



# Removal of contaminants through microalgae: towards the development of a <u>circular economy</u> Remoción de contaminantes a través de microalgas: hacia el desarrollo de una economía circular

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## Abstract

The occurrence of algal blooms has increased in both extent and frequency worldwide. However, it can become an effective alternative for wastewater treatment (WWT). It is important to note that conventional processes used in WWT facilities are mostly inefficient in removing high loads of nutrients. In this regard, the utilization of microalgae, such as *Chlorella* spp., emerges as an economically viable alternative that allows for effective removal of nutrients and ensures the production of an effluent that complies with permissible discharge limits to water bodies. Additionally, the biomass formed from microalgae treatment can be used as biofuels. This work describes the use of microalgae as a convenient and cost-effective biological process for the removal of nutrients and organic matter, even allowing for energy recovery through biomass valorization. Thus, the use of microalgae is proposed as a solution to water pollution with high nutrient loads.

**Keywords:** algae; alternative treatment; biological process; efficiency; emerging pollutant; microalgae; nutrient; viability; water pollution.

# Resumen

La presencia de floraciones algales ha aumentado en extensión y frecuencia a nivel mundial. No obstante, actualmente, más que una problemática puede convertirse en una alternativa eficaz de tratamiento de aguas residuales. Es importante señalar que los procesos convencionales con los que operan las instalaciones de tratamiento de aguas residuales, en su mayoría, son ineficientes en cuanto a la eliminación de altas cargas de nutrientes. En este contexto, el uso de microalgas como la especie *Chlorella* spp. surge como una alternativa económicamente viable que permite una remoción efectiva de nutrientes y asegura la obtención de un efluente que cumpla con los límites máximos permitidos de descarga a cuerpos de agua. Adicionalmente, la biomasa derivada del tratamiento de aguas residuales con microalgas puede ser utilizada como biocombustible. En este trabajo, se describe la utilización de microalgas como un proceso biológico adecuado y rentable para la eliminación de nutrientes, permitiendo incluso la recuperación de energía en forma de la

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valorización de la biomasa. Así, se propone el uso de microalgas con miras a dar solución a la contaminación de las aguas con altas cargas de nutrientes.

**Palabras clave:** alga; contaminación del agua; contaminantes emergentes; eficiencia; microalga; nutriente; proceso biológico; tratamiento alternativo; viabilidad.

### 1. Introduction

The pollution of wastewater (WW) is a serious environmental problem that affects many regions of the world [1]. The increase in population, urbanization, and economic development have contributed to a rise in the production of WW, leading to a higher organic and nutrient load in water bodies [2], [3]. This organic material may be biodegradable and undergoes decomposition through biological oxidation, resulting in a decrease in the available dissolved oxygen  $(O_2)$ , potentially causing adverse effects on aquatic life [3]. Additionally, nutrients refer to nitrogen (N) and phosphorus (P), crucial elements for plant growth and essential to maintaining a thriving aquatic ecosystem. Nevertheless, their discharge in high concentrations can lead to eutrophication, negatively impacting water quality and, subsequently, the aquatic flora and fauna [4]. To address the problem of WW pollution, it is necessary to implement appropriate management measures.

This includes the treatment of WW through WWTP that can reduce the organic and nutrient load before water is discharged to the environment [5]. Additionally, it is important to promote sustainable WW management practices, such as decentralized WW treatment in rural areas, reuse of treated water for several non-potable uses, and public education about the importance of water conservation.

Currently, non-conventional biological methods that employ microalgae are an alternative for tertiary treatments operating in WWTP, both domestic and industrial WW [6]. These types of waters, if discharged directly without a proper final treatment, would generate an adverse environmental impact on the receiving water bodies, by significantly increasing the content of organic matter in terms of biochemical oxygen demand (BOD<sub>5</sub>) and chemical oxygen demand (COD), as well as the content of solids, nitrates, nitrites and phosphates, which can cause acute toxicity problems at different trophic levels, in addition to eutrophication of aquatic ecosystems [7], [8].

