Advanced and Sustainable Technologies (ASET) eISSN 2976-2294
Volume 3 (Special Issue), March 2024 [11-17]

Performance of *Chlorella vulgaris* in Phycoremediation of Livestock Effluent

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Received 12 September 2023, Revised 22 September 2023, Accepted 25 September 2023

ABSTRACT

Livestock effluent is known to contain significantly higher concentrations of organic matter and challenging-to-degrade organic compounds compared to urban wastewater. This makes the effluent treatment challenging and adversely affects nearby aquatic environments if improperly treated. Phycoremediation uses microalgae in water and wastewater treatment. This research aim was to evaluate pollutant removal efficiencies, including chemical oxygen demand (COD), total suspended solids (TSS), nitrate (NO_3-N), turbidity, and phosphate (P), using microalgae Chlorella vulgaris (C. vulgaris) cultivation systems for livestock effluent treatment. The biomass weight of C. vulgaris in the cultivation systems was also observed. In this study, C. vulgaris was cultivated in closed cultivation systems in 5 L water bottles with different dilutions (100%, 75%, 50%, and 25%). The water effluent was compared to permissible values using the National Water Quality Standard (NWQS) class II for recreational water use. TSS was significantly removed by 61.97%, while COD and P were removed by 39.1% and 36.4%, respectively. The biomass growth was observed through the dry weight of the C.vulgaris. Therefore, the removal of nutrients from cattle farm effluent by phytoremediation using C. vulgaris demonstrates potential for treatment efficacy.

Keywords: Phycoremediation, *Chlorella vulgaris*, Livestock effluent.

1. INTRODUCTION

The demand for animal-based products has increased significantly, so the livestock industry has also increased substantially [1]. Non-ruminant livestock in Malaysia is the largest population dominated by chicken production, which is 95.9%, and 56.3% are ruminant farming, which is beef cattle farming that is located mostly in West Malaysia. Hence, the estimated manure excreted from this livestock in Malaysia is 17.745 million tons for 2020 [2]. Livestock wastewater is highly concentrated with organic matter, increased nutrients, and suspended solids. Significantly, the increased waste product due to livestock production has various implications and impacts on the environment, climate changes, and greenhouse gases such as water pollution and eutrophication [3]. Thus, the livestock wastewater or effluent required to go through the treatment process to reduce the environmental impact, mitigate potential risks to human health, and prevent further contamination of surface water and groundwater; it is crucial to address these activities as they involve direct discharge into these water sources.

Phycoremediation is one of the bioremediation processes specifically used to treat wastewater as a water treatment that uses algae since it provides tertiary bio-treatment at the same time while potentially producing valuable biomass that can help for various applications apart, while it removes organic compounds, pathogens, organic nutrient, emerging contaminants, and heavy

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metals [4]. Algae, known as eukaryotic species, is a media that has been used in phycoremediation treatment. Most algae, including Cyanobacteria and macroalgae in treatment wastewater including *Chlorella sp.*, *Chlamydomonas sp.*, *Spirulina sp.*, *Oscillatoria sp.*, *Nostoc sp.*, and *Scenedesmus sp*. In this study, C. *vulgaris* was used as a phycoremediator of livestock effluent. The phycoremediation process with the cultivation of algae in wastewater offers the combined advantages of greenhouse gas mitigation, wastewater treatment, and simultaneously creating algal biomass. This biomass can be utilized for various applications such as protein supplements, food additives, bioenergy resources (biogas and biofuels), pharmaceuticals, cosmetics, and other valuable chemicals [5].

Previous researchers also use *C. vulgaris* in livestock effluent, such as cattle manure and slaughterhouse wastewater [6]. During photosynthesis, *C. vulgaris* uses light and carbon dioxide (CO_2) as a source of energy and carbon source to uptake phosphorus and nitrogen for their cellular respiration [7]. Hence, it helps reduce the concentration of nutrients, increases the death of pathogenic organisms, and reduces the need for mechanical aeration during wastewater treatment to meet the dissolved oxygen (DO) requirement. Due to its robustness, high oil content, mixotrophic culturing environment, high growth rate under varied complicated settings, and tolerance to high levels of heavy metals, *C. vulgaris* offers enormous potential as future industrial bioenergy producers and for phycoremediation of different wastewater quality [8].

