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Efficiency of Microalga *Dunaliella tertiolecta* in Cultivation and Removal of Pollutants from Dairy Industry Wastewater

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Abstract

Dunaliella tertiolecta is increasingly recognized as a valuable bioindustry microalga due to its ability to produce high-value pigments and biologically active compounds. However, the high cost of conventional culture media remains a major challenge for its large-scale cultivation. To address this issue, using nutrient-rich industrial wastewaters, such as dairy effluent, offers a promising, sustainable, and economical alternative. This study investigated the growth performance and pigment production of *D. tertiolecta* cultivated for 21 days in five concentrations of dairy wastewater (0%, 25%, 50%, 75%, and 100%) under controlled laboratory conditions (light intensity of 2500 lux; salinity of 1.5 M; temperature of 25 ± 2°C; pH of 7.5 ± 0.15). Algal cell density, chlorophyll a, chlorophyll b, and carotenoid content were measured every three days, while levels of ammonia, nitrate, and phosphate were assessed every five days. The results showed that the 25% wastewater treatment (T_2) produced the highest cell density on day 12 ($54.20 \times 10^6 \pm 1$ cells/mL). This treatment also resulted in the highest pigment concentrations, with chlorophyll a at 2.76 ± 0.04 mg/mL, chlorophyll b at 7.24 ± 0.06 mg/mL, and carotenoids at 2.24 ± 0.06 mg/mL concentrations. In terms of nutrient removal, T_2 achieved the greatest reduction in phosphate (0.052 ± 0.02 mg/mL) and nitrate (0.059 ± 0.94 mg/mL), while the highest ammonia removal (0.062 ± 2.23 mg/mL) occurred in the 50% treatment (T_3). Overall, the findings indicate that dairy wastewater, when properly diluted, can serve as an effective and low-cost culture medium for *D. tertiolecta*, supporting both biomass production and wastewater bioremediation.

Keywords: *D. tertiolecta*, Wastewater, Pigments, Biological treatment, Nutrients

Introduction

Microalgae are increasingly recognized as functional food ingredients due to their rich content of bioactive compounds,

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such as carotenoids, polyunsaturated fatty acids, phycobiliproteins, and essential vitamins. These compounds contribute to antioxidant, anti-inflammatory, and immunomodulatory effects, aligning with the rising consumer demand for health-promoting and sustainable dietary sources. Among them, the *Dunaliella* genus of halophilic unicellular green microalgae has garnered attention for its ability to thrive in hypersaline environments, coastal lagoons, and rocky marine habitats, while producing high levels of β-carotene and other valuable metabolites. Martínez-Ruiz et al., 2025). Species within the genus *Dunaliella* demonstrate remarkable physiological plasticity, allowing them to thrive under extreme environmental conditions such as high salinity and variable light intensities. These adaptive traits—particularly the modulation of photosynthetic pigments and the production of osmoprotective metabolites—position *Dunaliella* as a perfect model for investigating stress tolerance mechanisms and cellular responses in plant-like systems (Barbosa et al., 2023; Mishra et al., 2008). *Dunaliella tertiolecta* has gained industrial and agricultural relevance due to its ability to synthesize valuable bioproducts, such as glycerol, β-carotene, single-cell proteins, and essential micronutrients, which are particularly beneficial in aquafeed formulations. Its biotechnological importance is further underscored by its exceptional capacity to accumulate high concentrations of β-carotene under stress conditions, making it one of the most efficient natural sources of this antioxidant pigment (Barbosa et al., 2023؛ Celente et al., 2024). Plants are a rich source of various carotenoids beyond β-carotene, including lutein, zeaxanthin, violaxanthin, α-carotene, and neoxanthin, which contribute significantly to human health due to their antioxidant, anti-inflammatory, and photoprotective properties (Demmig-Adams et al., 2020).

