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Research Article

Removal of Nitrogen and Phosphorus from Liquid Dairy Manure Using Microalgae

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Abstract: Animal production wastes and effluents are among the most highly produced wastewaters, containing high concentrations of nutrients and microbes that could lead to contamination and eutrophication of water sources. Large-scale enterprises in cattle breeding face challenges in storing and removing a substantial volume of liquid manure (LM). Therefore, the management of LM becomes an economic burden for producers. In this case, the question arises as to whether a more economical and sustainable treatment method can be employed by utilizing LM from animal production in algal growth, which has emerged as a renewable raw material source in recent years. In this study, a microalgae *Ankistrodesmus sp*. was employed for nutrient removal from dairy LM at concentrations of 10%, 20%, and 30% over 35 days. The total nitrogen reduction rates in the reactors with 10%, 20%, and 30% LM were 72.8%, 69.1%, and 71%, respectively, while the total phosphorus reduction rates were 65.7%, 52.6%, and 31.5%, respectively. Overall, integrating microalgae cultivation into wastewater treatment processes shows promise for nutrient removal and biomass production. By leveraging the nutrient-rich characteristics of LM from cattle farming, microalgae provide a sustainable and effective approach to reduce environmental pollution and enhance resource recovery in agriculture. Further research and development in this field are essential for optimizing treatment methods and improving the environmental sustainability of livestock operations.

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1. Introduction

Dairy production continues to increase due to meet the growing demand for beef and milk production. Dairy cattle breeding plays a significant role in the overall livestock industry in Türkiye, contributing to the country's agricultural and economic landscape. However, this intensive farming generates a large quantity of wastewater. The microorganisms and nutrients in the manure and wastewater cause pollution in surface and groundwater when the manure from animal production is not

stored or managed properly. The cattle breeding industry faces significant challenges in managing liquid manure removal and storage, primarily attributed to the substantial volume of manure wastewater generated by large-scale operations. Consequently, the management of LM wastewater becomes an economic burden for producers.

Livestock wastewater management and treatment methods are crucial for mitigating environmental pollution and ensuring public health. The substantial volume of manure and wastewater generated by large-scale livestock operations poses significant challenges in terms of effective treatment and disposal. Biological treatment methods have emerged as a common and robust technology for livestock wastewater treatment due to their cost-effectiveness and low environmental impact (Cheng et al., 2020). Additionally, innovative methods such as biochar adsorption and constructed wetlands have shown promise in removing pollutants and nutrients from livestock wastewater, offering potential solutions for sustainable treatment and resource recovery (Knight et al., 2000; Fang et al., 2014; Cakmakcı et al., 2017). The impact of livestock wastewater on environmental pollution, including the dissemination of antibiotic-resistant bacteria and genes, underscores the urgency of developing effective and affordable treatment methods, particularly in developing countries where wastewater treatment infrastructure may be limited (Martinez et al., 2009; Usui et al., 2022). Furthermore, the implementation of alternative technologies such as electrocoagulation and advanced pond systems has been explored to address the challenges associated with livestock wastewater treatment, emphasizing the need for sustainable and efficient solutions (Pinedo-Hernández et al., 2016; Hadi et al., 2020). As livestock wastewater management becomes an increasingly pressing concern, the development and implementation of innovative treatment methods are essential to minimize environmental pollution and safeguard public health.

In this case, the question arises as to whether a more economical and sustainable treatment method can be employed by utilizing liquid manure from animal production in algal cultivation, which has emerged as a renewable raw material source in recent years. Microalgae are very effective in reducing carbon dioxide emissions due to their high photosynthetic activity and faster growth rate compared to other plants. In addition, harvested algae can be used as biofuels, nutrients, and animal feed (Yen et al., 2015). Microalgae are frequently used as a biological treatment method for wastewater treatment (Osabutey et al., 2023; Ferreira et al., 2018; Coşkun et al., 2018; Ilgi and Sebnem, 2007). Microalgae have shown positive results in the treatment of industrial wastes in different sectors due to their adaptability to different environmental conditions, their fast and easy cultivation, and their ability to rapidly convert nitrogenous compounds into oxygen and biomass (Batista et al., 2015). Shelknanloymılan et al. (2012) investigated the removal of ammonium and phosphorus ions from wastewater containing synthetic and organic materials. They cultivated *C. vulgaris* in a photobioreactor for 30 days to remove nitrogen (NO_3-N) and phosphate (PO_4-P) and reported that *C.vulgaris* had a favorable effect on the removal of nitrogen and phosphate in wastewater. Akca (2015) investigated the optimal growth conditions for microalgae in anaerobic digester effluent through two experiments. The first part explores the growth of microalgae in varying dilutions of effluent with tap water under continuous light, where the highest cell density achieved was 0.625 g L^{-1} at 20% dilution. The second part investigates growth at the lowest effective dilution (40%) with added phosphorus due to initial phosphorus limitations, enhancing growth efficiency compared to the first experiment. However, issues such as pH drop, lack of buffer capacity, and competition with other microorganisms in non-sterile conditions were noted, affecting overall growth performance.

