Research Article

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Assessment of Nutrient Removal Efficiency of Marine Microalgae *Dunaliella salina* in Saline Food Industry Wastewater

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ABSTRACT

Industrial wastewater release into the environment is a critical global challenge leading to severe water pollution, adversely impacting terrestrial and aquatic ecosystems requiring effective solutions. The treatment, disposal, and recirculation of saline wastewater from food containing nitrate and phosphate poses significant health and environmental risks. This research aims to address the challenge by investigating the potential of using the marine microalgae *Dunaliella salina* for treating saline food industry wastewater, both in laboratory and pilot-scale settings using raceway tanks. In the laboratory study, a mixture of wastewater and F/2 medium was inoculated with *Dunaliella salina* to assess its potential for growth in saline wastewater, rich in nitrate and phosphate. The study observed a cell density of 5.5 x 10^5 cells/ml. Subsequently, a pilot-scale study was conducted, where the maximum cell density of 6.2 x 10^5 cells/ml was achieved. The efficacy of *Dunaliella salina* in removing nitrate and phosphorus from the culture was evaluated by measuring the levels of these compounds. A significant reduction in nitrate concentration from 146 to 22.16 mg/l was observed, with a removal efficiency of 84.82%; similarly, the phosphate concentration decreased from 55.3 to 10.79 mg/l, with a removal efficiency of 80.49 %. The reductions in nitrate and phosphate concentrations, along with the demonstrated growth of *Dunaliella salina* in saline industrial wastewater, confirmed the feasibility of employing these microalgae for bioremediation treatment in a sustainable way.

Keywords: *Dunaliella salina*; Wastewater treatment; Saline effluent; Marine microalgae; Bioremediation.

1. INTRODUCTION

Industrial wastewaters have high concentrations of pollutants. As the global population continues to expand, industries are generating increasingly larger volumes of high-strength effluents with various salinity and nutrient loads daily. The primary objective of wastewater treatment is to substantially decrease the levels of carbonaceous materials such as nitrogen (N), and phosphorus (P) compounds, before being discharged into receiving systems (Sharma *et al*. 2020). The uncontrolled release of untreated wastewater has adverse impacts on the levels of dissolved oxygen, and the health of aquatic fauna and flora (Ullah Bhat and Qayoom, 2022). High-strength wastewaters with variable salinity and nutrient loads were produced in several industrial processes.

The food industry utilizes high salt concentrations to preserve and enhance the texture of food products; as a result, the effluent released after processing frequently contains elevated salinity levels. Salinity serves as a crucial factor in wastewater treatment. High-saline water typically contains inorganic salt contents ranging from 1 to 3.5% w/w of sodium chloride (Zhao *et al*. 2020). Wastewaters from food processing industries are treated by different methods such as Combined physiochemical and biological methods, Reverse osmosis, Electrochemical, Chemical oxidation Electro-oxidation, Microbial fuel cells, Dissolved air floatation, Micro/ultra/nanofiltration, and Photocatalytic degradation methods; but these methods have their limitations like fouling issues, membrane clogging problems and high electricity consumption (Shrivastava *et al*. 2022).

Industries currently rely on Effluent Treatment Plants (ETPs) for treating their wastewater; however, this system has drawbacks such as increased costs, particularly due to high salinity levels. Effluents from the food industry are typically abundant in organic matter, phosphate, nitrate, ammonia, and industry-specific compounds, which can vary depending on the chemicals/methods used in their process. The discharge of untreated high-salinity wastewater poses significant risks of environmental pollution, impacting aquatic life, water quality, and agricultural suitability. Hence, it is necessary to implement effective methods for treating

high-salinity wastewater, aiming for zero discharge, and promoting water recycling (Guo *et al*. 2023). The Paris Climate Agreement has devised the 'Kyoto Protocol' and is signed by more than 190 nations across the globe, which emphasizes the reduction of industrial pollution as a measure to combat climatic changes. The high salinity of industrial wastewater poses challenges for biological treatment systems due to the elevated concentrations of salt (Uygur and Kargı, 2004); however, microalgae-based wastewater treatment offers a cost-effective solution that facilitates nutrient removal and CO_2 bio-fixation. Microalgae have the potential to utilize both organic and inorganic nitrogen, enhancing their versatility in wastewater remediation processes (Mohsenpour *et al*. 2021).

