T.C.
CANAKKALE ONSEKIZ MART UNIVERSITY
SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF ENVIRONMENTAL ENGINEERING

INTEGRATED BIOREFINERY APPROACH FOR WASTEWATER TREATMENT AND RESOURCE RECOVERY USING MIXOTROPHIC MICROALGAE

DOCTORAL THESIS

İREMSU KAYAN

Thesis Advisor
PROF. DR. NİLGÜN AYMAN ÖZ

ÇANAKKALE – 2025

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LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

İremsu KAYAN tarafından Prof. Dr. Nilgün AYMAN ÖZ yönetiminde hazırlanan ve **25/08/2025** tarihinde aşağıdaki jüri karşısında sunulan "**Integrated Biorefinery Approach for Wastewater Treatment and Resource Recovery Using Mixotrophic Microalgae**" başlıklı çalışma, Çanakkale Onsekiz Mart Üniversitesi Lisansüstü Eğitim Enstitüsü **Çevre Mühendisliği Anabilim**'nda **DOKTORA TEZİ** olarak oy birliği ile kabul edilmiştir.

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ETİK BEYAN

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ETHICAL STATEMENT

In line with the thesis preparation guidelines of Çanakkale Onsekiz Mart University Graduate School of Education, I hereby confirm that I have prepared this thesis work; The data, materials, and documents included in this thesis were gathered in full compliance with academic and ethical guidelines, and all relevant data, analyses, records, and findings have been reported in accordance with scientific ethical norms and scientific integrity principles, that all references and sources consulted in this study have been duly acknowledged and cited, that no alterations have been made to the original dataset employed in this study, and that the contents of this thesis reflect original research and findings. With this statement, I formally affirm and undertake that I acknowledge and take full responsibility for any legal outcomes that may result in the event of any violation of these principles.

(İmza)
İremsu KAYAN
25/08/2025

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I dedicate this doctoral thesis to my beloved daughter, Beril KAYAN, who has given meaning to my life and has always been my source of inspiration.

İremsu KAYAN

Çanakkale, August 2025

ÖZET

MİKSOTROFİK MİKROALGLER KULLANILARAK ATIKSU ARITIMI VE KAYNAK GERİ KAZANIMI İÇİN ENTEGRE BİYOREFİNERİ YAKLAŞIMI

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25/08/2025, 135

Tezde, miksotrofik bir deniz mikroalgi olan *Nannochloropsis sp.*'in hem evsel nitelikli atıksu hem de bir gıda endüstrisi atıksuyu olan peynir altı atıksuyunu (PAS) arıtma potansiyeli test edilmiştir. Ayrıca arıtım deneylerine ek olarak PAS ile kültive edilmiş mikroalg reaktörlerinden elde edilen biyokütlenin enerji ve sağlık uygulamalarında kullanım potansiyeli değerlendirilmiştir. Evsel atıksu ile kurulan kesikli reaktörlerde optimum koşullarda kimyasal oksijen ihtiyacı (KOİ) giderim verimi %53, toplam kjeldahl azotu (TKA) giderim verimi %87 ve ortofosfor giderim verimi %70 olarak elde edilmiştir. Seyreltilmiş PAS için ise optimum koşullar altında çalışan reaktörde yaklaşık %90 KOİ, %67 TKA ve %65 ortofosfor giderimi sağlanmıştır. Arıtma çalışmalarında deneysel koşulların ve mikroalg atıksu oranlarının belirlenmesinde Yanıt Yüzey Yöntemi, sonuçların doğruluğunun test edilmesinde ise karar ağacı kullanılmıştır. Anaerobik kesikli reaktörlerde sığır gübresine ek substrat olarak belirli oranlarda PAS ile kültive edilmiş mikroalg biyokütlesinin biyogaz oluşumuna etkisi incelenmiştir. %20 oranında mikroalg (PAS ile kültive edilen) içeren anaerobik reaktörde en yüksek biyogaz üretimi elde edilmiştir. Bu reaktöre paralel aynı oranda sadece PAS ve sadece mikroalg reaktörleri kontrol reaktörleri olarak işletilmiştir. Ayrıca tezde PAS ile kültive edilmiş mikroalg biyokütlesinin, saf mikroalg biyokütlesinin ve bu iki farklı biyokütle ile sentezlenen gümüş nanopartiküllerin antimikrobiyal, antioksidan ve DNA kırma potansiyelleri incelenmiştir. Sürdürülebilir Kalkınma Amaçları doğrultusunda döngüsel ekonomi prensiplerine uygun olarak mikroalg-

bazlı atıksuların arıtımı ve oluşan biyokütlenin çeşitli uygulamalarda kullanılabilme potansiyeli ortaya konmuştur.

Anahtar Kelimeler: Mikroalg, Atıksu Arıtımı, Biyogaz, Biyoaktivite, Sürdürülebilirlik, Döngüsel Ekonomi

ABSTRACT

INTEGRATED BIOREFINERY APPROACH FOR WASTEWATER TREATMENT AND RESOURCE RECOVERY USING MIXOTROPHIC MICROALGAE

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In the thesis, the treatment potential of *Nannochloropsis sp.,* a mixotrophic marine microalgae, was tested for its potential to treat both municipal wastewater and cheese whey wastewater (CWW), which is a food industry wastewater. In addition to the treatment experiments, the potential use of microalgae cultivated with CWW was evaluated in terms of energy and health applications. Under optimal conditions, in the microalgae-based batch reactors fed with municipal wastewater, removal efficiencies for chemical oxygen demand (COD), Total Kjeldahl Nitrogen (TKN) and orthophosphate were 53%, 87%, 70%, respectively. For diluted PAS, approximately 90% COD, 67% TKN, and 65% orthophosphate removal were achieved. In the treatment studies, the Response Surface Methodology was used to determine the experimental conditions and microalgae/wastewater ratios, while decision trees were used to test the accuracy. The effect of microalgae biomass cultivated with CWW on biogas formation in anaerobic batch reactors was investigated at different ratios as an additional substrate to cattle manure. The highest biogas production was obtained in the anaerobic reactor containing 20% microalgae (cultured with CWW). Reactors containing only CWW and only microalgae were operated in parallel with this reactor as control. Furthermore, the thesis investigated the antimicrobial, antioxidant, and DNA-breaking potentials of CWW-cultured microalgal biomass, pure microalgal biomass, and silver nanoparticles synthesized with these two biomass. In line with the Sustainable Development Goals, in accordance with circular economy principles, microalgae-based wastewater treatment and the potential for the biomass for various applications were demonstrated.

Keywords: Microalgae, Wastewater Treatment, Biogas, Bioactivity, Sustainability, Circular Economy

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NOMENCLATURE

CWW	Cheese Whey Wastewater
WW	Wastewater
RSM	Response Surface Methodology
AI	Artificial Intelligence
ML	Machine Learning
COD	Chemical Oxygen Demand
BOD	Biochemical Oygen Demand
TKN	Total Kjeldahl Nitrogen
Ortho-P	Orthophosphorus
T	Temperature
ANOVA	Analysis of Variance
CCD	Central Composite Design
BBD	Box-Behnken Design
OD	Optical Density
VS	Volatile Solids
TS	Total Solids
SS	Suspended Solids
N	Nitrogen
TN	Total Nitrogen
P	Phosphorus
TP	Total Phosphorus
Cr	Chromium
Cd	Cadmium
Cu	Copper
Pb	Lead
Fe	Iron
NH_3	Ammonia
EPA	Eicosapentaenoic Acid
PBR	Photobioreactors
AGS	Aerobic Granular Sludge
MBR	Membrane Bioreactors

Wos	Web of Science
MIC	Minimum Inhibitory Concentration
Df	Degrees of Freedom
SDGs	Sustainable Development Goals
LEDs	Light-Emitting Diodes
AD	Anaerobic Digestion
MA	Microalgae
MBBR	Moving bed biofilm reactor
BNR	Biological Nutrient Removal
SBR	Sequencing Batch Reactor
FAME	Fatty Acid Methyl Ester
MUFA	Monounsaturated Fatty Acids
PUFA	Polyunsaturated Fatty Acids
DHA	Docosahexaenoic Acid

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CHAPTER ONE
INTRODUCTION

The increasing population around the world accelerates the depletion of current environmental reserves. Given the ongoing climate emergency, issues such as the protection of existing water resources and the recycling of treated wastewater into the system need to be developed and managed correctly. Wastewater management is important in terms of health and environmental sustainability. However, particularly in underdeveloped and developing countries, a large portion of effluents originating from inadequate wastewater treatment is released into natural ecosystems due to high costs (Biggs et al., 2025). Inadequate wastewater management causes environmental threats in the short/long term. In many countries around the world, water scarcity is a serious issue that requires immediate attention. Additionally, due to the anticipated water crisis in the future, it will not be sufficient to simply treat wastewater. Treated water will need to be added to the usable water cycle or used for different purposes (energy, fertilizer, food). It is clear that improving traditional wastewater treatment methods and developing sustainable wastewater strategies is crucial to deal with this global water crisis. Therefore, implementing cost-effective and sustainable wastewater treatment systems that provide effective wastewater treatment is a global imperative.

Conventional wastewater treatment includes physical, biological, and chemical processes. In this context, methods such as sedimentation (Bruno et al., 2025), filtration (El Barkaoui et al., 2025), and flotation (Huang et al., 2025) are considered physical methods. Chemical methods like coagulation-flocculation (Suarez et al., 2009) and advanced oxidation (Zeng et al., 2025) can be preferred, especially for pollutants that cannot be efficiently degraded by biological methods. Biological processes consist of activated sludge (Henze, 1992), moving bed biofilm reactor (Santos et al., 2020), trickling filter (Żyłka et al., 2018), membrane-integrated biological reactor (Kitanou et al., 2021), and anaerobic digestion (Oz et al., 2004), which focus on organic compound degradation by microbial communities. These traditional biological treatment methods have some limitations in terms of nutrient removal. Nutrient removal systems are biological nutrient removal (BNR) (Carrera et al., 2004), anammox (Van Loosdrecht et al., 2004), membrane (Czerwionka et

al., 2012), and Bardenpho (Ashrafi et al., 2019). Biological systems that include carbon and nutrient removal systems are used in the treatment of wastewater, but real-scale systems for nutrient removal are not common enough, especially in developing countries and rural areas. Building advanced treatment facilities for nutrient removal requires significant financial investment, technical personnel, and space. Therefore, alternative treatment methods for nutrient removal need to be developed and disseminated. Researchers are conducting various studies on the efficiency of microalgae in removing nutrients and carbon.

Microalgae have the potential to provide an environmentally friendly approach to wastewater treatment systems. Microalgae incorporate nutrients into biomass formation. They also utilize carbon from the atmosphere or wastewater, improving the water quality of their surroundings and contributing to ecological balance. Using microalgae in wastewater treatment makes the process more effective and supports circular bioeconomy ideas by helping to recover valuable products. Especially in small settlements and rural areas where central treatment technology is not economically feasible, wastewater treatment applications incorporating microalgae may offer an alternative solution. These systems are easy to implement, as they provide effective carbon and nutrient removal while requiring minimal infrastructure costs. However, large-flow wastewater treatment plants may present some operational problems due to the availability of suitable and large land, the need for a large number of lights, and the difficulty of biomass harvesting. Therefore, while microalgae-based wastewater treatment systems provide significant advantages for decentralized rural areas, future studies should focus on increasing their applicability for large-scale facilities or integrating them into existing systems.

Microalgae have three different nutritional types: phototrophic, heterotrophic, and mixotrophic (Ahmad et al., 2021). Among these three nutritional types, mixotrophic microalgae possess mechanisms that enable them to remove both inorganic and organic carbon (Li et al., 2011). Thanks to these features, they can adapt to changing environmental conditions more easily and reduce cultivation costs (Cheirsilp et al., 2012). Mixotrophic microalgae offers a cost-effective alternative compared to traditional carbon and nutrient treatment methods, which have high operating costs and a high amount of biological sludge.

Nannochloropsis sp., which is among the mixotrophic microalgae species, can live in seawater but has been widely studied due to its high adaptability.

Belonging to the class Eustigmatophyceae, *Nannochloropsis sp.* comprises small (~ 2–8 μm), unicellular, and nonmotile eukaryotic microalgae (Esteves et al., 2025). Five types of *Nannochloropsis* have been identified: *Nannochloropsis oceanic*a, *Nannochloropsis australis*, *Nannochloropsis granulata*, *Nannochloropsis oculata*, and *Nannochloropsis limnetica*. It tolerates saline conditions and has a high lipid content. It has robust cell walls that make it resistant to environmental conditions. This species of mixotrophic microalgae has the potential to remove organic pollutants (Kayan and Oz, 2025a). Thanks to their mixotrophic properties, they can remove both organic and inorganic carbon. This will be advantageous for wastewater treatment. Optimization is crucial for the production of microalgal biomass, in addition to maintaining controlled and highly efficient treatment processes.

Optimization applied to enhance both biomass generation and water purification processes performance of microalgae-integrated treatment setups leads to positive outcomes in various areas, such as resource utilization and water management. Employing microalgae in conventional treatment of wastewater requires optimization because of some difficulties it may bring. While the univariate evaluation of working conditions and experimental designs allows for the assessment of individual parameter effects, these methods overlook the combined effects of multiple variables. This situation leads to inefficient results with respect to time and input requirements and generally prevents the discovery of true optimal conditions. Therefore, when determining the optimal values for working conditions and experimental designs, the relationships between the parameters should also be taken into account. The primary motivation for optimization parameters in microalgae applications is the cultivation conditions of microalgae, which are highly effective on treatment performance. Moustafa et al. (2023) revealed that determining microalgal layer thickness biomass affects wastewater treatment performance. Another parameter that affects microalgae biomass growth and treatment efficiency is the accurate determination of nutrient concentrations (Sadiq et al., 2018). Additionally, accurately determining and controlling

environmental and operating conditions is vital. These studies emphasize the need to identify and control various operating conditions for systems to operate at high performance.

Generally, researchers prefer single-variable approaches in optimization studies. This situation limits the explanation of the relationships between variables. The statistical method known as Response Surface Methodology (RSM) enables both modeling and optimization of response variables with multiple independent variables. This technique, which uses second-degree polynomial models derived from experimental data, makes it possible to optimize many variables at the same time and understand their relationships. RSM is a widely preferred, robust approach to enhance processes in both scientific and industrial fields. Major contributions of this technique include lessening the number of necessary tests, the ability to model interactions between variables, flexible experimental design options, and the attainment of reliable results (Elibol, 2002). This approach has been effectively utilized in various areas, for instance food technology (Ishiwu, 2022), wastewater treatment (Bashir et al., 2012), and engineering (Gupta and Navak, 2016).

RSM offers the possibility to analyze the behavior of multivariable systems with a reduced set of tests while also enabling the exploration of how independent parameters influence outcomes. With the help of contour and three-dimensional surface graphs used within the method, optimal working conditions can be visually evaluated. Additionally, the reliability and robustness of the model can be verified with statistical tests such as ANOVA (Analysis of Variance). The application of RSM includes some strategies. These steps include choosing the right experimental design (like Central Composite Design—CCD) (1), carrying out the experiments (2), creating a second-degree regression model with the collected data (3), checking the model using ANOVA (4), and finally, finding the best conditions (5) (Myers et al., 2016).

RSM has promising potential for microalgae application in treating wastewater, thus increasing treatment efficiency and reducing operating costs. In the literature, there are studies on microalgae with municipal wastewater (Zhai et al., 2017; Hossain et al., 2022), wastewater from palm oil production facilities (Khalid et al., 2019), and mining wastewater

(Yang et al., 2022). RSM has been used in developing models to predict the effect of various independent variables affecting microalgae-wastewater removal performance, including light duration (Khalid et al., 2019; Singh and Mishra, 2020), illumination level (Khalid et al., 2019), temperature (Zurera-Cosano et al., 2006), pH (Zurera-Cosano et al., 2006), initial microalgae concentration (Garcia-Gimeno et al., 2005) and nutrient levels (Yang et al., 2022). Related studies report that optimization has a positive effect, but traditional optimization methods may have some limitations in explaining complex algorithms. In this context, to evaluate the study more comprehensively, decision trees, which are among common machine learning (ML) approaches, were applied to test the accuracy level and generalizability of the models designed with RSM. This approach does not aim for optimization; it is used merely as a supportive tool to comparatively evaluate the predictive capability of RSM models.

ML methods, especially decision trees, are increasingly being incorporated into optimization research to enhance the prediction accuracy and reliability (Maleki et al., 2024). ML-based techniques are advantageous for continuous monitoring, process optimization, anomaly forecasting, and error identification in wastewater management (Sundui et al., 2021). Combining statistical approaches like RSM with ML techniques offers a comprehensive understanding of complex systems, thereby improving the overall performance and resilience of water purification systems (Razzak et al., 2024).

Microalgae biomass cultured in wastewater produced in reactors has various applications in energy (Mahdy et al., 2017) and health (Panis and Carreon, 2016), as well as the potential for wastewater treatment under optimal conditions using RSM and ML algorithms. Anaerobic digestion (AD), which is included in energy applications, is the conversion of organic matter into biogas by passing through biological stages. Integration of microalgae into anaerobic reactors contributes to the conversion of biomass into bioenergy and reclaiming energy. Certain preliminary techniques and co-digestion options exist to increase the volume of biogas produced in reactors. It may contribute to adjusting the C/N proportion effectively of microalgal biomass containing high nitrogen content with carbon-rich substrates such as cattle manure and to strengthening the operational stability of anaerobic digestion. Biomass derived through wastewater treatment of microalgae in

anaerobic reactors supports a circular bioeconomy and creates a renewable energy source for biogas production. Moreover, the contribution of microalgae biomass cultivated in wastewater to bioenergy production has the potential to provide added value in health and environmental biotechnology applications.

There are studies examining the biological contributions of microalgae, especially their antimicrobials (Gnanakani et al., 2019) and antioxidants (Wali et al., 2020). These studies stated that the growth of pathogenic microorganisms can be prevented and oxidative stress can be reduced (Sabatini et al., 2009). However, the studies are usually carried out with pure cultured microalgae biomass. Existing studies have not addressed the utilization of microalgal biomass grown with wastewater as a raw material in biotechnological fields such as antimicrobial, antioxidant, and DNA cleavage. Food industry wastewater can be used for the purpose. Testing the bioactivity of biomass produced by microalgae grown using wastewater can reduce waste generation and provide raw materials for biomaterial production.

Integrating microalgae in wastewater treatment systems promises to create sustainable solutions for water scarcity, energy demand, health, and climate change. Employing microalgae for wastewater treatment can be linked to some United Nations (UN) Sustainable Development Goals (SDGs). The capacity of microalgae to thrive in wastewater environments reduces operating costs by reducing the cost of additional nutrients and encourages water reuse. It falls within the scope of SDG 6 because it increases the potential for water treatment and reuse. Under the general heading of SDG 6, specifically subheading 6.3, this initiative is linked to the goal of halving the volume of untreated wastewater by 2030 and enhancing water quality for safe reuse. In addition, microalgae biomass has the potential to provide raw material support in areas such as fertilizer, feed and energy, thereby supporting SDG 12's fifth sub-goal of waste reduction and resource recovery. Microalgae co-substrate increases energy efficiency in anaerobic reactors. This feature aligns with SDG 7. This necessity is linked to the goal of increasing the proportion of renewables in energy use targeted for 2030, as outlined in subheading 7.2 under the general heading of SDG 7. The ability to reduce greenhouse gases emitted by traditional treatment and disposal methods with microalgae technology supports climate action (SDG 13). CWW-cultivated microalgae

are relevant to SDG 3, as they have the capacity to generate base materials for multiple uses in health- and food safety-related areas. Under the general heading of SDG 3, subheading 3.9 states that the number of diseases and deaths caused by water pollution and harmful chemicals should be greatly reduced by 2030. Considering all this, the versatile use of microalgae in wastewater systems is compatible with the UN SDG.

The present study primarily intends to assess the treatment capacity for a selected *Nannochloropsis sp.* strain on municipal and CWW through the application of various optimization and validation techniques. Also, the biomass produced from treating CWW with this microalgae is used as a feed in anaerobic reactors, and its impact on biogas production is studied. In addition, the biological activity of this biomass is to investigate its antimicrobial, antioxidant, and DNA cleavage properties. The scope objectives of this thesis can be summarized as follows:

- Cultivating *Nannochloropsis sp.* under laboratory conditions,
- Perform the necessary analyses of wastewater samples taken from relevant facilities in the laboratory,
- Determine the parameters and limit values for RSM optimization and establish reactor operating conditions,
- Evaluate RSM optimization to maximize microalgae biomass growth and pollutant removal efficiency, and verify it with the decision tree algorithm,
- Evaluate microalgae biomass growth and viability and pollutant removal,
- Test the produced microalgae biomass for co-digestion in biogas reactors,
- Investigate the usability potential of microalgae biomass in biotechnological research efforts.

CHAPTER TWO

THEORETICAL FRAMEWORK/PREVIOUS STUDIES

2.1. Conventional Wastewater Treatment Approaches: Principles, Benefits, and Limitations

Water pollution occurs when substances that pose environmental and public health risks are discharged into water bodies. Water pollution has various causes, including industrial and agricultural activities, mining, urban wastewater, energy use, and the disposal of radioactive waste (Crini and Lichtfouse, 2019). The use of water for these activities causes wastewater to be produced, thereby contributing to water pollution. Toxic substances resulting from the pollution of our world's water resources (rivers, seas and oceans) with wastewater cause accumulation in the food chain (Sharma et al., 2019). Therefore, protecting existing water resources and ensuring effective wastewater recovery and reutilization is of paramount importance.

The treatment of wastewater is essential for removing the detrimental impacts of potentially hazardous microorganisms and pollutants affecting both public health and ecosystems (Flannery et al., 2012). There are many wastewater treatment technologies available. Technologies differ based on the selected microorganism and reactor design. Biological processes are at the forefront in aerobic, anaerobic, and biofilm technologies. However, these treatment technologies, which are primarily aimed at carbon removal, have some limitations in removing other pollutants. Furthermore, the energy costs associated with traditional biological treatment systems are quite high. Excessive sludge production in facilities is another prevalent problem.

In addition to treatment, it is also important today to integrate wastewater systems into reclaimed water reuse schemes, which addresses concerns about water scarcity. These systems effectively treat wastewater on-site and ensure its reuse according to the region's needs (Lienhoop et al., 2013). This understanding has a different perspective from traditional methods. Traditional wastewater treatment systems have certain limits in nutrient removal

and recovery. Modern approaches, on the other hand, aim to transform wastewater into a resource through reuse and recycling.

Many studies in the literature treat wastewater using traditional methods. Table 1 presents the various treatment methods, initial concentrations of pollutants, and their removal efficiencies for municipal and dairy wastewater considered in this thesis.

Table 1

Comparison of initial concentrations and removal efficiencies of pollutants in various reactor types treating municipal and dairy wastewater

WW Type	Reactor Type	Initial Concentration (mg/L)	Removal Efficiency (%)	References
Municipal	Aerobic MBR*	COD: 84±21	COD: 63-78	Baek and Pagilla, 2006
Municipal	Anaerobic MBR*	COD: 84±21	COD: 55-68	Baek and Pagilla, 2006
Municipal	Anaerobic MBR*	COD: 252 ± 59	COD: 90 ± 2	Dong et al., 2016
Municipal	Anaerobic Filter	COD: 350–450	COD: 95	Bodkhe, 2008
Municipal	MBR*	COD: 647–771 TN: 51-74 TP: 17-23	COD: 94 TN: 91 TP: 85	Kitanou et al., 2021
Municipal	SBR* (anoxic-aerobic)	COD: 647–771 TN: 51-74 TP: 17-23	COD: 78 TN: 60 TP: 78	Kitanou et al., 2021
Municipal	BNR**	COD: 556 TN: 71.9 TP: 8.1	COD: 87 TN: 80 TP: 90	Demir et al., 2019
Municipal	Anammox	COD: 300 NH_4^+-N: 100-150	COD: 80 NH_4^+-N: 95	Leal et al., 2016

Table 1 continued

Synthetic	Bardenpho-MBBR*	COD: 700-800 TN: 35-40 TP: 7-8	COD: 98 TN: 93 TP: 95	Ashrafi et al., 2019
Synthetic	UASB*- Bardenpho	COD: 6000 TN: 205 TP: 124	COD: 99 TN: 99 TP: 100	Mazaheri and Doosti, 2024
Whey	UASB*	33,000	90	Zayed and Winter, 2000
Dairy	UASB*	2000-6000	>80	Ince, 1998
Dairy	SBR*	COD: 10,000 TKN: 780	COD: 80 TKN: 75	Li and Zhang, 2002
Dairy	MBR*	COD: 7067 NH_3-N: 500	COD: >90 NH_3-N:~80	Kumari et al., 2021
Dairy	SBR*	COD: 3350 TKN: 140 NH_3-N: 125 NO_3-N: 18 TP: 55	COD: 93 TKN: 47 NH_3-N: 47 NO_3-N: 33 TP: 49	Pannirselvam et al., 2015
Dairy	BNR*	COD: 87,640 ± 1273 NH_4^+-N: 87.7 ± 6.2 Ortho-P: 244.8 ± 16.4	COD: 94 NH_4^+-N: 98 Ortho-P: 96	Liu et al., 2017
Dairy	MBR*	COD: 921–9004	COD: 97-98	Farizoglu and Uzuner, 2011
Dairy	Microfiltration + Nanofiltration	COD: 3133 ± 438 TKN: 75 ± 12	COD: 51 TKN: 58	Bortoluzzi et al., 2016

* MBR: Membrane Bioreactors; SBR: Sequencing Batch Reactor; BNR: Biological Nutrient Removal; MBBR: Moving bed biofilm reactor; UASB: Upflow anaerobic sludge blanket

Table 1 displays the comparisons of municipal and dairy wastewater operating in various biological systems. The table also shows the initial concentrations of wastewater pollutants and their removal efficiencies. In addition, the table also includes some studies evaluating pollutant treatment performance in the Bardenpho (Ashrafi et al., 2019; Mazaheri and Doosti, 2024). However, synthetic wastewater was used in these studies. Furthermore, these studies employed hybrid systems instead of a single treatment process, resulting in quite high pollutant removal efficiencies. Carbon and nutrient removals can be realized through the Bardenpho treatment method. Therefore, it is in line with the objectives of this thesis. However, as can be seen, a single treatment system is not sufficient at high COD concentrations; it is operated together with processes such as MBBR or UASB. The same situation applies to the CWW used in the thesis. Because the COD concentration of raw CWW is quite high (~70,000 mg COD/L), hybrid treatment systems should be preferred.

2.2. Microalgae-Based Wastewater Treatment: A Sustainable Alternative

Microalgae are unicellular or multicellular species that can survive in both marine and freshwater environments, with approximately 30,000 identifiable species (Thoré et al., 2023). These organisms exhibit various biological mechanisms, including autotrophic, heterotrophic, and mixotrophic (Kurniawan et al., 2025). Determining the application of microalgae-based systems in wastewater remediation requires a correct understanding of these mechanisms. While autotrophic microalgae consume atmospheric carbon dioxide via photosynthesis (Sundaram et al., 2023), heterotrophic microalgae use organic carbon and do not need light at this stage (Kurniawan et al., 2025). Mixotrophic microalgae exhibit both autotrophic and heterotrophic metabolism and behave accordingly under varying conditions (Su et al., 2025). This helps them adapt to their environment and increases their range of use. In addition, mixotrophic microalgae can adsorb substances that are difficult to treat organically, thereby benefiting facilities that produce industrial wastewater containing these substances (Ravikumar et al., 2024). Considering all these benefits, the role of mixotrophic microalgae in environmental management and circular economy solutions is remarkable (Fuentes-Grünewald et al., 2021). In addition to the high efficiency of carbon and nutrient removal by microalgae, it would be useful to understand what mechanisms are involved in these pollutant removals. Table 2 shows the detailed removal mechanisms of microalgae for

carbon and nutrients (Gonçalves et al., 2017). Such processes support the removal of pollutants and explain the complex metabolic abilities of microalgae.

Table 2

Carbon and nutrient removal mechanisms of microalgae

Nutrient	Nutrient Type	Mechanism
Carbon	Carbon dioxide	Kelvin Cycle
	Organic Carbon	Respiration
Nitrogen	Nitrogen gas	Fixation
	Nitrate and nitrite	Ammonium reduction followed by amino acid conversion
	Ammonium	Direct amino acid conversion Air stripping
Phosphorus	Phosphate	Phosphorylation Chemical Precipitation

Among the microalgae species involved in wastewater treatment, *Nannochloropsis sp.* stands out with its high lipid content, mixotrophic feeding type, and wide range of working ability. In addition to being small, spherical, and flagellate, this microalgae species can produce high levels of chlorophyll-a (Kannah et al., 2021). In addition, it is frequently preferred in aquaculture because it contains elevated levels of vitamin B12 and eicosapentaenoic acid (EPA) (Lozano-Muñoz et al., 2020). It is capable of performing photosynthesis when exposed to a light source while also making use of organic carbon present in wastewater. Thanks to this feature, its metabolic flexibility provides an advantage in situations where light availability is limited, thus ensuring continuous nutrient removal and treatment of organic pollutants (Castillo et al., 2021). Furthermore, inclusion of mixotrophic microalgae strains in treatment processes can increase the operational efficiency of bioremediation by increasing the activity of bacterial communities due to their synergistic interactions (Abate et al., 2024). Table 3 summarizes the uses of *Nannochloropsis sp.* in the

literature. As indicated in the table, it has potential for biofuel, biogas, wastewater treatment, and pharmaceutical synthesis.

Table 3

Applications and functional potential of *Nannochloropsis sp*.: A literature-based overview

Usage Area	Operational Conditions	Key Findings	Reference
Biofuel/ Biomass Production	2000 L ponds; 1.4–2.8 rad/s paddlewheel; 25°C; 150 µmol m^2/s light	Max biomass (46 g/m^2) and lipid (52%) at 2.1 rad/s paddle speed	Moazami et al., 2012
Biofuel/Strain Selection	9 strains tested for growth, lipid productivity, fatty acid profile, biodiesel quality parameter	N. oceanica IMET1 showed highest lipid productivity (158.76 mg/L/day) and favorable biodiesel properties	Ma et al., 2014
Biofuel / Extraction and Catalysis	Sonication, microwave, reflux; solvents: methanol, chloroform/methanol, hexane; h-Beta zeolite as catalyst	Reflux with methanol gave highest lipid yield; h-Beta zeolite improved biodiesel conversion	Carrero et al., 2011
Biogas / Anaerobic Digestion	Batch and semi-continuous; corn silage + N. salina; 200+ days; 4.7 kg VS/m^3/d	Co-digestion improved stability and methane yield; prevented failure at high loading rates	Schwede et al., 2013
Biogas / Pretreatment Strategies	Anaerobic co-digestion with cow manure; pretreatments: ultrasonication, hot water, both	No-pretreatment gave more biogas; pretreatment increased methane ratio (up to 52%)	Saleem et al., 2020
Biogas/Residue Valorization	BMP tests; raw vs. lipid-extracted biomass; co-digestion with cellulose	Lipid-extracted residues gave higher methane yield than raw biomass	Barontini et al., 2016

Table 3 continued

Biomass / Sustainability / Biofuel	Cultivation with anaerobic digestate; N:P ratio adjusted; N-stress conditions	100% N and 16% P supplied by effluent; lipid up to 50%; >90% cost and impact reduction vs. fertilizers	Mayers et al., 2017
Biofuel / Wastewater Reuse	Growth in treated municipal wastewater and centrate; up to 75% v/v substitution	Growth and lipid production not inhibited; wastewater viable for nutrients; recycling still essential	Dong et al., 2014
Biofuel / Cultivation Optimization	*Nannochloropsis* in Green Wall Panel; different airflow & irradiance; N-starvation; use of industrial wastewater	Airflow tuning improves productivity at high light; nutrient stress boosts lipids; wastewater reduces costs	Biondi et al., 2013
Wastewater Treatment/Modeling	CWW up to 8000 mg COD/L; microalgae 2200 mg VS/L; RSM and decision tree	Up to 96% COD, 97% TKN, 99% Ortho-P removal; RSM and ML predictions matched	Kayan and Oz, 2025a
Wastewater Treatment/Modeling	Real wastewater; 21 h light, 8000 lux, 30 °C; RSM and decision tree	Max removal: COD 53%, TKN 87%, Ortho-P 70%; 49% increase in Chl-a	Kayan and Oz, 2025b
Pharmaceuticals / Antimicrobial	FAME extracted via lipase-catalyzed transesterification; tested at 10–30 µL/disc doses	FAME showed stronger inhibition against Gram-negative bacteria; DNA fragmentation confirmed activity	Surendhiran et al., 2014
Pharmaceuticals / Nutraceuticals	AME extract; LC-MS characterization; DPPH, H_2O_2, ABTS assays; MDA-MB-231 cell line	Strong antioxidant (IC_{50}: 52 µg/L), broad-spectrum antimicrobial, and dose-dependent anticancer activity	Wali et al., 2020

Table 3 continued

Nutraceuticals/Biofuel Co-production	Salinity: 20–40 g/L; Light: 60 & 150 µmol m^2/s; Photoperiod: 5 light/dark cycles	EPA up to 37.8% under 18h:6h light; low salinity increased pigment content; residual oil suitable for biodiesel	Mitra et al., 2015
Environmental Stress / Metal Tolerance	Cd exposure, oxidative stress markers, antioxidant enzyme & isozyme profiling	Cd increased lipid peroxidation and H_2O_2; APX and GPX upregulated; SOD and GR decreased	Lee and Shin, 2003

The limited nature of natural water resources necessitates the advancement of eco-friendly methods for wastewater. This context highlights the capacity of microalgae to assist in wastewater management. Numerous studies have reported the potential of microalgae for the reduction of nutrients from receiving environments (Tuantet et al., 2014). Moreover, these characteristics make microalgae notable for their broad biodiversity and widespread occurrence in aquatic ecosystems. Microalgae, municipal wastewater (Kayan and Oz, 2025a), brewery wastewater (Su et al., 2022), dairy industry wastewater (Kayan and Oz, 2025b), seepage water (Kholomyeva et al., 2020), palm oil wastewater (Resdi et al., 2021), and textile wastewater (Simsek and Sisman-Aydin, 2025). Table 4 summarizes the effectiveness of various microalgae species in different wastewaters in the literature. Each study has different operating conditions. In general, the evaluation is based on carbon and nutrient removal, as these parameters indicate whether the biological community in the wastewater is working efficiently. However, when evaluating the removal efficiencies, it is critical to know the initial concentrations of the pollutants. Because the removal of pollutants with low and high initial concentrations cannot be evaluated under the same conditions. Hena et al. (2018) reported the initial COD values of dairy wastewater to be between 119.21 and 140.17 mg/L in their study, Yılmaz and Budak (2018) reported the COD value as 48 mg/L in their study. These initial COD values are pretty low compared to real industrial wastewater, partially explaining the high removal efficiency obtained in these studies. However, in the studies conducted within the scope of this thesis, CWW wastewater taken from real-scale facilities was used, and the initial COD concentration for CWW was reported as 8000 mg/L. This value is relatively higher than in most studies in the literature.

Table 4

Studies using different microalgae in the treatment of various wastewaters

Type of Wastewater	Type of Microalgae	Removal Efficiency (%)	Reference
Municipal	*Chlorella vulgaris*	COD: 86	Zahmatkesh et al. (2022)
Municipal	*Chlorella zofingiensis*	N: 93, P: 90	Zhou et al. (2018)
Municipal	*Nannochloropsis sp.*	COD: 53, TKN: 87, Ortho-P: 70	Kayan and Oz (2025a)
Brewery	*Chlorella vulgaris*	COD: 40, TN: 94	Su et al. (2022)
Textile	*Neglectella solitaria*	COD: 90–98	Simsek and Sisman-Aydin (2025)
Textile	*Chlorella vulgaris*	COD: 94	Javed et al. (2022)
Textile	*Scenedesmus abundans*	COD: 87, N: 69, P: 71	Brar et al. (2019)
Textile	*Anabaena ambigua*	COD: 50, N: 53, P: 63	Brar et al. (2019)
Dairy	*Nannochloropsis sp.*	COD: 77–96, TKN: 51–97, Ortho-P: 60–99	Kayan and Oz (2025b)
Dairy	*Chlorella sorokiniana*	COD: 87, TP: 95, TN: 81	Kusmayadi et al. (2022)
Dairy	*Auxenochlorella protothecoides*	COD: 65, TN: 43, P: 77	Gramegna et al. (2020)
Dairy	*Chlorella vulgaris*	TN: 95, TP: 93	Yılmaz and Budak (2018)
Dairy	*Arthrospira platensis*	COD: 98, N: 99, P: 100	Hena et al. (2018)
Leachate	*Desmodesmus subspicatus*	Fe: 100, NH_3: 83	Kholomyeva et al. (2020)
Palm oil factory effluent	*Nannochloropsis*	COD: 48–55, N:46–60, P:44–62	Resdi et al. (2021)

As seen in Table 4, *Chlorella vulgaris* is commonly applied in the treatment of wastewater (Yılmaz and Budak, 2018; Javed et al., 2022; Su et al., 2022; Zahmatkesh et al., 2022). The reason for this use is that this microalgae species has a significant efficiency in nutrient assimilation (Kwon et al., 2020). A large part of the studies conducted with *Nannochloropsis sp.* in the literature are on biofuel production. This is because this

microalgae species can convert carbon dioxide into triacylglycerols (Kilian et al., 2011). Additionally, since this microalgae species can accumulate substantial amounts of EPA, it is also utilized as a raw material in fish farming (Ma et al., 2016). Its use in wastewater treatment is quite limited compared to all these other studies. Most of the studies conducted with *Nannochloropsis sp.* have been conducted using laboratory-scale and synthetic wastewater (Wijayanti and Ansori, 2021). Research involving full-scale wastewater treatment plants is still limited (Resdi et al., 2021). Therefore, determining and developing its potential in real wastewater treatment highlights the originality of this thesis.

