








Environment

Microalgae cultivation: from waste as a nutrient source to CO₂ mitigation – a review containing CFD modeling

Cultivo de microalgas: de resíduos como fonte de nutrientes até a mitigação de CO₂ – uma revisão contendo modelagem em CFD

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ABSTRACT

The increasing concern for environmental management promotes the development of new products and processes, aiming for economic progress through environmental protection. Through the cultivation of microalgae, photosynthetic organisms that convert water, CO₂ and light into oxygen and biomass, able to produce an unlimited amount of biocompounds. Factors such as temperature, pH, type of system, and culture medium used are essential for its development and biomass composition. These microbes can not only absorb inorganic matter from the effluent and assimilate these nutrients for its growth, minimizing the cost of nutrient addition, but also absorb CO₂ in the atmosphere or flue gas through photosynthesis. Thus, this work presents a review of the cultivation of microalgae using wastewater as a source of nutrients generating compounds with industrial interest and biotechnological applications, along with computational fluid dynamics (CFD) modeling for CO₂ reduction aiming for scale-up. The use of wastewater for the cultivation of microalgae has been studied for years, as has CO₂ mitigation, however, there is still much to be explored to obtain greater use of waste; and together with the increasing use of CFD techniques in bioprocess, they can contribute to process optimization, scale-up, and improvements in the environment.

Keywords: Microalgae; Cultivation; Nutrients; Wastewater; Bioproducts; CFD

RESUMO

A crescente preocupação com a gestão ambiental promove o desenvolvimento de novos produtos e processos, visando o progresso econômico com proteção ambiental. Através do cultivo de microalgas, organismos fotossintéticos que convertem água, CO₂ e luz em oxigênio e biomassa, são capazes de

produzir uma quantidade ilimitada de biocompostos. Fatores como temperatura, pH, tipo de sistema e meio de cultivo utilizado são essenciais para o seu desenvolvimento e composição da biomassa. Esses micróbios podem não apenas absorver matéria inorgânica do efluente e assimilar esses nutrientes para o seu crescimento, minimizando o custo de adição de nutrientes, mas também absorver CO₂ na atmosfera ou gases de combustão através da fotossíntese. Assim, este trabalho apresenta uma revisão sobre o cultivo de microalgas utilizando águas residuais como fonte de nutrientes gerando compostos de interesse industrial e aplicações biotecnológicas além da modelagem em Fluidodinâmica Computacional (CFD) para redução de CO₂ visando o aumento de escala. A utilização de águas residuárias para o cultivo de microalgas vem sendo estudada há anos, bem como a mitigação de CO₂, porém, ainda há muito a ser explorado para obter maior aproveitamento dos resíduos; e juntamente com o aumento da técnica de CFD aplicada a bioprocessos, podem contribuir para otimização destes, aumento de escala e melhorias no meio ambiente.

Palavras-chave: Microalgas; Cultivo; Nutrientes; Águas residuais; Bioprodutos; CFD

1 INTRODUCTION

Due to a growing concern of the industrial sector with environmental management, the development of new products, alternative processes, and efficient techniques are necessary for the economic advance with environmental protection, aiming at the reduction or remediation of pollution (Mittersteiner et al., 2017; Moreira et al., 2023; Yuan et al., 2023).

Waste management comprises the collection, transportation, processing, recycling or disposal, and residue monitoring. It encourages the reuse of matter within society, and through waste management, many governmental agencies and international organizations have established waste-related policies to reduce environmental impacts (Demirbas, 2011; Gentil et al., 2011; Moreira et al., 2023).

One of the main challenges for sustainable development is an integrated waste management system (Adeniran et al., 2017). Demirbas (2011) presents a hierarchy of waste management: discarding, energy recovery, recycling, reuse, reduction, and prevention, whereas disposal is the least favored option and prevention the most favored.

Microalgae culture can be used in more sustainable processes, which can use wastewater for its growth and production of bioproducts, such as biofuels, biofertilizers, and bioplastic production (Gouveia et al., 2016; Koçer et al., 2023; Maity et al., 2014;

Moreira et al., 2023; Razzak et al., 2013; Yuan et al., 2023). Microalgae are cultivated for more than a century. Diverse studies report on its biological and environmental aspects, theories and cultivation techniques, and economic applications (Banerjee & Ramaswamy, 2019; Razzak et al., 2013; Richmond, 2004; Saleem et al., 2024; Zhuang et al., 2018).

Microalgae can adapt to different environments, adjust, or change its internal structure to excrete a range of compounds. These microorganisms can change metabolism in response to changing environmental conditions (Kong et al., 2024; Mata et al., 2010; Villaró-Cos et al., 2024). They can exhibit a high growth rate, and some species can duplicate their cells several times a day (Arenas et al., 2017)

Some factors influence the production of biomass from microalgae cultivation, such as the composition of the culture medium, type of system cultivation, aeration, lighting, pH, and temperature (Ansari et al., 2017; Kong et al., 2024; Miao & Wu, 2004; Razzak et al., 2013; Villaró-Cos et al., 2024; Zhuang et al., 2018). For the cultivation of microalgae, various nutrients are necessary, but the use of wastewater can promote an economically feasible way, as it reduces the cost of adding nutrients (Gentili, 2014; Wan Mahari et al., 2022).

Besides, it can be included on CO₂ stabilization, in addition to its removal, contributing to the circular economy proposal. However, to implement the whole process is difficult, due to the need of large spaces to cultivation. Thus, as alternative, the use of bubble columns and fluidized beds, as bioreactors are increasing, but their used is limited in small scales (Gilbert-López et al., 2015; Wan Mahari et al., 2022).

In this review, we summarize the cultivation of microalgae using different wastewater as an alternative culture medium to demonstrate the potential use of microalgae for wastewater treatment and the production of biomass and other industrially relevant biocompounds. Furthermore, a brief presentation of the Computational Fluid Dynamics (CFD) modeling review for photobiorreactors.

