Makerere University Journal of Agricultural and Environmental Sciences
Vol. 15(1). pp. 98 - 113, 2026

pISSN: 1563-3721
eISSN: 2958-4795

https://doi.org/10.70060/mak-jaes-2026-186

Received: *05 January 2026*; *Accepted*: *14 June 2026*

Bioremediation potential of Lake Victoria microalgae species in the treatment of dairy processing facility wastewater

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Abstract

Dairy wastewater poses a threat to the health of aquatic ecosystems in Sub-Saharan Africa (SSA) due to its pollution potential. Microalgae present a promising bioremediation potential by removing nutrients and organic carbon from wastewater. However, there is paucity of information supporting the potential of microalgae in treatment of dairy wastewater. The study compared the efficiency of microalgae species in removing organic and nutrient pollutants from wastewater. Wastewater samples were collected from the Uganda Industrial Research Institute (UIRI) dairy processing plant. Microalgae genera were isolated from the samples *via* single-cell micropipette method. *Scenedesmus* spp., *Chlorella* spp. and *Selenastrum* spp. were successfully isolated and cultured. *Scenedesmus* spp. showed excellent performance in nutrient removal and biomass production, achieving reductions of 92.6% in chemical oxygen demand, 88.2% in organic carbon, 80.9% in total nitrogen, and 90.8% in total phosphorus from initial levels of 969.33, 148.94, 33.21 and 86.58 mgL^{-1}, respectively. Over a 12-day cultivation period, *Scenedesmus* spp. achieved a peak dry biomass yield of 1.04 gL^{-1}, at an average dry biomass generation rate of 0.087 g L^{-1} day^{-1}. *Chlorella* spp., performance was not far behind that of *Scenedesmus* spp. The results achieved highlight the potential of microalgae in treating diary wastewater with simultaneous biomass production. Microalgae based systems would be a sustainable solution for dairy wastewater treatment, aligning with Goal Six of the United Nation's Sustainable Development.

Key words: *Chlorella* spp., chemical oxygen demand, organic carbon, nutrients, *Scenedesmus* spp., *Selenastrum* spp.

Introduction

The dairy industry stands out as a significant contributor of pollution due to its large volumes of effluent, estimated at 10 liters per liter of processed milk (Akansha *et al*., 2020). Specifically, dairy wastewater is loaded with organic matter, i.e., fats and proteins; that elevate its chemical oxygen demand (COD) and concentration of N and P, all of which contribute to water pollution and eutrophication in receiving water bodies (Daneshvar *et al*., 2019; Gramegna *et al*., 2020*)*. Conventional wastewater treatment methods are effective at lowering COD, and suspended solids but often fail to adequately lower nitrogen and phosphorus. This highlights the need for innovative and sustainable approaches to achieve complete nutrient removal (Choi, 2016).

Wastewater bioremediation using microalgae treatment, has become a promising and eco-friendly alternative to conventional methods (Wang *et al*., 2016). Microalgae refers to microscopic, photosynthetic organisms that consist of a single cell; often referred to as phytoplankton. They inhabit almost all aquatic environments on earth; ranging from freshwater lakes to salty oceans (Choi, 2016). Microalgae assimilate nutrients such as N and P from wastewater and convert them into biomass *via* photosynthesis (Choi, 2016). This process not only lowers the pollution risk of wastewater, but also produces useful byproducts like biofuels, animal feed, and biofertilisers (Daneshvar *et al*., 2019). Conversely, microalgae need only minimal resources (water, light and CO) to thrive. Since these are plentiful in wastewater environments, this treatment approach is both cost-effective and sustainable (Abdel-Raouf *et al*., 2012).

Several studies have investigated dairy wastewater treatment using microalgae, each focusing on different aspects of the process. Choi (2016) assessed how effectively *Chlorella Vulgaris* treated dairy wastewater, noting that the process depended on the algae's growth rate, system conditions, and biomass harvesting. The main focus of their study was on producing biodiesel from the biomass obtained. Talapatra *et* *al*. (2023) investigated the co-cultivation of *Tetraselmis indica* with *Pseudomonas aeruginosa* in dairy wastewater, focusing on biomass production and chlorophyll-a extraction for biofuel. Al Ahmed (2014) explored the use of *Spirulina ssp.* to treat industrial dairy wastewater, successfully reducing nutrients, notably nitrates (80%) and phosphorus (72%). Most of these studies research concentrated on a single species. Gramegna *et al*. (2020) studied four species of *Chlorella* and a genetically modified strain of *Chlamydomonas reinhardtii* to assess their nutrient absorption from dairy wastewater. They found that *C. protothecoides* achieved the highest biomass output. Although these studies hinted at the effectiveness of different microalgae species in bioremediation of dairy wastewater, they primarily focused on biofuel production, biomass yield, or enzyme creation. There are few that have comprehensively assessed nutrient removal efficiencies across different species. This study aimed to assess the biomass accumulation of different locally sourced microalgae species, and their potential in bio-remediation of dairy wastewater.

