



FEDERAL RURAL UNIVERSITY OF PERNAMBUCO
PRO-RECTORATE OF GRADUATE STUDIES
GRADUATE PROGRAM IN FISHERY RESOURCES AND AQUACULTURE

**USE OF THE DINOFLAGELLATE *Durusdinium glynnii* FOR DOMESTIC EFFLUENT
TREATMENT AND OBTAINING OF BIOCOMPOUNDS**

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Thesis presented to the Graduate Program in Fishery Resources and Aquaculture of the Federal Rural University of Pernambuco as a requirement to obtain the title of Doctor.

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I dedicate this work to my family and friends.

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"If I have seen further, it is by standing on the shoulders of giants" – Isaac Newton."

Abstract

The cultivation of microalgae in wastewater has gained increasing interest among researchers due to its potential for the biological treatment of these effluents. In addition to bioremediation, cultivating microalgae in wastewater enables the production of high-value biocompounds from the resulting biomass. However, the improper disposal of these effluents in natural environments poses significant environmental challenges. For this reason, research in this field has been developed with the aim of providing a sustainable alternative for bioremediation, integrating waste treatment with the production of biomolecules with biotechnological applications. In this context, the present study was structured into three main parts: (i) a literature review on the use of microalgae in domestic effluents, (ii) an experimental investigation into the adaptability of the dinoflagellate *Durusdinium glynnii* to different salinity conditions, and (iii) the practical application of this organism in the treatment of domestic wastewater, focusing on bioremediation and the production of bioactive compounds. The literature review provides an updated survey on the cultivation of microalgae in domestic effluents, highlighting its biotechnological potential. Most studies address microalgae-bacteria consortia and mixed cultures, with an emphasis on *Chlorella vulgaris* and the Scenedesmaceae family. These microalgae are efficient in bioremediation and the production of biomass rich in lipids, proteins, and carbohydrates, with applications in the pharmaceutical, food, agricultural, and aquaculture industries. The primary studied application is biodiesel production; however, there is also potential for biofertilizers, bioplastics, and other bioproducts. Thus, the cultivation of microalgae in domestic effluents emerges as a sustainable alternative for the bioeconomy and waste treatment. Since marine species are not commonly used for the bioremediation of domestic effluents, which generally involve freshwater organisms, it is essential to evaluate their adaptability to this environment. Therefore, an experimental study was conducted to investigate strategies for adapting the dinoflagellate to low-salinity cultivation. This study evaluated the growth and cellular morphology of *D. glynnii* under two acclimation strategies: saline shock and gradual salinity reduction. The species exhibited optimal growth between 20 and 30 g L⁻¹, with adaptation down to 16 g L⁻¹ under the gradual strategy. Extreme salinities (10 and 40 g L⁻¹) compromised growth. Gradual salinity reduction induced the transition from motile cells to predominant spherical forms below 12 g L⁻¹. The results indicate that *D. glynnii* is euryhaline, with potential for cultivation in brackish waters. Finally, a direct application test was performed using domestic effluent in the cultivation of the dinoflagellate. The study evaluated nutrient removal efficiency, growth, and biocompound production by *D. glynnii* cultivated in autoclaved

(AUT) and microfiltered (MIC) domestic effluent. The removal of ammonia, nitrite, and orthophosphate reached maximum efficiencies of 83.64%, 100%, and 100%, respectively. Growth was not affected by the type or concentration of effluent ($p < 0.05$), indicating that domestic effluent can completely replace synthetic culture medium. The production of chlorophylls and carotenoids, particularly peridinin, a carotenoid with anticancer properties, increased with 50% effluent concentration. These findings highlight the potential of *D. glynnii* for bioremediation and the production of high-value biocompounds, contributing to the development of sustainable and biotechnological applications.

Keywords: biocompounds, bioremediation, dinoflagellate, domestic effluent, salinity.

Resumo

O cultivo de microalgas em águas residuais tem despertado crescente interesse entre pesquisadores devido ao seu potencial para o tratamento biológico desses efluentes. Além da biorremediação, o cultivo de microalgas em águas residuais permite a obtenção de biocompostos de alto valor agregado a partir da biomassa produzida. No entanto, o despejo inadequado desses efluentes nos ambientes naturais gera desafios ambientais significativos. Por essa razão, pesquisas nessa área têm sido desenvolvidas com o objetivo de oferecer uma alternativa sustentável para a biorremediação, integrando o tratamento de resíduos à produção de biomoléculas com aplicações biotecnológicas. Nesse contexto, o presente estudo foi estruturado em três partes principais: (i) uma revisão de literatura sobre o uso de microalgas em efluentes domésticos, (ii) uma investigação experimental sobre a adaptabilidade do dinoflagelado *Durusdinium glynnii* a diferentes condições de salinidade e (iii) a aplicação prática desse organismo no tratamento de águas residuais domésticas, com foco na biorremediação e na produção de compostos bioativos. A revisão bibliográfica apresenta um levantamento atualizado sobre o cultivo de microalgas em efluentes domésticos, destacando seu potencial biotecnológico. A maioria dos estudos aborda consórcios microalga-bactéria e culturas mistas, com ênfase em *Chlorella vulgaris* e na família Scenedesmaceae. Essas microalgas são eficientes na biorremediação e na produção de biomassa rica em lipídios, proteínas e carboidratos, com aplicações nas indústrias farmacêutica, alimentícia, agrícola e aquícola. A principal aplicação estudada é a produção de biodiesel, mas há potencial para biofertilizantes, bioplásticos e bioprodutos. Assim, o cultivo de microalgas em efluentes domésticos surge como uma alternativa sustentável para a bioeconomia e o tratamento de resíduos. Como espécies marinhas não são comumente usadas na biorremediação de efluentes domésticos, que geralmente envolvem organismos de água doce, é essencial avaliar sua adaptabilidade a esse ambiente. Por isso, foi conduzido estudo experimental investigou estratégias para adaptar o dinoflagelado ao cultivo em condições de baixa salinidade. Este estudo avaliou seu crescimento e morfologia celular do *D. glynnii* sob duas estratégias de aclimatação: choque salino e redução gradual da salinidade. A espécie apresentou melhor crescimento entre 20 e 30 g L⁻¹, com adaptação até 16 g L⁻¹ na estratégia gradual. Salinidades extremas (10 e 40 g L⁻¹) prejudicaram o crescimento. A redução gradual da salinidade induziu a transição de células móveis para formas esféricas predominantes abaixo de 12 g L⁻¹. Os resultados indicam que *D. glynnii* seja eurialino, com potencial para cultivo em águas salobras. E por fim foi feito o teste propriamente dito com uso de efluente doméstico no cultivo do dinoflagelado. O estudo avaliou a remoção de nutrientes, o crescimento

e a produção de biocompostos pelo *D. glynnii* cultivado em efluente doméstico autoclavado (AUT) e microfiltrado (MIC). A remoção de amônia, nitrito e ortofosfato atingiu eficiência máxima de remoção de 83,64%, 100% e 100%, respectivamente. O crescimento não foi afetado pelo tipo ou concentração do efluente ($p < 0,05$), indicando que ele pode substituir completamente o meio sintético. A produção de clorofilas e carotenoides, especialmente a peridinina, que é um carotenoide com propriedades anticâncer, aumentou com 50% de efluente. Diante disso, os resultados destacam o potencial de *D. glynnii* para a biorremediação e a produção de biocompostos de alto valor.

Palavras-chave: biocompostos, biorremediação, efluente doméstico, dinoflagelado, salinidade.

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1. Introduction

1.1- Research context

The environmental pollution caused by the disposal of domestic wastewater has affected water bodies, groundwater, and the environment in both developed and developing countries (Sabeen et al., 2018). Approximately half of the world's seas, rivers, and lakes have been contaminated with untreated domestic and industrial wastewater, which, when discharged into water bodies, leads to changes in pH and a decrease in dissolved oxygen concentration, resulting in the death of various aquatic life forms (Sabeen et al., 2018; Moondra et al., 2020) due to the process of eutrophication of aquatic ecosystems.

In Brazil, the municipal sanitation service accounted for 60.3% in 2017, representing an increase of 9.4% compared to the previous survey conducted in 2008. However, it is suggested that the growth of this service occurs more vertically than horizontally, meaning that the expansion takes place in areas already served rather than in new municipalities (IBGE, 2020). This fact is due to rapid population growth, which increases wastewater production and raises concerns about water scarcity, as the discharge of untreated effluent with high nutrient content leads to eutrophication and a decrease in the quality of water resources (Katam and Bhattacharyya, 2019).

To prevent pollution and eutrophication, urban and industrial wastewater must be treated before being discharged into the environment (Ruiz-Martinez et al., 2012). In treatment plants, wastewater undergoes three different processes, according to Arashiro et al. (2019) and Rout et al. (2021): (i) removal of organic and inorganic solids from raw water through sedimentation; (ii) biological removal of residual organics and a small amount of dissolved nutrients; and (iii) removal of nutrients, refractory organics, heavy metals, among others.

This highlights the importance of developing alternative techniques to reduce environmental impact through nutrient removal from wastewater and the application of circular bioeconomy through the recovery and reuse of nutrients from these sources, avoiding the depletion of finite resources (Rout et al., 2021). Over the years, efforts have been made to introduce technologies for wastewater treatment, such as conventional filtration, coagulation-flocculation, biological treatment systems, among others (Ezugbe and Rathilal, 2020). In this context, the application of a biological treatment system with microalgae in wastewater has been a promising alternative, as effluents contain nitrogen, phosphorus, carbon compounds, and other macro and micronutrients necessary for the growth of microalgae (Daneshvar et al., 2018), and the biochemical composition of microalgae enables biosorption processes (Dias et al., 2019).

Cultivating these organisms in nutrient-rich wastewater contributes to nutrient recovery and waste valorization (Assemany et al. 2020).

Despite the great versatility in the application of microalgal biomass, it has not yet been commercialized due to its high production cost (Assis et al. 2019). Therefore, this approach also emerges to reduce production costs in algae cultivation (i.e., alternative sources of nutrients and water), while providing a sustainable method for wastewater treatment (i.e., an alternative to conventional activated sludge) (Nguyen et al., 2022). In these cultivation systems, algae use macronutrients and micronutrients from wastewater for their metabolic activities and store excess energy in the form of lipids, carbohydrates, and proteins (Baldev et al., 2021), in addition to pigments and carotenoids of biotechnological interest.

Among the microalgae with potential for exploitation in aquaculture, we can mention the group of dinoflagellates. According to Oliveira et al. (2020), these organisms have the ability to produce allelopathic compounds and grow in a mixotrophic nutritional mode, making them highly suitable for wastewater treatment. Moreover, most of these photosynthetic organisms possess brownish-red chloroplasts containing chlorophylls *a* and *c*₂, β -carotene, a group of exclusive xanthophylls, including peridinin, and cellulose plates forming the theca (Cousseau et al., 2020). They are also a natural source of pigments, exclusive secondary metabolites, toxins, and bioactive molecules with potential applications in biomedical, toxicological, and pharmacological fields (Gallardo-Rodríguez et al., 2012; Langenbach and Melkonian, 2019).

However, few efforts have been dedicated to the controlled cultivation of dinoflagellates in bioreactors, with the main focus being studies on harmful algal blooms (*bloom* dynamics) (Gallardo-Rodríguez et al., 2012). It is known that the cultivation of dinoflagellates in closed photobioreactors is challenging due to the sensitivity of cultures to turbulence and shear forces (Langenbach and Melkonian, 2019), which is a limiting factor for the study of these organisms. However, research points to the feasibility of cultivating the dinoflagellate species *Symbiodinium voratum* in biofilm photobioreactors with cell immobilization and in dual-layer porous substrate bioreactors (Benstein et al., 2014; Langenbach and Melkonian, 2019).

Dinoflagellates from the Symbiodiniaceae family form symbiotic relationships with various hosts, including foraminifera, ciliophora, and mollusca. However, their role as endosymbionts of cnidarians is well known, as they play a fundamental role in coral reef building (Klueter et al., 2017). The species focused on in this study is *Durusdinium glynnii*, which demonstrates bioremediation and biotechnological potential (Oliveira et al., 2024), although research involving its cultivation is scarce. In contrast, published studies on the species focus on

genotypic diversity and spatio-temporal distribution (Pettay et al., 2011), and the description of population genetics, phylogenetics, ecology, and biogeography (Wham et al., 2017).

Given the scarcity of literature and its potential for aquaculture, this study aims to fill gaps related to the sustainable cultivation of the dinoflagellate *D. glynnii*. Accordingly, this thesis is structured into three parts. The first part is a literature review, which aims to address the key aspects related to the cultivation of microalgae in domestic effluents, identifying their potential, existing gaps, and future perspectives on the subject. The second part evaluated the adaptive capacity of the dinoflagellate subjected to salinity tests to identify the species potential to be cultivated in low-salinity wastewater. Finally, a bioremediation test was conducted using the dinoflagellate in domestic wastewater, aiming to assess its growth potential, nutrient removal efficiency, and production of biocompounds.

1.2. Objectives

General

To promote the bioremediation of domestic effluent through the cultivation of the dinoflagellate *Durusdinium glynnii*, aiming at the increase of cellular biomass and the accumulation of biocompounds of high biotechnological value.

Specific

- To conduct a literature review to understand the current scenario of microalgae cultivation in domestic effluents, identifying the main approaches, gaps, and perspectives.
- To evaluate the effect of different acclimation strategies for the dinoflagellate *Durusdinium glynnii* to low salinity cultivation, in order to identify the species' adaptability to cultivation in effluent with reduced salinity.
- To analyze the capacity of *Durusdinium glynnii* to remove nitrogenous and phosphorous compounds present in domestic effluents, aiming to determine its efficiency in bioremediation.
- To evaluate the growth kinetics of the dinoflagellate, including the determination of maximum cell density, the day of maximum cell density, growth rate, doubling time, and specific growth rate.
- To quantify the concentration of high-value biocompounds produced by the dinoflagellate, with an emphasis on the analysis of pigments and carotenoids.

2. Article 1: Microalgae-based domestic wastewater treatment: a review of biological aspects, bioremediation potential and production of biomass with biotechnological high-value

Scientific article published in the journal Environmental Monitoring and Assessment (ISSN: 1573-2959).

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Microalgae-based domestic wastewater treatment: a review of biological aspects, bioremediation potential and production of biomass with biotechnological high-value

Brandão et al., 2023

Abstract

This review aims to perform an updated bibliographical survey on the cultivation of microalgae in domestic wastewater with a focus on biotechnological aspects. It was verified that the largest number of researches developed was about cultures in microalgae-bacteria consortium and mixed cultures of microalgae, followed by researches referring to the species *Chlorella vulgaris* and to the family Scenedesmaceae. According to published studies, these microorganisms are efficient in the biological treatment of domestic wastewater, as well as in the production of high value-added biomass, as they are capable of biosorbing the organic and inorganic compounds present in the culture medium, thus generating cells with high levels of lipids, proteins, and carbohydrates. These compounds are of great importance for different industry sectors, such as pharmaceuticals, food, and also for agriculture and aquaculture. In addition, biomolecules produced by microalgae can be extracted for several biotechnological applications; however, most studies focus on the production of biofuels, with biodiesel being the main one. There are also other emerging applications that still require more in-depth research, such as the use of biomass as a biofertilizer and biostimulant in the production of bioplastic. Therefore, it is concluded that the cultivation of microalgae in domestic wastewater is a sustainable way to promote effluent bioremediation and produce valuable biomass for the biobased industry, contributing to the development of technology for the green economy.

Keywords: microalgae, domestic wastewater, effluent treatment, bioremediation, biotechnology, nutrients.

Introduction

Microalgae are single-celled organisms that grow in freshwater, saltwater, and even wetland environments. Although they lack complex structures and organs, they perform photosynthesis using light energy due to the photosynthetic pigments (i.e., chlorophyll) in their cells. In this process, carbon dioxide and water are converted into organic macromolecules through metabolic pathways analogous to those of higher plants (Tan et al., 2020). These organisms are sources of nutrients that include vitamins, minerals, proteins with essential amino acids, polyunsaturated fatty acids, dietary fiber, and structural biopolymers, such as proteins and carbohydrates (Bernaerts et al., 2019). For this reason, the cultivation of microalgae has potential for application in different areas of the economic sector, such as the pharmaceutical, cosmetic, food, and aquaculture industries.

Despite this, the amount of biomass produced is still insufficient to meet the needs of the market, with a total of 87,000 tons recorded in 2018 in eleven countries, a value that is still low considering the demand. This can be attributed to the fact that there is still a large gap between the technology currently available and the technology needed to meet the potential world demand (Acién et al., 2012; FAO, 2020; Kusmayadi et al., 2021). In addition, techno-economic, environmental, and social constraints and difficulties are constantly faced, as well as challenges in cultivation, separation, and associated downstream processes that hinder a commercial production of microalgae-derived products (Vuppaladadiyam et al., 2018a).

Microalgae require less land and fresh water than terrestrial plants, and because they do not depend on the availability of fertile lands, they can be cultivated close to industrial areas (Vuppaladadiyam et al., 2018b). The importance and potential of these organisms for the biobased industry has been demonstrated in recent research, and it is known that in a biobased economy, biomass is valued and used in the sustainable production of food, feed, fuel, energy, pharmaceuticals, and personal care (Barsanti & Gualtieri, 2018; Vuppaladadiyam et al., 2018b). Also, emerging applications based on microalgae include the production of biomaterials as an alternative to materials of fossil origin, such as biofertilizers, biostimulants, and biopesticides, making agriculture and energy matrices (i.e., biodiesel, bioethanol and biogas) more sustainable (Fernández et al., 2021).

In this context, researchers have played a fundamental role in the development of cheap and environmentally friendly technologies for the cultivation of microalgae, so these can be applied on a large scale. Thus, biological treatment systems with microalgae in wastewater

emerge as a promising alternative, since the effluents contain nitrogen, phosphorus, carbon compounds, and other macro and micronutrients necessary for the growth of microalgae (Daneshvar et al., 2018; Dias et al., 2019). Efficiently, microalgae remove nutrients from wastewater (which act as cost-free culture media) to convert them into valuable biomass for purposes such as bioenergy, biobased products, and materials that fall within the framework of bioeconomies (Fernández et al., 2021).

According to studies that focus on the cultivation of microalgae in wastewater, domestic effluent has been shown to be efficient for species such as *Desmodesmus subspicatus* (Bastos et al., 2019), *Scenedesmus acuminatus* (de Araujo Vidal et al., 2019), and *Chlorella vulgaris* (Trevisan et al., 2018). Besides remediation of organic and inorganic nutrients, biomass produced in wastewater can be used as a sustainable feedstock for the production of several biomolecules (Balamurugan et al., 2021). These biomolecules can be used as a low-cost source for the production of biodiesel (Mansour et al., 2019; Silambarasan et al., 2021), biostimulant, and biofertilizer (Li, Lens, et al., 2021); in addition, they can actively absorb CO₂ for photosynthesis and self-reproduction (Iglina et al., 2022) contributing to the reduction of carbon dioxide levels in the atmosphere.