2.3. Wastewater Treatment Technologies Employing Microalgae

Integrating microalgae into wastewater treatment systems offers promising benefits for both public health and sustainability. This integration can be incorporated into various stages of wastewater treatment processes, such as secondary or tertiary. Microalgae can be co-cultured with bacteria in the secondary stage of treatment systems. This microalgae-bacteria consortium can remove nutrients from wastewater while effectively reducing BOD and COD (Sánchez-Zurano et al., 2021). This method is especially beneficial because it uses the nutrients in the wastewater to help microalgae grow, which means it needs less energy than traditional treatment systems. Microalgae like *Chlorella vulgaris* (Khakimova et al., 2022) and *Scenedesmus sp.* (Arias et al., 2018) are often noted for doing well in these systems because they can grow on and utilize extra available nutrients while generating oxygen through photosynthesis, essential for the survival of bacteria.

Microalgae show promise for application in tertiary treatment processes to improve water quality (Gupta et al., 2016a). Microalgae incorporated into tertiary treatment can significantly contribute to achieving tight regulatory requirements with respect to effluent quality for discharge or further reuse. Incorporation of microalgae in tertiary treatment not only efficiently contributes to the elimination of pollutants but also makes it possible to generate biofuel-grade biomass to be harvested and used in different applications (Vandana and Rakesh, 2023). New knowledge highlights the potential role of microalgae in the inclusion of nutrient recycling strategies, making wastewater management practices even more sustainable (El Bakraoui et al., 2022).

Microalgae in advanced treatment are increasingly researched for their biosorption potential in the decontamination of a wide range of pollutants, including pharmaceuticals, from wastewater (Sarma et al., 2024). This property becomes relevant for the treatment of specialized treatment plants handling industrial wastewaters (Almomani and Bhosale, 2021) or domestic wastewaters (Ahiahonu et al., 2024), potentially containing complex organic substances. Microalgae can adapt to various types of wastewater, making them feasible candidates for advanced treatment processes because they are capable of removing organic contaminants for the growth of the biomass.

The integration of microalgae into wastewater treatment processes—whether at secondary, tertiary or advanced stages—requires a careful evaluation of the cultivation systems used. Therefore, to enhance the effectiveness of nutrient elimination and biomass production, it becomes essential to understand the advantages and disadvantages of microalgae cultivation under open or closed system conditions and make decisions accordingly. When choosing a photobioreactor system for microalgae cultivation, it is essential to evaluate many parameters, including its energy and carbon dioxide efficiency and biomass yield. No singular system can comprehensively satisfy all these criteria. The selection of microalgae and the reactor's intended use are essential for assessing the system's efficiency.

Raceway ponds and similar open culture systems are simple to set up and manage, offering economic benefits. In open systems, microorganisms can contribute to biomass growth by naturally using CO_2 (atmospheric or waste-sourced) and sunlight (Arsalan and Iqbal, 2023). These systems currently account for over 95% of global commercial microalgae production. High-rate algal reactor systems exhibit a high removal efficiency for nutrients through microalgae-bacteria symbiosis; they are also suitable for urban applications due to their small space requirement (Tangahu et al., 2018). Despite the advantages of open systems, they have several disadvantages, including inability to effectively regulate temperature and pH, high risk of fouling, inadequate distribution of light and gas, and insufficient available biomass (Carvalho et al., 2006). Possible contamination in open

systems can lower microalgal biomass because different organisms compete for nutrients (Wen et al., 2019). Improvements in open system design, such as raceway ponds, are being investigated to address these risks (Romagnoli et al., 2020). Open culture methods are inadequate for growing delicate strains or for processes that demand strict control parameters.

Closed photobioreactor systems (PBRs) can create a more controlled environment for important parameters like light, temperature, and CO_2 (Borowiak et al., 2020). Therefore, microalgal biomass is anticipated to grow more efficiently in closed systems than in open ones. Moreover, contamination and evaporation levels remain minimal in these systems, allowing for higher yields (Borowiak et al., 2020). Closed systems have various types, such as tubular structures, flat reactor plates, air-lift column reactors, polymeric reactor bags, and porous substrate bioreactors (PSBRs). Despite the various advantages of closed systems, significant capital and operating costs limit their applicability to large-scale operations (Parashar et al., 2023). Table 5 provides an overview, based on previous research, of the performance of microalgae strains in various bioreactor systems. This table helps understand which microalgae strains perform best in which bioreactor system. As indicated in the table, *Nannochloropsis sp.* has potential for use in photobioreactors.

Table 5

Performance of microalgae strains in various bioreactor systems: A literature-based overview

Microalgae Type	Reactor Type	Studied Parameters	Key Findings	Reference
Phormidium sp.	High Rate Algal Ponds	C:N:P ratios, retention time (4–7 days), synthetic wastewater	Dominated under high COD loading, regardless of retention time	Cromar and Fallowfield, 1997
Chlorella sp.	Parallel photobioreactor system	CO_2 concentration (2–15%), cell density, lipid/biomass productivity	Max growth at 2–5% CO_2 (0.66 day^{-1}); 6-unit PBR improved CO_2 reduction (0.573 g h^{-1}) and lipid/biomass yield	Chiu et al., 2008
Scenedesmus sp.	Outdoor raceway pond (7.2 m^2)	Anaerobic centrate (30 and 45%), culture depth, dilution rate, CO_2 from flue gas	Max 22.9 g m^{-2} day^{-1} biomass at 30% centrate, 15 cm depth; N and P removal 3 g N, 0.6 g P m^{-2} day^{-1}; 2.3 g lipid m^{-2} day^{-1}	Jebali et al., 2018
Scenedesmus obliquus	Flat-panel photobioreactor	Light (50–200 µmol $m^{-2}s^{-1}$), temperature (22–30 °C), pH, Tricine buffer	Max biomass 1.76 g L^{-1} at 24 °C and 150 µmol $m^{-2}s^{-1}$ with buffer; 33% lipid content; palmitic and linoleic acids dominant	Trivedi et al., 2021
Nannochloropsis sp.	Flat-panel photobioreactor	Light path (1.3–17 cm), daily harvesting	Optimal growth at 10 cm light path with 10% daily harvest	Richmond and Cheng-Wu, 2001
Nannochloropsis sp.	Airlift photobioreactor	Low-frequency blue LED flashing light	light cycle increased biomass generation	Yustinadiar et al., 2020

Table 5 continued

Nannochloropsis sp.	Annular column PBR (internal LED)	Blue & red LED, photoperiod (18:6 vs 12:12), light intensity	Higher cell density was achieved with the 18:6 light:dark cycle	Taisir et al., 2016
Nannochloropsis sp.	Helical-tubular photobioreactor	Temperature, natural/artificial light, CO_2-air distribution, cell monitoring	Max density at high temp + mixed light; 1.10–3.03 g L^{-1} day^{-1} productivity; daily harvesting ≥10% of total volume	Briassoulis et al., 2010
Nannochloropsis sp.	Flat-plate vertical PBR (10 cm light-path)	Outdoor conditions, mixing/cooling energy, harvesting regime, EPA productivity	Sealed system maintained monoalgal cultures year-round; 12 g m^{-2} day^{-1} biomass, 650 mg m^{-2} day^{-1} EPA yield	Cheng-Wu et al., 2001
Nannochloropsis sp.	Flat-panel PBR	Dissolved oxygen (20–250% air saturation), Rubisco inhibition, quantum yield	Growth inhibited above 75% O_2	Raso et al., 2012

Table 5 shows studies examining various microalgae species in different bioreactor systems. The different types of reactors (HRAP, flat panel, tubular systems, etc.), operating conditions (light intensity, temperature, CO_2 concentration, C:N:P ratios, LED light frequency, etc.), and the outcomes were assessed based on biomass yield or lipid generation. *Nannochloropsis sp.* is a preferred microalgae species among microalgae species and has a high potential for use, especially in closed systems. In large-scale studies of *Nannochloropsis sp.*, light cycle (Yustinadiar et al., 2020), light intensity (Taisir et al., 2016) and temperature (Briassoulis et al., 2010) are found to be the critical parameters affecting biomass production. In this respect, it is consistent with the parameters determined in the thesis. In addition,

studies conducted with synthetic wastewater or pure water have generally been included in the literature. However, within the scope of this thesis, experiments were conducted with real wastewater taken from real-scale facilities. According to Table 5, findings indicate that *Nannochloropsis sp.* achieves significant biomass production when operated under controlled conditions (Briassoulis et al., 2010; Taisir et al., 2016). This situation shows the importance of modeling and optimization approaches, which are the main part of the thesis.

2.4. Process Optimization in Microalgal Wastewater Treatment Systems

Current research demonstrates a growing interest in the application of statistical software and machine learning algorithms, which might increase performance in wastewater treatment plants and predict process outcomes. RSM is a statistical strategy toward enhancing the efficiency of wastewater treatment operations. RSM uses ANOVA to allow investigations into the importance of the variables with respect to the treatment process and the significance of these factors. Its primary objective is to optimize a dependent variable that is influenced by multiple independent factors, and it is valuable for developing empirical models, evaluating input effects, and identifying optimal conditions. Furthermore, RSM has been utilized to optimize wastewater treatment by adjusting various process parameters, such as the light cycle (Hossain et al., 2022), light intensity (Fan et al., 2020), temperature (Hossain et al., 2022), and nutrient concentration (Wu and Ying, 2019).

Table 6 provides a detailed comparison of several microalgae species that are primarily synthesized in the laboratory and are predominantly used for domestic wastewater treatment. The efficiency of pollutant removal is intimately associated with operating factors and the physicochemical characteristics of the wastewater. For example, under the optimal conditions for light, temperature, and nutrient ratios, *Chlorella kessleri* removed 99.96% of nitrogen and 95.92% of phosphorus from synthetic municipal wastewater (Hossain et al., 2022). Similarly, nutrient removal exceeding 99% was achieved with two species of microalgae grown in treated urban wastewater when light conditions and biomass levels were controlled (Fan et al., 2020). Conversely, real wastewater matrices, such as greywater or municipal wastewater, exhibited complex compositions while still achieving excellent removal efficiency.

Table 6

RSM-based assessment of microalgae for pollutant removal from diverse wastewaters

Microalgae	**Wastewater**	**Parameters**	**Removal Efficiency**	**References**
Chlorella kessleri	municipal wastewater (synthetic)	Temperature (°C), light-dark cycle (h/h), nitrogen-phosphorus ratio	N: 99.96%; P: 95.92%	Hossain et al., 2022
Spirulina platensis	domestic wastewater (sterilized)	Light intensity (lux), daily illumination time (h), biomass (mg/L)	TN: more than 99% TP: 88.6%	Fan et al., 2020
Scenedesmus obliquus	domestic wastewater (sterilized)	Light intensity (lux), daily illumination time (h), biomass (mg/L)	TN: 93.8% TP: more than 99%	Fan et al., 2020
Chlorella vulgaris	wastewater (synthetic)	COD (mg/L), NH_4^+–N (mg/L), TP (mg/L)	COD: 70%–83%; NH_4^+–N: 51%–91% ; TP: 30%–94%	Wu et al., 2019
Spirulina platensis	municipal wastewater (synthetic)	pH, light intensity (lux), daily illumination time (h)	TN: 92.58; TP: 94.13%	Zhai et al., 2017
Scenedesmus obliquus	municipal wastewater (real)	TP (mg/L), $FeCl_3 \cdot 6H_2O$ (mg/L)	TN/TP: >99%	Zhang et al., 2014
Chlorella sorokiniana	municipal wastewater (real)	Temperature (°C), light intensity (μμmol m^{-2} s^{-1}), MgAC (g L^{-1})	COD: 91.1% (highest)	Panbehkar Bisheh and Amini Rad, 2023
Arthrospira sp.	gray water (real)	Chloride, sulfate, surfactant	BOD: 96.8%; COD: 87.5%; TOC: 99.8%	Mukhopadhyay et al., 2022
Nannochloropsis sp.	municipal wastewater (real)	Light cycle (h/h), light intensity (lux), temperature (°C)	COD: 53%, TKN: 87%, Ortho-P: 70%	In this thesis
Nannochloropsis sp.	CWW (real)	Microalgae concentration (mgVS/L), wastewater concentration (mgCOD/L)	COD: 77-96%, TKN: 51-97%, Ortho-P: 60-99%	In this thesis

This thesis pinpoints the need to optimize the working conditions for each substrate engaged in treatment systems utilizing microalgae. However, studies with *Nannochloropsis sp.* using both real wastewater and optimization software to determine the best parameters are limited. While RSM can provide optimization for parameters, ML techniques also have

the potential to enhance the optimization process (Hashemi et al., 2022). This hybrid modeling enables optimization of the parameters and increases the predictive accuracy of the models, thus avoiding complex process challenges.

Machine learning can show how it interacts with the input and output of the process, which is very useful when the characteristics of the variables are studied, as is the case with wastewater (Singh et al., 2023; Singh and Mishra, 2021). Table 7 details how artificial intelligence (AI) and machine learning are used in treating wastewater with microalgae. Research comparing different machine learning approaches in wastewater treatment determined that the decision tree is the most suitable algorithm for classifying parameter data (Elsayed et al., 2022). A substantial dataset enhances the accuracy of decision trees, a type of machine learning model. For example, Singh et al. (2023) predicted the optimum combinations for increasing microalgae biomass production through a decision tree algorithm and achieved an overall accuracy of 81.25% in the 18 combinations identified. Nonetheless, generating these extensive datasets for real-scale applications is infeasible both economically and temporally (Oruganti et al., 2023). Therefore, our study identified the most significant parameters influencing microalgae systems based on a literature assessment, and the research was conducted within the confines of these parameters. Recently, decision tree models have been developed that enable prediction of initial inoculum level, temperature, N/P ratio, light intensity, and pH. These findings demonstrate that RSM and decision trees provide significant potential for use across various wastewater types and operational situations, particularly before the implementation of a novel microalgae in large-scale systems. Consequently, in our research, these two methodologies were employed as complementary to one another.

Table 7

Application of AI/ML algorithms in microalgal wastewater treatment systems

Type of microalgae	Type of wastewater	Employed AI/ML algorithms	Remarks	Reference
Spirulina platensis	Tofu wastewater (dairy industry)	ANN* and FBI*	The microalgae fuel performance had a 2.24% enhancement due to the combination of ANN and FBI	Sayed et al., 2024
Chlorophyceae, *Cyanophycean,* *Eustigmatophyceae*, *Trebouxiophyceae*, *Chlorodendrophyceae*, *Xanthophyceae*	Agriculture, Domestic, Municipal, Industrial, Livestock, Synthetic	Decision tree	The algorithms revealed 18 descriptor variable combinations that predict biomass output with 81.25% accuracy.	Singh et al., 2023
Nannochloropsis sp.	Municipal wastewater (real)	Decision tree	Light intensity above 6500 lux and a light cycle above 19.5 hours give the highest microalgal biomass production percentage of 56%.	In this thesis
Nannochloropsis sp.	Cheese whey wastewater (real)	Decision tree	Appropriate microalgae and wastewater concentrations for carbon (>90%) and nutrient (>70%) removal were determined.	In this thesis

*ANN: Artificial neural network; FBI : Forensic-based investigation

2.5. Assessment of Biogas Generation Potential from Wastewater-Derived Microalgal Biomass via Anaerobic Digestion

The increasing energy demand and interest in sustainable solutions emphasize the importance of resources that provide environmentally friendly energy. Developing countries should prioritize biogas generation through anaerobic processes (Rahman et al., 2015). The biogas systems, which produce renewable energy, decrease greenhouse gases, improve waste management, provide nutrient-rich fertilizer, enhance sustainable agriculture, support local economies, create jobs, and reduce pollution pressure from waste on land and surface waters, also serve most of the SDGs (Obaideen et al., 2022). Increasing regulatory pressures (Collivignarelli et al., 2015) and the cost of conventional wastewater treatment systems necessitate investigation of alternative processes such as microalgae. Biomass derived from microalgae reactors may serve as a co-substrate in anaerobic digestion systems to aid biogas formation.

The literature frequently emphasizes the inclusion of microalgae in anaerobic digestion (AD) for its potential to increase methane production. Microalgae, which can adapt to different environments (freshwater, saltwater, and wastewater) with autotrophic, heterotrophic, and mixotrophic feeding modes, stand out with their biomass production capacities and environmental adaptation characteristics (Xue et al., 2020). Biomass produced by microalgae increases the biodegradability of waste in addition to being an ideal substrate for bacteria in anaerobic reactors, thereby enhancing methane yield (Costa et al., 2022). It has been shown that protein-rich species, in particular, can accelerate the hydrolysis and methanogenesis stages (Magdalena et al., 2018).

A review of studies has indicated that microalgae like *Chlorella vulgaris* and *Scenedesmus* can boost methane production by making organic waste easier to break down. For example, the use of *Chlorella vulgaris* together with wastewater treatment sludge increases methane production while reducing ammonia toxicity, thereby improving process efficiency (Solé-Bundó et al., 2019a). Additionally, the mixture of *Chlorella vulgaris* biomass with cattle manure provides a high methane yield (Mahdy et al., 2017). Under

mesophilic conditions, *Scenedesmus* biomass has shown promising results with 70% COD degradation efficiency and substantial biogas yield (Greses et al., 2017). These findings indicate that both species can be effectively used in anaerobic reactors.

Studies have demonstrated the role of microalgae in generating renewable energy and biobased materials. However, the practical applications of microalgae for bioenergy purposes have mostly remained at the laboratory scale (Zhu et al., 2020). Despite limited full-scale applications, there is potential for microalgae to boost methane production in anaerobic reactors, and they can be used as a valuable substrate. Nevertheless, to increase methane yield, microalgae may need to be pretreated or co-digested with different substrates (Vargas-Estrada et al., 2022). Studies in the literature also include utilization of microalgal species with activated sludge, fertilizer, food residues, plant matter, glycerol, waste paper and industrial waste (Solé-Bundó et al., 2019b). Table 8 summarizes different investigations into anaerobic co-digestion processes involving microalgae.

Table 8

Different investigations into anaerobic co-digestion processes involving microalgae

Microalgal Strain	Co-digested	Operational Condition	Yield (mL/gVS)	Reference
Nannochloropsis (lipid extracted biomass)	fat, oil, and grease waste	Mixture ratio: 50:50 (VS) OLR:2gVS/L d T: 37°C time: 40 d	400 (methane)	Park and Li, 2012
Nannochloropsis gaditana	Cellulose	Mixture ratio: 50:50 (VS) T: 37°C 2L batch reactors time: 45 d	268 (methane)	Barontini et al., 2016
Nannochloropsis limnetica	Pig manure	Mixture ratio: 40:60 (VS) T: 53 °C OLR: 2 gVS/L	355 (methane)	Tsapekos et al., 2018

Table 8 continued

Nannochloropsis oculata	Cow manure	T:35±2 °C, pH:7.2, time: 90 d 550 mL microalgae+1000g cow manure +1000 mL sludge	300 (methane)	Saleem et al., 2020
Chlorella sp.	Activated sludge	Mixture ratio: 41:59 (TS) T: 37°C	468 (methane)	Wang et al., 2013
Chlorella sp.	Septic sludge	Mixture ratio:50:50 (VS) T: 35 °C initial C/N ratios 11:1 to 27:1 time: 30 d	547.3±35.6 (methane)	Lu and Zhang, 2016
Chlorella sp.	Chicken manure	Mixture ratio: (CM: Chl.) 0:10, 2:8, 4:6, 6:4, 8:2, and 10:0 T=35 ± 1 °C	238.71 (methane)	Li et al., 2017
Selenastrum capricornutum	Sewage sludge	Mixture ratio: 50:50 (VS), T:33 °C	392 (biogas)	Caporgno et al., 2015

Nannochloropsis is preferred for energy production in anaerobic reactors due to its resistance to environmental conditions and high levels of lipids (Bohutskyi et. al., 2015). *Nannochloropsis*, which is included in the thesis, has been tested with animal manure. In the scope of the thesis, CWW, which requires a high cost to be treated, has been preferred. In addition, anaerobic reactors were operated with cattle manure.

Microalgae are considered a prominent and beneficial material source not only in biogas systems but also in the production of biofuels and bio-products (Dolganyuk et al., 2020). The presence of biologically active compounds, such as macromolecules, micronutrients, and functional pigments in their structures, makes them important for many environmentally valuable applications (Dolganyuk et al., 2020). Furthermore, it has been shown that microalgae have higher CO_2 capture capacities compared to terrestrial plants (Cea-Barcia et al., 2018). The contribution of cultivating microalgae for treating wastewater, nutrient recovery, and CO_2 capture offers a synergistic approach for environmental sustainability.

2.6. The Biotechnological Potential of Wastewater-Derived Microalgal Biomass in Health and Medical Applications

Microalgae offer a lot of potential in biotechnology, in addition to helping the environment. These organisms can be used in cosmetics (Stolz and Obermayer, 2005), food supplements (Koyande et al., 2019), and medicine (Huang et al., 2024), among other things. Microalgae are useful in these sectors mainly because they can make proteins, vitamins, and other useful compounds (Wu et al., 2021). Because of these traits, microalgae contribute to the production of environmentally friendly and natural products.

Research has indicated that microalgae show broad potential for medical and health-related uses, including antibiotic production (Pratt et al., 1944), antioxidant (Panis and Carreon, 2016), antiviral (de Jesus Raposo et al., 2014), anticancer (Gerwick et al., 1994), and the isolation and production of anti-inflammatory compounds (Guzmán et al., 2003). Microalgae provide natural biological protection by preventing the development of pathogenic agents, including bacteria, fungi, and viruses, through bioactive components like fatty acids, peptides, and phenolic compounds (Pérez et al., 2016). Additionally, microalgae are known to generate various antioxidant metabolites (polyphenols, carotenoids) that have been associated with protection against illnesses like cancer and cardiovascular disease (Lauritano et al., 2016). In addition, bioactive peptides and proteins obtained from microalgae (*Chlorella* and *Spirulina*) have shown blood pressure-regulating, blood sugar-lowering, anti-tumor, and oxidative stress-reducing properties, which is an important development for health applications (Leong and Chang, 2024). Because of these valuable capabilities, microalgae are of critical importance in the healthcare sector. Being an organic material with multifunctional applicability, it holds promise for future research.

Another focus of studies conducted with microalgae is the evaluation of their antioxidant activities (Tedeschi et al., 2021). A practical and effective method utilized to determine antioxidant effectiveness associated with biologically active substances, including carotenoids and phenolic compounds in microalgal extracts, is the DPPH-based method for

assessing free radical inhibition, which serves as a method to determine antioxidant behavior (Papalia et al., 2019). Research has shown that *Nannochloropsis sp*. can be a natural reservoir of antioxidant compounds (Safafar et al., 2015; Santhar et al., 2021). However, these studies used a pure *Nannochloropsis sp.* culture, fed with a special medium. Cultivating *Nannochloropsis sp.* requires many additional costs (Faé Neto et al., 2018), but in this study, cheese whey wastewater, an industrial wastewater, was preferred instead of a special medium. Thus, wastewater reuse has been achieved, and by creating a sustainable cycle, the aim has been to utilize the wastewater in microalgae production.

In addition to the antimicrobial and antioxidant potentials of *Nannochloropsis sp.,* evaluating its DNA cleavage activity, especially in terms of contributing to studies in biotechnology and medicine, will help in the holistic consideration of this microalgal species. However, although there is no study directly addressing the DNA cleavage activity of *Nannochloropsis sp.*, several studies in the literature indicate that it may have potential regarding this specific research topic. Garcia-Parra et al. (2022) carried out a study in which the researchers indicated that the bioactive compounds formed could affect DNA integrity, even though they did not directly measure DNA cleavage. In another study on the use of *Nannochloropsis oculata* supplementation in livestock, it was stated that microalgae could reduce oxidative stress, and this condition could be explained by its association with DNA integrity and damage (El-Hawy et al., 2022). More research is needed to examine how *Nannochloropsis sp.* directly affects DNA cleavage to gain a clearer insight into and interpret how these mechanisms influence and interact within health-related fields.

2.7. Integrated Evaluation of Environmental Impact, Carbon Emissions, and Cost in Microalgal Wastewater Treatment

The inclusion of microalgae in wastewater treatment systems can be considered a sustainable alternative to traditional treatment methods. Microalgae are not only responsible for removing nutrients from wastewater but also play a role in the formation of bio-products (biofertilizers, biogas, and biofuels), thus contributing to the carbon cycle. Life cycle assessment (LCA) evaluated within the scope of resource recovery, are used to assess the applicability and sustainability of microalgae-based technologies. The objective of this

chapter is to compile and contrast information on scale/context, methods, valorization pathways, environmental findings, and economic findings obtained from studies in the literature. Table 9 presents the studies within this scope.

Table 9

A dual perspective assessment—environmental and economic—of wastewater treatment using microalgal systems

Scale/ Context	Methods	Valorization Pathways	Environmental Findinds	Economic Findings	Reference
Pilot HRAP treating municipal WW (p.e. ~1,500); small community	LCA (ReCiPe midpoints) + economic assessment	1) Biogas 2) Biofertilizer vs. activated sludge	Biofertilizer in warm climates lower eutrophication and toxicity	HRAP + biofertilizer most cost-effective vs. activated sludge	Arashiro et al., 2018
Urban vs. industrial WW; lab/pilot systems	LCA + bioproduct recovery	Pigments, biofertilizer, biogas	Industrial-WW scenario had up to 5× lower impacts vs. conventional medium; urban less efficient	Promising solution for circular bioeconomy	Arashiro et al., 2022
Pilot scale; olive mill wastewater rich in phytotoxins and organic load	LCA using EF 3.0 method; simplified economic analysis	Cultivation of A. platensis (Spirulina) for food; recycling nutrients from wastewater	Integrated system reduced impact by 75.95% vs. conventional treatment	Payback period ≈ 10 years;WW treatment cost more impactful than electricity or biomass sales	Moglie et al., 2024
Modification of existing full-scale sewage treatment plant; municipal sewage	Integrated LCA and Quantitative Microbial Risk Assessment; health impact measured in DALYs	Biomass valorized via: 1) Biodiesel, 2) Biocrude, 3) Biogas	Biocrude valorization pathway had lowest impact (0.015 DALYs/m^3); electricity use was major hotspot	Economic evaluation proposed but not yet conducted	Bhatt et al., 2024

Table 9 continued

Municipal wastewater; full-scale context implied	LCA across 16 environmental impact categories; scenario analysis with 4 valorization pathways	S1: Agriculture S2: Incineration S3: Cement fuel S4: Biostimulants	S4 showed improvements in 14/16 categories only if a 5% fertilizer reduction was assumed; otherwise, all scenarios had similar benefits; S1–S3 showed limited gains	Economic analysis not included	Crippa et al., 2025
Small communities; comparison across different climatic regions; municipal wastewater	LCA and cost analysis; compared two HRAP scenarios with an activated sludge system	1) Biogas production 2) Biofertilizer production	Biogas had lower impacts on environmental problems; biofertilizer better in some cases depending on climatic conditions; warm climates enhanced HRAP performance	Biofertilizer scenario was more economically feasible than biogas; HRAPs more cost-effective than activated sludge for small-scale systems	Vassalle et al., 2025

Table 9 shows that integrating microalgae into wastewater treatment systems is effective in reducing environmental impacts compared to traditional methods. In small communities in hot climates, using HRAPs instead of traditional wastewater management approaches and combining these systems with biofertilizer production can make wastewater treatment more sustainable and economically viable (Arashiro et al., 2018). Integrating *Spirulina* microalgae into traditional industrial wastewater treatment resulted in approximately a 70% reduction in negative environmental impact. In addition to environmental impacts, economic assessment (sufficient land and solar effect) provides positive results, especially for small-scale areas in rural or hot regions (Vassalle et al., 2025).

Wastewater treatment plants contain various units with high carbon footprints and energy consumption. These include the pump unit, aeration tank, sludge drying beds, and the system where treated wastewater is recycled (Singh et al., 2016). Li et al. (2023) examined three separate sections in calculating the total carbon emissions produced during wastewater processing activities: pretreatment (chemical use-related), biological treatment

(nitrogen removal units emitting nitrous oxide), and sludge treatment (electricity use-related carbon dioxide emissions). It was reported that emissions generated during biological treatment were approximately twice as high as those from other stages (Li et al., 2023). The microalgae-based nutrient removal included in this thesis may contribute to the development of studies aimed at reducing nitrous oxide emissions from nitrification-denitrification units in wastewater treatment plants that perform nitrogen removal.

In carbon footprint calculations, emissions must be examined under Scope 1 (direct), Scope 2 (indirect), and Scope 3 (through the supply chain). In their study, Wu et al. (2022) compared both anaerobic and aerobic processes in wastewater treatment plants under these three scopes. According to the results, there is a strong link between treatment plant design and carbon footprint, and emissions from scopes 2 and 3 can be reduced by selecting the right process, but reducing emissions from scope 1 is challenging (Wu et al., 2022). Additionally, the comparison of aerobic and anaerobic processes revealed that anaerobic systems emit fewer emissions and thus have a lower carbon footprint (Wu et al., 2022). This study assessed the carbon footprint based on Scope 2 emissions, focusing solely on the laboratory-scale operation of microalgal batch reactors.

2.8. Sustainability Framework: Alignment with SDGs

This thesis includes multiple fields of study, such as microalgae-assisted wastewater treatment, renewable energy production, and biomass utilization. These fields of study correspond to the SDGs' goals. When the keyword microalgal-supported systems for treating wastewater is searched in the literature, a strong relationship with the UN SDGs is found. In particular, these studies are reported to contribute to goals such as SDGs 3, 6, 7, 9, 11, 12, and 13. Table 10 explains the relevance of the central subjects of investigation determined within the scope of the thesis to the SDGs.

Table 10

Microalgae-related processes and their contribution to SDGs

Process	Related SDG	Description
Wastewater Treatment	3, 6, 11, 12	Carbon and nutrient removal, Protection of aquatic systems, Reduction of health risks, Efficient use and recycling of water resources
Biogas Production	7, 12, 13	Renewable energy from microalgal biomass, Climate-friendly energy source, Conversion of waste into energy
Antimicrobial, Antioxidant, DNA breakage	3, 9, 12	Natural compounds for disease prevention, Development of high-value bioproducts in health, Sustainable use of biomass for various applications

A literature search was conducted in the WoS database on June 27, 2025, with the keyword “microalgae-based wastewater treatment”. A total of 556 articles were identified following the search (WoS link). Table 11 provides the classification of the 556 publications according to the SDGs. Of these studies, 502 were associated with SDG 6, which addresses clean water access and sanitation; 57 with SDG 3 concerning health and well-being; 14 with SDG 7 aimed at clean and accessible energy; 9 with SDG 12 targeting sustainable production and consumption; and 7 each with SDG 11 addressing urban sustainability and resilience and SDG 14 concerning marine ecosystems. Although the thesis appears to focus on wastewater treatment, it covers a wide range of critical research topics that serve sustainability goals.

Table 11

Distribution of articles related to SDGs in microalgae-based studies

SDG number	Number of Articles
3	57
6	502
7	14
11	7
12	9
14	7

Numerous studies in the literature examine the potential of microalgae in wastewater treatment, biomass production, or biotechnological applications. However, these studies have generally focused on one or two topics. This thesis is a comprehensive study examining how the microalgae *Nannochloropsis sp.* can be used in both municipal and industrial wastewater treatment and how the biomass obtained from these industrial processes can be used for energy and health applications. The next section details the experimental design, materials, and methods used in this thesis.

CHAPTER THREE

RESEARCH METHODOLOGY/MATERIALS AND METHODS

This study was conducted with two different wastewaters, municipal wastewater and CWW. Both wastewaters were used to create a marine microalgae growth medium for *Nannochloropsis*. RSM was used to optimize the treatment performance of pollutants in the wastewater and also the microalgae biomass production. Decision tree was applied to evaluate the accuracy of the obtained results and to explain some parameter interactions that could not be explained by RSM. Biomass obtained from *Nannochloropsis* reactors cultivated with CWW was used both for biogas production in anaerobic reactors and for testing bioactivity potentials. During these studies, control experiments were conducted to interpret the treatment performance more clearly. This integrated approach is in line with various principles of sustainability development goals (SDG 3, SDG 6, SDG 7, SDG 9, SDG 11, SDG 12, SDG 13, and SDG 14) and circular bioeconomy principles. The general flow of this thesis is given in Fig. 1.

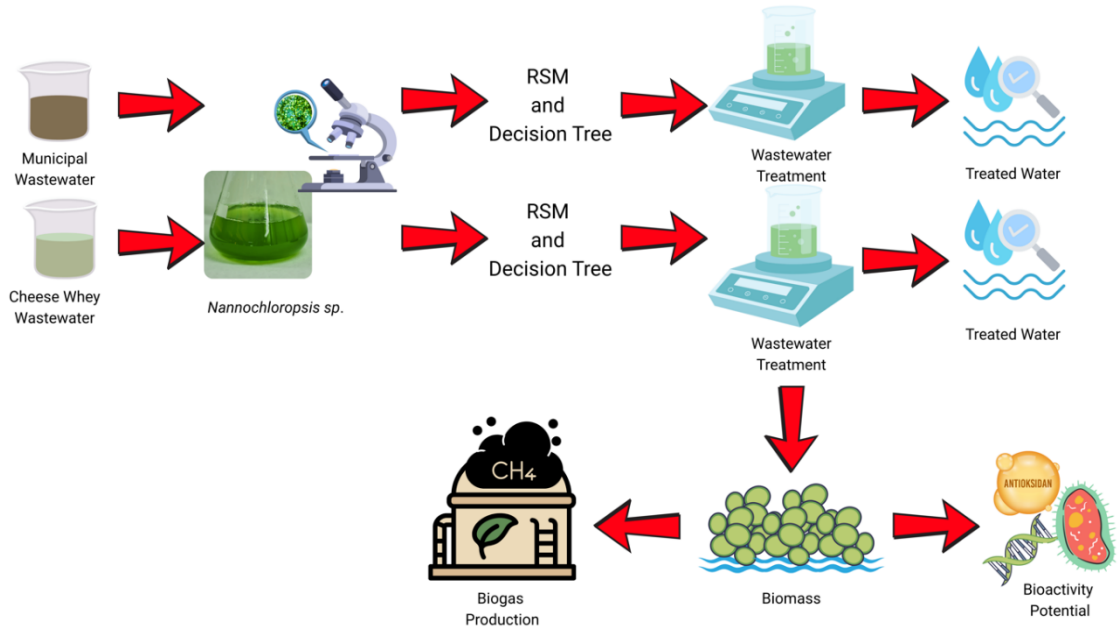


Fig. 1. General schematic workflow of the study.