Dunaliella salina exhibits a strong adaptability to high-salt conditions, making it an ideal candidate for wastewater treatment. It can thrive in salt concentrations ranging from 9 to 200 g/l (Oren, 2014), and it efficiently converts sunlight, warmth, water, and minerals into essential nutrients such as proteins, carbohydrates, vitamins, and amino acids (Moayedi *et al*. 2019). Under favorable conditions, *Dunaliella salina* exhibits remarkable productivity of high quantities of beta and alpha-carotene (deep orange-red pigments). Studies indicate that *Dunaliella salina* can effectively remove nitrate, ammonia, and phosphorus from wastewater, achieving removal rates ranging from 45 to 88% (Liu and Yildiz, 2018). Utilizing *Dunaliella salina* for wastewater treatment offers a promising avenue to address saline wastewater challenges and transform treatment processes into profitable ventures, fostering sustainable circular bio-economy development (de-Souza *et al*. 2024). Despite its potential, *Dunaliella salina* remains relatively unexplored in wastewater treatment; this research endeavors to investigate its utilization in treating saline food industry wastewater.

2. MATERIALS AND METHODS

2.1. Collection and Culture of Algae

Dunaliella salina was isolated from the mixed algal strains collected from the Salt Pan located in Vedaranyam, Tamilnadu, India. Standard plating techniques were used to isolate pure algal strain, so the samples were inoculated onto F/2 medium supplemented with 2% agar and incubated at 22 ± 2 °C with the light intensity of 100 µmol/m^2/s with photoperiod of 24 h. Subsequent streaking of isolated colonies onto fresh plates was repeated until mono-algal culture was obtained. The morphology traits of the algal isolates, comprising size, shape, and internal features such as cell wall, flagellate and chloroplast were meticulously assessed and documented through photography. The molecular examination was concurrently performed through 16S/18S rRNA gene sequence analysis to complement the morphological examination. The isolated cultures were inoculated on sterilized F/2 media containing 1 M NaCl and are maintained in a 1-liter Erlenmeyer flask with a light intensity of 100 µmol/m^2/s with a photoperiodism of 12 h of darkness and 12 h of light, at 27 °C (Achour *et al*. 2019).

2.2 Collection of Effluent

Raw wastewater samples were directly sourced from the discharge point of the food industry located in Erode, Tamilnadu, India, and collected using a sanitized container. The samples were transported to the laboratory under proper conditions for analysis. A comprehensive analysis was carried out for various physicochemical properties in accordance with the protocols outlined in APHA (1998) and the findings from these analyses are detailed in Table 1.

Table 1. Major parameters of food industry wastewater

Parameter	Unit	Value
pH	-	7.2
COD	mg/l	458
BOD	mg/l	1862
NO_3-	mg/l	146
PO_3-	mg/l	55.3
Salinity	ppt	30

Table 2. Batch culture trials

Trial	Microalgae Inoculum (ml)	Wastewater (WW) (ml)	F/2 Medium (ml)	MiR [WW/(WW + Medium)]
T1	50	0	450	0
T2	50	50	400	0.11
T3	50	100	350	0.22
T4	50	150	300	0.33
T5	50	200	250	0.44
T6	50	250	200	0.55
T7	50	300	150	0.66
T8	50	350	100	0.77
T9	50	400	50	0.88
T10	50	450	0	1

2.3 Batch Culture Experiments

An initial trial was performed to determine the optimum quantity of wastewater for promoting the growth of *Dunaliella salina* and COD removal. The salinity was maintained at 30 ppt for all the trials. A series of 10 trials (T1 through T10) were performed using 1-liter Erlenmeyer flasks, with each experiment replicated three times. The T1 trial was the control for the experiment and the rest of the trials with different mixed ratios were the tests as listed in Table 2. The experiment was carried out under controlled conditions with the parameters, viz., temperature, light intensity, salinity, and pH, at 25 °C, 100 µmol m^{-2} s^{-1}, 35 ppt, and 8.2, respectively. Adequate aeration was provided to the system continuously. In the whole experiment, no

supplementary nutrients were introduced to the system. The cell count was carried out using a Haemocytometer and optical microscope. The culture was assessed for cell growth and contaminations under the magnification of 40 X and 100 X.