The treatment of domestic effluents using microalgae is an emerging and innovative tool, as it is considered a sustainable and low-cost process, and, therefore, it has aroused the interest of researchers in the development of sustainable approaches. Therefore, the literature review becomes an important tool for understanding the current scenario, as well as identifying scientific gaps. Given this context, this review aims to perform an updated bibliographic survey regarding the use of microalgae for the biological treatment of domestic wastewater. Therefore, this article will discuss themes related to the following: (1) cultivation of microalgae in domestic effluents, (2) efficiency in removing nutrients present in domestic effluent, (3) biochemical composition of the produced biomass, and (4) its potential biotechnological applications.

Characteristics of domestic effluent and aspects of cultivation of the main cultivated species

At domestic effluent stations, the effluent is submitted to three different treatment processes, namely: primary, secondary, and tertiary. Nutrient concentrations vary at each effluent treatment stage (Table 1, Fig. 1).

Table 1 Main nutrients and their concentrations for the different domestic effluent treatment processes. N.D.: not determined.

Parameters	Primary	Secondary	Tertiary
Ammonia (mg L ⁻¹)	52.5	N.D.	0.4
Biochemical oxygen demand (mg L ⁻¹)	N.D.	71.5	N.D.
Chemical oxygen demand (mg L ⁻¹)	246.4	95.8	N.D.
Nitrate (mg L ⁻¹)	1.7	N.D.	8.5
Nitrite (mg L ⁻¹)	N.D.	N.D.	N.D.
pH	7.2	7.9	7.3
Phosphate (mg L ⁻¹)	5	N.D.	1.69
Total carbon (mg L ⁻¹)	N.D.	1.8	22.6
Total nitrogen (mg L ⁻¹)	73.5	21.96	8.7
Total phosphorus (mg L ⁻¹)	N.D.	1.8	1.71

According to Solís-Salinas et al. (2021), Eida et al. (2018) and Ji et al. (2013).

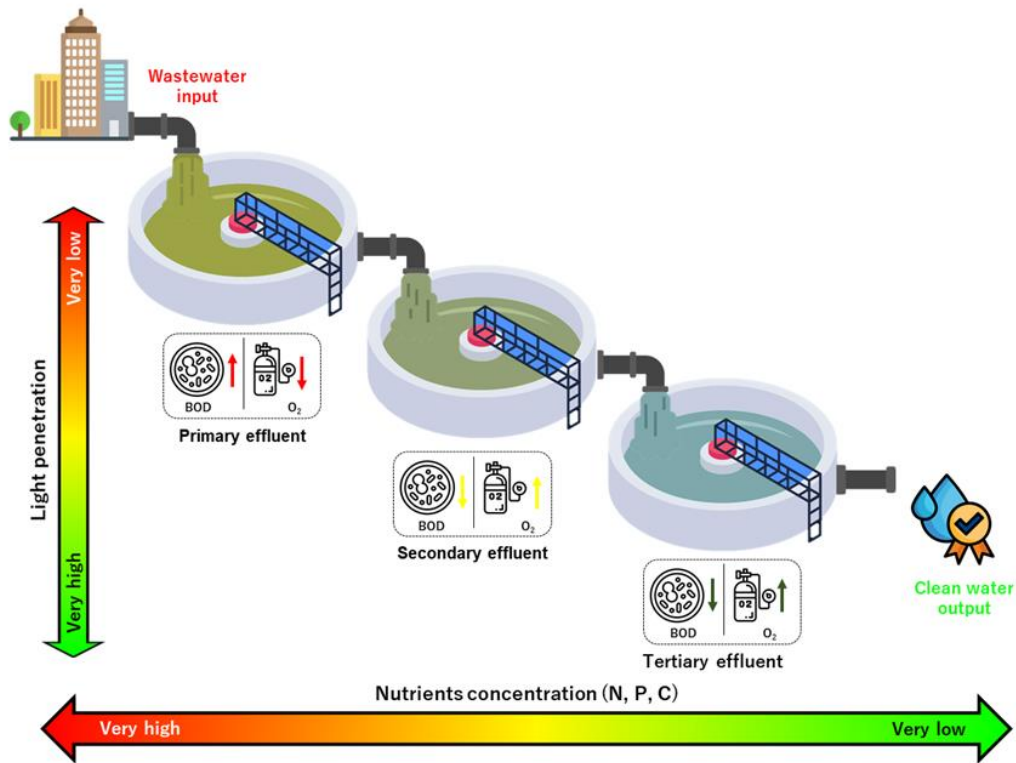


Fig. 1 Summary of the main characteristics of the three different effluent treatment stages.

Microalgae are among the most diverse groups of the world; however, few species have attracted the interest of the scientific community. Among these, the main ones used for the

biological treatment of domestic effluents are those belonging to the genera *Chlorella* and *Scenedesmus*, both for primary, secondary, and tertiary effluents. Regarding their biotechnological applications, these microalgae are widely studied for applications in the areas of biofuels, biofertilizers, medicines, and human food (Fig. 2).

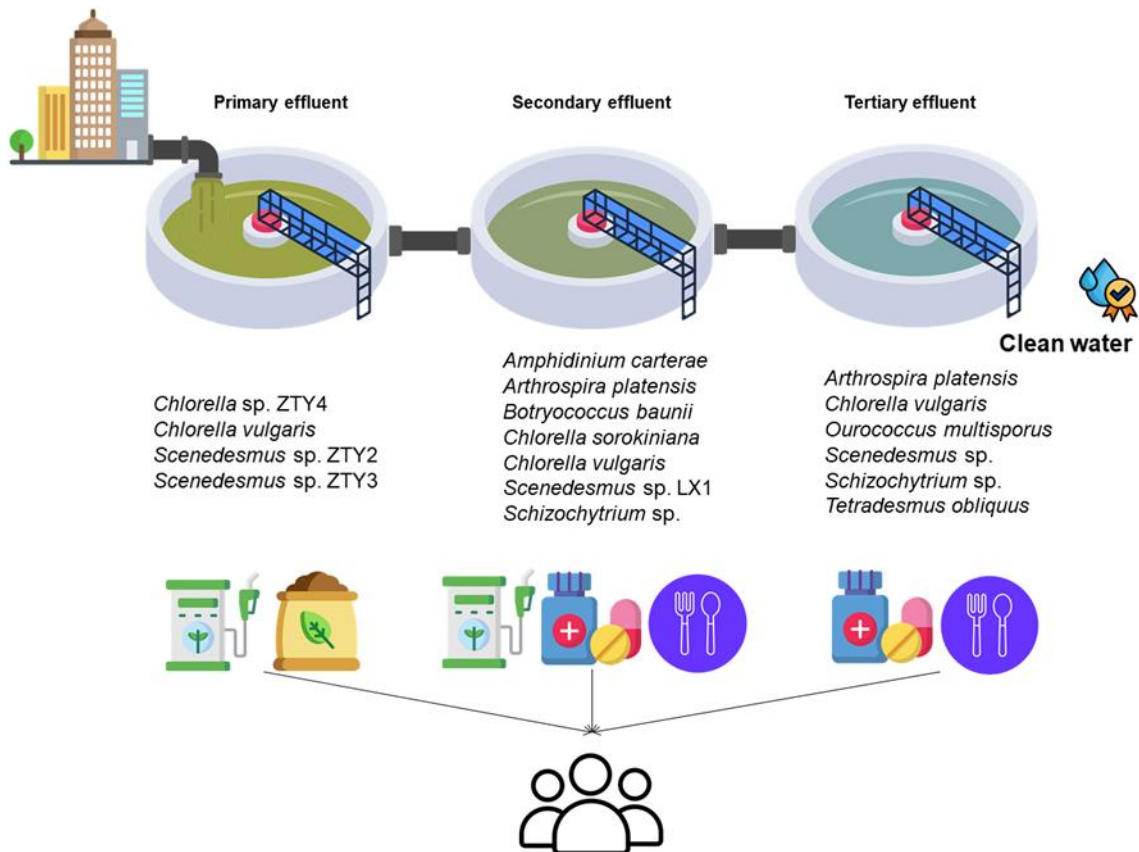


Fig. 2 Main species of microalgae cultivated in domestic effluent and their potential biotechnological applications.

Among these, the most studied in terms of cultivation in domestic effluent are listed below:

Microalgae-bacteria consortium

The use of microalgae and bacteria has been considered a promising consortium, since bacteria are capable of producing CO₂, which is one of the main limiting nutrients for microalgae growth. For the secondary treatment of domestic wastewater in high-rate algae ponds (HRAPs), this consortium was responsible for the significant degradation of the antibiotic ciprofloxacin

during the day, mainly with the reduction of the hydraulic retention time (Hom-Diaz et al., 2017). When a double-chamber biophotovoltaic cell was used in the treatment of domestic sewage, a synergistic interaction was observed between bacteria and microalgae capable of promoting the generation of bioelectricity, the production of biofuel, the sequestration of carbon dioxide (CO₂), and the treatment of wastewater (Chandra et al., 2017). In this case, there is the emergence of promising new technologies for microalgae cultivation that are environmentally friendly and capable of promoting the generation of raw material with economic value.

It is known that different cultivation conditions have a direct influence on the growth of microorganisms, and in this context, Kang et al. (2018) found that the microalgae-bacteria consortium in domestic wastewater can establish favorable conditions for growth and nitrogen removal by controlling aeration and light intensity. Besides cultivation conditions, operational strategies are also able to influence growth and biological bioremediation capacity. By investigating the symbiotic relationship between bacteria and microalgae, it was found that microalgae growth-promoting bacteria are a potential new strategy for large-scale microalgae cultivation in an economic and ecological way (Dao et al., 2018).

By investigating the influence of biogas flow and liquid/biogas ratio (L/G) on the quality of biomethane produced under the influence of two types of effluent (domestic versus concentrate) and the hydraulic retention time in the biogas upgrade and in the efficiency of nutrient recovery, a greater removal of CO₂ and H₂S, 99% and 100%, respectively, was observed at the highest L/G ratio (3.5) using concentrated effluent, with no significant influence of the biogas flow (del Rosario Rodero et al., 2019). Assis et al. (2019), when analyzing different support materials (i.e., cotton, nylon and polyester) for adherence of the biofilm of cells cultivated in domestic sewage, found a greater growth of microalgae and nitrifying bacteria with the use of polyester; however, the different materials did not interfere with the biochemical composition of the adhered biofilm.

In evaluating the potential for removing nutrients and organic pollutants present in domestic sewage through the microalgae/bacteria consortium, Fito and Alemu (2019) observed the maximum removal of TNK (69%), TP (59%), PO₄³⁻-P (73%), chemical oxygen demand (COD, 84%), and BOD₅ (85%), which allows the conclusion that this is a promising technology for industrial application. Regarding the bioprocesses that occur during the treatment of domestic effluent, Barreiro-Vescovo et al. (2020) verified important aspects of this symbiosis: (1) the oxygenation rates and kinetic responses of light intensity were similar to those reported for microalgae cultures grown in synthetic medium, (2) the endogenous respiration of microalgae

was impacted on the consumption of oxygen under dark conditions, and (3) limiting conditions of organic substrate selected bacteria with high substrate affinity.

As for the primary treatment of domestic wastewater in high-rate ponds, it was found that the semi-continuous feeding regime during the day was more advantageous than the continuous one, both in terms of microalgal biomass productivity and nutrient removal efficiency (Serejo et al., 2020). Still, according to this study, the authors concluded that for the removal of surfactants, a feed of only 0.1 h d^{-1} generates better results when the treated effluent is removed from the system before admitting new effluent (batch operation). In another semi-continuous operation for the primary treatment of domestic effluent in photobioreactors, the dominance of the wild strain *Chlorella sorokiniana* was observed, while the dominance of pigmented and photoheterotrophic groups was verified for the bacterial community, which reduced the metabolic capacity of the photobioreactor, resulting in insufficient effluent treatment (Barreiro-Vescovo et al., 2021).

Thus, it is possible to state that the cultivation conditions, the operational strategies, and the cultivation systems employed can influence the removal of nutrients and the production of microalgae biomass. In this context, a study that evaluated the influence of the microalgae-bacteria consortium, aeration, carbon dioxide supplementation, photoperiod, and cultivation systems (open or closed) in the removal of pathogens present in domestic wastewater was carried out by Ruas et al. (2021). These authors verified that certain pathogenic bacteria have specific inactivation mechanisms and that not all of them can be inhibited by allelopathy. They also found that light, pH, and dissolved oxygen, or any combination of these, have a greater influence on the bacterial community than the presence of the microalgae (Ruas et al., 2021). From an ecological point of view, Plouviez et al. (2019) performed the first monitoring of nitrous oxide (N_2O) emission during the primary treatment of domestic wastewater by the microalgae-bacteria consortium in an HRAP and observed an emission range between 25 and 75% (v/v).

The results of the microalgae-bacteria symbiotic relationship evaluated in a study conducted by Katam et al. (2021, b) demonstrated efficiency in removing carbon and nutrients from domestic wastewater, as well as the ability to remove organic micropollutants of different classes (e.g., pharmaceuticals, personal care products, pesticides, herbicides, and fungicides). Initially, 106 micropollutants were detected, and at the end of the treatment, only 17 remained in the effluent, proving the removal effectiveness of this environmentally friendly and sustainable alternative. Similarly, a methodology that uses a microbial fuel cell (MFC), which is a device that uses bacteria as catalysts to oxidize organic matter and generate electric current, combined

with a strain of *Chlorella vulgaris*, proved to be effective in the treatment of effluents, resulting in better removal of total phosphorus (up to 97%) and ammonia (up to 99%), proving to be promising for the treatment of wastewater (Jiang, 2017).

Mixed culture of microalgae

Among the important microorganisms for the bioremediation of domestic wastewater, mixed cultures of microalgae have wide application in scientific studies in the area. However, from an ecological point of view, little has been studied about the phototrophic communities present in waste stabilization ponds. In this regard, Eland et al. (2018) identified that *Chlorella* species and *Planktosphrix*, a cyanobacteria, dominated in domestic effluent systems and in mixed effluent (industrial/domestic). However, the two systems showed differences in the proportion of photosynthetic and non-photosynthetic organisms, as well as in the patterns of diversity in longitudinal succession due to the low levels of oxygen detected. In the same context, the genera *Euglena* and *Chlorella* were more frequent in stabilization ponds for the treatment of domestic sewage (Florentino et al., 2019). Furthermore, it was found that in both surveys, microalgae from the genus *Chlorella* were among the dominant organisms, probably due to its cosmopolitan characteristic. It can also be stated that the diversity of organisms in waste treatment systems is directly related to the physicochemical variables of the environment, and therefore, the communities of microorganisms will never present an exact pattern.

To determine the viability of effluent treatment and the use of the resulting biomass as a source of renewable energy, Do et al. (2019) cultivated four species of microalgae from a municipal wastewater treatment and observed that the strain of *Desmodesmus* sp. was the one that best adapted to the local climatic conditions, being responsible for the removal of 99.10% of the ammoniacal nitrogen, 91.31% of the total nitrogen, and 95.67% of the total phosphate. This corroborates the information that the physicochemical conditions, which can be altered by the local climate, interfere with the composition of microalgae in the environment. In 20 days, a study carried out in the Yamuna River polluted with effluent from different sources (domestic, industrial and agricultural) concluded that microalgae have a significant and low-cost capacity for treating wastewater. Additionally, it was found that this treatment reduced the biological and chemical demand for oxygen by approximately 37% and increased dissolved oxygen by approximately 116% (Shrestha et al., 2020). In a study aiming at the production of starch and

microalgae biomass related to *Chlorella*, Noguchi et al. (2021) verified that the accumulation of this carbohydrate in the cells required a relatively low concentration of nitrogen, while its growth was inhibited by the biological contamination present in the treated domestic effluent. In order to control the negative effect of microbial contamination, these same authors investigated the effect of disinfection of wastewater by chlorination and observed the effectiveness of this treatment at 2.0 mg Cl/L for 15 min, with a high proportion of microalgae and high concentration of carbohydrates, especially in the intermediate phase of the culture (days 14–27).

The microalgae biomass produced in domestic sewage with replacement of 10% of its volume by olive mill effluent obtained a yield of 0.10 m³ CH₄ Kg⁻¹ of volatile solids and a 61% increase in methane production (Assemany et al., 2020). Still, according to the same authors, the biochemical constitution of the microalgae biomass is mainly associated with the type of medium in which they are cultivated. A study on the energy potential of mixed biomass showed high calorific value (20.36 MJ Kg⁻¹), volatile matter (69%), carbon fixation (8.2%), and ash content (22.8%) for the effluent of industrial origin. As for biomass cultivated in domestic effluent, the values for the aforementioned parameters, respectively, were approximately 13.21 MJ Kg⁻¹, 54%, 6%, and 39%, demonstrating that the industrial effluent is the best alternative for thermal generation (de Oliveira Costa et al., 2017). For the best use of microalgae biomass cultivated in high-rate ponds using domestic effluent as a culture medium, it was observed that the content of apolar lipids in the dry biomass represented 7.8%, reaching a daily lipid productivity of 0.2 g m⁻² day⁻¹ and a biogas production potential of 0.20 m⁻³ kg⁻¹ solids. Thus, it was concluded that all energy routes studied had higher energy input than output, indicating that the HRAP needs intervention to improve energy performance and increase biomass productivity (Assemany et al., 2018). These results demonstrate that the bioenergetic potential of microalgae depends not only on the type of effluent in which they are grown but also on the interference in their metabolic pathways.

The production of 1 kg of biomass cultivated in high-rate ponds with domestic effluent as the culture medium resulted in at least a 30% reduction in environmental impacts, while supplying industrial CO₂ and hybrid systems to increase biomass productivity did not compensate in environmental terms, increasing impacts by up to 227% (Magalhães et al., 2021). The production of microalgae biomass briquettes using domestic effluent stood out for its environmental gains compared to the synthetic culture medium. These are able to reduce electricity consumption, due to the natural decrease in moisture present in the microalgae biomass, in addition to being able to replace charcoal briquettes (Marangon et al., 2021).

Pharmaceutical components present in domestic wastewater, such as antiepileptics, lipid-lowering agents, tranquilizers, analgesics, and anti-inflammatories, showed a percentage reduction greater than 50%, reducing the ecological risk of these compounds in a naturally colonized high-rate lake (Jiménez-Bambague et al., 2020). It is noteworthy that this is an approach that has not yet been studied and that requires substantial advances.

There are several physical factors that can affect biomass production and domestic wastewater treatment by microalgae. Therefore, Couto et al. (2021) evaluated the effect of depth in high-rate ponds with and without CO₂ addition and found that 40-cm ponds provided algal biomass growth similar to 30-cm ponds. These results are interesting, since it becomes possible to reduce the area required for the cultivation system without compromising the production of biomass and the efficiency of the effluent bioremediation. Fal et al. (2021) investigated the integrated cultivation between domestic effluent treatment and production of lipid-rich biomass and observed a 99.15% reduction in total phosphorus and an increase in efficiency by 99.97% in terms of total nitrogen. Of the evaluated species, the microalga *Chlorella sorokiniana* had the highest lipid content (36.93%), demonstrating potential for the treatment of domestic effluent and biodiesel production, in addition to having other applications such as CO₂ biosequestration and the elaboration of value-added products.

