Revalorization of Microalgae Biomass for Synergistic Interaction

Subjects: Environmental Sciences

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Microalgae and cyanobacteria are photosynthetic microorganisms' sources of renewable biomass that can be used for bioplastic production. These microorganisms have high growth rates, and contrary to other feedstocks, such as land crops, they do not require arable land. In addition, they can be used as feedstock for bioplastic production while not competing with food sources (e.g., corn, wheat, and soy protein).

Keywords: microalgae ; biomass ; bioplastics ; polyhydroxyalkanoates ; environmental impact

1. Introduction

Plastics are high molecular mass synthetic organic polymers mainly derived from hydrocarbons obtained from fossil fuels, such as crude oil and natural gas. Plastics are used for several purposes, including packaging, which typically is not recycled but ends up as waste. In 2015, it was estimated that of the 6300 million metric tons of plastic generated, around 9% was recycled, 12% incinerated, and 79% deposited in landfills or the natural environment [1][2]. If the current production and waste management continue according to these trends (annual growth rate of plastic production around 8.4%), around 12,000 million metric tons of plastic waste will be deposited in landfills or the natural environment by 2050. Furthermore, it is important to note that plastic degradation can range from 58 to 1200 years [3]. Together with the high rate of plastic use, this fact has caused the accumulation of plastics on the planet to be an environmental problem that requires urgent attention.

Plastic accumulation in the aquatic environment is one issue of emerging concern because of its possibility of being ingested throughout the food web and accumulated by living organisms [4]. To visualize the large consumption of plastics and their global impact, the UN General Assembly has reported that 13 million metric tons of plastic leak into the ocean per year. It has been reported that particles of plastic-related products have several negative effects in living organisms, for instance, on the gut, intestine, lung, and liver. Polystyrene induces responses, such as oxidative stress, mitochondrial dysfunction, and inflammation representing a risk factor for the kidneys [4]. In general, microplastics can be ingested by humans and organisms ranging from plankton and fish to birds and mammalians throughout the aquatic environment. Additionally, plastics can absorb or contain several chemical additives acting as vectors for multiple organic pollutants [5][6][7].

Multiple efforts are needed to deal with plastic waste generation and to reduce its presence in the aquatic and soil environments. Actions taken worldwide against plastic generation include new policies on plastic prohibition. For instance, the UK government implemented a plastic packaging tax in 2022, applicable to all packaging plastic (manufactured or imported into the UK) that does not contain at least 30% of recycled plastic [8]. In Spain, the free delivery of plastic bags to consumers at sale points of products was prohibited except for plastic bags made of 70% recycled plastic [9]. In New Jersey (USA), starting 2022, a law will be implemented prohibiting selling or providing single-use plastic carryout bags [10]. In Mexico City, these laws have also been established, where single-use plastic carryout bags and plastic straws are prohibited [11].

2. Phycoremediation of Wastewater

Microalgae species belonging to different genera, such as *Chlorella* and *Scenedesmus,* have been reported to treat wastewater effluents by assimilation of micro- and macronutrients and adsorption of organic and nonorganic pollutants [12][13]. Table S1 (Supplementary Materials) compiles the most recent advances in the phycoremediation of different wastewaters. *Chlorella* species are recognized for their metabolic plasticity. They can grow fast in wild environment conditions and can exhibit heterotrophic, photoautotrophic, or mixotrophic growth according to the medium requirements

[14]. Diverse studies have evaluated the potential of *Chlorella* in wastewater treatment (WWT), in either synthetic or raw wastewater. For instance, synthetic textile wastewater was remediated by *Chlorella vulgaris*, and after 13 days of cultivation, 99% of methylene blue was degraded [15]. These studies have in common that the chemical oxygen demand (COD), nitrogen, and phosphorus removal reached about 70–95%. The removal of pollutants by *Chlorella* species and their feasibility in producing biomass using wastewater as a nutrient source make them a promising candidate for establishing a large-scale wastewater phycoremediation system.