3.1 . Material

3.1.1. Cultivation of Microalgae

Nannochloropsis sp. was sourced from an aquaculture facility located in Çanakkale. Table 12 shows the concentrations of various parameters of *Nannochloropsis sp.* The cultivation reactors were maintained at a steady orbital agitation of 300 rpm in a 24:0 light:dark cycle (hour) without additional aeration at room temperature (25°C) and pH 8±0.2. The cultivation reactors with a working capacity of 400 ml were measured for two weeks with optical density (OD) at 680 nm daily with a DR 5000 spectrophotometer. Before inoculation, microalgae were grown separately and allowed to reach an exponential growth phase. The linking of the amount of volatile solids in biomass measured in mg L^{-1} and the optical density during mixotrophic growth was determined using Equation (3.1). VSS analysis was performed following the procedures outlined in Standard Methods (SM 2540-E).

$$\text{Volatile solids (mg } L^{-1}) = 193.49 \times OD680 + 81.298 , \; R2 = 0.9933 \quad (3.1)$$

Table 12

Characterization of the saline water microalga *Nannochloropsis sp.*

Parameters	Concentration (mg L^{-1})
COD	455
TKN	2.8
Ammonium	1.4
Nitrate	575
Nitrite	37.6
Organic Phosphorus	97.2
Ortho-Phosphorus	8.1
Total Solid (TS)	19,553
Volatile Solid (VS)	5220
Suspended Solid (SS)	297.5
Volatile Suspended Solid (VSS)	217.5

3.1.2. Municipal Wastewater

Municipal wastewater was taken from the inlet unit of a wastewater treatment plant in Çanakkale. Batch reactors of *Nannochloropsis sp.* and municipal wastewater were carried out under laboratory conditions. RSM was used to determine the optimum operational conditions. Experimental setup in the laboratory for batch reactors cultivated with municipal wastewater. The active volumes of the reactors are 400 mL. The reactors were kept under light at 170 rpm in an orbital stirrer for 5 days. The room temperature, light cycle, and light intensity were adjusted for each reactor according to the RSM output. Using the decision tree algorithm, we verified the microalgae growth in the reactors operating under optimal conditions. Pollutant removal efficiencies such as carbon and nutrients were evaluated. After the process, the quality of the treated water was evaluated to determine its reusability, and it was shown that this approach has positive environmental impacts that support the SDGs. The results show that microalgae can contribute to sustainable water management and provide a biologically efficient way of treating municipal wastewater. Fig. 2 summarizes the

studies conducted in this thesis with municipal wastewater and *Nannochloropsis sp.* microalgae.

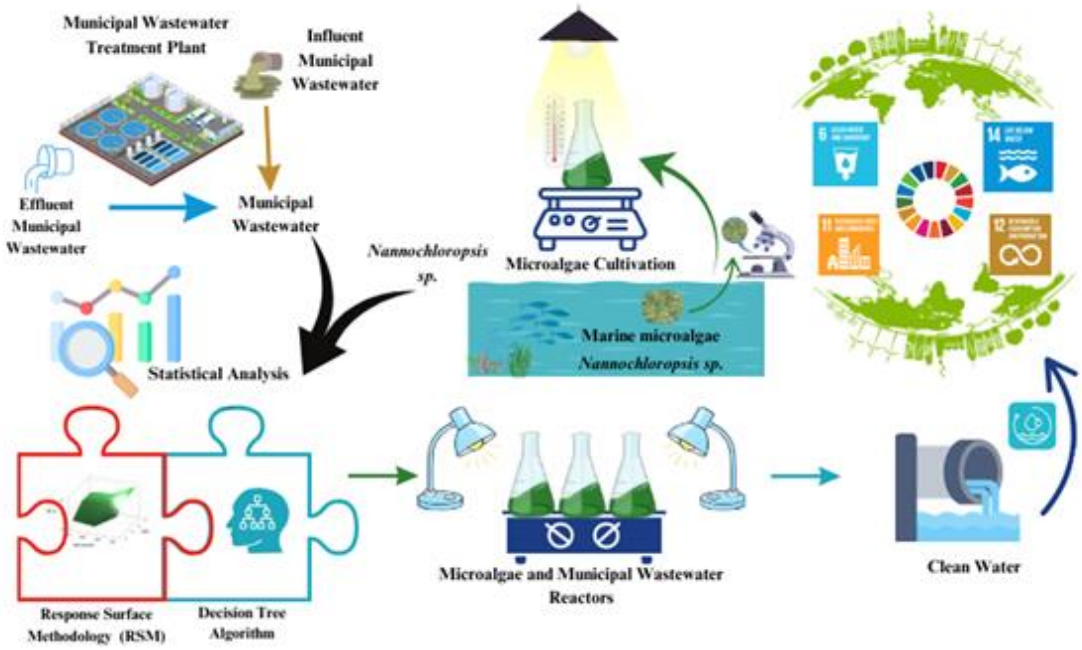


Fig. 2. Integrated wastewater treatment process using municipal wastewater and *Nannochloropsis sp.* microalgae.

Wastewater of municipal origin was sourced from a biological plant in Çanakkale (40.09566, 26.38179). Both influent and effluent points of the facility were sampled. Table 13 shows the characteristics of influent and effluent municipal wastewater.

Table 13

Municipal wastewater (influent and effluent) composition

Parameter	Units	Influent	Effluent
pH		7.35	7.5
Conductivity	ms/cm	3.41	2.34
TDS	g L^{-1}	2.18	1.49
Turbidity	NTU	131	29.9
Color	PtCo	334	146
sCOD	mg L^{-1}	226	33.5
TKN	mg L^{-1}	13	6
Ammonium	mg L^{-1}	8.5	4
Nitrate	mg L^{-1}	0.8	0.4
Nitrite	mg L^{-1}	2.3	1.8
Ortho-P	mg L^{-1}	2.9	0.9
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

3.1.3. Cheese Whey Wastewater

The study explores the use of *Nannochloropsis sp.* for treating CWW derived from a cheese manufacturing unit located in Çanakkale. First, a culture of microalgae was grown in a lab. Then, limiting concentrations of CWW COD and microalgae VS concentrations were determined using preliminary studies and literature reviews. After determining the limiting concentrations, Minitab software was used. This software created the reactor set. As a result of approximately one month of operation of the reactors, the pollutant removal efficiencies and microalgae biomass growths were evaluated. Finally, the process data were thoroughly examined using RSM and decision tree analyses, revealing the key parameters affecting the

treatment performance. Fig. 3 shows the studies conducted with CWW and *Nannochloropsis sp*.

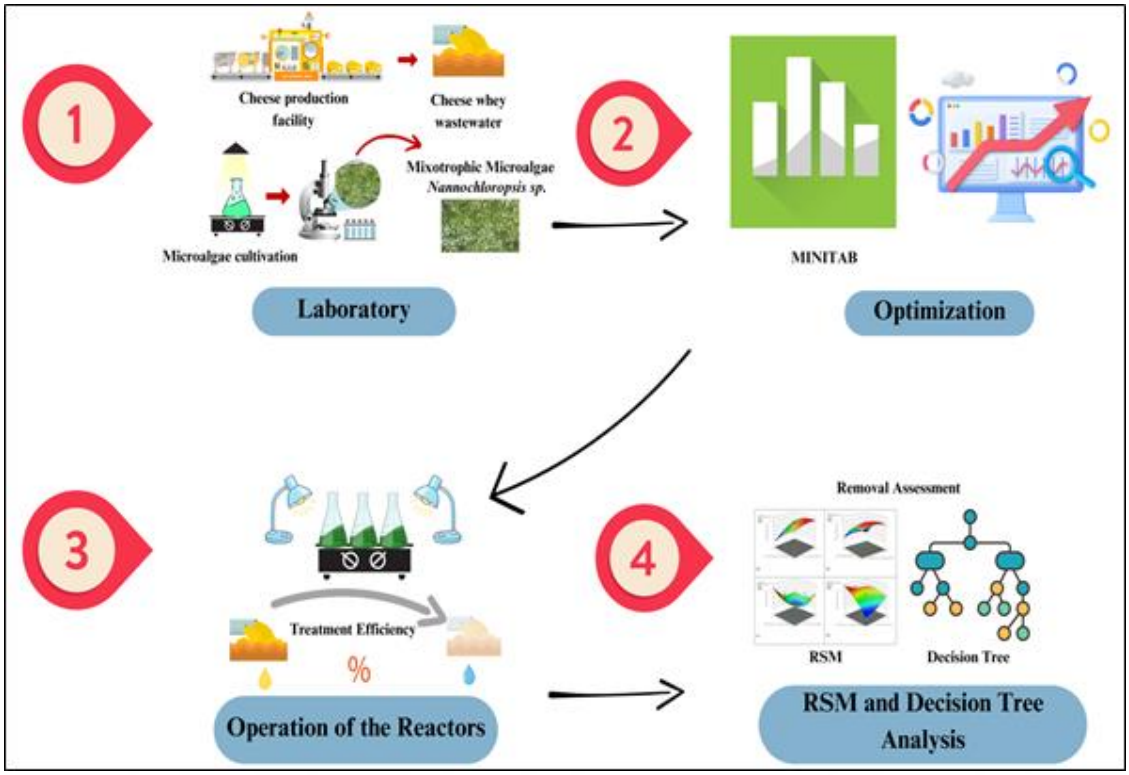


Fig. 3. Integrated wastewater treatment process using cheese whey wastewater and *Nannochloropsis sp.* microalgae.

The biomass of *Nannochloropsis sp.* was cultivated in the reactors using CWW. CWW samples were taken from a cheese production facility located in the Ezine, Çanakkale. Table 14 shows the values of this CWW sample, which correspond to different parameters. These values belong to raw CWW. In preliminary studies, it was found that raw CWW samples negatively affected microalgae growth. Therefore, reactors were operated at the highest COD concentration (8000 mg L^{-1}) at which microalgae would not be negatively affected. Concentrations of parameters such as TKN and Ortho-P also decreased at the same rate.

Table 14

Various parameter concentrations of raw CWW

Parameter	Concentration* (mg L^{-1})
COD	74,820
TKN	1410
Ortho-P	442
Cl^-	19,994
TS	63,580
VS	57,880

*CWW used in microalgae reactors was diluted.

In this experiment, *Nannochloropsis sp.* and CWW were utilized. The cultivation was conducted in glass reactors with a working volume of 250 mL. Experimental conditions included continuous illumination at 5000 lux for 24 hours, mixing at 170 rpm, and room temperature over four weeks. Reactor optimization in studies of microalgae and wastewater has been conducted by evaluating parameters like light intensity, photoperiod, temperature, and concentration. In research examining light intensity, this figure typically ranges from 1000 to 8000 lux. A beneficial impact on microalgae culture was noted particularly at 5000 lux (Zhai et al., 2017; Fan et al., 2020). Consequently, 5000 lux was selected as the light intensity for this experimental configuration. Moreover, numerous research studies in the literature address photoperiod, which is an additional determining factor. Generally, it has been observed that the light cycle is more favorable for microalgae growth than the dark cycle (Zhai et al., 2017; Fan et al., 2020; Hu et al., 2025). Consequently, in this experimental configuration, the reactors were maintained under continuous light for 24 hours.

In the thesis, the CWW concentration range used was between 1000- 8000 mgCOD/L. Similarly, the microalgae VS concentration was 1000-8000 mgVS/L. The CWW concentration range was determined based on preliminary experiments. The upper value for the microalgae VS concentration was also determined in preliminary studies. However, the lower value, 1000 mgVS/L, was determined based on literature studies. Arias et al. (2018)

reported that in a study where wastewater was treated with microalgae, a VS concentration of microalgae of approximately 1 g/L positively affected pollutant removal performance. However, these concentration values vary based on the preferred microalgae and wastewater type as well as environmental conditions. Therefore, instead of selecting a single value and testing only one, studies were carried out in a wide range of 1000-8000 mgVS/L. The reason for the upper limit of 8000 mgVS/L is that *Nannochloropsis sp.* microalgae can only be grown up to this concentration in the laboratory.

Time-dependent samples were taken from each reactor. OD 680 nm and chlorophyll-a analyses were performed to determine microalgae biomass change and viability. COD was also used for carbon removal, while TKN and Ortho-P were used for nutrient removal.

3.1.4. Biogas Generation via Anaerobic Reactors

The inoculum material required for inoculating the reactors under anaerobic conditions is available in our laboratory. The reactors will be operated under the operating conditions given in Table 15. Anaerobic reactors will be run at a pH of 6.8-7.2, a temperature of 37ºC, and a stirring speed of 90 rpm. In anaerobic experiments, anaerobic conditions will be created by passing nitrogen gas through the solution. The alkalinity of the anaerobic reactor will be adjusted to 1000 mg $NaHCO_3$/L by sodium bicarbonate feeding. In addition, control reactors will be operated simultaneously. COD, pH, total nitrogen, and total phosphorus tests will be performed on the samples taken at regular intervals. The parameters of total and volatile matter also determine the properties of the anaerobic biomass.

Table 15

Operating conditions of anaerobic reactors

Parameters	Range of values
pH	6.8-7.2
Temperature (ºC)	35±2
Mixing (rpm)	90
Alkalinity	1000 $NaHCO_3$/L
Volume (L)	1 or 2
Nutrient solution (g/L)	KH_2PO_4 ; 2.5, $K_2HPO_43H_2O$; 1.31, NH_4Cl; 1, $MgCl_{2}6H_2O$; 0.22

Anaerobically digested sludge taken from a biogas plant operating in Şanlıurfa was used in the reactors. It was characterized in the laboratory. As a result of the analysis of the anaerobic sludge, the total solids (TS) content was measured as 18.257 mg/L and the volatile solids (VS) content as 10.092 mg/L. Around 55% of the TS was composed of organic matter, while the dry matter content of the sludge was measured at 2%.

3.1.5. Manure

Cattle manure was collected from a livestock farm in Çanakkale. It was characterized in the laboratory. The TS amount of the cattle manure sample was determined as 204.735 mg/L and the VS content as 176.257 mg/L. In other words, approximately 86% of the total solids are organic components. This value indicates that it can provide high biogas potential for anaerobic reactors. The dry matter ratio was determined to be 17%.

3.2. Analytical Methods

Sample analysis methods are presented in Table 16. COD analyses were conducted using closed reflux. The "SM 5220 D" standard method was used for this analysis. Samples were filtered through 0.45 filter paper. Potassium dichromate and silver sulfate sulfuric acid were added to each sample. Then, they were held in the thermoreactor (at 148°C) for 2 hours. The samples were measured using a spectrophotometer (Hach, USA) at 600 nm.

Different analyses were performed for different forms of nitrogen. For the TKN, a combination of digestion and distillation units was used, while for the ammonium form, only the distillation unit was used. For digestion, sulfuric acid was added to 20 mL of sample. Additionally, a tablet was added for the TKN analysis. The digestion was carried out at 365°C for two hours. Then, distillation was applied to boric acid, and the collected samples were titrated with 0.02 N H_2SO_4, and the consumption was recorded. For nitrate analysis, HCl was added to each sample and the blank. Color developer was introduced for nitrite analysis. A DR 5000 spectrophotometer was used for nitrate (220 nm) and nitrite (543 nm).

Total phosphorus was analyzed using the standard method (SM 4500-P B). For ortho-P analyses, tin chloride and ammonium molybdate were added to the samples, and after waiting for 10 minutes, measurements were performed using a DR 5000 spectrophotometer (690 nm).

Glass filter paper with a pore size of 0.45 μm is used to filter the samples through the filtration system. For the analysis of residual solids on the filter, SS analysis, the filter paper is dried in an oven at 105-107°C for 1 hour. To determine the content of VSS, SS analysis was followed by a muffle furnace (T=550-600°C). Similarly, for total solids, a hot plate followed by oven treatment (T=105-107°C) was carried out. To determine the VS content, total solids analysis was followed by a muffle oven (T = 550–600°C).

Table 16

Analytical methods used in the characterization of wastewater and sludge and monitoring of experiments

Parameter	Sample	Method	Instrument
pH	Wastewater, Microalgae	4500-H B Method Electrometric (APHA, AWWA-WPCF-1992)	ORION SA 520 pH meter
TSS and TDS	Wastewater	Standard Method-2540-D	Filtering set and oven
VSS	Wastewater	Standard Method-2540 D	Filter set, oven, and muffle furnace
Conductivity	Wastewater	2510 B Metod (APHA, AWWA-WPCF-1992)	Conductivity meter
COD	Wastewater	5220 D Metod, Kapalı Reflux, Kolorimetrik, (APHA, AWWA-WPCF-1992)	DR 5000 spectrophotometer
Nitrogen (Total Kjeldahl Nitrogen, nitrite, nitrate, ammonium)	Wastewater	LCK 339 (1-50 mg/L NO3)	DR 5000 spectrophotometer, Eflab burned and distillation equipment
Phosphorus (organic and inorganic)	Wastewater	LCK 349 (0,15-4,50 mg/L PO4)	DR 5000 spectrophotometer
Biogas (CH_4, CO_2, H_2S)	Reactor	Gas measurement	Miligas Counter
TS	Biomass	Standard Method-2540-B	Oven
VS	Biomass	Standard Method-2540-E	Oven, and muffle furnace
Activity Test	Biomass	Standard Method-2540-E	Specific Methanogenic Activity Test
Microscope	Wastewater	Moticam1 1280*720	Motic

Biogas is a significant energy source generated through the functioning of anaerobic reactors. Anaerobic reactors have a volume of 1 or 2 liters and are operated under the operating conditions of the reactors given in Table 5. The reactors are connected to the biogas counter (Milligas Counter) by a pipe system (Fig. 4). In this way, the amount of biogas that will be formed in the reactor can be known. Microorganisms called inoculate sludge are used in anaerobic reactors.

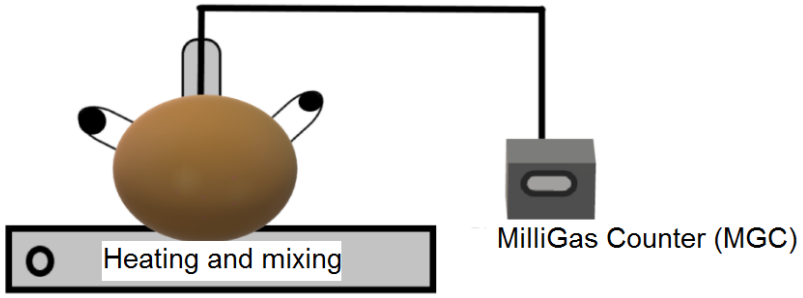


Fig. 4. Anaerobic batch reactor and biogas counter unit.

3.3. Microalgal Biomass Growth Analysis

3.3.1. Spectrophotometer

A single wavelength absorbance measured at 680 nanometers was used on a DR 5000 spectrophotometer to monitor changes in microalgae biomass daily.

3.3.2. Chlorophyll-a

Chlorophyll-a is an analysis performed to assess the activity of microalgal biomass. For this analysis, 10 ml samples were taken from each reactor and subjected to centrifugation at a speed of 3000 rpm for 1 minute. At the end of this process, the upper liquid phase was carefully separated. 5 ml acetone was used to extract the pigments from the microalgal biomass remaining in the lower section. After shaking for a while, the reading was taken on a spectrophotometer. The sample was analyzed at three distinct wavelengths—630, 647, and 664 nm. The results obtained are placed in the corresponding place in Equation (3.2). (Toyub et al., 2008).

$$\text{Chlorophyll-a (mg L}^{-1}) = 11.85\ (OD\ 664)\text{-}1.54\ (OD\ 647)\text{-}0.08\ (OD\ 630) \quad (3.2)$$

3.4. Data Analysis and Statistical Methods

3.4.1. Response Surface Methodology

RSM represents a hybrid approach involving mathematical and statistical tools for experimental modeling and optimization. RSM serves to analyze and enhance the interaction among multiple input parameters and their corresponding results. In this way, it can determine the optimum parameter combination that yields the most meaningful result (Mayers et al., 2017). Minitab (Version 20.4) software was used for RSM analysis.

There are two different designs based on RSM. One is CCD, and the other is the Box-Behnken Design (BBD). BBD was used in this study. BBD consists of three levels (low-medium-high) (Hossain et al., 2018). CCD consists of five levels (including axial points) (Njoku and Otisi, 2023). The lower number of levels in BBD reduces factor confounding and thus decreases the total number of experiments (BBD provides thirteen experimental runs while CCD provides fifteen to twenty 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 (Tekindal et al., 2014).

Cheese Whey Wastewater

Table 17 shows the Minitab output of the concentration values determined for CWW and *Nannochloropsis*. In total, 13 reactors were identified. This table presents the concentration values as COD for CWW and VS for *Nannochloropsis*.

Table 17

Experimental design proposed by Minitab

No	CWW concentration (mgCOD L^{-1})	*Nannochloropsis sp.* concentration (MA) (mgVS L^{-1})
1	4500	4500
2	6975	2025
3	2025	2025
4	2025	6975
5	4500	4500
6	4500	1000
7	4500	4500
8	6975	6975
9	8000	4500
10	1000	4500
11	4500	4500
12	4500	8000
13	4500	4500

Municipal Wastewater

The ranges and values of the independent variables included in the experimental design are provided in Table 18. Each parameter was assigned three coded levels (-1, 0, +1) in accordance with the BBD used within the RSM framework. The minimum and maximum values for the parameters of light cycle, light intensity, and temperature were determined based on relevant literature and subsequently entered into Minitab software. The software coded the minimum value as “-1”, the maximum as “+1”, and the midpoint between these as “0”. In microalgae-based wastewater treatment systems, these three variables—light cycle, light intensity, and temperature—are considered among the most critical operational factors. Based on previous studies, the selected ranges were 6–24 hours for the light cycle (Zhai et al., 2017; Fan et al., 2020), 2000–8000 lux for light intensity

(Fan et al., 2020), and 20–30°C for temperature (Hossain et al., 2022). The software generated outputs by assigning these three coded levels for each parameter.

Table 18

Range and concentrations of independent test variables in the experiment

Variable	Symbol	Coded factors and levels		
		-1	0	+1
Light Cycle (h)	*x1*	6	15	24
Light Intensity (lux)	*x2*	2000	5000	8000
Temperature (°C)	*x3*	20	25	30

All data were processed and optimized using RSM with Minitab v.20.4 software. The relationship between the growth rates of *Nannochloropsis sp.* and optical density 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 below 0.05 being regarded as statistically significant. Equation (3.3) describes a formula for calculating the relative error in percentage between experimental and predicted biomass growth rates.

$$\text{Relative Error (\%)} = (Y_{exp.} - Y_{p.})/Y_{exp.} * 100 \quad (3.3)$$

Where:

Yexp. denotes the biomass growth rate obtained from experimental data (%);

Yp indicates the estimated growth rate from the model (%).

Generally, a lower p-value shows a greater statistical importance of the related factor. When the p-value is below 0.05 are considered highly statistically meaningful at a 5% significance threshold or covered by the 95% confidence range, if the p-value ranges from

0.05 to 0.1 are deemed indicating statistical significance at the 10% level or within a 90% certainty range. Conversely, a term is regarded as insignificant if its p-value exceeds 0.1.

3.4.2. Decision Tree

The data modeling process was carried out using the decision tree algorithm within RapidMiner Studio (version 10.3, RapidMiner Inc., USA). Fig. 5 indicates the process undertaken in the development of the decision tree algorithm. Initially, the dataset was imported into the workspace using the "Retrieve" operator. The role of each attribute was then defined, with the target variable (label) being assigned as the parameter to be predicted specifically, a selected removal efficiency value. Following this, the dataset was partitioned into training and testing subsets using a 60:40 ratio to ensure appropriate model validation and to minimize overfitting. The "Multiply" operator was utilized to duplicate the dataset stream for parallel processing or additional analysis steps. Subsequently, the "Select Attributes" operator was applied to define the input variables for the model. At this stage, it included different data for both wastewaters (CWW and municipal). Decision trees were created for CWW based on the removal performance of the pollution parameters. The independent variables in this decision tree were CWW COD and *Nannochloropsis sp.* VS concentration. In municipal wastewater, a decision tree was created with temperature, light cycle, and light intensity variables based on the biomass production efficiency of *Nannochloropsis sp.* microalgae. Finally, the "Decision Tree" operator was executed to construct a classification model. The algorithm generated a hierarchical tree structure that enabled understanding how input variables are related to the target variables. This model served to identify the most significant predictors and decision rules contributing to optimal removal efficiency in the given experimental setup.

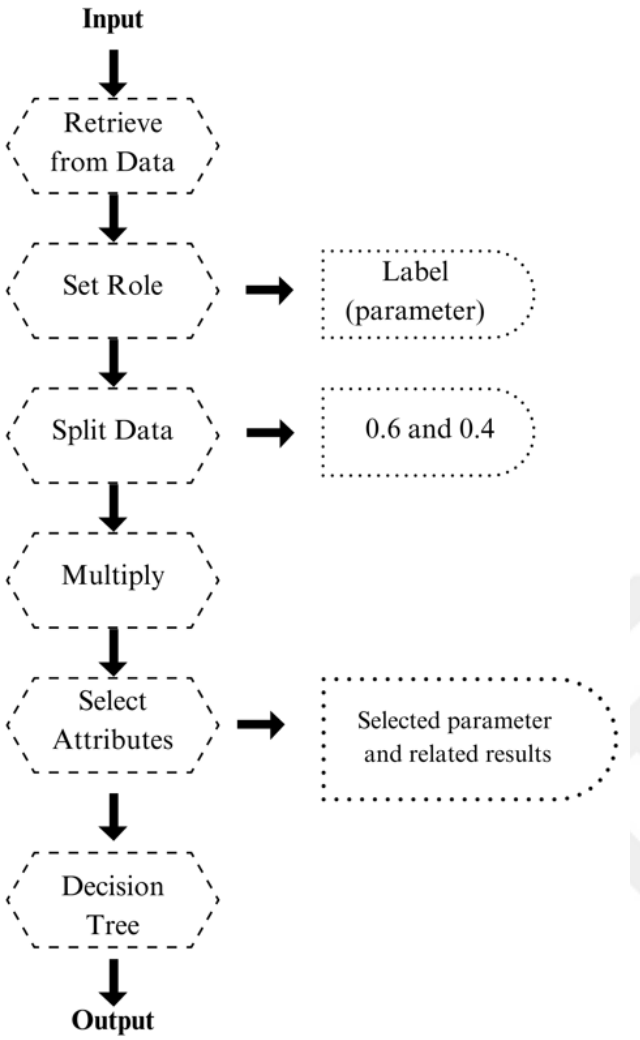


Fig. 5. Decision tree flowchart.

3.4.3. Bibliometric Analysis

Using VOSviewer version 1.6.19 software, the data was examined for the most productive countries, the most cited documents, co-occurrence networks, keyword clusters of authors, publication years, and major countries involved in research. These components provide a comprehensive perspective on the state of research, highlighting the significance of bibliometric studies. The VOS viewer software used in the study is an open-source and user-friendly program widely used for network creation and visualization of bibliometric data (Van and Waltman, 2010).

3.5. Bioactivity Assessment of *Nannochloropsis sp.* Biomass

Antimicrobial, antioxidant, and DNA interference potentials of pure *Nannochloropsis sp.* cultures and CWW-fed microalgae biomass were evaluated. Antimicrobial activity was analyzed by agar disc diffusion and broth microdilution methods; antioxidant capacity by the DPPH free radical scavenging method; and DNA breakage potential by agarose gel electrophoresis using pBR322 plasmid DNA.

3.6. Fatty Acid Composition Analysis of Microalgal Biomass

The fatty acid composition of both CWW-cultured and pure microalgal biomass was determined by converting the extracted lipids into fatty acid methyl esters (FAME). For this purpose, 0.1 g of lipid sample was thoroughly mixed with 10 mL of n-hexane. Then, 0.5 mL of 2N methanolic KOH solution was added, and the mixture was left to stand in the dark for 2 hours. The upper phase containing the FAME was collected and analysed using a Shimadzu GCMS-QP2010 ULTRA system equipped with an RTX-2330 capillary column (60 m × 0.25 mm × 0.2 μm). Helium was used as the carrier gas. The oven temperature was programmed at a rate of 4°C/minute between 100°C (5 minutes) and 240°C and held at 240°C for 15 minutes. Injection was performed in split mode at 250°C (1 μL, split ratio 50:1). The interface and ion source temperatures were 250°C and 200°C, respectively. USP FAME Mix was used as the reference standard to identify saturated and unsaturated fatty acids, including EPA and Docosahexaenoic Acid (DHA). Fatty acid composition analyses were conducted at the Faculty of Marine Sciences and Technology at Çanakkale Onsekiz Mart University.

CHAPTER FOUR
RESEARCH FINDINGS

4.1. Treatment of Municipal Wastewater with Microalgae

4.1.1. Bibliometric Analysis

A bibliometric analysis was performed to explore the role of microalgae in the treatment of municipal wastewater. Web of Science (WoS) was used in this study. The database search was executed using this author-specified keywords: *microalgae*, *wastewater treatment*, "*domestic wastewater"*, or "*municipal wastewater"*. The analysis considered publications up to the year 2025. To ensure consistency and relevance, only documents categorized as "articles" were included, while all other document types were excluded. Additionally, the language filter was set to English, excluding publications in other languages. The search resulted in access to 498 articles (Access link). To gain further insight into the use of optimization techniques within this research field, an additional search was performed by including the term *optimization* in "All Fields." This refinement reduced the number of relevant publications to 133 (Access link). Furthermore, more specific optimization approaches were explored: when *Response Surface Methodology* was included as a search term in "All Fields," 26 articles were identified (Access link); similarly, when *Decision Tree* was searched, 5 articles were retrieved (Access link).

Fig. 6 shows a visual representation of how often the keywords related to microalgae and municipal wastewater treatment appear together, created with VOSviewer. The keyword microalgae appears as the central and most frequently used term, indicating its primary role in the research field. Frequently co-occurring terms include municipal wastewater, bioremediation, nutrient removal, biomass production, and biofuel, highlighting the main research themes, such as wastewater treatment, biomass generation, and energy recovery.

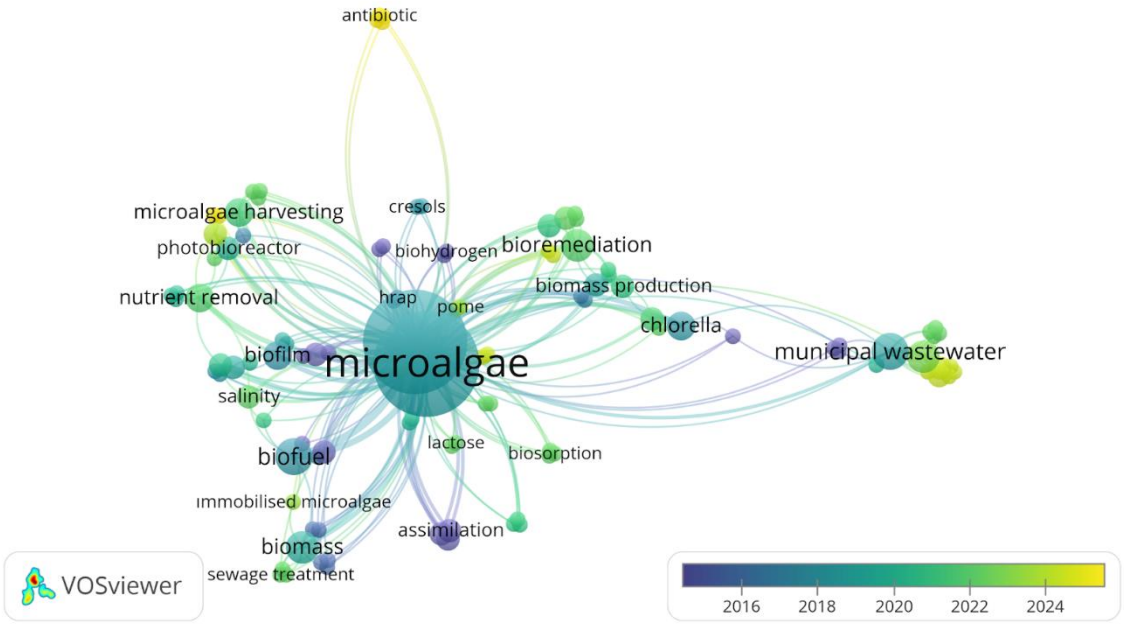


Fig. 6. Overlay network of author keywords in microalgae applications for municipal (or domestic) wastewater treatment.

Fig. 6 illustrates a color gradient corresponding to the publication years. Blue tones indicate older research, while yellow tones indicate more recent studies. Keywords such as microalgae harvesting, municipal wastewater, and antibiotics are among the more recent trends. Furthermore, it is notable that optimization-related terms (e.g., optimization, RSM, decision tree) do not appear in the network, suggesting that these approaches are either underrepresented or not commonly used as author keywords in this context.

4.1.2. Preliminary Studies

Researchers tested preliminary experiments to monitor the development and biomass increase of the marine mixotrophic microalga *Nannochloropsis sp.* in municipal wastewater 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 fed with the wastewaters. Time-dependent growth curves of *Nannochloropsis sp.* were determined in reactors operated with wastewater samples taken from both input and output stages of the treatment plant. The experiments indicated that *Nannochloropsis sp.* can only grow in the influent wastewater.

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 municipal wastewater. There are very few studies with *Nannochloropsis sp.* using raw influent domestic wastewater (Gupta et al., 2016b). However, when the studies with high removal efficiencies are examined, it is seen that the influent COD has very low initial values (COD: 24-99 mg/L) (Şirin and Sillanpää, 2015) compared to the composition of the raw wastewater utilized in the present study.

Testing Microalgae with Influent Municipal Wastewater at Different Ratios

The optimal ratio of *Nannochloropsis sp*. to municipal wastewater was determined after the preliminary studies selection of influent municipal wastewater 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 three-day period. The highest biomass growth percentage (39%) with respect to the initial concentration of *Nannochloropsis sp.* was observed in the 1:1 ratio reactor. Consistent with these findings, Hammad et al. (2019) 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 point out the importance of optimizing the wastewater-to-microalgae ratio for effective pollutant removal.

The effectiveness of treatment processes for wastewater methods based on microalgal organisms is largely determined by the ratio of microalgae to wastewater. In the study conducted by Onay (2018), 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 most amount of bioethanol. Phosphorus and nitrogen concentrations present in the wastewater affect the nutrient removal performance

of microalgae (Singh et al., 2017), and microalgal development and treatment performance may be affected by the presence of carbon and nitrogen in particular ratios (Torres-Franco et al., 2021).

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. (2020) suggested that screening microalgae strains that can respond effectively to shifting environmental conditions and wastewater quality in wastewater treatment systems is essential. Careful management of temperature, pH, and nutrients is also critical to maximize microalgae growth. Rodríguez-Miranda et al. (2020) 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 (Barceló-Villalobos et al., 2019).

4.1.3. Optimization with RSM

Table 19 presents the experimental and predicted percentages of biomass growth rates for *Nannochloropsis sp.*, derived from the BBD-based RSM model. The calculated relative error values for each reactor are also presented. The anticipated biomass growth rate was determined for each reactor utilizing Equation (4.1).

$$\text{Biomass production rate} = 14.9 + 4.18\times A - 0.03160\times B + 3.53\times C - 0.2270\times(A)^2 + 0.000002\times(B)^2 - 0.245\times(C)^2 + 0.000055\times A\times B + 0.1877\times A\times C + 0.000806\times B\times C \quad (4.1)$$

*Where;

A represents light duration,

B is light intensity,

C is temperature.

Table 19

Experimental design and results

No	Light Cycle (Light hour)	Light Intensity (lux)	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

Table 19 shows the operating conditions of all reactors and the growth percentages of microalgae biomass, both experimentally and according to RSM predictions, and the difference between these values. According to Fig. 7, the maximum biomass growth rate of *Nannochloropsis* sp. was reached under high levels of light cycle, light intensity, and temperature. 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). The interaction plot further illustrates the joint influence of pairs of independent input parameters in relation to the

response variables. Parallel lines in the respective interaction graphs indicate a lack of interaction, while a steeper slope reflects a stronger interaction. As shown in Fig. 8, all input factors interact with each response.