2 MICROALGAE CULTIVATION

Microalgae are photosynthetic organisms that, through the photosynthesis process, convert water, carbon dioxide (CO₂), and light into oxygen and biomass (Costa & de Morais, 2011; Kong et al., 2024; Villaró-Cos et al., 2024; Wan Mahari et al., 2022). The biomass composition gives microalgae attractive qualities for application in human and animal nutrition, incorporation into cosmetics, as a source of valuable molecules such as polyunsaturated fatty acids, and use in pigments (Spolaore et al., 2006).

The growth and the composition biomass of microalgae is a result of the interaction between biological factors, physical and chemical. To attain the maximum benefit from microalgae culture, it is an essential selection of adequate species or strains. Microalgae culture consists of a strain selected for producing the desired product and the most beneficial outcome of the culture process (Razzak et al., 2013; Saleem et al., 2024; Villaró-Cos et al., 2024).

High light intensities in the cultivation of microalgae can lead to two phenomena unfavorable to growth. One is photoinhibition, which occurs when the flow of photons absorbed in thylakoids causes an excessive concentration of high energy electrons in the cell to be consumed by the Calvin Cycle. These high energy electrons react with water and form hydrogen peroxide, toxic to cells (Chojnacka & Noworyta, 2004). Photolimitation is another unfavorable phenomenon that occurs due to the shading that the surface cells cause in the deeper layers of the culture media (Varshney et al., 2015; Wan Mahari et al., 2022).

pH control is one of the most critical parameters in microalgal growth (Kong et al., 2024). Microalgae growth better in pH values close to 7.0 in most cases, but there are exceptions. For example, for microalga *Dunaliella salina* the optimal pH is around 11.5, while for *Dunaliella acidophila* it is between 0.0 and 3.0 (Varshney et al., 2015).

Microalgal growth rates increase also with the increase of temperature, but only up to a limit. Singh and Singh (2015) present the optimum temperature for

different algae species growth. Examples include the blue-green algae (cyanobacteria) photosynthesis, whose optimum temperature was 0-20°C from June to November and 20-30°C during summer. The growth of *Scenedesmus* species occur in the ranges from 10 to 40°C. *Spirulina* species can grow in temperatures ranging from 20 to 40 °C, but the protein and carbohydrate levels were affected by the temperature.

During microalgae cultivation, the culture media must provide the necessary nutrients for their development. Organic carbons (sugars, proteins, and fats), ionic salts, and other nutrients (nitrogen and phosphorus) are essential for the growth of microalgae (Kong et al., 2024; Mata et al., 2010; Wan Mahari et al., 2022).

Macronutrients needed for microalgae are composed of carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, iron, potassium, and magnesium. Trace metals, such as copper, zinc, molybdenum, vanadium, boron, cobalt, calcium, sodium, manganese, selenium, and nickel, are called micronutrients (Richmond, 2004; Sniegoňová et al., 2023).

There is a great diversity of algal groups, which may be related to some factors, such as chemical composition, morphological and cellular structure, reproduction, and development. Microalgae have high nutritional quality, being a source of carbohydrates, lipids, proteins, pigments, minerals, and vitamins (Becker, 2007; Kong et al., 2024; Saleem et al., 2024; Sniegoňová et al., 2023; Villaró-Cos et al., 2024). These constituents could change by relying on the media and conditions of environments producing the microalgae biomass (Stengel et al., 2011).

Algae cultivation requires large amounts of water and nutrients. Calixto et al. (2016) and Jebali et al. (2015) mention the potential of using wastewater to obtain microalgal biomass economically. Media prepared from different types of wastewater were used for the cultivation of microalgae, showing that wastewater could minimize the costs of obtaining the microalgal biomass and high-value bioproducts (G. F. Ferreira et al., 2019; Gentili, 2014; Ho et al., 2011).

3 WASTE AS AN ALTERNATIVE CULTIVATION MEDIUM

The use of wastewater for microalgae cultivation has benefits, such as minimization of freshwater usage, reduction of the costs, and some nutrients from wastewater and production of biomass for high-value products (Chiu et al., 2015; Wan Mahari et al., 2022). Algal cells can absorb inorganic nutrients such as nitrogen and phosphorus from wastewater and assimilate them for their growth (Aslan & Kapdan, 2006; Lau et al., 1995; Moreira et al., 2023; Villaró-Cos et al., 2024).

The use of wastewater to grow algae could simultaneously solve problems related to freshwater demand, high nutrient cost, and the need to remediate waste. Algae can convert organic pollutants into cellular constituents such as lipids and carbohydrates, thereby reducing the wastewater polluting potential (Bhatnagar et al., 2011; Kong et al., 2024; Wan Mahari et al., 2022).

Rural waste, such as bone meal, urine, or wastewater from biogas digesters, can be used to grow microalgae, as well as municipal, brewery, aquaculture, and other wastewaters (Ansari et al., 2017; Becker & Venkataraman, 1984; Villaró-Cos et al., 2024). Table 1 shows that numerous studies already evaluated the use of different residues to produce microalgal biomass.