2.4 Design and Fabrication of 500 L Mini Raceway Tanks

The Mini Raceway tanks (9 ft. x 3 ft.) were designed with a central division and fabricated with fiber-reinforced plastic. The tanks were designed as an oval raceway compact system with the pond's length-to-width ratio of 3:1 (L/D ratio = 3) and height of 0.3 m with a central division to create a loop/raceway flow (Bosma *et al*. 2014). The tank's total capacity is 1 m^3 with a working volume of 0.5 - 0.7 m^3. A paddle wheel with eight blades was installed 5 cm from the bottom and operated at 15 - 20 rpm controlled by variable frequency drive.

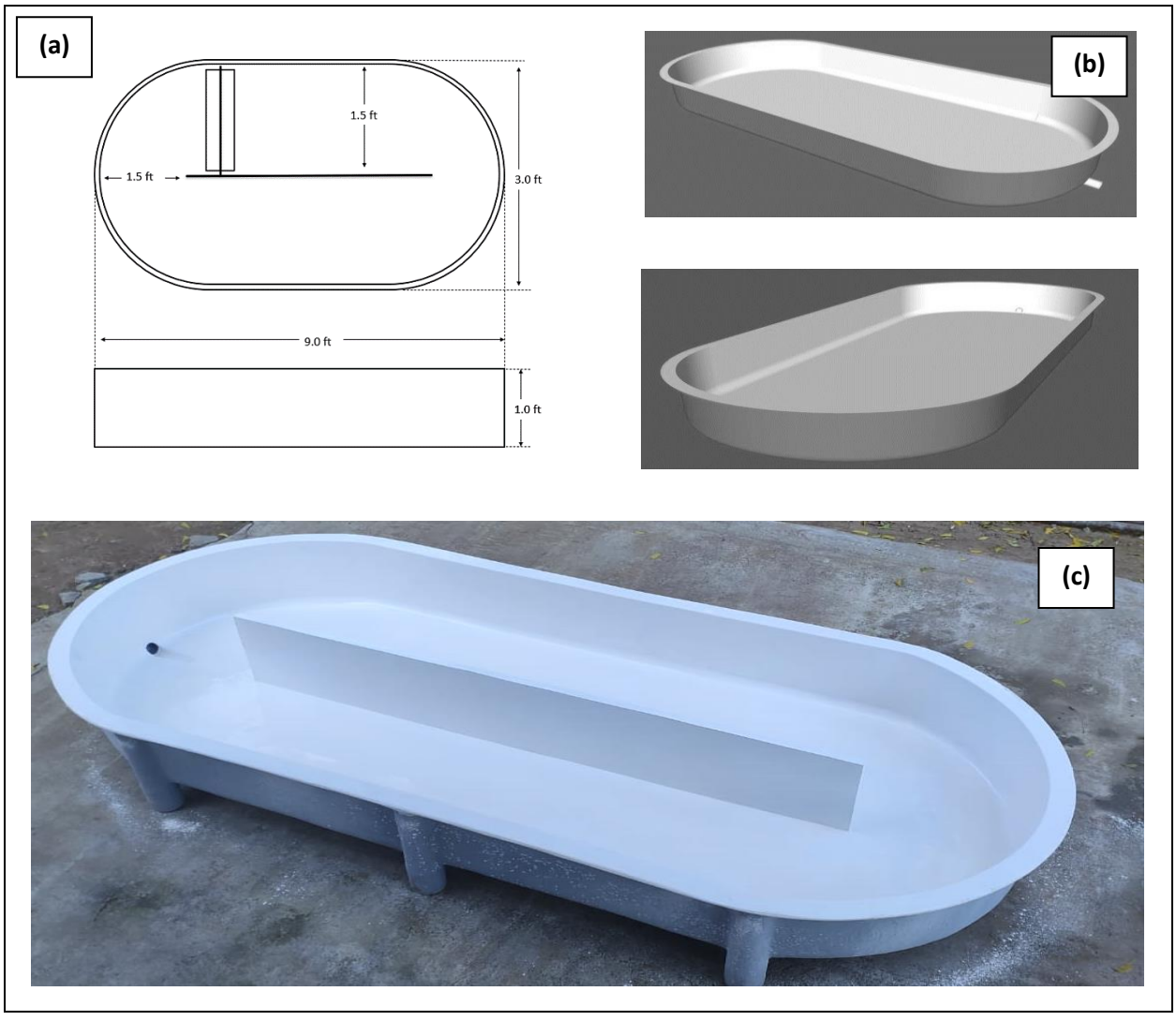


Fig. 1: a) Design diagram of Mini Raceway tanks with dimensions b) Computed design model of Mini Raceway tank and c) Mini Raceway tank fabricated with fiber-reinforced plastic

2.5 Growth of Microalgae in Mini Raceway Tanks

The wastewater was filtered and pretreated by chlorine (10 ppm) for 24 h with continuous air sparging. The treated wastewater of 500 L was used for the cultivation of microalgae *Dunaliella salina* in the raceway tank. The raceway tank was inoculated with 50 L of *Dunaliella salina* culture with a cell density of 8 x 10^6 cells/ml. The paddle was operated with a velocity of 0.25 m/s. *Dunaliella salina* was grown in wastewater for 30 days to determine the efficiency of algae. The experiment was carried out outdoors, and the light was limited (200 – 600 µmol m-2 s-1) to the algae using shade

nets. The parameters such as temperature and pH were measured using electrodes, the light was measured with a light meter and the cell count was monitored with a hemocytometer and a microscope. The nitrate and phosphate values were measured using a Spectrophotometry assay. Nitrate and phosphate removal efficiencies were calculated using the following equation:

$$Removal\ efficiency\ (\%) = \frac{100 \times (C_{Initial} - C_{Final})}{C_{Initial}}$$

where, $C_{Initial}$ (mg l^{-1}) is the initial concentration and C_{Final} (mg l^{-1}) is the final concentration at the end of the experiment.

3. RESULTS AND DISCUSSION

3.1 Growth of *Dunaliella salina*