In 1970s, the first studies on the algae cultivation for the tertiary treatment of WW were reported [9]. Considering that the conventional activated sludge process is not efficient in nutrient removal, algae pond process was introduced in order to avoid eutrophication when the

treated WW effluent was discharged in a natural water body. It was observed that the use of the microalgae system was a proper treatment from an economical and technical point of view. Additionally, the introduction of a biological floc is not required [10], [11], [12], resulting in a decrease in the operating costs of the system. Since then, the use of microalgae as an alternative biosystem has been the subject of numerous research due to its photosynthetic capabilities, which allow to remove significant amounts of nutrients, including P and N, during its growth after processing contaminated effluents. Furthermore, the recovery of energy through the production of valuable products from the algal biomass and the extraction of metabolites can be taken advantage. This represents a potential source of bioproducts that include a wide variety of compounds with various applications, including the generation of biofuels and biosorbents for the economical, effective, and ecologically safe removal of several types of pollutants [11], [12], [13].

In this context, the present investigation aims to clarify the use of microalgae as a feasible choice for treating water with elevated concentrations of nutrients. The goal is to devise an effective method that streamlines the recovery of treated wastewater, thereby contributing to the progression of sustainable development.

## 2. Bioremediation: cultivation and metabolism

Bioremediation involves the use of living organisms or their derivatives to remove or transform pollutants in the environment. In the context of WW treatment, this might include employing bacteria, fungi, or algae to break down organic matter and uptake nutrients into harmless substances [14]. A particularly promising approach to bioremediation in WW is the utilization of microalgae. These microorganisms can engage in photosynthesis, converting inorganic carbon into organic matter through light energy, while simultaneously removing nutrients and contaminants from the water. This not only results in the production of  $O_2$  but also generates valuable biomass that can be harvested for various purposes, including biofuel production and the extraction of high-value compounds [15].

WW treatment systems utilizing microalgae offer numerous advantages compared to conventional operational units or treatment approaches. Primarily, they demand reduced energy and chemicals, establishing them as a more sustainable and economically viable choice [6]. Additionally, they can be easily integrated into existing WW treatment infrastructure, reducing the need for expensive retrofitting. Finally, they offer a high degree of flexibility, as microalgae can be tailored to the specific needs and conditions of the WW being treated [16], [17].

Currently, there are several techniques of microalgae biomass cultivation that are applied for large-scale production. Ponds and lakes are used as open cultivation systems of microalgae. In turn, closed cultivation systems, including photobioreactors (PBRs), are also used for this purpose. It is highlighted that open cultivation systems are cheaper and easier than closed ones; nonetheless, closed systems offer better control opportunities and a higher biomass production [18]. A PBR consists of a vessel that is partially or fully closed, where photosynthesis occurs. In these reactors, electric lights are used for providing the energy required by microorganisms. Lights are usually located within the reactor, but they can be installed outside the reaction chamber, where the cultivation medium is enclosed. The walls of the PBR are transparent and the microalgae are placed in plates or tubes, whose main objective is to reduce costs. In this regard, the use of catalysts, PBR configuration, environmental parameter control during cultivation, and aseptic designs must be optimized; even operational parameters such as the solution temperature and pH are vital, as well as the gas diffusion control [19].

The typical open pond cultivation system is comprised of a basic water pond, where natural sunlight is crucial for photosynthesis, and  $CO_2$  is sourced from the surrounding atmosphere. The pond is typically designed in a raceway format, with a paddlewheel facilitating the circulation and mixing of nutrients and algal cells throughout the loop (as shown in Figure 1), which effectively reduces the amount of space occupied by the pond.

The main components for the microalgae growth include a medium of growth with adequate nutrients and a source of light to carry out the process of photosynthesis. Additionally, a source of  $CO_2$  or air flow must be guaranteed, as well as an adequate temperature and solution pH, so that the microalgae growth is not limited [18], [20].

Homogenization is also needed, and predators or invasive species are required to be at minimum. These growth factors can be categorized into three groups as indicated in Table 1. It is pointed out that these factors may vary from species to species and, therefore, should be particularized according to the purpose.



Туре	Factors	
Environmental factor	Nutrients Temperature	
	pH	
Operating factor	Light type and intensity	
	Mixing	
Biotic factor	Predators	
	Invasive species	

Table 1.	Microalga	e growth	factors
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Three categories of strategies used for microalgae cultivation are recognized according to nutritional requirements: matrotrophic, heterotrophic and autotrophic. The former has gained a great attention because of the advantages associated over other cultivation systems [21], [22]. Autotrophic algae require a light source as energy for photosynthesis, while heterotrophs use for growth organic carbon substrates to achieve energy and sunlight is not required. Nonetheless, certain species of algae can function in both autotrophic and heterotrophic modes, contingent upon the resources that are available. Under mixotrophic conditions, microalgae use both organic and inorganic carbon simultaneously for their biochemical processes and biomass production [23]. Considering the time used for growing, mixotrophic and heterotrophic algae are of utmost importance, since they can grow 24 h/d in contrast to autotrophic algae, which only grow for 12 h/d [16], [18]. However, depending on their application, each cultivation approach is introduced to ensure the most adequate conditions to achieve maximum microalgae biomass production.