Table 1 – Cultivation of microalgae using waste as an alternative source of nutrients

(continue)

Microalga	Residue	Aim	Reference
<i>Chlorella pyrenoidosa</i> and <i>Scenedesmus</i> sp.	Sewage stabilized and activated	Removal of nutrients from wastewater by <i>Chlorella pyrenoidosa</i> and <i>Scenedesmus</i> sp	Tam and Wong, 1989
<i>Chlorella pyrenoidosa</i>	Sewage stabilized and activated	Compare the growth and nutrient removal efficiency of microalgae <i>Chlorella pyrenoidosa</i> in stabilized and activated sewage	Tam and Wong, 1989

Table 1 – Cultivation of microalgae using waste as an alternative source of nutrients

(continue)

Microalga	Residue	Aim	Reference
<i>Botryococcus braunii</i> UTEX 572	Swine wastewater	Determine the optimum wastewater conditions for microalgae culture, evaluating the removal of phosphorus and nitrogen, hydrocarbon productivity and biomass	An et al., 2003
<i>Azolla microphylla</i>	Municipal wastewater	Evaluate the use of municipal wastewater in the cultivation of <i>Azolla microphylla</i>	Arora; Saxena, 2005
<i>C. vulgaris</i>	Synthetic wastewater	Analyze the removal kinetics of nitrogen and phosphorus from synthetic waste water by <i>C. vulgaris</i>	Aslan; Kapdan, 2006
<i>Haematococcus pluvialis</i>	Wastewater from a sewage treatment plant generated by a rural community and swine wastewater.	Treatment of wastewater and production of a carotenoid, astaxanthin.	Kang et al., 2006
<i>Chlorella vulgaris</i>	Municipal wastewater	Investigate the nutrient removal and growth of <i>Chlorella vulgaris</i> in residual wastewater	Sreesai and Pakpain, 2007
<i>Spirulina maxima</i>	Beet vinasse	Analyze the viability of the use of beet vinasse as a culture medium	Barrocal et al., 2010
<i>Chlorella sp.</i>	Dairy manure	Assess the efficiency of the cultivation of <i>Chlorella sp.</i> , using as supplement dairy manure	Wang et al., 2010
<i>Chlorella sp.</i>	Municipal wastewater	Evaluate the growth of algae and verifying the removal of nitrogen, phosphorus, chemical oxygen demand (COD) and metal ion from wastewater	Wang et al., 2010
<i>Chlamydomonas globosa</i> , <i>Chlorella minutissima e</i> <i>Scenedesmus bijuga</i>	Municipal and industrial wastewater	Evaluate the mixotrophic growth of microalgae in media with organic carbon and wastewater	Bhatnagar et al., 2011
<i>Chlorella vulgaris</i>	Artificial wastewater	Evaluate the cultivation of <i>Chlorella vulgaris</i> in artificial wastewater for the production of lipids	Feng et al., 2011

Table 1 – Cultivation of microalgae using waste as an alternative source of nutrients

(continue)

Microalga	Residue	Aim	Reference
<i>Chlorella sp.</i>	Municipal wastewater	Determine the growth rate, biomass yield, nutrient removal efficiency and biodiesel productivity	Li et al., 2011 and Yuan et al., 2023
<i>Scenedesmus obliquus</i>	Chicken residue, fish pond disposal and municipal discharges of secondary settling tanks	Simultaneous production of biodiesel and waste recycling	Mandal and Mallick, 2011
<i>Scenedesmus obliquus</i>	Brewery wastewater	Analyze the potential use of the microalgae <i>Scenedesmus obliquus</i> for the treatment of brewery wastewater and biomass production	Mata et al., 2012
<i>C. vulgaris</i> (UTEX-265) and <i>Chlorella sp</i>	Brewery waste	Treat wastewater and maximize lipid productivity	Farooq et al., 2013
<i>Chlorella vulgaris</i>	Wastewater mixtures of a municipal treatment plant	Cultivate <i>Chlorella vulgaris</i> in wastewater for the production of biodiesel	He et al., 2013
<i>Chlamydomonas polypyrenoideum</i>	Wastewater from the dairy industry	Reduce the pollution load of wastewater, generating biomass and demonstrating biomass potential as a source of biofuel	Kothari et al., 2013
<i>Chlorella vulgaris</i>	Sugarcane vinasse	Verify the potential of vinasse as an alternative source of nutrients for microalgae cultivation to produce biomass and algae oil	Marques et al., 2013
<i>Chlorella zofingiensis</i>	Swine waste	Measure the pollutants removal of wastewater and the microalgae biomass for value-added energy applications	Zhu et al., 2013
<i>Chlorella vulgaris</i>	Mixture of municipal wastewater	Investigate the removal of nutrients from municipal wastewater by <i>Chlorella vulgaris</i> , biomass and lipid production	Ebrahimian et al., 2014
<i>Scenedesmus dimorphus</i> , <i>Selenastrum minutum</i> and <i>Scenedesmus sp.</i>	Wastewater (dairy sludge, paper and cellulose inflows, municipal inflows and dairy wastewater)	Cultivate microalgae in wastewater to simultaneously treat wastewater and produce biomass and lipids	Gentili, 2014

Table 1 – Cultivation of microalgae using waste as an alternative source of nutrients

(continue)

Microalga	Residue	Aim	Reference
<i>Scenedesmus</i> sp.	Ethanol vinasse	Evaluate the viability of microalgae production using vinasse as an alternative culture medium	Ramirez et al., 2014
<i>Chlorococcum</i> sp. RAP13	Dairy wastewater	Evaluate the use of dairy wastewater in the cultivation of microalgae, to produce a significant amount of biomass and lipids	Ummalyima and Sukumaran, 2014)
<i>Chlorella vulgaris</i> , <i>Scenedesmus obliquus</i> and a consortium of naturally occurring algae	Municipal wastewater	Simultaneous treatment of urban wastewater and energy recovery of the biomass obtained	Batista et al., 2015
<i>Spirulina platensis</i>	Beet vinasse	Analyze the vinasse addition on the concentration of biomass and protein productivity	Coca et al., 2015
<i>Chlorella protothecoides</i> UTEX-1806	Brewery wastewater	Evaluate the cultivation of <i>Chlorella protothecoides</i> in brewery wastewater, the treatment of wastewater and the production of biomass for production of biodiesel	Darpito et al., 2015
<i>Chlorella sorokiniana</i> and <i>Scenedesmus obliquus</i>	Municipal wastewater	Assess wastewater treatment and biomass production for biofuels	Gupta et al. 2016; Koçer et al., 2023
<i>C. saccharophila</i> UTEX 2911, <i>C. pseudococcum</i> UTEX 214, <i>Scenedesmus</i> sp UTEX1589, <i>N. oleoabundans</i> UTEX 1185 and a consortium of 10 isolates from wastewater	Dairy residue	Algae culture in dairy residue for biodiesel production	Hena et al., 2015)
<i>Scenedesmus</i> sp, <i>Chlorella</i> sp and <i>Chlorella vulgaris</i>	Wastewater from a wastewater treatment plant	Evaluate the biomass productivity and the nutrient removal capacity of each microalgae strain	Jebali et al., 2015)
<i>Chlorella</i> sp. GD	Swine wastewater	Cultivate <i>Chlorella</i> sp. GD in wastewater for the production of biomass and oil for the production of biodiesel	Kuo et al., 2015
<i>Chlorella</i> sp. GD	Piggery wastewater	Biomass and lipid production	Kuo et al., 2015