The growing global population and the expansion of the food industry, especially the dairy sector, have produced large amounts of wastewater rich in organic matter and nutrients. Discharging this wastewater directly into the environment poses a serious threat to water resources and ecosystem health (Costa et al., 2021). On the other hand, microalgae are microorganisms with high potential for absorbing nutrients, producing biomass, and synthesizing bioactive compounds such as pigments. These features have attracted wide interest in biotechnology, agriculture, and environmental fields (Yang et al., 2011). Wastewater is typically defined as water that contains a mixture of organic matter, pathogens, nutrients, and chemical pollutants. When released untreated into natural ecosystems, it poses significant risks to public health and environmental integrity, including contamination of drinking water sources, eutrophication, and the spread of waterborne diseases (Lin et al., 2022؛ Babuji et al., 2023؛ Jayaswal et al., 2017). Dairy wastewater is a significant environmental concern due to its high organic load and nutrient content. With the global rise in dairy consumption, the volume of wastewater generated by dairy processing facilities has increased substantially. This effluent typically contains elevated levels of proteins, soluble carbohydrates, nitrogenous compounds, phosphorus, and other nutrients, which can lead to eutrophication and oxygen depletion in receiving water bodies if not properly treated (Jimeto et al., 2025; Tayawi et al., 2025).

Although various chemical and physical techniques have been explored for treating dairy wastewater, many of these approaches remain economically unfeasible due to high operational and maintenance costs (Al-Tayawi et al., 2023; Radwan, 2020). Several studies have explored the cultivation of microalgae in wastewater. For example, Khalaji et al. (2019) used two concentrations of *Chlorella vulgaris* in dairy wastewater at different dilution levels. Their results showed that as the concentration of microalgae increased, nutrient uptake decreased, but nitrate absorption improved. In another study, *Chlamydomonas polypyrenoideum* was grown in diluted dairy wastewater mixed with distilled water. The findings indicated that dairy wastewater, due to its high nutrient content, can serve as a valuable medium for microalgal biomass production (Rodrigues-Sousa et al., 2021). Recent investigations have demonstrated that cultivating *Chlorella vulgaris* in dairy wastewater using a tubular photobioreactor significantly enhances nitrogen removal efficiency while simultaneously improving both the yield and biochemical composition of the algal biomass. These findings highlight the dual benefit of microalgae-based treatment systems: effective nutrient recovery and cost-efficient biomass generation for downstream applications (Sudhanthiran et al., 2022).

Although species like *C. vulgaris* have been widely studied, *D. tertiolecta* remains underexplored in the context of dairy wastewater cultivation. Therefore, in this study, attempted to different concentrations of dairy wastewater were used as a cost-effective, nutrient-rich culture medium to grow the microalga *D. tertiolecta*. The aim was to evaluate its effects on pigment content, growth, and nutrient uptake efficiency, to identify the most suitable wastewater concentration for optimal cultivation of *D. tertiolecta*.

Material and methods

Microalgae stock preparation and cultivation conditions

A pure stock culture of *D. tertiolecta* was obtained from the Food Industry Biotechnology Research Institute in Tabriz, Iran. The strain had been taxonomically confirmed at both the genus and species levels using molecular methods and was reactivated by a specialist at the institute. Initial cultivation was carried out using Zarrouk medium (Richmond, 2003). The microalgae were grown in 500 mL (50 mL alga + 450 mL culture medium) glass flasks without shake under the following conditions: continuous gentle and continuous aeration, salinity of 1.5 M (equivalent to 87 g/L NaCl), temperature of 25 ± 1 °C, light intensity of 2500 lux with a light/dark cycle of 16:8 h, and a pH of 7.5 (Martinez et al., 2000).

Dairy wastewater collection and preparation

Dairy wastewater was collected after equipment washing at the Kerman Dairy

Factory, located in Zahak County, Sistan and Baluchestan Province, Iran. (Table 1). The wastewater was stored in polyethylene containers and transported to the laboratory at 4 °C (Hamoun International Wetland Research Institute, Zabol, Iran). Before experimentation, large particulate matter was removed using Whatman filter paper. The filtered wastewater was then sterilized in an autoclave at 120 °C and 1.6 atm pressure for 20 min, followed by a second filtration through Whatman paper to ensure clarity.

Experimental design

The experimental treatments included volumes of 5 mL culture medium and 50 mL *D. tertiolecta* inoculum, but with different concentrations of dairy wastewater: 0%, 25%, 50%, 75%, and 100%, each diluted with distilled water. The total duration of the experiment was set to 21 days. It should be noted that all sampling was performed with three replicates.

