Research Article

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A dual approach using response surface methodology and machine learning for optimization and enhancement of microalgae-based municipal wastewater treatment

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Abstract

BACKGROUND: Municipal wastewater comprises both organic and inorganic contaminants. Especially in rural areas, conventional municipal treatment plants primarily focus on carbon removal; therefore, nutrient removal should be prioritized for preventing environmental pollution. Mixotrophic microalgae such as *Nannochloropsis* sp. have significant potential for both carbon and nutrient removal. However, microalgae-based wastewater systems can be affected by many parameters and, using response surface methodology and decision tree, a machine learning model can help to determine the optimal conditions for the systems to operate more efficiently.

RESULTS: The optimal removal conditions were determined by response surface methodology to be a light period of 21 h at an intensity of 8000 lx and a temperature value of 30 °C. Under these optimal conditions, the respective removal efficiency for chemical oxygen demand, total organic carbon, total Kjeldahl nitrogen, and orthophosphate was 53%, 34%, 87%, and 70%, respectively. Chlorophyll-a concentration increased by as much as 49%. Real municipal wastewater was used instead of synthetic wastewater, yielding closer approximations to real situations.

CONCLUSION: The present study has underscored innovative, data-driven approaches as core in ensuring sustainable wastewater management and sets a useful framework for future research, which could be done with the aim of refining the methods to enhance efficiency in treatment.

Supporting information may be found in the online version of this article.

Keywords: municipal wastewater; microalgae; *Nannochloropsis* sp.; response surface methodology; decision tree; optimization

INTRODUCTION

Management of municipal wastewater is a significant issue due to the high volume of wastewater produced daily and the high cost of effective methods for treating nutrients and sludge.[1] Alternative systems need to be investigated and optimized for effective treatment. The annual amount of municipal wastewater is 267.5 billion m^3, of which only 54.7% is treated, while the other 45.3% is discharged into nature without treatment.[2] Especially in rural areas, there is a lack of a proper municipal wastewater treatment system for nutrient removal, and discharging it into the environment without removing key pollutants leads to environmental threats[3] such as eutrophication and mucilage. The mucilage formation, which is closely linked to nutrient enrichment, has caused environmental threats in habitats such as the Adriatic Sea,[4] Ligurian Sea,[5] and Sea of Marmara.[6] Therefore, alternative processes should be prioritized[7] to treat nutrient-rich wastewater without high treatment costs. Microalgae-based wastewater treatment systems have great potential in small settlements where nutrient treatment systems are limited due to tight budgets for plants and lack of technical staff.[8] The use of microalgae for wastewater treatment has been reported in different studies.[9–11] The production of alternative materials from this microalgae production could be a key to a circular economy and resource recovery.[12]

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The microalgae can develop in three ways in the receiving environment: phototrophic, heterotrophic, and mixotrophic.[13] Mixotrophic microalgae such as *Nannochloropsis* sp. have enormous potential in wastewater treatment due to their ability to remove both nutrients and organic matter.[14] Contrary to conventional aerobic biological wastewater treatments, which are generally associated with high operation costs and production of biological sludge-demanding further treatment, microalgae treatment by mixotrophic species may have lower operational costs.[15,16] Studies related to municipal wastewater treatment using *Nannochloropsis* sp. are limited in the literature[17,18] compared to other microalgae strains. Moreover, studies have mostly examined the effluent from domestic wastewater treatment facilities as feed.[18,19] It has been stated that processes fed with effluent wastewater constrain microalgal growth. In this respect, it is necessary to test actual influent municipal wastewater and determine the potential of microalgae under different operating parameters. Moreover, despite microalgae-based systems representing a promising approach for wastewater treatment, their full-scale application is still limited,[20] because these systems are affected by various parameters, such as fluctuating wastewater volumes, microalgae species selection, and the optimization of operational conditions.[21] Therefore, it is a challenge to determine optimal values of the parameters for different wastewater types. *Nannochloropsis* sp. is a marine microalga, which has had only limited tested with wastewaters compared to other species, and optimal conditions have not been investigated in detail within the wastewater treatment processes. In this respect, testing the potential of microalgae under different operating parameters is essential for the systems. For this purpose, further studies are needed for process optimization and/or prediction of optimal conditions.

Recent studies indicate that there is an interest in the application of statistical software and machine learning (ML) algorithms to optimize and predict process outcomes.[22] Response surface methodology (RSM) is a statistical strategy for optimization of the processes[23,24] and can be utilized to optimize wastewater treatment by adjusting various process parameters, such as the light cycle,[23–25] light intensity,[24–26] temperature,[23,26] and nutrient concentration.[27] While RSM can provide optimization for parameters, ML also has the potential to enhance the process.[28]

ML can effectively elucidate the interactions between the input and output of the process, as exemplified in wastewater management.[21,29] Nonetheless, generating these extensive datasets for real-scale applications is infeasible both economically and temporally.[30] In a study comparing many ML models for wastewater treatment, it was concluded that a decision tree was the most suitable algorithm for classifying parameter data.[29] For decision trees, a substantial dataset is preferred to enhance accuracy. For example, Singh *et al.*[29] predicted the optimal combinations for increasing microalgae biomass production through a decision tree algorithm and achieved an overall accuracy of 81.25% in the 18 combinations identified. Through the analysis of variable features, ML may offer insights that improve the comprehension and optimization of processes.[31] Moreover, it is advantageous for identifying patterns that may not be readily apparent through traditional methods. The decision tree was identified as the most appropriate algorithm for classifying parameter data in a study that compared various ML models for wastewater treatment.[32] Recently, decision tree models have been used for the prediction of initial inoculum level, temperature, nitrogen/phosphorus ratio, light intensity, and pH.[29] ML can be combined with other statistical approaches in order to gain a deeper comprehension of complex systems.

The integration of RSM and ML methods in a hybrid approach has the potential to enhance the predictive accuracy of optimization models aimed at maximizing system performance, particularly regarding biomass growth and/or wastewater treatment efficiency. This dual approach effectively overcomes the technical challenges associated with microalgae cultivation in wastewater.[30,33] It has been stated that the economic viability of microalgae-based treatment systems can be significantly improved by optimizing operational parameters using RSM[34] and ML.[29] Recently, a limited number of studies that utilized both RSM and ML for various goals have been reported. RSM and artificial neural network algorithms have been applied to biodiesel production using *Chlorella pyrenoidosa*, resulting in more precise predictions by reducing trial-and-error costs.[35] In another study, RSM, analysis of variance (ANOVA), and deep neural networks were combined to optimize photobioreactor conditions, where *Chlorella vulgaris* was used for bioremediation.[36] The combined use of AdaBoost and XGBoost algorithms with RSM in wastewater has only been studied in a work where microalgae were used in gray water, examining the effects of initial organic and nutrient concentrations.[33] However, the literature reveals that no research has evaluated the optimization of microalgae-based wastewater treatment using both RSM and ML algorithms.

