Microalgae Cultivation Technologies

Subjects: Biotechnology & Applied Microbiology
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Microalgal biomass is currently considered as a sustainable and renewable feedstock for biofuel production (biohydrogen, biomethane, biodiesel) characterized by lower emissions of hazardous air pollutants than fossil fuels. Photobioreactors for microalgae growth can be exploited using many industrial and domestic wastes. It allows locating the commercial microalgal systems in areas that cannot be employed for agricultural purposes, i.e., near heating or wastewater treatment plants and other industrial facilities producing carbon dioxide and organic and nutrient compounds.

Keywords: microalgal biomass ; microalgae cultivation ; biofuels ; advantages ; limitations

1. Introduction

Microalgae are single-cell organisms that convert solar radiation energy into chemical energy via photosynthesis [1]. Controlled production of microalgal biomass is a fast-growing technology, as microalgae can be used to produce a wide range of commercially valuable cellular metabolites, including high-quality proteins, lipids, carbohydrates, dyes, and vitamins for the food/feed industry and the broad cosmetic industry.

The fact that microalgae represent an alternative and competitive source of biomass is due to their advantage over typical terrestrial and energy plants [2]. Algae possess very high photosynthetic efficiency [3], can relatively fast build biomass [4], are resistant to various contaminants [5], and can be grown on land that is unsuitable for other purposes [6]. Microalgae production systems can also be used in environment-protecting technologies [7], including sewage and leachate treatment [8], neutralization of waste and sludge [9], carbon dioxide biosequestration, biogas upgrading, and flue gas treatment [10]. This makes it possible to select and adapt specific strains for individual applications, including energy carrier production, environmental protection, and environmental engineering technologies [11]. Given these considerations, algae may provide a viable alternative to traditional energy crops [12].

2. Microalgal Biomass as a Source of Biofuels

Microalgae can serve as a potential source of many different types of biofuels (Figure 2). Examples include anaerobic digestion of biomass into biogas, production of biodiesel from lipids stored in algae cells and hydrogen from photobiological conversion, and lastly, gasification, pyrolysis, or direct combustion of the harvested algal biomass [13][14].

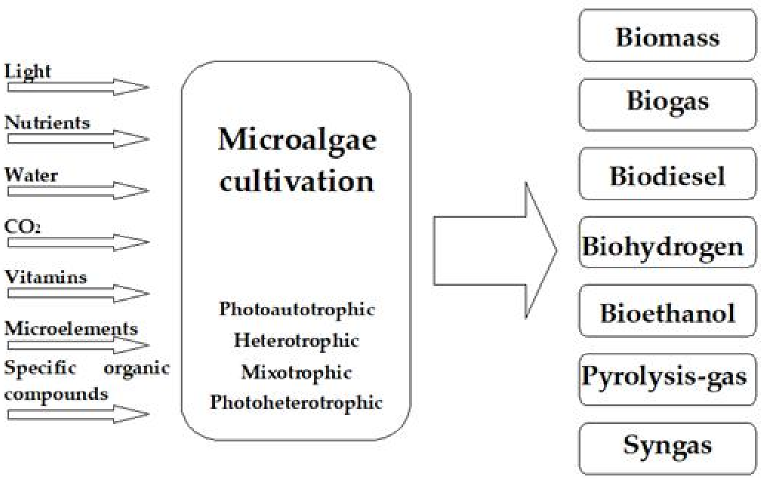

Figure 2. Available mechanisms for producing biofuel with microalgae.