Cell counting

Cell counts were performed every three days using a light microscope and a Neubauer counting chamber at 40× magnification. Five squares of the chamber were counted, and cell density was calculated using Eq. (1) (Mokhberi et al., 2015). It should be noted that all cells were counted visually.

$$\text{Number of cells/mL} = \text{Average cell count} \times 10^4 \times \text{Dilution factor} \quad (1)$$

Measurement of chlorophyll (a, b) and total carotenoids

The concentrations of chlorophyll a, chlorophyll b, total chlorophyll (a + b), and total carotenoids were measured every three days using a UV/Vis spectrophotometer (UV/Vis 2100, Unico). For each measurement, 5 mL of the algal culture was centrifuged at 5000 rpm for 10 min. The supernatant was carefully removed using a micropipette. Then, 5 mL of 85% acetone was added to the pellet. After vortexing, the tubes were kept in the dark for 5 minutes. The mixture was centrifuged again at 5000 rpm for another 10 minutes. The supernatant was then transferred to a cuvette, and absorbance was measured

Table 1. Physicochemical properties of dairy wastewater

Compound	Concentration (mg/L)
Sulfate (SO_4)	185
Magnesium carbonate ($MgCO_3$)	38
Manganese (Mn)	0.001
Calcium carbonate ($CaCO_3$)	4540.3
Copper (Cu)	0.74
Total copper (Total Cu)	1.52
Potassium (K)	34.92
Iron (Fe)	0.42
Mg as $CaCo_3$	257.8
Magnesium (Mg)	54.52

at wavelengths of 452, 644, and 633 nm using the spectrophotometer. Chlorophyll a, chlorophyll b, and total carotenoid concentrations (mg/mL) were calculated using the following equations. (2-4) (Frank and Wegmann, 1979).

$$C_{chla}=(10.3\times E_{633}) - (0.918 \times E_{644}) \quad (2)$$

$$C_{chl\ b}= (19.7 \times E_{644}) - (3.87 \times E_{633}) \quad (3)$$

$$C_{car}=(4.20\times E_{452})-(0.0264 \times C_{chl\ a})- (0.496 \times C_{chl\ b}) \quad (4)$$

Measurement of nutrients (Ammonia, nitrate, and phosphate)

The concentrations of phosphate, ammonia, and nitrate were measured every five days throughout the experiment. On day one, after mixing the designated ratios of distilled water, culture medium, and dairy wastewater, the initial concentrations of nitrate, ammonia, and phosphate were measured using standard reagent tablets and a photometer (Palintest 8000). Subsequent measurements were taken every five days. For each time point, a portion of the sample was transferred into a test tube using a pipette. After centrifugation, the algal biomass was separated, and the supernatant was used to determine nutrient concentrations. To assess nutrient removal efficiency, equation (5) was used (Hen et al., 2015).

$$W\%= (C_i-C_o)/C_o \times 100\% \quad (5)$$

where W% represents the nutrient removal percentage, C_0 is the initial concentration, and C_i is the concentration at the time of measurement.

Statistical analysis

All data were analyzed using SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (One-Way ANOVA) was applied to determine statistical differences among treatments.

Mean comparisons were performed using Duncan's multiple range test at a 95% confidence level. Graphs were generated using Microsoft Excel 2016.

Results

Cell counting

According to Table 2, the treatment with 25% wastewater (T2) showed a significant increase in cell density from day 3 to day 12 compared to the other treatments (p <