In the present work, therefore, the efficacy of microalgae, specifically *Nannochloropsis* sp., has been investigated in treating raw municipal wastewater, while the operating conditions are optimized using RSM and a decision tree analysis with an integrated approach in order to maximize microalgal growth. In this study, we contribute to the research gap in the field by utilizing the synergy of RSM and ML techniques to optimize microalgae-based wastewater treatment. The use of the hybrid methodology not only improves the efficiency of process optimization but also eliminates the cost that is associated with optimization.

MATERIALS AND METHODS

Cultivation

A microalgae strain including *Nannochloropsis* sp. was obtained from a fish farm in Çanakkale (Turkey). The inoculum culture was maintained in saltwater enriched with 100 g L^{-1} $MgSO_4$, 100 g L^{-1} $MgCl_2$, 150 g L^{-1} $CaCl_2$, 100 g L^{-1} KNO_3, 4 g L^{-1} $NaHCO_3$, and 7 g L^{-1} KH_2PO_4 for *Nannochloropsis* sp. under constant orbital shaking (300 rpm) on a 24/0 h light/dark cycle at room temperature (25 °C) and pH 8 $\pm$ 0.2 without additional aeration. In a study conducted on the effect of the different types of light sources (yellow, white, red, and blue) on *Nannochloropsis oceanica*, the highest microalgal biomass growth (2.1 g L^{-1}) was found under white light.[37] Therefore, in our study, white light-emitting diodes (LEDs) were chosen as a light source in microalgal cultivation. LEDs have been thoroughly investigated owing to their small size, light weight, durability, efficiency in terms of longer working life, and benefits in enhancing growth and biomass yield.[38] LEDs create a regulated light environment that can be customized to meet the specific requirements of microalgae.[39]

For 2 weeks, microalgal growth experiments were carried out in 500 mL Erlenmeyer flasks (working capacity = 400 mL). A DR 5000 spectrophotometer (Hach, USA) was used to measure optical density (OD) at 680 nm on a daily basis. Before inoculation, microalgae were cultivated individually and allowed to reach an exponential growth phase.

Municipal wastewater characteristics

Municipal wastewater was gathered from a biological wastewater treatment facility in Çanakkale (latitude 40.09566, longitude

Table 1. Municipal wastewater (influent and effluent) composition

Parameter	Units	Influent	Effluent
pH		7.35	7.5
Conductivity	ms cm^{-1}	3.41	2.34
TDS	g L^{-1}	2.18	1.49
Turbidity	NTU	131	29.9
Color	Pt-Co	334	146
COD	mg L^{-1}	226	33.5
TOC	mg L^{-1}	25.21	8.73
TKN	mg L^{-1}	12.69	5.64
Ammonium	mg L^{-1}	8.46	4.23
Nitrate	mg L^{-1}	0.8	0.4
Nitrite	mg L^{-1}	2.26	1.73
Orthophosphate	mg L^{-1}	2.84	0.92
TS	mg L^{-1}	2276	1392
VS	mg L^{-1}	764	348
SS	mg L^{-1}	420	42
VSS	mg L^{-1}	96	26.4

Abbreviations: TDS, total dissolved solids; COD, chemical oxygen demand; TOC, total organic carbon; TKN, total Kjeldahl nitrogen; TS, total solids; VS, volatile solids; SS, suspended solids; VSS, volatile suspended solids.

26.38179). This biological wastewater treatment plant is only capable of carbon removal. Samples were taken from the influent and effluent sections of the facility. The wastewater was stored at 4 °C. Table 1 shows the characteristics of influent and effluent municipal wastewater.

ANALYTICAL METHODS

Biomass growth analysis

The growth of *Nannochloropsis* sp. was monitored daily by measuring the OD of the culture at 680 nm. The correlation between the concentration of volatile suspended solids (VSS) in the biomass and the optical density during mixotrophic growth was established using Eqn (1). The VSS analysis was conducted following the procedures outlined in the standard methods (SM 2540-E):

$$\text{VSS}\left(\text{mg}\,\text{L}^{-1}\right)=193.49\times\text{OD}680+81.298, R^2=0.9933 \quad (1)$$

In the present study, various ratios of municipal influent wastewater to *Nannochloropsis* sp. were tested. *Nannochloropsis* sp. was taken from the stock solution and fed with actual domestic wastewater at different ratios. The volume ratios of municipal wastewater to *Nannochloropsis* sp. were 1:4, 1:3, 1:2, 4:1, 3:1, 2:1, and 1:1, respectively, with reactors operated over a 3-day period. These ratios represent the volumes determined for wastewater and microalgae within the total reactor volume. The microalgae volatile solids (VS) varied based on the wastewater:microalgae ratio as 1962 mg L^{-1} (at a 1:4 ratio), 1845 mg L^{-1} (at a 1:3 ratio), 1650 mg L^{-1} (at a 1:2 ratio), 560 mg L^{-1} (at a 4:1 ratio), 677 mg L^{-1} (at a 3:1 ratio), 871 mg L^{-1} (at a 2:1 ratio), and 1261 mg L^{-1} (at a 1:1 ratio), respectively. The VS of *Nannochloropsis* sp. in the stock solution was measured at 2525 mg L^{-1}, and the VSS of wastewater was assessed at 96 mg L^{-1}.

Carbon and nutrient concentration

Samples were filtered with a 47 mm glass fiber filter. The VSS analysis was carried out using standard methods (SM 2540-E). Chemical oxygen demand (COD) was assessed using a DR 5000 spectrophotometer (Hach Lange) following the standard method (SM 5220 D). Total Kjeldahl nitrogen analysis, which includes organic nitrogen and ammonium determination, was conducted using a combustion unit and distillation unit in accordance with the standard method (SM 4500-Norg B). Total phosphorus was analyzed following the standard method (SM 4500-P B). Percent removal (%R) for each parameter was calculated using Eqn (2):

$$\%R=(C_0-C_1)/C_0\times 100 \quad (2)$$

where C_0 represents the concentration of all parameters before treatment (mg L^{-1}), C_1 indicates the concentration of all parameters after treatment (mg L^{-1}), and %R denotes the percentage removal of the selected parameters.