The inoculums of bacteria, microalgae, protozoa, and small metazoans were used in a mixture of agricultural runoff and domestic wastewater in photobioreactors and demonstrated the efficiency of microalgae-based technologies for nutrient removal, reaching an average efficiency of around 65% (García et al., 2018). The treatment of kitchen effluent with a mixed culture of microalgae was able to remove 88% of the chemical demand for oxygen and 85% of the total nitrogen, while in an anaerobic bacterial system, the removal of the chemical demand for oxygen was 89%, and the total nitrogen was 48%. This demonstrates that the first system is capable of replacing aerobic bacteria in the biological treatment of effluents in certain cases (Katam & Bhattacharyya, 2018). To determine the efficiency during the harvest of a mixed culture of microalgae, a comparison was made between natural and chemical coagulants, showing that, among natural coagulants, neem seed was the most efficient in removing microalgae from the medium with domestic wastewater, being capable of removing 94% or more of the cultured cells (Ali et al., 2019). Domestic wastewater treatment by co-culture of immobilized microalgae showed a high lipid content, ranging from 28 to 40%, and was efficient in removing carbon (88%), nitrogen (91%), and phosphorus (93%) (Katam & Bhattacharyya, 2019).

A mixed culture of *Chlorella* sp., *Scenedesmus quadricauda*, *Anabaena variabilis*, *Chlorococcus* sp., and *Spirulina* sp., inoculated in a photobioreactor supplied with domestic effluent and enriched with ammonia and high concentrations of dissolved oxygen (0.4–1.0 mg L⁻¹), was able to inhibit bacterial growth (Mukarunyana et al., 2018). According to Li et al. (2021, b), microalgae cultivated in consortium and in selenium-enriched domestic effluent offer a promising alternative for transforming low-value resources into high-value food supplements. In addition, they can also be used as a biofertilizer or biostimulant and have high levels of selenium removal (43%), ammonia (93%), TP (77%), and COD (70%). The mixed consortium of microalgae enriched with carbohydrates in semi-continuous operation with low loads of nutrients and carbon from domestic wastewater demonstrated that the microalgae were able to adapt to the restriction conditions, being able to accumulate a high carbohydrate content (about 57% of cell dry weight) due to imbalance in N/P ratios (Solís-Salinas et al., 2021).

Assis et al. (2020) proposed a hybrid wastewater treatment system, in which a biofilm reactor (BR) functions as a microalgal biomass production and harvesting unit connected to an HRAP. The results were promising, and about 2.6× more biomass was produced compared to the conventional system without BR. In addition, the harvest efficiency was 61% in the hybrid system, against 22% obtained with the conventional system. In this same context, a hybrid system of vertical flow was built for the treatment of domestic effluent and the cultivation of microalgae, which resulted in lower capital costs and in the production of value-added products, such as the harvest of 1 kg of microalgae biomass. This amount of biomass could produce 0.16 kg of lipids, 0.26 kg of ethanol, and 0.2 kWh of energy (Chavan & Mutnuri, 2021), with these obtained bioproducts of great importance for the biobased industry.

Microalgae co-cultures were inoculated in photobioreactors of different colors (white, blue, and green) and exposed to natural environmental conditions in order to increase the productivity and quality of the produced biomass. In this process, it was shown that most of the organic and solid content of the domestic effluent was removed, in addition to suggesting that the white photobioreactor increased the productivity of microalgae by 2.3 to 3.5 times compared to the blue and green PBRs (Khalekuzzaman et al., 2021). Two mixed cultures of microalgae, one dominated by *Spirulina maxima* and the other by *Euglena sanguinea*, were sampled from a domestic wastewater treatment plant to verify the effectiveness of three extraction techniques (conventional, ultrasonication, and microwave) on lipid yield and the fatty acid profile. Palmitic acid (C16) was identified as the main fatty acid present in the two mixed cultures applying the three techniques. For ultrasound, the highest percentage was palmitic acid for the cultures

of *S. maxima* (43.2%) and *E. sanguinea* (53.5%). On the other hand, for the microwave technique, the highest amount was of oleic acid (C18:1) with values of 20.9% for *S. maxima* and 13.7% for *E. sanguinea* (Mansour et al., 2019).

Two other mixed cultures of microalgae (MAC1 and MAC2) from a sewage treatment plant demonstrated that CO₂ enrichment was efficient in removing nitrogenous and phosphate nutrients, as well as COD. Furthermore, it also significantly increased lipid production in MAC1 and MAC2, to 12.29% and 11.37%, respectively, while the biomass yield reached 0.36 g L⁻¹ in both cultures. The total contents of chlorophyll and protein were 14.27 and 12.28 µgmL⁻¹ in MAC1 and 0.13 and 0.15 µgmL⁻¹ in MAC2 (Sharma et al., 2020a). These same mixed cultures described above were efficient in removing heavy metals present in domestic wastewater, with a removal percentage of 85.06% Cu, 75.2% Cr, 98.2% Pb, and 99.6% Cd (Sharma et al., 2020b). These findings demonstrate the ability of microalgae to bioremediate domestic effluent and produce biomass rich in secondary metabolites of commercial interest.

With the aim of determining the cheapest coagulant and flocculant reagents for harvesting microalgae present in domestic wastewater, in order to use it for irrigation, the toxicity of the effluent treated with aluminum and chitosan was tested on different organisms such as *Daphnia magna*, *Allium cepa*, *Lactuca sativa*, and *Triticum aestivum*. It was shown that chitosan was the least toxic flocculation method when compared to aluminum coagulation, even though both microalgae harvesting methods changed the chemical composition of the treated water (van den Berg et al., 2020). Marella et al. (2019) reported a dominance by pennate diatoms during urban domestic wastewater treatment; during the winter, the diatom biomass showed a high content of eicosapentaenoic acid (EPA) while during the summer, the biomass showed suitable characteristics for biodiesel production (high content of saturated fatty acids). This suggests that climate variation directly interferes with the production of metabolites by microalgae, especially in regions with well-defined seasons.

Chlorella vulgaris

Among the main species of the *Chlorella* genus, the most studied is *Chlorella vulgaris*. When using different strains of *C. vulgaris* for the secondary treatment of domestic wastewater enriched with Bayfolan® fertilizer and without enrichment, it was found that the enriched treatment significantly increased the biomass production (from 1.1–1.5 g L⁻¹ to 1.9–2.2 g L⁻¹), pigments (going from values between 4.4 and 8.7 mg L⁻¹ to 35.08 and 61.2 mg L⁻¹), and proteins

(going from values between 14.6 and 27.9% to 26.9 and 47.67%) (Fernández-Linares et al., 2017). When evaluating the influence of different concentrations of CO₂ on the growth of *C. vulgaris* cultivated in domestic wastewater, it was found that the concentration of 20% CO₂ drastically decreased the pH, altering the photosynthetic pathways. On the other hand, the highest final biomass concentration and the fastest phosphorus and nitrogen removal were achieved at 10% CO₂ concentration, followed by 5% (Liu et al., 2017).

Chlorella vulgaris was able to grow under the conditions of 0.02 v/v of domestic effluent, initial pH of 3 and 0.03 v/v of microalgae initial inoculum. Although the effluent was diluted, thus presenting a low concentration of nutrients, the stress condition induced the cells to increase the lipid yield to 32.7%. However, when large amounts of wastewater were used, an increase in microalgae decomposition due to the presence of biotic contaminants was observed (Lam et al., 2017). It is known that the concentration of nutrients in the culture medium is capable of influencing the production of biomass, and it is also known that an effluent has loads of nutrients and pollutants that are different from each other due to physical-chemical and environmental factors. In the case of the aforementioned study, the presence of contaminants in the effluent acted as an inhibitor of the growth of the microalgae culture. In this context, the results of the comparison between filtered (2 µm membrane) and unfiltered domestic wastewater suggest that the cultivation of *C. vulgaris* in filtered wastewater is an option for the treatment of domestic effluent to improve its quality, since it was able to absorb about 94.18% of ammonia and 97.69% of orthophosphate (Mayhead et al., 2018).

A microbial photoautotrophic fuel cell based on immobilized microalga (*C. vulgaris*) was developed, and the results showed its feasibility for the treatment of domestic effluent by removing about 93.2% of sCOD, 95.9% of ammonia, 95.1% of total nitrogen, and 82.7% phosphate, as well as producing 466.9 mW m⁻³ of maximum power density (Wang et al., 2019). The same species cited above was subjected to anaerobic digestion in the absence and presence of co-substrates (kitchen waste and residual activated iodine), and the results showed that the microalga was able to efficiently remove 99.6% of nitrogen and 91.2% of phosphorus in municipal wastewater, while the biomethane yield was improved by co-digestion of both co-substrates (Calicioglu & Demirer, 2019).

When evaluating different N/P ratios and CO₂ concentrations in the growth kinetics of *C. vulgaris* collected from a domestic settling pond, it was verified that the medium supplemented with 16% CO₂ and the N/P ratio of 10:1 presented the most productive cultivation in terms of maximum biomass concentration, specific growth rate, biomass productivity, and rate

of CO₂ biofixation, which were 0.7900 g L⁻¹, 0.4170 d⁻¹, 0.08500 g L⁻¹ d⁻¹, and 0.1430 gCO₂ L⁻¹ d⁻¹, respectively. The species also showed adaptability and adequate growth under CO₂ levels of 24% in domestic wastewater and was also able to absorb nitrogen and phosphorus in the range of 70–95% (Molazadeh et al., 2019). The cultivation of the microalga reduced the concentration of nitrates (41%) and orthophosphates (37%) when this occurred in domestic sewage, while the removal efficiency of these same parameters was 36% and 23%, respectively, for cultivation in distilled water. It is also worth mentioning that the maximum biomass productivity was higher in wastewater (6768 mg L⁻¹) when compared to distilled water (3308 mg L⁻¹) (Ali et al., 2021). This can be explained by the fact that distilled water undergoes a distillation process where toxic substances, impurities, and minerals are eliminated, making it poor in nutrients when compared to domestic wastewater, naturally rich in organic and inorganic compounds that favor the growth of microalgae. Under different concentrations of *C. vulgaris* (20, 30, and 40%) and two hydraulic retention times (HRT) (8 and 24 h), the concentration of 30% provided maximum removal in the two HRT for the treatment of domestic effluent, reaching values of 87.67% in the removal of phosphate, 96.88% of ammonia, and 80.39% of DOC in unfiltered samples. As for samples filtered at 8 h of HRT, the values were 96.32, 100, and 83.64% of phosphate, ammonia, and DOC removal, respectively (Moondra et al., 2020).

Family Scenedesmaceae

Species of the Scenedesmaceae family are also widely studied. Eida et al. (2018) tested different levels of addition of domestic wastewater (0, 25, 50, 75, and 100%) to the Bold's Basal (BBM) culture medium for the cultivation of the microalga *Tetradismus obliquus* (formally known as *Scenedesmus obliquus*). The authors and obtained results that demonstrated that when cultivated in the proportion of 25% of residual water and 75% of BBM medium, the species efficiently used 95.2% of phosphorus and 78.5% of nitrogen. Although the lipid content was higher in the 100% BBM medium, reaching 25.2% of cellular dry biomass, the highest percentage of C16-C18 fatty acids (54.76% of total lipids) was observed in microalgae grown in 100% water waste, which means that the synthetic culture medium can be replaced by domestic effluent without affecting biomass production. In another study conducted by Ruiz-Marin et al. (2018), the cell density obtained for *T. obliquus* cultivated photoautotrophically and mixotrophically using domestic wastewater was 13×10^6 cell mL⁻¹ and 14×10^6 cell mL⁻¹, while

the nitrogen limitation (from 90 to 20 mg N L⁻¹) was able to increase in only 5% the lipid productivity under mixotrophic metabolism.

T. obliquus microalgae cells immobilized in 2% calcium alginate for the treatment of secondary domestic effluent under different hydraulic retention times (HRT) (3, 6, 12, and 20 h) improved performance in the removal of orthophosphate (PO₄-P) and nitrate (NO₃-N) with increasing HRT. Values ranged from 0.3–3.1 mg L⁻¹ in 3 h to 0.01–0.2 mg L⁻¹ in 20 h for PO₄-P and ≤ 19.3 mg L⁻¹ in 3 h and ≤ 0.4 mg L⁻¹ in 20 h for NO₃-N (Whitton et al., 2018). Angioni et al. (2018), when studying the species *Scenedesmus acutus*, evaluated how stress conditions in a microbial fuel cell reactor affect the metabolism and accumulation of secondary metabolites. The authors observed accumulation of significant amounts of lutein, violaxanthin, astaxanthin, and canthaxanthin, as well as about 49% dw of accumulated lipids and a fatty acid profile mainly composed of those with 16 and 18 carbons (C16-C18), which are the ones indicated for the production of biofuels. These results corroborate the hypothesis that microalgae have the ability to act as bioremediators of domestic effluents, as well as for the production of biomass of biotechnological interest.

Pandian and Thomas (2019) tested the immobilized microalga *Scenedesmus rubescens* (alginate and alginate-carrageenan) in the phycoremediation of domestic wastewater and found a reduction in phosphate (98%) and nitrate (75%) levels. Under optimized growth conditions in medium with domestic and industrial wastewater, this species showed three significant variables, nitrate (0.2%), phosphate (0.018%), and inocula (7.5%), which yielded a biomass greater than 0.73 g L⁻¹, about 5.4 times more than when grown in regular medium (Sureshkumar & Thomas, 2019). A laboratory-scale hybrid air transport photobioreactor was used for the cultivation of the microalga *Scenedesmus abundans* in domestic wastewater, and the remediation of the chemical demand for oxygen, dissolved inorganic nitrogen, and dissolved inorganic phosphorus was observed at values close to 80.19%, 90.73%, and 86.31%, respectively. Furthermore, hydrothermal liquefaction (HTL) of algal biomass yielded about 35.5% by weight of bio-oil and the carbon mass balance between product streams demonstrated that CO₂ was sequestered by bio-char (0.46 g), bio-gas (0.15 g), bio-oil (0.963 g), and post-HTL wastewater product streams (0.35 g) (SundarRajan et al., 2020). This is interesting because, in addition to contributing to the cell's photosynthesis process, since carbon is the main macronutrient used in the growth of microalgae, it also contributes to reducing the levels of this gas in the atmosphere.

When evaluating different flocculants in the biomass recovery of a *Scenedesmus* culture grown in medium with domestic wastewater, it was observed that flocculation by chitosan and cationic polymer kept the species whole and intact, while cell lysis occurred during flocculation with alum (Kumar Gupta et al., 2018). Under condition of pH increase during microbial activities, Mohamed et al. (2018) verified an increase in the percentage of removal of nutrients present in a domestic secondary effluent. In this condition, the microalga *Scenedesmus* sp. was able to remove 91.86% of total nitrogen, 98.15% of total phosphorus, and 93.11% of total organic carbon. Additionally, it reduced the BOD by 86.5%, the COD by 69.9%, and the total suspended solid by 76%. Ratnawati et al. (2020) stated the process of phycoremediation of water from a retention pond using *Scenedesmus* sp. when adding 3% of potassium and 29.41 mg L⁻¹ of carbon, which also resulted in higher concentrations of dissolved oxygen (7.70 mg L⁻¹) and chlorophyll (8.88 mg L⁻¹).

The cultivation of *Scenedesmus* sp. in different non-sterile domestic wastewater found that the primary effluent was the most suitable substrate for microalgae growth compared to the secondary effluent, reaching a biomass concentration of 0.69 g L⁻¹ and 166.7 mg L⁻¹ in lipid yield. Moreover, ultrasonic exposure at the optimal frequency of 18 Hz, 20 W power, and 10 min time was able to increase the accumulation of biomass and lipids to 1.56 g L⁻¹ and 240 mg L⁻¹, respectively. Simultaneously, this species was able to remove 96.8% of the total nitrogen and 97.7% of the total phosphorus (Ren et al., 2019). These findings may be associated with a higher load of nutrients and organic matter present in the primary effluent compared to the secondary effluent. Baldev et al. (2021), when submitting the microalga *Scenedesmus* sp. to wastewater from different locations (S1, S2 and S3) in order to produce biofuels, observed higher biomass production (0.84 g L⁻¹) and lipid productivity (8.6 mg L⁻¹ day⁻¹) for location S1. Regarding the fatty acid profile (FA) of this green microalga under these conditions, the authors reported 43.3% of saturated FA, 44.4% of monounsaturated FA, and 12.3% of polyunsaturated FA, which confirms the possibility of oil production from this species. Concerning *Scenedesmus* sp. cultivation in different concentrations of domestic sewage (0, 20, 40, 60, 80, and 100% v/v), a maximum biomass productivity of 62 mg L⁻¹ day⁻¹ was verified in 100% v/v of sewage, as well as a lipid productivity of 19 mg L⁻¹ day⁻¹. These values were favorable to the production of bioethanol (10.4 g L⁻¹ after distillation) and to the bioremediation of the effluent (Thangam et al., 2021). Finally, the results listed so far confirm that this biological treatment process is environmentally friendly and also contributes to the biobased industry.

***Botryococcus* spp.**

Still little applied in studies with domestic effluent, species of the genus *Botryococcus* represent one of the most promising organisms for the production of biofuels. Regarding the process of harvesting *Botryococcus* sp. biomass grown in domestic effluent, Gani, Mohamed Sunar, et al. (2017) used the coagulants alum and chitosan to evaluate the efficiency of this process and found a higher harvest value (99.3%) at an optimal dosage of 177.74 mg L⁻¹ and pH 8.24 with alum, while chitosan reached a biomass recovery rate of 94.2% at a dosage of 169.95 mg L⁻¹ at pH 12. By immobilizing *Botryococcus* sp. in alginate for the treatment of domestic effluent, Sunar et al. (2019) proved that this microalga was efficient in removing pollutants and nutrients present in wastewater, reducing 77.44% of the BOD and 66.57% of the COD.

The microalga *Botryococcus* sp. also proved to be effective for the phycoremediation of domestic wastewater, as there was a significant reduction in the load of pollutants in the effluent, with a reduction of approximately 93.9% in the COD, 69.1% in BOD, 59.9% in total nitrogen, 54.5% total organic carbon, and 36.8% phosphate. Also, the biomass of this species cultivated in the effluent showed potential for the production of bioplastic, containing 71.6% of the total hydrocarbon compounds (Gani, Sunar, et al., 2017). Applying the concept of biorefinery, *Botryococcus* sp. was cultivated using domestic effluent as nutrient source, and in order to reduce the cost of harvesting the biomass, an autoflocculation technique was established which yielded 94.8% of harvested biomass without the addition of flocculants. Furthermore, the extraction of biodiesel through a zirconia tungstate catalyst was also analyzed, which produced 94.1 wt% biodiesel yield (Ashokkumar et al., 2019).