The treatment of liquid manure from animal production with microalgae by photobioreactor systems contributes to the reduction of environmental pollution and provides valuable biomass, which is a renewable energy source of liquid manure. It also contributes to the reduction of pollutant gases released during the storage and processing of manure.

The utilization of dairy LM for the cultivation of microalgae presents a promising avenue for the removal of nitrogen and phosphorus from agricultural waste streams. Cattle manure is indeed a rich source of nitrogen and phosphorus, which are essential nutrients for microalgae growth (Halim et al., 2016). Research has shown that microalgae can efficiently remove large amounts of nitrogen and phosphorus from wastewater, serving as a sustainable method for nutrient removal and biomass production (Zieliński et al., 2018). Additionally, the use of anaerobically digested cattle manure as a growth medium for microalgae cultivation has demonstrated significant nitrogen removal and substantial phosphorus removal, indicating the potential for effective nutrient uptake by microalgae

(Thomas et al., 2017; Psenovschi et al., 2019). Liquid digestate, a byproduct of the anaerobic digestion of cattle manure, has also been explored as a cost-effective approach for nutrient removal and biomass production (Kisielewska et al., 2020). The potential of cattle manure as a nutrient source for microalgae cultivation has been further supported by studies demonstrating the effectiveness of cattle manure in providing nutrients for the growth of microalgae (Izmailov et al., 2021).

This study aimed to advance the development of wastewater treatment processes based on microalgae by addressing the questions outlined earlier. The specific objective was to remove nitrogen, phosphorus, and other nutrients from raw dairy liquid manure to reduce the environmental effects on dairy farms. Microalgae grown in photobioreactor systems were fed with liquid manure and the effect of microalgae on total nitrogen and phosphorus removal in LM was investigated. LM samples collected from a commercial livestock barn were concentrated by 10%, 20%, and 30% and microalgae growth was investigated for 35 days. The total nitrogen and phosphorus removal efficiencies of microalgae in liquid manure were evaluated by analyzing the nutrient values in the liquid manure at the beginning and end of the experiments.

2. Material and Methods

2.1. Dairy liquid manure (LM)

The dairy LM sample was acquired from a commercial dairy cattle farm operating in Karacabey, Bursa, Türkiye. The dairy barn was naturally ventilated with a free-stall design. The outdoor exercise area featured a solid, non-sloping sand foundation, overlaid with a material derived from the solid-liquid separation process. The manure accumulated on the floor was mechanically scraped and gathered approximately every 5–6 days, and subsequently stored at the east corner of the barn in an area measuring 800 m^2 with a height ranging from 0.7 to 1.2 m. The wastewater generated from washing milking houses and other areas of the farm was gathered and directed into a sedimentation tank to extract the supernatant for subsequent solid-liquid separation processes. The solids obtained from the separation process were repurposed as padding material in the outdoor exercise area, while the liquid fraction was transferred to a storage tank located on the north side of the farm for use in washing the floors.

Samples were collected in May 2023 from the liquid manure collection pit and analyzed for total nitrogen, total phosphorus, and pH (using a Hanna HI98128 pH meter) levels before use. Afterward, 3 different samples containing the concentration of 10%, 20%, and 30% liquid manure were prepared for use in the experiments. In order to carry out the experiments in triplicate, each sample was divided into 3 equal parts, and 3 separate experiments were carried out with a total of nine photobioreactors (PBRs).