Chlorophyll-a

Chlorophyll-a analysis was conducted to examine the photosynthetic activities and growth of microalgae. A volume of 10 mL was obtained from each sample, followed by centrifugation at 3000 rpm for 1 min to separate the supernatant. Acetone was added to the remaining solid fraction, and the samples were analyzed using a spectrophotometer at 664 nm, 647 nm, and 630 nm. The obtained results were inserted into Eqn (3) provided, below, and the final results were obtained in mg L^{-1}:[40]

$$\text{Chlorophyll–a}\left(\text{mg}\,\text{L}^{-1}\right)=11.85\,(\text{OD}\,664)-1.54\,(\text{OD}\,647)-0.08\,(\text{OD}\,630) \quad (3)$$

Statistical analysis

RSM is a mathematical and statistically integrated method for empirical modeling and optimization. There are two fundamental designs: central composite design (CCD) and Box–Behnken design (BBD). In this study, BBD was chosen over CCD. Both approaches provide results that are nearly identical, since BBD often requires fewer tests than CCD. BBD consists of three levels

Table 2. Range and concentrations of independent test variables in the experiment

Variable	Symbol	Coded factors and levels −1	0	1
Light cycle (h)	*x*1	6	15	24
Light intensity (lx)	*x*2	2000	5000	8000
Temperature (°C)	*x*3	20	25	30

(low–medium–high).[41] CCD consists of five levels (including axial points).[42] The lower number of levels in BBD reduces factor confounding and thus decreases the total number of experiments (BBD provides 13 experimental runs, while CCD provides 15–20 experimental runs). Despite BBD suggesting fewer experimental sets, it provides sufficient information to fit a second-order model and is highly efficient as a source of information without compromising model quality.[43] Table 2 shows each independent variable's level and range. Each variable has three coded points (−1, 0, +1) according to the RSM with BBD. The minimum and maximum values for the light cycle, light intensity, and temperature parameters were determined through a literature review and entered into the Minitab software. The program codes the minimum value as '−1', the maximum value as '1', and the average value between these two as '0'. In the microalgae-based wastewater treatment systems, the most critical parameters are determined as light cycle, light intensity, and temperature. Based on the literature, the limits for the parameters have been selected as 6–24 h for the light cycle,[25,44] 2000–8000 lx for light intensity,[26,44] and 20–30 °C for temperature.[23,26] The program generated output by identifying three points for each parameter and encoding these values as '−1, 0, 1'.

All data were processed and optimized using RSM with Minitab v.20.4 software. The relationship between the growth rates of *Nannochloropsis* sp. and OD at 680 nm was determined through bivariate analysis. Based on these values, 15 reactors were operated for each domestic influent wastewater sample. ANOVA was implemented to evaluate the differences between reactors operating under ideal circumstances, with *P*-values of less than 0.05 being regarded as significant. Equation (4) describes a formula for calculating the relative error as a percentage between experimental and predicted biomass growth rates:

$$\text{Relative error}\,(\%) = \left(Y_{\exp} - Y_{\mathrm{p}}\right)/Y_{\exp} \times 100 \qquad (4)$$

where $Y_{\exp}$ is the experimental biomass growth rate (%) and Y_{p} is the predicted biomass growth rate (%).

Generally, a lower *P*-value signifies a higher significance of the corresponding term. Terms with a P-value < 0.05 are considered highly significant at the 5% level or within a 95% confidence interval, while terms with $0.05 < P$-value < 0.1 are deemed significant at the 10% level or within a 90% confidence interval. Conversely, a term is regarded as insignificant if its *P*-value exceeds 0.1.

The decision tree algorithm was applied using RapidMiner Studio version 10.3 for the data modeling process. The microalgal growth performance of *Nannochloropsis* sp. in municipal wastewater was measured with the OD 680 nm parameter. A decision tree model was created by considering variables such as temperature, light cycle, and light intensity. This model was utilized in data classification and analysis.

RESULTS AND DISCUSSION

Preliminary study on the growth ability of *Nannochloropsis* sp. in municipal wastewater

Preliminary experiments for monitoring the growth of the mixotrophic marine microalga *Nannochloropsis* sp. in municipal wastewater were tested under different operational conditions. Municipal wastewater samples were collected from both influent and effluent units of a biological wastewater treatment facility, and *Nannochloropsis* sp. was evaluated in these wastewaters.

The growth curves of *Nannochloropsis* sp. fed with wastewater collected from the influent and effluent units were determined over time. The experiments showed that *Nannochloropsis* sp. can only grow in the influent wastewater. An increase in biomass concentration and pigment in *Nannochloropsis* sp. was observed in the trials conducted with influent wastewater, as indicated by the absorbance at OD 680 nm. It is known that high absorbance at 680 nm indicates a high concentration of pigment in microalgae.[45] However, *Nannochloropsis* sp. did not grow in the reactors fed with effluent municipal wastewater. This could be due to the low organic content of the effluent wastewater. In a study by Velichkova *et al*.,[46] mixotrophic microalgae (*Nannochloropsis oculata* and *Tetraselmis chuii*) were used and tested together with wastewater from a semi-closed aquaculture system. The results indicated that microalgae require carbon, nitrogen, phosphorus, and other micronutrients for growth. It has been shown that mixotrophic *Nannochloropsis* sp. is sensitive to the concentrations of carbon, nitrogen, and phosphorus in wastewater[47] and that *Nannochloropsis* sp. cannot survive at low concentrations of organic carbon.[48] Therefore, in this study, influent municipal wastewater was selected as a feed.

In the literature, it is seen that mostly synthetic wastewater is used for wastewater treatment studies, and the wastewaters do not fully reflect the character of complex domestic wastewater. There are very few studies with *Nannochloropsis* sp. using raw influent domestic wastewater.[17,49] However, when the studies with high removal efficiencies are examined, it is seen that the influent COD has very low initial values (24–99 mg L^{-1})[17] compared to the characteristics of the influent wastewater used in our study.

