella* sp. augmented. On the other hand, the removal rates of the leather industry WW was 14% higher compared than piggery WW, since the microalgae cultivated by leather WW uptake higher amount of the nitrogen as the nutrient than the leather WW based microalgae. With regard to the phosphorous, the identical behavior has been witnessed. Similar to the total nitrogen, the phosphorus removals rates were larger for the leather WW than piggery WW. The cumulative difference between both the WW was 15%, hence mixing both WW to reduce the total nitrogen and phosphorous content in the wastewater in reasonable pace. Among the different concentration, L75:P25 sample reported 25% of the total nitrogen removal rates and 50% reduction in the total phosphorous. On the other hand, the dry biomass and lipid content were also higher for the leather WW than piggery WW. The maximum dry biomass was obtained for L25:P75 and lipid content for the sample L75:P25. Based on the various results, L75:P25 is more sustainable and it can be a viable solution for WW treatment.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

No conflict of interest and no funding received. All the data were presented inside the manuscript and readers can have them according to request. No animals and humans are involved in the study.

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