Table 1 – Cultivation of microalgae using waste as an alternative source of nutrients

(continue)

Microalga	Residue	Aim	Reference
<i>Chlorella</i> sp., <i>Chlamydomonas</i> sp., <i>Lagerheimia longiseta</i> and <i>Pediastrum tetras</i>	Fruit / horticultural waste, sugar cane residues and vinasse, chicken droppings, raw chicken manure and municipal sewage	Evaluate cell growth, productivity, biochemical compositions and the ester profiles of their biomasses	Calixto et al., 2016
<i>Chlorella vulgaris</i> , <i>Scenedesmus obliquus</i> and other strains such as <i>Chlorella</i> , <i>Chaetophora</i> , <i>Scenedesmus</i> and <i>Navicula</i> .	Municipal wastewater	Evaluate the best microalga in terms of recover wastewater, generation of biomass and productivity for uses, such as biofuels, biofertilizers and bioplastic production	Gouveia et al., 2016
<i>Spirulina maxima</i>	Sugar cane vinasse	Analyze the feasibility of <i>Spirulina maxima</i> cultivation using sugar cane vinasse as a supplement in the medium	Santos et al., 2016
<i>Chlorella</i> sp. MM3	Brewery wastewater	Investigate a sustainable approach for iron nanoparticles synthesis	Subramaniam et al., 2016
<i>Scenedesmus obliquus</i> , <i>Chlorella sorokiniana</i> and <i>Ankistrodesmus falcatus</i>	Aquaculture wastewater	Cultivate microalgae using aquaculture wastewater for the integrated generation of biomass and subsequent removal of nutrients	Ansari et al., 2017; Koçer et al., 2023
<i>Scenedesmus obliquus</i>	Brewery wastewater	Evaluate biomass productivity, CO ₂ biofixation rate and wastewater treatment efficiency	ferreira et al., 2017
<i>Scenedesmus</i> sp.	Tanning wastewater	Associate wastewater treatment to biomass production	Fontoura et al., 2017
<i>Chlorella</i> sp.	Starch wastewater	Recover wastewater through an anaerobic fermentation followed by microalgae cultivation	Qi et al., 2017
<i>Chlorella vulgaris</i>	Central wastewater with glycerol	Investigate the potential use of residual water for algae growth	Ren et al., 2017a
Several species	Textile wastewater	Production of biodiesel	Fazal et al., 2018

Table 1 – Cultivation of microalgae using waste as an alternative source of nutrients
(conclusion)

Microalga	Residue	Aim	Reference
<i>Micractinium</i> sp. CCAP 211/92			
<i>Chlorella sorokiniana</i> UTEX 1665			
<i>Chlorella</i> sp. KU211a	Textile wastewater	Generation of biomass and biodegradation of chromogenic substances and heavy metals	Oyebamiji et al., 2019
<i>Chlorella</i> sp. KU211b			
<i>Chlorella sorokiniana</i> UTEX 246			
<i>Chlorella</i> sp. CB4			
<i>Chlorella vulgaris</i>	Dairy products	Integrating BG-11 medium (IBG), with industrial dairy powder waste as nutrient medium	Peter et al., 2023

Few studies dealt with the use of microalgae for brewery wastewater treatment and biomass generation and other products (Darpito et al., 2015; Farooq et al., 2013; A. Ferreira et al., 2017; Mata et al., 2012; Subramaniam et al., 2016). Ferreira et al. (2017) studied the brewery wastewater treatment by *Scenedesmus obliquus* and evaluated the resulting biomass and bioenergy production. The authors verified that it is possible to integrate sustainable processes for resulting efficient nutrients recycle and obtain energy from algal biomass, such as an energy carrier bioH₂ (67.1 mL H₂ g⁻¹), bio-oil, bio-char and bio-gas from a single pyrolysis process in the percentages of 64%, 30% and 6%, respectively.

Darpito et al. (2015) evaluated the cultivation of *Chlorella protothecoides* associated to brewery wastewater anaerobically treated in replacement to the BG-11 medium. The results showed that total nitrogen and phosphorus were removed in levels of about 96 and 90%, respectively. Besides, *C. protothecoides* accumulated 1.88 g L⁻¹ of biomass with an increase of the total fatty acid content by 1.84-fold (35.94 ± 1.54% of its dry cell weight), relative to that of BG-11. These results indicate that the use of wastewater as an alternative medium for biodiesel production from *C. protothecoides* is cost-effective.

Other studies highlight the relevance of municipal wastewater treatment

associated with the cultivation of microalga (Batista et al., 2015; Ebrahimian et al., 2014; Gouveia et al., 2016; Gupta et al., 2016; He et al., 2013). Gouveia et al. (2016) used a vertical tubular photobioreactor integrated into a wastewater treatment plant and inoculated with *Chlorella vulgaris*, *Scenedesmus obliquus* and *Consortium C* (*Chlorella*, *Chaetophora*, *Scenedesmus*, and *Navicula*), isolated from the effluent. The experiments achieved volumetric productivities of 0.1 g/L·d (*Chlorella vulgaris*), 0.4 g/L·d (*Scenedesmus obliquus*), and 0.9 g/L·d (*Consortium C*). The maximum removals attained by *Consortium C* with 98% for total nitrogen, 100% for phosphorus, and 64% for chemical oxygen demand. The results indicated that water obtained after treatment could be used without restrictions or discharge in water flow, and the biomass could be processed to make biofuels, biofertilizers, biopolymers, or biofilms.