Materials and methods

Dairy wastewater characterization

Dairy wastewater samples were taken from the outlet of the Uganda Industrial Research Institute (UIRI) dairy processing facility in Uganda (0°20'10.0"N, 32°37'31.0"E). The main products at UIRI are pasteurized milk, yoghurts, ice creams, cottage cheese and soy products. Wastewater sampling followed the U.S. EPA's LSASD standard procedure (McHugh, 2023), whereby, three wastewater samples were collected on three different days with a two days interval, each in triplicates. Five-liter samples were collected using clean plastic scoops, labeled and kept in cooler boxes for laboratory analysis. The characterization followed the methodologies of Leonard *et al*. (2018), and Alalam *et al*. (2021). The wastewater samples were laboratory tested for pH, temperature, dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS), chemical oxygen demand (COD), total nitrogen, total phosphorus, and total organic carbon following standard procedures in Table 1.

Isolation of microalgae species

Freshwater samples were collected from Ggaba, a landing site on the shores of Lake Victoria, Uganda, at coordinates 0.258208°N, 32.637251°E. This site was selected based on the presence of a greenish coloration, indicating the potential presence of microalgae. Freshwater sampling followed the procedure for lake or stream surface water chemistry (Musselman, 2012).

Microalgae isolation was carried out using single-cell micropipette isolation techniques, under a Hund Wetzlar inverted microscope (PH40, bino, 100x-400x). The target

Table 1. Procedures for characterization of the dairy wastewater used in this study

Parameter	Method used	Brief procedure	Equipment used	Reference
pH	Instrumental field measurement	The pH of the sample was measured onsite using the probe of a calibrated Aquaread Aquameter. The probe was rinsed with distilled water before and after each measurement to ensure accuracy. Readings were taken after stabilization of the display.	Aquaread Aquameter AP-800-D, England	House (2024)
Temperature	Instrumental field measurement	The temperature was recorded simultaneously with pH using the same multimeter. The sensor was submerged in the wastewater sample, and the temperature reading was noted after stabilisation.	Aquaread Aquameter AP-800-D, England	House (2024)
Dissolved Oxygen (DO)	Instrumental field measurement	DO was measured in situ using the dissolved oxygen probe of the Aquameter. The sensor was calibrated beforehand, submerged in the sample, and allowed to stabilise before recording the value.	Aquaread Aquameter AP-800-D, England	House (2024)
Electrical Conductivity (EC)	Instrumental field measurement	EC was determined by immersing the EC probe of the Aquameter into the wastewater sample. Readings were automatically adjusted for temperature and displayed directly on the meter screen.	Aquaread Aquameter AP-800-D, England	House (2024)

Table 1. Contd.

Parameter	Method used	Brief procedure	Equipment used	Reference
Total Dissolved Solids (TDS)	Instrumental field measurement	TDS was indirectly obtained from EC measurements using built-in conversion settings in the multimeter. The EC probe was rinsed and dipped into the sample, and the TDS value displayed on screen.	Aquaread Aquameter AP-800-D, England	House (2024)
Chemical Oxygen Demand (COD)	Standard method 5220D - Closed Reflux Colorimetric	A 2.5 mL sample was placed into a digestion tube, mixed with 1.5 mL of potassium dichromate digestion solution and 3.5 mL of H_2SO_4/Ag_2SO_4. Tubes were tightly sealed and heated at 150°C for 2 hours. After cooling, the absorbance was read at 600 nm using a spectrophotometer. COD was calculated using a calibration curve prepared from KHP standards.	Digestion tubes, Oven, Spectrophotometer	Baird *et al*. (2012)
Total Nitrogen (TN)	Kjeldahl Method	A 20 mL sample was digested with concentrated H_2SO_4 and a catalyst (K_2SO_4/Se) at 370°C for 3 hours. The digest was cooled, diluted, and distilled in the presence of NaOH. Ammonia was captured in boric acid and titrated with 0.01N H_2SO_4 to pH 5.0 using a pH meter. The volume titrated was used to calculate total nitrogen.	Block digester, Distillation unit, pH meter	Estefan (2013)

Table 1. Contd.