Testing microalgae with influent municipal wastewater at different ratios

The optimal ratio of *Nannochloropsis* sp. to municipal wastewater was established after the preliminary studies. The influent municipal wastewater was selected as the growing medium for mixotrophic microalgae. The study tested different ratios of municipal wastewater to *Nannochloropsis* sp., (1:4, 1:3, 1:2, 4:1, 3:1, 2:1, and 1:1) in the reactors operated over a 3-day period. The most significant growth, relative to the initial concentration of *Nannochloropsis* sp., was observed in the 1:1 ratio reactor. Consistent with these findings, Hammad *et al*.[50] tested temperature, pH, and municipal wastewater-to-microalgae (*Chlorella vulgaris*) ratios, determining that a municipal wastewater-to-microalgae ratio of 1 was most effective in reducing pollutants in wastewater. These results underscore the importance of optimizing the wastewater-to-microalgae ratio for effective pollutant removal. The efficiency of wastewater treatment methods based on microalgae is largely determined by the ratio of microalgae to wastewater. In the study conducted by Onay,[51] the impact of different concentrations of *Nannochloropsis gaditana*, combined with municipal wastewater at varying proportions (0%, 30%, 60%, and 100%), on bioethanol production was systematically investigated. The findings showed that the reactor using 30% wastewater produced the highest amount of bioethanol. However, the literature lacks specific studies that determine the optimal ratio of *Nannochloropsis* sp. to municipal wastewater for treatment purposes using this microalga. The concentration of phosphorus and nitrogen in the wastewater has an effect on the nutrient removal efficiency of microalgae.[9] It has been reported that the growth and treatment effectiveness of microalgae can be impacted by the presence of carbon and nitrogen in particular ratios.[52] Wu and Ying[27] investigated optimal concentrations of COD, NH_4-N, and total phosphorus in synthetic wastewater treated with mixotrophic microalgae (*Chlorella vulgaris*). The study identified optimal concentration ranges for *Chlorella vulgaris* as 500–1500 mg L^{-1} for COD, 20–40 mg L^{-1} for NH_4-N, and 8–20 mg L^{-1} for total phosphorus. These findings highlight the necessity of maintaining wastewater parameters within specific concentration limits to optimize microalgae-based treatment efficiency.

Scaling up microalgae-based systems depends on the right selection of strains and optimization of operating conditions. For obtaining effective wastewater treatment performance, alternative microalgae strains should be investigated. Xu *et al.*[53] suggested that screening microalgae strains that can adapt to changes in environmental conditions and wastewater quality in wastewater treatment systems is essential. Careful management of temperature, pH, and nutrients is also critical to maximize microalgal growth. Rodríguez-Miranda *et al.*[54] stated that microalgae species have different temperature preferences, and this impacts the microalgae biomass yield in full-scale reactors. On the other hand, to achieve high biomass production in real-scale photobioreactors, it is necessary to select strains that grow rapidly and can easily adapt to different culture conditions.[55]

In addition, the economic sustainability of microalgae production depends on factors such as operating costs and biomass recovery efficiency. A study by Díez-Montero *et al.*[56] suggested that the use of low-cost nutrient sources can increase the economic feasibility of microalgae production. In this study, the use of domestic wastewater for microalgae culture will provide benefits in terms of sustainability.

RSM model

The optimal RSM model was created using the experimental data gathered from *Nannochloropsis* sp. growth runs conducted in the laboratory. Table 3 presents both the observed and predicted percentage growth rates for *Nannochloropsis* sp. as calculated with the RSM model, along with the corresponding relative error values.

These data were used to create the RSM with BBD-based models. The results were examined using regression analysis. The predictive models for *Nannochloropsis* sp. growth are defined using Eqn (5), as follows:

$$\begin{aligned}\text{Biomass production rate} = {} & 14.9 + 4.18\times A - 0.03160\times B + 3.53\times C \\ & - 0.2270\times A^2 + 0.000002\times B^2 - 0.245\times C^2 \\ & + 0.000055\times A\times B + 0.1877\times A\times C \\ & + 0.000806\times B\times C\end{aligned} \tag{5}$$

where *A* represents light duration, *B* is light intensity, and *C* is temperature.

Table 3 presents the microalgal growth rate in 15 reactors, as indicated by RSM outputs based on the three selected parameters (light cycle, light intensity, and temperature) in wastewater treatment processes. These percentages include both the experimental data and the rates predicted by the model. When the data were examined, it was observed that some values were positive while others were negative. Negative values indicate that there is no increase in microalgal biomass and that after a certain period the microalgae are unable to function in the

Table 3. Experimental design and results

No.	Light cycle (light hour)	Light intensity (lx)	Temperature (°C)	Experimental biomass growth rate (%)	Predicted biomass growth rate (%)	Relative error (%)
1	6	8000	25	34.6	34.13	1.38
2	15	5000	25	29.2	28.91	0.99
3	24	8000	25	65.9	79.17	−16.76
4	15	2000	30	−8.6	−8.8	−2.27
5	15	8000	30	61	71.63	−14.84
6	15	8000	20	59	66.2	−10.87
7	15	2000	20	35.8	34.13	4.91
8	15	5000	25	28.9	28.91	−0.04
9	24	5000	30	24.7	24.51	0.77
10	24	2000	25	20	19.95	0.24
11	6	5000	30	−34	−34.46	−1.32
12	15	5000	25	27.3	28.91	−5.58
13	6	2000	25	−19.5	−19.15	1.83
14	6	5000	20	1.16	1.18	−1.86
15	24	5000	20	26.4	26.36	0.14

environment.[57] A general consistency has been observed between the experimental and predicted percentage values. This situation supports the accuracy and reliability of the model.

Figure 1 shows the relationship between the maximum growth of *Nannochloropsis* sp. and independent variables (light cycle, light intensity, and temperature). The constructed RSM models are applicable across the full range of each independent variable. Main effect and interaction plots were utilized to assess how environmental factors – light cycle, light intensity, and temperature – affect the growth of *Nannochloropsis* sp. (Fig. 1). This pattern reveals that all three environmental factors affect the response parameter (growth rate of *Nannochloropsis* sp.), with their relative influence ranked as follows: light intensity ($x2$) > light cycle ($x1$) > temperature ($x3$). Generally, variations in these independent variables impact the response differently. When the main impact line remains parallel to the horizontal axis, it