Gupta et al. (2016) evaluated the use of *C. sorokiniana* and *S. obliquus* to wastewater treatment without the requirement for any additional treatment. *S. obliquus* showed better efficiency for removing nutrients and comparable pathogens removal, already, *C. sorokiniana* demonstrated greater adaptability to physiological stresses. The authors emphasized that the selection of algal species with better stress resistance is important to extend their applicability for comprehensive wastewater treatment and lipid production (Koçer et al., 2023).

Concerning studies related to the treatment of textile effluent, the research developed by Oyebamiji et al. (2019) evaluated the growth capacity of six microalgae of the *Chlorellaceae* family. The authors also verified that the reduction/removal of heavy metals as well as chromogenic substances from the textile wastewater was reduced by 47.10–70.03%. They concluded that textile wastewater produces microalgae biomass useful for biofuels production, minimizing the impact of wastewater on the environment.

A study developed by Fazal et al. (2018) approached the textile wastewater as potential source of nutrients and water, the bioremediation of textile wastewater by different algal species, different cultivation systems, harvesting and production of biodiesel. Moreover, the authors suggest future research and development challenges

for textile wastewater treatment coupled with microalgal biodiesel production.

Peter et al. (2023) proposed a integrate system to process food waste as an organic carbon source with BG-11 medium for *Chlorella vulgaris*. They conclude that comparing the non-integrated medium, the mixture of dairy waste as growth media offers the greatest microalgae production ($146.63\text{mg L}^{-1} \text{d}^{-1}$), lipid yield (238.20 mg/g), protein (227.45 mg/g), and carbohydrate concentration (314.01 mg/g). As compared to the BG-11 culture media, the integration of organic medium significantly increases biomass production, lipid, protein, and carbohydrate content by 44%, 11%, 20% and 57%, respectively.

Not all types of wastewater are suitable algae cultivation (Gentili, 2014). According to Chiu et al. (2015), an important issue to be evaluated in crops using wastewater is biological pollution, which can significantly restrict the growth of microalgae. Hence, some measures of biological pollutants control can be executed, such as filtration, the addition of chemical additives, alterations of the environmental conditions (Zhu et al., 2013).

Guldhe et al. (2017) mention that wastewater may contain organisms, such as bacteria, fungi, and microalgae, which may interfere in the desired microalga growth. Hence, it is essential to remove or reduce their presence in wastewater. Several pretreatment methods have been used in recent years to control the presence of these organisms in wastewater, such as filtration and autoclaving.

The influence of using wastewater as a nutrient source in the final product quality must be assessed to guarantee this integration. Other essential factors to be evaluated are environment and economical advantages (G. F. Ferreira et al., 2019).

The type of cultivation system used is the other factor that needs to be analyzed to obtain a better conversion of nutrients present in wastewater to biomass. There is a wide range of microalgae cultivation systems reported in the literature. These systems differ mainly depending on the cost, the type of desired products, the source of nutrients, and the CO₂ capture (Razzak et al., 2013).

4 CULTIVATION SYSTEMS

The cultivation of microalgae at a large scale is possible in closed and in open systems (Ansari et al., 2017). A comparison of the capabilities of each cultivation system is required to select a suitable microalgal biomass production method.

Open systems are the most commonly used configurations. Systems of this type are usually composed of a closed-loop channel, where a paddlewheel promotes mixing and circulation, and baffles guide the flow (Chisti, 2007). This system has the advantage that they are easier to construct and operate than most closed systems (Parmar et al., 2011). These advantages lead to the perception that they are less expensive than photobioreactors, although the latter provides higher biomass productivity in comparison.

The operation in open systems is carried out during daylight with a continuous culture feeding, and the broth is harvested behind on completion of the circulation loop. The paddle wheel must operate all the time to prevent sedimentation. Open systems enable the consumption of carbon dioxide from the atmosphere. However, mass transfer is much less efficient than in photobioreactors, and water losses through evaporation can be significant. Moreover, poor mixing makes it challenging to obtain high biomass concentration. Moreover, productivity is also affected by competition with unwanted algae predator organisms (Chisti, 2007).

There are several configurations for open systems of cultivation (Kligerman & Bouwer, 2015):

1. Open ponds have paddlewheels or other devices to promote mixing and aeration. Raceway, circular, inclined, and unmixed are common types of open ponds.
2. Wastewater stabilization ponds promote biological treatment by a symbiosis between bacteria and algae. Hence wastewater is stabilized, and pathogens are reduced. This system is composed commonly by a series of anaerobic, facultative and maturation ponds.

3. Advanced integrated wastewater pond system consists of four lagoon types, in this order: a facultative one with fermentation pits, an algal high rate pond, algal settling pond, and then a maturation pond.
4. Algal high rate ponds consist of a shallow pond operating at shorter hydraulic retention time. They have the same shape of raceways, in which a large paddle wheel vane pump provides gentle mixing.

Open systems also release valuable gases such as biomethane to the atmosphere, which impedes their certification for carbon emission reduction trading (Lam & Lee, 2011). As an alternative, closed ponds make it easier to control the environment (Parmar et al., 2011). Biogas can be captured and directly used for flaring, boiler fuel, or power generation in these types of systems. They allow the growth of more species, which stay dominant in the cultivation broth for the prolonged duration (Lam & Lee, 2011). In arid areas, where traditional terrestrial agriculture is not feasible, they also have the benefit of preventing evaporation. Closed pond systems have higher costs than open ponds, but they are considerably cheaper than photobioreactors for similar areas of operation (Parmar et al., 2011).