Parameter	Method used	Brief procedure	Equipment used	Reference
Total Phosphorus (TP)	Colorimetric Method	A 10 mL sample was digested with nitric and perchloric acid at 180°C until clear. After cooling and dilution, the digest was reacted with ammonium molybdate-ascorbic acid reagent. The developed blue color was measured at 882 nm using a spectrophotometer. Phosphorus was quantified from a calibration curve prepared using standard solutions.	Block digester, Spectrophotometer	Estefan (2013)
Total Organic Carbon (TOC)	Potassium Dichromate Oxidation-Titration	A 0.5 mL sample was digested with 5 mL of 1N K, Cr, O‡ and 7.5 mL H, SO,, at 155°C for 30 minutes. After cooling, the digest was titrated with 0.2 M ferrous ammonium sulfate using ferroin indicator until a color change indicated the endpoint. TOC was calculated from the volume of titrant used.	Block digester, Magnetic stirrer, Burette	Okalebo *et al.* (2002)

cells were collected from freshwater samples; with micropipettes and transferred into sterile growth media (Cell-hi WP, Varicon Aqua Solutions) on a petri dish, following standard algal culturing methods (Andersen, 2005). The isolated cells were cultured in sterile conditions, within a laminar flow hood, using Cell-hi WP growth media contained in conical flasks for growth.

Microalgae were identified to genera following their morphological characteristics under a Hund Witzlar inverted microscope (PH40, bino, 100x-400x). Identification followed standard guides, including a guide to the identification of microscopic algae in South African freshwaters (Van Vuuren *et al*., 2006), an annotated key to algae of the United States (Greeson, 1982), and microalgae strain catalogue (Figueroa-Torres *et al.*, 2016). Key criteria included cell shape, size, wall structure, chloroplast arrangement, and colony formation (Figueroa-Torres *et al.*, 2016).

Comparative nutrient removal efficiencies by selected microalgae genera

Of the recovered microalgae species, three genera *Chlorella* spp*., Scenedesmus* spp*., and Selenastrum* spp. were selected for further inquiry. The experiment was conducted using synthetic photobioreactors. For each species, one liter as the volume, containing dairy wastewater and 10% microalgae inoculum made up a treatment. Treatments were laid out in a completely randomised experimental design. The treatments were tested against a control which was dairy wastewater without microalgae. This was maintained to account for any nutrient removal by abiotic processes. These photobioreactors were maintained under controlled environmental conditions, including light intensity (150 µmol m^{-2} s^{-1}) and temperature (25/ ±/ 2°C). Each treatment was replicated three times. The study was repeated two times under similar environmental conditions.

Nutrient removal efficiency was evaluated by pollutant concentrations before and after the experiment; and calculated using Equation 1.

Removal efficiency: $RE(\%) = \frac{(C_i - C_f)}{C_i} \times 100$ .... Equation 1

Where: C_i = initial concentration; and C_f = final concentration of target pollutants

The resulting data were used to determine the most effective microalgae species for nutrient removal from dairy wastewater.

Microalgae biomass yield

The biomass yield was measured using the APHA method 2540 D for total suspended solids, dried at 103-105 °C (Baird *et al.*, 2012). The samples were collected, filtered and the microalgae biomass oven dried at 105 °C for one hour. The total biomass content was determined from the dry weight of the filtered microalgae cells.

Data analysis

The data collected were analysed using R Statistical software, Version 4.4.2. Prior to analysis, the Shapiro-Wilk test was conducted to assess the normality of the dataset. Where assumptions of normality were not met, data were standardised appropriately. A one-way ANOVA was used to test for differences in nutrient remediation among the microalgae genera. Where significant differences were found, a Tukey's HSD post-hoc test was applied to identify exactly which pair of group means differ. Pearson's correlation was performed to assess the link between nutrient removal efficiency and microalgae biomass yield. All analyses used a significance level of 0.05.

Results

Genera of microalgae in Lake Victoria freshwater

Six microalgae genera were identified from the freshwater samples collected from Lake Victoria. These included *Scenedesmus* spp., *Ankistrodesmus* spp., *Selenastrum* spp., *Chlorella* spp., *Nitzschia* spp., *and Navicula* spp. After isolation and cultivation, only *Scenedesmus* spp., *Chlorella* spp., *and Selenastrum* spp. showed growth potential in the laboratory, making them suitable candidates for further experiments.