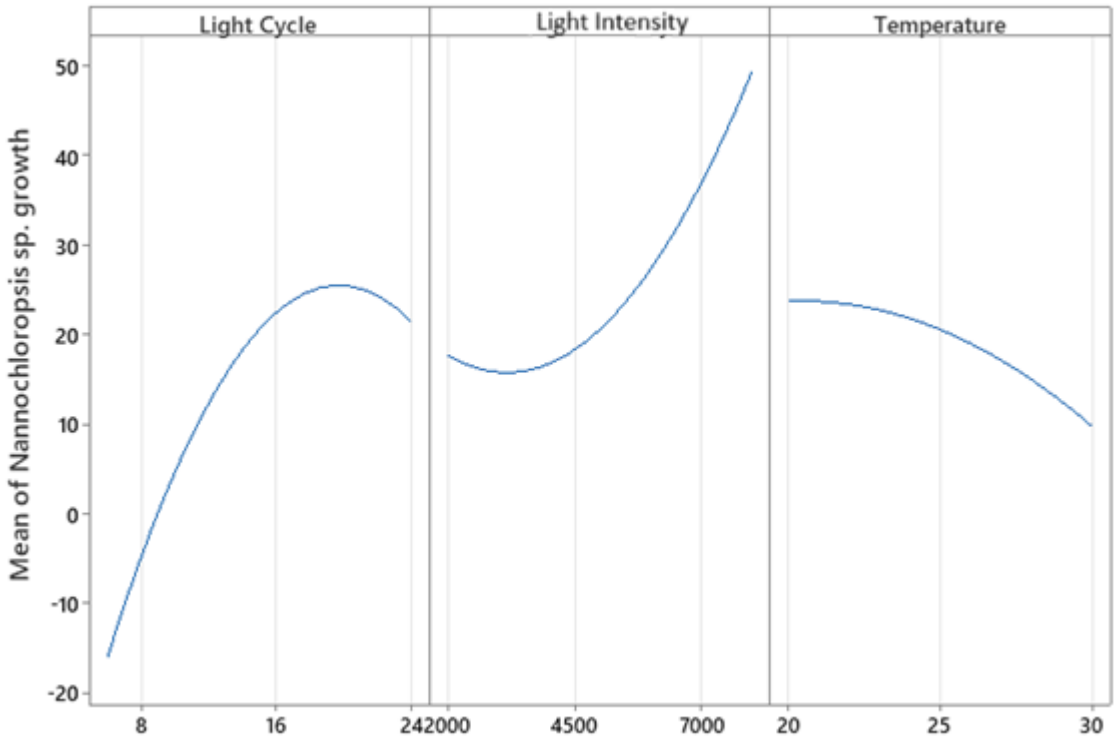


Fig. 7. Relationship between maximum growth of *Nannochloropsis sp.* and independent variables (light cycle, light intensity and temperature).

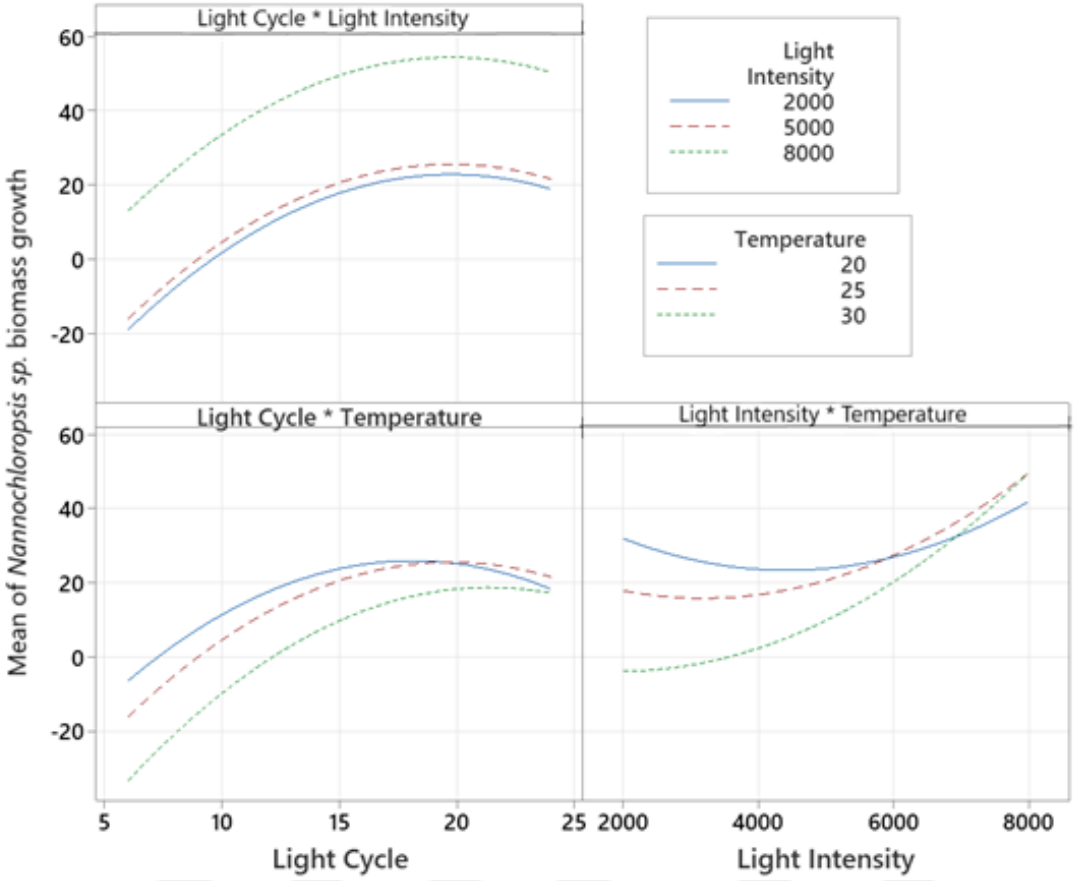
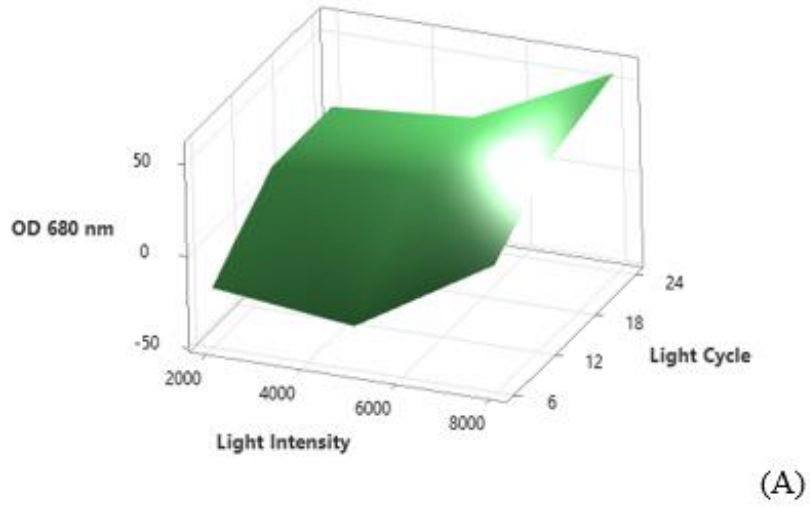


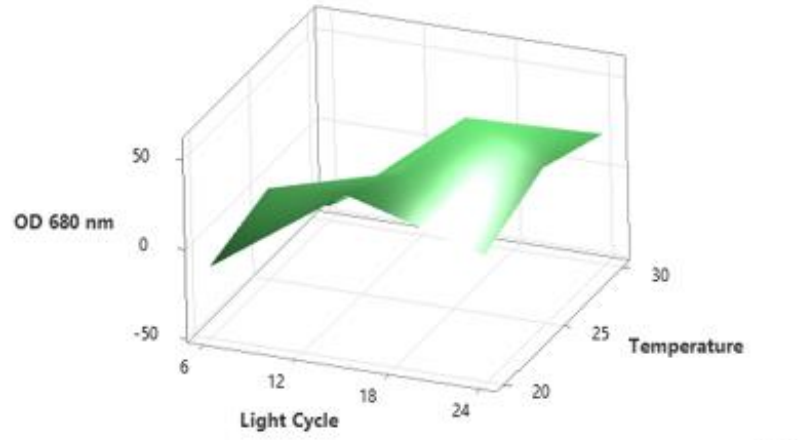
Fig. 8. Interaction effects of input variables (light cycle, light intensity and temperature) on both outputs.

Three-dimensional surface plots were made for both responses using Minitab software (v-20.4) to investigate more comprehensively the connection between independent factors and responses, as illustrated in Fig. 9. The results indicate that all input variables influence the response, the growth of *Nannochloropsis sp.* If we consider the independent variables as binary, as seen in Fig. 9(a), the area where microalgae biomass shows a positive increase is the area where the light cycle and light intensity are close to the upper limit. It is observed that when the light cycle and light intensity are close to the upper limit, the change in the temperature parameter has less impact on the graph (Figs. 9(b) and (c)). Additionally, comparable interdependent influences among variables were identified, as discussed in Fig. 8. While 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

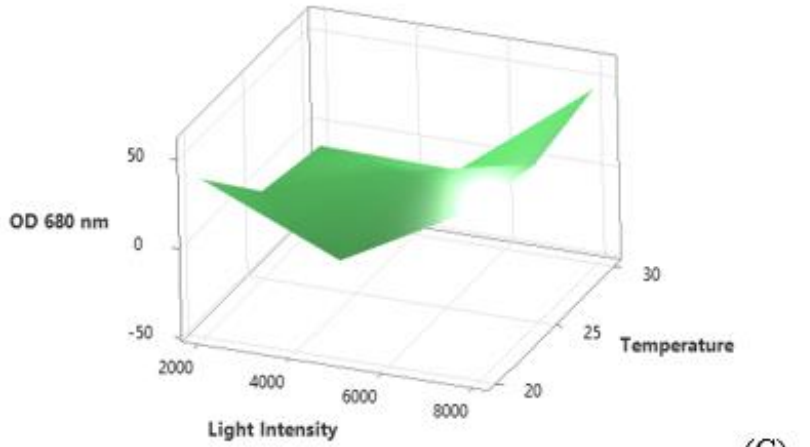
regression model outcomes for *Nannochloropsis sp*. as derived from the analysis are presented in Table 20. The significance of each model term was determined according to the corresponding p-values listed in the ANOVA output. Generally, a lower p-value signifies a higher significance of the corresponding term. Terms with a p-value < 0.05 are considered within a 95% confidence interval, while terms with 0.05 < p-value < 0.1 are deemed within a 90% confidence interval. Conversely, a term is regarded as insignificant if its p-value exceeds 0.1. 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 greater than 0.001, along with corresponding p-values > 0.05, demonstrate there is no statistically relevant lack of fit, confirming that the model can reliably predict the response values.



(A)



(B)



(C)

Fig. 9. Three-dimensional surface plot illustrating the interaction between dependent (optical density at 680 nm) and independent variables (A: light cycle and light intensity, B: temperature and light cycle, C: temperature and light intensity).

Table 20

ANOVA of the model for municipal wastewater

Source	DF	Adj SS	Adj MS	F-value	*p*-value
Model	9	10061.6	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
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		
Cor Total	14	10230.7			

Fig. 10 shows that the forecasted outcomes and observed data points 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 be 0.9971, which substantiates a strong correlation with the experimental data. Additionally, the R^2adj 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. Using experimentally obtained values, Design Expert Software conducted statistical analysis, as demonstrated below:

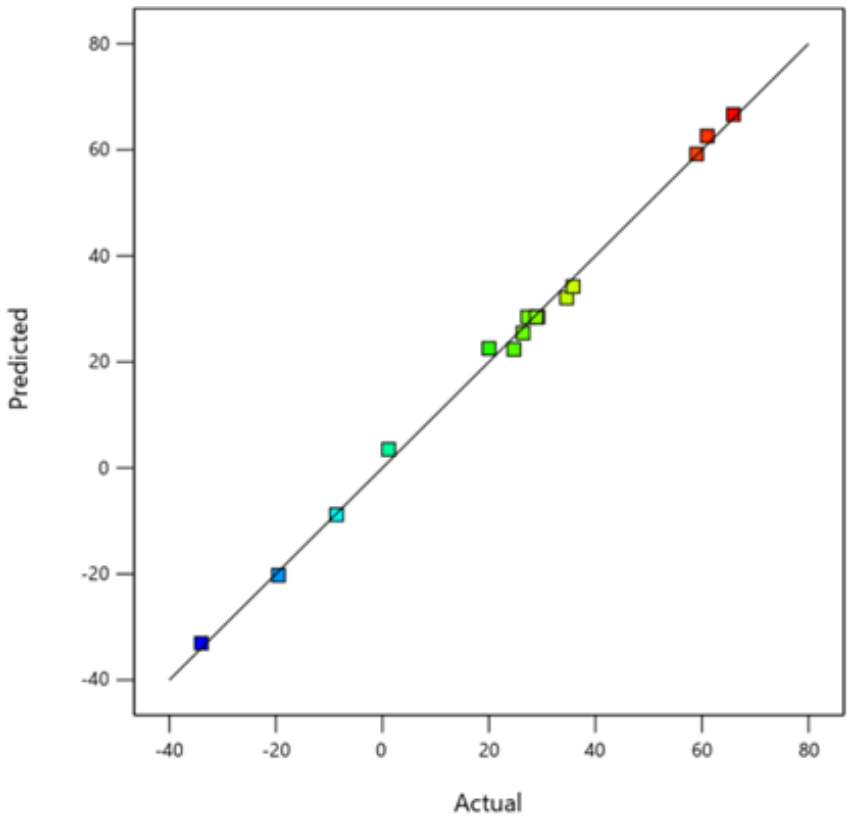
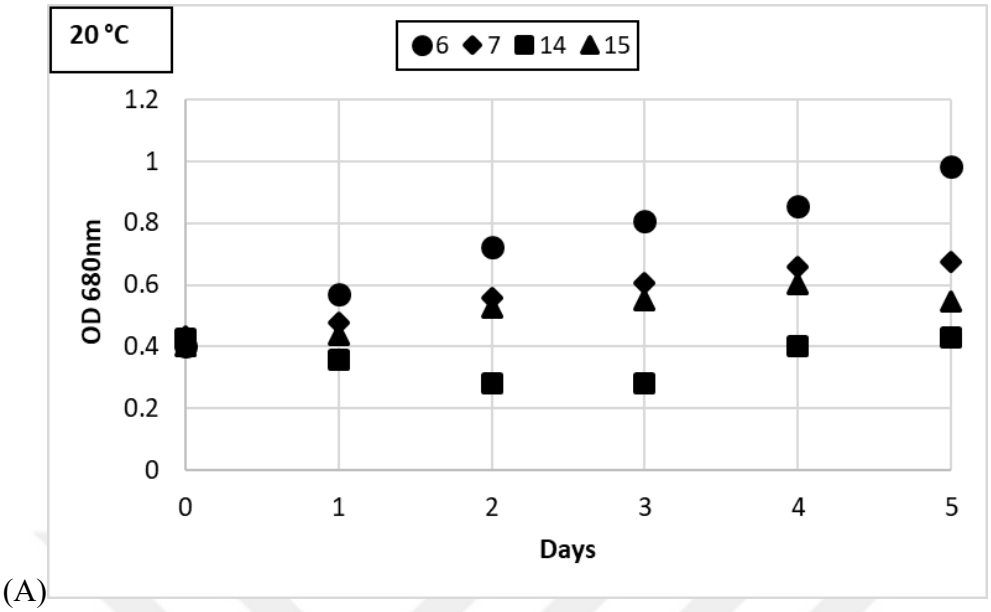
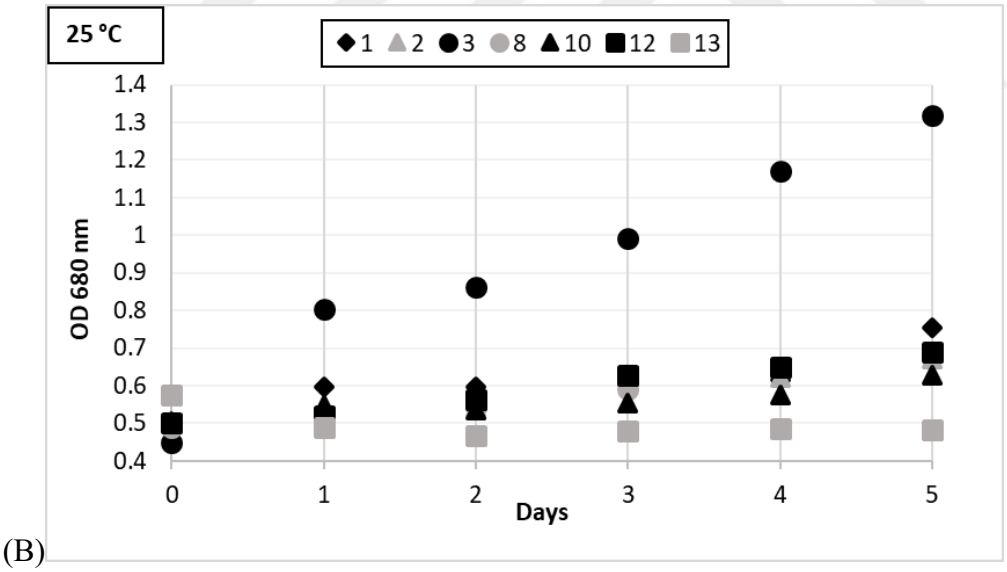


Fig. 10. Predicted and actual values of biomass production.

Fig. 11(A, B, C) 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. Maximum biomass production of microalgae occurred in reactors 3, 5, and 6, which shared the common characteristic of having the maximum light intensity of 8000 lux. In contrast, no significant biomass growth was observed in reactors 11 and 13, where the common factor was the shortest light duration of 6 hours. These results suggest that both light duration and intensity are important in promoting microalgae biomass growth.



(A)



(B)

Fig. 11 continued.

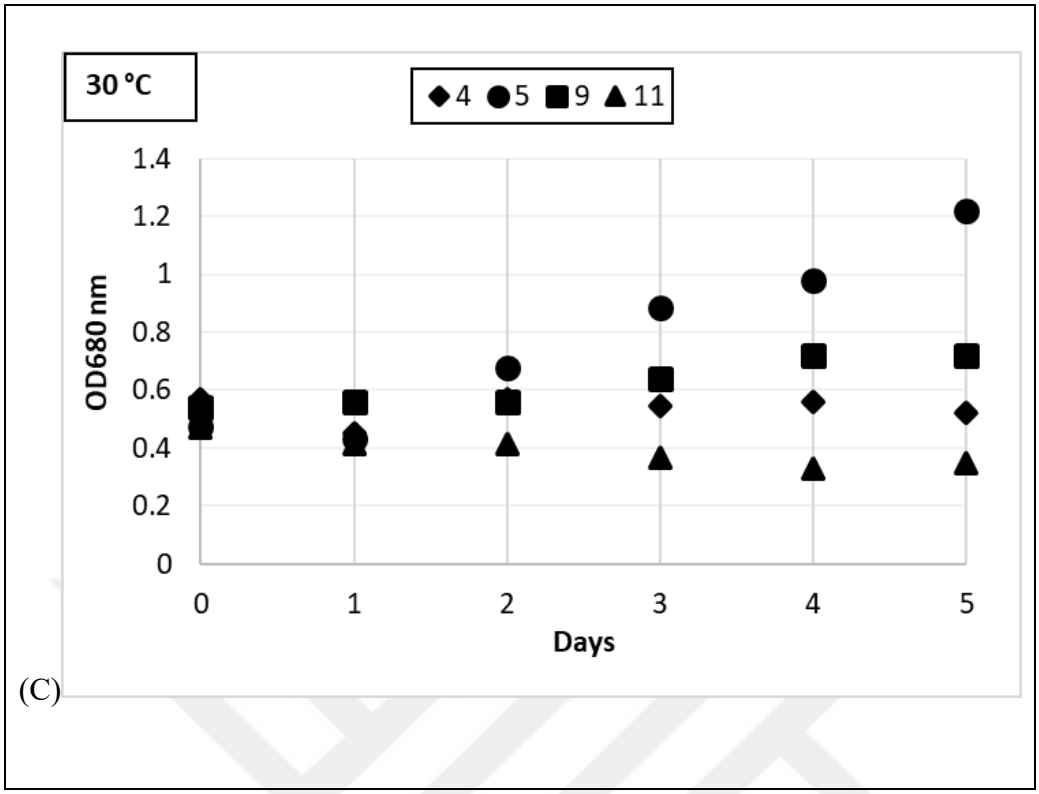


Fig. 11. Changes in optical density at 680 nm across 15 reactors as determined by response surface methodology during the operational phase ((A) 20 °C; (B) 25 °C; (C) 30 °C).

4.1.4. Evaluation with Decision Tree

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, with the findings displayed in Fig. 12.

The decision tree shown in Fig. 12 highlights the influence of various independent variables on *Nannochloropsis sp.* biomass increase. 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 (Barten et al., 2022), 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 lux and the light cycle surpassed 19.5 hours. This result aligns well with the optimal conditions established through RSM optimization for microalgal biomass growth, specifically a 21-hour light cycle and 8000 lux light intensity. In the reactor where biomass growth does not occur, light intensity is below 6500 lux, and the light cycle is below 10.5 hours. This result clearly demonstrates the critical role of light intensity and light cycle in microalgae biomass growth. RSM graphs also support these results.

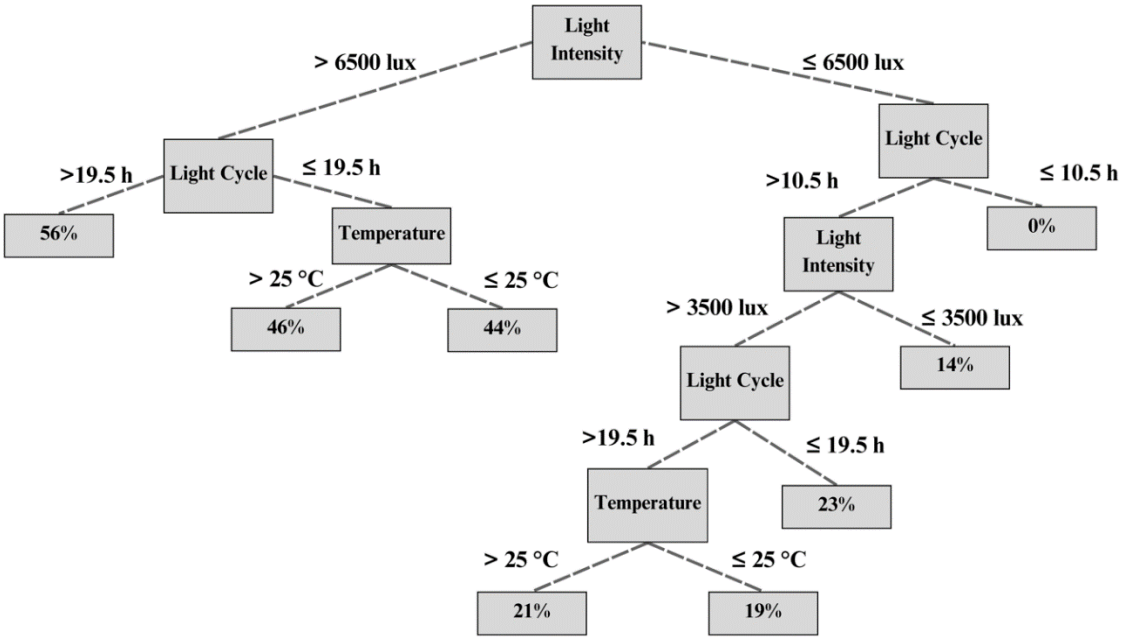


Fig. 12. Decision tree model with independent variables affecting *Nannochloropsis sp.* biomass growth.

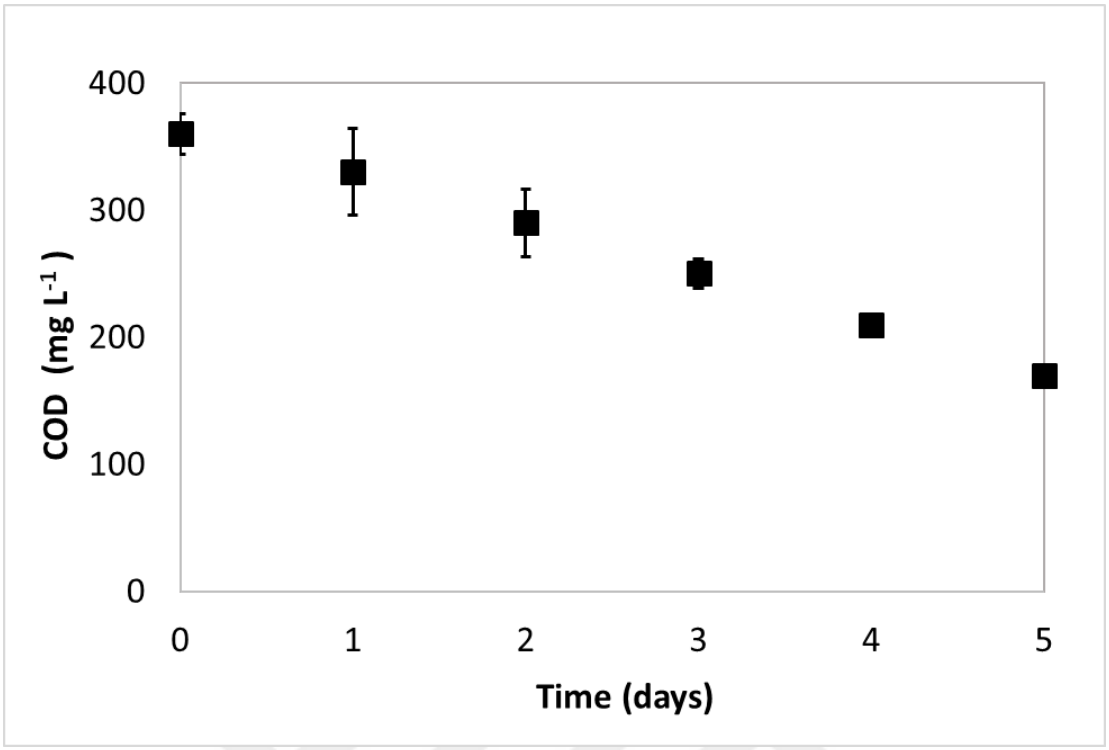
Advanced process control technologies are required for efficient removal of pollutants from municipal wastewater. ML can estimate wastewater treatment systems process costs (Torregrossa et al., 2018), reduce energy consumption (Zhao et al., 2012), and improve operational efficiency. The development of such plants requires the interaction of different disciplines such as statistics, computer science, and biotechnology (Sundui et al., 2021). 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 (Oruganti et al., 2023; Singh and Mishra, 2022), but there is no decision tree application adapted for specific microalgae/wastewater combinations yet.

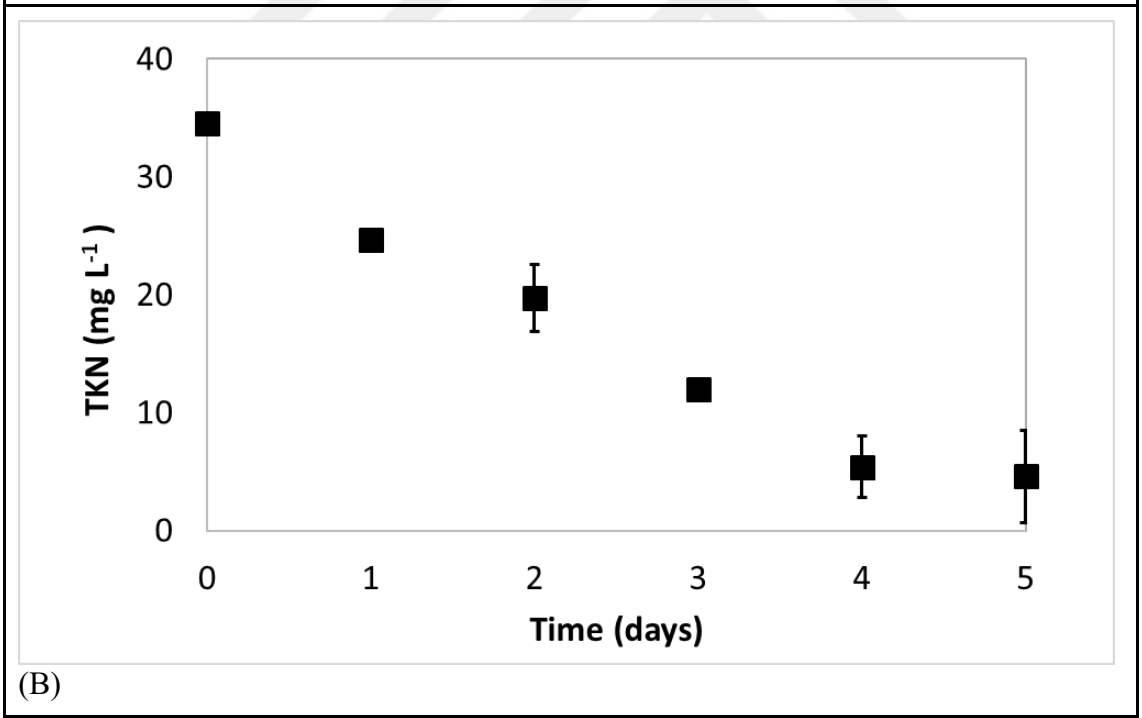
4.1.5. Carbon and Nutrient Removal Performance

The current study explores the ability of *Nannochloropsis sp.* removing carbon and nutrients from municipal wastewater. The time-dependent removal of COD, TKN, Nitrate and Ortho-P in the reactor operated under optimal conditions (21-hour light cycle, 8000 lux light intensity, and a temperature of 30°C) is presented in Fig. 13. In this figure, 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), 87% TKN removal (B), Nitrate (C), 70% Ortho-P removal (D), and 56% OD680 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 (Kim et al., 2014; Wijayanti and Ansori, 2021). Other sources of wastewater include palm oil mills (Resdi et al., 2021), the pharmaceutical industry (Adenigba et al., 2020), the dairy industry (Gurumoorthy and Saravanan, 2016), the pulp and paper industry (Polishchuk et al., 2015), and municipal wastewater (Gupta et al., 2016b). Among the studies focusing on domestic wastewater, only a limited number have specifically targeted nutrient and carbon removal (Şirin and Sillanpää, 2015; Gupta et al., 2016b). 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.

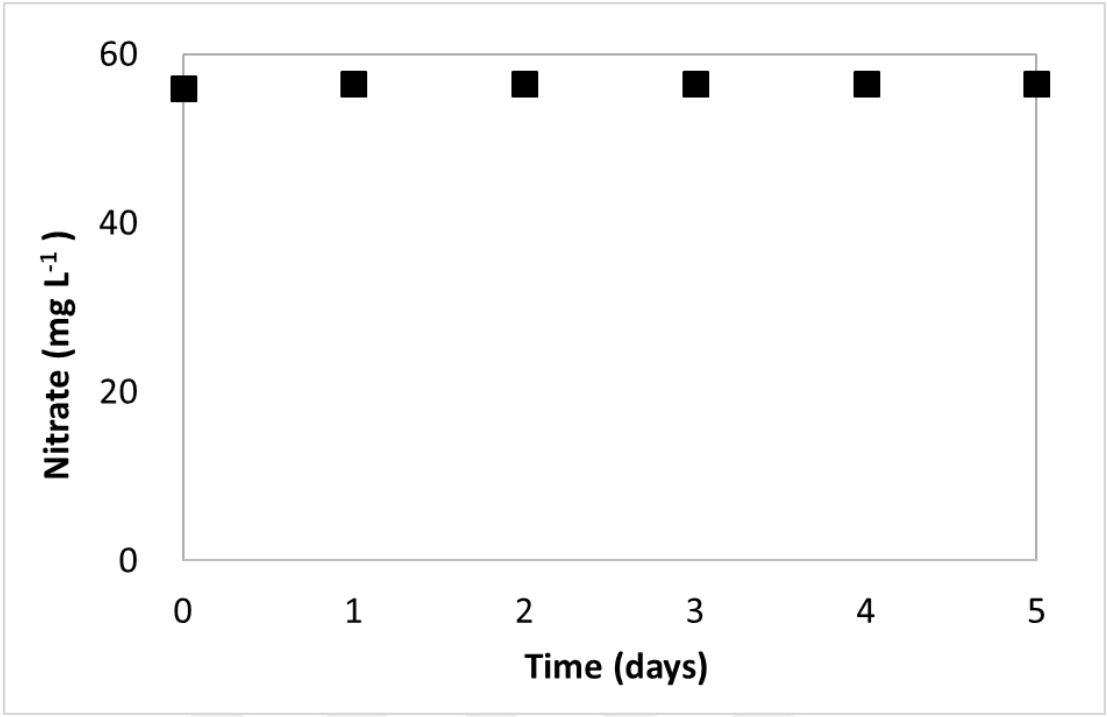


(A)

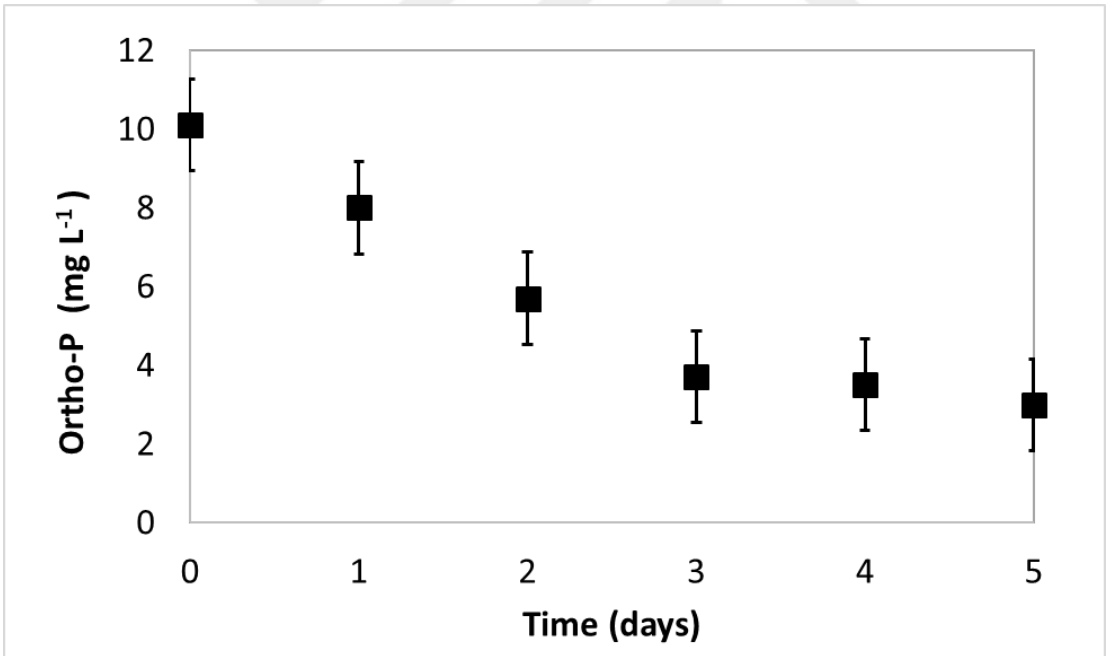


(B)

Fig. 13 continued.

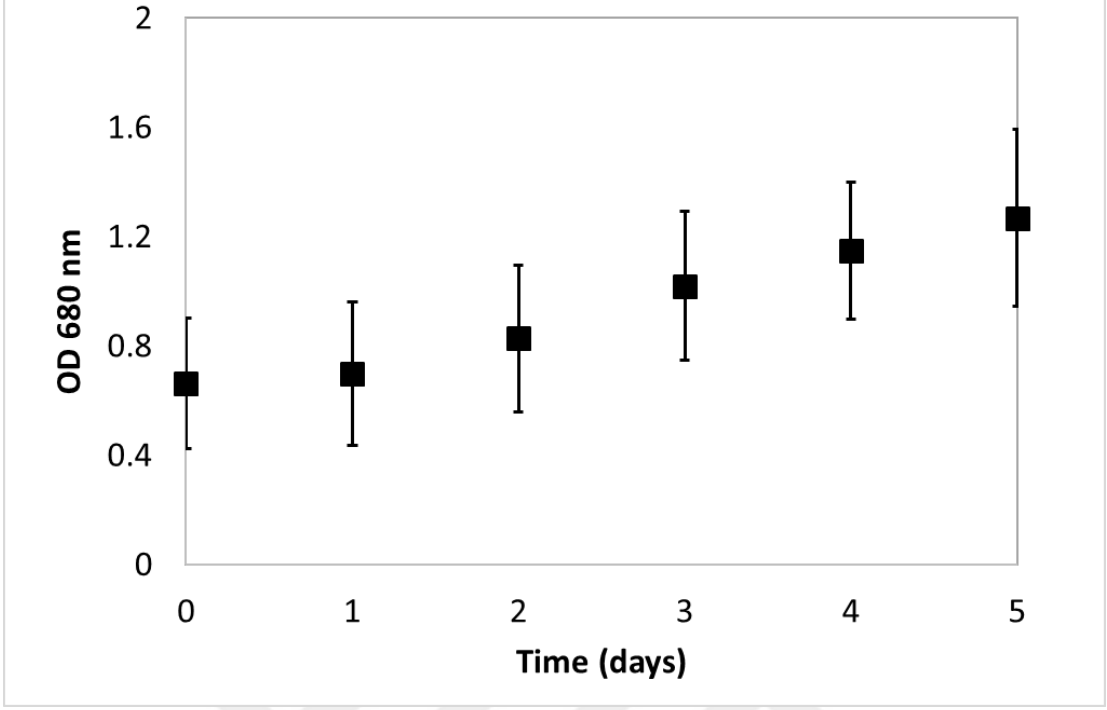


(C)



(D)

Fig. 13 continued.



