REVIEW ARTICLE

Integrated wastewater treatment and CO_2 capture by microalgae-based system

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In a socio-environmental landscape that looks towards eco-sustainability and renewable energy, the use of photosynthetically active organisms such as microalgae has gained considerable attention in recent years. Microalgae can be cultivated in combination with wastewater bioremediation and carbon dioxide capture systems. The microalgal biomass produced is rich in bioactive compounds including lipids, carbohydrates, proteins, and antioxidants, which can be employed for a variety of biotechnological applications including bioenergy, pharmaceutical, food, and cosmetic production. This study explored the current research and potential applications of integrating wastewater treatment and carbon capture into a single closed-loop system with the aim of protecting the environment while also yielding high-value compounds. The implications of this innovative approach to eco-sustainability and renewable energy were also discussed.

Keywords: microalgae; wastewater treatment; renewable energy; high-value compounds; carbon capture; single closed-loop system.

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Introduction

Wastewater, the water used in domestic, industrial, or agricultural human activities, often contains organic and inorganic pollutants that can be dangerous for human health or for the environment. Wastewater treatment involves removing different categories of pollutants in the residual water before being discharged into natural water bodies. Wastewater contains a great diversity of contaminants due to their origin, which can be generally domestic or agro-industrial [1]. In general, nitrogen and phosphorus are the main source of contamination as their accumulation results in eutrophication of aquatic environments, but heavy metals can also be found, which over time tend to accumulate inside the animal cell, causing the phenomenon of biomagnification [2]. The removal of pollutants can be achieved through various strategies (physical, chemical, or biological) and depends on the complexity of the wastewater to be treated. A combination of different processes is often required [3]. Conventional wastewater treatments are based on a sequence of aerobic and anaerobic processes that convert the contaminants present into inert derivatives, allowing water to be disposed of or reused. However, these conventional processes have a non-negligible environmental impact due to greenhouse gas emissions into the atmosphere, the need for large areas of land destined for these treatments, and the high energy cost [4, 5]. Wastewater treatment plants conventionally involve a succession of different processes that are usually classified as preliminary, primary, secondary, tertiary, and quaternary treatment [6]. The preliminary (physical) phase eliminates large materials such as branches, gravel, waste, or even fats and oils by flotation or sedimentation. In the primary treatment (physical-chemical) the sedimentation of the suspended particles takes places, using sieves with different pore diameters or sedimentation tanks with a laminar flow regime for sand and gravel [7]. After sedimentation, chemical precipitation, adsorption, and other physical-chemical applications, secondary (biological) treatment takes place with the aim of eliminating organic matter through the use of microorganisms [8]. One of the most commonly used strategies is activated sludge, which uses aerobic microorganisms to eliminate organic matter. If the concentration of organic matter is too high, an anaerobic process is preferred. The last step concerns the removal of those pollutants that cannot be removed by aerobic and anaerobic processes [5, 6].

Microalgae

Microalgae are a diverse group of photosynthetic organisms that inhabit both terrestrial and aquatic environments including marine and freshwater. Taxon of microalgae include both eukaryotic microorganisms and prokaryotic cyanobacteria with an estimated total number of species exceeding 200,000. Microalgae are more efficient than plants at converting solar energy into biomass due to their simpler cellular structure and direct immersion in an aqueous environment, allowing for more efficient access to water, CO_2, and other nutrients. The increasing interest in microalgae bio-refineries has led to the recognition that domestic and agro-industrial wastewater contain alternative nutrients at low cost, and CO_2 from atmospheric and exhaust gases can be utilized to grow microalgae. Integration of microalgae biorefineries is a promising solution to address environmental issues while simultaneously producing biofuels and other value-added compounds [8].