Dunaliella salina strain was isolated from the mixed culture by using standard methods and it was named as *Dunaliella salina* . Microscopic examination revealed that the cells exhibited an ovoid to spherical morphology and were motile due to the presence of two flagella. Cultivation experiments were conducted using an F/2 medium supplemented with 1 M NaCl, indicating the halotolerant nature of *Dunaliella salina* . The average cell size ranged from 15.2 ± 0.1 to 9.0 ± 0.3 µm (Borowitzka and Siva, 2007). Confirmation of the algal species was achieved through 16s/18s RNA sequencing. Fig 3.1 displays a microscopic image of *Dunaliella salina* at a magnification of 40X.

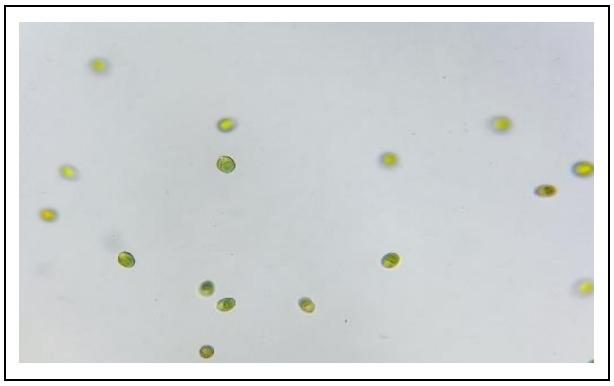


Fig. 2: Microscopic image of *Dunaliella salina* under 40X magnification

3.2 *Dunaliella salina* Growth in Different Concentrations of Effluent

Batch experiments were conducted to assess the potential for cultivating *Dunaliella salina* in saline food industry wastewater. A series of trials were designed with varying concentrations of wastewater combined with F/2 medium, and the algae's growth was monitored over 15 days. Fig. 2 illustrates the growth patterns, displaying cell counts over time for trials labeled T1 through T10. In the control trial (T1) conducted without wastewater, *Dunaliella salina* exhibited rapid growth with virtually no lag phase. Conversely, trials T2 to T9, which involved varying mixtures of wastewater and F/2 medium, demonstrated that the strain adapted quickly to the environmental conditions, and the lag phase was observed in these trials due to the nature of the wastewater. However, trial T10, utilized only wastewater, exhibited limited growth due to the higher concentration of pollutants present. Cell density began to increase notably from day 5 in all the trials. The change in coloration observed in the cultures from day 5 was indicative of the increased number of cells suspended in the medium. These findings provide further support that saline food industry wastewater can serve as a viable nutrient source for the growth of *Dunaliella salina*.