(E)

Fig. 13. Time-dependent concentration changes of pollution parameters and biomass growth of microalgae.

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 (2020) showed that this hybrid approach optimizes microalgae dewatering processes, increasing efficiency and reducing costs. It enables the creation of forecasting models capable of adjusting to variations in environmental parameters, which offers significant advantages in full-scale applications.

4.1.6. Chlorophyll-a

Chlorophylls are key pigments in photosynthesis, capturing light, transferring energy, and converting it into chemical energy (Hosikian et al., 2010). Through this process, chlorophyll enables plants, including microalgae, to produce the energy they need for growth

and survival. Unlike higher plants, marine microalgae exhibit specific biochemical characteristics that make them particularly efficient in this process (Rasmussen and Morrissey, 2007).

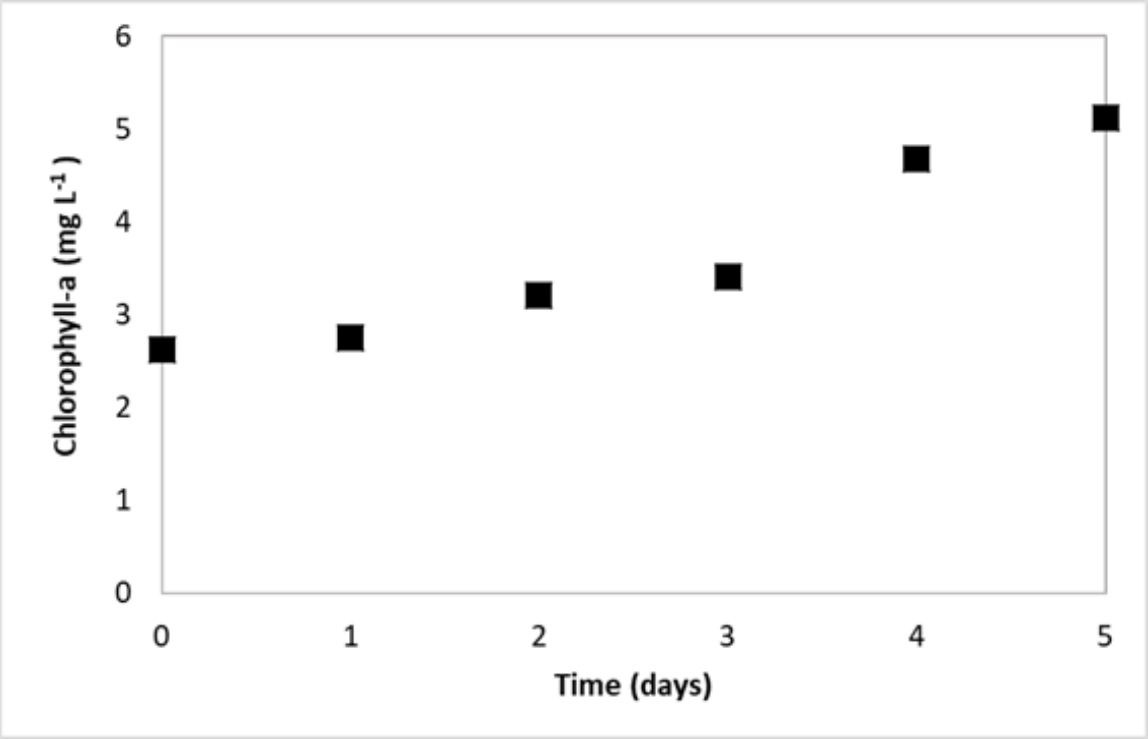


Fig. 14. Time-dependent changes in chlorophyll-a concentration of *Nannochloropsis sp.* in municipal wastewater under optimal conditions.

Fig. 14 shows the chlorophyll-a values of microalgae-municipal wastewater reactors over a period of 5 days under ideal conditions. 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.

4.2. Treatment of Cheese Whey Wastewater with Microalgae

4.2.1. Bibliometric Analysis

A bibliometric analysis was conducted to examine studies involving the combined use of microalgae and CWW. The search was performed using the author keywords “microalgae”, “wastewater treatment”, “cheese whey wastewater”, or “dairy wastewater”. All studies published up to 2025 were included in the analysis. Only documents classified as articles were considered, while all other document types were excluded. Furthermore, only publications indexed in the resources indexed in SCI-Expanded and ESCI were taken into account, and the language filter was set to English. As a result, a total of 487 publications were identified (link).

To further investigate the application of optimization techniques in this field, an additional search was conducted using the term optimization in all fields, yielding 55 relevant publications (link). When the term RSM was used instead, only 28 articles were found (link). Interestingly, a search for the term decision tree within this context yielded no publications (link). These findings show that although researchers have looked into combining microalgae with CWW or dairy wastewater, there hasn't been much use of optimization techniques like RSM. Additionally, the lack of studies using decision tree methods point to a big gap in the current research. This situation clearly underscores the novelty and originality of the present study.

Fig. 15 presents the overlay visualization derived from author keywords using a co-occurrence analysis using VOSviewer. The term “wastewater treatment” emerged as the most prominent and central keyword, indicating its strong co-occurrence with a wide range of other terms in the field. Other frequently associated keywords include “*Chlorella vulgaris*”, “nitrogen removal”, “biosorption”, and “microalgae harvesting”, reflecting the core focus areas in microalgae-based treatment systems.

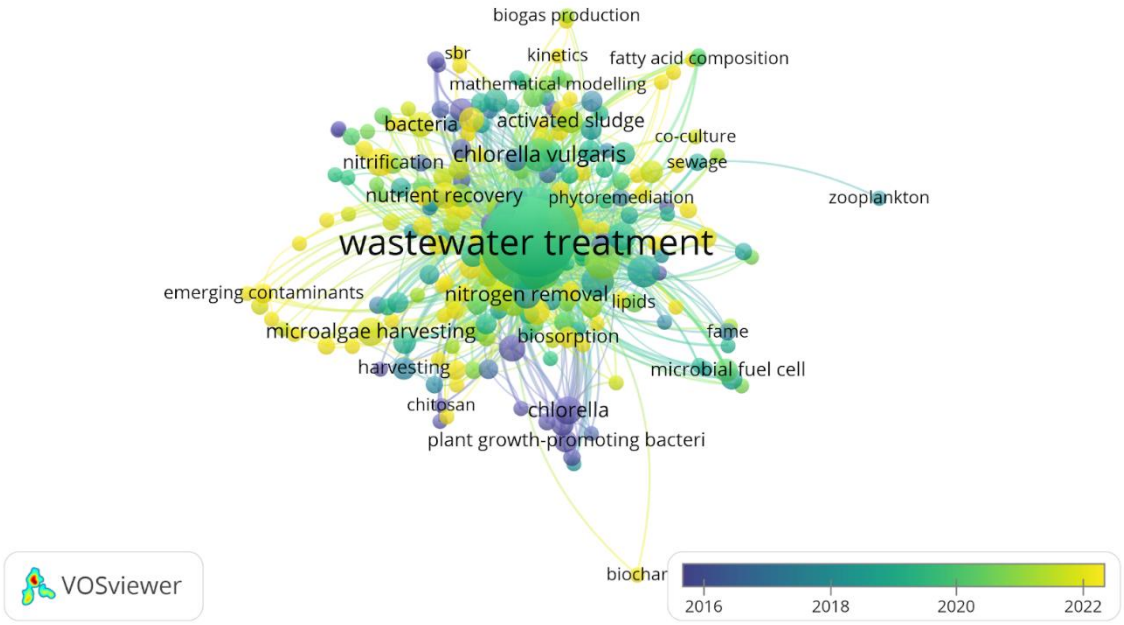


Fig. 15. Overlay network of author keywords in microalgae applications for cheese whey (dairy) wastewater treatment.

Fig. 15 displays earlier publications in purple and more recent studies in yellow. Notably, keywords such as "*chlorella*", "chitosan", and "biosorption" are associated with earlier studies (2016–2018), suggesting that these topics have been extensively explored in the past. In contrast, terms like "emerging contaminants", "biochar", "plant growth-promoting bacteria", and "microalgae harvesting" appear in yellow, indicating a shift in research interest towards more recent and emerging themes. This trend highlights an increasing focus on advanced treatment applications, resource recovery, and integrated environmental solutions recently.

Overall, the visualization reveals not only the dominant themes in the literature but also the transition toward more sustainable and innovative approaches in microalgae-based wastewater treatment. These insights are instrumental in identifying research gaps and aligning future studies with current scientific and environmental priorities.

4.2.2. Preliminary Studies

CWW was not used raw in this study due to its high organic content. However, preliminary studies were conducted to determine the highest COD concentration that could be used with microalgae. Within the scope of these studies, *Nannochloropsis sp.* VS concentration was kept constant (2600 mgVS/L) and CWW concentrations of 1000-20000 mgCOD/L were tested. These reactors were operated under 24-hour light, at room temperature, with continuous mixing, and in 100 ml volume reactors. Daily changes in microalgae biomass were monitored for four days (at 680 nm using a DR spectrophotometer). The obtained data are presented in Fig. 16. By the conclusion of the third day, no growth was observed at COD concentrations greater than 8000 mg/L. Therefore, it was decided to use a CWW concentration of 8000 mgCOD/L in subsequent experiments.

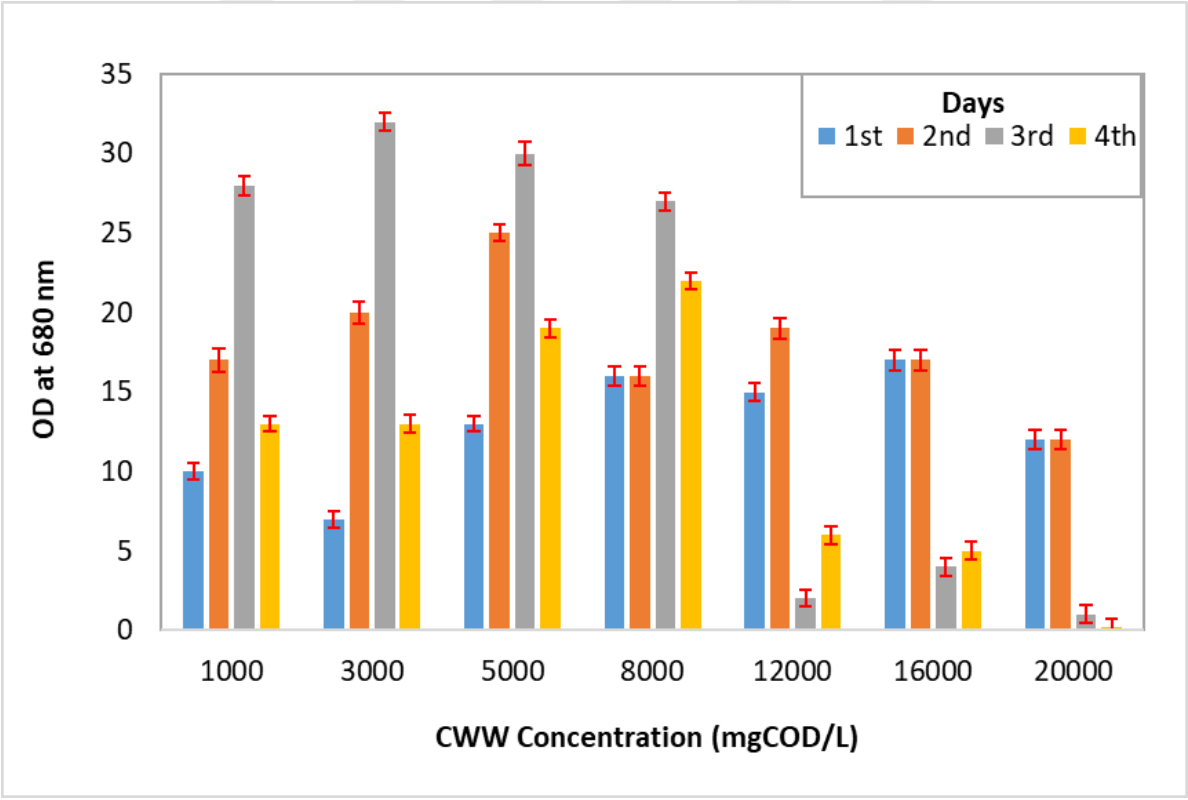
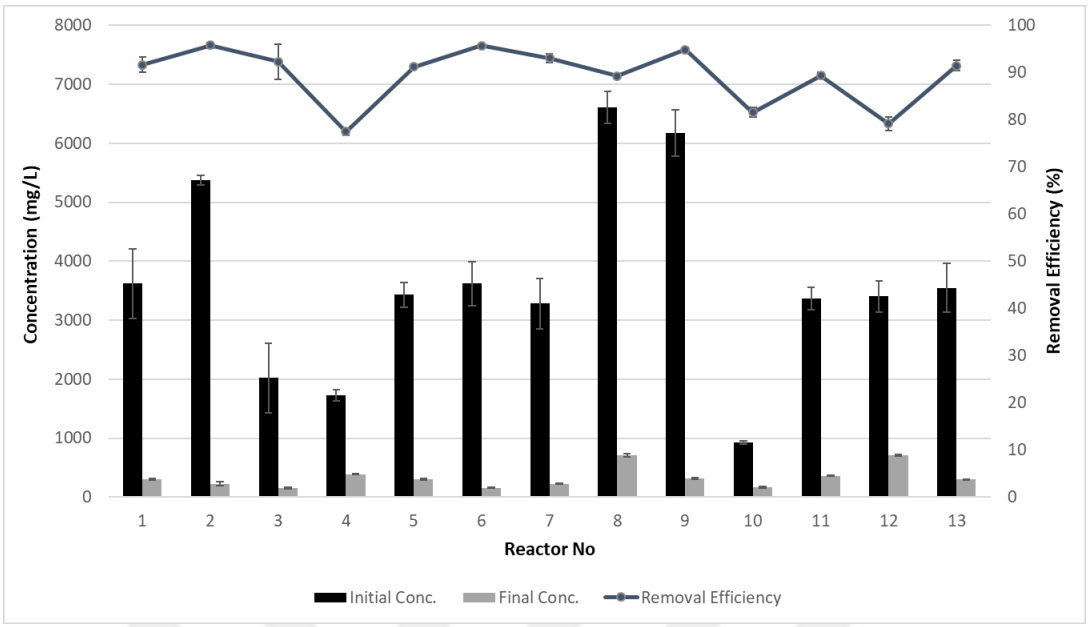


Fig. 16. Time-dependent changes in microalgae biomass with cheese whey wastewater ranging from 1000 to 20,000 mg/L.

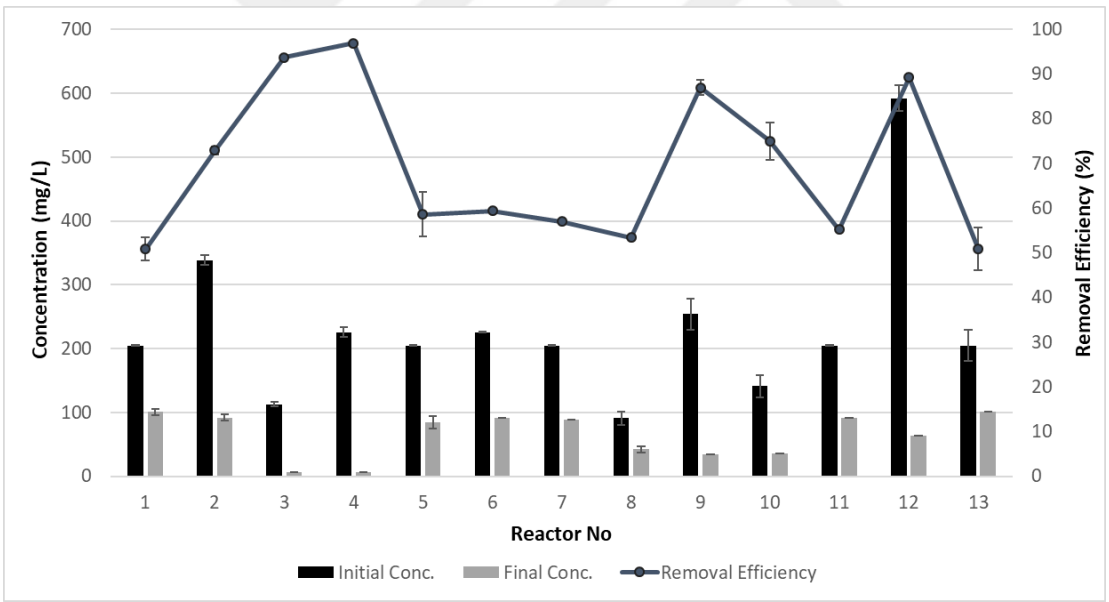
4.2.3. Optimization with RSM

Optimizing microalgae-based treatment systems in terms of concentration is essential for achieving efficient operation and maximizing treatment performance. Preliminary studies were carried out. A wide range of CWW COD concentrations was determined (1000 mgCOD/L-20000 mgCOD/L) and microalgae reactors were operated with COD values in this range. The reactors with the best microalgae growth were determined to be 5000 mgCOD/L and 8000 mgCOD/L. The concentration of 8000 mg/L was selected for the experimental setup in this research. Minitab software then generated concentrations for 13 reactors. Table 17 provides comprehensive information on the operational parameters of the reactors. Each reactor was operated according to the specific combination of CWW and *Nannochloropsis sp.* concentrations recommended in the design. Measured parameters included organic content, and nutrient concentrations were monitored regularly during the reactor operation.

Fig. 17 illustrates how effectively the reactors removed COD, TKN, and Ortho-P. The results indicate that carbon removal is prominent in the reactors. This finding is explained by the fact that as a mixotrophic species, *Nannochloropsis sp.* is capable of utilizing organic carbon for its metabolic needs. Additionally, the influence of microbial contamination on overall removal performance in the reactors was tested. In this context, wastewater sterilization was applied, and the removal efficiencies in sterilized and unsterilized reactors were compared. Analysis revealed no meaningful variation was observed in microalgal biomass production or pollutant reduction capacity between them. Therefore, sterilization, an additional process, was not included in the experimental setup.

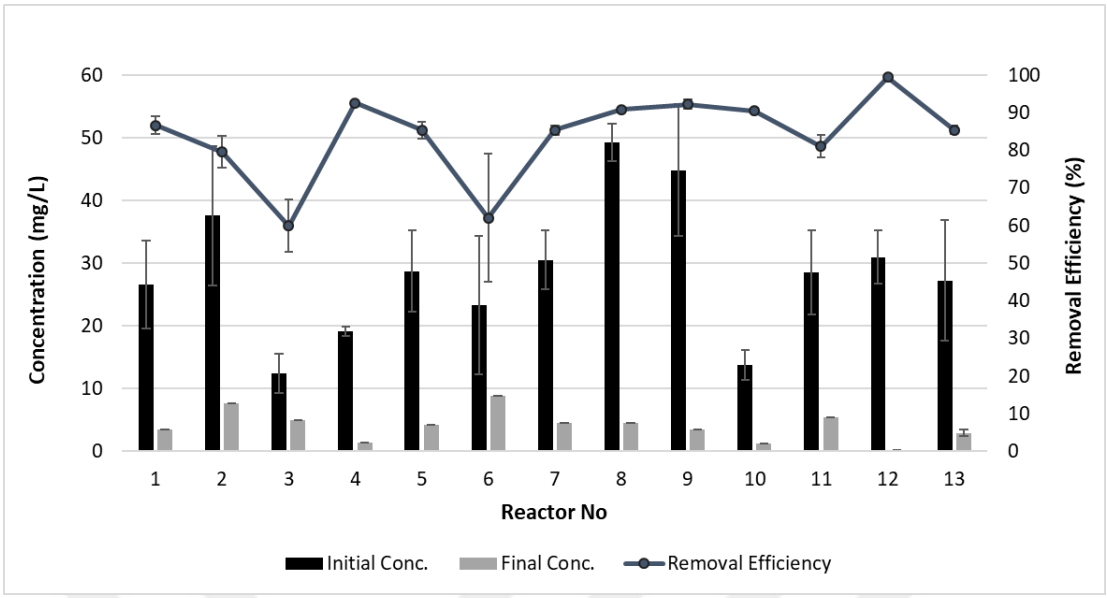


(A)



(B)

Fig. 17 continued.



(C)

Fig. 17. The temporal trends and treatment performances of COD (A), TKN (B), and Ortho-P (C) in various reactors using CWW and *Nannochloropsis sp.*

Fig. 18 shows 3D surface graphs of target parameters removal in reactors containing *Nannochloropsis sp.* and CWW. The removal of these parameters is 77-96%, 95-98%, 51-97%, and 60-99% for COD, TKN, and Ortho-P, respectively. In light of previous studies, carbon removal increases as the COD concentration of CWW increases.

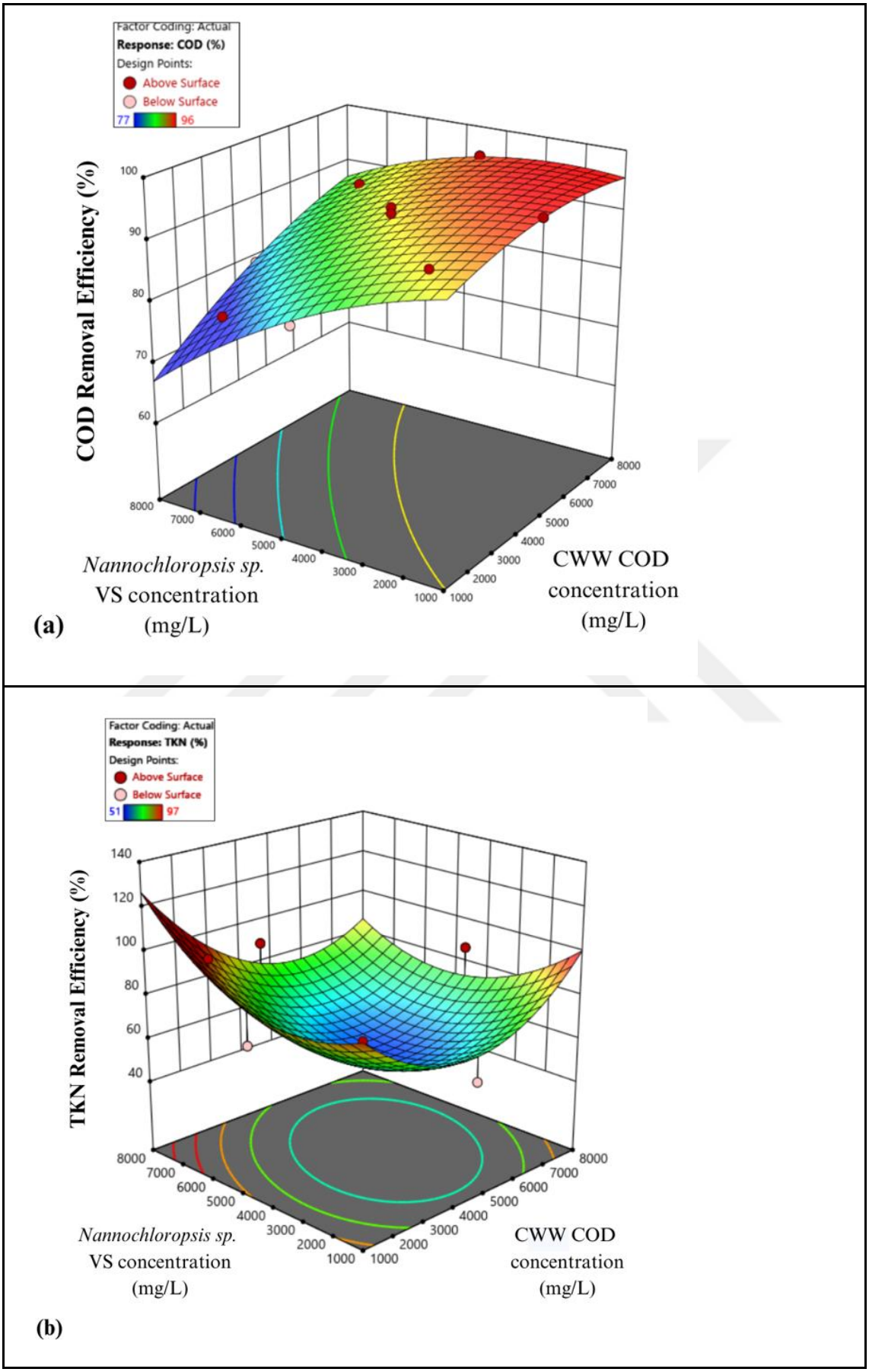


Fig. 18 continued.

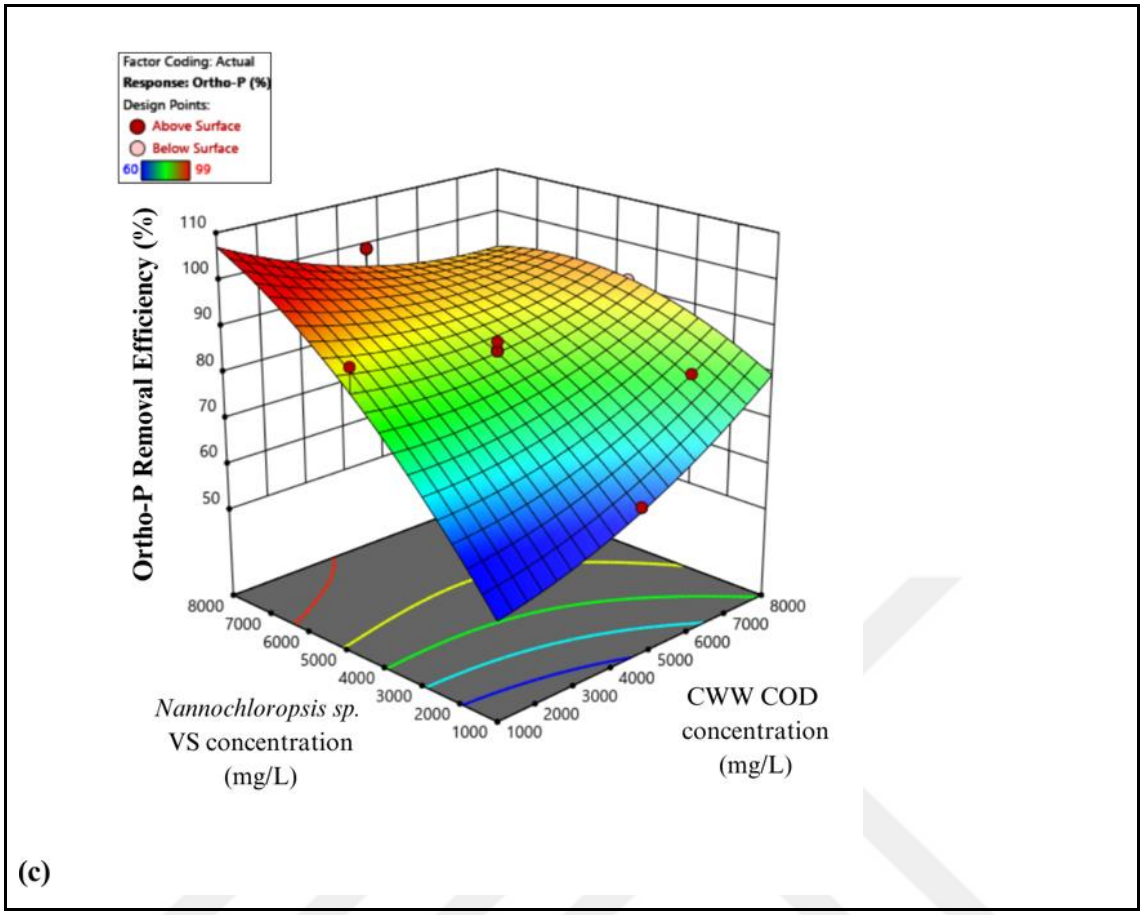


Fig. 18. 3D surface plots of *Nannochloropsis sp.*-CWW reactor COD (a), TKN (b), and Ortho-P (c) removal rates.

Fig. 19 shows how the real and expected removal rates of the parameters in reactors with *Nannochloropsis sp.* and CWW match up. In these graphs, values aligning along the same linear line indicate a consistency between the experimental and model-predicted values. Fig. 19 provides graphs that show a concentration of values along the linear line, with the exception of the TKN graph. The R^2 values supporting this result were found to be approximately 0.97, 0.63, and 0.92 for COD, TKN, and ortho-P, respectively. These R^2 values show how well the model matches the experimental studies, and it has been found that the TKN values are lower than the others. This result means that, overall, the TKN parameter in biological systems has lower R^2 values than COD and is harder to predict (Wang et al., 2020). If the R^2 value is higher than 0.5, the model can be considered acceptably accurate for microalgae-based wastewater treatment studies (Padri et al., 2020). RSM optimization is useful for planning experiments and is dependable for many factors, but it has some drawbacks in completely understanding the complicated and nonlinear relationships between those factors (Raj et al., 2021; İmamoğlu, 2024).

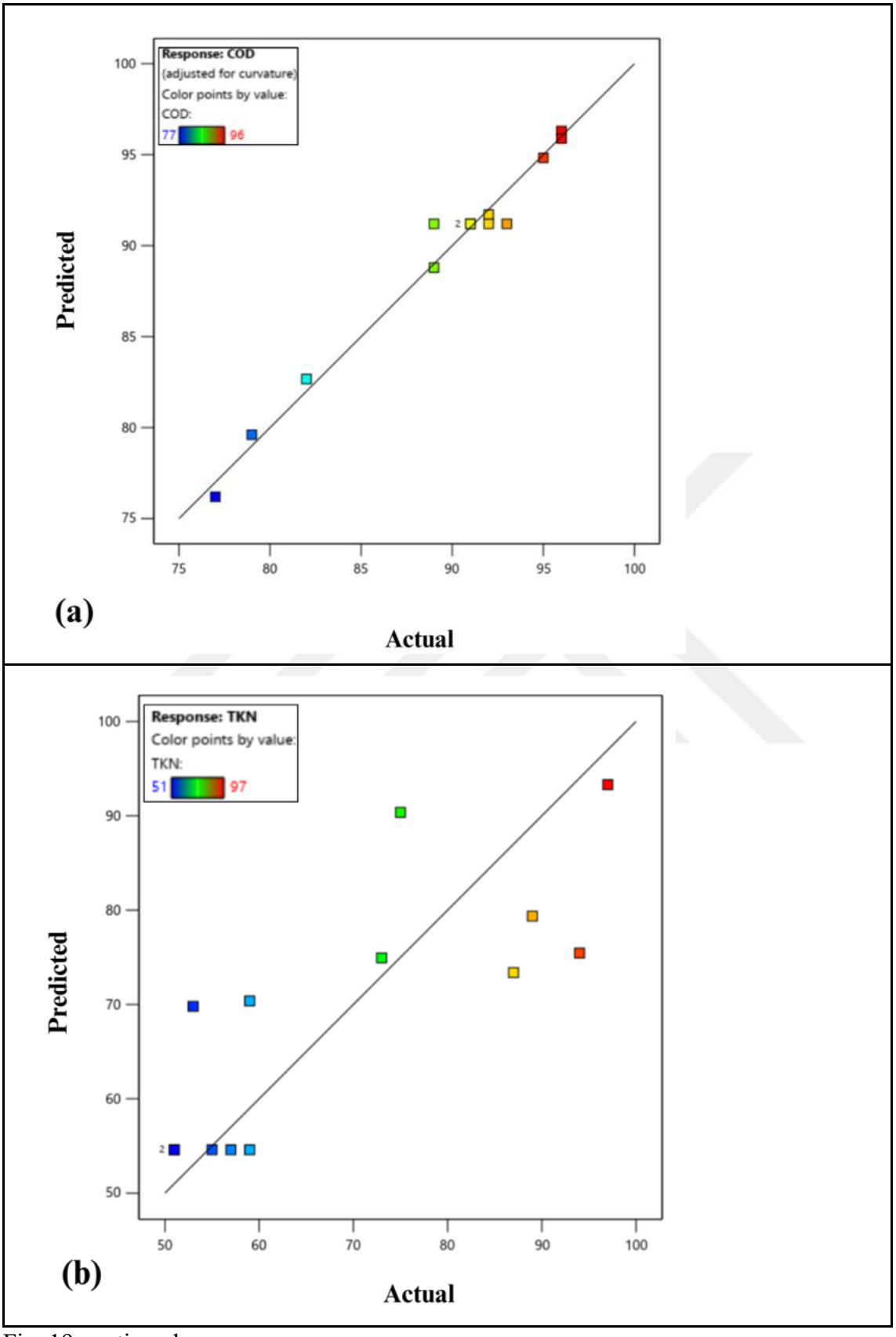


Fig. 19 continued.

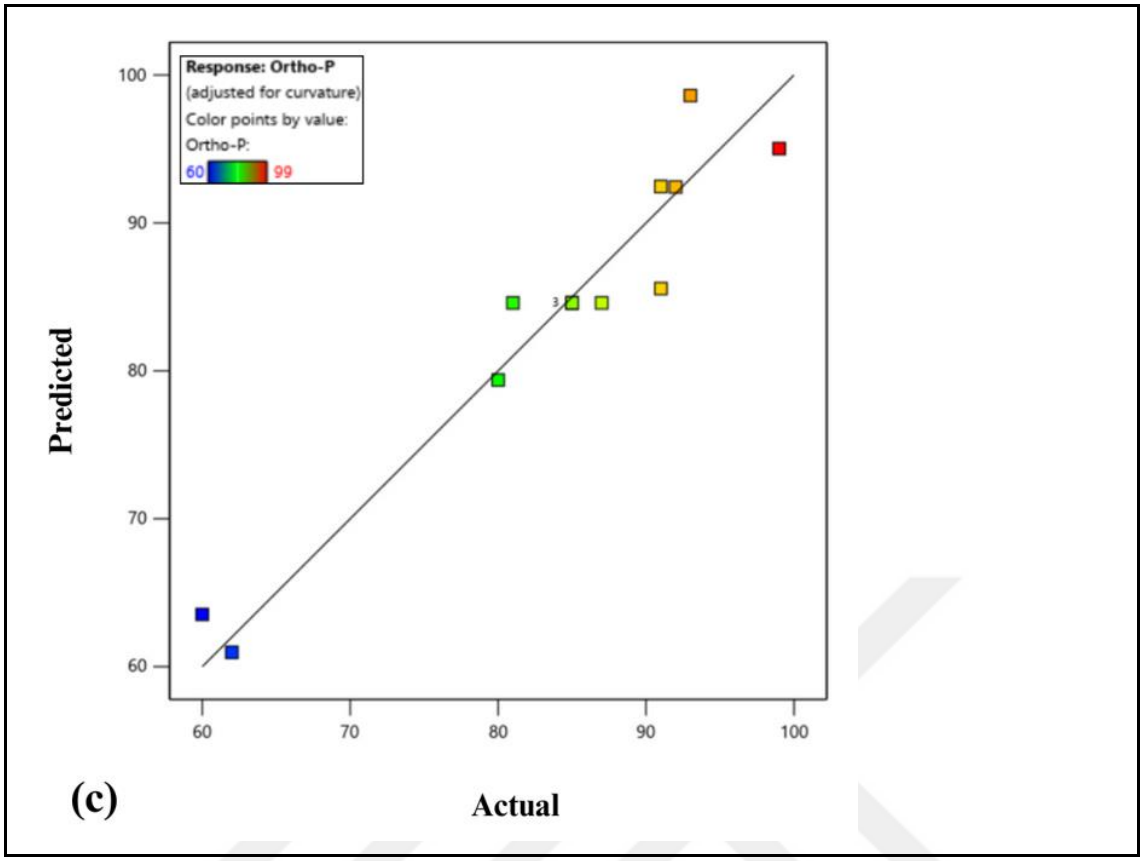


Fig. 19. Comparison between predicted and actual removal efficiencies for (a) COD, (b) TKN, and (c) Ortho-P.

Table 21 presents a comprehensive overview of the ANOVA findings and correlation indicators related to the developed predictive model. The lack-of-fit test provides more information about the model's suitability. To determine how well the model fits the data, you look at the lack-of-fit value in the ANOVA table. A value higher than 0.05 indicates that the model fits the data well. This suitability applies to COD and Ortho-P in batch reactors operated with CWW. Another model value is the p-value. This value questions whether the model is meaningful. If this value is less than 0.05, it indicates that the model is significant. In this way, the effect of the independent variables are determined. Here too, it is concluded that this value is significant for COD and ortho-P. This model needs to be developed for TKN. Consistent with the standards of statistical reliability, p-values below 0.05 were observed for all parameters except TKN, indicating that the model predictions were statistically significant at a 95% confidence level. The observed exception for the TKN parameter indicates potential model inadequacies in predicting nitrogen dynamics, a phenomenon frequently reported in the literature due to the complex biological

transformations associated with nitrogen removal processes (Padri et al., 2020). The distinction between R^2 and predicted R^2 values is noteworthy; while R^2 measures the proportion of variance explained by the model within the sample data, predicted R^2 assesses how well the model is expected to perform when applied to new, independent datasets. In this context, the slightly lower predicted R^2 compared to the R^2 value suggests a reasonable but not overly optimistic model fit, which aligns with best practices in predictive modeling to avoid overfitting.