2.2. Algal cultivation

Ankistrodesmus sp. strain (AQUAMEB-33), obtained from the AQUAMEB Culture Collection of Algae and Cyanobacteria (Aquatic Microbial Ecology and Biotechnology Laboratory, Bursa, Türkiye), was chosen for its high growth rate, adaptability to various environmental conditions, and ability to absorb nitrogen at high rates (Uguz and Sozcu, 2023). The algal strain was cultivated in an autoclaved (20 min at 121 ºC) bold basal medium (BBM) in a 1-L Erlenmeyer flask. The BBM was composed of $CaCl_2*2H_2O$ (25 mg L^{-1}), K_2HPO_4 (75 mg L^{-1}), $MgSO_4*7H_2O$ (75 mg L^{-1}), K_2HPO_4 (175 mg L^{-1}), $NaNO_3$ (250 mg L^{-1}), EDTA $C_{10}H_{16}N_2O_8$ (50 mg L^{-1}), MoO_3 (1.42 mg L^{-1}), NaCL (25 mg L^{-1}), H_2SO_4 (1 ul), KOH (31 mg L^{-1}), H_3BO_3 (11.42 mg L^{-1}), $FeSO_4*7H_2O$ (4.98 mg L^{-1}), and a trace metal solution [1 mL L^{-1}; including $MnCl_2*4H_2O$ (1.44 g L^{-1}), $ZnSO_4*7H_2O$ (8.82 g L^{-1}), $Co(NO_3)_2*6H_2O$ (0.49 g L^{-1}), $CuSO_4*5H_2O$ (1.57 g L^{-1})] (Uguz et al., 2022). The Erlenmeyer flasks that contain algal cultures were placed in front of daylight LED lights (ACK Lighting, İstanbul, Türkiye).

2.3. DW concentrations

Figure 1 shows the experimental setup in this study. A total of nine photobioreactor tanks were used for three different experimental groups with three replicates each. *Ankistrodesmus sp*. culture and dairy LM were mixed at a concentration of 10%, 20%, and 30% LM (Table 1). Microalgae growth was studied in reactors containing LM (e.g. 1700 mL DI water + 200 mL LM + 100 mL algae culture for 10% LM) for 35 days. All reactors were supplied with 0.5 LPM (vvm) of air. All reactors were run under

the illumination of the daylight LED lamps (200 µmol $s^{-1}m^{-2}$) from one side of the reactors. Environmental conditions, including pH, water level, aeration, temperature, and light intensity were monitored at 24-hour intervals throughout the experimental period. The water level was maintained by adding autoclaved deionized water. For microalgae cell counts, 30 mL of liquid was sampled from each reactor at 24-hour intervals.

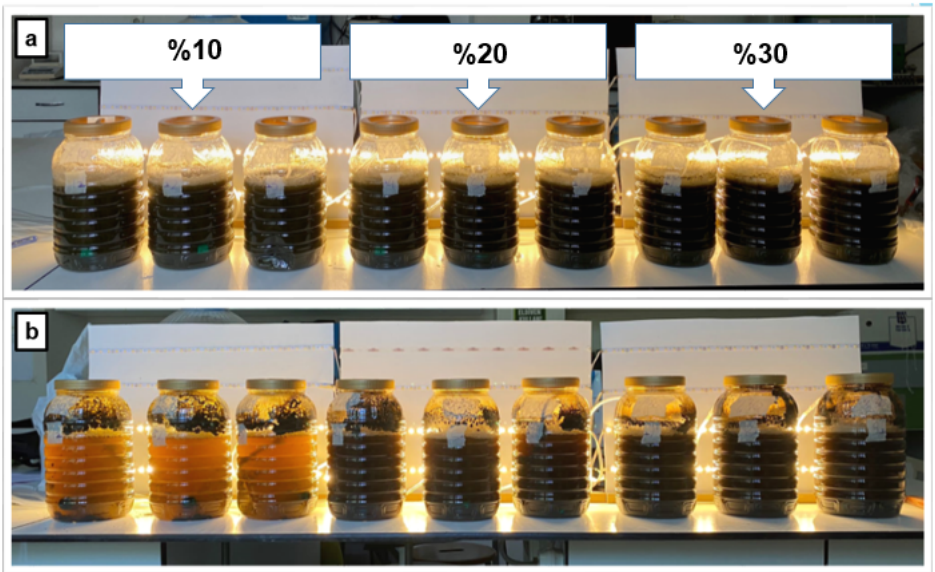

Figure 1. The photobioreactors (PBRs) containing 10%, 20%, and 30% liquid dairy manure on the first (a) and last day (35^{th} day) (b) of the experiment.

In the study, the temperature and pH values of the microalgae culture medium grown in LM were not kept constant and their changes were monitored throughout the experiment. At 24-hour intervals, the temperature and pH values of the algal culture medium were measured with a Hanna Model HI98128 temperature and pH meter.