Wastewater treatment using microalgae

The treatment of wastewater through microalgae was first studied in the 1950. Microalgae were used in a "virtuous circle" to produce oxygen for bacteria, which using oxygen mineralized organic matter, releasing in turn carbon dioxide that was used by microalgae for the photosynthesis process [9, 10]. Microalgae can be used effectively to remove nutrients in wastewater during tertiary or quaternary treatments. The composition of wastewater is very similar to that of culture media usually used for the microalgae growth since they contain, besides to unwanted compounds such as heavy metals and emerging pollutants, carbon, nitrogen, phosphorus, and other minor components necessary for their growth compounds [5, 11]. The composition of the effluent in which the microalgae are grown depends on the stage of treatment to which it has been subjected. Wastewater from secondary or tertiary treatments usually contains a carbon defect and an excess of nitrogen and phosphorus. Therefore, an additional carbon input in the form of carbon dioxide or bicarbonate will be required to allow the complete assimilation of nitrogen and phosphorus during microalgal metabolism [5, 12]. As for nitrogen and phosphorus, the main sources are agriculture, food industry, livestock, and municipal drainage. Nitrogen is found in wastewater mainly in the form of ammonium (NH_4^+) and nitrate (NO_3^-), while the most common form of phosphate is orthophosphate [11, 13]. Inorganic nitrogen compounds such as ammonium, nitrate, nitrite, and ammonia are transformed by microalgae into organic compounds such as proteins, enzymes, or chlorophylls. Phosphorus can be involved in the formation of proteins, lipids, and carbohydrate intermediates. Microalgae can incorporate inorganic phosphate compounds such as hydrogen phosphate ($H_2PO_4^-$ and HPO_4^-) forming organic species through phosphorylation (ATP) [5, 13]. Furthermore, phosphate tends to accumulate in the cytosol in the form of polyphosphate granules, and some microalgae are able to use phosphorus, forming organic esters useful for algal growth [14]. Nitrogen consumption rates can reach values close to 40 mg/L·d, and phosphorous higher than 4 mg/L·d [13]. Several studies reported the effectiveness of the use of microalgae in the bioremediation of wastewater. *Nannochloropsis salina* sp. grown in bench-scale batch reactors using anaerobically digested municipal wastewater effluent as a nutrient source, showed the highest biomass concentration of 0.92 g/L and nitrogen and phosphorus removal rate of 35.3 mg/L·d and 3.8 mg/L·d, respectively [15]. In another study, where *Chlorella vulgaris* sp. was grown in wastewater from tertiary phase treatment, total nitrogen and phosphorus consumption during microalgal growth were observed [13]. Other micronutrients such as trace elements and metals usually present in the wastewater can be metabolized by microalgae during growth, when present at low concentrations. As far as heavy metals are concerned, several species of microalgae capable of accumulating them are currently being studied [16, 17]. Furthermore, new studies are underway on the elimination of emerging pollutants by means of microalgae, for example by considering the removal of antibiotics and care products that persist after disposal in aquatic ecosystems [18].

Carbon dioxide biofixation by microalgae

Although industrialization has improved our daily lives, the impact on the environment in terms of uncontrolled carbon dioxide emissions has worsened. The contribution of fossil fuel plants is about 40% of total global emissions of carbon dioxide, to which must be added the combustion of fossil fuels for transport. In heavy industries, carbon dioxide emissions are a by-product of chemical reactions that do not involve combustion, but carbon dioxide emissions indirectly produced by electricity production must also be considered [19, 20]. Microalgae are studied not only from the point of view of wastewater bioremediation, but also due to their ability to biofixate carbon dioxide [21]. In fact, through photosynthesis, microalgae capture carbon dioxide from the atmosphere or from flue gas emissions, converting it into biomass, whose lipid, protein, and vitamin rich content can be used for applications in the energy, food, and pharmaceutical/cosmeceutical industries [22]. Microalgae have been demonstrated to exhibit high carbon dioxide biofixation capabilities, which are 10 to 50 times more effective than those of terrestrial plants [23]. As the biomass of the microalgae is composed of up to 50% carbon, it has been estimated that around 1 kg of microalgae can absorb 1.83 kg of carbon dioxide, representing 40% of the global sequestration rate [24]. Optimising the carbon fixation efficiency of microalgae requires consideration of multiple variables including the microalgal strain employed, which must be able to tolerate high levels of carbon dioxide as well as the physical and chemical process parameters and culture system used [25]. Considering multiple variables, a general comparative study of the most suitable microalgae strain for carbon dioxide bioconversion would be challenging [23]. For instance, *Botryococcus braunii* SAG-30.81 grown in a lab-scale fermenter with an initial concentration of 5% carbon dioxide reached a maximum biomass concentration of 3.11 g/L and carbon dioxide biofixation rate of 0.5 g/L·d [26]. A separate study revealed the highest biomass concentration and carbon dioxide biofixation rates for *Scenedesmus obliquus* SJTU-3 (1.84 g/L and 0.29 g/L·d, respectively) and *Chlorella pyrenoidosa* SJTU-2 (1.55 g/L and 0.26 g/L·d, respectively) when 10% carbon dioxide was blown up during microalgae growth [27]. Moreover, the effect of different elevated carbon dioxide concentrations on *Chlorella* sp. and *Tetraselmis suecica* was investigated [28]. It was found that the maximum biomass productivity and carbon dioxide biofixation for *Chlorella* sp. of 0.64 g/L and 0.10 g/L·d were obtained with 5 and 15% carbon dioxide, respectively. On the other hand, the maximum biomass productivity and carbon dioxide biofixation for *T. suecica* of 0.72