Carbon is a crucial component for the growth of microalgae, as it is an essential element in proteins, lipids, and carbohydrates. Insufficient carbon levels can lead to a reduction in biomass productivity and hinder the accumulation of lipids in the cells. Likewise, N is necessary for protein synthesis, as well as the composition of genetic materials such as DNA and RNA, and energy-storing molecules such as ATP and ADP. Therefore, enhancing N uptake and assimilation within algae cells could boost intracellular metabolism and promote algal growth. Nitrate, ammonium, and organic N are all potential N sources for microalgae cells; however, their availability in WW depends on their generation within specific industries. Additionally, P plays a vital role in regulating algae metabolism, despite its low content within algal biomass [18].

Under this scenario, for the microalgae cultivation, the aim is to use nutrients from primary and secondary WW effluents, as well as anaerobic digestion concentrate (ADC), to replace chemical fertilizers [24]. Li et al. [25]

sought to identify robust algae strains for the concentrated cultivation system and analyze the impact of environmental factors, such as light and dark cycles, light intensity and exogenous CO<sub>2</sub> concentration, on N and P removal from WW, biomass accumulation and biofuel generation. Results indicated that 14 algae strains from Haematococcus spp., Chlorella spp., Scenedesmus spp., Chloroccum spp. and Chlamydomonas spp. could thrive in the concentrated WW stream. Chlorella kessleri achieved the highest net biomass accumulation (2.01 g/L), followed by Chlorella prototecoides (1.31 g/L), and both displayed the capacity for mixotrophic growth when cultured in concentrates. Environmental factors had a significant effect, with higher light intensity and exogenous CO<sub>2</sub> concentration, and a longer illumination period promoting biomass accumulation, N and COD elimination, while a lower exogenous CO<sub>2</sub> concentration promoted P removal [25]. Likewise, Bohutskyi et al. [26] analyzed the influence of ADC dose and light intensity on the growth and productivity of microalgae Chlorella spp. and Scenedesmus spp., where the supplementation with ADC elevated nutrient concentrations and improved the ratio N:P. Nonetheless, an ADC dose of 20% led to the microalgae growth inhibition; probably due to the potential toxicity ascribed to ammonia (NH<sub>3</sub>). Regarding N and P removal, Morales-Amaral et al. [24] found that over 90% was exceeded, and the COD in the effluent was less than 100 mg/L. Above 50% of ADC, toxicity existed, and crop yield decreased. Muriellopsis spp. proved to be the most robust strain that tolerates higher concentrations of ammonium  $(NH_4^+)$  and achieves a higher yield [24]. It is noteworthy that stress-tolerant microalgae strains are highly efficient to produce raw material for biofuels in WW, so the aim is to understand the conditions for their proper growth and adaptability [27], [28].

In general, ADC represents an economic substitute that provides several necessary nutrients for microalgae growth and improves biofuel sustainability [26]. Nonetheless, as previously mentioned, microalgae have highly varying tolerance levels to NH<sub>4</sub><sup>+</sup> or NH<sub>3</sub> toxicity, and although it plays a crucial role in their metabolism, high NH4<sup>+</sup> or NH3 concentrations cause toxicity and can limit their growth. To address this, several strategies have been proposed. The simplest consists of dilution; in fact, in manure, domestic and food processing WW, NH3 toxicity has been mitigated using this strategy. However, the disadvantage lies in the high composition of water. On the other hand, Park et al. [29] developed a vacuum NH<sub>3</sub> extraction system to pretreat high NH<sub>3</sub> concentration manure waters for microalgae cultivation. It has also been demonstrated that an efficient way to reduce NH<sub>3</sub> toxicity is by removing this chemical species through pumping air into the culture medium or WW [29].