Table 1. LM concentrations in the photobioreactor system

LM concentrations	LM (mL)	DI water (mL)	Algal Culture (mL)
%10	200	1700	100
%20	400	1500	100
%30	600	1300	100

2.4. Algal growth

In cell counting; samples taken from the photobioreactor with the help of a 100 µL pipette were transferred into the hemocytometer slide and cells were counted under a microscope at 24-hour intervals. Cell concentrations were determined by calculating the number of cells per mL. Specific growth rate and doubling time were calculated by the following equations (Becker, 1994);

$$\mu = \frac{L_n N_t - L_n N_0}{t - t_0} \quad (1)$$

$$t_d = \frac{Ln_2}{\mu} \quad (2)$$

Where; µ represents the specific growth rate (day^{-1}), Nt_1 and Nt_0 shows the cell concentration (cells mL^{-1}) on day-t, and t_d is the doubling time (day).

2.5. Total nitrogen and phosphorus determination and removal rate

The nitrogen content (%) was determined by DIN 38409 Part 28:1992-04 method. For the distillation process, the FOSS Kjeltec 8200 device was used. To initiate the analysis, a 50 mL aliquot of the algal sample was introduced into the combustion tube, followed by the addition of 2 g of potassium sulfate, 0.2 g of Devarda alloy, and 5 mL of concentrated sulfuric acid. Subsequently, the combustion tube was vigorously agitated and allowed to stand for a minimum of 1 hour. The temperature of the combustion unit was adjusted to 200 °C, and the tubes were positioned and maintained at this temperature for 30 minutes. The temperature was then elevated to 360 °C in the incinerator, and the incineration process was sustained at this temperature for 1 hour. Upon completion of the combustion phase, the tubes were transferred to the distillation apparatus. In the distillation setup, 25 mL of a 4% boric acid solution containing an indicator was introduced into the flask, which was then inserted into the device. Upon sealing the tube protective cap, water, and NaOH solution were automatically dispensed into the tube, initiating the distillation process within the apparatus. Following the distillation cycle, the contents of the flask were titrated until a grayish-pink hue was achieved using approximately 0.02 N HCl solution. The determination of total nitrogen content was derived from the volume of HCl solution consumed during the titration process.

Total phosphorus determination was carried out using 1000 mg/L standard phosphorus solution (Sigma-Aldrich, Supelco, Canada) in an ICP-OES device. It was prepared daily using 0.3% HNO_3 according to the working standards. Before starting the analysis, the IC-AN6-1 Multiple Anion Standard (HPS, USA) was checked with 9.8 ppm and then the analysis of the samples was started. For phosphorus, the calibration curve was linearly plotted with 6 different standard solutions in the range of 1-20 mg L^{-1}.

In the experimental procedure, a volume of 50 mL of liquid algae sample was extracted for analysis, following the wet digestion method as outlined. The sample underwent a heating process on a heater, during which 5 mL of nitric acid (HNO_3) and 1 mL of hydrogen peroxide were successively added until the volume was reduced to 25 mL. Subsequently, the solution was allowed to cool to room temperature, followed by filtration and transfer into 50 mL falcon tubes. The sample was then adjusted to a final volume of 50 mL using deionized distilled water. Calibration solutions and other samples were subjected to analysis using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) instrument, operating under specific experimental conditions (Table 2).

Table 2. ICP-OES operating conditions and measurement parameters

RF power (W)	1300
Plasma	15 L min^{-1}
Aux.	1 L min^{-1}
Nebulizer	0.5 L min^{-1}
Processing mode	Area
Sample flow	0.8 ml min-1
Wavelength	213.617 nm

2.6. Statistical Analysis

The study data was presented as mean ± standard deviation. Statistical analyses, including Analysis of Variance (ANOVA) and t-tests, were conducted using JMP software (version 13.0) as appropriate. The least significant difference (LSD) Student's comparison test was used to compare the differences between experiments where significant differences were observed. A confidence level of 95% was chosen to accurately assess significance. Statistical significance was considered present for $p < 0.05$.