g/L and 0.11 g/L·d were obtained by using 15 and 5% carbon dioxide [28].

Combining microalgae cultivation with wastewater treatment and carbon dioxide biofixation

The integration of systems for the cultivation of microalgae such as the treatment of wastewater on one hand and the sequestration of carbon dioxide on the other is a promising option for achieving eco-sustainability and bioremediation of the environment (Figure 1) [29-31]. In this process, microalgae use wastewater as a source of nitrogen and phosphorus, and carbon dioxide as a source of carbon, in order to growth and produce microalgal biomass. This results in an environmentally sustainable method for removing nutrients from wastewater, capturing carbon dioxide, and producing biomass that can be used for the production of biofuels, biogas, and biofertilizers [32]. Furthermore, the addition of carbon dioxide from combustion gases which contain a high percentage of this gas directly increases the production of microalgal biomass, thereby improving the treated wastewater and contributing to the reduction of excess carbon dioxide in the atmosphere [33].

Figure 1. Wastewater bioremediation combined with carbon dioxide biofixation by microalgae.

Several studies have demonstrated the feasibility of using municipal, agricultural, and industrial wastewater, combined with different percentages of carbon dioxide. In one study involving municipal wastewater, the effects of different municipal wastewater ratios and 15% of carbon dioxide aeration on the growth of *Nannochloropsis* sp. were investigated. The optimal growth occurred in a 50% municipal wastewater ratio and was further improved by aeration with 15% of carbon dioxide. The biomass concentration and total lipid content increased from 0.71 to 2.23 g/L and from 33.8 to 59.9%, respectively, after the lipid accumulation phase [30]. When *Scenedesmus obliquus* was cultivated in municipal wastewater containing 0.03 - 15% of carbon dioxide, the maximum biomass and lipid productivity were 0.577 and 0.016 g/L·d, respectively, with 5% carbon dioxide. The maximum carbon dioxide biofixation rate was 0.256 g/L·d and the maximum removal efficiencies of total nitrogen and total phosphorus were 97.8% and 95.6%, respectively [34]. *Chlorella vulgaris* ATCC 13482 and *Scenedesmus obliquus* FACHB 417 were cultivated in municipal wastewater within a 7 L airlift bubble column photobioreactor supplied with 5% carbon dioxide. The initial concentration of ammonia was reduced from 43.7 mg/L to 2.9 and 3.7 mg/L by *C. vulgaris* and *S. obliquus*, respectively. Also, an initial concentration of phosphate of 18.5 mg/L was decreased to 1.1 and 1.6 mg/L by *C. vulgaris* and *S. obliquus*, respectively. The biomass production and carbon dioxide fixation rates for *C. vulgaris* and *S. obliquus* were 0.94 and 0.86 g/L, as well as 0.14 and 0.13 g/L·d for carbon dioxide fixation [35]. Additionally, *Chlorella* sp. GD was cultivated in aquaculture wastewater aerated with boiler flue gas, with biomass productivity and carbon dioxide fixation efficiency of 1.296 g/L·d and 2.333 g/L·d, respectively. This microalga was also capable of removing up to 90% nitrogen and 99% phosphorus [36]. *Scenedesmus* sp. 336 was investigated in the hybrid system of brewery wastewater supplemented with 15% carbon dioxide. The results showed that the dry weight of microalgae biomass was 1.02 g/L, nitrogen and phosphorus removal rates were 75.96% and 95.71% respectively, with lipid productivity of 0.04 g/L·d [37]. Successful implementation of growth of microalgae by utilizing nutrients from industrial wastewater and carbon dioxide biofixation has been achieved as follows. *Chlorella vulgaris* NIOCCV was cultivated in seafood processing industry wastewater, reaching the optimum biomass productivity, carbon dioxide fixation efficiency, and lipid content of 0.26 g/L·d, 0.43 mg/L·d, and 38%, respectively, when carbon dioxide supply was 10% [36]. Nutrients from industrial wastewater with flue gas containing 5% carbon dioxide were used for the cultivation of *Chlorella* sp., giving the highest biomass growth and carbon dioxide fixation of 1.52 g/L and 0.18 g/L·d, respectively, with more than 70% nutrient removal [38]. An example of a species of blue-green microalgae that has been studied for its potential to remove nutrients from wastewater and fix carbon dioxide is *Phormidium valderianum* BDU 20041. When grown in an open tank in ossein effluent using flue gas, this species showed a biomass productivity, carbon dioxide fixation rate, and lipid content of 30 mg/L·d, 56.4 mg/L·d, and 12.74%, respectively [39]. Further investigation into other species of microalgae is required to optimize integrated systems for the bio purification of wastewater and bioremediation of the atmosphere [40].