Table 21

ANOVA of the model responses for CWW

	COD	*TKN*	*Ortho-P*
Model			
Sum of Squares	456.54	2289.68	1452.67
Df	5	5	5
Mean Square	91.31	457.94	290.53
F-value	60.68	2.39	18.12
p-value	< 0.01	0.14	0.01
Std. Dev.	1.23	13.83	4
R^2	0.97	0.63	0.92
R^2 *(adjusted)*	0.96	0.36	0.87
R^2 *(predicted)*	0.94	-1.54	0.56
Lack of fit	0.85	0.01	0.05

The forecasting models for the removal of COD (Equation 4.2), TKN (Equation 4.3), and Ortho-P (Equation 4.4) were given, respectively, as follows:

$$\text{COD \%} = 90.40 + 0.002171*CWW - 0.001215*MA \quad (4.2)$$

$$\text{TKN \%} = 119.5 - 0.01819*CWW - 0.00959*MA + 0.000002*CWW*CWW + 0.000002*MA*MA - 0.000001*CWW*MA \quad (4.3)$$

$$\text{Ortho-P \%} = 35.90 + 0.00205*CWW + 0.01376*MA + - 0.000001*MA*MA - 0.000001*CWW*MA \quad (4.4)$$

* MA: *Nannochloropsis sp.* VS concentration

Response optimization of pollutant parameters was performed using Minitab. Based on the experimental design and statistical analysis, the optimum concentration for CWW was 8000 mg COD/L and 2200 mg VS/L for *Nannochloropsis*. A graph showing the desirability values (d) for each parameter is presented in Fig. 20. The desirability values were determined to be 0.99 for COD, 0.78 for TKN, and 0.63 for Ortho-P. It is clear that carbon removal has a higher desirability score. However, the desirability scores of 0.78 for TKN and 0.63 for Ortho-P indicate the system's ability to reduce these pollutants.

Throughout the optimization process, COD concentration in the influent CWW and the VS content of microalgae were considered as key variables influencing the removal efficiencies of all targeted pollutants. A key point to highlight is that further optimization of additional process elements may enhance the overall treatment performance and potentially improve the desirability scores for all response variables. Therefore, a comprehensive optimization strategy considering both biological and operational parameters could contribute to the further advancement of microalgae-driven wastewater treatment systems.

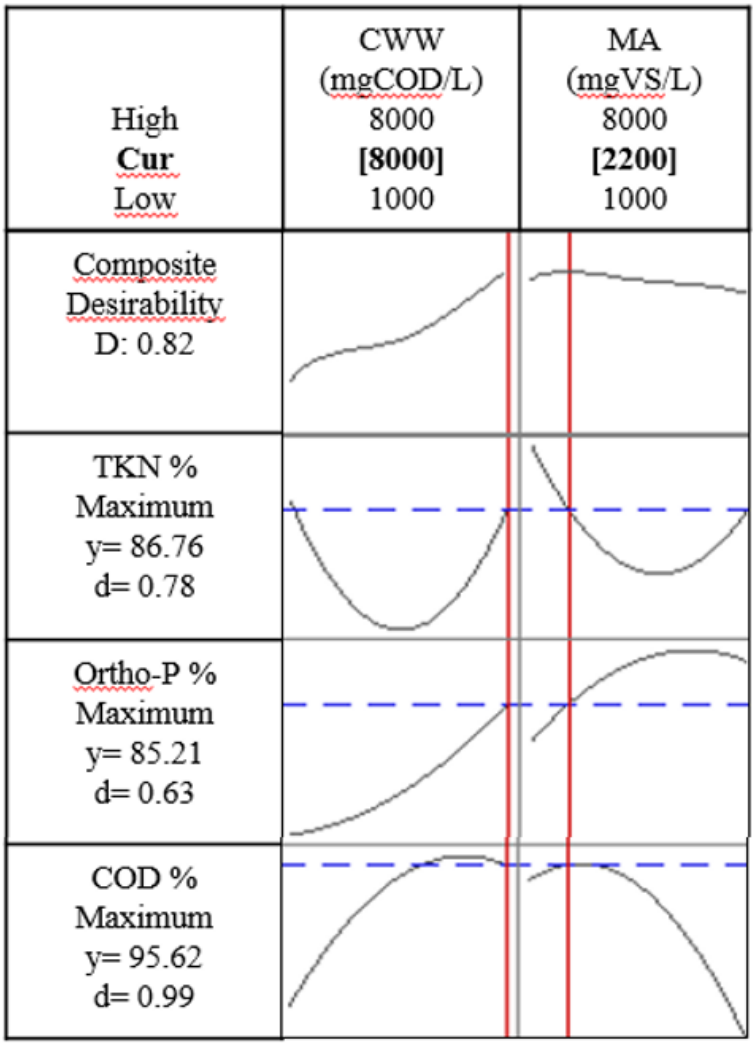


Fig. 20. Response optimization performed at the optimum condition to reach the highest objective.

The RSM model was created using data obtained from experimental reactor sets. Table 22 shows the relative error percentages (Equation 4.6) (Hossain et al., 2022) for the parameters using the RSM model, as well as the experimental and predicted %R (Equation 4.7) (Hossain et al., 2022) values. The table indicates that the model yields the smallest relative errors for Ortho-P and TKN.

$$\%R = (C_0\text{-}C_1)/C_0*100 \quad (4.6)$$

where;

C_0 represents the concentration of all parameters before treatment (mg/L).

C_1 indicates the concentration of all parameters after treatment (mg/L).

% R refers to the proportion of removal achieved for the targeted parameters.

Relative Error (%) = (*Yexp.*-*Yp.*)/*Y exp.**100 (4.7)

Table 22

Observed and estimated %R values for each parameter, along with their relative errors

Parameter	Experimental value (%R)	Predicted value (%R)	Relative Error (%)
COD	90.2 ± 1.3	105.09*	-16.51
TKN	66.7 ± 8.6	72.96	-9.38
Ortho-P	64.8 ± 2.9	60.13	7.2

*The maximum achievable COD removal rate is 100%.

In the present study, RSM, a technique frequently utilized in optimization research, was employed to identify key factors influencing pollutant removal. The results show that while carbon removal is dependent on CWW COD levels, nutrient removal is influenced by both CWW COD levels and microalgae VS concentration. Although RSM successfully identified the major influential parameters for carbon and nutrient removal, it demonstrated limitations in defining the precise critical concentration thresholds necessary for optimal performance.

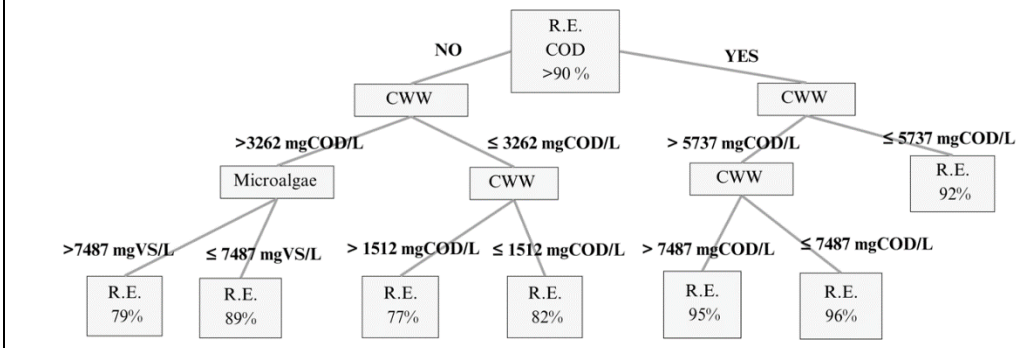
In contrast, the decision tree algorithm has the potential to provide a more detailed interpretation in determining the critical concentration levels of variables. This approach offered enhanced clarity regarding operational thresholds, which RSM alone could not determine. A similar methodology was reported by Sabour and Amiri (2017), where the application of RSM began with optimizing management of leachate effluents, and subsequently, the predictive capability and model accuracy were evaluated through the integration of an Artificial Neural Network (ANN) algorithm. Their results indicated that utilizing ANN following RSM significantly improved the model's predictive performance. Based on this precedent, the decision tree model was incorporated in the present study to

assess and validate the predictive accuracy of the RSM outcomes, thereby strengthening the reliability of the developed optimization model.

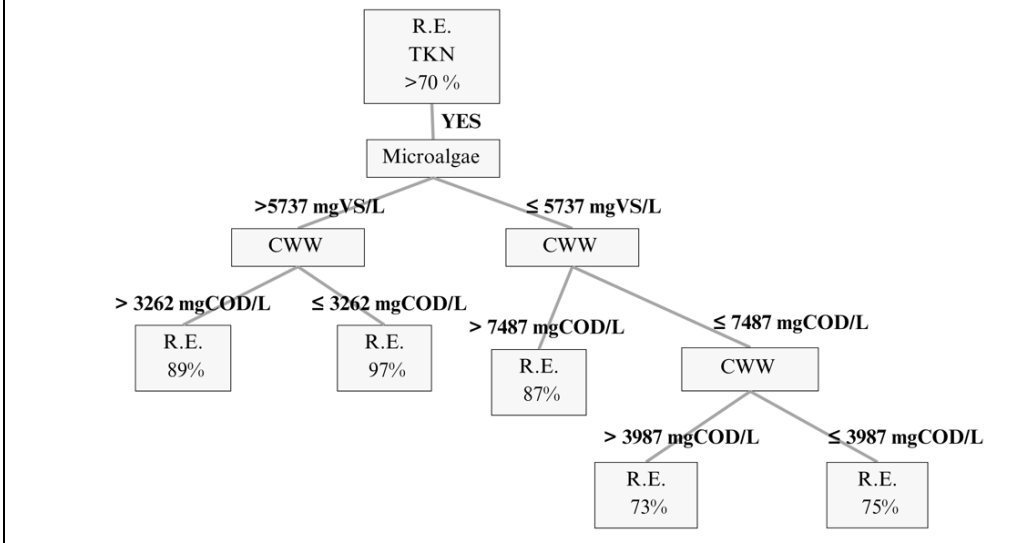
4.2.4. Evaluation with Decision Tree

RSM has certain drawbacks; hence, in this work, a decision tree has been utilized in addition to it to assess the model's accuracy and gather more information. By studying the interactions between variables in a hierarchical structure, decision trees can contribute to a more thorough examination of RSM's anticipated outcomes, as well as improve the model's dependability (Zhang and Wu, 2021).

Fig. 21 shows that in decision tree analysis, a study was performed to determine the most effective concentrations of *Nannochloropsis sp.* and CWW by evaluating the pollutant removal efficiencies achieved in the treatment system. In this analysis, CWW concentration and microalgal biomass concentration were tested as the critical variables influencing biomass growth. The model showed that COD removal reached its maximum (96%) at around 7500 mg COD/L CWW concentration, while the concentration of *Nannochloropsis sp.* showed minimal influence on COD removal under these conditions. In contrast, the trends in nutrient elimination differed significantly from those seen in carbon removal. Efficient nutrient elimination efficiency was positively influenced by lower CWW concentrations combined with higher *Nannochloropsis sp.* concentrations. Specifically, TKN removal peaked at 97% when the microalgae level surpassed 5700 mg VS/L while CWW dropped beneath 3250 mg COD/L. Ortho-phosphate removal exceeding 93% was linked to *Nannochloropsis sp.* levels higher than 5700 mg VS/L and CWW levels below 4500 mg COD/L.

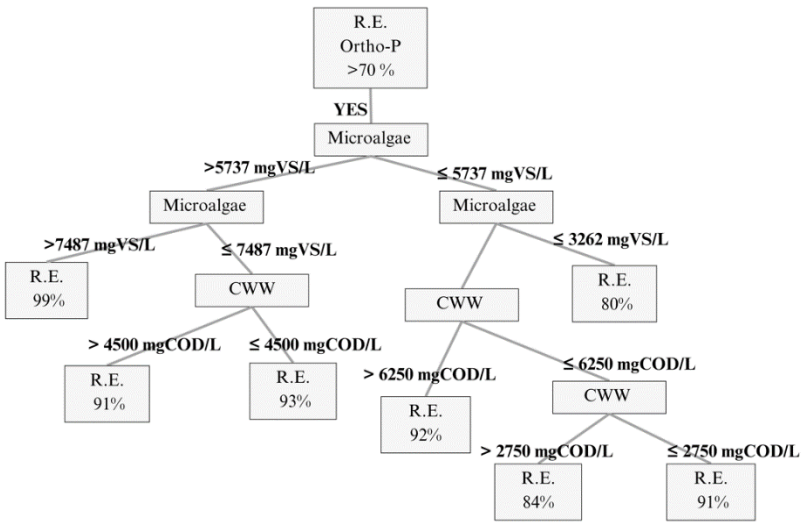


(A)



(B)

Fig. 21 continued.



(C)

Fig. 21. Decision trees showing the removal efficiencies of COD, TKN, and Ortho-P pollutant parameters (A, B, C), respectively

These findings demonstrate that, while carbon removal was predominantly governed by a single factor—CWW concentration—nutrient removal required the simultaneous optimization of both microalgal biomass and influent carbon concentrations. This divergence is explained by the distinct physiological pathways underlying each process: nutrient (nitrogen and phosphorus) removal in mixotrophic microalgae primarily relies on direct nutrient assimilation (Plouviez and Guieysse, 2023), whereas carbon removal is closely linked to photosynthetic activity and the availability of organic carbon sources (Saha and Murray, 2018).

Notably, microalgae such as *Nannochloropsis sp.* are capable of activating carbon removal pathways even at relatively low biomass concentrations (Khan et al., 2018), which supports the observed high carbon removal efficiencies in this study. While RSM analysis could identify influential parameters, it fell short in precisely determining the critical concentration thresholds required for nutrient removal. The decision tree approach, however,

successfully elucidated these concentration-dependent effects. In this system, where carbon removal efficiency was already high, the prominence of CWW concentration in the optimization results further confirmed its central role in driving carbon removal performance.

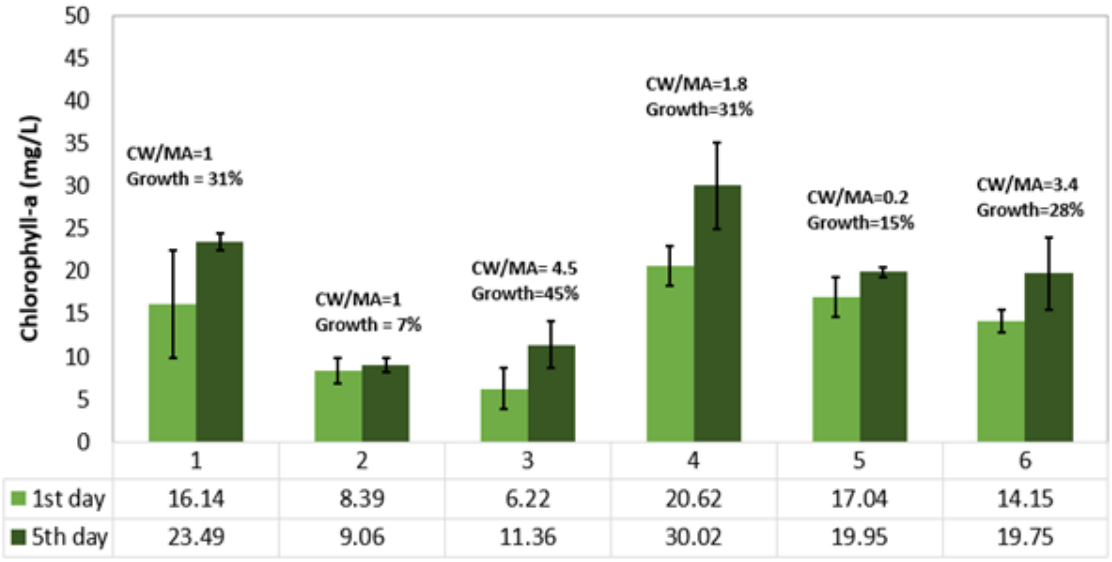
As mentioned earlier, environmental factors and working conditions significantly affect the pollution removal performance of microalgae. Determining the optimal values of these parameters correctly through various methodological approaches is thought to improve the general sensitivity and effectiveness of the treatment process. However, due to the complexity and time-consuming nature of experimentally evaluating each parameter, this study has adopted the previously reported optimal values for key operational conditions. Specifically, the illumination level (5000 lux) and thermal condition (24°C) parameters determined for *Nannochloropsis sp.* in the literature have been used as fixed parameters.

Focusing particularly on the impact of concentration variables, the present study has modeled and predicted the potential for efficient pollution removal by *Nannochloropsis sp.* cultivated in CWW. This method is quite different from the research done by Singh et al. (2023) because it looks at different types of wastewater, focuses on different goals, and uses a specific type of microalgae. The decision tree algorithm shows its benefits by offering organized solutions for treating wastewater with high organic content, which is usually tough to handle with standard methods. Additionally, it significantly reduces the time required to identify the most effective operational factors and presents a valuable guideline for the future management of other complex wastewater.

4.2.5. Chlorophyll-a

In this study, chlorophyll-a concentrations of samples taken from reactors were determined. In Fig. 22, a general increase in chlorophyll-a was observed in all reactors, but this increase varied depending on the CWW/MA ratio. The most significant rise was detected at a 4.5 ratio between CWW and MA, calculated from 4500 mgCOD/L CWW and 1000 mgVS/L MA. Notably, this finding aligns with the ratio corresponding to the concentrations

identified as optimal (CWW: 8000 mg COD/L; MA: 2202 mg VS/L), where the ratio of CWW to MA was nearly 3.63.



	1	2	3	4	5	6
1st day	16.14	8.39	6.22	20.62	17.04	14.15
5th day	23.49	9.06	11.36	30.02	19.95	19.75

Fig. 22. Chlorophyll-a concentrations in reactors (mg/L).

Regarding nutrient removal, our findings corroborated previous observations emphasizing the critical role of microalgae concentration in ammonia (TKN) removal (Yang et al., 2022). In reactors that received the same amount of wastewater (4500 mg COD/L), the removal of TKN increased a lot from 51% with 4500 mg VS/L of microalgae up to 89% as the microalgal concentration was elevated to 8000 mg VS/L, showing a clear link between the amount of microalgae and how well nitrogen is removed. Nevertheless, it should be emphasized that excessively low or excessive algal concentrations have been reported to limit ammonia removal efficiency (Yang et al., 2022).

Additionally, the influent CWW concentration was found to be a decisive factor: at a constant microalgae concentration (6975 mg VS/L), TKN removal efficiency reached 97% at a lower CWW concentration (2025 mg COD/L), but dropped to 53% at a higher CWW concentration (6975 mg COD/L). These results suggest that both the initial microalgae biomass and the influent wastewater strength must be carefully balanced to achieve optimal nitrogen removal.

4.2.6. Determination of Bioactivity *Nannochloropsis sp.* Biomass

Antimicrobial activities

The antibacterial activity of microalgae extracts and nanoparticle-supported formulations was tested using agar disk diffusion and broth microdilution. Pure *Nannochloropsis sp*. extract had little inhibitory effect on microorganisms. However, CWW-fed *Nannochloropsis sp.* extract exhibited stronger antibacterial activity. Microalgae-metal nanoparticle compositions exhibited the strongest antibacterial properties. These combinations are most effective against Gram-negative bacteria and yeast species. These findings imply that nanoparticles and microalgae-derived chemicals may be synergistic. Although they are less effective than conventional antibiotics and antifungals, they are a biotechnological alternative. Minimum inhibitory concentration (MIC) values obtained by broth microdilution demonstrate that nanoparticle-supported formulations exhibit greater antibacterial efficacy. Nanoparticles increase the surface area, facilitating contact with microorganisms.

Finally, formulations of nanoparticle-supported and CWW-fed *Nannochloropsis sp.* outperform pure microalgae extract in terms of antibacterial activity. These results suggest that microalgae supported by nanotechnology may serve as alternative antimicrobials.

Antioxidant activities

The DPPH free radical scavenging technique was used to measure antioxidant activity. The synthetic antioxidant reference molecule exhibited maximum antioxidant capacity at all doses. Among the natural samples, the *Nannochloropsis sp.* extract in the CWW medium scavenged DPPH the best. The antioxidant activity of this extract with nanoparticles has increased, demonstrating that nanoparticles can stabilize certain antioxidant components. However, pure *Nannochloropsis sp.* extract exhibited decreased antioxidant capacity at all doses. While microalgae-based natural antioxidants are less

effective than synthetic ones, *Nannochloropsis sp.*, when fed with CWW and its nanoparticle-supported variants, are a sustainable and natural source of antioxidants.

DNA Cleavage Activities

The DNA fragmentation test examined the effects of microalgae derivatives on DNA under hydrolytic and oxidative conditions. Not much DNA degradation occurred under hydrolytic conditions. This suggests that microalgae do not damage DNA. However, under oxidative stress, *Nannochloropsis sp.* extract supplemented with CWW was found to fragment DNA more effectively than the pure extract alone. This impact was amplified in nanoparticle-supported formulations, disrupting the DNA structure. This suggests that the extracts' phenolic chemicals and metal ions may oxidize DNA.

Ultimately, the *Nannochloropsis sp.* extract cultured in the CWW medium, as well as its nanoparticle-supported forms, degraded DNA more effectively. These findings demonstrate the potential for increasing the biological production of microalgal derivatives for biotechnological applications.

4.2.7. Determination of the Fatty Acid Profile of Microalgal Biomass

The fatty acid composition of microalgal biomass differed significantly between pure *Nannochloropsis sp.* culture and microalgal biomass cultivated with CWW. The total saturated fatty acid content increased from approximately 32% in the pure culture to 63% in the biomass cultivated in CWW. Similarly, monounsaturated fatty acids (MUFA) increased from 7.1% to 9%. In polyunsaturated fatty acids (PUFA), a notable increase was observed, rising from 39% in the pure microalgae culture to 72% in the microalgae culture cultivated with CWW. Furthermore, EPA and DHA, which were not detected in the pure microalgae culture, were detected in the biomass cultivated with CWW at 2.6% and 2.1%, respectively. This indicates that the biosynthesis of high-value omega-3 fatty acids increases under mixotrophic conditions. This situation demonstrates that feeding *Nannochloropsis sp.* with food industry wastewater creates potential for its use in the nutrition and industrial sectors.

4.3. Biogas Production by Using Microalgae as Co-digestion

4.3.1. Bibliometric Analysis

The present study delivers a thorough bibliometric investigation on related to microalgal utilization in AD systems, centering around global research trends, publication metrics, and technological advancements. The publications included in the bibliometric analysis were obtained through a structured search in the WoS database (link to dataset). Table 23 presents a summary of the top 10 most efficient sources in publishing literature focused on AD with microalgae. This summary includes the total citation count, sources, type of microalgae and co-digestion, and finally, the highest methane yield.

Table 23

The top 10 most cited articles on the role of microalgae in anaerobic digestion

Ranking	Citation	Source	Type of microalgae	Waste type use in the co-digestion	Highest Methane yield	References
1st	130	Bioresource technology	*Chlorella*	Waste sludge	468 mL/g VS	Wang et al., 2013
2nd	92	Science of the Total Environment	*Chlorella sp.*	Primary sludge	-	Solé-Bundó et al., 2017
3rd	91	Bioresource technology	*Microalgae mixture*	Sewage sludge	408 ± 16 Ncm^3 g VS^{-1}	Olsson et al., 2014
4th	85	Energy Conversion and Management	*Scenedesmus*	Opuntia maxima	308 ± 22 LCH_4 $kgVS^{-1}$	Ramos-Suárez et al., 2014
5th	82	Renewable Energy	*Isochrysis galbana & Selenastrum capricornutum*	Sewage sludge	566 ± 5 mLBiogas/gSV	Caporgno et al., 2015
6th	73	Bioresource Technology	-	Straw, silage, and seaweed	404 L_N kg^{-1} VS	Herrmann et al., 2016
7th	62	Bioresource technology	*Nannochloropsis salina*	Corn silage	0.33 m^3 kg VS^{-1}	Schwede et al., 2013

8th	57	Renewable and Sustainable Energy Reviews	-	-	-	Solé-Bundó et al., 2019b
9th	56	Bioresource Technology	*Dunaliella salina*	Olive mill solid waste	48.1 mL CH_4/(g VS day)	Fernández-Rodríguez et al., 2014
10th	54	Waste Management	*Chlorella sp.*	Chicken manure	31.62 $mL \cdot g^{-1}$ VS	Li et al., 2017

Table 23, which presents the top 10 most cited articles, also highlights the microalgae species preferred in the studies. In order to examine the effective role of microalgae in AD in more detail, the most preferred microalgae species among all bibliometric data (n=228) are given in Fig. 23. Publications on AD by microalgae generally focused on *Chlorella* microalgae. 48 articles preferred this microalgae species. *Scenedesmus, Nannochloropsis, Tetraselmis, Spirulina platensis* and *Chlamydomonas* microalgae, which closely follow this microalgae species, were included in 23, 13, 4, 3 and 3 publications, respectively. *Isochrysis galbana, Dunaliella salina, Selenastrum capricornutum, Pinnularia sp., Synechococcus sp.* and D*esertifilum tharense* microalgae are reported in less than 3 publications.

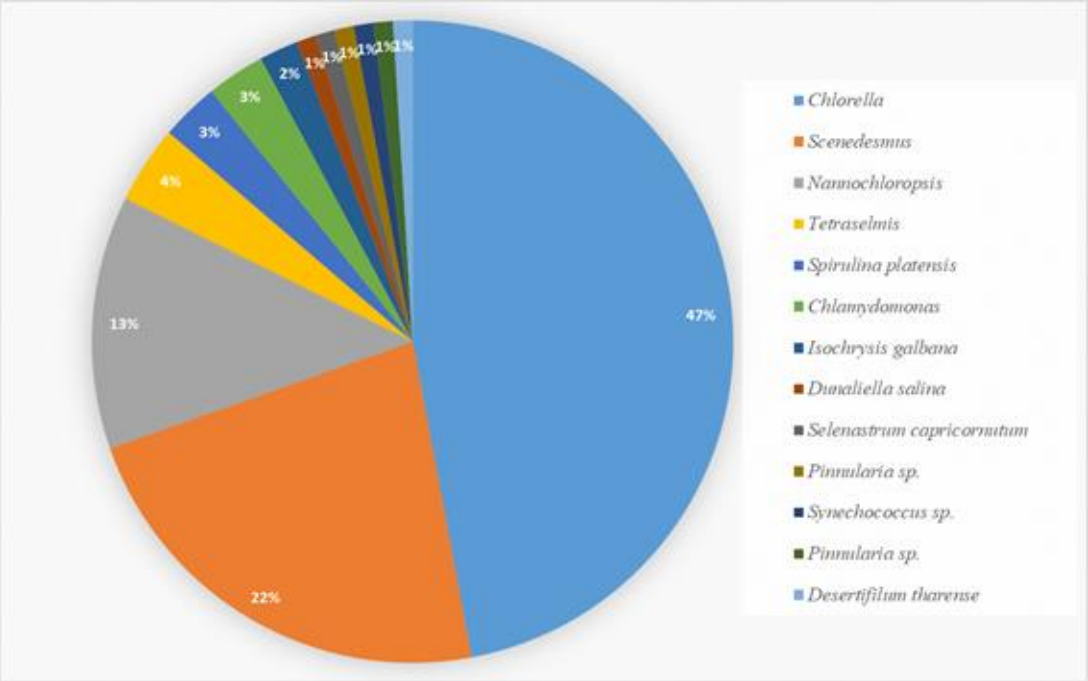


Fig. 23. Distribution of microalgae used in studies on the role of microalgae in anaerobic digestion.

In light of the data obtained for WoS search, the co-digestion studies focused mainly on *Chlorella* and *Scenedesmus* species, with a smaller number of studies testing *Nannochloropsis* and *Tetraselmis* microalgae. However, considering that microalgae are very diverse with different characteristics, it is important to evaluate different species of microalgae within anaerobic digestion. The impact of various microalgae species on methane yield in anaerobic co-digestion systems has not yet been fully studied. In addition, this study was conducted only for the WoS database. Future studies can also be conducted to include other databases.

Table 24 shows the natural habitats of microalgae preferred in the studies obtained as a result of bibliometric analysis. Generally, microalgae species living in freshwater were preferred in the studies. Since they are widely used, the selection of certain microalgae species can affect the efficiency of anaerobic systems. Therefore, it seems that the selection of both microalgae species and waste type in anaerobic co-digestion plays a crucial role in enhancing system performance.

Table 24

Habitats of microalgae were included in the studies used in bibliometric analysis

Types of microalgae	Number of documents	Freshwater	Saline water
Chlorella	48	+	
Scenedesmus	23	+	
Nannochloropsis	13		+
Tetraselmis	4		+
Spirulina platensis	3	+	
Chlamydomonas	3	+	
Isochrysis galbana	2		+
Dunaliella salina	1		+
Selenastrum capricornutum	1	+	
Pinnularia sp.	1	+	
Synechococcus sp.	1	+	+
Desertifilum tharense	1	+	

4.3.2. Anaerobic Co-digestion Performance of *Nannochloropsis sp.* Cultivated with CWW

Anaerobic co-digestion has several advantages for material digestion, including increased cost-efficiency, dilution of inhibitory compounds, degradation of products such as lipids, and biogas production (Dai et al., 2016). In section 2.5, there is a table in which microalgae is used as co-substrate in anaerobic reactors and its effect on biogas volume is described in detail (Table 8). *Nannochloropsis* is also included in these studies. However, pure culture was used in these studies. In this thesis, Nannochloropsis cultivated with CWW was used as a co-substrate. In addition, reactors were operated with only CWW and only *Nannochloropsis* biomass as control reactors. Therefore, this thesis differs from other anaerobic reactor studies using microalgae.

In this thesis, reactors were tested in anaerobic biological systems using *Nannochloropsis sp.* microalgae grown with CWW at various ratios (0%, 10%, 15%, 20%, and 30%) as biomass. The time-dependent biogas volumes of these reactors are shown in Fig. 24. The biogas volumes produced in these reactors were 11663, 8611, 14455, 15856, and 11647 mL, respectively, for the 0%, 10%, 15%, 20%, and 30% CWW+MA biomass reactors. The results show that the reactor with the highest biogas volume is the 20% reactor. Additionally, the VS removal rates of these reactors are 33, 28, 35, 30, and 32, respectively. The decision was made based on biogas volume among reactors with similar VS removal efficiencies. Therefore, the reactor containing 20% CWW+MA biomass was selected. At this stage, control reactors containing only *Nannochloropsis sp*. and only CWW were established at the same ratio (20%).

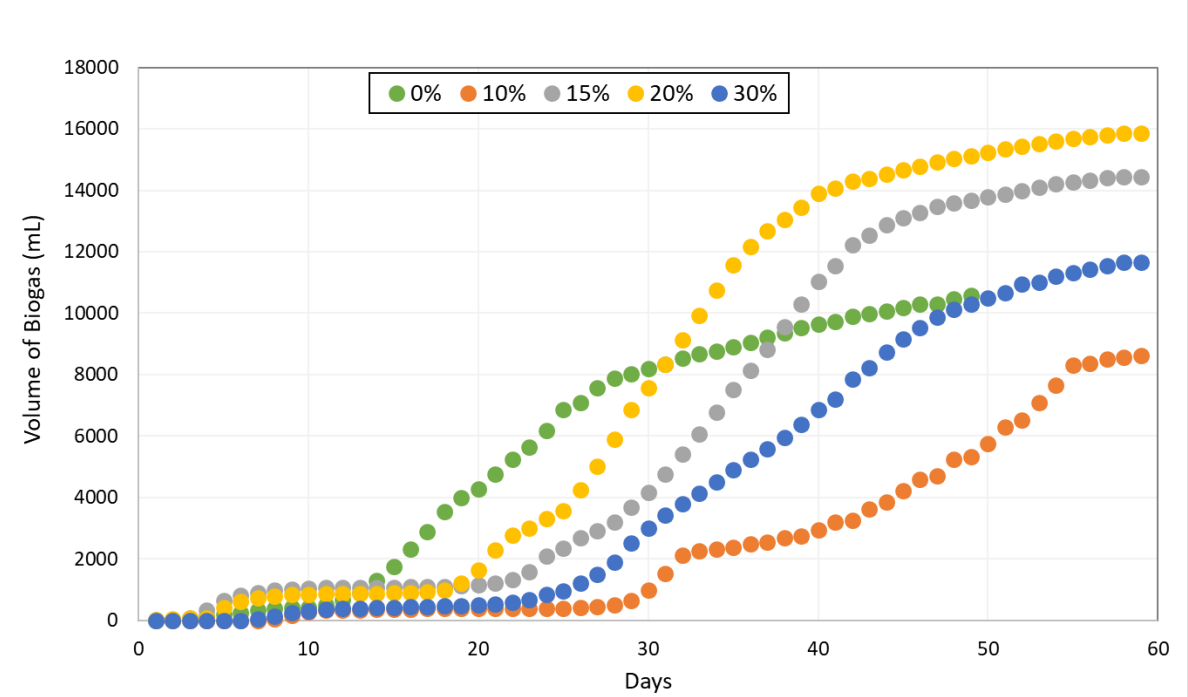


Fig. 24. Time-dependent biogas production from microalgae cultivated with CWW at different percentages

As can be seen in Fig. 24, using 20% of CWW-cultivated microalgae biomass in addition to cattle manure as a co-substrate in anaerobic reactors gave the highest biogas volume. After this stage, control reactors were installed. In these control reactors, the same

proportion (20%) of CWW-only and microalgae-only anaerobic reactors were operated. Fig. 25 shows the time-dependent biogas production volume of these reactors. It can be seen that by far the microalgae biomass cultivated with CWW increases the biogas production to high levels. In the reactor containing only CWW, biogas production ceased after approximately two weeks of reactor operation. This highlights the importance of microalgae in anaerobic reactors.

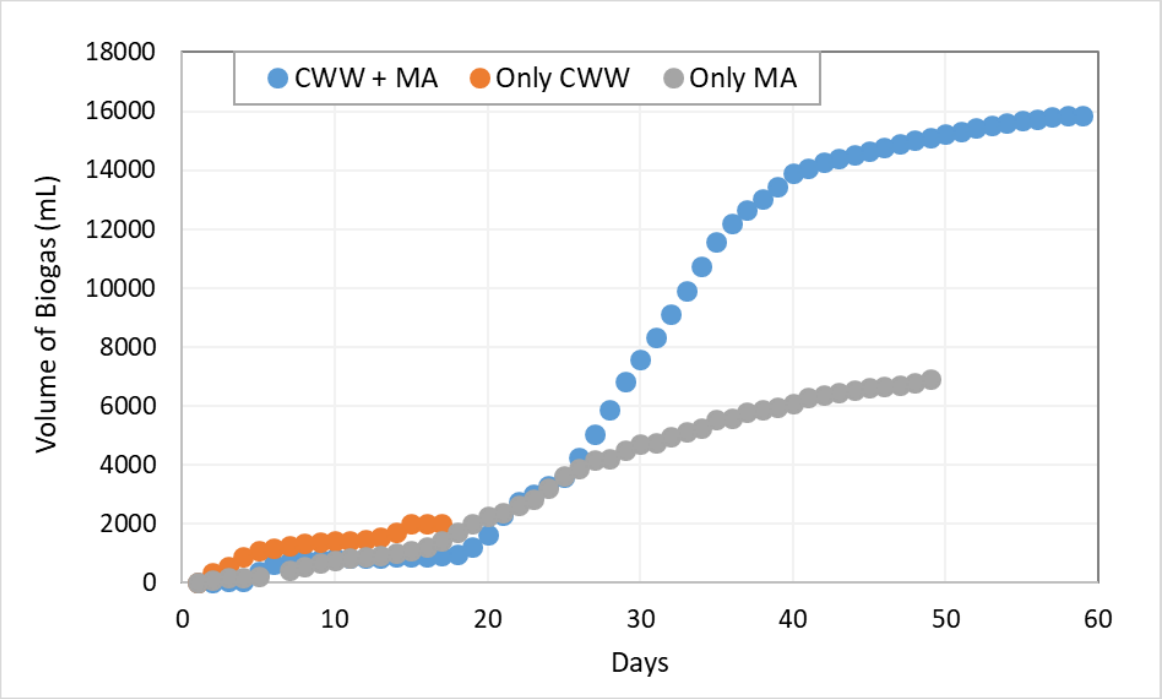


Fig. 25. Time-dependent biogas production at 20% in only CWW, only microalgae, and CWW+microalgae anaerobic reactors.