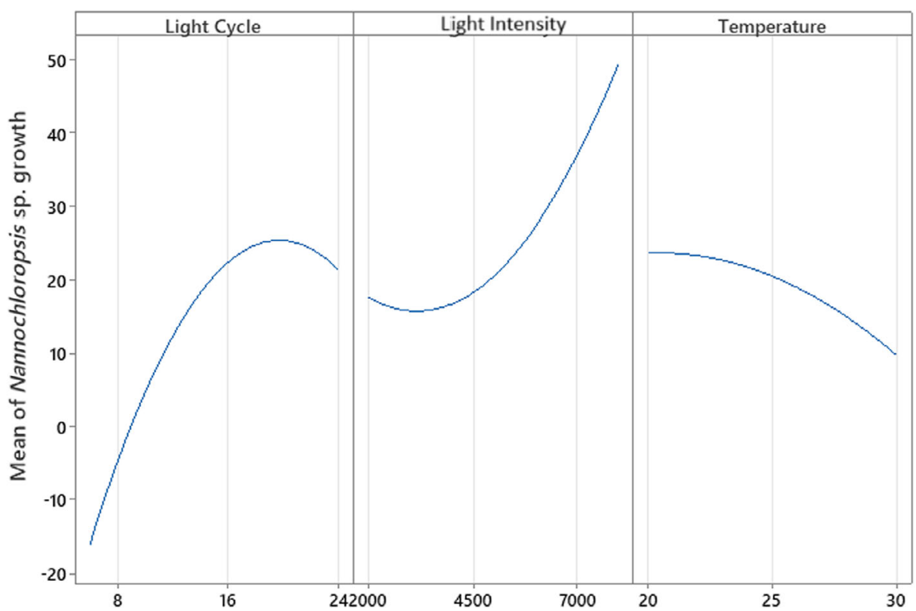

Figure 1. Relationship between maximum growth of *Nannochloropsis* sp. and independent variables (light cycle, light intensity, and temperature).

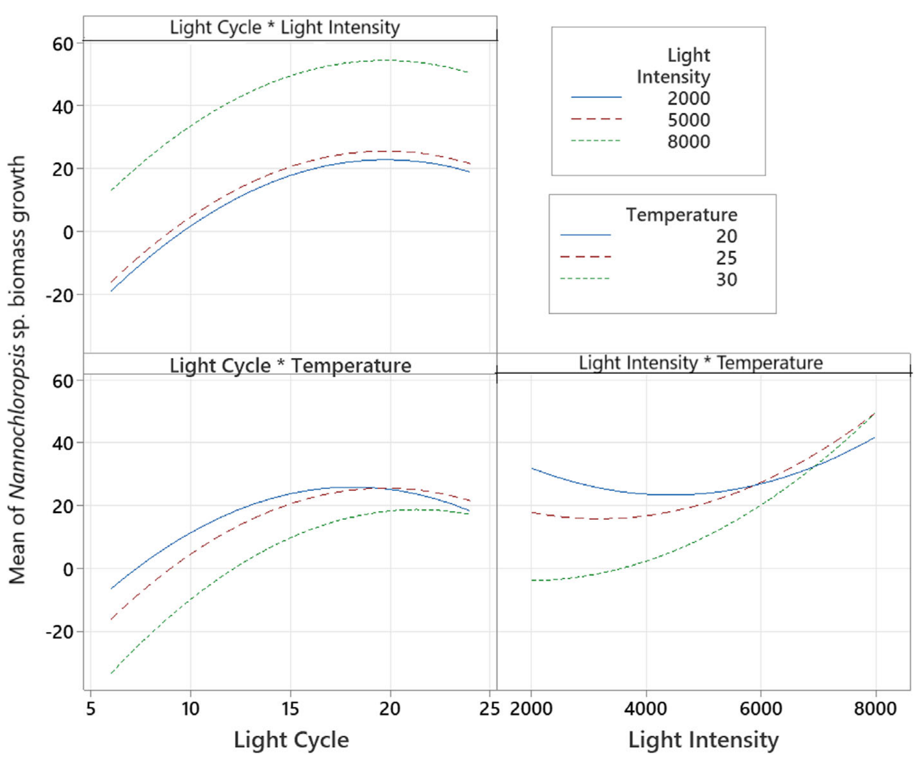

Figure 2. Interaction effects of input variables (light cycle, light intensity, and temperature) on both outputs.

Table 4. ANOVA of the model

Source	Degrees of Freedom	Adjusted Sum of Squares	Adjusted Mean Square	*F*-value	*P*-value
Model	9	10 061.6	1117.96	33.05	0,001
A	1	3538.7	3538.74	104.61	0.0000
B	1	2573.1	2573.07	76.06	0.0000
C	1	700.7	700.66	20.71	0.006
AB	1	8.9	8.94	0.26	0,629[b]
AC	1	285.3	285.28	8.43	0.034
BC	1	585.1	585.12	17.3	0.009
A^2	1	1248.8	1248.82	36.92	0.002
B^2	1	822.8	822.8	24.32	0.004
C^2	1	138.5	138.51	4.09	0.099[b]
Error	5	169.1	33.8		
Lack of fit	3	134.5	44.83	2.59	0.291
Pure error	2	34.7	17.33		
Corrected total	14	10 230.7			

suggests that the response is unaffected by that specific input variable. Conversely, a steep main effect line indicates that the response is significantly influenced by the variable in question. In this figure, the response of *Nannochloropsis* sp. growth to the parameters (light cycle, light intensity, and temperature) has been shown in terms of the singular parameters. Figure 1 also indicates that there was a decrease in microalgal growth with increasing temperature. *Nannochloropsis* sp. can grow between 15 and 30 °C, but the optimum development temperature is approximately 25 °C.[58] When the temperature reaches 30 °C, *Nannochloropsis* sp. may experience a decrease in growth rate due to physiological stress.[59] The main reason for this decrease is that the respiration rate of microalgae exceeds their photosynthesis rate at high temperatures.[60]

Figure 2 shows the effect of the interaction between parameters on the biomass growth of *Nannochloropsis* sp. It shows that the interaction between light cycle and light intensity is significant. However, it is very clear that the interaction between 8000 lx light intensity and long light cycle is significant because the distance between the curves representing other light intensities is substantial.[61] When the light cycle and temperature parameters are examined together, it is clear that, as the light cycle is extended, there is a general increase in biomass. When the light intensity and temperature parameters are examined together, the trend of biomass increase is observed for each temperature as the light intensity increases. In this study, the algorithm identified 30 °C as the optimal temperature. Nevertheless, the algorithm provided the optimal value for the parameters not as a singular parameter but as a collective optimal value alongside other factors influencing microalgal growth. The interaction plot further illustrates the combined effect of pairs of independent variables on the responses. Parallel lines in these plots indicate a lack of interaction, while a steeper slope reflects a stronger interaction. As shown in Fig. 2, all input factors interact with each response.

Using Minitab software (v-20.4), three-dimensional surface plots were generated for both responses to examine the relationship between dependent and independent variables, as illustrated in Supporting Information, Fig. S1. The results indicate that all input variables influence the response, specifically the growth of *Nannochloropsis* sp. Additionally, comparable interaction effects between variables were observed, as discussed in Fig. 2. Since each operating factor analyzed plays a crucial role in the growth of *Nannochloropsis* sp. in municipal wastewater, it is essential to assess the statistical significance of these parameters and the models employed. Accordingly, ANOVA was conducted, and the ANOVA results of the regression models for *Nannochloropsis* sp. growth are presented in Table 4. The significance of each model term was determined based on the respective *P*-values in the ANOVA table.