Unlike open systems, photobioreactors permit a single-species culture of microalgae for an extended growing period essentially. A standard configuration is a tubular photobioreactor, which consists of an array of transparent tubes. Their diameter is limited by light penetration, which is affected by culture broth turbidity (Chisti, 2007). The flow is maintained under turbulent conditions, to prevent biomass sedimentation in the tubes.

Flow is produced using either mechanical or airlift pumps. Mechanical pumps are easy to design, as well as to install and operate, but they can damage the biomass. Airlift pumps may be used to minimize cellular damage, but they require a supply of air to operate and are less flexible than mechanical pumps (Chisti, 2007).

A disadvantage of closed photobioreactors is the accumulation of oxygen generated, in levels enough to inhibit photosynthesis. Furthermore, the combination of

a high concentration of dissolved oxygen and intense sunlight produces photooxidative damage to algal cells. Therefore, the length of the tubes is limited to the maximum extension allowed by the continuous operation before the removal of oxygen in a degassing zone is required (Chisti, 2007).

Disregarding losses to the atmosphere, both raceways and tubular photobioreactors can biofix the same amount of carbon dioxide (Chisti, 2007). Despite their differences, both raceway and photobioreactor cultivation methods are technically feasible for waste treatment.

5 BIOTECHNOLOGICAL APPLICATIONS OF MICROALGAE CULTIVATED IN WASTEWATER

Due to their physical and nutritional characteristics, microalgae present numerous applications, such as biofuel and biosurfactants production, human and animal nutrition, aquaculture, extraction of substances of pharmaceutical importance, production of food colorings, production of hydrogen and methane, wastewater treatment (Fernandes de Carvalho et al., 2018; Kong et al., 2024; Moreira et al., 2023; Richmond, 2004; Schmitz, 2012; Spolaore et al., 2006; Stengel, Connan, & Popper, 2011; Villaró-Cos et al., 2024). Moreover, according to Santos et al., (2016) the biofixation of CO₂ by microalgae is a promising way to mitigate emissions of greenhouse gases, to obtain 1 kg of biomass, 1.83 kg of carbon dioxide is consumed.

Suganya et al. (2016) analyzed the production of high-value microalgal by-products and their commercial applications, according to the researchers, these commercial applications are dominated by four strains: *Arthrospira* (also known as *Spirulina*), *Chlorella*, *Dunaliella salina*, and *Aphanizomenon*.

The microalga *Spirulina* presents economic, ecological, and nutritional importance, being the focus of relevant biotechnological research (Antelo et al., 2010; Yamaguchi et al., 2019). It also has applications in wastewater treatment and water renewal. Depraetere et al. (2013) evaluated color removal from the piggery wastewater

and the enhancing of growth rate, also verified some methods for remove phosphate.

Zhou et al. (2017) studied the use of effluents and *Spirulina* biomass yield in the treatment of saline wastewater. The results demonstrated the efficiency of saline wastewater to obtaining biomass, with the optimal ratio of 7:3 of black seawater water and freshwater washing wastewater. Zhai et al. (2017) verified that glucose enhanced the *Spirulina* production, nutrients recovery and/or removal, and mitigated the nutrients limitation. These strategies can be a useful reference for the improvement of microalgal production, as well as to its application for the wastewater treatment in large-scale under a controllable environment.

Subramaniyam et al., 2016 investigated an integrated and sustainable approach for iron nanoparticle synthesis using *Chlorella sp.* MM3 biomass cultivated with the use of brewery wastewater. From this study, it is observed that *Chlorella sp.* MM3 grows well in brewery wastewater by removing pollutants such as nitrogen, phosphorus, and organic carbon (Yuan et al., 2023). Moreover, the obtained biomass was used to create stable, reactive, economic, and environmentally safe iron nanoparticles.

D'Alessandro and Antoniosi Filho (2016) presented concepts and studies of lipids and pigments obtained from microalgae. According to the authors, at least three classes of pigments occur in microalgae: phycobilins, chlorophylls, and carotenoids. Kang et al. (2006) proposed a system using the microalga *Haematococcus pluvialis* for the treatment of biological wastewater and for the production of astaxanthin, which is a valuable carotenoid, from the microalgal biomass.

Kuo et al. (2015) evaluated *Chlorella sp.* GD biomass and lipid production using piggery wastewater. The results show that microalgae grow efficiently in wastewater from pig farms. In addition, a stable growth performance was achieved for cultivation of microalgae in a semi-continuous culture for an extended period.

The microalgae can be used as raw material for biofuels production (Lam & Lee, 2011; X. Li et al., 2007; Moreira et al., 2023; Ramirez et al., 2014). Gao et al., (2010) mention that microalgal lipids are suitable for the production of biodiesel.

Xin et al. (2010) mention the coupling of biodiesel production and microalgae-based wastewater treatment as a promising approach to dealing with the energy crisis. Renewable energy resources provide clean alternatives to fossil fuels. Biofuels derived from microalgae were proposed as they represent an alternative free from impacts on agriculture (CHIU et al., 2015).

Several studies have been carried out aiming used wastewater, such as for obtain biofuels (A. Ferreira et al., 2017; Gouveia et al., 2016; Gupta et al., 2016; Kligerman & Bouwer, 2015; Lam & Lee, 2011) for producing biomass and lipid (Ebrahimian et al., 2014; Feng et al., 2011; Gentili, 2014; Kuo et al., 2015; Marques et al., 2013; Ummalyma & Sukumaran, 2014), for biodiesel production and nutrient removal (Darpito et al., 2015; Fazal et al., 2018; Kuo et al., 2015; Y. Li et al., 2011; Mandal & Mallick, 2011) Due to the increased demand for natural products, studies on microalgae cultivation are even more promising. It is essential to favor the viability of its use, such as the cultivation of microalgae in sustainable media and improving its photosynthetic efficiency through CO₂ consumption (Batista et al., 2015; Ferreira et al., 2019).