2. MATERIAL AND METHODS

2.1 Experimental setup

In this research, a closed system using 5 L water bottles was set up to cultivate *C. vulgaris*. Four bottles were used, each with different ratios of filtrated water to livestock effluent for dilution, as shown in Table 1. Treatment 1 (T1) was served as a control with no livestock effluent added. Dilution is required to avoid inhibiting microalgae growth due to high organic concentration in the effluent [9]. The experimental setup is illustrated in Figure 1. About 25 mL volume of *C. vulgaris* was added to each bottle to cultivate. Aeration is provided by an air pump connected to an air-line tube with an air stone, creating oxygen-filled bubbles for circulation. To facilitate photosynthesis, LED lights were used for a consistent 10-hour per day using a timer. Temperature was controlled with a thermostat and fan, maintaining an optimal temperature range of 25 °C - 28 °C. The duration of cultivation was set for 12 days, followed by *C. vulgaris* harvesting. The experiment was replicated twice.

Table 1: Ratio (%) of treatment water.

Treatment	Filtered water	Livestock effluent
T1	100% (5 L)	0%
T2	75% (3.75 L)	25% (1.25 L)
T3	50% (2.5 L)	50% (2.5 L)
T4	25% (1.25 L)	75% (3.75 L)

2.2 Samples and Analysis

The livestock effluent was collected from a buffalo pond at Taman Agrovet in Padang Besar, Perlis. The samples were then examined for their physiochemical properties, including pH, chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), turbidity, dissolved oxygen (DO), phosphate (P), and nitrate (NO_3-N) before being used in the microalgae cultivation systems. The samples were analyzed using a multiparameter probe (Hydrolab DS5X and YSI) and HACH Spectrophotometer DR2900 according to APHA procedures [10].

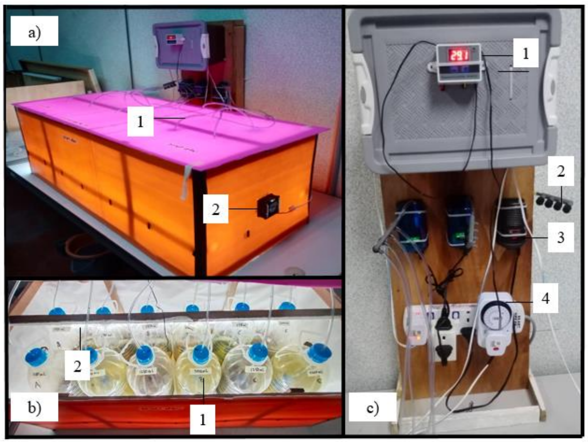

Figure 1: Experimental setup, a) Outer look of closed-system: 1. Air tube line, 2. Fan 5V, b) Inside of closed-system: 5 L culture bottle, 2. LED light, c) Control panel: 1. Thermostat, 2. Control airflow valve, 3. Air pump, 4. Timer.

During the *C. vulgaris* cultivation, the samples were collected and examined for pH, COD, P, and NO_3-N removal every three days until the end of the cultivation duration. By the end of 12 days, the *C. vulgaris* were harvested by filtration method using a Whatman glass microfiber filter with a diameter of 25 mm and a gooch crucible of 25 mL to analyze the biomass weight. Most microalgae strains are harvested using filtration techniques, which produce about 5-27% of solid concentration after harvest and recover 70–90% of the algal biomass [11]. The COD, P, and NO_3-N removal efficiency (RE) were evaluated using the following formula:

$$RE\ (\%) = \frac{(Co - Ci)}{Co} x\ 100\% \qquad (1)$$

where *Co* is the nutrient concentration of the influent, and *Ci* is the nutrient concentration of the effluent. The significance of the treatments was analyzed using an ANOVA statistical analysis.