3. Results and Discussions

Raw liquid dairy manure, a byproduct of dairy farming operations, is a complex and nutrient-rich effluent that requires careful management due to its unique characteristics. This type of manure is commonly utilized in agricultural practices as a source of nutrients for crops, serving as an alternative to chemical fertilizers (Chang et al., 2022). However, raw liquid dairy manure can potentially harbor

various pathogens such as Shiga toxin-producing Escherichia coli (STEC), Salmonella spp., and Listeria spp., which pose risks to both human health and the environment (Sheng et al., 2019). The composition of raw liquid dairy manure includes a significant amount of organic matter, nitrogen, and phosphorus, making it a valuable resource for soil fertility improvement (Regueiro et al., 2020). Additionally, the presence of antibiotic-resistance genes in dairy manure highlights the importance of understanding and managing the microbial community within this waste stream (Wang et al., 2020). Understanding the properties and behavior of raw liquid dairy manure is essential for developing effective strategies for its treatment, utilization, and environmental impact mitigation.

In this study, the liquid manure samples (LM) were collected from a commercial barn to investigate the effect of algal cultivation on the removal of N and P concentrations in liquid dairy manure. The LM samples were analyzed for pH, conductivity, TOC, COD, TKN, NH_4-N, and TP. Table 3 shows the characteristics of raw liquid dairy manure. TN and TP concentrations in the liquid manure were respectively 2165 and 200.3 mg L^{-1}. Chiue et al. (2015) documented that the typical concentrations of total nitrogen (TN) and total phosphorus (TP) in wastewater originating from livestock farming and agricultural activities generally range from 185 to 3213 mg L^{-1} for TN and approximately 30 to 987 mg L^{-1} for TP. This includes various sources such as anaerobically digested poultry litter effluent, as well as swine or dairy manure wastewater.

Table 3. Characteristic of raw dairy liquid manure (LM)

pH	6.9±0.2
Conductivity (mS cm^{-1})	7.75±0.03
TOC (mg L^{-1})	2896±142
COD (mg L^{-1})	11648±784
TN (mg L^{-1})	2256±84
NH_4-N (mg L^{-1})	607±36
TP (mg L^{-1})	200.3±18

Note: Data were presented in mean±standard deviation.

Liquid manure samples taken from the farm were added to the photobioreactor system at the rates of 10%, 20%, and 30%, and microalgae growth was examined for 35 days. Total nitrogen and phosphorus analyses were performed on the samples taken on the first and last day of each experiment and nitrogen and phosphorus reduction efficiencies were examined. Figure 2 shows the photobioreactors containing 10%, 20%, and 30% liquid dairy manure on the last day of the experiment.

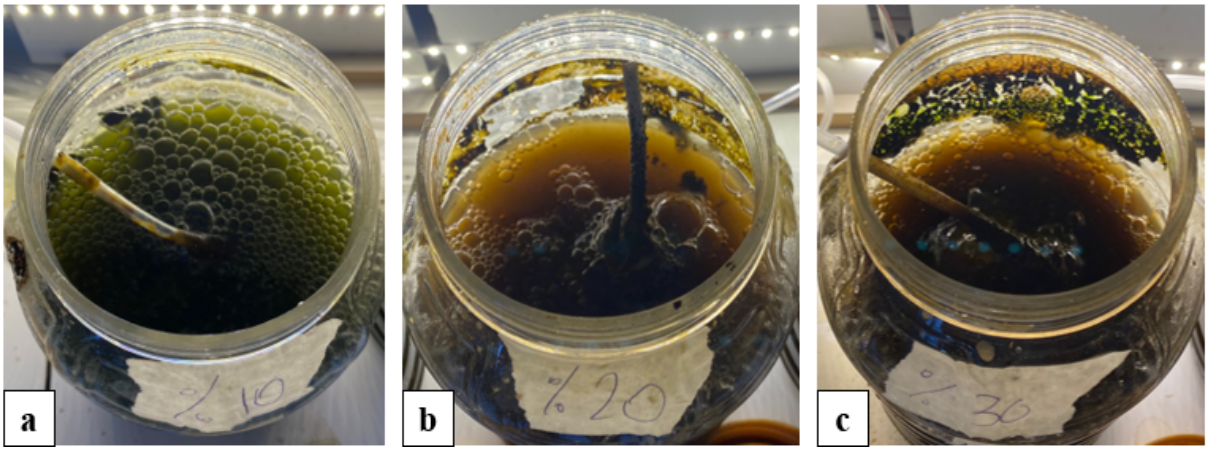

Figure 2. The 10% (a), 20% (b), and 30% (c) liquid dairy manure on the last day (35th day) of the experiment.

pH is one of the most important factors directly affecting the biomass yield and the specific growth rate of algae. pH also plays an effective role in the transport of nutrients in the culture medium (Uguz et al., 2022). The pH range required for algal growth is generally between 7-9 (Hodaifa et al.,

2009). In this study, the pH values of the culture medium in the reactors were not kept stable during the experiment, and its change was monitored throughout the experiment. Figure 3 shows the pH values in the reactors during the 35-day experiment.