Sun et al. (2022) proposed a cyclic cultivation technology to reduce the water footprint and environmental pollution for microalgae production and improve resource efficiency. They established a novel growth model for simulation and optimization of the operational scheme. After techno-economic analysis, they found that the cyclic technique is vastly superior to the non-cyclic by enabling the high-level recovery resources.

According to Cecchin et al. (2018) and Ren et al. (2017) with the appropriate supplementation of inorganic carbon sources, the organic sources present in wastewater can be used by microalgae increasing the biomass production.

Improving the CO₂ capture by microalgae can enhance biomass production, wastewater treatment efficiency, and reduce the CO₂ aeration cost (Kong et al., 2024). Cheng et al., (2021) reported that the CO₂ removal efficiency of *Chlorella vulgaris* in open and closed systems are 40.64% and 73.07%, respectively.

For improving the mass transfer, (Cheng et al. (2020) proposed a three-stage-

shear-serrated aerator to generate smaller bubbles; biomass production increased by 46.8%. However, mass transfer efficiency was only improved by 25.5%, probably because the diameter was not reduced enough (2.4mm). Thus, with a jet aeration, microbubbles were formed (0.37mm), which increased mass transfer by 4.6 times, and the microalgae biomass production by 49.4% (J. Cheng et al., 2019).

Another alternative to improve CO₂ absorption is to increase bubble retention time inside the bioreactor. It depends on the rising pathway, bubbles velocity and CO₂ solubility; Xia et al. (2018) used flat plate PBRs with inclined baffles to culture microalgae and found that the CO₂ retention time was extended from 0.448s to 256s, which increase the concentration of microalgae and the CO₂ fixation rate by 26.0% and 26.2%, respectively. Xu et al.,2020) designed a helical baffle and central hollow tube in the CO₂ dissolver to improve microalgae growth. With it, it was possible to increase bubble retention time by 190.2% and enhanced the mass transfer coefficient by 69.2%, which led also to an increase of microalgae biomass production by 40.8% in the horizontal tubular photobioreactor. According to (Dasan et al., 2020) the CO₂ fixation efficiency was 3.78-fold higher in a sequential-flow PBRs system than in a single column PBR.

These methods to improve the CO₂ capture by microalgae are suitable for closed systems and low CO₂ concentrations, because microalgae have a limited carbon capture rate, thus with high concentrations, CO₂ molecules will be released from the liquid phase.

6 COMPUTATIONAL FLUID DYNAMICS MODEL FOR CO₂ REDUCTION

The review of Computational Fluid Dynamics (CFD) modeling in this case aims to present the studies related to the initial conditions and parameters for modeling CO₂ reduction.

The increasing concentration of CO₂ in the atmosphere is considered the main cause of global warming and can have catastrophic consequences for the

environment. Thus, the analysis of the feasibility, precautions and complications of microalgae cultivation can be developed through mathematical models that consider the simultaneous effects of light intensity and nutrient concentration, with the goal of optimizing the cultivation conditions to achieve maximum microalgae growth and CO₂ biofixation (Chang et al., 2016).

Some studies have analyzed the feasibility of microalgae on reduced scales, using photobioreactors (Amini et al., 2016; Guo et al., 2017; Pfaffinger et al., 2016). However, the development and optimization of these reactors are not simple tasks.

In this context, Computational Fluid Dynamics (CFD) emerges as a promising tool for characterizing and modeling the mechanisms and flows of the studied processes. It is widely used in various areas, such as aerospace, automotive and assessment of alternative projects, as it allows simulating costly prototypes. The ease of understanding, optimization projections and process conditions are some of the advantages of this tool (Ferrua & Singh, 2013).

There are some crucial steps for the development of a CFD model, such as pre-processing. In this step, it is necessary to create the geometry, discretize the domain of the equations governing the transport phenomena and establish a convergence criterion. In addition, it is necessary to define a computational mesh and the initial conditions of the model. In the post-processing, it is essential to analyze and interpret the obtained data, with the aim of extracting meaningful results for the study in question (Sadino-Riquelme et al., 2018).

One of the factors of greatest influence on microalgae production is light, which provides the energy necessary for the maintenance of these organisms' metabolism (Cardoso, 2011; Wan Mahari et al., 2022). Light interacts with the biological structures of microalgae, triggering the photosynthesis process.

According to the study Chisti (2007) photosynthesis results in the release of oxygen (O₂) in an average ratio of 1 mole of O₂ for each mole of carbon dioxide (CO₂) consumed, although this ratio can vary by up to 20%, depending on the intensity of

the light. It is important to note that during photosynthesis, O₂ needs to be removed from the environment, as high concentrations can inhibit growth and cause cell damage under intense light conditions. Furthermore, when the concentration of O₂ is significantly higher than that of CO₂, cellular metabolism can be diverted to a process called photorespiration, in which the cell's energy reserves are used as a source of carbon and cellular energy in the presence of light (Bitog et al., 2011). Therefore, it is crucial that the concentration of O₂ does not exceed 400% of the value corresponding to the concentration in water in equilibrium with air at an atmospheric pressure of 1 atm, and this limit may be even lower for some species (Chisti, 2007).

Microalgae prefer wavelength ranges in the blue ($\lambda \sim 420\text{-}470$ nm) and red ($\lambda \sim 660$ nm) of the spectrum (Glemser et al., 2016; Wang et al., 2014).

Several studies have presented mathematical models for the growth of *Chlorella* from light intensity. These models have been applied by other researchers in the cultivation of new species (Chae, Hwang & Shin, 2006). In addition, empirical models have been developed that use light intensity as a parameter (Chalker, 1980).

The contemplation of the phenomena of emission, absorption, reflection and scattering can be observed in the differential equations resulting from the radiation balance, which is derived from the energy balance function (Amini et al., 2016).