Table 2. Growth rate of *D. tertiolecta* algae under different treatments

Day	Treatment (mg/L)				
	T_1	T_2	T_3	T_4	T_5
1st	2.93 ± 0.12^{J}	3.07 ± 0.31^{F}	3.13 ± 0.31^{FJ}	2.73 ± 0.61^{E}	3.40 ± 0.20^{D}
3rd	5.87 ± 0.50^{dF}	9.73 ± 0.31^{bE}	8.47 ± 0.31^{cE}	12.87 ± 0.42^{aB}	5.47 ± 0.23^{dC}
6th	10 ± 0.20^{dE}	30.13 ± 0.50^{aC}	30.80 ± 0.40^{aC}	17.07 ± 0.31^{bA}	12.53 ± 0.50^{cA}
9th	16.73 ± 0.12^{cD}	50.20 ± 2.40^{aAB}	43.07 ± 1.70^{bA}	11 ± 1.22^{dC}	7.80 ± 0.40^{eB}
12th	26.87 ± 0.70^{cC}	54.20 ± 1.10^{aA}	36.87 ± 0.42^{bB}	7.13 ± 0.70^{dD}	3.87 ± 2.23^{eD}
15th	32.53 ± 0.64^{bB}	47.40 ± 0.60^{aB}	16.07 ± 1.10^{cD}	3.67 ± 0.23^{dE}	2.47 ± 0.23^{eE}
18th	32.93 ± 0.83^{aB}	20.27 ± 1.22^{bD}	3.87 ± 0.42^{cF}	1.67 ± 0.12^{dF}	-
21th	33.87 ± 0.61^{aA}	11.67 ± 7.10^{bE}	2.20 ± 0.40^{cJ}	-	-

0.05), reaching the highest value of 54.2 × 10^4 cells/mL on day 12. The treatment with 50% wastewater (T3) also exhibited a significant upward trend from day 3 (8.47 × 10^4 cells/mL) to day 9 (43.10 × 10^4 cells/mL) ($p < 0.05$); however, a decline in cell density was observed in both T2 and T3 treatments thereafter. In contrast, the 75% (T4) and 100% (T5) wastewater treatments showed only a slight increase in cell density on day 6, reaching 17.07 × 10^4 and 12.53 × 10^4 cells/mL, respectively, followed by a continuous decrease that approached zero by day 18. Interestingly, the control treatment (T1) exhibited a delayed but steady increase in cell density from day 6 (10 × 10^4 cells/mL) to day 21 (33.87 × 10^4 cells/mL), with statistically significant differences compared to the other treatments from day 18 to day 21 ($p < 0.05$).

Chlorophyll a and b analysis

Based on Figures 1 and 2, the highest levels of chlorophyll a and b were observed in treatment T_2 on day 12, with mean values of 2.76 ± 0.04 mg/mL and 7.24 ± 0.06 mg/mL, respectively, compared to other treatments. Similarly, treatment T_3 showed peak concentrations on day 9, with chlorophyll a at 2.45 ± 0.10 mg/mL and chlorophyll b at 7.05 ± 0.05 mg/mL. Following these peak values, both pigments exhibited a declining trend. The lowest concentrations of chlorophyll a and b were recorded in treatments T_4 and T_5.

Carotenoid analysis

According to Figure 3, different concentrations of wastewater had a significant effect on carotenoid content. On day 18, treatment T_2 exhibited the highest carotenoid concentration (4.24 ± 0.06 mg/mL), significantly exceeding the values observed in the other treatments. In contrast, the lowest carotenoid level was recorded in treatment T_5.

Phosphate analysis

According to Table 3, increasing the dilution of wastewater led to a decrease in the initial

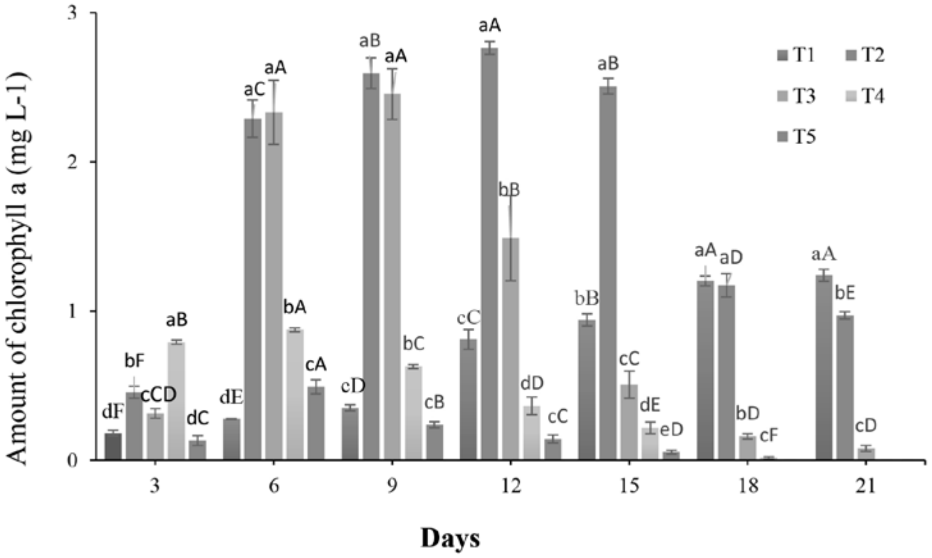

Fig. 1. Chlorophyll a content of microalgae *D. tertiolecta* in different treatments (lowercase and uppercase letters indicate significant differences ($p< 0.05$) on one and different days between treatments, respectively))