## 3. Mixed consortia of algae and bacteria

Chlorella spp. and Scenedesmus spp., as well as some species of the cyanobacteria group, have been described in the treatment of different types of WW, especially those from conventional treatment plants, industrial and urban origins, and those derived from animal excreta [8], [30], [31]. Species of *Chlorella* spp. algae have been widely applied due to their proven capabilities in removing COD, N and P with different hydraulic retention times (HRT) between 10 h and 42 d, with or without mixing with bacteria 10. As observed by Hongyang et al. [32], the morphotypes that could grow in mixotrophy and were resistant to WW belong mostly to the chlorophyte group, possibly to the Chlorella spp. genus; algae that were able to grow in media with 100% (v/v) WW [32]. However, it should be mentioned that many of the resistance mechanisms used by algae to tolerate high concentrations of non-domestic WW are unknown [28].

In contrast, multiple studies are involved in the formation of mixed algae consortia, obtaining favorable results compared to other cultures, such as the reduction in nutrient and organic matter content, efficient removal of heavy metals, and increased lipid content [33]. In this regard, a study conducted by Szwaja et al. [34] involved cultivating a blend of microalgae in several closed vertical PBR using various light sources. The objective was to investigate how the light source impacted the taxonomic structure and chemical makeup of the harvested biomass, as well as the generation of fermentative biogas/methane (B/M). The researchers analyzed the efficiency of anaerobic digestion by performing respirometry measurements, and а significantly higher B/M production was found in variants that tested biomass with predominance of cyanobacteria. Additionally, about the biogas produced had 55% of methane (CH<sub>4</sub>) content and volatile solids (VS) ranging from 383.2 L/kg to 400.8 L/kg VS. Lower VS and CH<sub>4</sub> content were generated for those variants whose chlorophytes was the predominant taxonomic group [34]. Furthermore, other authors propose the synergistic interaction between bacteria and microalgae to enhance the treatment of WW [35].

On the other hand, as observed, filamentous cyanobacteria and particularly *Spirulina* spp. [36], appears to be a promising option for cultivation in WW and agro-industrial waste, since they generate a satisfactory amount of biomass and can be easily harvested due to their structure and size. Additionally, their biomass composition can be altered through several operational and environmental factors to produce biomass with specific qualities [31], [37].

The employment of cyanobacteria in WW treatment has recently garnered attention due to their capacity to eliminate contaminants. However, their utilization also introduces various concerns that need attention [38]. A primary apprehension revolves around the potential production of toxins, as certain cyanobacteria species are recognized for generating harmful substances that pose risks to both humans and animals. Additionally, the rapid proliferation of cyanobacteria may deplete dissolved O<sub>2</sub> in the water, adversely impacting aquatic life. Furthermore, the release of excess nutrients from cyanobacteria during treatment can contribute to eutrophication in receiving waters, fostering harmful algal blooms, and worsening water quality issues. Therefore, while the use of cyanobacteria in WW treatment holds promise, careful consideration of their potential risks and limitations is imperative before implementation.

Finally, it is noteworthy to underscore the merits of adopting a biological treatment for wastewater decontamination. In contrast to physicochemical treatments, biological processes prove to be more economical, efficient (through bioadsorption), and, significantly, result in fewer toxic by-products. This aspect becomes particularly crucial when addressing the treatment of drinking water through physicochemical treatment systems [39].

# 4. Future perspectives: towards achieving a sustainable development

Given the worldwide shortage of fossil fuels, specifically oil and natural gas, there has been a notable emphasis on producing renewable biofuels [40]. Algae are involved in the production of a high amount of oil than other types of crops; therefore, these organisms are a promising feedstock. At the same time, there is concern about the increasing emission of  $CO_2$  into the atmosphere due to the rise in the burning of fossil fuels, which will continue to increase whether viable energy sources for replacement are not found. In this regard, algae can assimilate CO<sub>2</sub> photoautotrophically or mixotrophically, making them an ideal candidate for carbon sequestration and the reduction of both greenhouse gases and pollutants. As a result, WW treatment with microalgae (phytoremediation) offers a higher rate of atmospheric carbon fixation, with an average value of 1.83 kgCO<sub>2</sub>/kg biomass. In addition, there is a faster rate of biomass productivity (40-50% greater than that of land-based crops), allowing for the simultaneous removal of pollutants between 80 and 100%.