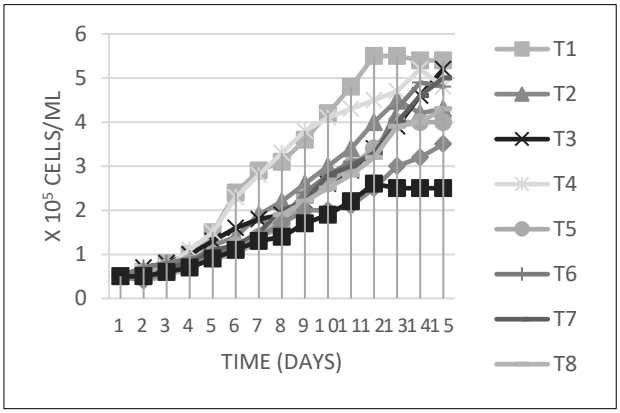
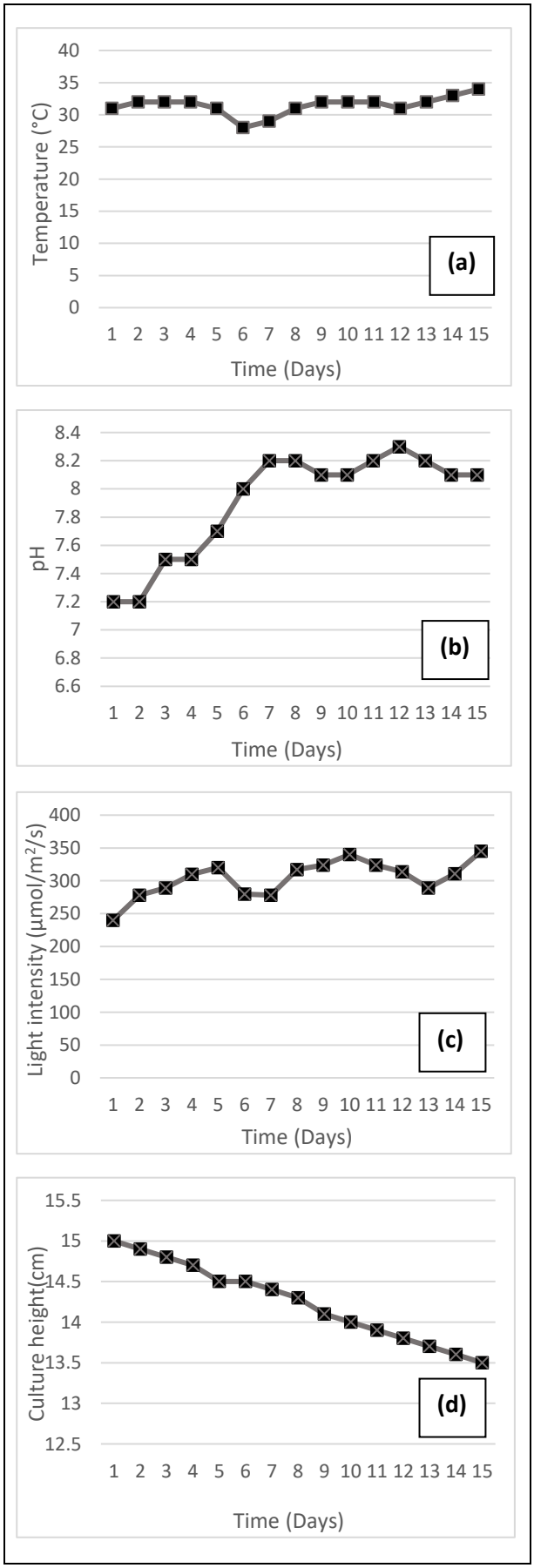


Fig. 3: Growth curve of *Dunaliella salina* with different concentrations of wastewater