The simulation of radiative transport within a photobioreactor is computationally challenging due to its spatial, directional and wavelength dependencies resulting from complex interactions of radiation with water, algae, and air. Due to the complexity of the system of equations obtained through the radiation balance, two simplification strategies are usually used to solve the model: two fluxes and discrete orders. The two-flux simplification model assumes that scattering only occurs in one direction, either positive or negative (Cornet & Dussap, 2009; Singh Khichi, Anis & Ghosh, 2018).

Simplification of discrete ordinates defines a limited number of directions for the light beam, differential equations are solved for each of these directions, approach used in some works that made use of CFD (Amini et al., 2016; W. Guo et al., 2019).

However, modern commercial CFD tools allow us to make use of wavelength dependent radiative transfer in well-resolved complex geometries of algae cultivation systems (Wheaton & Krishnamoorthy, 2012). CFD models can be used to predict the patterns of multiphase flow and light intensity distribution in open channel pond light transfer (Huang et al., 2015; Liffman et al., 2013).

The conditions for the formulation of the mathematical model were taken from the literature describing CFD simulation in algal cells and culture medium using a multiphase Eulerian model. Mathematically, the phases of the cells and the culture medium can be treated as continuous phases, incorporating concepts of solid phase viscosity and solid phase pressure for the algal cells (Almohammed et al., 2014). In this context, the culture medium can be considered as the primary fluid phase, while the algal cells are treated as the secondary, dispersed phase within the primary phase.

The intensity of light radiation transfer in open channel ponds is highly dependent on the spatial position and angular direction. The radiation transfer to the medium that absorbs, scatters, and does not emit light can be modeled using discrete ordinates (Berberoglu, Pilon, & Melis, 2008).

For the development of the model, certain optical properties such as specific scattering, absorption, and extinction coefficients must be established. This information can be obtained from the literature (Hannis Ruud Van Ommen & Robbert Kleerebezem Robert Mudde, 2013), since the radiation characteristics of microorganisms are directly related to their concentration (Berberoglu, Pilon, & Melis, 2008). Thus, the average medium characteristics in the visible light range (400-700 nm) were used to determine the light intensity by Hannis et al. (2013).

The lower and lateral walls of the lagoon were considered as opaque, while the upper wall was treated as semitransparent. A light intensity radiation of $w\ w/m^2$ was applied at the top of the free surface of the fluid on the upper wall (Amini et al., 2016).

To determine the pseudo-steady state condition, the flow was considered a transient phenomenon, with the simulations starting from an initial velocity of zero.

The pseudo-steady state condition was defined as that in which there are no significant changes in the maximum velocity and in the velocity profile as the simulation time increases. The minimum mixing time required was established as the simulation time required to reach the pseudo-steady state condition. Once this state was reached, the three-dimensional velocities were predicted at each measurement point for the next 20 seconds of simulation, at 0.25 second intervals (Amini et al., 2016).

Solar radiation in the culture medium and CO₂ consumption are the external variables of the model. CO₂ consumption is directly related to solar radiation, that is, when radiation decreases, CO₂ consumption also decreases. According to the study by Ferreira et al. (2017), the highest CO₂ consumption occurred at highest O₂ concentration. A daily consumption of 0.4324 kg of CO₂ was estimated, resulting in an annual consumption of 5.2 kg per photobioreactor.

Otsuki (2001) developed a system for CO₂ biofixation through the intensive cultivation of microalgae in plate photobioreactors. The study determined a positive balance for CO₂ fixation, as well as for the energy consumption of the system and the production of energy from biochemical synthesis.

Sadeghizadeh et al. (2018) used a three-phase and three dimensional CFD model to validate the hydrodynamics and mass transfer in an internal loop airlift reactor. Results shown that for lower gas superficial velocity, the CO₂ removal efficiency was higher, presenting a good agreement with experimental data with 10% of error.

CFD modeling was employed for characterizing the flow hydrodynamics and energy dissipation rates by López-Rosales et al. (2019). They verified that gas holdup, energy dissipation and mass transfer correlated well with Froude number. Besides, they found that *K. veneficum* growth was limited by CO₂ transfer depending on the threshold of energy dissipation and a fluid dynamic stress.

Wang et al. (2021) developed a novel flat-plate photobioreactor with inclined baffles, in which the internal structure was optimized using computational fluid dynamics model. Numerical results showed that a small angle could improve the

swirl flow and light regime performance of the reactor, and the ratio of the baffle opening distance to the reactor width was the key parameter for the light regime performance. Radial velocity along light gradient direction and light/dark cycle frequency increased with the decrease of r . The optimal parameters were: angle of 10° and ratio of 0.25. Experiments showed that the maximum algal biomass using the optimal structure was increased by 45.7 and 25.8% compared with the reactor without baffles and with horizontal baffles, respectively.

7 CONCLUSIONS

The cultivation of microalgae using wastewater provides a sustainable solution for carbon neutrality in waste treatment plants, in addition to better use of waste, reduction of fresh water utilization and the cost of adding nutrients, pollutants removal and production of biomass for obtaining products of high added value. Thus, it is a subject studied for years, however, microalgae-based wastewater treatment technology is mostly conducted at the lab-scale or pilot scale, and there are still many problems that need to be solved to achieve large-scale applications. Microalgae growth are dependent on the wastewater diversity of nutrients, needing a well-balanced medium for cultivation, and the identification of microalgae species which can improve the specific waste treatment.

However, scale-up and improve the treatment performance require high cost of equipment and experiments, thus computational fluid dynamics (CFD) arises as a promising tool to model this type of process, aiming its employment in large scale. CFD is already used in several industrial applications as aerospace, automotive, oil and gas, etc. Nevertheless, modeling the whole process involving wastewater treatment with CO_2 absorption in a photobioreactor is a huge challenge, once there is not proper models available at the moment.

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