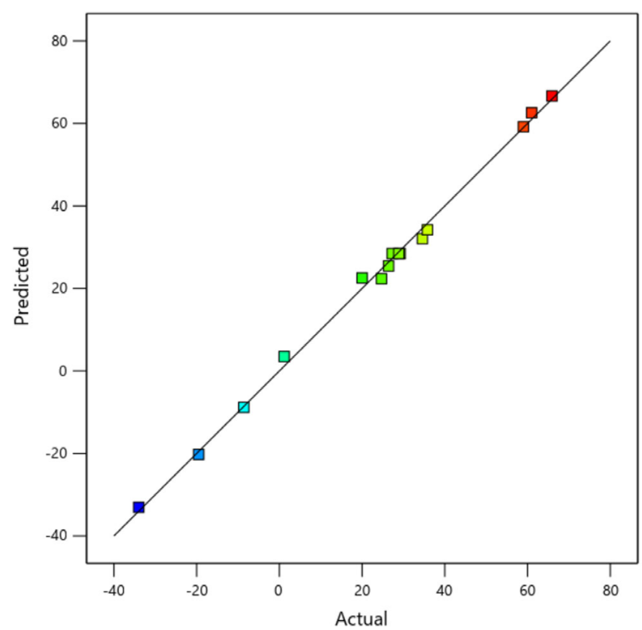

Figure 3. Predicted and actual values of biomass production.

The model was determined to be statistically significant, as *P*-values were found to be less than 0.05, indicating that the model is explainable within a 95% confidence interval. *F*-values >0.001, along with corresponding *P*-values >0.05, demonstrate that the lack of fit is insignificant and that the model can reliably predict the response values.

Figure 3 shows that the predicted and actual values for microalgal biomass production align closely around the linear line, forming a curve on the same plane. The model's R^2 value was found to

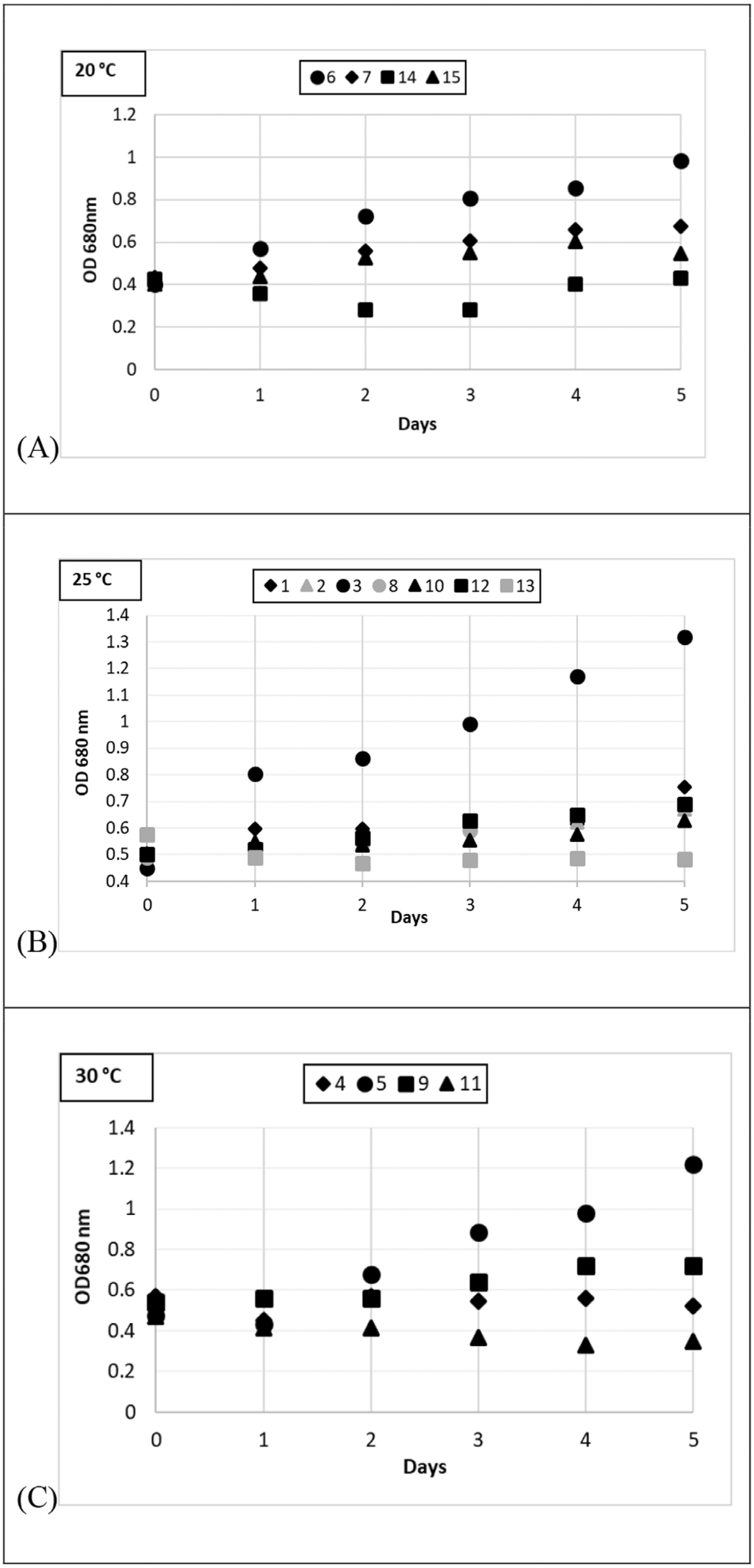

Figure 4. Changes in optical density (OD) at 680 nm across 15 reactors as determined by response surface methodology during the operational phase: (A) 20 °C; (B) 25 °C; (C) 30 °C.