Efficiency of microalgae in nutrient removal

The efficiency in removing nutrients present in wastewater indicates the potential that each species of microalgae has to bioremediate the effluent. That is, this potential is relative to the ability of each organism to biosorb the organic and inorganic compounds present in the environment, reducing its polluting load and ensuring that water resources do not suffer from eutrophication, ensuring the survival of various forms of life that depend on natural environments to remain in balance. Under certain cultivation conditions, technology and management directly contribute to optimizing the bioremediation capacity of effluents by microalgae. For this reason, according to what appears in the literature, an overview of the main species of microalgae and

their respective maximum efficiencies in the removal of nutrients of domestic wastewater is shown below.

The treatment of domestic wastewater via nitrification and denitrification in an anoxic-aerobic algae-bacterial photobioreactor coupled to a biogas scrubbing an increase in the removal of TN, NH_4^+ , and P-PO_4^{3-} through CO_2 supplementation, which favored almost complete nitrification of the NH_4^+ into NO_3^- and promoted microalgae growth, while carbon removal occurs constantly regardless of CO_2 addition (García et al., 2017). When the effluent was enriched with Fe, Mg, Mn, S, and EDTA, the microalga *T. obliquus* was able to treat domestic wastewater, when subjected to batch experiments, reaching removal levels above 90% for nitrogenous and phosphate compounds (Foix-Cablé et al., 2018). Another species that showed high efficiency in the treatment of secondary domestic effluent was *Oscillatoria tenuis* in a batch experiment, being able to remove nitrogen and phosphorus, to reduce the COD at levels above 80% (Cheng et al., 2018). An open raceway pond was used for the tertiary treatment of domestic wastewater using the cyanobacterium *Arthrospira platensis* where the removal of nitrogenous and phosphate compounds and COD was observed (Chavan & Mutnuri, 2018). However, the percentages found in this study are below those observed in other literature; the authors attribute this fact to a shorter residence time (4 days in this study). The cultivation of *Botryococcus* sp. in vertical tubular photobioreactors was able to reduce TN, $\text{PO}_4\text{-P}$, C, and COD to levels of 59.9%, 36.8%, 54.5%, and 93.9%, respectively (Gani, Sunar, et al., 2017), demonstrating the efficiency of microalgae in removing pollutants from domestic effluents.

The microalgae *Chaetophora elegans* and *Oscillatoria lutea* were tested in raw sewage and effluent, and the results observed were that *O. lutea* showed greater efficiency in removing COD and phosphate when cultivated in raw sewage, whereas *C. elegans* was more efficient in removing COD from raw sewage, while phosphate showed greater efficiency when cultivated in effluent (Najimi et al., 2017). *Chlorella* sp. was able to remediate COD, nitrate, and phosphate in domestic effluents both on a batch scale and through cultivation in an oxidation ditch reactor (Farahdiba et al., 2020; Hidayah & Cahyonugroho, 2021). For example, *C. sorokiniana* was capable of bioremediating domestic effluent under different cultivation conditions. Mixotrophic metabolism in anaerobic tanks resulted in lower levels of ammonia and phosphate absorption when compared to heterotrophic metabolism, as well as cultivation in a flat panel photobioreactor proved to be efficient in reducing the reduction of nitrogen and phosphorus in blackwater (Moges et al., 2020; Ramsundar et al., 2017). *Chlorella minutissima* was also able to remove nutrients from wastewater and the capture of CO_2 when grown in mixotrophic mode (Dineshkumar et

al., 2020). Despite this, the microalga *Chlorella variabilis* achieved bioremediation levels above 80% in light graywater when subjected to heterotrophic cultivation mode (Oktor & Çelik, 2019). *C. vulgaris* was efficient in domestic wastewater treatments when enriched with Bayfolan®, cultivated in a photobioreactor (Fernández-Linares et al., 2017), in a bubble column (Mayhead et al., 2018) and in lagoon systems under different ratios of nitrogen/phosphorus (N/P) and CO₂ concentration (Molazadeh et al., 2019). When cultivated in a consortium, microalgae also demonstrate efficiency in the phycoremediation of domestic wastewater, such as *C. vulgaris*-*C. protothecoides* (Oberholster et al., 2021), *C. vulgaris*-*Desmodesmus asymmetricus* (Oscanoa Huaynate et al., 2021), and *Chlorella* sp.-*Scenedesmus* sp. (Silambarasan et al., 2021). The immobilized functional microalgae were studied in *Desmodesmus* sp. which demonstrated high performance in removing phosphorus and nitrogen in domestic effluent (Wang et al., 2020). *Monoraphidium* sp. was efficient in removing pollutants from domestic sewage wastewater, presenting results above 80% (Mishra & Mohanty, 2019). Secondary wastewater from domestic sewage was bioremediated by *Pseudokirchneriella subcapitata* through glucose and nitrogen supplementation (Conceição et al., 2019).

A lab-scale hybrid loop airlift photobioreactor was used to cultivate the microalgae *Scenedesmus abundans* in domestic wastewater, and in this condition, it was found that *S. abundans* was able to remove COD, nitrogen, and phosphorus (SundarRajan et al., 2020). A culture of *S. acutus* also maintained in a photobioreactor demonstrated great potential for wastewater treatment, being able to remove more than 87% of COD (Angioni et al., 2018). The species *S. obliquus* proved to be efficient in the treatment of domestic wastewater both when cultivated in 150-mL flasks and in a photobioreactor under a cell immobilization mechanism and different hydraulic retention times (Eida et al., 2018; Whitton et al., 2018). In response to wastewater treatment, the microalgae *S. rubescens* removed almost all the phosphorus and nitrogen present in domestic effluent after 20 days of cultivation (Sureshkumar & Thomas, 2019). *Scenedesmus* sp. proved to be efficient in treating effluent when cultivated in a raceway pond (Kumar Gupta et al., 2018), in photoreactor tanks (Mohamed et al., 2018), bubble column photobioreactor (Ren et al., 2019), and in a batch system (Baldev et al., 2021). The resistance of the *Synechocystis* sp. strain to stress in the removal of nutrients from mixed domestic and industrial wastewater was investigated, and it proved to be efficient in removing nitrogen, phosphorus, and COD (Abedi et al., 2019). The cultivation of *T. obliquus* in a column-type photobioreactor was efficient in removing 80% of ammonia present in domestic effluent

(Ma et al., 2020). These, and others, values for nitrogen and phosphorus removal are summarized in Table 2.

It is worth mentioning that the values presented in this article refer to the highest values found in the specialized literature. Also, these results should not be considered a standard, since these values can be changed according to the cultivation system, life cycle, cultivation time, and environmental conditions, such as light, temperature, and pH, among other variables. That is, the removal efficiency varies according to the physical, chemical, and biological parameters in which the organism will be submitted.

Table 2 Efficiency in the removal of nutrients present in domestic wastewater by different species of microalgae.

Species	Removal efficiency (%)								References
	TN	NH ₄ -N	NO ₃ -N	NO ₂ -N	TP	PO ₄ -P	C	COD	
Microalgal-cyanobacterial consortium	81	97	–	–	–	64	90	–	(García et al., 2017)
<i>Acutodesmus obliquus</i>	94	100	–	–	98	–	–	–	(Foix-Cablé et al., 2018)
<i>Arthrospira platensis</i>	15.47	17.16	23.49	–	–	14.57	–	17.88	(Chavan & Mutnuri, 2018)
<i>Botryococcus</i> sp.	59.9	–	–	–	–	36.8	54.5	93.9	(Gani et al., 2017b)
<i>Chaetophora elegans</i>	–	–	–	–	–	93	–	30	(Najimi et al., 2017)
<i>Chlorella</i> sp.	–	–	63.64	–	–	66.15	–	50	(Farahdiba et al., 2020; Hidayah et al., 2021)
<i>Chlorella sorokiniana</i>	98	94.29	56.25	75.0	–	87.82	–	59.42	(Ramsundar et al., 2017; Moges et al., 2020)
<i>C. minutissima</i>	–	99.1	99.5	–	–	–	–	98.18	(Dineshkumar et al., 2020)
<i>C. variabilis</i>	85.6	–	–	–	> 97	–	–	92.3	(Oktor & Çelik, 2019)
<i>C. vulgaris</i>	99.31	100	49.9	–	96.5	97.69	–	100	(Fernández-Linares et al., 2017; Mayhead et al., 2018; Molazadeh et al., 2019)
<i>C. vulgaris</i> - <i>C. protothecoides</i> consortium	73.1	99.4	–	–	74.5	87	69	75	(Oberholster et al., 2021)
<i>C. vulgaris</i> - <i>Demodesmus asymmetricus</i> consortium	–	24.02	100	100	–	77.11	–	–	(Oscanoa Huaynate et al., 2021)
<i>Chlorella</i> sp.- <i>Scenedesmus</i> sp. consortium	94	98	96	–	–	95	86	83	(Silambarasan et al., 2021)
<i>Desmodesmus</i> sp.	86	–	–	–	72	–	–	–	(Wang et al., 2020)
<i>Monoraphidium</i> sp.	–	99.5	89	–	–	100	–	88.44	(Mishra & Mohanty, 2019)
<i>Oscillatoria tenuis</i>	–	96.1	–	–	82.9	–	–	92.6	(Cheng et al., 2018)
<i>Oscillatoria lutea</i>	–	–	–	–	–	98.66	–	73.33	(Najimi et al., 2017)
<i>Pseudokirchneriella subcapitata</i>	98.33	–	–	–	–	> 98	–	–	(Conceição et al., 2019)
<i>Scenedesmus abundans</i>	90.73	–	–	–	86.31	–	–	80.19	(SundarRajan et al., 2020)
<i>Scenedesmus acutus</i>	–	–	–	–	–	–	–	> 87	(Angioni et al., 2018)
<i>Scenedesmus obliquus</i>	78.5	70	–	–	95.2	> 80	–	–	(Eida et al., 2018; Whitton et al., 2018)
<i>Scenedesmus rubescens</i>	–	–	100	–	–	99.95	–	–	(Sureshkumar & Thomas, 2019)
<i>Scenedesmus</i> sp.	96.8	80	86	99	97.7	83.1	93.1	69.9	(Kumar Gupta et al., 2018; Mohamed et al., 2018; Ren et al., 2019; Baldev et al., 2021)
<i>Synechocystis</i> sp.	–	99.44	94.14	–	–	97.53	–	41	(Abedi et al., 2019)
<i>Tetradesmus obliquus</i>	–	80	–	–	–	–	–	–	(Ma et al., 2020)

Biochemical Composition

The biochemical composition of microalgae varies according to the species and physical and chemical variables, depending on the cultivation conditions; these organisms can biosynthesize secondary metabolites such as lipids, proteins, and carbohydrates. These compounds are of great importance for the biotechnology industry. The data with the highest values on the percentage of lipids, proteins, and carbohydrates found in recent literature are shown in Table 3. In general, the variation in the rates of these compounds represents in dry weight 10.6–72.5% (Assis et al., 2019; Gani, Sunar, et al., 2017), 11.5–61.31% (Ashokkumar et al., 2019; Molazadeh et al., 2019), and 12.4–93.5% (Dineshkumar et al., 2020; Kumar Gupta et al., 2018), respectively, with the highest values represented by species from the genera *Botryococcus* and *Chlorella*. These compounds are the basis for the production of several biologically based by-products, and currently, the main focus of research involves the extraction of lipids for the production of biodiesel. However, these compounds have applications in several areas, such as the pharmaceutical and food industries, as well as in agriculture and aquaculture.

The results cited above are subjective, since the biochemical composition of the biomass of a microalga species will vary according to the conditions to which it will be subjected. Thus, it is not possible to infer which species produces more or less nutrients but to use the results presented as parameters for understanding which factors can influence the metabolism of microalgae. Another thing to be considered is the amount of nutrients present in the domestic effluent, which depends on the amount of organic matter and does not have a pre-established standard. In addition, when subjected to some treatment process in an effluent treatment station, the wastewater will reduce its load of organic and inorganic compounds present. Consequently, the use of primary, secondary, or tertiary effluent will also influence the biosynthesis of these compounds by microalgae.

Maximizing the use of algal biomass used in effluent treatment leads to increased revenue through value-added bioproducts and bioenergy (Chavan & Mutnuri, 2018). In most biochemical analyses of microalgae, the protein content of the biomass is high; however, for strains of *Tetradismus obliquus*, *Asterarcys quadricellulare*, *Desmodesmus* sp., and *Pseudopediastrum* sp., the protein content exceeded the carbohydrate and lipid content; this approach has potential use for animal feedstock or bio-fertilizer applications (Do et al., 2019). *Botryococcus* sp. showed potential as new green sources of hydrocarbons due to its high lipid biosynthesis (Gani, Sunar, et al., 2017). Species of the *Chlorella* genus demonstrated high potential in the production of lipids, carbohydrates, and proteins (Dineshkumar et al., 2020;

Fal et al., 2021; Lam et al., 2017; Molazadeh et al., 2019; Ramsundar et al., 2017; Tran et al., 2020). The *Chlorella-Phormidium* consortium presented molecules rich in lipid and protein; when cultivated in a modified algal biofilm reactor, it favored the remediation of domestic effluent and the production of biomethane (Choudhary et al., 2017). According to Silambarasan et al. (2021), the use of intercropping is not only a potential biological resource for wastewater treatment and biodiesel production but also an effective biofertilizer for sustainable agricultural production. The results obtained with the cultivation of *Monoraphidium* sp. confirmed its potential as a bioenergy feedstock (Mishra & Mohanty, 2019).

Table 3. Gross biochemical composition (% of dry biomass) of microalgae grown in domestic wastewater.

Species	Lipids	Proteins	Carbohydrates	References
<i>Arthrospira platensis</i>	26.65	41.24	34.8	(Chavan & Mutnuri, 2018)
<i>Asterarcys quadricellulare</i>	16.99	35.78	26.98	(Do et al., 2019)
<i>Botryococcus</i> sp.	72.5	11.5	34.1	(Gani et al., 2017b; Ashokkumar et al., 2019)
<i>C. sorokiniana</i>	36.93	15.32	24.08	(Ramsundar et al., 2017; Fal et al., 2021)
<i>C. variabilis</i>	26.12	37.34	35.23	(Tran et al., 2021)
<i>C. vulgaris</i>	32.7	61.31	–	(Lam et al., 2017; Molazadeh et al., 2019)
<i>Chlorella</i> and <i>Phormidium</i> consortium	38	44	–	(Choudhary et al., 2017)
<i>Chlorella minutissima</i>	23.7	–	93.5	(Dineshkumar et al., 2020)
<i>Chlorella</i> sp. and <i>Scenedesmus</i> sp. consortium	34.83	–	–	(Silambarasan et al., 2021)
<i>Desmodesmus</i> sp.	23.51	39.66	24.41	(Do et al., 2019)
Microalgae-bacteria consortium	10.6	25.6	21.2	(Assis et al., 2019)
Mixed microalgae culture	40	48.4	56	(Katam & Bhattacharyya, 2018, 2019; Solís-Salinas et al., 2021; Li et al., 2021b)
<i>Monoraphidium</i> sp.	30.07	35.81	30.37	(Mishra & Mohanty, 2019)
<i>Pseudopediastrum</i> sp.	26.45	32.15	27.86	(Do et al., 2019)
<i>Scenedesmus abundans</i>	33.8	43.6	20.15	(SundarRajan et al., 2020)
<i>Scenedesmus acutus</i>	49	–	–	(Angioni et al., 2018)
<i>Scenedesmus</i> sp.	30.5	28	12.4	(Kumar Gupta et al., 2018; Thangam et al., 2021)
<i>Tetradasmus obliquus</i>	22.38	34.29	27.24	(Do et al., 2019)

Biotechnological applications

Of the articles considered for this research, a total of 34 were listed, most of which focus on the production of biofuels from microalgae produced in domestic wastewater, being biodiesel (17 articles), bioenergy (5 articles), and biogas (4 articles) the most studied (Table 4). It can also be seen in Table 3 that there are other emerging areas in the field of biotechnology that are still little explored, such as bioethanol, bio-oil, biostimulants, biofertilizers, biochar, and bioelectricity. In addition to these, obtaining polyhydroxyalkanoates (PHA) and hydrocarbons, which can be used in the manufacture of bioplastics, is still an underdeveloped activity.

Table 4. Absolute number of articles published for each area of biotechnological application.

Biotechnological applications	Number of articles	References
Biodiesel	17	(Lam et al., 2017; Eida et al., 2018; Ruiz-Marin et al., 2018; Mansour et al., 2019; Do et al., 2019; Ali et al., 2019; Marella et al., 2019; Katam & Bhattacharyya, 2019; Dineshkumar et al., 2020; Sharma et al., 2020a, b; Khalekuzzaman et al., 2021; Silambarasan et al., 2021; Fal et al., 2021; Ali et al., 2021; Baldev et al., 2021; Tran et al., 2021)
Bioenergy	5	(Chandra et al., 2017; Angioni et al., 2018; Mishra & Mohanty, 2019; Wang et al., 2019; Marangon et al., 2021)
Biogas	4	(Chavan & Mutnuri, 2018; Cheng et al., 2018; Rodero et al., 2019; Assemany et al., 2020)
Bioethanol	2	(Chavan & Mutnuri, 2021; Thangam et al., 2021)
Bio-oil	1	(SundarRajan et al., 2020)
Biostimulant and Biofertilizer	1	(Li et al., 2021a)
Polyhydroxyalkanoates	1	(Almeida et al., 2021)
Biochar	1	(Ashokkumar et al., 2019)
Hydrocarbons	1	(Gani et al., 2017b)
Bioelectricity	1	(Jiang, 2017)

In percentage terms, the results presented above correspond to 50% of articles focusing on biodiesel production, 14.71% on bioenergy production, 11.76% on obtaining biogas, and the other areas represent 2.94% each (Fig. 3). Despite being economical and ecologically friendly, it

is clear that the use of domestic wastewater to obtain microalgae biomass with biotechnological value still lacks information.

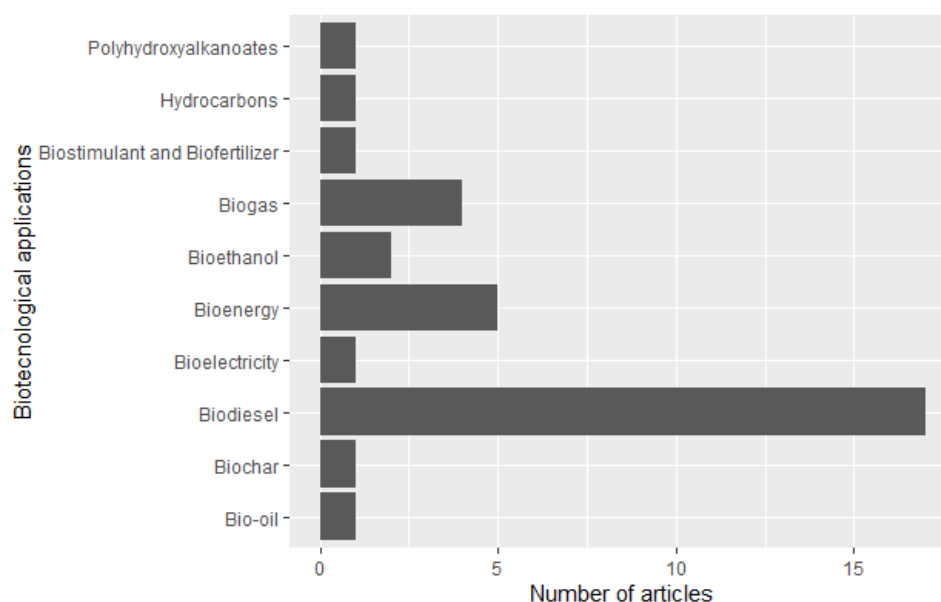


Fig. 3 Percentage of articles published according to biotechnological applications.