Scenedesmus species are also used for wastewater phycoremediation. They can assimilate nitrogen, phosphorus, organic carbon and reduce COD. Besides that, *Scenedesmus* can survive in low-light and polluted environments, such as wastewater from industrial processes, while showing excellent phycoremediation efficiency [16]. For instance, reports on *Scenedesmus* include olive oil mill effluent [17], palm oil mill effluent [18], domestic wastewater [19], industrial wastewater, and brewery effluent [20]. *Scenedesmus obliquus* was cultivated using municipal wastewater, and 0.88 g L^{-1} of microalgal biomass was reached [21]; however, it obtained a lower biomass production compared with BG11 medium [1.3 g L^{-1}]. These studies show the potential of these species to phycoremediate wastewater due to its high removal rate of COD, phosphorus, and nitrogen. These results show a promising adaptability of this microalgae to different types of wastewaters.

Phycoremediation of wastewater can be implemented in actual wastewater treatment plants in different steps, such as after a grit and grease removal process, as a primary and secondary wastewater treatment process, and as a third treatment before chlorination**.** Few studies are dedicated to studying WWT by microalgae after grit pretreatment. For instance, in a study of Choong et al. (2020) the grease wastewater used as a culture medium enhanced lipid content in *Scenedesmus* and *Tetraselmis* [22]. Additionally, *Ochromonas danica* grown with waste grease as a culture medium accumulated intracellular lipids between 48% and 79% w w^{-1} [22][23][24]. Nevertheless, attention should be paid to the wastewater turbidity and pollutant concentration. Additionally, the residence time should be considered when treating wastewater with microalgae. For instance, in López-pacheco et al. [25], when using wastewater after grit and grease removal, microalgae growth occurred at a maximum of 75% of raw wastewater [25]. This is because a high concentration of raw wastewater has high turbidity and organic load that hinders microalgal cell growth. Therefore, microalgae have a higher potential for WWT as secondary and tertiary treatments.

2.1. Disinfection Process in Wastewater by Phycoremediation

Stress can play an important role in microalgal production of antibacterial compounds. Some of these antibacterial compounds (e.g., chlorellin, linolenic acid, phycobiliproteins) are secondary metabolites that have been a valuable source in developing new pharmaceuticals, such as antibiotic, anti-inflammatory, and anticancer drugs [26]. The production of these antibacterial agents depends on the microalgae species; for instance, *Chlorella* species demonstrated antibacterial activity against some bacteria (*Vibrio* bacterial strains) [27]. This antibacterial potential of microalgae culture can possibly be associated with microalgae excretion of substances that inhibit the growth of bacterial strains, such as fatty acids. For instance, in a study by Juttner [28], it was shown that microalgae (diatom consortium mainly composed of *Diatoma elongatum*) release fatty acids as a defense mechanism against grazing predators (e.g., *Favella ehrenbergii*) [28]. *Phaeodactylum tricornutum* was also studied to determine this phenomenon. It was found to liberate fatty acids (capric acid, lauric acid, myristoleic acid, and palmitoleic acid) through lipase action after cell lysis [29][30]. Additionally, in *Chlorella* species, a fatty acid (lipophilic substance) has been identified and named chlorellin, which is excreted during the initial phase of culture growth. The liberation of these fatty acids and lipophilic substances is induced by cell lysis of microalgae already damaged by predators or pathogens. These sacrificial cells protect the culture from further damage since they act as signals or precursors that activate downstream systemic defense responses; this mechanism has also been shown in *Phaeodactylum tricornutum* cultures [31].