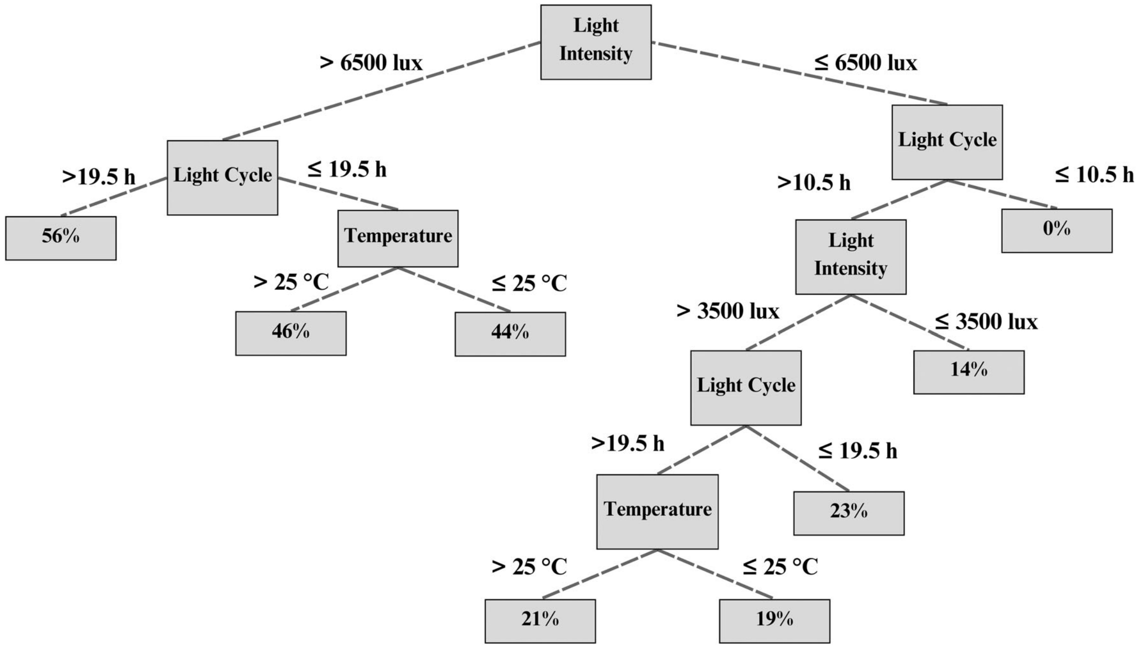

Figure 5. Decision tree model with independent variables affecting *Nannochloropsis* sp. biomass growth.

be 0.9971, which substantiates a strong correlation with the experimental data. Additionally, the R^2_{adj} value was calculated as 0.9917, showing less than a 2% decrease compared to R^2, indicating the model's accuracy in predicting the range of response values. The Adeq. Precision ratio was 46.832, and since this value exceeds 4 it confirms that the model has sufficient precision in predicting responses. The literature and many statistical software programs (e.g., Design-Expert) reported that an Adeq. Precision value above 4 indicates that the model has a reliable prediction accuracy.[62] In this study, the Adeq. Precision ratio value is 46.832, indicating that the model has a very high signal-to-noise ratio. In other words, the model can explain systematic variation (signal) very well. The effect of random variation (noise) remains quite low.

Figure 4 illustrates the changes in OD 680 nm observed during the operation of 15 reactors as determined by RSM. While some reactors exhibited microalgal growth, others showed a decline, indicating varying responses to the experimental conditions. The highest microalgal biomass growth was observed in reactors 3, 5, and 6, which shared the common characteristic of having a maximum light intensity of 8000 lx. In contrast, reactors 11 and 13, which shared the shortest light duration of 6 h, exhibited no significant biomass growth. These results suggest that both light duration and intensity are important in promoting microalgal biomass growth.

It should be also noted that, under optimal conditions, changes in the chlorophyll-a content of *Nannochloropsis* sp. in influent municipal wastewater were observed over a 5-day period. The data indicate a 49% increase in chlorophyll-a concentration by the end of the fifth day, suggesting that *Nannochloropsis* sp. remained viable and exhibited growth throughout the experimental period.

Development of ML algorithm

A constructed dataset that represented the wastewater treatment process based on microalgae was analyzed using a decision tree. *Nannochloropsis* sp. biomass rate of growth was calculated using the dataset's median. The tree was constructed using the 'RapidMiner' function, and Fig. 5 illustrates the decision tree based on independent variables that enable *Nannochloropsis* sp. to increase biomass in municipal wastewater. The presence of a parameter in the first step of the decision tree indicates that this variable plays a key role in explaining the system and determining the results. In this context, light intensity is the most fundamental factor for *Nannochloropsis* sp. biomass growth,[63] and the influence of other factors can only be assessed through this fundamental distinction.

It was determined that temperature, as an independent variable, had no substantial effect on maximal biomass growth. The highest biomass growth (56%) was achieved when the light intensity exceeded 6500 lx and the light cycle surpassed 19.5 h. This result aligns well with the optimal conditions established through RSM optimization for microalgal biomass growth, specifically a 21-h light cycle and 8000 lx light intensity. In the reactor where biomass growth does not occur, light intensity is below 6500 lx, and the light cycle is below 10.5 h. This result demonstrates the critical role of both light intensity and light cycle in microalgal biomass growth. RSM graphs also support these results.

Advanced process control technologies are required for efficient removal of pollutants from municipal wastewater. ML has the ability to estimate wastewater treatment system process costs,[64] reduce energy consumption,[65] and improve operational efficiency. The development of such plants requires the interaction of different disciplines such as statistics, computer science, and biotechnology.[66] The decision tree algorithm is an interpretable method that can model complex relationships between input–output variables and is widely used in analyzing wastewater treatment data,[30,67] but there is no decision tree application adapted for specific microalgae/wastewater combinations yet.

Carbon and nutrient removal performance

The current study explores the ability of *Nannochloropsis* sp. to remove carbon and nutrients from municipal wastewater. The time-dependent removal of COD, total organic carbon, total