Microalgae are considered raw material for the production of third-generation biofuels and can be grown in wastewater to conserve freshwater and also help mitigate climate change by capturing carbon dioxide from the atmosphere (Ali et al., 2021). However, the fatty acid composition of oil extracted from microalgae varies from strain to strain and according to the nutrients supplemented in the medium (Baldev et al., 2021). For biodiesel production, the fatty acid fraction (FA) is transesterified into fatty acid methyl esters (FAME). The FAME profile plays a critical role in determining the properties of the biodiesel produced, being able to determine the suitability of a microalga for the production of this biofuel (Lam et al., 2017; Silambarasan et al., 2021). Therefore, the determination of an appropriate species and cultivation conditions are crucial factors to produce a high-quality biodiesel through the use of domestic effluent.

Marangon et al. (2021), when replacing charcoal briquettes with microalgae biomass briquettes in a cogeneration plant, found a reduction in charcoal extraction and bioremediation of domestic effluent, thus benefiting the environment. In general, studies in the area of microalgae biorefinery demonstrate the sustainability of the use of these organisms. However, it

is important that research continue in the area in order to integrate products and co-products, develop accessible technologies for large-scale application, and thus promote the development of technology for the green economy.

Final considerations

The cultivation of microalgae in domestic wastewater is a low-cost and technically viable alternative for implementation in traditional wastewater treatment plants worldwide. The results summarized in this review demonstrate the potential of these organisms both for phycoremediation and for obtaining high-value biomass for the biotechnology industry, since different bioactive compounds of commercial importance can be extracted from microalgae biomass, such as lipids, proteins, and carbohydrates. Species of the genus *Chlorella* and the family Scenedesmaceae are highly applied in this field of research and demonstrate to be robust and highly efficient in the treatment of domestic wastewater. However, knowledge about the bioremediation potential of other species is limited, as most studies focus on a few species.

According to the research findings, it is evident that further testing is imperative for the successful commercial adoption of this cultivation technology. This need for additional testing is particularly pronounced in emerging domains like microalgae biorefinery and bioenergy. Additionally, we recommend delving into less-studied species, as different strains may require acclimatization to local cultivation conditions. Another underexplored aspect pertains to the utilization of treated effluent, which could serve various purposes such as irrigation and potential reuse in aquaculture.

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3. Article 2: New findings on the survival of *Durusdinium glynnii* under different acclimation methods to low salinities

New findings on the survival of *Durusdinium glynnii* under different acclimation methods to low salinities

Brandão et al., 2025

Abstract

Durusdinium glynnii is an endosymbiotic dinoflagellate associated with corals. The knowledge regarding the physiological responses of this species to long-term saline stress has, thus far, been scarcely explored in the scientific literature. In this context, the present study evaluated the influence of salinity on the growth and cell morphotype of the dinoflagellate *D. glynnii* under two acclimation strategies: abrupt saline shock and gradual salinity reduction. The results demonstrated optimal growth rates within a salinity range of 20 to 30 g L⁻¹ ($\mu = 0.22$ to 0.35 day⁻¹). The S2 acclimation strategy facilitated adaptation to salinities as low as 16 g L⁻¹, maintaining significantly higher cell densities when compared to the S5 strategy. In contrast, extreme salinities, such as 10 and 40 g L⁻¹, compromised growth, thereby highlighting the preference of this dinoflagellate for intermediate salinity conditions. Gradual salinity reduction had a direct impact on the cellular morphotype, promoting the transition from motile cells (mastigotes) to spherical symbiotic forms (coccoid), which were predominant at salinities below 12 g L⁻¹. The findings indicate that *D. glynnii* is a euryhaline species capable of adapting to moderate salinity variations but limited under extreme conditions. This study provides essential insights into the physiology of *D. glynnii*, underscoring its potential for cultivation in brackish water environments and for the production of biomass with biotechnological applications. The data presented herein form the basis for future investigations into the molecular and biochemical mechanisms that underpin salinity tolerance.

Keywords: *Durusdinium glynnii*, dinoflagellates, salinity stress, cell morphology, density.

Introduction

Microalgae are important microorganisms within the Protista kingdom. Among them, dinoflagellates form a monophyletic group distinguished by unique features such as flagellar insertion, specific pigmentation, organelles, and nuclear characteristics that set them apart from other groups (Carty and Parrow 2015). They are among the most diverse unicellular groups on the planet, contributing significantly to both primary production and global biogeochemical processes. The species *Durusdinium glynnii* from the family Symbiodiniaceae, formerly referred to as *Symbiodinium* clade D (LaJeunesse et al. 2014, 2018; Kang et al. 2020; Kannan et al. 2023), holds particular importance due to its mutualistic relationships with invertebrates and reef ecosystems, especially hard corals. This relationship is essential for the formation and maintenance of tropical and subtropical coral reef habitats worldwide (Gordon and Leggat 2010; LaJeunesse et al. 2018).

Saline stress is a common factor in natural environments; however, microalgae develop survival strategies to maintain a balance between growth and stress responses, as eukaryotic algae exhibit high plasticity and adaptability to most abiotic stressors (Ren et al. 2021; Shetty et al. 2019). It is also known that the adaptability of microalgae to saline stress varies according to the species' tolerance level (El-Sheekh et al. 2024). Coastal environments, being highly dynamic, exhibit abrupt variations in the physicochemical properties of water, with salinity emerging as one of the main stressors for the biological community, influenced by rainfall, tidal cycles, and evaporation. This suggests that *D. glynnii* is naturally subjected to salinity fluctuations in its environment; however, knowledge gaps remain regarding the ecophysiological impacts of this variable on the population of this dinoflagellate species.

Salinity is one of the factors that significantly influence the biology and physiology of dinoflagellates (Abadie et al. 2016). These organisms, in particular, are capable of crossing the marine/freshwater barrier more frequently than other protist groups, such as chlorophytes and rhodophytes, due to their ability to adapt to new conditions more rapidly and frequently than anticipated (Čalasan et al. 2019; Annenkova et al. 2020). In this context, physiological studies emerge as important tools to determine the conditions under which algae thrive. It is known that changes in salinity can alter the morphology of protists (Tawong et al. 2015; Heyerhoff et al. 2019), potentially slowing cell division, reducing size, disrupting motility, and inducing the formation of palmelloid structures in microalgae. However, some of these changes are species-specific (Kholssi et al. 2023), and studies investigating the effects of low salinity concentrations on marine microalgae remain limited in the scientific literature (Chaloub et al. 2023).

In summary, understanding how salinity influences the growth and cellular morphology of *D. glynnii* expands our knowledge of the biology of these organisms and generates insights that are directly applicable to cultivation and biotechnology. Comprehending how variations in salinity affect dinoflagellates is essential for optimizing cultivation conditions in laboratory and industrial environments, where stable conditions are critical for the production of high-quality biomass. In addition to advancing the scientific understanding of these organisms, this research provides a robust foundation for innovations and improvements in cultivation practices and biotechnological applications. For this purpose, the present study evaluated the impact of salinity on the growth and cellular morphology of *D. glynnii*, employing two strategies: salinity shock and gradual salinity reduction.

Materials and methods

Biological material

The *Durusdinium glynnii* strain (BMK 211) was obtained from the Live Feed Production Laboratory at the Federal Rural University of Pernambuco, where it is maintained in filtered and autoclaved seawater with a salinity of 30 g L⁻¹, enriched with f/2 culture medium (Guillard 1975). The cultures were acclimated to a controlled temperature of 22±1°C, an irradiance of approximately 150 μmol photons m⁻² s⁻¹, and a 12:12 h light:dark photoperiod.

Experimental design and cultivation conditions

The dinoflagellate pre-culture, initially cultivated in f/2 medium, was transferred to modified Conway medium (Walne 1966). Following the transfer, the strain underwent an adaptation period to adjust to the new nutritional conditions, ensuring proper cell development under optimal conditions for the subsequent experiments.

Experiment 1: This study aimed to evaluate salinity shock stress, where the dinoflagellate was subjected to salinity levels of 10, 20, 30, and 40 g L⁻¹, with 30 g L⁻¹ serving as the control treatment. The experimental design was completely randomized, with three replicates per treatment, totaling 12 experimental units over a duration of 18 days. The assay was conducted in 50 mL screw-cap test tubes, which were manually agitated daily to maintain the cells in suspension. The treatment that demonstrated the best growth conditions in this experiment was used as the starting point for the second phase of the research.

Experiment 2: The objective was to examine the growth capacity of *D. glynnii* under a long-term acclimation strategy. Two strategies for gradual salinity reduction were applied, with intervals of 2 g L⁻¹ (S2) and 5 g L⁻¹ (S5). The experimental design was also completely

randomized, with four replicates per treatment, totaling eight experimental units. The cultures were maintained in borosilicate bottles with a total volume of 1 L and an initial culture volume of 250 mL, under constant aeration. Dilutions were performed weekly, whenever the microalgae reached the exponential growth phase, until either a salinity of 0 g L⁻¹ was reached or total cell mortality occurred.

Both experiments were conducted under a semi-continuous system with controlled temperature (22 ± 1°C), irradiance of approximately 150 μmol photons m⁻² s⁻¹, a 12:12 h light:dark photoperiod, and an initial inoculum density of 5 × 10⁴ cells mL⁻¹.

Growth parameters

To evaluate microalgal growth in the conducted studies, 1.5 mL samples were periodically collected from the experimental units and fixed with formaldehyde (4%) for cell quantification using a hemocytometer under a binocular optical microscope. Based on the average density values, logistic growth curves were plotted for each treatment and adjusted according to Pindich and Rubinfeld (1981). These values were also used to calculate the growth rate (K, divisions day⁻¹), doubling time (DT, days division⁻¹), and specific growth rate (μ, day⁻¹) (Stein 1973). For the maximum cell density (MCD, ×10⁴ cells mL⁻¹), the highest average cell concentration observed during cultivation was considered, and the day of maximum cell density (dMCD, day) corresponded to the cultivation day on which the MCD was observed.

Cell morphotype

Weekly, prior to each dilution, 1.5 mL samples were collected from each experimental unit to estimate the number of cells in the motile stage (mastigote) and symbiotic stage (coccoid). For this purpose, two types of sample preparations were performed: unfixed samples, which allowed the counting of coccoid cells present within the hemocytometer grid, and fixed samples, which were used to quantify the total average number of cells. The difference between the quantifications of fixed and unfixed samples corresponded to the density of mastigote cells (Equation 1).

$$D_{mast.} = D_{total} - D_{coc.} \quad (1)$$

Where: $D_{mast.}$ represents the density of cells in the mastigote stage, D_{total} is the total density of coccoid and mastigote cells, and $D_{coc.}$ represents the density of coccoid cells only.

Statistical analyses

The results were expressed as mean \pm standard deviation. Data were tested for normality and homogeneity using the Shapiro-Wilk and Cochran tests, respectively. Since the data met the assumptions of a normal distribution, they were subjected to analysis of variance (ANOVA), followed by Tukey's test for mean comparisons. To evaluate the influence of gradual salinity reduction on the cell density of the dinoflagellate, a quadratic regression analysis was performed. A significance level of $p < 0.05$ was considered for all analyses.

Results

Growth kinetics of Durusdinium glynnii under different salinities

The results of the salinity shock study demonstrate that this variable influences the biological parameters related to the cell production of *Durusdinium glynnii*. At the highest salinity, the dinoflagellate exhibited the highest division rate (0.42 ± 0.18 div. day⁻¹) in the shortest time (2.70 ± 0.96 days div.⁻¹), reaching an average density of $30.44 \pm 6.34 \times 10^4$ cells mL⁻¹ at a specific growth rate of 0.62 ± 0.11 day⁻¹ on the sixth day of cultivation. Conversely, at the lowest salinity, the dinoflagellate showed a low cell division rate (0.27 ± 0.05 div. day⁻¹) and a high doubling time (3.89 ± 0.81 days div.⁻¹), achieving a cell density of $27.25 \pm 9.28 \times 10^4$ cells mL⁻¹ at 0.19 ± 0.06 day⁻¹ on the eighteenth day of cultivation. The salinity of 20 g L⁻¹ did not differ statistically from the control (30 g L⁻¹) in any growth parameter. Despite the lower division rate and longer doubling time, both treatments numerically exhibited the highest cell densities, ranging from 35.38 ± 12.79 to $34.38 \pm 13.42 \times 10^4$ cells mL⁻¹ for salinities of 20 and 30 g L⁻¹, respectively (Table 1).

Table 1. Growth parameters of the dinoflagellate *Durusdinium glynnii* subjected to stress from different salinities.

Parameters	Salinity (g L ⁻¹)			
	10	20	30	40
K (divisions day ⁻¹)	0.27 \pm 0.05a	0.23 \pm 0.02a	0.23 \pm 0.05a	0.42 \pm 0.18a
DT (days division ⁻¹)	3.89 \pm 0.81ab	4.32 \pm 0.32ab	4.42 \pm 0.88a	2.70 \pm 0.96b
μ (day ⁻¹)	0.19 \pm 0.06c	0.35 \pm 0.03b	0.22 \pm 0.03bc	0.62 \pm 0.11a

MCD ($\times 10^4$ cells mL ⁻¹)	27.25 \pm 9.28a	35.38 \pm 12.79a	34.38 \pm 13.42a	30.44 \pm 6.34a
dMCD (day)	18	6	6	6

Mean \pm standard deviation (n = 3). Mean values within the same row with different letters indicate significant differences according to an ANOVA followed by Tukey's test ($p < 0.05$). K, growth rate; DT, doubling time; μ , specific growth rate; MCD, maximum cell density; dMCD, day of maximum cell density.

The trends observed in the growth curves indicate that, at a salinity of 40 g L⁻¹, the system reached the stationary phase starting from the 6th day, showing growth inhibition in comparison to the other treatments. The salinity of 10 g L⁻¹, in turn, exhibited slower growth, not reaching the stationary phase by the end of the cultivation period. However, it demonstrated a greater growth potential compared to the salinity of 40 g L⁻¹, achieving higher cell densities, albeit with a longer growth period. The curve corresponding to the salinity of 20 g L⁻¹ was the one that most closely resembled the control treatment. The results indicate that the dinoflagellate *D. glynnii* is capable of adapting to a direct shock with a salinity of 20 g L⁻¹ without impairing its growth (Fig. 1).

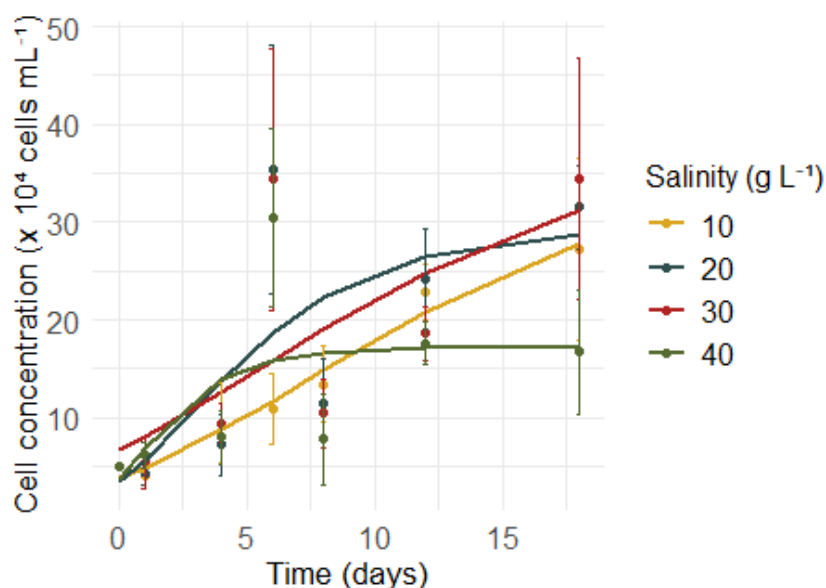


Fig. 1 Logistic growth curve of the dinoflagellate *Durusdinium glynnii* subjected to stress from different salinities.

Growth of D. glynnii under strategies of gradual salinity reduction

Given that *D. glynnii* maintains its growth pattern at a salinity of 20 g L⁻¹, the second experiment was initiated under this condition. Strategy S2 exhibited an initial growth trend during the early stages of the experiment, continuing to increase as salinity was gradually reduced to 18 g L⁻¹. Upon reduction to a salinity of 16 g L⁻¹, a slight decrease in cell density was observed, which remained stable over time, culminating in a peak in density at the end of this period, with an average value of 109.63 x 10⁴ cells mL⁻¹. When the salinity was reduced to 14 g L⁻¹, a marked decrease in cell density occurred, which later stabilized (Fig. 2A).

At a salinity of 12 g L⁻¹, a less pronounced initial reduction in density was observed, followed by a significant increase over time, resulting in a second peak at the end of this stage, with an average of 92.75 x 10⁴ cells mL⁻¹. Upon reduction to 10 g L⁻¹, another sharp decline in cell density was observed, which remained stable until the transition to a salinity of 6 g L⁻¹. From this point onward, density began to gradually decrease until reaching a salinity of 2 g L⁻¹, where no cells were observed in suspension at the end of the experiment on day 60 (Fig. 2A).

Strategy S5 exhibited an initial growth trend during the early stages of cultivation, continuing to increase after the salinity was reduced to 15 g L⁻¹. At this stage, the population density rapidly recovered over time, reaching a maximum average value of 50.42 x 10⁴ cells mL⁻¹. Upon reducing salinity to 10 g L⁻¹, a sharp decline in density was observed. Despite this, *D. glynnii* gradually recovered, eventually reaching the highest average density observed throughout the cultivation for this strategy (75.63 x 10⁴ cells mL⁻¹). On the other hand, when salinity was reduced to 5 g L⁻¹, a further significant decrease in density occurred, which, in this case, failed to recover over time. Finally, upon reduction to a salinity of 0 g L⁻¹, there was a further pronounced decrease in density, culminating in the absence of cells in suspension by the end of the experiment on day 35 (Fig. 2B).