There are some studies on the antibacterial capacity of microalgae in aquaculture systems. For instance, *Chaetoceros calcitrans* and *Nitzchia* sp. completely inhibited a *Vibrio* population (*Vibrio harveyi*) within 24 h of exposure in tiger shrimp (*Penaeus monodon*) culture. In the same conditions, *Leptolyngbya* sp. (cyanobacteria])also reduced *Vibrio harveyi* population from 10^4 to 10^1 CFU mL^{-1} [32]. Therefore, this type of coculture can help reduce bacterial diseases in aquaculture systems and bacterial load in wastewater. Additionally, there are studies reporting the removal of total and fecal coliforms by microalgae. For instance, *Chlorella sorokiniana* removed 68% of total coliforms (log inactivation: 0.76) and 99% of *Escherichia coli* (log inactivation: 2.73) from a mixture of sanitary wastewater and swine manure [33]. In domestic wastewater, *Chlorella* sp. removed 99% of *Pseudomonas aeruginosa* (log inactivation: 2.5), 99% of total coliforms log inactivation: 2.8), 99% of *Enterococci* (log inactivation: 2.6), and 98% of *Escherichia coli* (log inactivation: 2.2) [34]. This remotion of coliforms is related to an increase in pH during the photosynthetic activity. A pH above 9 is no longer optimal for aerobic and facultative bacteria activity. During cultivation, H^+ is consumed during the conversion of bicarbonate into CO_2, and the produced hydroxyl ions accumulate in the medium, causing an increase in the pH and inactivating by this way coliforms; this process is one of the major mechanisms for fecal bacteria remotion in microalgae ponds [35].

Microalgae have also been reported to interact with plants for wastewater phycoremediation. Vetiver-*Dictyosphaerium* sp. c-culture was used in swine wastewater treatment, where about 35 genera of bacteria were detected; of these, 31 genera decreased throughout this treatment process. Specifically, some of the bacteria decreased from approximately 2000 operational taxonomic units (OUT) to zero or near zero (1–228 OUT) (e.g., *Methanosaeta*, *Escherichia*, *Paenibacillus*, *Rhodococcus*, *Ralstonia*, and *Citrobacter*). Additionally, *Escherichia* spp. was completely removed by day 15 of wastewater treatment [36].

2.2. Biomass Harvesting

Biomass harvesting is a unique process to consider in microalgae production. In this way, there are different techniques to recover as much biomass as possible from the culture system. These methods include flocculation, flotation, centrifugation, and filtration. The harvesting process may depend on the biomass application and culture scale. For instance, when using membrane filtration, biomass of good quality with no chemicals is generated [37]. This is similar to centrifugation, where biomass of good quality is obtained and has a high recovery efficiency (>90%). On the other hand, flocculation can be considered a lower-cost alternative. In fact, flocculation has been used for microalgae harvesting on a large scale and is a common harvesting method in conventional wastewater treatment [38][39].

3. Genetic Engineering to Increase Bioplastic Yield in Microalgae and Cyanobacteria

Genetic engineering is a potential method for modifying the genes of microalgae and cyanobacteria to improve the synthesis of desired polymers, such as starch, TAGs, or PHB [40]. For instance, TAG production in *Neochloris oleoabundans* was increased by co-overexpression of lipogenic genes (plastidial lysophosphatidic acid acyltransferase and endoplasmic-reticulum-located diacylglycerol acyltransferase 2). With this transformation, *Neochloris oleoabundans* increased 1.6-fold the lipid content and 2.1-fold TAG production [41]. The researchers also reported a long-term stability of the modified strain since this productivity was maintained for 4 years.