3.3 Microalgal Growth in Mini Raceway Tanks

In this batch experiment, 450 l of pre-treated saline food industry wastewater was filled into mini raceway tanks with a capacity of 1000 l and a working volume of 500 l. These tanks were inoculated with 50 l of *Dunaliella salina*, which had been grown in F/2 medium with an inoculum density of 6 lakhs cells/ml. Therefore, the initial cell density (on Day 1) was calculated as 60,000 cells/ml. The batch experiment was conducted for 15 days in an outdoor environment, during which parameters such as temperature, pH, light intensity, and culture height were monitored and depicted in Figures 4 a - 4 e. Algal growth was observed from Day 2 of inoculation, as indicated by the recorded data. The maximum cell growth of approximately 6.2 lakhs cells/ml was achieved on Day 10, after which no further growth was observed, and the cell count remained stationary. Subsequently, the final concentrations of nitrate and phosphate were analyzed to determine the removal efficiency by *Dunaliella salina* . On Day 15, the concentration of nitrate was reduced from 146 to 22.16 mg/l, with a removal efficiency of 84.82%. Similarly, the phosphate concentration decreased from 55.3 to 10.79 mg/l, with a removal efficiency of 80.49%.



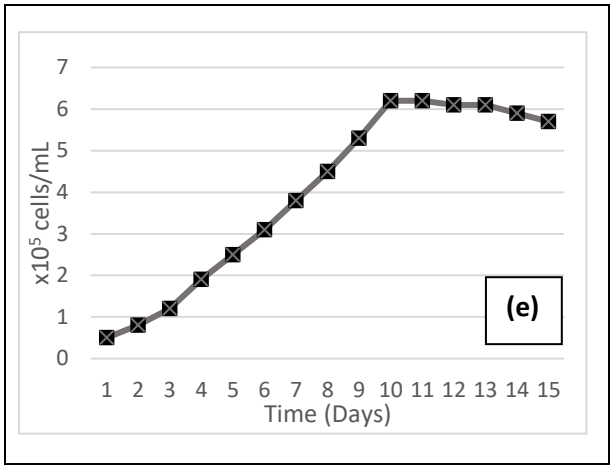


Fig. 4: Major parameters of *Dunaliella salina* grown in wastewater in Mini Raceway tanks (a) Culture temperature, (b) pH, (c) Light Intensity, (d) Culture height and (e) Cell density

Moreover, *Dunaliella salina* growth in the saline wastewater resulted in a reduction of COD by 72.48%, with the concentration decreasing from 458 to 126 mg/l. Overall, these results demonstrate the effectiveness of *Dunaliella salina* in treating saline food industry wastewater, as evidenced by significant reductions in nitrate, phosphate, and COD concentrations, highlighting its potential for environmental remediation.

Table 3. Nutrient Removal efficiency of *Dunaliella salina*

Parameters	Initial concentration (mg/L)	Final concentration (mg/L)	Removal Efficiency (%)
COD	458	126	72.48
NO^{3+} (N)	146	22.16	84.82
PO^{4+} (P)	55.3	10.79	80.49

4. CONCLUSION

High saline wastewater poses significant challenges in terms of treatment and disposal, making it imperative to explore alternative methods for its utilization. *Dunaliella salina*, a type of microalgae, shows promise as a solution for treating high saline wastewater, especially in industries such as food processing where salt is used extensively for processing and preservation. Overall, the findings of the experiment confirm the efficacy of *Dunaliella salina* in treating saline food industry wastewater. The significant reductions observed in nitrate, phosphate, and COD concentrations demonstrate the algae's ability to effectively remediate pollutants in such environments. With its ability to efficiently remove pollutants from wastewater, particularly in saline environments,

Dunaliella salina holds promise as a sustainable and effective solution for mitigating the environmental impact of industrial wastewater discharge. Thus, this study concludes that *Dunaliella salina* represents a viable option for targeted bioremediation efforts in saline industrial wastewater treatment processes. Implementing the process of using *Dunaliella salina* for circularly treating saline food industry wastewater holds great promise for industrial sustainability; furthermore, it can yield additional value-added products, such as biofuels, animal feed supplements, or high-value compounds like antioxidants or pigments. By extracting these products from the algae, the industry can generate additional revenue streams while simultaneously reducing waste.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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