2.3 Chlorella vulgaris Biomass

The *C. vulgaris* biomass was observed by using the dry weight method. First, a pre-weighed filter paper is prepared by weighing a clean and dry filter paper using a precision balance, and its initial weight is recorded. Then, a 20 mL volume of the *C. vulgaris* culture was collected and adjusted based on the expected biomass concentration. The culture was then poured onto the pre-weighed filter paper, allowing the liquid to pass through while retaining the biomass on the filter. The filter paper with the biomass was then carefully transferred to a drying oven, set at a temperature of 60 °C. The filter paper with the biomass was dried in the oven until a constant weight was achieved, which may take several hours. After cooling to room temperature, the filter paper with the dried biomass was reweighed using the precision balance, and the final weight was recorded. The dry weight of the *C. vulgaris* biomass was calculated by subtracting the initial weight of the filter paper from the final weight. This calculation provides an accurate measure of the solid component of the biomass without the influence of water content. The dry weight measurement obtained from the sample represents the mass of *C. vulgaris* biomass [12].

3. RESULTS AND DISCUSSION

3.1 Livestock effluent physicochemical properties

Table 2 presents the physicochemical properties of the livestock effluent collected from Taman Agrovet, Padang Besar, Perlis. The parameters examined include pH, DO, COD, TSS, turbidity, P, and NO_3-N. The concentration values were compared with the National Water Quality Standard

(NWQS) class II for recreational water use as regulated by the Department of Environment (DOE), Malaysia [13].

The pH and TSS values of the livestock effluent samples were within the acceptable range set by NWQS. However, the concentration of NO_3-N, P, COD, and turbidity values exceed the NWQS class II limit, indicating a nonconformity of the requirements. Therefore, the livestock effluent samples require further treatment to enhance the pollutant water quality. The availability of nutrients in the samples offers the suitability of the effluent to be utilized as a nutrient source in *C. vulgaris* cultivation.

Table 2: Initial concentration reading for each parameter of physicochemical properties compared to National Water Quality Standard Malaysia class II.

Parameter	Unit	National Water Quality Standard	Initial concentration
pH	-	6-9	8.14±0.48
Turbidity	NTU	50	2673±0
TSS	mg/L	50	0.0058±0.0017
NO_3-N	mg/L	7	36.43±1.41
P	mg/L	0.2	3.35±0.26
DO	mg/L	5-7	4.39±0.19
COD	mg/L	25	596±244

3.2 Phycoremediation treatment efficiency

Livestock effluent wastewater commonly contains high concentrations of COD [14]. The presence of high COD concentration may result in oxygen depletion, which can harm aquatic organisms and impact the taste and odor of the water. Figure 2 displays a line graph representing COD concentration over 12 days in phycoremediation treatment using *C. vulgaris*. The graph shows a significant removal in COD during the initial three days for all systems and achieved the highest removal of nitrate in the T3 treatment with 39.10% removal. In contrast, T1 maintains relatively stable COD removal in the control systems, indicating minimal reduction since no effluent is present. The removal for T2 and T4 demonstrate noticeable declines in COD values over time. This indicates that *C. vulgaris* might assimilate the organic compounds in the effluent, resulting in a drop in COD concentration within the three days of the cultivation. The removal percentage was significantly lower than [14], where the COD achieved 62.30% COD removal by cattle farm effluent. Therefore, the COD in livestock effluent was utilized by *C. vulgaris*. Statistical analysis ($P \leq 0.05$) confirms a significant difference in COD reduction among the treatment percentages.

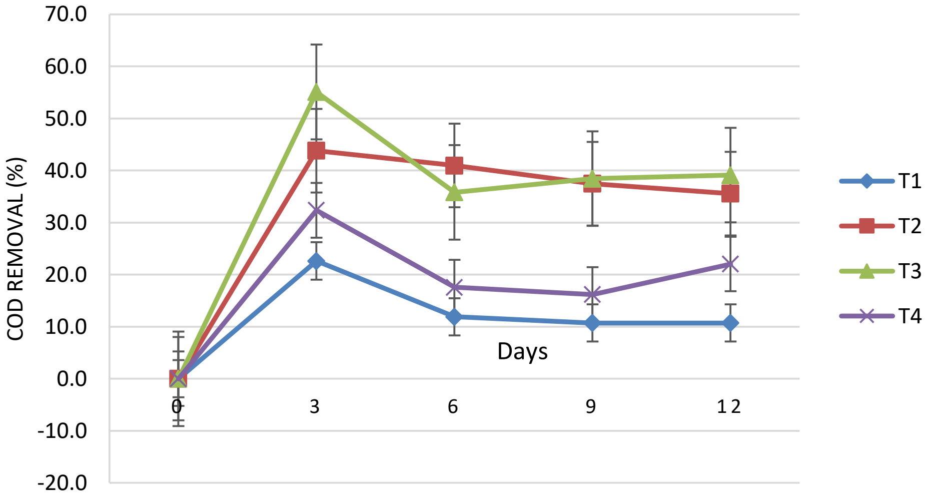

Figure 2: COD removal.