Table 25 shows in detail the TS and VS concentrations during the initial and final phases of the anaerobic reactors, conducted within the scope of this thesis. The VS/TS ratio is approximately 80% for each reactor. This indicates that the contents of the reactors are suitable for biogas formation. Although the VS/TS ratio was similar in all reactors, it was determined that the solids values in the starting reactors were higher than those in the final reactors. This is due to the breakdown of organic substances within the reactor into biogas caused by microbial activity during the anaerobic digestion process.

Table 25

Changes in solid content of CWW, microalgae and their different percentages over time

Percent	Content	Time	TS (mg/L)	VS (mg/L)
%0	CWW and MA	Start date	85,284	71,960
%10	CWW and MA	Start date	81,928	66,144
%15	CWW and MA	Start date	83,584	66,900
%20	CWW and MA	Start date	78,512	64,156
%30	CWW and MA	Start date	84,504	66,228
%0	CWW and MA	End date	66,100	48,416
%10	CWW and MA	End date	60,136	47,912
%15	CWW and MA	End date	56,212	43,716
%20	CWW and MA	End date	58,292	44,988
%30	CWW and MA	End date	57,700	45,132
%20	only CWW	Start date	89,780	76,032
%20	only MA	Start date	81,440	64,684
%20	only CWW	End date	73,564	60,756
%20	only MA	End date	56,704	44,632

Table 26 shows the VS removal efficiency and biogas production obtained at the final stage of the anaerobic reactors. Here, it is determined that there are close values in terms of VS removal in CWW cultivated reactors. However, there is a difference in biogas production volumes. Therefore, the evaluation was made according to biogas production, and it was determined that 20% was the highest-performing reactor.

Table 26

Volatile solids removal and biogas production in anaerobic reactors containing CWW, microalgae, and their different percentages

Percent	Content	VS removal (%)	Biogas volume (mL)
%0	CWW and MA	33	11,663
%10	CWW and MA	28	8,611
%15	CWW and MA	35	14,455
%20	CWW and MA	30	15,856
%30	CWW and MA	32	11,647
%20	only CWW	20	2,025
%20	only MA	31	7,769

4.4. Carbon Footprint Analysis

Fig. 26 shows the system boundaries and process flow of the experimental study of wastewater batch reactors with *Nannochloropsis sp.* Dashed lines illustrate the system boundaries. The study includes direct energy and material inputs. Nutrient input is provided from the wastewater. Carbon dioxide, another input, was not required in the microalgae-based system. Since *Nannochloropsis sp.* is mixotrophic, it is capable of using carbon from both organic and inorganic sources. There is no need for chemical additions during reactor operation. The largest input in the system is the electricity consumption used for mixing, lighting, and air conditioning. Initially, the "Microalgae cultivation" process was carried out to produce pure microalgae biomass. This process was later connected to the "Operation of Microalgae Batch Reactors" subprocess. At this stage, both wastewater and electrical energy were incorporated into the experimental procedure. Both the treatment of wastewater and generation of biomass were accomplished in the batch reactors at the end of this process. The microalgae obtained from the process can function as a raw input in biogas and nanomaterial manufacturing without any additional processing. This system does not include the sludge that forms at the end of wastewater treatment processes, which is costly to dispose

of. The biomass produced at the end of the system can be used as a raw material in various applications.

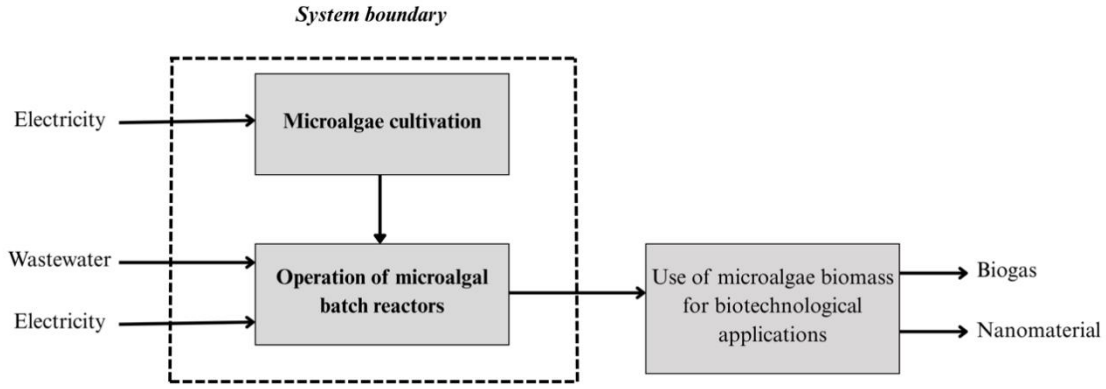


Fig. 26. System boundaries for the study.

4.4.1. Microalgae Cultivation

The beginning stage of this investigation was microalgae cultivation, which enables the formation of pure microalgae biomass. In this stage, batch reactors were operated for two weeks with an active reactor volume of 400 ml. A 24-hour light source was used throughout the study. Calculations were made separately for illumination, mixing, and air conditioning. The coefficient of 0.426 kg CO_2-eq/kWh shown in Table 27 represents the carbon dioxide equivalent emissions resulting from electricity generation. In other words, approximately 0.426 kg of CO_2 equivalent greenhouse gases are released into the atmosphere as a result of consuming 1 kWh of electricity. A total carbon emission of 1.71 kg CO_2-eq was calculated for illumination, which was 4.29 kg CO_2-eq/L per liter. For mixing, the total carbon emission was 5.72 kg CO_2-eq, which was 14.31 kg CO_2-eq/L per liter. For air conditioning, the total value was 128.82 kg CO_2-eq, which was 322.05 kg CO_2-eq/L per liter. The highest carbon emission came from air conditioning. Table 27 provides detailed calculations of electricity consumption and carbon emissions generated during the operation of batch reactors in microalgae cultivation.

Table 27

Calculations of electricity consumption and carbon emissions in microalgae cultivation

	Lighting	Mixing	Air conditioning	Total
Total electricity consumption (kWh)	0.012kW × 336 h= 4.032	0.04kW×336 h=13.44	0.9kW×336h=302.4	~320
Total carbon emission (kg CO_2-eq)	4.032 kWh × 0.426= 1.71	13.44 ×0.426=5.72	302.4×0.426=128.82	136.25
Total carbon emission per liter (kg CO_2-eq/L)	1.71 / 0.4 =4.29	5.72 / 0.4 = 14.31	128.82 / 0.4 = 322.05	340.65

4.4.2. Operation of Microalgae Batch Reactors

Cheese Whey Wastewater

Electricity consumption accounts for the largest share of the carbon footprint in the operation of batch reactors. Electricity consumption comes from mixing, lighting, and heating/cooling systems. For lighting, four 6W bulbs were used for 7 days, remaining on 24 hours a day. The active volume of the batch reactors is 250 mL. A total of 13 batch reactors were calculated using RSM output. However, since replicates and control reactors were also operated, a total of 30 reactors were operated. Considering this data, we can calculate the carbon footprint of operating CWW with microalgae. A total of 24W of lighting power is required to illuminate all reactors. This system, which is on 24 hours a day for 7 days, requires 168 hours of electricity. The total electricity consumption for this period is 4.03 kWh. This electricity consumption results in carbon emissions of 1.72 kg CO_2-eq. This value per liter was calculated as 0.23 kg CO_2-eq/L. A 40 W orbital mixer was used for mixing. The duration, reactor volume, and emission factor were the same as those used for lighting. Total electricity consumption was calculated as 6.72 kWh. Carbon emissions were 2.9 kg CO_2-eq. Carbon emissions per liter of this value were calculated as approximately 0.39 kg CO_2-eq/L. Air conditioning was used to control the temperature of the batch reactors. An air conditioner with a capacity of approximately 9,000 BTU/h consumes 0.9 kW of electrical power. Total electricity consumed during reactor operation was determined to be 151.2 kWh. The carbon emissions from this consumption are 64.41 kg CO_2/kWh. This value corresponds to 8.6 kg

CO_2-eq/L per liter. The carbon emission equivalent per litre of electricity consumption from lighting, mixing and air conditioning in batch reactors is 9.2 kg CO_2-eq/L. Table 28 provides detailed calculations of electricity consumption and carbon emissions generated during the operation of batch reactors running microalgae and CWW together.

Table 28

Calculation of electricity consumption and carbon emissions of CWW-microalgae batch reactors

	Lighting	Mixing	Air conditioning	Total
Total electricity consumption (kWh)	0.024kW×168h=4.03	0.04kW×168h=6.72	0.9kW×168h=151.2	~162
Total carbon emission (kg CO_2-eq)	4.03×0.426=1.72	6.72×0.426=2.9	151.2×0.426=64.41	69
Total carbon emission per liter (kg CO_2-eq/L)	1.72 / 7.5 = 0.23	2.9 / 7.5 = 0.39	64.41 / 7.5 = 8.6	9.2

Municipal Wastewater

Calculations were made for lighting, mixing and climate control in batch reactors containing municipal wastewater and microalgae, as in all other batch reactors. For lighting, four 6W bulbs were used, which remained on for 24 hours a day for 5 days. The active volume of the batch reactors was 400 mL. A total of 15 batch reactors were calculated using RSM output. However, since replicas and control reactors were also run, a total of 30 reactors were operated. Considering these data, we can calculate the carbon footprint of operating CWW with microalgae. A total of 24 W of lighting power is required to illuminate all reactors. The total electricity consumption for lighting over 5 days is 2.88 kWh. This electricity consumption results in 1.23 kg CO_2-eq carbon emissions. This value is calculated as 0.1025 kg CO_2-eq/L per liter. A 40 W orbital mixer was used for mixing. The duration,

reactor volume, and emission factor are the same as those used for lighting. The total electricity consumption is calculated as 4.80 kWh. Carbon emissions are 2.05 kg CO_2-eq. The carbon emissions per litre are approximately 0.1708 kg CO_2-eq/L. An air conditioner was used to control the temperature of the batch reactors. An air conditioner with a capacity of approximately 9,000 BTU/h consumes 0.9 kW of electrical power. The total electricity consumed during the operation of the reactor was determined to be 108 kWh. The carbon emissions resulting from this consumption are 46.01 kg CO_2/kWh. This value corresponds to 3.83 kg CO_2-eq/L per liter. Table 29 provides detailed calculations of electricity consumption and carbon emissions generated during the operation of batch reactors running microalgae and municipal wastewater together.

Table 29

Calculation of electricity consumption and carbon emissions of municipal wastewater-microalgae batch reactors

	Lighting	Mixing	Air conditioning	Total
Total electricity consumption (kWh)	0.024×120=2.88	0.04×120=4.8	0.9×120=108	~116
Total carbon emission (kg CO_2-eq)	2.88×0.426=1.23	4.8kWh×0.426=2.05	108kWh×0.426=46.01	49.3
Total carbon emission per liter (kg CO_2-eq/L)	1.23 / 12 L = 0.1025	2.05 / 12 L = 0.1708	46.01 / 12 = 3.83	4.1

CHAPTER FIVE
CONCLUSION AND SUGGESTIONS

Within the scope of this thesis, the treatment of both municipal wastewater and a food industry wastewater known as CWW has been investigated using the mixotrophic marine microalgae *Nannochloropsis sp*. Additionally, a comprehensive examination has been conducted on the biogas formation and utilization materials in the health sector from the biomass of *Nannochloropsis sp.* microalgae cultivated with CWW obtained from bioreactors.

This work aims at building a system that is able to serve as a basis for real-scale applications using a sustainable and circular approach with microalgae and wastewater. In this context, optimization and validation mechanisms have been used to enhance the system's performance. For the optimization of bioreactors, RSM was used, and decision tree algorithms were tested for model validation. The combined use of these two methods has been implemented to enhance system performance. Additionally, a single type of industrial wastewater has been used in this thesis, but the information obtained here will serve as a guide for other industrial wastewaters.

The dairy and dairy products industry holds a significant share in industrial activities worldwide. In our country, especially in the Çanakkale region, the activities of this sector are quite intensive. There are wastewaters with various compositions coming from production facilities. This thesis focuses on CWW. However, the information obtained from studies on CWW has the potential to meet the needs of this sector. CWW is a type of wastewater with high organic pollutant concentrations, and it is not possible to integrate it into microalgae systems in its raw form. Therefore, in this thesis, the COD value of 8000 mg/L, which is the maximum at which *Nannochloropsis sp.* can operate, was reached, and experiments were conducted at this concentration. However, it has been demonstrated that for wastewater within this sector, but with an organic content not as high as CWW, the systems can be used effectively with other raw wastewaters. According to the results of RSM

and the decision tree, the critical parameter in the treatment of CWW with *Nannochloropsis sp.* is the CWW COD concentration. As the COD concentration increased, there was an increase in the COD removal efficiency. However, this situation does not apply to nutrient removal. High COD concentration can reduce nutrient removal performance. Optimization and validation applications for CWW have been conducted based on concentration for both wastewater and microalgae. In order to elevate the system performance to higher levels, future studies can conduct a more extensive evaluation by considering working conditions such as light intensity, illumination duration, and temperature.

Another wastewater examined within the scope of the thesis is municipal wastewater. Using the RSM and decision tree algorithms, the effects of the independent variables of illumination time, light intensity, and temperature on the growth of microalgal biomass were examined. According to the findings of the evaluation of the reactor experiments, the optimum conditions were determined to be a 21-hour illumination duration, 8000 lux light intensity, and 30°C. The parameter that most affects the activity of microalgae has been determined to be high light intensity during long illumination periods. According to the interpretation of the RSM and decision tree algorithms, nutrient removal in *Nannochloropsis sp.* and municipal wastewater treatment has shown higher performance than carbon removal. This situation is an important outcome for places where nutrient removal is inadequate. This study is a solution for rural areas, small businesses with industrial sources, sparsely populated settlements, holiday resorts, or remote settlements that cannot be integrated into a central wastewater system. Large-scale facilities may face difficulties in achieving full compliance due to high flow rates, operational, and installation costs. While there are sometimes difficulties in carbon removal even in these mentioned settlement areas, the microalgae-based wastewater treatment system aims to achieve both carbon and nutrient removal.

Another objective of the thesis is to produce microalgal biomass and transform it into valuable compounds applicable in areas such as energy and health-related fields. Creating potential for renewable energy sources is another focus of the study. In this context, the anaerobic reactor used cow manure, known for its biogas potential, as the main substrate,

with microalgal biomass cultivated with CWW as an additional substrate. The reactors were prepared considering the dry matter percentage. Microalgae biomass percentages were prepared to be 0, 10, 15, 20, and 30. The highest biogas production was obtained from the reactor with 20%. As a control experiment, anaerobic reactors containing 20% *Nannochloropsis sp.* and 20% CWW were operated. The results have clearly shown that the biogas volume of the reactor containing microalgae cultivated with CWW was higher than that of the other reactors. This indicates that there is potential for using microalgae as a supporting substrate in biogas-producing systems. This method is quite functional in terms of sustainability and circular economy, as it both reduces organic wastewater and possesses the characteristics of renewable energy.

The production of materials for health applications, which is another area of use for microalgal biomass, is another work package carried out within the scope of this thesis. In the literature, there are many research areas for microalgae, such as antimicrobial, antioxidant, and anti-cancer properties. However, in all of these studies, pure culture microalgae were used. In this thesis, both pure culture microalgae and microalgae cultivated with CWW have been used. Additionally, a comparison was made by synthesizing silver nanoparticles to enhance the activity. With these prepared biomasses, antimicrobial activity, antioxidant capacity, and DNA cleavage potentials were tested. As a result of the antioxidant and DNA cleavage analyses, the highest yield was found in the microalgae biomass treated with CWW and ash. As a result of the antimicrobial activity evaluation, the pure microalgae biomass was obtained from the biomass synthesized with silver nanoparticles. While microalgae is an important material for all three applications, its significance in wastewater for antioxidant and DNA cleavage is quite clear. This result shows that this biomaterial, which can be produced at a low cost, can be used in potential food applications, primarily in health.

This study also contributes to the 17 SDGs: SDG (3, 6, 7, 9, 11, 12, and 13). In addition, the study used real wastewater rather than synthetic wastewater, which cannot be fully adapted to real-world applications. The increasing demand for sustainable wastewater treatment solutions requires the integration of advanced statistical and computational

techniques in microalgae-based systems to improve sustainability, scalability, and efficiency. Studies on species selection, optimization, and adaptive management strategies should continue to improve microalgae cultivation in real-world applications.

REFERENCES

Abate, R., Oon, Y. S., Oon, Y. L. and Bi, Y. (2024). "Microalgae-bacteria nexus for environmental remediation and renewable energy resources: Advances, mechanisms and biotechnological applications". *Heliyon*.

Adenigba, V. O., Omomowo, I. O., Oloke, J. K., Fatukasi, B. A., Odeniyi, M. A. and Adedayo, A. A. (2020). "Evaluation of microalgal-based nanoparticles in the adsorption of heavy metals from wastewater". In IOP Conference series: Materials Science and Engineering (Vol. 805, No. 1, p. 012030). IOP Publishing.

Ahiahonu, E. K., Anku, W. W., Roopnarain, A., Serepa-Dlamini, M. H. and Govender, P. P. (2024). "Evaluation of green microalgal strains for simultaneous municipal wastewater treatment and heavy metal bioremediation". *Journal of Chemical Technology & Biotechnology*, 99(9), 2035-2046.

Ahmad, I., Abdullah, N., Koji, I., Yuzir, A. and Mohamad, S. E. (2021). "Potential of microalgae in bioremediation of wastewater". *Bulletin of Chemical Reaction Engineering & Catalysis*, 16(2), 413-429 .

Ajala, S.O. and Alexander, M.L. (2020). "Multi-objective optimization studies of microalgae dewatering by utilizing bio-based alkali: a case study of response surface methodology (RSM) and genetic algorithm (GA)". *SN Appl Sci,* 2:387.

Almomani, F. and Bhosale, R. R. (2021). "Bio-sorption of toxic metals from industrial wastewater by algae strains Spirulina platensis and Chlorella vulgaris: Application of isotherm, kinetic models and process optimization". *Science of the Total Environment*, 755, 142654.

Arashiro, L. T., Montero, N., Ferrer, I., Acién, F. G., Gómez, C. and Garfí, M. (2018). "Life cycle assessment of high rate algal ponds for wastewater treatment and resource recovery". *Science of the Total Environment*, 622, 1118-1130.

Arashiro, L. T., Josa, I., Ferrer, I., Van Hulle, S. W., Rousseau, D. P. and Garfí, M. (2022). "Life cycle assessment of microalgae systems for wastewater treatment and bioproducts recovery: Natural pigments, biofertilizer and biogas". *Science of The Total Environment*, 847, 157615.

Arias, D. M., Solé-Bundó, M., Garfi, M., Ferrer, I., García, J. and Uggetti, E. (2018). "Integrating microalgae tertiary treatment into activated sludge systems for energy and nutrients recovery from wastewater". *Bioresource technology*, 247, 513-519.

Arsalan, S. and Iqbal, M. J. (2023). "Evaluating optimal cultivation sites for microalgae as a sustainable biofuel energy resource". *Environmental Research Communications*, 5(10), 105014.

Ashrafi, E., Zeinabad, A. M., Borghei, S. M., Torresi, E. and Sierra, J. M. (2019). "Optimising nutrient removal of a hybrid five-stage Bardenpho and moving bed biofilm reactor process using response surface methodology". *Journal of Environmental Chemical Engineering*, 7(1), 102861.

Australian Government. The Biology of Nannochloropsis Oceanica Suda & Miyashita (a Microalga); Australian Government: Canberra, ACT, Australia, 2019

Baek, S. H. andPagilla, K. R. (2006). "Aerobic and anaerobic membrane bioreactors for municipal wastewater treatment". *Water environment research*, 78(2), 133-140.

Barontini, F., Biagini, E., Dragoni, F., Corneli, E., Ragaglini, G., Bonari, E. and Nicolella, C. (2016). "Anaerobic digestion and co-digestion of oleaginous microalgae residues for biogas production". *Chemical Engineering Transactions*, 50, 91-96.

Bhatt, A., Sahu, N., Dada, A. C., Prajapati, S. K. and Arora, P. (2024). "Assessing sustainability of microalgae-based wastewater treatment: environmental considerations and impacts on human health". *Journal of Environmental Management,* 354, 120435.

Balouiri, M., Sadiki, M. and Ibnsouda, S. K. (2016). "Methods for in vitro evaluating antimicrobial activity: A review". *Journal of pharmaceutical analysis*, 6(2), 71-79.

Barceló-Villalobos, M., Serrano, C. G., Zurano, A. S., García, L. A., Maldonado, S. E., Peña, J. and Fernández, F. A. (2019). "Variations of culture parameters in a pilot-scale thin-layer reactor and their influence on the performance of Scenedesmus almeriensis culture". *Bioresource Technology Reports,* 6, 190-197.

Barten, R., Chin-On, R., de Vree, J., van Beersum, E., Wijffels, R. H., Barbosa, M. J. and Janssen, M. (2022). "Growth parameter estimation and model simulation for three

industrially relevant microalgae: Picochlorum, Nannochloropsis, and Neochloris". *Biotechnology and bioengineering,* 119(6), 1416-1425.

Bashir, M. J., Aziz, H. A., Aziz, S. Q. and Amr, S. A. (2012). "An overview of wastewater treatment processes optimization using response surface methodology (RSM)". In The 4th International Engineering Conference–Towards engineering of 21st century (pp. 15-16).

Biggs, T., Mladenov, N., Garcia, S., Yuan, Y., Sousa, D., Grant, A. and Liden, D. (2025). "Fluorescence-Based Indicators of Escherichia coli and Untreated Wastewater: Turbidity Correction and Comparison of In Situ and Benchtop Fluorometers in a Sewage-Polluted Urban River". *ACS Es&t Water*.

Biondi, N., Bassi, N., Chini Zittelli, G., De Faveri, D., Giovannini, A., Rodolfi, L. and Tredici, M. R. (2013). "Nannochloropsis sp. F&M-M24: Oil production, effect of mixing on productivity and growth in an industrial wastewater". *Environmental Progress & Sustainable Energy*, 32(3), 846-853.

Bodkhe, S. (2008). "Development of an improved anaerobic filter for municipal wastewater treatment". *Bioresource technology*, 99(1), 222-226.

Bohutskyi, P., Chow, S., Ketter, B., Betenbaugh, M. J. and Bouwer, E. J. (2015). "Prospects for methane production and nutrient recycling from lipid extracted residues and whole Nannochloropsis salina using anaerobic digestion". *Applied Energy*, 154, 718-731.

Borowiak, D., Pstrowska, K., Wiśniewski, M. and Grzebyk, M. (2020). "Propagation of inoculum for Haematococcus pluvialis microalgae scale-up photobioreactor cultivation system". *Applied Sciences*, 10(18), 6283.

Bortoluzzi, A. C., Faitão, J. A., Di Luccio, M., Dallago, R. M., Steffens, J., Zabot, G. L. and Tres, M. V. (2017). "Dairy wastewater treatment using integrated membrane systems". J*ournal of environmental chemical engineering*, 5(5), 4819-4827.

Brar, A., Kumar, M., Vivekanand, V. and Pareek, N. (2019). "Phycoremediation of textile effluent-contaminated water bodies employing microalgae: nutrient sequestration and biomass production studies". *International Journal of Environmental Science and Technology*, 16(12), 7757-7768.

Briassoulis, D., Panagakis, P., Chionidis, M., Tzenos, D., Lalos, A., Tsinos, C. and Jacobsen, A. (2010). “An experimental helical-tubular photobioreactor for continuous production of Nannochloropsis sp”. Bioresource Technology, 101(17), 6768-6777.

Bruno, P., Bruno, F., Di Bella, G., Napoli, E. and De Marchis, M. (2025). “CFD study on the effect of the baffles geometry in sedimentation efficiency in wastewater treatments through Large Eddy Simulations”. *Journal of Environmental Management*, 373, 123536.

Castillo, T., Ramos, D., García-Beltrán, T., Brito-Bazan, M. and Galindo, E. (2021). “Mixotrophic cultivation of microalgae: an alternative to produce high-value metabolites”. *Biochemical Engineering Journal*, 176, 108183.

Carrera, J., Vicent, T. and Lafuente, J. (2004). “Effect of influent COD/N ratio on biological nitrogen removal (BNR) from high-strength ammonium industrial wastewater”. *Process Biochemistry*, 39(12), 2035-2041.

Carrero, A., Vicente, G., Rodríguez, R., Linares, M. and Del Peso, G. L. (2011). “Hierarchical zeolites as catalysts for biodiesel production from Nannochloropsis microalga oil”. *Catalysis Today*, 167(1), 148-153.

Carvalho, A. P., Meireles, L. A. and Malcata, F. X. (2006). “Microalgal reactors: a review of enclosed system designs and performances”. *Biotechnology progress*, 22(6), 1490-1506.

Cea-Barcia, G., López-Caamal, F., Torres-Zúñiga, I. and Hernández-Escoto, H. (2018). “Biogas purification via optimal microalgae growth: A literature review”. *Biotechnology progress*, 34(6), 1513-1532.

Cheirsilp, B. and Torpee, S. (2012). “Enhanced growth and lipid production of microalgae under mixotrophic culture condition: effect of light intensity, glucose concentration and fed-batch cultivation”. *Bioresource Technology*, 110, 510-516.

Cheng-Wu, Z., Zmora, O., Kopel, R. and Richmond, A. (2001). “An industrial-size flat plate glass reactor for mass production of Nannochloropsis sp.(Eustigmatophyceae)”. *Aquaculture*, 195(1-2), 35-49.

Chiu, S. Y., Kao, C. Y., Chen, C. H., Kuan, T. C., Ong, S. C. and Lin, C. S. (2008). "Reduction of CO2 by a high-density culture of Chlorella sp. in a semicontinuous photobioreactor". *Bioresource technology*, 99(9), 3389-3396.

Choi, H. J. (2016). "Dairy wastewater treatment using microalgae for potential biodiesel application". *Environmental Engineering Research*, 21(4), 393-400.

Collivignarelli, M. C., Abbà, A., Padovani, S., Frascarolo, M., Sciunnach, D., Turconi, M. and Orlando, M. (2015). "Recovery of sewage sludge on agricultural land in Lombardy: current issues and regulatory scenarios". *Environmental Engineering & Management Journal (EEMJ)*, 14(7).

Costa, J. A. V., Bezerra, P. Q. M., Moreira, J. B., Molina, A. N. and de Morais, M. G. (2022). "Biogas from microalgae: Production approaches and strategies for process optimization". *Role of Microbes in Industrial Products and Processes*, 305-329.

Crini, G. and Lichtfouse, E. (2019). "Advantages and disadvantages of techniques used for wastewater treatment". *Environmental chemistry letters*, 17, 145-155.

Crippa, I., Dolci, G., Grosso, M. and Rigamonti, L. (2025). "Life Cycle Assessment of Microalgal Biomass Valorization from a Wastewater Treatment Process". *Waste and Biomass Valorization*, 16(1), 525-541.

Cromar, N. J. and Fallowfield, H. J. (1997). "Effect of nutrient loading and retention time on performance of high rate algal ponds". *Journal of Applied Phycology*, 9, 301-309.

Czerwionka, K., Makinia, J., Pagilla, K. R. and Stensel, H. D. (2012). "Characteristics and fate of organic nitrogen in municipal biological nutrient removal wastewater treatment plants". *Water Research,* 46(7), 2057-2066.

de Jesus Raposo, M. F., de Morais, A. M. M. B. and de Morais, R. M. S. C. (2014). "Influence of sulphate on the composition and antibacterial and antiviral properties of the exopolysaccharide from Porphyridium cruentum". *Life sciences*, 101(1-2), 56-63.

Demir, M. N., Debik, E., Ozkaya, B. and Coskun, T. (2019). "Determination of microbial community in a pilot scale two-stage step-feed biological nutrient removal process". *Global Nest Journal*, 21, 211-221.

Dolganyuk, V., Belova, D., Babich, O., Prosekov, A., Ivanova, S., Katserov, D. and Sukhikh, S. (2020). “Microalgae: A promising source of valuable bioproducts”. *Biomolecules*, 10(8), 1153.

Dong, B., Ho, N., Ogden, K. L. and Arnold, R. G. (2014). “Cultivation of Nannochloropsis salina in municipal wastewater or digester centrate”. *Ecotoxicology and environmental safety*, 103, 45-53.

Dong, Q., Parker, W. and Dagnew, M. (2016). “Long term performance of membranes in an anaerobic membrane bioreactor treating municipal wastewater”. *Chemosphere*, 144, 249-256.

El Bakraoui, H., Slaoui, M., Mabrouki, J., Hmouni, D. and Laroche, C. (2022). “Recent trends on domestic, agricultural and industrial wastewaters treatment using microalgae biorefinery system”. *Applied Sciences*, 13(1), 68.

El Barkaoui, S., Mandi, L., Fichera, M., Ryah, H., Baçaoui, A., Del Bubba, M. and Ouazzani, N. (2025). “Optimizing biochar-based column filtration systems for enhanced pollutant removal in wastewater treatment: A preliminary study”. *Chemosphere*, 372, 144067.

El-Hawy, A. S., Abdel-Rahman, H. G., El-Bassiony, M. F., Anwar, A., Hassan, M. A., Elnabtiti, A. A. S. and Kamel, S. (2022). “Immunostimulatory effects of Nannochloropsis oculata supplementation on Barki rams growth performance, antioxidant assay, and immunological status”. *BMC Veterinary Research*, 18(1), 314.

Elibol, M. (2002). “Response surface methodological approach for inclusion of perfluorocarbon in actinorhodin fermentation medium”. *Process Biochemistry*, 38(5), 667-673.

Elsayed, A., Siam, A. and El-Dakhakhni, W. (2022). “Machine learning classification algorithms for inadequate wastewater treatment risk mitigation”. *Process Safety and Environmental Protection*, 159, 1224-1235.

Esteves, M. V., Marques, D. M., de Almeida, J. D., Faria, N. T. and Ferreira, F. C. (2025). “Marine Microalgae–Microorganism Co-Cultures: An Insight into Nannochloropsis sp. Use and Biotechnological Applications”. *Foods*, 14(9), 1522.

Faé Neto, W. A., Borges Mendes, C. R. and Abreu, P. C. (2018). “Carotenoid production by the marine microalgae Nannochloropsis oculata in different low-cost culture media”. *Aquaculture research*, 49(7), 2527-2535.

Fan, H., Wang, K., Wang, C., Yu, F., He, X., Ma, J. and Li, X. (2020). “A comparative study on growth characters and nutrients removal from wastewater by two microalgae under optimized light regimes”. *Environmental Technology & Innovation*, 19, 100849.

Farizoglu, B. and Uzuner, S. (2011). “The investigation of dairy industry wastewater treatment in a biological high performance membrane system”. *Biochemical Engineering Journal*, 57, 46-54.

Flannery, J., Keaveney, S., Rajko-Nenow, P., O'Flaherty, V. and Doré, W. (2012). “Concentration of norovirus during wastewater treatment and its impact on oyster contamination”. A*pplied and environmental microbiology*, 78(9), 3400-3406.

Fuentes-Grünewald, C., Gayo-Peláez, J. I., Ndovela, V., Wood, E., Kapoore, R. V. and Llewellyn, C. A. (2021). “Towards a circular economy: a novel microalgal two-step growth approach to treat excess nutrients from digestate and to produce biomass for animal feed”. *Bioresource Technology*, 320, 124349.

García, D., Posadas, E., Grajeda, C., Blanco, S., Martínez-Páramo, S., Acién, G. and Muñoz, R. (2017). “Comparative evaluation of piggery wastewater treatment in algal-bacterial photobioreactors under indoor and outdoor conditions”. *Bioresource Technology*, 245, 483-490.

Garcia-Parra, J., Fuentes-Grünewald, C. and Gonzalez, D. (2022). “Therapeutic potential of microalgae-derived bioactive metabolites is influenced by different large-scale culture strategies”. *Marine Drugs*, 20(10), 627.

García-Gimeno, R. M., Barco, E., Rincón, F., Zurera-Cosano, G. (2005). “Response Surface Model for the Estimation of Escherichia coli O 157: H7 Growth under Different Experimental Conditions”. *Journal of food science*, 70(1), M30-M36.

Gerwick, W. H., Roberts, M. A., Proteau, P. J. and Chen, J. L. (1994). “Screening cultured marine microalgae for anticancer-type activity”. *Journal of Applied Phycology*, 6, 143-149.

Gnanakani, P. E., Santhanam, P., Premkumar, K., Kumar, K. E. and Dhanaraju, M. D. (2019). "Nannochloropsis extract–mediated synthesis of biogenic silver nanoparticles, characterization and in vitro assessment of antimicrobial, antioxidant and cytotoxic activities". *Asian Pacific journal of cancer prevention: APJCP*, 20(8), 2353.

Gonçalves, A. L., Pires, J. C. and Simões, M. (2017). "A review on the use of microalgal consortia for wastewater treatment". *Algal Research*, 24, 403-415.

Gökpınar, Ş., Işık, O., Göksan, T., Durmaz, Y., Uslu, L., Ak, B., Önalan S.K. and Akdoğan, P. (2013). "Studies in Algal Biotechnology". *Aquaculture Studies*, 13(4), 021-026.

Gramegna, G., Scortica, A., Scafati, V., Ferella, F., Gurrieri, L., Giovannoni, M. and Benedetti, M. (2020). "Exploring the potential of microalgae in the recycling of dairy wastes". *Bioresource Technology Reports*, 12, 100604.

Gurumoorthy, P. and Saravanan, A. (2016). "Biofuel Production from Microalga Nannochloropsis oculata using Dairy Industry Waste Water". *International Journal of ChemTech Research*, 9(5), 346-351.

Guzmán, S., Gato, A., Lamela, M., Freire-Garabal, M. and Calleja, J. M. (2003). "Anti-inflammatory and immunomodulatory activities of polysaccharide from Chlorella stigmatophora and Phaeodactylum tricornutum". *Phytotherapy Research*, 17(6), 665-670.

Greses, S., Gaby, J. C., Aguado, D., Ferrer, J., Seco, A. and Horn, S. J. (2017). "Microbial community characterization during anaerobic digestion of Scenedesmus spp. under mesophilic and thermophilic conditions". *Algal research*, 27, 121-130.

Gupta, P. L., Lee, S. M. and Choi, H. J. (2016a). "Integration of microalgal cultivation system for wastewater remediation and sustainable biomass production". W*orld Journal of Microbiology and Biotechnology*, 32, 1-11.

Gupta, P. L., Choi, H. J. and Lee, S. M. (2016b). "Enhanced nutrient removal from municipal wastewater assisted by mixotrophic microalgal cultivation using glycerol". *Environmental Science and Pollution Research,* 23, 10114-10123.

Gupta, P. and Nayak, K. K. (2016). “Optimization of keratin/alginate scaffold using RSM and its characterization for tissue engineering”. *International journal of biological macromolecules,* 85, 141-149.

Hammad, M. M. H., Hameed, K. W. and Sabti, H. A. (2019). “Reducing the pollutants from municipal wastewater by Chlorella vulgaris microalgae”. *Al-Khwarizmi Engineering Journal,* 15(1), 97-108.

Han, M., Zhang, C. and Ho, S. H. (2023). “Immobilized microalgal system: An achievable idea for upgrading current microalgal wastewater treatment”. *Environmental Science and Ecotechnology*, 14, 100227.

Hashemi, A., Basafa, M. and Behravan, A. (2022). “Machine learning modeling for solubility prediction of recombinant antibody fragment in four different E. coli strains”. *Scientific reports*, 12(1), 5463.

Hena, S., Znad, H., Heong, K. T. and Judd, S. (2018). “Dairy farm wastewater treatment and lipid accumulation by Arthrospira platensis”. *Water research*, 128, 267-277.

Henze, M. (1992). “Characterization of wastewater for modelling of activated sludge processes”. *Water Science and Technology,* 25(6), 1-15.

Hosikian, A., Lim, S., Halim, R. and Danquah, M. K. (2010). “Chlorophyll extraction from microalgae: A review on the process engineering aspects”. I*nternational journal of chemical engineering*, 2010(1), 391632.