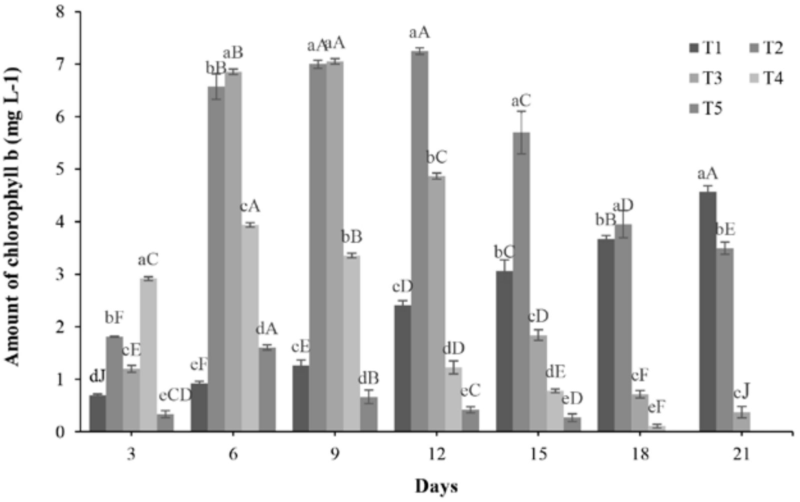

Fig. 2. Chlorophyll b content of microalgae *D. tertiolecta* in different treatments (lowercase and uppercase letters indicate significant differences ($p < 0.05$) on one and different days between treatments, respectively)

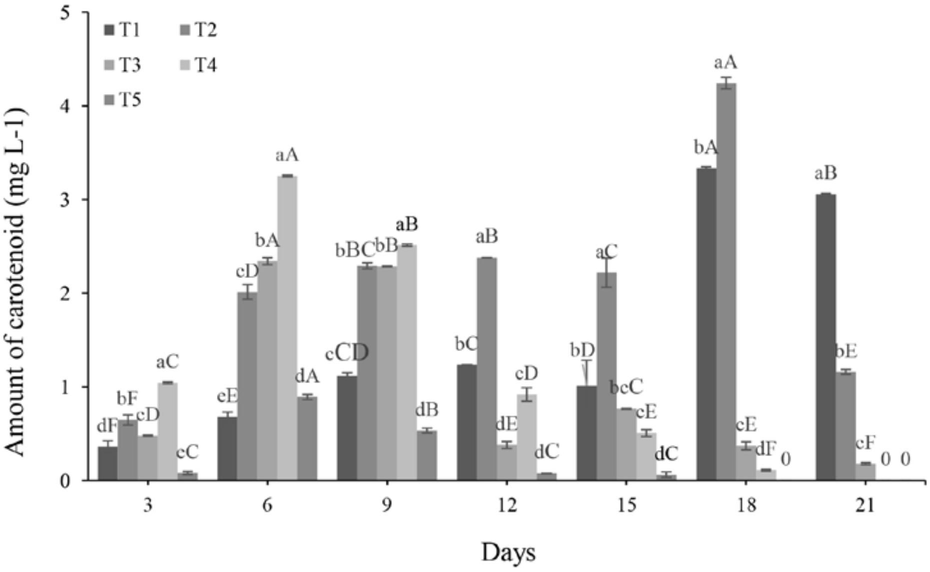

Fig. 3. Total carotenoid content of microalgae *D. tertiolecta* in different treatments (lowercase letters indicate significant differences ($p<0.05$) on one day between different treatments, and uppercase letters indicate significant differences ($p<0.05$) between each treatment on different days)