Naturally, nitrogen is accessible in the form of nitrate, urea, ammonium, and peptones. This study examines the nitrate concentration as a nitrogen function [15]. Nitrogen is a macronutrient for the growth of microalgae. Figure 3 displays the nitrate removal during the phycoremediation treatment using different dilutions. The removal starts growing after 3 days for T3 and T4. The T2 treatment shows the highest nitrate removal efficiency with 68.3%, while T4 has the lowest removal efficiency with 36.7%. Even if the effluent is unavailable in T1, the nitrate concentration rises slightly until day 9. Microalgae function to convert inorganic nitrogen to organic form by an assimilation process that can be performed by all eukaryotic microalgae, which require inorganic nitrogen in the form of nitrite (NO_2^-), nitrate (NO_3^-), and ammonia (NH_4)[11]. The maximum nitrate removal rate can be achieved up to 84.68% by cultivating *C. vulgaris* in distillery wastewater [16]. Statistical analysis suggests no significant difference ($p>0.95$) among the treatments.

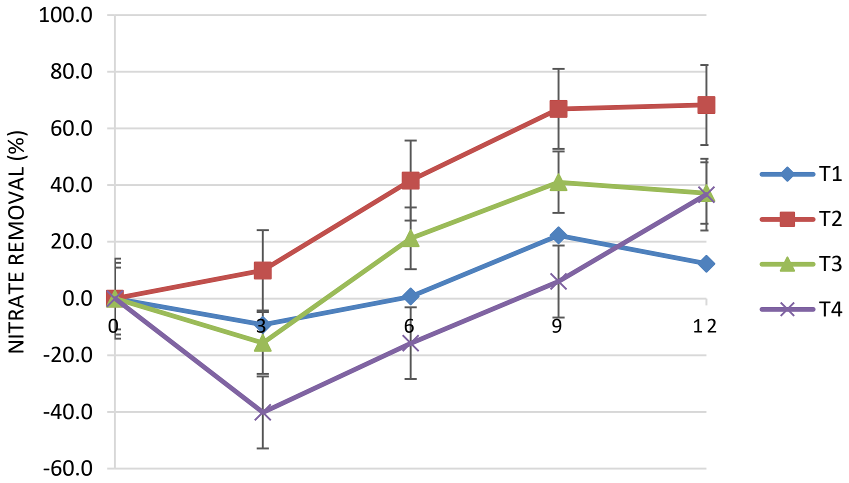

Figure 3: Nitrate removal.

Phosphorus is another key element for microalgae growth and other cellular activities[16]. Figure 4 displays a bar graph illustrating P concentration in livestock effluent on the initial day (Day 0) and Day 12. By Day 12, the T1 treatment decreased slightly to 2.96 with 17.78% removal efficiency. Significant decreases were observed in the T2 and T3 treatments, with 90.67% and 36.39% removal, respectively. The T4 treatment showed the lowest removal, with 20.97% removal. The bar graph indicates that T2 and T3 effluent treatments were more effective in reducing P levels, with no significant difference among treatment percentages according to the ANOVA test ($p> 0.05$). The substantial reduction in phosphorus levels in the *C. vulgaris* cultivation systems is because this nutrient has been absorbed by *C. Vulgaris* microalgae as a necessary nutrient for its growth. Furthermore, it can be said that phosphorus concentration is often a limiting nutrient in microalgae growth [12], and the cells can integrate and accumulate this nutrient, declining the amount of phosphate in the wastewater.