Hossain S.Z., Alnoaimi A., Razzak S.A., Ezuber H., Al-Bastaki N. and Safdar M. (2018). “Multiobjective optimization of microalgae (Chlorella sp.) growth in a photobioreactor using Box-Behnken design approach”. *The Canadian Journal of Chemical Engineering*, 96:1903–1910.

Hossain, S. Z., Sultana, N., Jassim, M. S., Coskuner, G., Hazin, L. M., Razzak, S. A. and Hossain, M. M. (2022). “Soft-computing modeling and multiresponse optimization for nutrient removal process from municipal wastewater using microalgae”. *Journal of Water Process Engineering*, 45, 102490.

Hu, G., Zhang, X., Gao, R., Liang, J., Liu, J., Kong, D. and Wang, Y. (2025). “Mechanism of photoperiod regime and fermentation mode effects on wastewater treatment by Chlorella pyrenoidosa”. *Algal Research*, 87, 103976.

Huang, H., Lang, Y. and Zhou, M. (2024). “A comprehensive review on medical applications of microalgae”. *Algal Research*, 103504.

Huang, Q., Jiang, F., Wang, L. and Yang, C. (2017). “Design of photobioreactors for mass cultivation of photosynthetic organisms”. *Engineering*, 3(3), 318-329.

Huang, Y., Wen, S., Wan, Z., Xin, H. and Li, K. (2025). “Study on treating oil-containing wastewater from substations based on combined air flotation coupled with multi-stage fine filtration technology”. *Discover Applied Sciences*, 7(4), 1-15.

Imamoglu E. (2024). “Artificial intelligence and/or machine learning algorithms in microalgae bioprocesses”. *Bioengineering*, 11(11), 1143.

Ince, O. (1998). “Potential energy production from anaerobic digestion of dairy wastewater”. *Journal of Environmental Science & Health Part A*, 33(6), 1219-1228.

Ishiwu, C. N. (2022). “Response Surface Methodology (RSM): Review of the practical approach in food science and technology research”. *International Journal of Agriculture, Food and Biodiversity,* 1(1), 52-59.

Javed, F., Rehman, F., Khan, A. U., Fazal, T., Hafeez, A. and Rashid, N. (2022). “Real textile industrial wastewater treatment and biodiesel production using microalgae”. *Biomass and Bioenergy*, 165, 106559.

Jebali, A., Acién, F. G., Barradas, E. R., Olguín, E. J., Sayadi, S. and Grima, E. M. (2018). “Pilot-scale outdoor production of Scenedesmus sp. in raceways using flue gases and centrate from anaerobic digestion as the sole culture medium”. *Bioresource Technology*, 262, 1-8.

Kannah, R. Y., Kavitha, S., Karthikeyan, O. P., Rene, E. R., Kumar, G. and Banu, J. R. (2021). “A review on anaerobic digestion of energy and cost effective microalgae pretreatment for biogas production”. *Bioresource technology*, 125055.

Kayan, I. and Oz, N. A. (2025a). “A dual approach using response surface methodology and machine learning for optimization and enhancement of microalgae-based municipal wastewater treatment”. *Journal of Chemical Technology & Biotechnology*, 100(6), 1244-1256.

Kayan, I. and Ayman Öz, N., (2025b). "Integrating response surface methodology and decision tree algorithms for valorization of cheese whey wastewater". *Desalination and Water Treatment*, vol.322, no.101129, 1-15.

Khalid, A. A. H., Yaakob, Z., Abdullah, S. R. S. and Takriff, M. S. (2019). "Analysis of the elemental composition and uptake mechanism of Chlorella sorokiniana for nutrient removal in agricultural wastewater under optimized response surface methodology (RSM) conditions", *Journal of Cleaner Production,* 210, 673-686.

Khakimova, N., Maravić, N., Davidović, P., Blagojević, D., Bečelić-Tomin, M., Simeunović, J. and Mišan, A. (2022). "Sugar beet processing wastewater treatment by microalgae through biosorption". *Water*, 14(6), 860.

Khan M., Shin J. and Kim J. (2018). "The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products". *Microbial Cell Factories*, 17(1).

Kholomyeva, M., Vurm, R., Tajnaiová, L., Šír, M., Maslova, M. and Kočí, V. (2020). "Phycoremediation of landfill leachate with Desmodesmus subspicatus: a pre-treatment for reverse osmosis". *Water*, 12(6), 1755.

Kilian, O., Benemann, C. S., Niyogi, K. K. and Vick, B. (2011). "High-efficiency homologous recombination in the oil-producing alga Nannochloropsis sp". *Proceedings of the National Academy of Sciences*, 108(52), 21265-21269.

Kim C.W., Sung M.G., Nam K., Moon M., Kwon J.H. and Yang J.W. (2014). "Effect of monochromatic illumination on lipid accumulation of Nannochloropsis gaditana under continuous cultivation". *Bioresource Technology*, 159, 30–35.

Kitanou, S., Ayyoub, H., Touir, J., Zdeg, A., Benabdallah, S., Taky, M. and Elmidaoui, A. (2021). "A comparative examination of MBR and SBR performance for municipal wastewater treatment." *Water Practice & Technology*, 16(2), 582-591.

Koyande, A. K., Chew, K. W., Rambabu, K., Tao, Y., Chu, D. T. and Show, P. L. (2019). "Microalgae: A potential alternative to health supplementation for humans". *Food Science and Human Wellness*, 8(1), 16-24.

Kumari, R., Ankit, H. and Basu, S. (2021). “Reclamation of water from dairy wastewater using membrane bioreactor (MBR)–Membrane filtration processes”. *Materials Today: Proceedings*, 47, 1452-1456.

Kurniawan, S. B., Čížková, M., Ahmad, A., Wibowo, Y. G., Said, N. S. M., Jusoh, H. H. W. and Imron, M. F. (2025). “Autotrophic vs. heterotrophic microalgae: Juxtaposition of performances in treating organic-rich effluent”. *Desalination and Water Treatment*, 101159.

Kusmayadi, A., Lu, P. H., Huang, C. Y., Leong, Y. K., Yen, H. W. and Chang, J. S. (2022). “Integrating anaerobic digestion and microalgae cultivation for dairy wastewater treatment and potential biochemicals production from the harvested microalgal biomass”. *Chemosphere*, 291, 133057.

Kwon, G., Nam, J. H., Kim, D. M., Song, C. and Jahng, D. (2020). “Growth and nutrient removal of Chlorella vulgaris in ammonia-reduced raw and anaerobically-digested piggery wastewaters”. *Environmental Engineering Research*, 25(2), 135-146.

Lauritano, C., Andersen, J. H., Hansen, E., Albrigtsen, M., Escalera, L., Esposito, F. and Ianora, A. (2016). “Bioactivity screening of microalgae for antioxidant, anti-inflammatory, anticancer, anti-diabetes, and antibacterial activities”. *Frontiers in marine science*, 3, 68.

Leal, C. D., Pereira, A. D., Nunes, F. T., Ferreira, L. O., Coelho, A. C. C., Bicalho, S. K. and de Araújo, J. C. (2016). “Anammox for nitrogen removal from anaerobically pre-treated municipal wastewater: effect of COD/N ratios on process performance and bacterial community structure”. *Bioresource technology*, 211, 257-266.

Lee, M. Y. and Shin, H. W. (2003). “Cadmium-induced changes in antioxidant enzymes from the marine alga Nannochloropsis oculata”. *Journal of Applied Phycology*, 15, 13-19.

Leong, Y. K. and Chang, J. S. (2024). “Proteins and bioactive peptides from algae: Insights into antioxidant, anti-hypertensive, anti-diabetic and anti-cancer activities”. *Trends in Food Science & Technology*, 145, 104352.

Li, Y., Gu, H., Zhao, G., Li, H., Zhang, M., Yang, X. L. and Song, H. L. (2023). "Carbon accounting of A2O process based on carbon footprint in a full-scale municipal wastewater treatment plant". *Journal of Water Process Engineering*, 55, 104162.

Li, Y., Chen, Y. F., Chen, P., Min, M., Zhou, W., Martinez, B. and Ruan, R. (2011). "Characterization of a microalga Chlorella sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production", *Bioresource Technology*, 102(8), 5138-5144.

Li, X. and Zhang, R. (2002). "Aerobic treatment of dairy wastewater with sequencing batch reactor systems". *Bioprocess and biosystems engineering*, 25, 103-109.

Lienhoop, N., Al-Karablieh, E. K., Salman, A. Z. and Cardona, J. A. (2014). "Environmental cost–benefit analysis of decentralized wastewater treatment and re-use: a case study of rural Jordan". *Water Policy*, 16(2), 323-339.

Liu, H., Chen, Y. and Wu, J. (2017). "Municipal wastewater biological nutrient removal driven by the fermentation liquid of dairy wastewater". *Environmental Technology*, 38(21), 2639-2649.

Lozano-Muñoz, I., Muñoz, S., Díaz, N. F., Medina, A., Bazaes, J. and Riquelme, C. (2020). "Nutritional enhancement of farmed salmon meat via non-GMO Nannochloropsis gaditana: eicosapentaenoic acid (EPA, 20: 5 n-3), docosapentaenoic acid (DPA, 22: 5 n-3) and vitamin D3 for human health". *Molecules*, 25(20), 4615.

Ma, Y., Wang, Z., Yu, C., Yin, Y. and Zhou, G. (2014). "Evaluation of the potential of 9 Nannochloropsis strains for biodiesel production". *Bioresource technology*, 167, 503-509.

Ma, X. N., Chen, T. P., Yang, B., Liu, J. and Chen, F. (2016). "Lipid production from Nannochloropsis". *Marine drugs*, 14(4), 61.

Maadane, A., Merghoub, N., El Mernissi, N., Ainane, T., Amzazi, S., Wahby, I. and Bakri, Y. (2017). "Antimicrobial activity of marine microalgae isolated from Moroccan coastlines". *The Journal of Microbiology, Biotechnology and Food Sciences*, 6(6), 1257.

Magdalena, J. A., Ballesteros, M. and González-Fernandez, C. (2018). "Efficient anaerobic digestion of microalgae biomass: proteins as a key macromolecule". *Molecules*, 23(5), 1098.

Mahdy, A., Fotidis, I. A., Mancini, E., Ballesteros, M., González-Fernández, C. and Angelidaki, I. (2017). "Ammonia tolerant inocula provide a good base for anaerobic digestion of microalgae in third generation biogas process". *Bioresource Technology*, 225, 272-278.

Maleki, S., Karimi-Jashni, A. and Mousavifard, M. (2024). "Removal of Ni (II) ions from wastewater by ion exchange resin: Process optimization using response surface methodology and ensemble machine learning techniques", *Journal of Environmental Chemical Engineering,* 12(2), 112417.

Mayers J.J., Nilsson A.E., Albers E. and Flynn K.J. (2017). "Nutrients from anaerobic digestion effluents for cultivation of the microalga Nannochloropsis sp.—impact on growth, biochemical composition and the potential for cost and environmental impact savings". *Algal Research*, 26:275–86.

Mazaheri, A. and Doosti, M. R. (2024). "Enhancing synthetic vinasse treatment efficiency using an integrated UASB-Modified Bardenpho Process". *Bioresources and Bioprocessing*, 11(1), 1-16.

Mendoza, J. L., Granados, M. R., De Godos, I., Acién, F. G., Molina, E., Banks, C. and Heaven, S. (2013). "Fluid-dynamic characterization of real-scale raceway reactors for microalgae production". *Biomass and Bioenergy*, 54, 267-275.

Mitra, M., Patidar, S. K., George, B., Shah, F. and Mishra, S. (2015). "A euryhaline Nannochloropsis gaditana with potential for nutraceutical (EPA) and biodiesel production". *Algal research*, 8, 161-167.

Moazami, N., Ashori, A., Ranjbar, R., Tangestani, M., Eghtesadi, R. and Nejad, A. S. (2012). "Large-scale biodiesel production using microalgae biomass of Nannochloropsis". *Biomass and bioenergy*, 39, 449-453.

Moglie, M., Biancini, G. and Cioccolanti, L. (2024). "Environmental and economic analysis of an olive mill wastewater treatment system integrated with microalgae production". *The International Journal of Life Cycle Assessment*, 29(6), 1000-1020.

Moustafa, A. M., ElNadi, M. E. H., Abdelmomen, M. M. and Nagy, A. M. (2023). "Impact of microalgae layer thickness on the treatment performance of drain water". *Scientific Reports*, 13(1), 20785.

Myers, R. H., Montgomery, D. C. and Anderson-Cook, C. M. (2016). "Response surface methodology: process and product optimization using designed experiments". *John Wiley & Sons*.

Njoku C.N. and Otisi S.K. (2023). "Application of central composite design with design expert v13 in process optimization, in Response Surface Methodology Research Advances and Applications". *IntechOpen*, London, UK.

Obaideen, K., Abdelkareem, M. A., Wilberforce, T., Elsaid, K., Sayed, E. T., Maghrabie, H. M. and Olabi, A. G. (2022). "Biogas role in achievement of the sustainable development goals: Evaluation, Challenges, and Guidelines". *Journal of the Taiwan Institute of Chemical Engineers*, 131, 104207.

Onay, M. (2018). "Bioethanol production from Nannochloropsis gaditana in municipal wastewater". *Energy Procedia*, 153, 253-257.

Oruganti, R. K., Biji, A. P., Lanuyanger, T., Show, P. L., Sriariyanun, M., Upadhyayula, V. K. and Bhattacharyya, D. (2023). "Artificial intelligence and machine learning tools for high-performance microalgal wastewater treatment and algal biorefinery: A critical review". S*cience of The Total Environment*, 876, 162797.

Oz, N. A., Ince, O. and Ince, B. K. (2004). "Effect of wastewater composition on methanogenic activity in an anaerobic reactor". J*ournal of Environmental Science and Health*, Part A, 39(11-12), 2941-2953.

Padri M., Boontian N. and Piasai C. (2020). "The Potency of Municipal and Industrial Waste and Wastewater as Substrate for Microalgae". *SUT International Virtual Conference on Science and Technology*.

Pane, G., Cacciola, G., Giacco, E., Mariottini, G. L. and Coppo, E. (2015). "Assessment of the antimicrobial activity of algae extracts on bacteria responsible of external otitis". *Marine drugs*, 13(10), 6440-6452.

Panis, G. and Carreon, J. R. (2016). “Commercial astaxanthin production derived by green alga Haematococcus pluvialis: A microalgae process model and a techno-economic assessment all through production line”. *Algal research*, 18, 175-190.

Pannirselvam, R., Ibrahim, Y. and Gopalakrishnan, A. N. (2015). “Simultaneous carbon and nutrient removal from dairy wastewater in sequencing batch reactor (SBR)”. *Global Nest Journal.*

Papalia, T., Sidari, R. and Panuccio, M. R. (2019). “Impact of different storage methods on bioactive compounds in Arthrospira platensis biomass”. *Molecules*, 24(15), 2810.

Parashar, A., Shah, N., Rane, M. and Shastri, Y. (2023). “Biogas-Assisted Growth of Chlorella vulgaris in an Open Raceway Pond: Proof of Concept and Economic Assessment”. *Chemical Engineering & Technology*, 46(7), 1455-1463.

Pereira, L. T., Lisboa, C. R., Costa, J. A., da Rosa, L. M. and de Carvalho, L. F. (2022). “Evaluation of protein content and antimicrobial activity of biomass from Spirulina cultivated with residues from the brewing process”. *Journal of Chemical Technology & Biotechnology*, 97(1), 160-166.

Pérez, M.J., Falqué, E. and Domínguez, H. (2016). “Antimicrobial action of compounds from marine seaweed”. *Marine Drugs*, 14, 52.

Plouviez, M. and Guieysse, B. (2023). “Microalgae cultivation: closing the laboratory-field yield gap”. *Frontiers in Bioengineering and Biotechnology.*

Polishchuk A., Valev D., Tarvainen M., Mishra S., Kinnunen V. and Antal T. (2015). “Cultivation of Nannochloropsis for eicosapentaenoic acid production in wastewaters of pulp and paper industry”. *Bioresource Technology*, 193, 193,469–476.

Pratt, R., Daniels, T. C., Eiler, J. J., Gunnison, J. B., Kumler, W. D., Oneto, J. F. and Strain, H. H. (1944). “Chlorellin, an antibacterial substance from Chlorella”. *Science*, 99(2574), 351-352.

Rahman, M. H., Mia, S. H., Nessa, A., Badhan, M. A. and Islam, M. A. (2015). “Reducing the Volume of Garbage and Production of Biogas through Anaerobic Decomposition”. *Journal of Environmental Science and Natural Resources*, 8(2), 33-36.

Raj J.V.A., Kumar R.P., Vijayakumar B., Gnansounou E. and Bharathiraja B. (2021). "Modelling and process optimization for biodiesel production from Nannochloropsis salina using artificial neural network". *Bioresource Technology*, 329, 124872.

Rasmussen, R. S. and Morrissey, M. T. (2007). "Marine biotechnology for production of food ingredients". *Advances in food and nutrition research*, 52, 237-292.

Raso, S., Van Genugten, B., Vermuë, M. and Wijffels, R. H. (2012). "Effect of oxygen concentration on the growth of Nannochloropsis sp. at low light intensity". J*ournal of applied phycology*, 24, 863-871.

Ravikumar, M., Velmurugan, K., John, A. and Selvarajan, E. (2024). "Microalgae to remove pharmaceutical and personal care products (PPCPs) from wastewater". *Biocatalysis and Agricultural Biotechnology*, 103415.

Razzak, S. A., Alam, M. S., Hossain, S. Z. and Rahman, S. M. (2024). "Tree-based machine learning for predicting Neochloris oleoabundans biomass growth and biological nutrient removal from tertiary municipal wastewater", *Chemical Engineering Research and Design,* 210, 614-624.

Resdi, R., Lim, J. S. and Idris, A. (2021). "Batch kinetics of nutrients removal for palm oil mill effluent and recovery of lipid by Nannochloropsis sp.". *Journal of Water Process Engineering*, 40, 101767.

Richmond, A. and Cheng-Wu, Z. (2001). "Optimization of a flat plate glass reactor for mass production of Nannochloropsis sp. outdoors". Journal of biotechnology, 85(3), 259-269.

Rodríguez-Miranda, E., Acién, F., Guzmán, J., Berenguel, M. and Visioli, A. (2020). "A new model to analyze the temperature effect on the microalgae performance at large scale raceway reactors". *Biotechnology and Bioengineering.* 118(2), 877-889.

Romagnoli, F., Ievina, B., Perera, W. A. A. R. P. and Ferrari, D. (2020). "Novel stacked modular open raceway ponds for microalgae biomass cultivation in biogas plants: preliminary design and modelling". *Rigas Tehniskas Universitates Zinatniskie Raksti*, 24(2), 1-19.

Sabatini, S. E., Juarez, A. B., Eppis, M. R., Bianchi, L., Luquet, C. M. and de Molina, M. D. C. R. (2009). "Oxidative stress and antioxidant defenses in two green microalgae exposed to copper". *Ecotoxicology and environmental safety*, 72(4), 1200-1206.

Sabour M.R. and Amiri A. (2017). "Comparative study of ANN and RSM for simultaneous optimization of multiple targets in Fenton treatment of landfill leachate". Waste Management, 65, 54–62.

Sadiq, U. A. F. M., Yow, M. E. and Jamaian, S. S. (2018). "The extended Monod model for microalgae growth and nutrient uptake in different wastewaters". *International Journal of Engineering & Technology*, 7(4.30), 200.

Saha S. and Murray P. (2018). "Exploitation of microalgae species for nutraceutical purposes: cultivation aspects". *Fermentation*, 4(2), 46.

Safafar, H., Van Wagenen, J., Møller, P. and Jacobsen, C. (2015). "Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater". *Marine drugs*, 13(12), 7339-7356.

Saleem, M., Hanif, M. U., Bahadar, A., Iqbal, H., Capareda, S. C. and Waqas, A. (2020). "The effects of hot water and ultrasonication pretreatment of microalgae (Nannochloropsis oculata) on biogas production in anaerobic co-digestion with cow manure". *Processes*, 8(12), 1558.

Sánchez-Zurano, A., Rodríguez-Miranda, E., Guzmán, J. L., Acién-Fernández, F. G., Fernández-Sevilla, J. M. and Molina Grima, E. (2021). "ABACO: a new model of microalgae-bacteria consortia for biological treatment of wastewaters". *Applied Sciences*, 11(3), 998.

Santhar, D. T., Haq, M. B., Marudhupandi, T., Vaseeharan, B., Rajan, D. K. and Moovendhan, M. (2021). "Evaluation of chemical compositions and antioxidant potential of marine microalgae of the genus Nannochloropsis". *Biomass Conversion and Biorefinery*, 1-7.

Santos, A. D., Martins, R. C., Quinta-Ferreira, R. M. and Castro, L. M. (2020). "Moving bed biofilm reactor (MBBR) for dairy wastewater treatment". *Energy Reports*, 6, 340-344.

Sarma, U., Hoque, M. E., Thekkangil, A., Venkatarayappa, N. and Rajagopal, S. (2024). "Microalgae in removing heavy metals from wastewater–An advanced green technology for urban wastewater treatment". *Journal of Hazardous Materials Advances*, 15, 100444.

Sayed, E. T., Rezk, H., Abdelkareem, M. A. and Olabi, A. G. (2024). "Artificial neural network based modelling and optimization of microalgae microbial fuel cell". *International Journal of Hydrogen Energy*, 52, 1015-1025.

Schwede, S., Kowalczyk, A., Gerber, M. and Span, R. (2013). "Anaerobic co-digestion of the marine microalga Nannochloropsis salina with energy crops". *Bioresource technology*, 148, 428-435.

Seger M., Unc A., Starkenburg S.R., Holguin F.O. and Lammers P.J. (2019). "Nutrient-driven algal-bacterial dynamics in semi-continuous, pilot-scale photobioreactor cultivation of Nannochloropsis salina CCMP1776 with municipal wastewater nutrients". *Algal Research*, 39,101457.

Shaima, A. F., Yasin, N. H. M., Ibrahim, N., Takriff, M. S., Gunasekaran, D. and Ismaeel, M. Y. (2022). "Unveiling antimicrobial activity of microalgae Chlorella sorokiniana (UKM2), Chlorella sp.(UKM8) and Scenedesmus sp.(UKM9)". *Saudi journal of biological sciences*, 29(2), 1043-1052.

Sharma, N., Singh, A. and Batra, N. (2019). "Modern and emerging methods of wastewater treatment". *Ecological wisdom inspired restoration engineering*, 223-247.

Simsek, K. and Sisman-Aydin, G. (2025). "The potential of growth and pollutant removal of green microalgae Neglectella solitaria for real textile wastewater". *Environmental Technology & Innovation*, 37, 104014.

Singh, P., Kansal, A. and Carliell-Marquet, C. (2016). "Energy and carbon footprints of sewage treatment methods". *Journal of Environmental Management*, 165, 22-30.

Singh, R., Birru, R. and Sibi, G. (2017). "Nutrient removal efficiencies of Chlorella vulgaris from urban wastewater for reduced eutrophication". *Journal of Environmental Protection,* 8(01), 1.

Singh, V. and Mishra, V. (2020). "Enhanced biomass production and nutrient removal efficiency from urban wastewater by Chlorella pyrenoidosa in batch bioreactor

system: optimization and model simulation", *Desalination and Water Treatment* 197, 52-66.

Singh, V. and Mishra, V. (2021). "Exploring the effects of different combinations of predictor variables for the treatment of wastewater by microalgae and biomass production". *Biochemical Engineering Journal*, 174, 108129.

Singh, V. and Mishra, V. (2022). "Evaluation of the effects of input variables on the growth of two microalgae classes during wastewater treatment". *Water Research,* 213, 118165.

Singh, V., Verma, M., Chivate, M. S. and Mishra, V. (2023). "Machine learning-based optimisation of microalgae biomass production by using wastewater". *Journal of Environmental Chemical Engineering*, 11(6), 111387,

Solé-Bundó, M., Garfí, M., Matamoros, V. and Ferrer, I. (2019a). "Co-digestion of microalgae and primary sludge: Effect on biogas production and microcontaminants removal". *Science of the total environment*, 660, 974-981.

Solé-Bundó, M., Passos, F., Romero-Güiza, M. S., Ferrer, I. and Astals, S. (2019b). "Co-digestion strategies to enhance microalgae anaerobic digestion: A review". *Renewable and Sustainable Energy Reviews*, 112, 471-482.

Stolz, P. and Obermayer, B. (2005). "Manufacturing microalgae for skin care". *Cosmetics and toiletries*, 120(3), 99-106.

Su, Y., Zhu, X., Zou, R. and Zhang, Y. (2022). "The interactions between microalgae and wastewater indigenous bacteria for treatment and valorization of brewery wastewater". *Resources, Conservation and Recycling*, 182, 106341.

Su, B., Huang, J., Zhang, J., Cao, L., Zhang, R., Wang, H. and Wang, X. (2025). "Development of a novel sterilized thin-film photobioreactor for efficient microalgae cultivation by mixotrophic mode". *Aquacultural Engineering*, 110, 102539.

Suarez, S., Lema, J. M. and Omil, F. (2009). "Pre-treatment of hospital wastewater by coagulation–flocculation and flotation". *Bioresource technology*, 100(7), 2138-2146.

Sundaram, T., Rajendran, S., Gnanasekaran, L., Rachmadona, N., Jiang, J. J., Khoo, K. S. and Show, P. L. (2023). “Bioengineering strategies of microalgae biomass for biofuel production: recent advancement and insight”. *Bioengineered*, 14(1), 2252228.

Sundui, B., Ramirez Calderon, O. A., Abdeldayem, O. M., Lázaro-Gil, J., Rene, E. R. and Sambuu, U. (2021). “Applications of machine learning algorithms for biological wastewater treatment: updates and perspectives”. *Clean Technologies and Environmental Policy,* 23, 127-143.

Surendhiran, D., Vijay, M., Razack, A., Subramaniyan, T., Shellomith, A. S. and Tamilselvam, K. (2014). “A green synthesis of antimicrobial compounds from marine microalgae Nannochloropsis oculata”. *Journal of coastal life medicine*, 2(11), 862-859.

Şirin, S. and Sillanpää, M. (2015). “Cultivating and harvesting of marine alga Nannochloropsis oculata in local municipal wastewater for biodiesel”. *Bioresource technology,* 191, 79-87 .

Tangahu, B. V., Triatmojo, A., Purwanti, I. F. and Kurniawan, S. B. (2018). “The effect of light-emitting diodes illumination period and light intensity on high rate algal reactor system in laundry wastewater treatment”. J*ournal of Ecological* Engineering, 19(6).

Taisir, M., Teo, C. L., Idris, A. and Yusuf, A. M. (2016). “Cultivation of Nannochloropsis sp. using narrow beam angle light emitting diode in an internally illuminated photobioreactor”. Bioresources and Bioprocessing, 3, 1-8.

Tedeschi, A. M., Di Caprio, F., Piozzi, A., Pagnanelli, F. and Francolini, I. (2021). “Sustainable bioactive packaging based on thermoplastic starch and microalgae”. *International Journal of Molecular Sciences*, 23(1), 178.

Tekindal M.A., Bayrak H., Özkaya B. and Yavuz Y. (2014). “Second-order response surface method: factorial experiments an alternative method in the field of agronomy”. Turkish Journal Of Field Crops, 19, 35–45.

Thoré, E. S.J., Muylaert, K., Bertram, M. G. and Brodin, T. (2023). “Microalgae”. *Current Biology,* 33 (3), R91 - R95

Torregrossa, D., Leopold, U., Hernández-Sancho, F. and Hansen, J. (2018). "Machine learning for energy cost modelling in wastewater treatment plants". *Journal of Environmental Management,* 223, 1061-1067.

Torres-Franco, A., Passos, F., Figueredo, C., Mota, C. and Muñoz, R., (2021). "Current advances in microalgae-based treatment of high-strength wastewaters: challenges and opportunities to enhance wastewater treatment performance". *Reviews in Environmental Science and Bio/Technology,* 20, 209-235 .

Toyub M.A., Miah M.I., Habib M.A.B. and Rahman M.M. (2008). "Growth perfor mance and nutritional value of Scenedesmus obliquus cultured in different concentrations of sweetmeat factory waste media". Bangladesh Journal of Animal Science, 37, 86–93.

Trivedi, J., Atray, N. and Agrawal, D. (2021). "Enhanced biomass production of Scenedesmus obliquus in a flat-panel photobioreactor, grown in photoautotrophic mode". *Biofuels,* 12, 53–59.

Tuantet, K., Janssen, M., Temmink, H., Zeeman, G., Wijffels, R. H. and Buisman, C. J. (2014). "Microalgae growth on concentrated human urine". *Journal of applied phycology*, 26(1), 287-297.

Van Eck, N. and Waltman, L. (2010). "Software survey: VOSviewer, a computer program for bibliometric mapping". *Scientometrics,* 84(2), 523-538.

Vandana, R. and Rakesh, S. (2023). "Phyco-remediation of sewage wastewater by microalgae". *In Sewage Management*. IntechOpen.

Van Loosdrecht, M. C. M., Hao, X., Jetten, M. S. M. and Abma, W. (2004). "Use of Anammox in urban wastewater treatment". *Water Science and Technology: Water Supply*, 4(1), 87-94.

Vargas-Estrada, L., Longoria, A., Arenas, E., Moreira, J., Okoye, P. U., Bustos-Terrones, Y. and Sebastian, P. J. (2022). "A review on current trends in biogas production from microalgae biomass and microalgae waste by anaerobic digestion and co-digestion". *BioEnergy Research*, 15(1), 77-92.

Vassalle, L., Ferrer, I., Passos, F., Mota Filho, C. R. and Garfí, M. (2023). "Nature-based solutions for wastewater treatment and bioenergy recovery: A comparative Life Cycle Assessment". *Science of The Total Environment*, 880, 163291.

Vehapi, M., İnan, B., Yılmaz, A. and Ozcimen, D. (2021). "Antimicrobial Activity of Algal Extracts Against Foodborne Pathogens". *Avrupa Bilim ve Teknoloji Dergisi*, (27), 36-43.

Wali, A. F., Al Dhaheri, Y., Ramakrishna Pillai, J., Mushtaq, A., Rao, P. G., Rabbani, S. A. and Farraj, D. A. A. (2020). "Lc-ms phytochemical screening, in vitro antioxidant, antimicrobial and anticancer activity of microalgae Nannochloropsis oculata extract". *Separations*, 7(4), 54.

Wang L., Chen L. and Wu S. (2020). "Nutrient reduction of dairy manure through solid-liquid separation with flocculation and subsequent microalgal treatment". *Applied Biochemistry and Biotechnology*, 190, 1425–37.

Wen, X., Tao, H., Peng, X., Wang, Z., Ding, Y., Xu, Y. and Li, Y. (2019). "Sequential phototrophic–mixotrophic cultivation of oleaginous microalga Graesiella sp. WBG-1 in a 1000 m 2 open raceway pond". *Biotechnology for Biofuels*, 12, 1-14.

Widowati, I., Zainuri, M., Kusumaningrum, H. P. and Hardivillier, Y. (2021). "Antibacterial Activity of Microalgae Dunaliella salina, Tetraselmis chuii and Isochrysis galbana Against Aquatic Pathogens". *Indonesian Journal of Marine Sciences.*

Wijayanti, T. A. and Ansori, M. (2021). "Application of modified green algae Nannochloropsis sp. as adsorbent in the simultaneous adsorption of Methylene Blue and Cu (II) cations in solution".*Sustainable Environment Research*.

Wu, H. and Ying, W. (2019). "Benchmarking machine learning algorithms for instantaneous net surface shortwave radiation retrieval using remote sensing data". *Remote Sensing*, 11(21), 2520.

Wu, W., Hu, Y. and Wang, X. C. (2019). "Mixotrophic cultivation of Chlorella vulgaris for efficient wastewater treatment: optimization using response surface methodology (RSM)", *Desalination Water Treatment,* 171, 369-378.

Wu, J., Gu, X., Yang, D., Xu, S., Wang, S., Chen, X. and Wang, Z. (2021). "Bioactive substances and potentiality of marine microalgae". *Food Science & Nutrition*, 9(9), 5279-5292.

Wu, Z., Duan, H., Li, K. and Ye, L. (2022). "A comprehensive carbon footprint analysis of different wastewater treatment plant configurations". *Environmental Research*, 214, 113818.

Xu, M., Zeng, Q., Li, H., Zhong, Y., Tong, L., Ruan, R. and Liu, H. (2020). "Contribution of glycerol addition and algal–bacterial cooperation to nutrients recovery: a study on the mechanisms of microalgae-based wastewater remediation". *Journal of Chemical Technology & Biotechnology,* 95(6), 1717-1728.

Xue, Z., Yu, Y., Yu, W., Gao, X., Zhang, Y. and Kou, X. (2020). "Development prospect and preparation technology of edible oil from microalgae". *Frontiers in Marine Science*, 7, 402.

Yang, L., Geng, Y., Cui, D., Liu, Z., Xiong, Z., Pavlostathis, S. G. and Luo, X. (2022). "Corrected response surface methodology for microalgae towards optimized ammonia nitrogen removal: A case of rare earth mining tailings wastewater in Southern Jiangxi, China". *Journal of Cleaner Production,* 343, 130998.

Yılmaz, S. and Budak, F. (2018). "Endüstriyel Atıksularda Mikroalgal Nütrient Giderimi ve Biyodizel Üretim Verimliliği: Süt Endüstrisi Örneği". *Çukurova Üniversitesi Mühendislik-Mimarlık Fakültesi Dergisi*, 33(4), 89-96.

Yustinadiar, N., Manurung, R. and Suantika, G. (2020). "Enhanced biomass productivity of microalgae Nannochloropsis sp. in an airlift photobioreactor using low-frequency flashing light with blue LED". Bioresources and Bioprocessing, 7, 1-15.

Zaharieva, M. M., Zheleva-Dimitrova, D., Rusinova-Videva, S., Ilieva, Y., Brachkova, A., Balabanova, V. and Kroumov, A. D. (2022). "Antimicrobial and antioxidant potential of Scenedesmus obliquus microalgae in the context of integral biorefinery concept". *Molecules*, 27(2), 519.

Zahmatkesh, S., Far, S. S. and Sillanpää, M. (2022). "RSM-D-optimal modeling approach for COD removal from low strength wastewater by microalgae, sludge, and activated carbon-case study Mashhad". *Journal of Hazardous Materials Advances*, 7, 100110.

Zayed, G. and Winter, J. (2000). “Inhibition of methane production from whey by heavy metals–protective effect of sulfide”. *Applied microbiology and biotechnology*, 53, 726-731.

Zeng, W., Yao, B., Zhou, Y., Yang, J. and Zhi, D. (2025). “Combination of electrochemical advanced oxidation and biotreatment for wastewater treatment and soil remediation”. *Journal of Environmental Sciences*, 150, 36-53.

Zhang, C., Zhang, Y., Zhuang, B. and Zhou, X. (2014). “Strategic enhancement of algal biomass, nutrient uptake and lipid through statistical optimization of nutrient supplementation in coupling Scenedesmus obliquus-like microalgae cultivation and municipal wastewater treatment”. *Bioresource Technology*, 171, 71-79.

Zhang Y. and Wu Y. (2021). “ Introducing machine learning models to response surface methodologies”. *In Response Surface Methodology in Engineering Science*.

Zhai, J., Li, X., Li, W., Rahaman, M. H., Zhao, Y., Wei, B. and Wei, H. (2017). “Optimization of biomass production and nutrients removal by Spirulina platensis from municipal wastewater”. *Ecological engineering*, 108, 83-92.

Zhao, L. J. and Chai, T. Y. (2012). “Yuan, D. C., Selective ensemble extreme learning machine modeling of effluent quality in wastewater treatment plants”. *International Journal of Automation and Computing,* 9(6), 627-633.

Zhou, W., Wang, Z., Xu, J. and Ma, L. (2018). “Cultivation of microalgae Chlorella zofingiensis on municipal wastewater and biogas slurry towards bioenergy”. *Journal of bioscience and bioengineering*, 126(5), 644-648.

Zhu, Z., Jiang, J. and Fa, Y. (2020). “Overcoming the biological contamination in microalgae and cyanobacteria mass cultivations for photosynthetic biofuel production”. *Molecules*, 25(22), 5220.

Zurera-Cosano, G., García-Gimeno, R. M., Rodríguez-Pérez, R. and Hervás-Martínez, C. (2006). “Performance of response surface model for prediction of Leuconostoc mesenteroides growth parameters under different experimental conditions”. *Food Control,* 17(6), 429-438.

Żyłka, R., Dąbrowski, W., Gogina, E. and Yancen, O. (2018). “Trickling filter for high efficiency treatment of dairy sewage”. *Journal of Ecological engineering*, 19(4), 269-275.