Table 3. Phosphate concentrations (mg L^{-1}) in different treatments

Phosphate	Day				
	1st	5th	10th	15th	20th
T_2	368.2 ± 0.4^{dA}	272.1 ± 0.3^{cB}	234.8 ± 0.5^{dC}	198.8 ± 0.4^{dD}	176.6 ± 0.3^{bE}
T_3	387.8 ± 0.4^{cA}	264.5 ± 0.4^{dB}	249.3 ± 0.4^{cC}	228.4 ± 0.3^{cD}	219.4 ± 0.5^{aE}
T_4	394.3 ± 0.5^{bA}	320.8 ± 0.4^{bB}	310.9 ± 0.4^{bC}	308.2 ± 0.3^{bD}	-
T_5	405.7 ± 0.5^{aA}	382.4 ± 0.3^{aB}	368.4 ± 0.5^{aC}	361.5 ± 0.4^{aD}	-

(Uppercase letters indicate significance ($p < 0.05$) in each row, and lowercase letters indicate significance ($p < 0.05$) in each column) phosphate concentration. The highest initial phosphate level was observed in the treatment with 100% wastewater (T5), measuring 405.7 mg/mL.There were only slight changes in phosphate removalafter day 10 in treatments T4 and T5. However, treatments T2 (234.8 mg/mL) and T3 (249.3 mg/mL) demonstrated a greater capacity for phosphate reduction compared to the other treatments. Overall, the results indicate that phosphate concentrations significantly decreased over time across all treatments ($p < 0.05$).

Nitrate analysis

Nitrate concentrations across the different treatments during the experimental period are presented in Table 4. The results indicated a decreasing trend in nitrate levels over time in all treatments. The highest initial nitrate concentration was recorded in treatment T5 on day 1, with a value of 53.4 mg/mL. All treatments, except T3, showed statistically significant differences in nitrate levels on days 10, 15, and 18 ($p < 0.05$).

Ammonia analysis

According to Table 5, the ammonia levels in all experimental treatments exhibited a significant reduction from day 1 to day 10. Following day 10, the ammonia removal rate declined. Statistically significant differences ($p < 0.05$) were observed in all treatments except T_3 on days 10 and 15, as well as treatments T_2 and T_3 on day 20.

Phosphate, nitrate, and ammonia removal

Table 4. Nitrate concentrations (mg L^{-1}) in different treatments

Nitrate	Day				
	1st	5th	10th	15th	20th
T_2	28.6 ± 0.8 ^{dA}	19.7 ± 0.3 ^{cB}	14.8 ± 0.4 ^{dC}	12.6 ± 0.4 ^{dD}	11.8 ± 0.6 ^{bD}
T_3	32.8 ± 0.4 ^{cA}	18.7 ± 0.5 ^{dB}	17.2 ± 0.3 ^{cC}	16.8 ± 0.3 ^{cD}	16.2 ± 0.5 ^{aC}
T_4	48.2 ± 0.5 ^{bA}	36.8 ± 0.5 ^{bB}	32.4 ± 0.3 ^{bC}	30.8 ± 0.3 ^{bD}	-
T_5	53.4 ± 0.5 ^{aA}	46.2 ± 0.4 ^{aB}	44.7 ± 0.4 ^{aC}	41.5 ± 0.4 ^{aD}	-

(Uppercase letters indicate significance ($p< 0.05$) in each row, and lowercase letters indicate significance ($p< 0.05$) in each column)

Table 5. Ammonia concentrations (mg L^{-1}) in different treatments

Ammonia	Day				
	1st	5th	10th	15th	20th
T_2	14.2 ± 0.4 ^{dA}	12.4 ± 0.5 ^{dB}	8.9 ± 0.3 ^{dC}	6.6 ± 0.3 ^{dD}	5.8 ± 0.4 ^{aE}
T_3	18.6 ± 0.3 ^{cA}	15.2 ± 0.3 ^{cB}	11.6 ± 0.3 ^{cC}	10.2 ± 0.3 ^{cC}	7.1 ± 2.2 ^{aD}
T_4	26.4 ± 0.3 ^{bA}	23.6 ± 0.3 ^{bB}	20.8 ± 0.4 ^{bC}	19.4 ± 0.5 ^{bD}	-
T_5	29.8 ± 0.4 ^{aA}	25.9 ± 0.3 ^{aB}	24.6 ± 0.3 ^{aC}	23.8 ± 0.4 ^{aD}	-