3.3 Biomass weight observation

Figure 5 presents a graph depicting the dry weight of *C. vulgaris* biomass in livestock effluent during phycoremediation, considering different concentrations. Analysis reveals trends in biomass growth and the influence of livestock effluent dilution on dry weight. Initially, all treatments displayed low biomass compared to the control, T1. By Day 3, biomass increased, particularly in the T2, T3, and T4 concentrations. On Day 6, biomass continued to rise, with the T4 concentration exhibiting the highest dry weight. On Day 12, biomass levels stabilized, with T3 and T4 displaying similar dry weights. These findings suggest that higher livestock effluent concentrations positively affect *C. vulgaris* biomass growth during phycoremediation. The statistical analysis P-value supports the significance of this treatment.

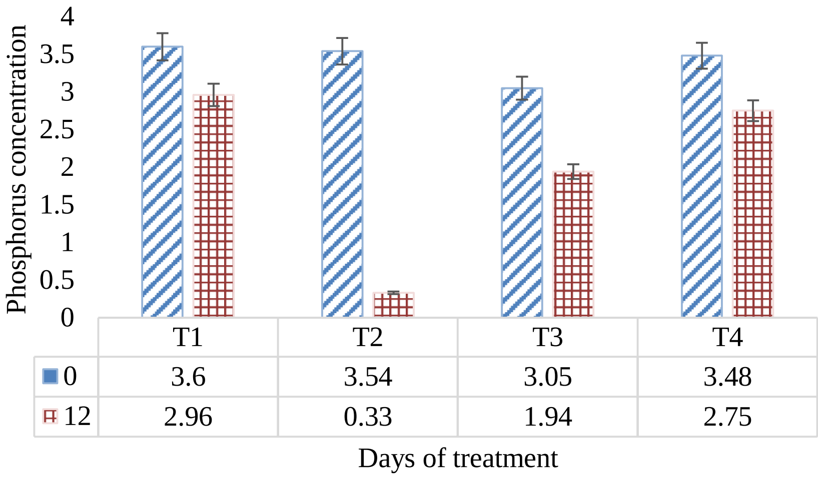

Figure 4: Phosphorus concentration.

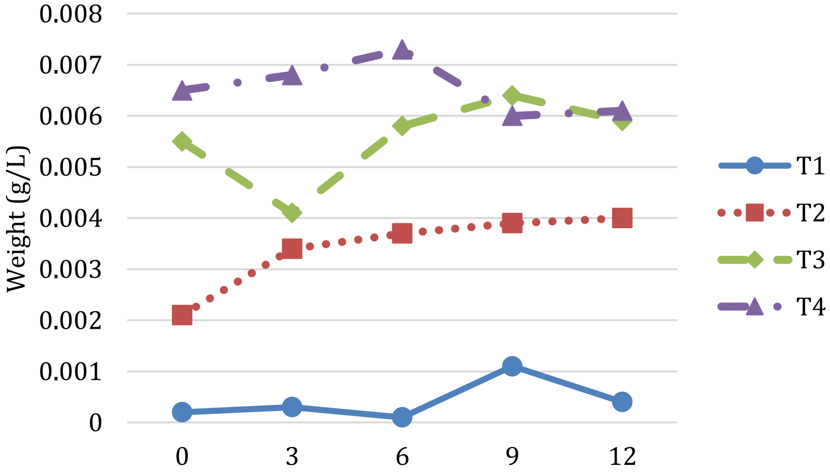

Figure 5: Biomass Dry weight *C. vulgaris.*

4. CONCLUSION

The experiment treated livestock effluent using *C. vulgaris* for phycoremediation. Physicochemical properties were characterized and compared to National Water Quality Standard class II. Parameters assessed included pH, nitrate, turbidity, TSS, TDS, P, COD, and DO. pH, TSS, and DO met standards, while further treatment was needed for other parameters. The phycoremediation treatment of livestock effluent using *C. vulgaris* showed promising results with 39.10% COD removal, 68.3% nitrate removal, and 90.67% phosphorus removal. Biomass production of *C. vulgaris* was highest on day 6. Overall, the experiment demonstrated the potential of phycoremediation using *C. vulgaris* for treating livestock effluent. It highlighted the influence of various factors on the growth and removal efficiency of pollutants, emphasizing the importance of proper dilution levels and favorable conditions for successful remediation.

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