The increase in PHB by using genetic engineering tools has also been reported. In order to induce the production of PHB in *Chlamydomonas reinhardtii*, Chaogang et al. [42] utilized two expression vectors containing the *phbB* and *phbC* genes from *Ralstonia eutropha*, both encoding PHB synthase [42]. The presence of PHB granules in the cytoplasm of the transgenic cells resulted in a favorable outcome, producing 6 µg g^{-1} of PHB compared with no PHB production in the wild-type strain. *Synechocystis* sp. PCC6803 also was modified for enhanced PHB production by the overexpression of a heterologous phosphoketolase (*XfpK*) from *Bifidobacterium breve*, which is used as a strategy to improve acetyl-CoA levels. Using this technique, a PHB production of 232 mg L^{-1} (12% w w^{-1}) was obtained under nitrogen depletion conditions, greater PHB production from *Synechocystis* sp. without mutation (1.8% w w^{-1}) [43]. Additionally, overexpression of the *phaAB* gene in *Synechocystis* sp. PCC 6803 enhanced PHB production, obtaining a PHB concentration of 35% w w^{-1} growth in nitrogen-deprived medium; also, this technique increased acetyl-CoA levels [44].

Most algal transgenics now employ constitutive promoters to express the recombinant gene throughout algal biomass synthesis, which might have a detrimental influence on growth due to the increased metabolic burden or a potential toxicity on the cell [45]. Thus, a preferable technique is to activate the expression of genes near the end of the growth phase by utilizing tightly controlled promoters with a wide dynamic range in conjunction with good codon optimization, boosting the development efficiency and ultimate production of the targeted gene output. Another problem is the urgent need to create effective chloroplast and mitochondria transformation procedures for most useful microalgal species, as these organelles play critical roles in cellular metabolism. Despite the fact that many projects are underway to generate genome, transcriptome, and proteome information for many microalgal species, it is indeed essential to decode the full annotation of genes and the connectivity of biosynthetic processes in order to fully exploit the prospects of microalgae species.

4. Bioplastics from Microalgae and Cyanobacteria Grown in Wastewater

The synthesis of bioplastics from biomass of microalgae and cyanobacteria can be complemented with wastewater treatment. This would allow to grow cellular biomass without requiring synthetic culture medium while also treating wastewater [46]. Nevertheless, up to date, there are few studies that have evaluated this approach. In a study by López

Rocha et al. [47], blends of microalgae biomass grown in municipal wastewater were prepared with glycerol. The consortium evaluated in the study included *Scenedesmus obliquus*, *Desmodesmus communis*, *Nannochloropsis gaditana*, and *Arthrospira platensis*. Following injection molding of the blends at 140 °C, bioplastic materials were obtained. Further characterization of the bioplastics formed showed that they had a high thermal stability with low water absorption [47]. In another study, *Desmodesmus* sp. and *Tetradesmus obliquus* biomass grown in municipal wastewater was also evaluated to produce bioplastics [48]. In that study, microalgae biomass and glycerol were mixed. The results showed that these bioplastics had similar mechanical properties to bioplastics derived from soy and rice proteins [48].

Most studies on wastewater treatment by microalgae and/or cyanobacteria have focused on PHB production. For instance, PHB from *Botryococcus braunii* grown in sewage wastewater obtained a final PHB of 247 mg L^{-1} [49]. *Synechocystis salina* cultivated in digestate from an anaerobic reactor fed with thin stillage was also evaluated for PHB production [50]. Results showed that at the pilot scale (200 L), 4.8% w w^{-1} of PHB accumulated in *Synechocystis*, similar to that in control cultures grown in synthetic medium [50]. Thus, the PHB production by microalgae/cyanobacteria using wastewater as a culture medium could be feasible.

Although not many studies have considered the production of bioplastics from other macromolecules besides PHB from wastewater-grown microalgae and/or cyanobacteria, it is possible discuss their potential based on the carbohydrate (including starch and glycogen) and lipid (TAGs) content reported in the literature. For instance, *Chlorella vulgaris* grown in aquaculture wastewater obtained a cell density of 3.2 g L^{-1} with a high accumulation of carbohydrates (39% w w^{-1}) [51]. *Isochrysis galbana* reached a cell density of 3.2 g L^{-1} with an accumulation of 37% w w^{-1} carbohydrates grown in aquaculture wastewater [51], and *Desmodesmus* spp. grown in landfill leachate and municipal wastewater accumulated 41% w w^{-1} of carbohydrates and 20% w w^{-1} of lipids [52]. Additionally, in that study, it was determined that low concentrations of nitrogen enhance starch production of microalgae culture growth in treated wastewater.