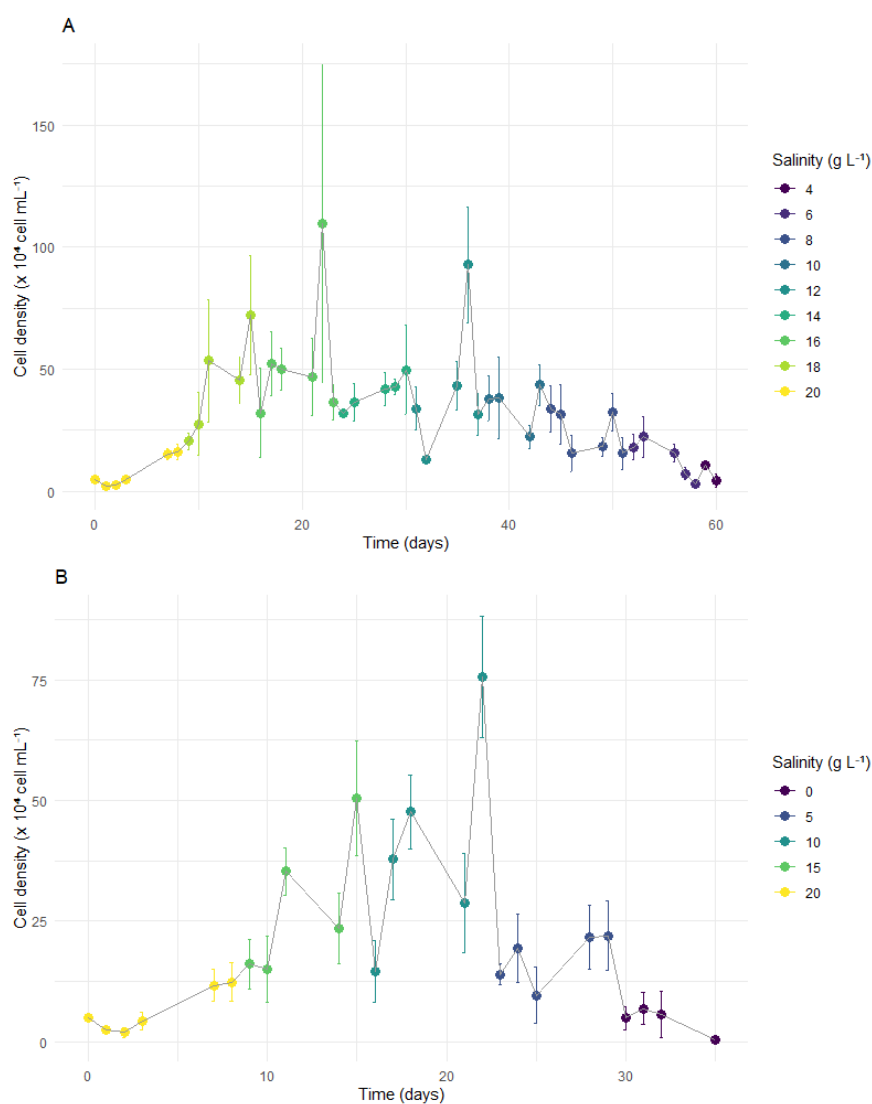


Fig. 2 Cell concentration of *Durusdinium glynnii* subjected to two strategies of gradual salinity reduction. Reduction in intervals of S2 (A) and S5 (B).

The analysis of the density curve as a function of salinity revealed that strategy S2 achieves higher densities in a shorter time compared to strategy S5. However, both curves exhibit an intersection point at a salinity of 12 g L⁻¹. Beyond this point, the density in strategy S2 gradually decreases, falling below the values observed for S5. On the other hand, strategy S5 reaches its highest density at a salinity of 10 g L⁻¹, before the cell density begins to decrease (Fig. 3). These results are consistent with the quadratic regression performed between density and salinity, where the vertex of the parabola passes through the salinity of 12 g L⁻¹. The model fit was moderate ($R^2 = 0.24$), indicating a limited explanation of the data variability by the

regression. Nevertheless, the analysis was significant based on the Pearson correlation ($p = 0.027$), indicating that this correlation is statistically relevant (Fig. 4).

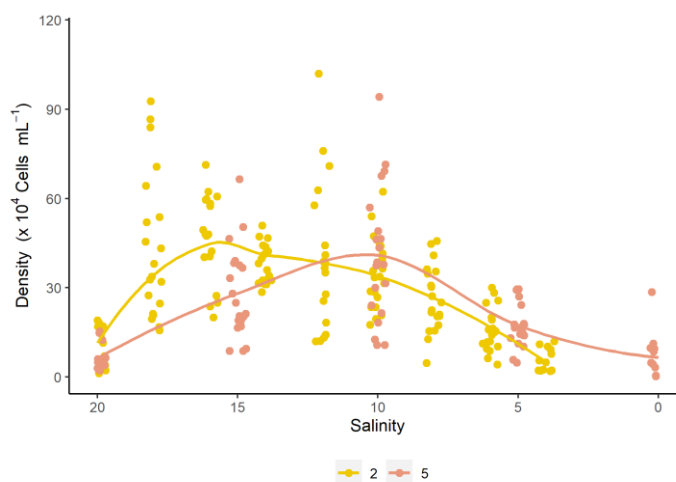


Fig. 3 Correlation between cell density and salinity for the different salinity reduction strategies (S2 and S5) for the dinoflagellate *Durusdinium glynnii*.

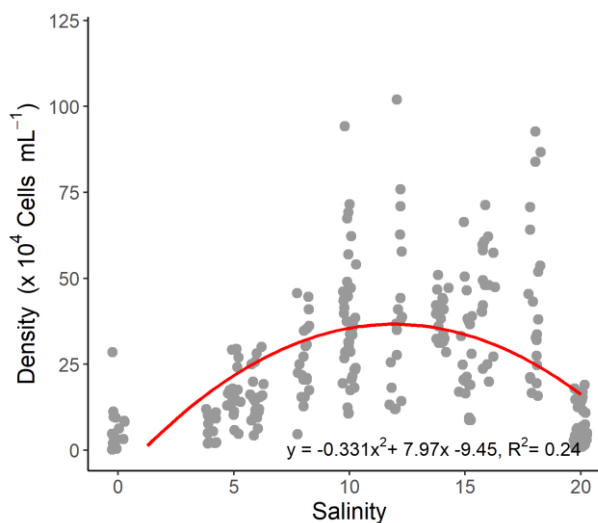


Fig. 4 Quadratic regression analysis of the cell density of *Durusdinium glynnii* as a function of salinity.

Cell morphotype

In the classification of cell morphotypes of *D. glynnii*, strategy S2 exhibited both coccoid cells and mastigotes at salinities ranging from 20 to 12 g L⁻¹. Initially, the cells were

predominantly mastigotes (approximately 70% of the total population), and as the dilutions progressed, coccoid cells became more prominent. At a salinity of 10 g L⁻¹, mastigotes were no longer present, and the population consisted exclusively of coccoid cells, reaching 100% of this morphotype at salinities equal to or below 10 g L⁻¹. In strategy S2, all salinities showed statistically significant differences between coccoid and mastigote cell morphotypes, with the exception of salinities 16 and 12 g L⁻¹, where no significant differences between these two morphotypes were observed (Fig. 5A).

In strategy S5, an inverse pattern was observed, with coccoid cells being dominant throughout the cultivation period. Statistically significant differences between coccoid and mastigote cell morphotypes were observed at salinities 10 and 5 g L⁻¹. At salinities 20 and 15 g L⁻¹, no significant differences between these two morphotypes were found. Furthermore, at a salinity of 5 g L⁻¹, mastigote cells were no longer detected, with 100% of the cell population consisting of coccoid cells (Fig. 5B).

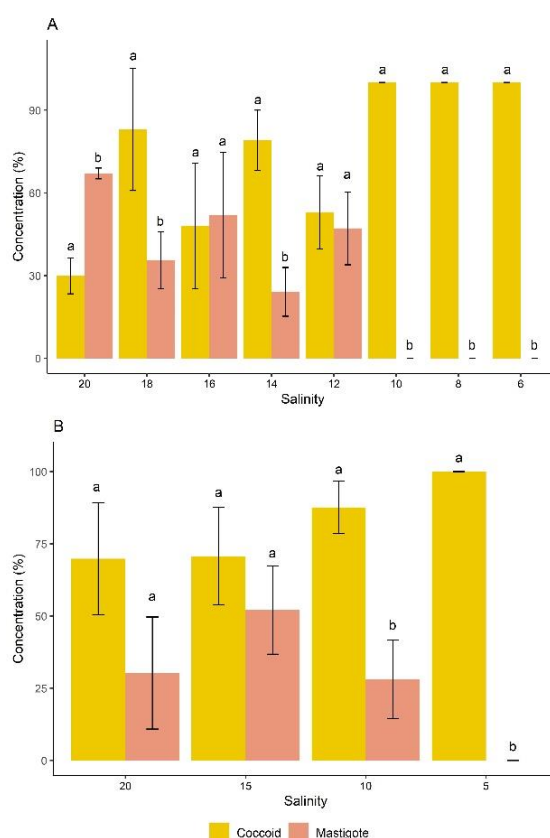


Fig. 5 Distribution of cell morphotypes of *D. glynnii* in strategies S2 (A) and S5 (B) at different salinities.

Discussion

Currently, there is limited literature available on isolated cultures of *Durusdinium glynnii*, and our study evaluated, for the first time, the long-term responses of this dinoflagellate to stress caused by salinity variations, expressing its tolerance and survival at low salinity. We observed that, even under stress induced by salinity shock, *D. glynnii* was able to grow in salinities between 10 and 40 g L⁻¹, although with reduced growth at 40 g L⁻¹ and a longer exponential phase at 10 g L⁻¹. A similar behavior was observed for the species *Akashiwo sanguinea*, which exhibited growth within the same salinity range (Matsubara et al. 2007). On the other hand, the species *Alexandrium minutum* exhibited growth between 12 and 37 g L⁻¹, with its optimal growth range between 20 and 30 g L⁻¹, classifying it as euryhaline (Grzebyk et al. 2003; Lim and Ogata 2005). Strains of *Ostreopsis* spp. grew within a salinity range of 20 to 40 g L⁻¹ (Tawong et al. 2015). *D. glynnii* exhibited an optimal growth range between salinities of 20 and 30 g L⁻¹, resembling *A. minutum*. This plasticity suggests that the dinoflagellate we studied may also be euryhaline, which enables it to adapt to varying salinity conditions.

Despite the reported results, *D. glynnii* showed a clear preference for higher salinity conditions, as evidenced by the reduction in growth rate at lower salinities. At high salinities, it is known that enzymes can be deactivated, leading to a reduction in the photosynthetic rate and consequent loss of cellular water, factors that contribute to decreased cell density (Zafar et al. 2021). This mechanism explains the short exponential phase observed at a salinity of 40 g L⁻¹, with growth inhibition from the sixth day of cultivation, even with a relatively high specific growth rate ($0.62 \pm 0.11 \text{ day}^{-1}$). On the other hand, exposure to low salinities is often associated with oxidative stress in dinoflagellates (Bui et al. 2022), which may have contributed to the slower adaptation and reduced densities observed in our study at a salinity of 10 g L⁻¹ ($0.27 \pm 0.05 \text{ div. day}^{-1}$). *A. minutum* exhibited similar behavior to *D. glynnii* when cultivated at 10 g L⁻¹, with very slow growth ($\sim 0.15 \text{ divisions day}^{-1}$) and higher growth rates at elevated salinities (Grzebyk et al. 2003).

Quantifying the growth rate provides valuable information regarding overall health and response to specific conditions, as it allows for the assessment of the organism's ability to adapt to different environments (Klueter et al. 2017). Within the optimal growth range (20 and 30 g L⁻¹), the specific growth rate (μ) of *D. glynnii* ranged from 0.22 to 0.35 day⁻¹. The dinoflagellate *Alexandrium tamiyavanichii*, which also grew well between 20 and 30 g L⁻¹ ($\mu = 0.25$ to 0.35 day⁻¹) (Lim and Ogata 2005), exhibited similar results. For *Vulcanodinium rugosum*, the salinities that favored these results were 25 and 40 g L⁻¹ ($\mu = 0.21 - 0.39 \text{ day}^{-1}$) (Abadie et al. 2016). *Prorocentrum micans* showed better growth at a salinity of 30 g L⁻¹ ($\mu = 0.38 \text{ day}^{-1}$) and low

growth at 25 g L^{-1} ($\mu = 0.15 \text{ day}^{-1}$), while *P. obtusum* exhibited similar growth (Guimarães and Rorig 2004). This comparison highlights that, although these dinoflagellate species share similar specific growth rates, their salinity preferences vary, reflecting species-specific adaptations.

During the salinity shock experiment, *D. glynnii* reached a maximum cell density of $35.38 \pm 12.79 \times 10^4 \text{ cells mL}^{-1}$. Previous studies reported that this same species reached a maximum density of $70.25 \pm 2 \times 10^4$ and $91.83 \pm 16.83 \times 10^4 \text{ cells mL}^{-1}$ (Oliveira et al. 2022, 2023). A reasonable explanation for the lower density observed in the present study is that the salinity shock experiment was conducted in test tubes without aeration, reducing the oxygen concentration in the culture medium, which likely favored slower growth or lower cell density. Regarding gradual acclimation, the highest cell density in strategy S2 was observed at a salinity of 16 g L^{-1} , while in strategy S5, it occurred at a salinity of 15 g L^{-1} . Although these salinities are similar, S2 achieved more than double the cell density compared to S5, suggesting that more gradual salinity reductions are more effective in promoting biomass production.

During the gradual acclimation process, it was observed that, starting at a salinity of 6 in strategy S2 and at a salinity of 5 g L^{-1} in strategy S5, there was a more pronounced decline in the growth curve. Under these conditions, the species was no longer able to adapt, indicating that this may be the lethal salinity threshold for *D. glynnii*. This behavior may be related to the osmoregulation process, which requires high energy expenditure from the cells, compromising growth rates (Martens et al. 2016). Another important factor to consider is the age of the cultures, as the gradual salinity reduction test in the present study lasted between 35 and 60 days. Aged cultures of dinoflagellates show a decrease in the production of genes related to stress/stimulus responses when exposed to abiotic stress conditions, resulting in increased cell mortality in response to external stress in marine microalgal populations with the advancing chronological age of the culture (Johnson et al. 2012; Gao and Erdner 2022). Based on this, we hypothesize that the aging of *D. glynnii* cultures, combined with extreme salinity reduction, reduces cell motility, compromises stress responses, and increases cell mortality. This suggests that cells progressively become less adaptable and more vulnerable as they age, which may affect the survival of the dinoflagellate.

Most symbiotic species have the potential to transition between a free-living motile stage (mastigote) and a spherical symbiotic stage (coccoid) (González-Pech et al. 2021). Microalgae respond to abiotic stress through various mechanisms, with morphological changes such as cell size alterations, flagellar loss, and reduction of motile cells as culture age increases (Novosel et

al. 2022; Jeong et al. 2013). This pattern was corroborated by the present study, which observed both cell morphotypes, with coccoid cells being predominant. This predominance progressively increased, reaching 100% coccoid cells as the stress caused by decreasing salinity intensified, demonstrating a clear transition from mastigote to coccoid cells in both strategies S2 and S5. A previous study indicated that under optimal growth conditions, *D. glynnii* was composed predominantly of mastigote cells (Oliveira et al. 2023). The same authors further reported that thermal stress reduced this percentage to below 90%, except when there was a high urea concentration, which favored mastigotes (>95%). Conversely, low nitrate concentrations induced a predominance of coccoid cells during thermal stress. Despite the application of different types of stress, both studies indicate a transition between morphotypes, with environmental stress favoring the formation of coccoid cells, but suggesting that the factors inducing this transition vary depending on the type of stress applied.

Conclusions

Salinity has been shown to be a key variable for the growth of the dinoflagellate *Durusdinium glynnii*, with an optimal range between 20 and 30 g L⁻¹. The experimental results suggest that this species can survive and maintain competitiveness in brackish water habitats, with the potential for adaptation to salinities up to 16 g L⁻¹ through strategy S2, without significantly compromising cellular growth. Although it does not guarantee maximum performance under low salinity conditions, cultivation under these conditions holds potential for optimizing the production of biocompounds of biotechnological interest. These findings provide fundamental insights into the ecophysiological behavior of *D. glynnii* in response to salinity variations, an area still underexplored in the scientific literature. This preliminary study establishes basic parameters for the growth and adaptation of the species, which may serve as a foundation for future, more in-depth investigations. Subsequent research could explore the molecular, biochemical, and metabolic mechanisms underlying the observed plasticity, thus broadening the potential applications of this dinoflagellate in biotechnology and ecological applications.

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4. Article 3: Bioremediation of domestic effluent, growth, and production of biocompounds by the dinoflagellate *Durusdinium glynnii*

Bioremediation of domestic effluent, growth, and production of biocompounds by the dinoflagellate *Durusdinium glynnii*

Brandão et al., 2025

Abstract

The study investigated the efficiency of nutrient removal, growth kinetics, and production of bioactive compounds by the dinoflagellate *Durusdinium glynnii* cultivated in different proportions of autoclaved (AUT) and microfiltered (MIC) domestic effluent. Ammonia, nitrite, and orthophosphate removal varied according to effluent conditions, reaching maximum values of 83.64% for ammonia, 100% for nitrite, and 100% for orthophosphate. Growth did not show a significant difference ($p < 0.05$) due to the effluent pretreatment (AUT and MIC), effluent concentration, or the interaction between these factors. This indicates that it is possible to completely replace the synthetic culture medium with domestic effluent in the cultivation of *D. glynnii*. The production of photosynthetic biocompounds showed an increase in the concentrations of chlorophylls and total carotenoids in the treatment with 50% effluent. The correlations between the pigments indicated interdependence between chlorophyll *a*, chlorophyll *c*, and peridinin. The results suggest that the dinoflagellate *D. glynnii* is a promising option for the bioremediation of domestic effluents and the production of biomass rich in high-value biotechnological compounds.

Keywords: dinoflagellate, effluent, nutrients, pigments.

Introduction

The current challenges arising from population growth, energy scarcity, global warming, and climate change have placed pressure on freshwater supplies, rendering water a scarce resource (Abedi et al. 2019; Ashokkumar et al. 2019). As a result of the population growth in urban areas, there has been a considerable increase in water consumption, which has led to the growing discharge of wastewater into the environment, often untreated (Abdel-Raouf et al. 2012; Dineshkumar et al. 2020; Do et al. 2020). One of the main sources of water pollution is the uncontrolled release of human waste (Abdel-Raouf et al. 2012). Approximately half of the world's seas, rivers, and lakes have been contaminated with untreated domestic and industrial wastewater, which, upon reaching water bodies, leads to changes in pH and a decrease in dissolved oxygen concentration, resulting in the death of various aquatic life forms (Sabeen et al. 2018; Moondra et al. 2020) due to the eutrophication process in aquatic ecosystems.