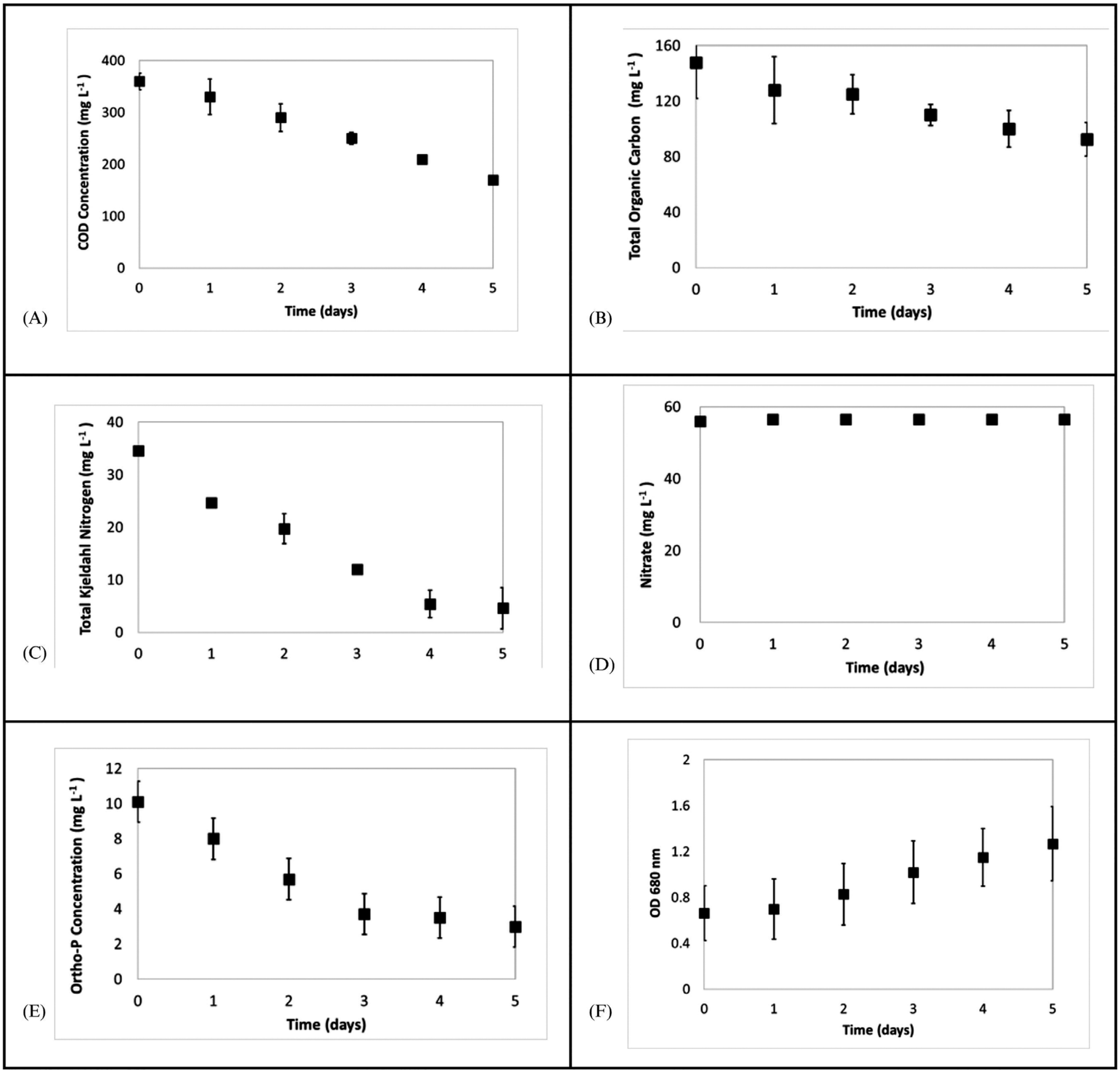

Figure 6. Time-dependent concentration changes in (A) chemical oxygen demand (COD), (B) total organic carbon, (C) total Kjeldahl nitrogen, (D) nitrate, (E) orthophosphate (Ortho-P), and (F) biomass of *Nannochloropsis* sp. at optical density (OD) 680 nm.

Kjeldahl nitrogen, nitrate, and orthophosphate in the reactor operated under optimal conditions (21 h light cycle, 8000 lx light intensity, and a temperature of 30 °C) is presented in Fig. 6. The optimal conditions are given in Supporting Information, Fig. S2. In Fig. 6, the concentration variations over time and resultant yields obtained from operating *Nannochloropsis* sp. and municipal wastewater under optimal conditions are presented as follows: 53% COD removal (A), 34% total organic carbon removal (B), 87% total Kjeldahl nitrogen removal (C), nitrate (D), 70% orthophosphate removal (E), and 56% OD at 680 nm, *Nannochloropsis* sp. biomass growth (F). No considerable nitrate removal was detected, owing to the preferential utilization of oxygen as an electron acceptor over nitrate.

In the literature, studies involving *Nannochloropsis* sp. have employed various types of wastewater, most of which were synthetically prepared under laboratory conditions.[68–72] Other wastewater sources explored include industrial effluents such as those from palm oil mills,[73] the pharmaceutical industry,[74] the dairy industry,[75] and the pulp and paper industry,[76] as well as domestic wastewater.[17–19,49,51,77] Among the studies focusing on domestic wastewater, only a limited number have specifically targeted nutrient and carbon removal.[17,18,49] None of these studies, however, have involved optimization of the operational conditions. This study is unique in that it uses real municipal wastewater applied to *Nannochloropsis* sp. and conducts a detailed optimization of numerous environmental parameters (temperature, light cycle, and light intensity) using both RSM and ML. The lack of extensive prior research in this area restricts the depth of discussion that can be provided.

In this context, the combination of RSM and ML can make optimization processes more efficient. The optimization methodologies offered valuable insights into the processes, as well as advanced modeling skills that allowed for the prediction of the growth of biomass. Ajala and Alexander[78] showed that this hybrid approach optimizes microalgae dewatering processes, increasing efficiency and reducing costs. It allows the development of predictive models that can adapt to changing environmental conditions, which offers significant advantages in full-scale applications.

The study highlights the potential of the mixotrophic marine microalga *Nannochloropsis* sp. in wastewater management with optimization techniques such as RSM and ML and ensures the sustainability of wastewater treatment processes with more efficient resource use while maximizing biomass.

CONCLUSIONS

The integration of both RSM and decision tree in this study highlights their complementary roles in optimizing wastewater treatment processes. These optimization approaches provide valuable insights into the best conditions for biomass growth. Our study demonstrates that municipal wastewater can be effectively treated using *Nannochloropsis* sp. It also underscores the importance of data-driven methods for efficient wastewater management, emphasizing the importance of adopting innovative, data-driven approaches. This study also contributes to the sustainable development goals (SDG); such as, clean water and sanitation (SDG6), sustainable cities and communities (SDG11), responsible production and consumption (SDG12), and life in water (SDG14). Moreover, in this study, instead of using synthetic wastewater, which cannot be fully adapted to real-scale applications, real municipal wastewater was used. The growing demand for sustainable wastewater treatment solutions necessitates the integration of advanced statistical and computational techniques in microalgae-based systems for enhancing sustainability, scalability, and efficiency. Studies on strain selection, optimization, and adaptive management strategies should continue to improve microalgae cultivation in real-world applications.

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Data will be made available on request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONSENT FOR PUBLICATION

The authors consent to publish the article on acceptance.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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