(Uppercase letters indicate significance ($p< 0.05$) in each row, and lowercase letters indicate significance ($p< 0.05$) in each column)

Table 6 indicates that the highest nitrate removal rate (58.75%) was observed in the 25% wastewater treatment (T2), while the lowest rate (22.28%) was recorded in the 75% treatment (T5). A statistically significant difference was observed among all treatments. Phosphate removal percentages also showed significant differences across treatments ($p < 0.05$), with the highest and lowest removal rates corresponding to T2 (52.3%) and T5 (10.89%), respectively. Regarding ammonia, the highest removal rates were observed in T3 (61.54%) and T2 (59.18%). However, no significant differences were found between T2 and T3, nor between T4 and T5.

Discussion

Biomass Production

The growth of microalgae and biomass productivity is significantly influenced by environmental parameters, including salinity, light intensity, temperature, pH, total dissolved solids (TDS), and nutrient availability. These factors play a crucial role in regulating photosynthetic efficiency and cellular metabolism, ultimately determining the success of algal cultivation systems (Peralta, 2023). In the present study, the highest biomass yield was observed in the treatment containing 25% dairy wastewater. This dilution appears to strike an optimal balance between nutrient availability and the mitigation of potential toxicity. At higher concentrations (75% and 100%), growth declined earlier, likely due to excessive organic load, oxygen depletion, pH fluctuations, and nutrient exhaustion. These findings align with those of Khalaji et al. (2019), who reported enhanced biomass production of *Chlorella vulgaris* in 25% dairy wastewater. Similarly, Salla et al. (2016) found that supplementing *Spirulina* cultures with whey protein concentrate significantly boosted biomass. Salgueiro et al. (2016) observed that *C. vulgaris* initially underwent an adaptation phase in wastewater, followed by exponential growth, and biomass increased from 0.05 g/L to 0.75 g/L within 9 days before declining due to environmental limitations. Lu et al. (2017) studied *Spirulina platensis* in brewery wastewater and reported that a 50% concentration caused excessive turbidity, reducing light penetration and photosynthetic efficiency. This is consistent with the reduced growth observed in treatments T4 and T5 of the present study. They also noted that while 10% dilution limited growth due to insufficient nutrients, a 20% concentration provided optimal conditions—closely mirroring the performance of the 25% treatment in this research.

Pigment Dynamics

Chlorophyll a and chlorophyll b are the primary photosynthetic pigments in algae, essential for capturing light energy and driving the photosynthetic process (Robertson, 2021; Nave, 2023). Chlorophyll b serves not only as an accessory pigment that broadens the spectrum of light absorption in photosynthetic organisms, but also plays a regulatory role in modulating the activity of other photoreceptors. Under environmental stress conditions, such as reduced light intensity, chlorophyll b contributes to cellular defense mechanisms by minimizing oxidative damage through enhanced energy dissipation and photoprotection (Nave, 2023).