Although conventional technologies (physical and chemical) for treating these effluents are effective, they are not economical, are poorly scalable, and often produce toxic byproducts. For this reason, biological treatment techniques emerge as a cheap and environmentally safe alternative (Yadav et al. 2020). Wastewater is rich in organic matter and nutrients, and when considering domestic wastewater, there are advantages in its use as a medium for microalgae cultivation, such as low toxicity, minimization of freshwater use, and high concentrations of nutrients such as nitrogen, phosphorus, and trace elements (Soydemir et al. 2016; Wang et al. 2022). The use of effluents as culture media for microalgae has attracted interest from the scientific community because, according to Rempel et al. (2023), this approach presents economic and environmental potential, enabling biomass generation, nutrient recovery, and, consequently, the treatment of these effluents.

The genera *Scenedesmus* and *Chlorella* are widely cited for their ability to reduce the organic load of domestic effluents and their resistance to nutritional alterations, allowing them to develop in media with various contaminants (Rempel et al. 2023). Freshwater species are primarily used for the treatment of domestic effluents. However, despite being unconventional, studies have also been conducted using marine microalgae for the bioremediation of these effluents, such as *Dunaliella viridis* and *Tetraselmis chuii* (Costa et al. 2004), *Tetraselmis indica* (Amit et al. 2017), and *Amphidinium carterae* (López-Rosales et al. 2022). In this context, the marine dinoflagellate *Durusdinium glynnii* emerges as a potential species for the treatment of domestic effluent and the production of biomass with biotechnological value.

Dinoflagellates are organisms capable of producing allelopathic compounds and growing under mixotrophic nutrition, which is why they hold great potential for wastewater treatment (Oliveira et al. 2020). Moreover, most of these photosynthetic organisms possess brown-red chloroplasts containing chlorophylls *a* and *c*₂, beta-carotene, a group of unique xanthophylls, including peridinin, and cellulose plates forming the theca (Cousseau et al. 2020). Carotenoids and chlorophylls are the most abundant natural pigments, and among dinoflagellates, carotenoids in the form of the Peridinin-Chlorophyll *a*-Protein (PCP) complex have exhibited significant anti-tumor and anti-inflammatory properties (Supasri et al. 2021). Thus, in addition to their potential for wastewater treatment, dinoflagellates stand out as a promising source of bioactive compounds.

Based on this information, it can be concluded that the use of domestic effluent as a culture medium for dinoflagellates with biotechnological potential presents an economic and sustainable alternative. The present study aims to investigate the effects of different concentrations of domestic effluent, treated by microfiltration and autoclaving, on the growth of the dinoflagellate *Durusdinium glynnii*, with the goal of increasing carotenoid, chlorophyll, and antioxidant compound production, as well as determining the absorption of nutrients present in the effluent.

Materials and Methods

Biological Material and Collection of Domestic Effluent

The strain of *Durusdinium glynnii* (BMK 211) was obtained from the strain bank of the Live Feed Production Laboratory at the Universidade Federal Rural de Pernambuco (UFRPE), where it is maintained in filtered and autoclaved seawater with a salinity of 30 g L⁻¹, enriched with f/2 culture medium (Guillard, 1975). The cultures were acclimatized to a controlled temperature of 22 ± 1°C, an irradiance of approximately 150 μmol photons m⁻² s⁻¹, and a photoperiod of 12:12 hours light:dark. The secondary domestic effluent was obtained from the waste treatment station at UFRPE. The composition of the f/2 culture medium, as well as that of the domestic effluent subjected to two pre-treatment processes, is described in Table 1.

Table 1. Initial characterization of the f/2 culture medium and domestic effluent used in the cultivation of *D. glynnii*.

Nutrients	f/2 Medium	Autoclaved Effluent	Microfiltered Effluent
Amônia ($\mu\text{g L}^{-1}$)	137.76	158.48	53.91
Nitrito ($\mu\text{g L}^{-1}$)	60.41	64.37	21.62
Ortofosfato ($\mu\text{g L}^{-1}$)	249.99	1706.33	3353.99

Experimental Design and Cultivation Conditions

The treatments were distributed in a completely randomized experimental design with a 2x6 factorial scheme, all performed in triplicate, totaling 36 experimental units. The first factor involved domestic effluent treated by microfiltration (MIC) and autoclaving (AUT), and the second factor consisted of a comparative control of 100% f/2 culture medium and five others with different concentrations of effluent added to the culture medium, namely: 6.25% (6.25E), 12.5% (12.5E), 25% (25E), 50% (50E), and 100% (100E). A brief schematic flow of how the effluent treatment was carried out for the development of this study is presented in Figure 1.

D. glynnii was inoculated at an initial concentration of 10×10^4 cells mL^{-1} , and the experimental cultures were maintained in flasks with 1 L of usable volume, under constant aeration, at an ambient temperature of $22 \pm 1^\circ\text{C}$, with an approximate light intensity of $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, a 12:12 hours light:dark photoperiod, and initial salinity of 25 g L^{-1} . The culture was conducted for 24 days, at which point all experimental units reached the stationary growth phase.

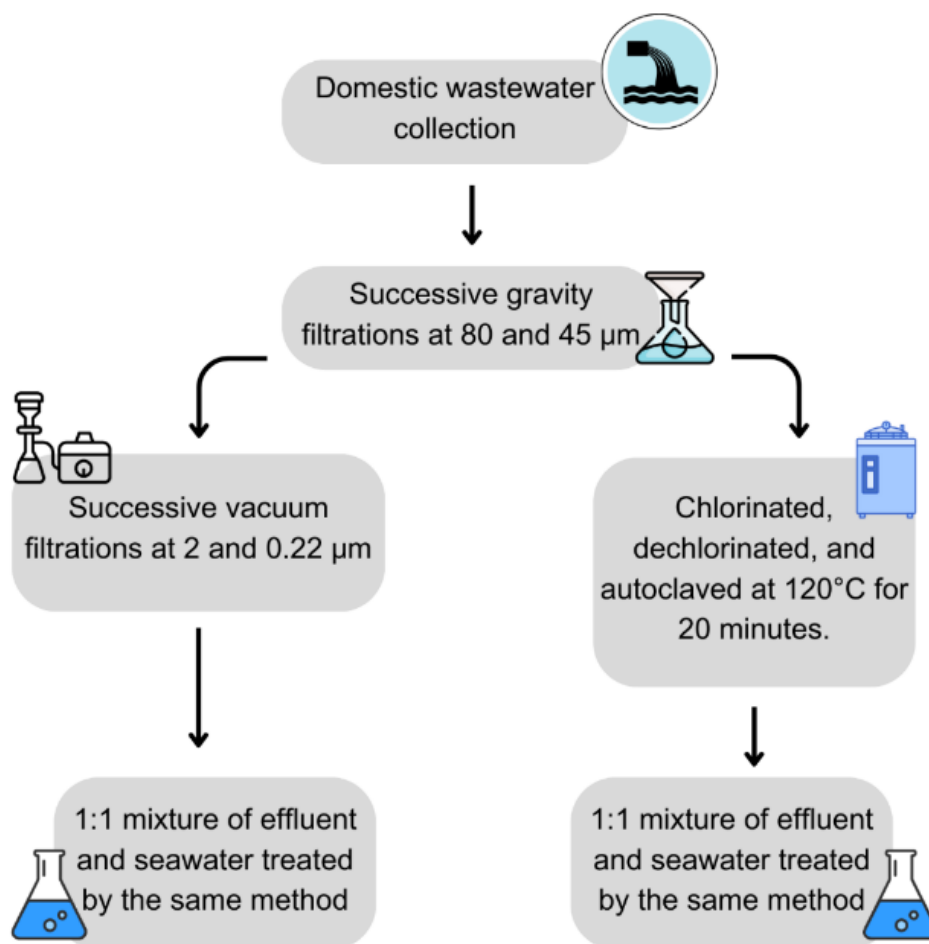


Fig. 1 Flowchart of domestic effluent treatment used in the cultivation of *Durusdinium glynnii*.

Nutrient Removal Efficiency

The water quality variables nitrite, ammonia, and orthophosphate were analyzed in samples of the initial and final culture media. Aliquots of 5 mL, previously filtered using a microfilter (0.22 μm), were then used to determine the concentrations of nitrogenous and phosphorous compounds (APHA, 2012). The nutrient removal rate was determined at the end of the experiment, following the method proposed by Moraes et al. (2024).

Growth Kinetics

Samples of 1.5 mL were collected and fixed with a 4% formaldehyde solution for growth analysis on days 0, 1, 2, 3, 6, 9, 12, 15, 18, 21, and 24. Cell quantification was determined by

counting using a Neubauer hemocytometer coupled with a binocular optical microscope. The cell count corresponding to the average of the three counts was applied in the adjustment of the logistic growth curve for each treatment, according to Pindich and Rubinfeld (1981). The average of these values was also applied in the determination of the maximum cell density (MCD, $\times 10^4$ cells mL^{-1}) and the day of maximum cell density (dMCD, day). The growth rate (K, divisions day^{-1}), doubling time (DT, days division^{-1}), and specific growth rate (μ , day^{-1}) were calculated using the equations proposed by Stein (1973).

Production of Photosynthetic Biocompounds

At the end of the bioassay, 15 mL samples were collected from each experimental unit to determine the concentrations of photosynthetic pigments. The samples were centrifuged at 3000 rpm for 10 minutes, and the biomass was then washed twice with distilled water to eliminate salt residues. Pigment extraction was performed using 90% acetone (Strickland and Parsons 1972). The levels of chlorophylls a and c, as well as total carotenoids, followed the method described by Parsons et al. (1984). Peridinin was measured according to the description of Prézelin (1976).

Statistical Analyses

The results were subjected to the Shapiro-Wilk normality test and the Levene test to check for data homogeneity. As the data met the assumptions of a normal distribution, two-factor ANOVA analysis and Tukey's test for mean comparison were applied. A significance level of 5% was considered for all analyses. To identify the relationship between the variables pigments and carotenoids produced, a correlation matrix analysis was applied.

Results and Discussion

Nutrient Removal Efficiency

The efficiency of nutrient removal did not show a defined pattern between the autoclaved and microfiltered effluents, nor in relation to the different levels of domestic effluent inclusion in the culture medium. For ammonia, a removal of 74.34% was observed in the treatment with 6.25% AUT, while in the treatment with 6.25% MIC, the removal reached 83.64%. In general, a sharp decline in ammonia removal efficiency was observed with the inclusion of 50% autoclaved

effluent. On the other hand, in the microfiltered treatment, no clear pattern was identified in the removal rate of this nutrient (Fig. 2).

For nitrite, complete removal was observed only in the condition with f/2 AUT culture medium. In the MIC treatment, 100% bioremediation occurred under the conditions with 12.5%, 50%, and 100% inclusion of domestic effluent. Nitrite showed the largest discrepancy in the treatment with 6.25% autoclaved effluent compared to the other treatments. In contrast, in the treatments subjected to microfiltration, a more consistent pattern was observed, with low variation between the tested conditions. The only exception was the treatment with 25E, which recorded the lowest removal value, reaching 83.59% (Fig. 2).

For orthophosphate, bioremediation was 100% in the treatment with f/2 autoclaved culture medium and 87.34% in the treatment with 100% microfiltered effluent. This nutrient was the only one that showed relative constancy in bioremediation across all treatments, especially those subjected to microfiltration (Fig. 2).

The literature suggests that the composition of wastewater has a significant impact on the development of microalgae and the rate of pollutant removal (Srimongkol et al. 2022). Furthermore, the physiology and morphology of each microalga differ in their efficacy and efficiency in the removal of nitrogen and phosphorus from different wastewater sources (Nguyen et al. 2022). In summary, the results observed in this study vary according to the physicochemical characteristics of domestic effluent, the pretreatment methods, and the physiology of *D. glynnii*.

According to Su et al. (2021), microalgae utilize ammonia more rapidly compared to other nitrogen sources due to the lower energy demands for its metabolism, and phosphorus uptake by microalgae is usually excessive in wastewater treatment systems, enhancing phosphorus removal. However, phosphorus uptake by algae is not necessarily stoichiometric and may be affected by factors beyond the physiology and concentration of this nutrient, such as its chemical forms, light intensity, pH, and temperature (Choi and Lee, 2015). In contrast, *D. glynnii* demonstrated higher efficiency in the uptake of nitrite compared to ammonia, yet our results demonstrate the dinoflagellate's significant ability to absorb phosphate compounds under all conditions studied.

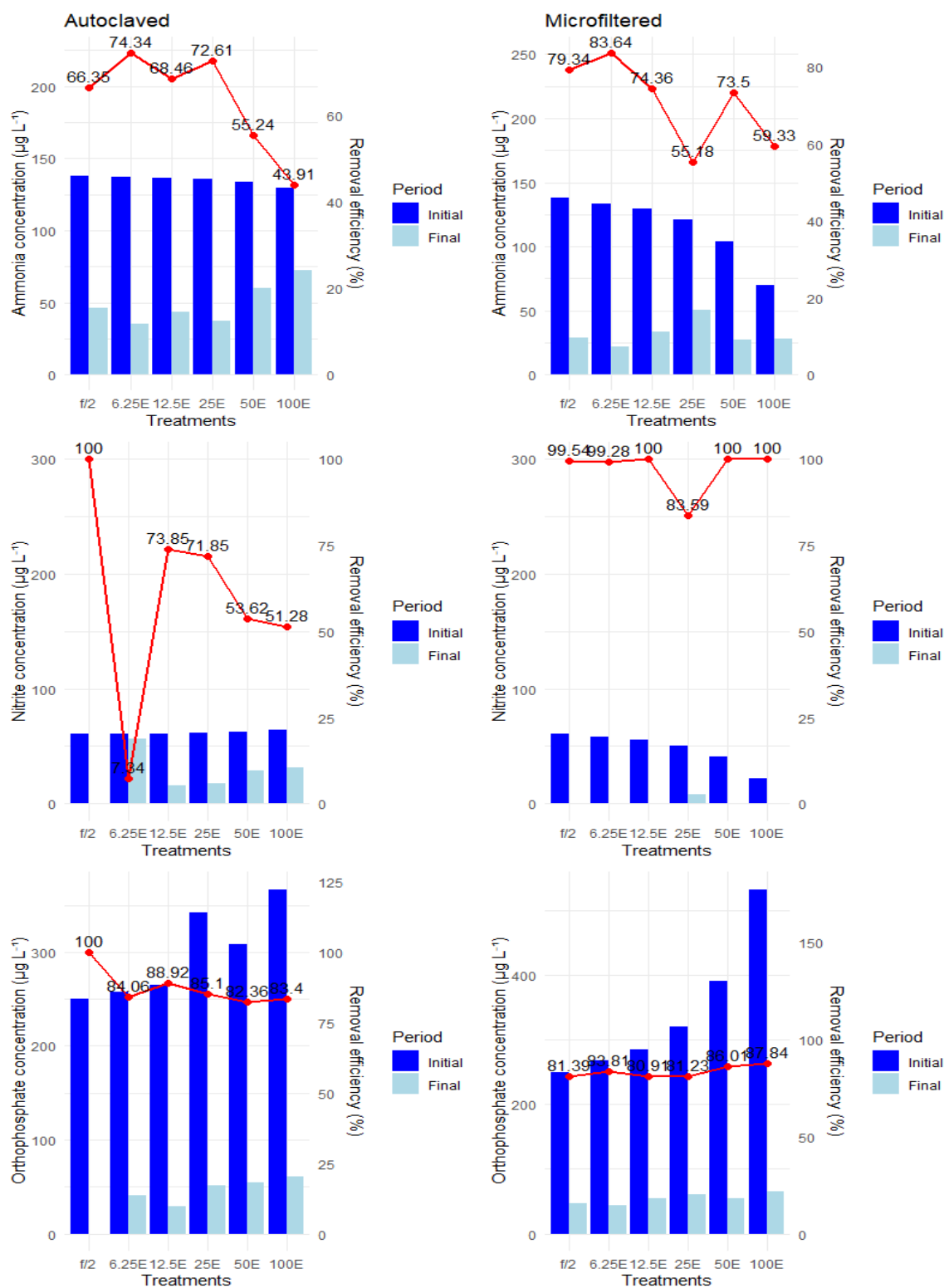


Fig 2. Nutrient concentration and efficiency of ammonia, nitrite, and orthophosphate removal for the cultivation of *Durusdinium glynnii* with different additions of domestic effluent to the culture medium.

Growth Kinetics

Durusdinium glynnii was able to grow in the different treatments and concentrations of domestic effluent. There is no evidence that the effluents, AUT and MIC, their concentrations, and the interactions had a significant impact on K, DT, μ , and MCD ($p > 0.05$), indicating that the treatment applied to the effluent, as well as its concentration, did not negatively interfere with the growth of the dinoflagellate. Although no statistical differences were observed, the 50% MIC treatment showed greater potential for biomass production ($146.08 \pm 8.61 \times 10^4$ cells mL⁻¹). The complete (100%) replacement of synthetic culture medium with domestic effluent is viable, as relatively high cell densities were observed in 100% AUT and MIC effluent (Table 2). In contrast, the dinoflagellate *Prorocentrum minimum* showed better growth performance when subjected to a low concentration of autoclaved urban wastewater (10%), whereas in the non-autoclaved condition, growth showed instability (Ho et al. 2013). This demonstrates the greater adaptability of *D. glynnii* for cultivation in domestic effluent.

In this study, *D. glynnii* was cultivated for the first time in domestic effluent, and the results are quite promising. When compared to marine species, *Dunaliella viridis* and *Tetraselmis chunii* reached densities of approximately 153.63×10^4 cells mL⁻¹ and 131.63×10^4 cells mL⁻¹, respectively, when cultivated in 40% secondary urban effluent (Costa et al. 2004), values that are close to the maximum density observed in the present study. However, when compared to a freshwater species like *Scenedesmus* sp., which reached maximum densities of 3.6×10^6 and 1.9×10^6 cells mL⁻¹ when cultivated in primary and secondary domestic wastewater, respectively (Zhang et al. 2014), the dinoflagellate showed lower values. The densities found in our study are consistent for the species, as the literature reports peaks of $91.83 \pm 16.83 \times 10^4$ cells mL⁻¹ for *D. glynnii* when cultivated under optimal conditions (Oliveira et al. 2023). This finding demonstrates that the dinoflagellate could be a viable option for cultivation in domestic effluents, paving the way for sustainable and economic applications in the biomass production of this microalga.

Another relevant aspect is that cultivation in 100% AUT effluent reached maximum cell density on day 12, while in 100% MIC effluent, this peak occurred only on day 18. This reduction in cultivation time is a positive factor, as it contributes to reducing operational costs, making the microalgae biomass production process more efficient and economically viable (Table 2). When cultivated in wastewater from shrimp farming, *D. glynnii* reached maximum cell density on day 12 in all treatments (Oliveira et al. 2024). In contrast to the results observed in the present study,

where this parameter was variable, this implies that different nutrient sources affect the microalgae's growth efficiency.

Table 2. Growth kinetics of the dinoflagellate *Durusdinium glynnii* cultivated in different proportions of domestic effluent.