Pollutants removal efficiencies

The results of bioremediation performance of the tested microalgae genera are presented in Table 2. The results demonstrated significant reductions in both organic and inorganic contaminants, with all species exhibiting notable removal efficiencies. Organic pollutants were represented by chemical oxygen demand (COD), and organic carbon (OC). COD is an analytical measurement used to quantify the amount of organic and inorganic pollutants in water. The test primarily targets organic pollutants (such as sugars, fats, proteins, and synthetic chemicals) and certain oxidizable inorganic substances. A high COD value directly indicates a heavy load of these pollutants. The value of COD of 969.33 mg L^{-1} of the initial sample was very high by allowable standards. The results show that chemical oxygen demand and organic carbon, were effectively reduced by the microalgae. Total Dissolved Solids (TDS), total Nitrogen (N), and total Phosphorus (P) were the inorganic pollutants measured, and they were variably removed by the studied microalgae (Table 2).

Table 2. Wastewater pollution removal efficiencies of the different microalgae genera

No.	Parameter	Initial concentration (mg L^{-1})	Nutrient removal efficiency (%) by microalgae Genera			
			Control	*Chlorella*	*Scenedesmus*	*Selenastrum*
1.	DO	1.46	8.0 ± 0.9^{b}	27.1 ± 1.1^{a}	26.0 ± 0.3^{a}	27.1 ± 0.7^{a}
2.	TDS	216.67	8.4 ± 1.6^{c}	33.7 ± 0.6^{a}	26.4 ± 1.2^{b}	25.6 ± 1.3^{b}
3.	COD	969.33	24.4 ± 1.8^{b}	92.8 ± 0.2^{a}	92.6 ± 1.2^{a}	91.0 ± 0.2^{a}
4.	Total nitrogen	33.21	18.2 ± 0.3^{c}	75.3 ± 0.7^{a}	80.9 ± 1.2^{a}	45.5 ± 1.9^{a}
5.	Total phosphorus	86.58	75.1 ± 1.6^{b}	89.3 ± 0.4^{a}	90.8 ± 0.6^{a}	88.8 ± 1.5^{a}
6.	Organic carbon	148.94	26.2 ± 0.9^{c}	84.2 ± 1.8^{b}	88.1 ± 1.2^{a}	82.5 ± 1.7^{b}

Values are mean ± standard deviation. Means within the same row followed by different superscript letters are significantly different at $P < 0.05$ (Tukey's HSD test). COD: chemical oxygen demand; DO: dissolved oxygen; TDS: Total Dissolved Solids

Microalgae biomass yield

The cultivation of microalgae in dairy wastewater resulted in varying biomass accumulation over time among the three species (Fig. 1). *Scenedesmus* spp. exhibited the highest biomass yield, reaching 1.04 g L^{-1} by day 12. This was followed by *Chlorella* spp. at 0.84 g L^{-1} and lastly by *Selenastrum* spp. at 0.60 g L^{-1}. The control group showed minimal biomass accumulation, trailing at 0.28 g/L by day 12. *Scenedesmus* spp. was the most promising genera for biomass production in dairy wastewater.

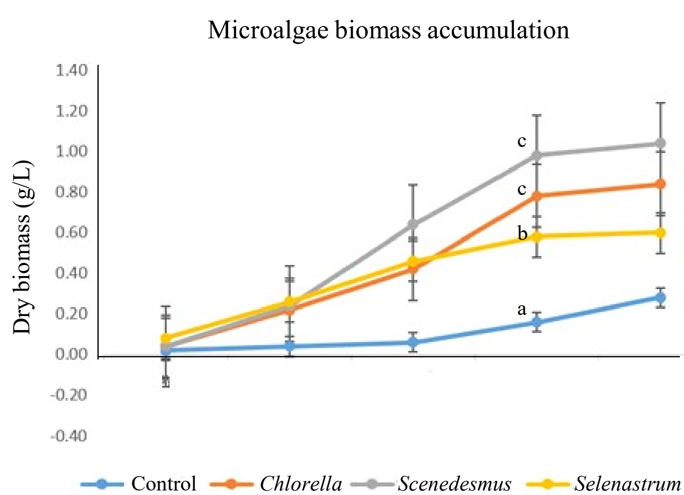

Figure 1. Biomass accumulation of microalgae species cultivated in dairy wastewater over 12 days. Values represent mean ± standard deviation. Different letters at each time point indicate statistically significant differences among treatments ($P < 0.05$, Tukey's HSD test).