Chlorella sp. and *Scenedesmus* sp. grown in domestic wastewater were able to achieve cell growth of 1.78 g L^{-1} and accumulated 34 % w w^{-1} of lipids [53]. *Tetraselmis* sp. grown in municipal wastewater achieved 1.57 g L^{-1} of microalgae biomass with 38% w w^{-1} of lipids [54]. Additionally, *Chlorella sorokiniana* accumulated 43% w w^{-1} of lipids when grown in aquaculture wastewater [55]. *Chlorella* sp. grown in swine wastewater (wastewater characterized for having a high organic load) increased lipid production, including triacylglycerols (2.5 higher times compared with standard medium) [56]. In addition, as it was mentioned before, the used grease wastewater as culture medium enhanced lipid content in microalgae biomass [57]; hence, the use of this wastewater for microalgae culture can also increase the production of bioplastics from these cultures.

5. Environmental Impact of Bioplastics

The environmental impacts of plastics are extremely important and have become a scientific, social, and political issue. Common plastics of petrochemical origin are widely used in different applications due to their low price, durability, and strength. In the last years, derived from their high demand and incorrect disposal, environmental problems have risen due to their accumulation and persistence in terrestrial and aquatic ecosystems. Bioplastics have emerged as an alternative to conventional plastics. They can be produced from materials of biological origin and have a lower impact on the environment [58][59]. Bioplastics are classified in three categories: (1) those that are biobased and biodegradable, (2) fossil-based and biodegradable, and (3) biobased and not biodegradable. Standard plastics (i.e., fossil-based and nonbiodegradable) are not bioplastics. Biodegradable bioplastics can be decomposed by the environment and microorganisms and are thus reintegrated into the ecosystem. For instance, starch-based bioplastics, PLA, and PHA/PHB can be easily degraded in small fragments that are digested by microorganisms. Production of bioplastics is, however, a relatively recent development, and there are still some constrains to be solved, as discussed below.

Land plant crops, such as corn, are currently used for bioplastic production. However, the use of these biomass feedstocks is controversial. They require large areas of cultivation, time, water, fertilizers, and pesticides. These grains are no longer used as food source but in the production of bioplastics and biofuels (e.g., ethanol). In fact, estimations report that a quarter of the cultivated land is currently used to produce biofuels and bioplastics, which has generated a marked increase in the prices of basic foods [60]. Walker and Rothman [60] compared bioplastics from PHB and PLA with plastics of petrochemical origin and determined that the production of bioplastics was more polluting due to the use of fertilizers and pesticides in crops [60]. Nevertheless, Elsawy et al. [61] reported that bioplastics, such as PLA, which are biodegradable and compostable, produce 70% less greenhouse gas emissions during their manufacturing compared with conventional plastics [61]. Therefore, the use of renewable biomass or organic waste can be a strategy to produce ecological bioplastics with lower greenhouse gas emissions [62].

The production of bioplastics using the biomass of microalgae produced from wastewater generates at least two positive impacts on the environment. Microalgae can reduce more than 80% of the nitrogen and COD present in the wastewater; likewise, different types of wastewaters can be used in this process, which demonstrates the versatility of the process [20] [21]. Additionally, microalgae could be produced in established wastewater treatment plants, which would not mean the use of arable land for this purpose [63]. On the other hand, the cultivation of microalgae can help reduce the concentration of CO_2 in the atmosphere, since for every kg of biomass of microalgae produced, 1.8 kg of CO_2 can be captured in the process [64]. In addition, as stated above, the rate of decomposition of a bioplastic is lower than that of conventional plastics [65], so its use would reduce the production of garbage and reduce the use of land for landfills.

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