Effluent treatment	Effluent concentrations (%)	K (div. day ⁻¹)	DT (day ⁻¹ div.)	μ (day ⁻¹)	MCD (x 10 ⁴ cells mL ⁻¹)	dMCD (day)
AUT	f/2	0.138±0.014	7.293±0.699	0.221±0.028	126.17±3.99	18
	6.25E	0.179±0.013	5.592±0.396	0.167±0.012	81.50±9.50	12
	12.5E	0.140±0.010	7.151±0.498	0.223±0.018	98.50±15.50	24
	25E	0.153±0.019	6.597±0.839	0.203±0.042	112.50±9.50	18
	50E	0.151±0.013	6.643±0.610	0.208±0.018	116.25±8.00	12
	100E	0.167±0.003	6.006±0.093	0.208±0.010	104.00±0.50	12
MIC	f/2	0.162±0.015	6.190±0.557	0.210±0,013	79.25±9.55	12
	625E	0.170±0.012	5.900±0.443	0.181±0,012	101.83±19.34	18
	12,5E	0.171±0.023	5.914±0.804	0.175±0,047	108.75±15.20	12
	25E	0.137±0.004	7.305±0.206	0.221±0,010	122.50±9.00	18
	50E	0.140±0.006	7.172±0.317	0.232±0,016	146.08±8.61	18
	100E	0.177±0.013	5.662±0.387	0.180±0.016	106.58±13.13	18
p- value	T	0.453	0.465	0.429	0.523	-
	C	0.269	0.231	0.302	0.837	-
	T x C	0.628	0.602	0.562	0.864	-

Mean ± standard deviation (n = 3) subjected to two-factor ANOVA followed by Tukey's *post hoc* test ($p < 0.05$). Where T represents factor 1 (treated effluent); C represents factor 2 (effluent concentration); T x C represents the interaction between the two factors.

Regarding the growth curve, it was observed that the treatments with 50% and 100% autoclaved effluent exhibited a shorter exponential phase compared to the others, which concluded on the ninth day; however, this did not negatively affect biomass production. The other treatments, both MIC and AUT, entered the stationary phase starting from the 12th day. (Figs. 3 and 4). This suggests that, despite a shorter growth phase, the dinoflagellate was able to adapt to conditions of high concentrations of domestic effluent. Research suggests that this occurs due to the adequate supply of nutrients necessary for growth, as algae are capable of efficiently redirecting the compounds present in wastewater to the produced biomass, promoting circular economy and process sustainability (Su 2021; Amenorfenyo et al. 2022; Srimongkol et al. 2022). Therefore, we suggest that, despite the reduced exponential phase, the system was not compromised over time, ensuring efficient biomass production.

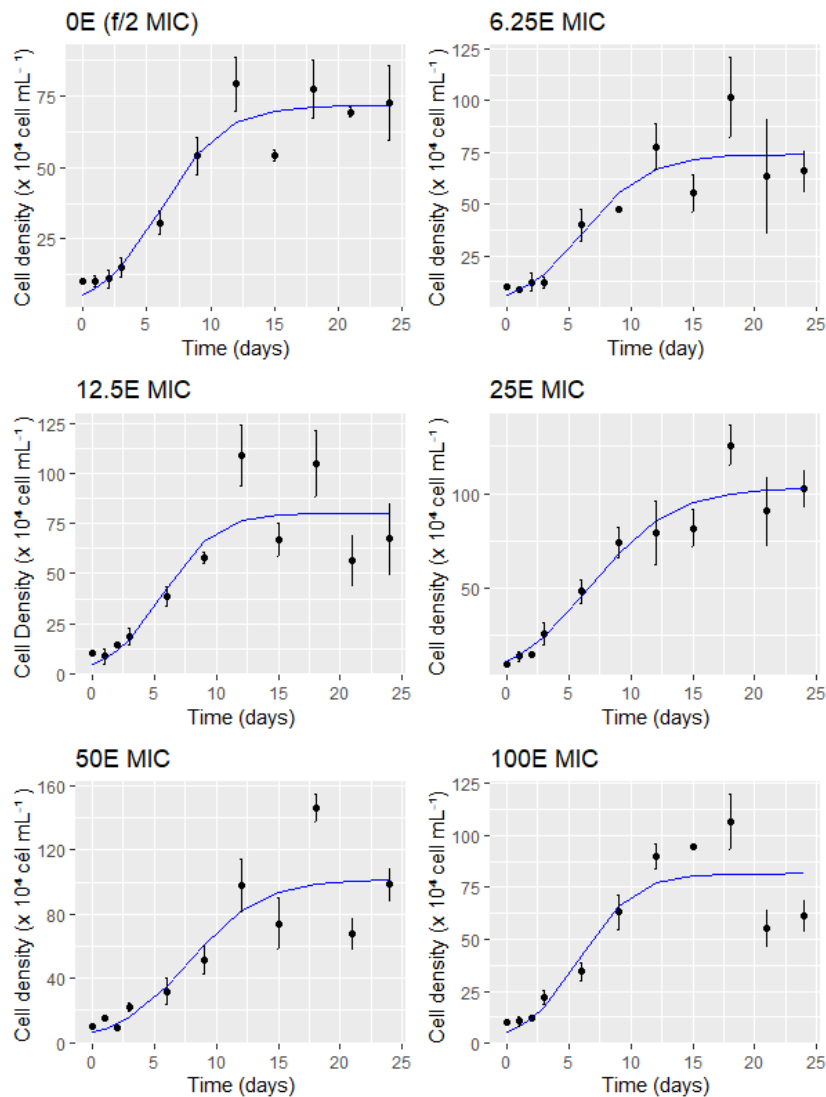


Fig. 3 Logistic growth curve of *Durusdinium glynnii* cultivated in medium with domestic effluent treated by microfiltration (MIC).

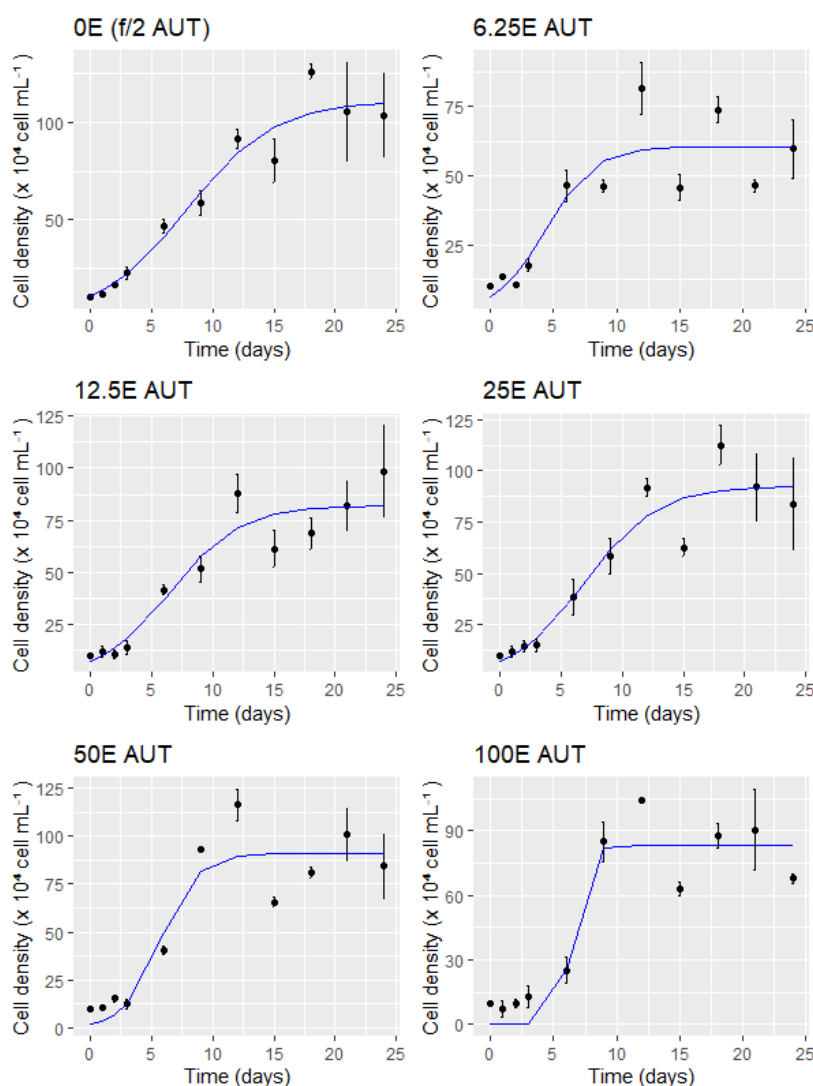


Fig. 4 Logistic growth curve of *Durusdinium glynnii* cultivated in medium with domestic effluent treated by autoclaving (AUT).

Production of Photosynthetic Biocompounds

The concentration of chlorophyll *a* showed a statistically significant difference only in the treatment with 12.5% and 50% effluent (Fig. 5A). Chlorophyll *c*, in turn, showed a significant difference exclusively in the treatment with 50% effluent (Fig. 5B). Total carotenoids showed a significant difference only in the treatment with 25% effluent (Fig. 5C). Peridinin did not exhibit a significant difference in any of the treatments, regardless of the effluent conditions (AUT or MIC) (Fig. 5D). All concentrations of the analyzed pigments reached their highest values in the 50% treatment, while the lowest values were observed in the 25% treatment, both under the microfiltration condition. The concentrations of chlorophylls *a* and *c*, total carotenoids, and

peridinin varied between $234.21 \pm 5.87 \mu\text{g L}^{-1}$ and $58.46 \pm 17.52 \mu\text{g L}^{-1}$, $106.6 \pm 7.16 \mu\text{g L}^{-1}$ and $22.03 \pm 7.98 \mu\text{g L}^{-1}$, $98.64 \pm 1.04 \mu\text{g L}^{-1}$ and $22.81 \pm 11.35 \mu\text{g L}^{-1}$, and $7.27 \pm 1.88 \mu\text{g mL}^{-1}$ and $2.02 \pm 0.84 \mu\text{g mL}^{-1}$, respectively (Fig. 5). Chloroplasts containing the carotenoid peridinin and chlorophylls *a* and *c* are a characteristic of most photosynthetic dinoflagellates (Dizaji et al. 2021).

Carotenoids and chlorophylls derived from microalgae are bioactive substances with multiple industrial and health applications; however, the use of peridinin is limited by the lack of reliable sources of the natural pigment (Sathasivam and Ki 2018; Langenbach and Melkoniano 2019; Sun et al. 2024). As demonstrated in previous research, chlorophylls and their derivatives possess health-beneficial properties, in addition to being used as natural colorants in foods and pharmaceuticals, while peridinin exhibits antioxidant, antitumor, and anti-inflammatory activities against cancer cells (Supasri et al. 2021; Sun et al. 2024). In this context, *D. glynnii* presents itself as a potential source for the extraction of biocompounds of biotechnological interest.

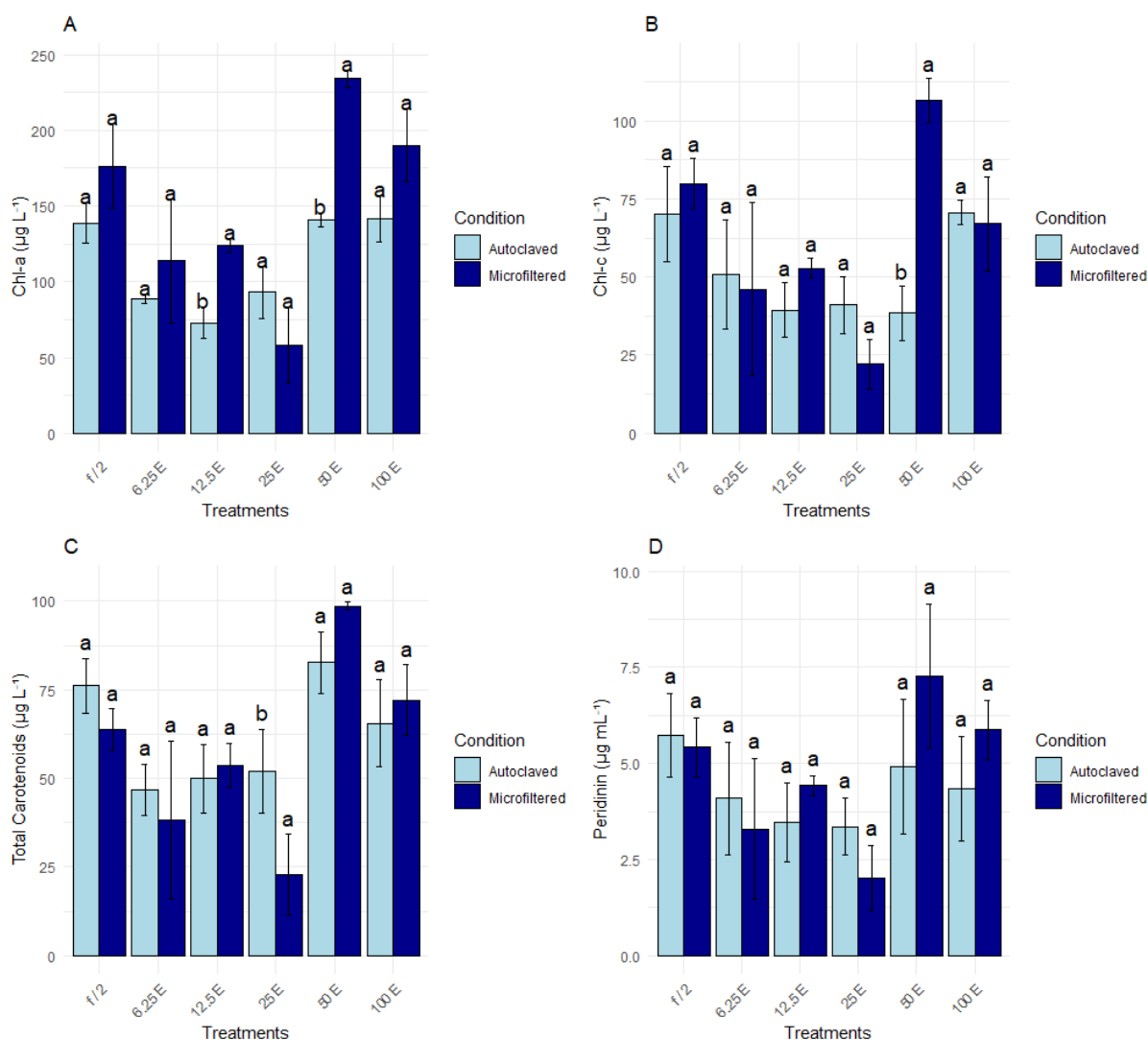


Fig. 5 Concentration and composition of the photosynthetic pigments chlorophyll *a* (A), chlorophyll *c* (B), total carotenoids (C), and peridinin (D) produced by the dinoflagellate *Durusdinium glynnii* under different medium conditions with domestic effluent. Different letters indicate a significant difference between the autoclaved and microfiltered conditions for each treatment, according to Tukey's *post hoc* test ($p < 0.05$).

The correlation between the variables was positive in all cases, indicating that an increase in one variable tends to be associated with an increase in the other. The highest correlations were observed between chlorophyll *a* and peridinin ($r = 0.93$), as well as between carotenoids and peridinin ($r = 0.94$), suggesting a close relationship between these pigments. Furthermore, the strong correlation between chlorophyll *a* and chlorophyll *c* ($r = 0.89$) indicates a significant interdependence between these two forms of chlorophyll. These results suggest that the

concentrations of the different pigments in the dataset are interrelated, possibly due to biological processes shared by the species, despite variations in the cultivation conditions (Fig. 6).

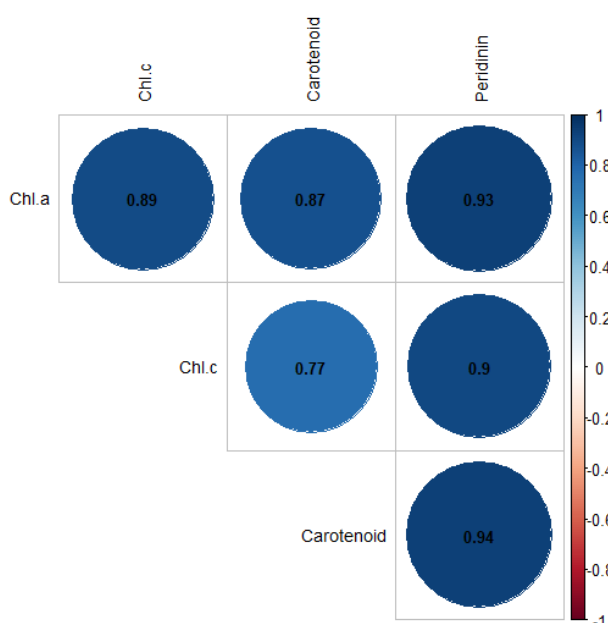


Fig. 6 Correlation matrix of the photosynthetic pigments chlorophyll *a* (Chl.a), chlorophyll *c* (Chl.c), total carotenoids, and peridinin produced by *D. glynnii* cultivated in domestic effluent. The values at the center of the matrix represent the correlation coefficients (*r*).

Conclusion

The cultivation of *Durusdinium glynnii* in domestic effluents has demonstrated potential for the bioremediation of these wastes, in addition to enabling the sustainable production of biomass with high biotechnological value. This approach eliminates the need for synthetic culture media, as it is possible to replace it with up to 100% effluent, the composition of which includes reagents that, if discarded into aquatic environments, could contribute to increased pollution. Although the inclusion of domestic effluent did not significantly interfere with the algal growth parameters, a potential increase in biomass production was observed in the 50% MIC treatment, with a peak density of $146.08 \pm 8.61 \times 10^4$ cells mL⁻¹. This same treatment proved to be the best condition for the production of biocompounds, as it exhibited higher numerical concentrations of chlorophylls and carotenoids, especially peridinin.

Additionally, the species demonstrates a high capacity for the removal of nitrogen and phosphorus nutrients, achieving up to 100% absorption of these compounds under specific conditions. Moreover, this approach contributes to the reduction of the water footprint of cultivation systems, as there is no need for the use of potable water. In this context, we suggest that future research explore the application of raw domestic effluent, aiming at the scalability of the process to industrial levels.

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5. Final Considerations

The results of this study reinforce the potential of microalgae cultivation, particularly *Durusdinium glynnii*, as a sustainable approach for the treatment of domestic effluents and the production of high-value biocompounds. The research presents *D. glynnii* as a euryhaline species capable of adapting to moderate salinity variations, which expands its cultivation possibilities in brackish water environments. However, further research is necessary to optimize cultivation conditions and to better explore the physiological and biochemical mechanisms involved in salinity tolerance and the synthesis of bioactive compounds.

Although the bioremediation potential is species-specific and also depends on the variable composition of the effluent, the viability of using domestic effluent as a culture medium has been demonstrated, as the growth of the microalga was not compromised by the replacement of the synthetic medium. Furthermore, the efficient removal of ammonia, nitrite, and orthophosphate confirms the role of *D. glynnii* in bioremediation, as it effectively reduced the nutrient load in wastewater. The production of bioactive compounds, such as peridinin, highlights its potential for biotechnological applications. Thus, this study contributes to the advancement of the bioeconomy by integrating waste treatment with the production of biomolecules for industrial applications.

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