The Pearson correlation coefficients (r) for the relationship between nutrient removal efficiency and microalgae biomass yield are presented in Table 3. The results revealed a strong positive correlation between biomass yield and the removal efficiencies of Total N, Total P, and Organic Carbon.

Table 3. Correlation between nutrient removal efficiency and microalgae biomass yield in a wastewater bioremediation experiment ($P<0.05$)

Parameter	Correlation coefficient (r)	Sig. (P)
Dissolved oxygen (DO)	-0.85	0.1208
Total dissolved solids (TDS)	0.14	0.0049
Chemical oxygen demand (COD)	0.85	0.0016
Total nitrogen	0.95	0.000025
Total phosphorus	0.95	0.0107
Organic carbon	0.96	0.0047

Discussion

After isolation and cultivation, only *Scenedesmus* spp., *Chlorella* spp., *and Selenastrum* spp. showed growth potential in the laboratory, making them suitable candidates for further experimentation. The successful isolation and subsequent cultivation of these three microalgae species, point to their adaptability and resilience in varied aquatic environments. Their ability to thrive in both lake freshwater and culture media environments, highlights their potential for wastewater treatment applications. Several other studies have worked with microalgae isolated from freshwater for wastewater treatment. Gumbi *et al*. (2021) isolated *Chlorella* spp. from a freshwater stream and used it to treat poultry and dairy wastewater. *Chlorella* spp. showed phytoremediation potential achieving removal efficiencies of 85-95% for N and 35-93% for P, and biomass yields of up to 1.28 g L^{-1}. This highlights the potential for indigenous micro algae species for wastewater treatment applications. Similarly, Morando-Grijalva *et al*. (2020) isolated *Chlorella vulgaris* and *Chlamydomonas sp.* from a freshwater turtle hatchery and cultivated them in aquaculture wastewater. The study reported high nutrient removal efficiencies, with nitrate and phosphate removal reaching up to 94.6 and 97.9%, respectively for *Chlorella vulgaris*, and biomass productivity of 0.10 g L^{-1} d^{-1}. These results demonstrate the effectiveness of freshwater microalgae in utilising wastewater as a growth medium; while simultaneously reducing nutrient loads. In addition, Kumar *et al*. (2017) and El-Sheekh *et al*. (2018) successfully isolated *Scenedesmus sp.* from freshwater environments and evaluated their growth performance under varying culture conditions. Kumar *et al*. (2017) identified *Scenedesmus rubescens* and reported optimal biomass productivity of up to 1.39 mg ml^{-1}. In this study, *Scenedesmus* spp. yielded an average biomass of 1.04 g L^{-1} in 12 days.

This superior performance of *Scenedesmus* spp. suggests that it should be prioritized in large-scale wastewater treatment systems, particularly in high-rate algal ponds and photobioreactors, where rapid nutrient removal and biomass generation are required. These results conform to those of Silambarasan *et al*. (2023) and Pandey *et al*. (2024) who reported maximum biomass yields of 3.44 and 1.93 g L^{-1}, respectively, demonstrating *Scenedesmus'* high nutrient assimilation efficiency. The results obtained further align with findings from Talapatra *et al*. (2023) who reported a maximum biomass yield of 1.454 g L^{-1} for *Tetraselmis indica* and *Pseudomonas aeruginosa* in dairy wastewater, and Choi (2016) who achieved a biomass yield of 1.23 g L^{-1} using *Chlorella vulgaris*. Pandey *et al*. (2019) and Handayani *et al*. (2020) also reported maximum biomass yields of 1.2 g L^{-1}. These findings suggest that *Scenedesmus* spp. are robust and can be optimized for high productivity, making them suitable for large-scale applications such as biofuel production and wastewater treatment. The successful isolation and growth of *Scenedesmus* spp. from Lake Victoria align with these findings, further confirming its suitability as a candidate genera for nutrient removal and biomass generation in dairy wastewater systems. The consistency between the present findings and previous studies underscores the importance of isolating locally adapted microalgae species. Such species are more likely to perform effectively under region-specific environmental conditions, thereby enhancing the efficiency and sustainability of wastewater treatment processes.