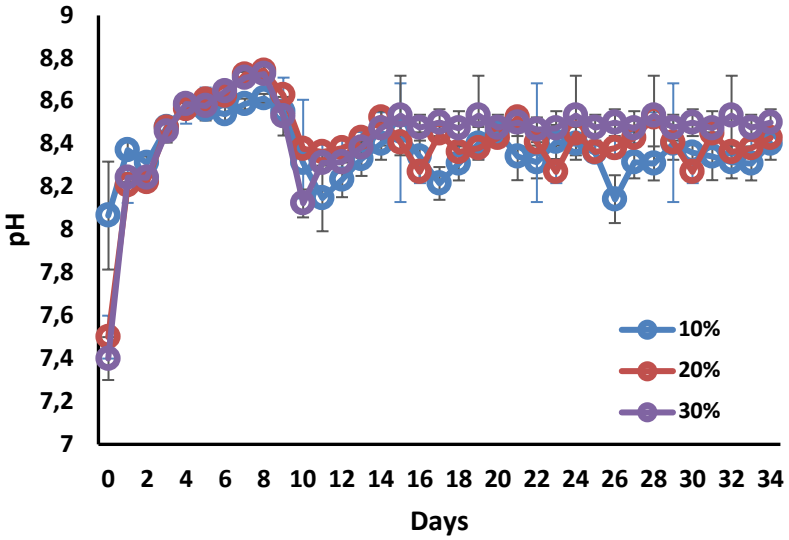

Figure 3. pH values of the algal culture in the reactors during the 35-day experiment. Error bars show the standard deviations calculated from replicate reactors.

At the beginning of the experiment, the pH values of the culture medium were around 8.1 in 10% reactors and 7.5 in 20% and 30% reactors. In the first 8 days of the experiment, the pH values increased to a maximum of 8.8 in all reactors and remained around 8.5 on average between days 10-35. This value shows that the pH range required for the growth of microalgae remains in the range of 7-9. Understanding how the pH of algal cultures changes in liquid manure is crucial for optimizing cultivation conditions and enhancing nutrient removal efficiency. The elevated NH_4^+-N concentration present in wastewater acts as a growth-inhibiting factor for microalgae, potentially correlating with variations in pH levels. Tam and Wong (1996) demonstrated that the growth of Chlorella was hindered when the NH_4^+-N concentration exceeded 700 mg L^{-1}, coupled with a pH below 7. At pH values higher than 8, NH_4^+ dissociates from NH_3. A decrease in pH inhibits the generation of NH_3, causing nitrogen to predominantly exist in the NH_4^+ ion form, which is less harmful to microalgal cells. When the nitrogen source is in the form of NH_3, it can lead to a decrease in pH within microalgal cells due to the high membrane permeability of NH_3 (Chiu et al., 2015). Consequently, the cellular mechanisms involved in pH regulation may facilitate the assimilation of NH_3 in wastewater utilized by microalgae.

In the study, algal growth was examined by cell counting in samples taken from the reactors at 24-hour intervals during the experiment. Figure 4 shows the time-dependent variation of cell concentrations in the reactors during the 35-day experiment. As can be seen in the figure, no cell growth was observed in the first 14 days in all three of the 10%, 20%, and 30% reactors, and at the end of the 14th day, an increase in the number of cells was observed only in the 10% reactors. *Ankistrodesmus sp.* remained in the adaptation phase for the first 14 days and then cell growth increased in 10% reactors, while there was no increase in the number of cells in 20% and 30% reactors. According to the data obtained, the average cell concentrations in reactors containing 10%, 20%, and 30% dairy liquid manure were 2.7 × 106, 1.9 × $10^{5,}$ and 1.3 × 10^5 cells mL^{-1}, respectively. The reactors containing 10% LM reached the maximum cell concentration on the 33rd day of the experiment. Table 4 shows the cell concentrations in reactors containing 10%, 20%, and 30% LM.