Chlorophyll is a nitrogen-rich pigment, with each molecule containing four nitrogen atoms embedded within the porphyrin ring. Under nitrogen-deficient conditions, plants mobilize internal nitrogen reserves to sustain essential processes such as cell division and photosynthesis. This limitation reduces photosynthetic efficiency and modifies the relative concentrations of chlorophyll a and b. Conversely, elevated levels of nitrogen and magnesium—such as those found in dairy wastewater—can promote chlorophyll biosynthesis and enhance photosynthetic performance through facilitating enzyme activation and pigment production (Chen, 2024). In this study, chlorophyll levels initially increased due to elevated nitrogen and magnesium in the dairy wastewater, which promote chlorophyll biosynthesis and enzymatic activity However, as cultivation progressed, nutrient depletion, temperature stress, and increased turbidity from biomass accumulation led to a decline in chlorophyll a. Dickinson et al. (2014) reported that *Scenedesmus* sp. grown in municipal wastewater exhibited chlorophyll a levels 2.5 times higher in nutrient-rich treatments, but light limitation due to biomass build-up eventually reduced pigment content. De Francisci et al. (2017) found that *C. sorokiniana* cultivated in mixed municipal-industrial wastewater produced average pigment levels of 0.44 mg/g β-carotene and 11.82 mg/g chlorophyll (dry weight), indicating wastewater can enhance pigment synthesis. Carotenoids absorb light in the 400–550 nm range and protect cells from oxidative stress and high radiation. *Dunaliella tertiolecta* is particularly adept at increasing carotenoid synthesis under stress conditions such as high salinity, nutrient deficiency, and intense lightup to 42 pg/cell (Trenkenshu, 2005). In the present study, the highest carotenoid content was observed in the 25% wastewater treatment, significantly higher than the control ($p < 0.05$). Seo et al. (2024) observed that *Halochlorella rubescens* grown in pig wastewater exhibited elevated chlorophyll and carotenoid levels on day 4, with carotenoids doubling compared to initial values. Additionally, Zhang et al. (2017) demonstrated that *C. vulgaris* increases carotenoid synthesis under gradual nitrogen depletion. These findings reinforce the stress-induced pigment dynamics observed in *D. tertiolecta*. Microalgae's ability to absorb nutrients from dairy wastewater makes it a cost-effective medium for biomass production and wastewater treatment (Costa et al., 2021). In this study, nutrient removal efficiency varied by dilution level. The 25% wastewater treatment achieved the highest nitrate and phosphate removal by the end of cultivation, while the 50% treatment showed the highest ammonia removal. These results suggest that moderate dilution optimizes nutrient uptake by reducing toxicity and maintaining sufficient nutrient levels. Khalaji et al. (2019) conducted a study on *Chlorella* at two different cell densities (13 and 26 million cells/mL) and three varying wastewater concentrations (25%, 50%, 75%). At the lower cell density, they observed a phosphate removal rate of 51.84% in 25% wastewater, a nitrate removal of 57.01% in 50%, and an ammonia removal rate of 44.25% in 50%, which closely matches the present findings. At lower cell density, Brar et al. (2019) compared

Chlorella, *Scenedesmus*, and *Anabaena* in the context of dairy wastewater, revealing that nitrate removal efficiencies of 88.91%, 84.72%, and 89.52%, respectively, alongside phosphate removal rates of 86.51%, 79.02%, and 87.83%. Interestingly, it was observed that higher wastewater concentrations improved phosphate removal, which stands in contrast with the current study. This discrepancy may stem from differences in algal species, wastewater composition, or experimental conditions. Ahmed et al. (2014) reported that *Spirulina* removed 72% phosphate and 80% nitrate from dairy wastewater, with nitrate removal consistently outperforming phosphate, similar to the trend observed in this study. Khemka & Safraf (2017) found that *Desertifilum tharense* removed up to 98% phosphate and 94% nitrate, significantly higher than the rates in this study, likely due to species-specific traits and optimized conditions.

Conclusion

The results demonstrated that the highest cell density was achieved in the 25% wastewater treatment (T2) on day 12, reaching 54.20 × 10^4 ± 1 cells/mL. Similarly, the highest concentrations of chlorophyll a (2.76 ± 0.04 mg/mL), chlorophyll b (7.24 ± 0.06 mg/mL), and carotenoids (2.24 ± 0.06 mg/mL) were also observed in T2. Regarding nutrient removal, T2 showed the most effective reduction in phosphate (0.052 ± 0.02 mg/mL) and nitrate (0.059 ± 0.94 mg/mL), while the highest ammonia removal (0.062 ± 2.23 mg/mL) was recorded in the 50% wastewater treatment (T3). Subsequently, the findings of this study highlight *Dunaliella tertiolecta* as a promising candidate for integrated wastewater treatment and biomass production. Its superior performance in the 25% dairy wastewater treatment, reflected in biomass yield, pigment synthesis, and nutrient removal, demonstrates its resilience and adaptability to semi-stressful environments. The 25% dilution level provides a strategic balance: it minimizes toxicity from organic overload while maintaining sufficient nutrient concentrations and light penetration. This balance supports robust growth and metabolic activity, making it ideal for scalable applications. Compared to other species such as *Chlorella vulgaris* and *Spirulina*, *D. tertiolecta* exhibits enhanced carotenoid synthesis and efficient nutrient uptake under moderate stress. These traits underscore its potential for use in photobioreactors, biofuel production, and eco-friendly wastewater remediation.

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