The reductions in pollutants achieved in this study exceeded those reported by Handayani *et al*. (2020) who evaluated simultaneous dairy wastewater treatment and biomass production using *Chlorella vulgaris.* In their study, removal efficiencies of 77% for total nitrogen and 78% for total phosphorus were achieved within 10 days, alongside a maximum biomass yield of 1.2 g L^{-1} attained after 18 days. In this study, *Scenedesmus* spp. showed a removal efficiency of 80.9% and 90.8% for Total N and P, respectively. These findings further highlight the comparatively superior performance of the genera in dairy wastewater treatment. *Chlorella* spp. also exhibited substantial reductions in Total N and P, with values of 75.3 and 89.3%, respectively; while *Selenastrum* spp. showed relatively lower N removal but maintained high P uptake. These findings are comparable to those of Handayani *et al*. (2020) and Al Ahmed (2014) who obtained reductions of 80% and 72% in nitrate and phosphorus, respectively. All the three microalgae genera reduced COD by >90%, which was higher than the 77 % reported by Al Ahmed (2014). These results highlight the potential of microalgae as a highly efficient and sustainable strategy for bioremediating dairy wastewater prior to being discharged into other fresh water bodies. Their ability to significantly reduce pollutant loads; while maintaining favourable water chemistry;

highlights their applicability in mitigating dairy waste-induced pollution in aquatic ecosystems.

The cultivation results show that *Scenedesmus* spp. experienced a rapid growth rate, suggesting the genus' superior capacity for nutrient uptake and biomass conversion. This enhanced performance can be attributed to its robust physiological adaptability, efficient light utilisation, and high tolerance to elevated organic loads and nutrient concentrations typically present in dairy effluents (Rawat *et al*., 2011, Abdel-Raouf *et al*., 2012). Additionally, *Scenedesmus* spp. is known to possess a high surface-area-to-volume ratio and effective enzymatic systems, which facilitate rapid assimilation of nitrogen and phosphorus, thereby promoting accelerated biomass accumulation (Xin *et al*., 2010). In contrast, the comparatively lower biomass yields observed in *Selenastrum* spp. may be attributed to slower growth kinetics, reduced nutrient uptake efficiency, or limited tolerance to the high organic and nutrient loads present in dairy wastewater. To enhance its performance, strategies such as nutrient optimization, co-cultivation with more robust species, or acclimatization under gradually increasing wastewater concentrations could be explored.

The strong positive correlation between biomass yield and the removal efficiencies of Total N, Total P, and Organic Carbon indicates that effective nutrient assimilation directly supports microalgae growth, aligning with findings of Gupta *et al*. (2024) and Magyar *et al*. (2024). These nutrients are critical for cellular metabolism, with nitrogen and phosphorus serving as key components of nucleotides, proteins, and phospholipids, while organic carbon provides an essential energy source (Yaakob *et al*., 2021). The positive correlation observed for COD suggests that the enhanced breakdown of organic matter facilitates biomass accumulation, likely by supplying readily available carbon substrates for metabolic activity. In contrast, TDS removal exhibited a weak correlation with biomass yield, implying that its reduction had a negligible effect on microalgal growth. Notably, dissolved oxygen removal showed a negative correlation, suggesting that increased biomass production may have enhanced photosynthetic activity, thereby elevating oxygen levels rather than depleting them. Studies conducted by Yousif *et al*. (2022) and Jakhwal *et al*. (2024) also demonstrated a strong positive correlation between nutrient uptake and biomass yield. These studies reinforce the notion that efficient nutrient removal, particularly of nitrogen and phosphorus, is closely linked to enhanced microalgal biomass production. The strong positive correlations observed in this analysis align with these findings, underscoring the potential of microalgae in wastewater treatment applications where nutrient removal and biomass generation are desired outcomes.

Conclusion

This study has identified three genera (*Scenedesmus* spp.*,* *Chlorella* spp. and *Selenastrum* spp.) of microalgae in Lake Victoria freshwater at Ggaba landing site in Uganda to be highly efficacious bioremediation, with *Scenedesmus* spp. having an edge over the others, with nutrient removal capacities of 80.9% and 90.8% for total nitrogen and total phosphorus, respectively. *Scenedesmus* spp. and *Chlorella* spp. are viable agents for mitigating dairy waste induced pollution. The high nutrient removal efficiencies and substantial biomass yields by these locally sourced microalgae is compelling evidence for its integration into wastewater treatment systems, capable of offering both ecological sustainability and resource recovery in the context of dairy wastewater management.

Acknowledgement

The authors sincerely acknowledge the Uganda Industrial Research Institute (UIRI) for the support and permission granted to collect dairy wastewater samples from their dairy processing facility. We further express our profound gratitude to the Department of Agricultural and Biosystems Engineering, Makerere University, for providing the laboratory facilities and experimental equipment essential for the successful execution of this research.

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