Table 4. The cell concentrations in reactors containing 10%, 20%, and 30% LM

	Cell Concentration (cells mL^{-1})		
Days	**%10 Mean**	**%20 Mean**	**%30 Mean**
0	950000	630000	473333.3
1	753333.3	303333.3	306666.6
2	786666.7	343333.3	226666.6
3	653333.3	396666.6	176666.6
4	523333.3	226666.6	180000
5	473333.3	216666.6	200000
6	603333.3	263333.3	86666.6
7	573333.3	243333.3	133333.3
8	626666.6	340000	276666.6
9	603333.3	323333.3	236666.6
10	520000	250000	123333.3
11	603333.3	320000	266666.
12	766666.6	366666.6	283333.3
13	796666.6	306666.6	196666.6
14	686666.6	376666.6	360000
15	436666.6	150000	40000
16	840000	60000	66666.6
17	1760000	70000	56666.6
18	2486666	56666.6	56666.6
19	2483333.3	70000	56666.6
20	2561428.5	70000	56666.6
21	3546666.6	113333.3	46666.6
22	2406666.6	90000	33333.3
23	2860000	90000	33333.3
24	3140000	63333.3	60000
25	3647500	60000	60000
26	3569853.7	76666.6	60000
27	3817763.0	63333.3	60000
28	6846666.6	60000	60000
29	6960835.6	76666.6	60000
30	7146428.5	63333.3	60000
31	8886666.6	60000	60000
32	9057759.5	76666.6	60000
33	9747133.1	63333.3	60000
34	9379166.6	60000	60000
35	9590259.5	60000	60000

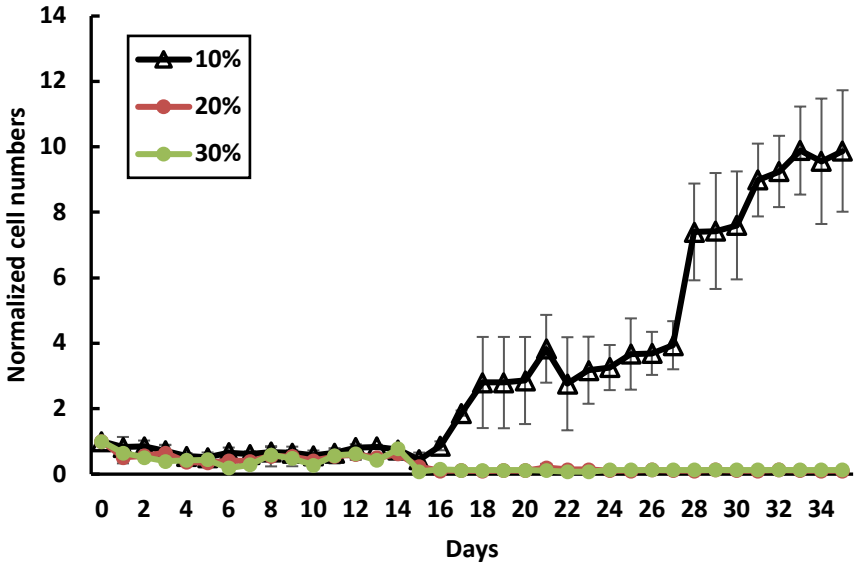

Figure 4. *Ankistrodesmus sp*. growth in reactors containing 10%, 20%, and 30% liquid manure (LM), (cell concentrations were normalized by dividing to initial cell concentrations). Error bars show the standard deviations calculated from replicate reactors.

Microalgae exhibit efficient growth and nutrient utilization capabilities in wastewater, offering a cost-effective approach to microalgae cultivation. However, it is imperative to acknowledge the significance of microalgal resilience to biotic pollution as a crucial factor influencing their growth in wastewater environments (Chiu et al., 2015). The growth of microalgae can at times be constrained by the presence of bacteria and protozoa in wastewater. According to the results obtained in the study, no algal growth was observed in the reactors containing 20% and 30% LM, but microalgae growth was observed in the reactor containing 10% LM. This circumstance may elucidate the absence of algal proliferation in Photobioreactors (PBRs) containing 20% and 30% liquid manure. The liquid manure samples in this study were not autoclaved before using in the experiments. Much of the documented data has been acquired under sterilized conditions, wherein the wastewater undergoes treatment through autoclave sterilization and/or centrifugation before its application in microalgae cultivation. While sterilizing wastewater for microalgal culture in laboratory settings is relatively straightforward, the complexity and cost escalate significantly when transitioning to large-scale microalgal cultivation utilizing wastewater. To ensure consistent microalgal growth in wastewater and mitigate potential biotic contamination, it becomes imperative to advance control methodologies for managing biotic pollution (Wang et al., 2013).