Recent developments in microalgae biotechnology are significant in establishing a biorefinery approach for WW treatment. This involves integrating biomass conversion processes and equipment to produce fuels, energy and chemicals from microalgae. The use of WW and waste as a medium and source of nutrients for algae cultivation offers a low-cost method for biomass generation [41]. Microbial lipids are highly desirable for biofuel production from microalgae. While various microorganisms such as bacilli, fungi and yeasts can accumulate oils, not all are suitable for large-scale production [40]. The competition for the generation of lipids by microalgae depends largely on the reactor design cost, energy demand, the wide range of nutritional substrates, and the different functions of metabolic pathways that demonstrate their potential scalability, taking into account the success obtained for the algal growth in chemically different WW [41], [42]. Microalgae have a high amount of lipids; nonetheless, a larger surface area for cultivation and longer fermentation periods are required compared to bacteria. While bacteria accumulate fewer lipids than microalgae, they have faster growth rates and can reach maximum biomass concentration in only 12-24 h, making them easier to cultivate.

Even though microalgae production is considered a sustainable resource for large-scale biofuel production, it is still limited due to the influence of lipid content, biomass productivity and water used in cultivation, as described above. However, above all, lipid extraction technologies and biomass harvesting processes are paramount. Recent advances have been made in the use of microalgae harvesting technologies, including physical, biological and chemical methods, which must meet certain ideal characteristics [43]. The expense associated with recovering microalgae is anticipated to constitute 20-30% of the total cost of biomass production [44]. Conventional methods for harvesting microalgae, including centrifugation, filtration, and flotation, are energy-intensive and contribute to 90% of the overall cost for biomass recovery from open ponds. Consequently, flocculation is a favored option due to its effectiveness and simplicity. However, the use of chemical flocculants presents health risks and can impede biofuel production due to their toxicity. Therefore, the careful selection of the harvesting process is pivotal to enhance recovery and diminish operational costs. This underscores the importance of examining various charge neutralization and exchange mechanisms, as well as exploring innovative approaches like the application of magnetic nanomaterials for efficient microalgae collection [43], [44], [45], [46], [47], [48].

Notwithstanding these benefits, there remain hurdles that need to be overcome to fully exploit the potential of microalgae-based WW treatment. These obstacles encompass the need to fine-tune cultivation conditions for optimizing nutrient removal and biomass production, ensuring the steady fastness and dependability of the addressing treatment process, and potential environmental risks such as the occurrence of algal blooms or the inadvertent release of genetically modified organisms into the environment [49], [50], [51]. However, overall, bioremediation, particularly through the application of microalgae, emerges as a hopeful and sustainable resolution to the challenges associated with WW treatment, charting a course toward a more robust and environmentally conscious future.

#### 5. Conclusions

Microalgae can effectively remove pollutants and nutrients from WW through photosynthesis, transforming them into biomass with added value for applications such as soil restoration, animal feed, biofuel production, and more. Additionally, this process yields valuable compounds like antioxidants, Omega-3 fatty acids, and pigments, which find high-value uses in industrial and nutritional sectors. This exemplifies the circular economy principles, wherein waste is converted into a resource, resulting in reduced waste and the preservation of natural resources. Furthermore, microalgae cultivation requires minimal land and freshwater, positioning it as a sustainable alternative to traditional WW treatment methods, which are both energy- and resource-intensive.

It is worth noting that an optimal integration of microalgae-based bioproducts and WW treatment can be achieved, using only WW as a nutrient source and providing a low-cost cultivation alternative. Environmental factors significantly affect both biomass and biofuel production as well as nutrient removal by microalgae from WW. Furthermore, nutrient stress from WW can manipulate the metabolite content of microalgae for further use. Additionally, microalgae offer a sustainable bioprocess for environmental remediation by removing contaminants and mitigating atmospheric carbon through CO2 fixation. Lastly, additional benefits are observed from mixed-culture cultivation, enhancing the synergistic interaction of bacteria and microalgae for efficient WW treatment.

Therefore, the application of microalgae in treating WW presents a promising opportunity to support the attainment of sustainable development objectives, including responsible production and consumption, climate action, and clean water and sanitation.

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#### **Autor Contributions**

J.M. Loaiza-González: Data curation, formal analysis, investigation, conceptualization, methodology, writing original draft. A. Rubio-Clemente: Conceptualization, formal analysis, methodology, writing original draft, writing – review & editing. G. Peñuela: writing -review & edition, funding acquisition, project manager.

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The authors reported no potential conflict of interest.

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## **Declaration of Competing Interest**

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