Bioreactors used in integrated systems with microalgae

The cultivation of microalgae in integrated systems for the purification of wastewater and the capture of carbon dioxide can take place in outdoor tanks or in closed photobioreactors (PBRs). Open tanks are more commonly employed because of their lower investment and maintenance costs compared to closed PBRs. Yet, they are susceptible to environmental factors such as light or temperature and can be prone to contamination [41]. The most frequently used tank configurations for microalgae cultivation are raceway (an oval or a track channel stirred by an aerator) and circular ponds (stirred by a rotatory agitator). Given the increased operational and management efficiency, raceway ponds are now preferred over the open circular ponds for large-scale outdoor microalgae cultivation, mostly using for *Chlorella* sp., *Spirulina platensis*, and *Hematococcus* sp. [42]. Closed PBRs have been developed to overcome the drawbacks of open tank systems and to facilitate the long-term cultivation of microalgae with well-controlled conditions such as temperature, light, pH, carbon dioxide concentration and mixing speed [40]. These PBRs are classified in flat panel, horizontal tube, bubble column and airlift PBRs, as well as PBRs plastic bags. *Chlorella* sp. and *Scenedesmus* sp. are commonly grown in closed PBRs. Although closed PBRs have advantages such as high cellular productivity, fast growth, and a small working area, they also have drawbacks including high production and operating costs, and difficulty in cleaning. Thus, the development of cost-efficient and easy-to-clean PBRs is necessary in order to facilitate large scale microalgae production and the integration of technologies for microalgal growth under controlled conditions [43].

Conclusion

The concept of circular bioeconomy, recently established, highlights the need for the transformation of biomass into high value-added products. The closed biorefinery concept, which combines wastewater treatment with carbon dioxide reduction, is of great importance for the bioeconomy and sustainability, as it allows the recovery and reuse of resources [44]. The microalgal biomass resulting from the integrated wastewater treatment process in the presence of fuel gas can be used to extract lipids, which can be used as row material for the production of renewable energy such as biofuels. In addition, the treated wastewater can be reused for agricultural or industrial purposes [44]. Optimizing of laboratory-scale research is essential for industrializing the integrated process. Hence, it is important to explore different microalgal strains that can tolerate high carbon dioxide concentrations, high temperatures, and possible pollutants present in wastewater and fuel gas [34]. Once the most suitable microalgae are identified, further studies on scaling up the process are required. It is necessary to design and develop cheaper and more efficient PBRs an engineering approach. The integrated process of wastewater treatment, carbon dioxide abatement, exhaust gas reduction, and by-products production through microalgal cultivation is economically viable and environmentally friendly. Thus, it is essential to focus on sustainable development and the circular economy due to industrialization issue. Microalgae, in combination with the integrated processes of wastewater treatment and carbon dioxide reduction, can provide a substantial response, even though it is still under development. The combination of microalgal culture with wastewater purification ha potential to reduce both CO_2 emissions and the cost of biodiesel production (and other valuable products) from microalgae. Wastewater provides water and nutrients needed for microalgal growth, reducing production costs. Additionally, credits for wastewater remediation and CO_2 emissions mitigation can be attained. Thus, this technique could make biodiesel production from microalgae more viable.

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