The concentrations of total nitrogen (TN) and total phosphorus (TP) in wastewater exhibit notable variations based on the type of wastewater. Among nitrogen-containing compounds found in wastewater, ammonia and nitrates are prevalent. Ammonium stands out as one of the primary chemical forms of nitrogen that can be easily assimilated by a wide range of microalgal species and strains (Chiu et al., 2013). In this study, the highest total nitrogen reduction rate was in the PBRs containing 10% LM (Table 5). On the first day of the experiment, the total nitrogen concentrations in the reactors were in the range of 248.2-740.7 mg L^{-1}, while at the end of the experiment (35th day), the total nitrogen concentrations in the reactors were in the range of 67.5-214.5 mg L^{-1}. At the end of the 35-day experiment, the total nitrogen reduction rates in the reactors containing 10%, 20%, and 30% LM were 72.8%, 69.1%, and 71%, respectively. Total nitrogen values on the first and last day in the reactors containing 10%, 20%, and 30% LM are given in Table 4. A previous study (Zhu et al., 2013) reported that *C. zofingiensis* grown in autoclaved pig wastewater removed 82.70% TN and 98.17% TP.

Table 5. TP and TP concentrations on the first and last day in reactors containing 10%, 20%, and 30% LM

LM conc. in PBRs	Total N			Total P		
	Day 1 (mg L^{-1})	Day 35 (mg L^{-1})	Removal rate (%)	Day 1 (mg L^{-1})	Day 35 (mg L^{-1})	Removal rate (%)
%10	248.2^{c}	67.5	72.8^{a}	49.3^{c}	16.9	65.7^{a}
%20	481^{b}	148.5	69.1^{a}	72.4^{b}	34.3	52.6^{b}
%30	740.7^{a}	214.3	71^{a}	109.2^{a}	74.8	31.5^{c}
SEM	±2.33		±6.06	±0.67		±0.61
P value	<0.0001*		0.6442	<0.0001*		<0.0001*

* $^{a-c}$ Differences in letters within columns indicate significant differences among the PBRs.

Phosphorus stands as a vital element crucial for the growth and metabolic processes of microalgae. It serves as an essential component, playing a significant role in the formation of ATP within microalgal cells. Consequently, the availability of phosphorus profoundly influences the growth of microalgae, particularly impacting processes like photosynthesis (Razzak et al., 2013). In wastewater, phosphorus is commonly present in the form of inorganic anions, such as $H_2PO_4^-$ and HPO_4^{2-}, which are crucial for the nutrient uptake and metabolic functions of microalgae (Chiu et al., 2015).

In this study, on the first day of the experiment, the total phosphorus (TP) amounts in the reactors were in the range of 49.3-109.2 mg L^{-1}, while at the end of the experiment (day 35) the TP amounts in the reactors were in the range of 16.9-74.8 mg L^{-1}. At the end of the 35-day experiment, the TP reduction rates in the reactors containing 10%, 20%, and 30% LM were 65.7%, 52.6% and 31.5%, respectively.

TP values on the first and last day in the reactors containing 10%, 20%, and 30% DW are given in Table 5.

Conclusion

Microalgae have demonstrated effectiveness in removing nitrogen and phosphorus from liquid manure, offering a sustainable method for nutrient removal and valuable biomass production. The research involved cultivating *Ankistrodesmus sp*. in photobioreactor systems fed with liquid manure and evaluating the microalgae's efficiency in removing total nitrogen and phosphorus from the liquid manure. The results indicated significant reductions in total nitrogen and phosphorus concentrations in the reactors containing different concentrations of liquid manure.

In conclusion, the integration of microalgae cultivation with wastewater treatment processes presents a promising solution for nutrient removal. By harnessing the nutrient-rich properties of liquid manure from cattle farming, microalgae offer a sustainable and efficient method for mitigating environmental pollution and promoting resource recovery in agricultural settings. Further research and development in this area are crucial for optimizing treatment processes and enhancing the environmental sustainability of livestock operations. Nevertheless, further research endeavors are imperative to enhance the economic viability and sustainability of microalgal production utilizing liquid manure as a nutrient resource.

Ethical Statement

Ethical approval is not required for this study because there are no certain types of studies involving humans or animals.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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Author Contributions

Conceptualization, S.U., and E.S.; methodology, S.U., E.S.; collection of data, N. K., S.B., E.C; investigation and data analysis, S.U. and E.S.; preparation, S.U., E.S., and E.Y.; writing-review and editing, S.U., E.S., and E.Y. All authors have read and agreed to the published version of the manuscript.

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