The simplest way to use microalgae for fuel purposes involves the combustion or co-combustion of their pre-dried biomass [15]. However, this solution is rarely practiced, most often in cases where the biomass of microalgae cannot be used to produce more advanced biofuels [16]. Biogas and biomethane are produced during controlled, anaerobic degradation of microalgal biomass by fermentation bacteria [17]. Methane fermentation is a cascade of successive biochemical transformations, including hydrolysis, acidogenesis, and methanogenesis, which are carried out by specialized consortia of microorganisms [18]. In turn, biodiesel is produced via the transesterification of bio-oil extracted from microalgal biomass. This process involves the reaction of triglyceride molecules, bio-oil components, with low-molecular-weight alcohols in the presence of catalysts [19]. Hydrogen production by microalgae is based on direct biophotolysis, which involves the photosynthetic production of hydrogen from water, which uses the energy of light to break down the water molecule into hydrogen and oxygen. The process is mediated by hydrogenase—a metal enzyme that catalyzes the reversible oxidation of H2 and releases gaseous hydrogen by reducing protons [20]. The basic technology for bioethanol production from microalgae entails a biochemical process in which bacteria hydrolyze the biomass and then yeast convert the sugars present in the biomass into alcohol, which is then distilled and dehydrated [21]. In turn, syngas and pyrolytic gas are produced via the endothermal conversion of biomass into gas, which mainly consists of hydrogen, carbon monoxide, carbon dioxide, methane, and low-molecular-weight hydrocarbons [22]. The contribution of individual products, including their qualitative composition, depends mainly on the process conditions, such as temperature, reaction time, pressure, and biomass characteristics [23].

Many researchers have argued that methane fermentation is the most promising and effective method for producing energy from algae. Sialve et al. (2009) found that, given suitable operating conditions, methane fermentation as a primary method of algal biomass processing is more economical than systems that incorporate lipid extraction and anaerobic processing of post-extraction residues [24]. Other findings suggest that the balance of methane fermentation unit operations is the most effective in terms of both the economy of the process and the pollution levels [25]. Studies have indicated that methane fermentation may be the most practical means of converting algal biomass into energy. However, Börjesson and Berglund (2006) noted that energy inputs and environmental impact varied greatly between the different methane fermentation technologies [26]. As such, an environmental life-cycle assessment (LCA) is necessary for a complete and objective evaluation of each process [27].

To meet the current challenges related to the circular bioeconomy, it is necessary to change the approach to biorefinery processes [28]. Technological, economic, and environmental efficiency improvements can be achieved by simultaneously producing many high-value products other than biofuels [29][30]. Research and development works must, therefore, be focused on finding new, more complex, and integrated production processes. Although various strategies have been proposed for converting algal biomass into fuel and fine chemicals, none have been proven to be economically viable and energy balanced [186]. Therefore, other, valuable biological products should also be searched for. In this context, the concept of microalgae biorefineries emerged with the concept of recovering multiple products from one operating process. Considering the biorefinery complexity index (BCI) as an indicator of technical and economic risk, one of the most promising seems to be the biorefinery platform based on microalgal biomass conversion into fuels, food, dietary and feed supplements, fertilizers, and pharmaceuticals [31]. A schematic diagram of a comprehensive biorefinery approach to the processing of microalgal biomass is presented below (Figure 3).

Figure 3. A schematic diagram of a comprehensive biorefinery approach to microalgal biomass processing.

3. Systems of Microalgae Species Cultivation for Biofuel

The growth rate of microalgae and their composition is influenced by the growth conditions and the species employed [32][33][34]. Many classification schemes categorize methods and technologies used to cultivate algae for biofuel [35][36]. Due to the specific nature of microalgae, the most important scheme divides the systems on the basis of the nutrient source and the type of biochemical processes used to grow the algal biomass rapidly. With this criterion in mind, cultures can be divided into four main types: photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic [37].

4. Strengths and Weaknesses of Different Technologies for Producing and Utilizing Microalgal Biomass

Microalgae-based technologies of sewage treatment, pollutant degradation, and biofuel production were described in detail in scientific papers, patent claims, and performance data from existing installations [38][39][40]. Microalgal biomass has been demonstrated to be one of the most efficient and environmentally friendly alternative energy sources, as it is a promising and sustainable source of bio-oil, methane, and biohydrogen, i.e., fuels that can help reduce atmospheric greenhouse gas emissions [41][42]. Microalgae represent an alternative to terrestrial vascular plant species commonly used as a biofuel feedstock, such as rapeseed, soybean, and oil palm [43]. Literature data indicate that the annual hectare yield of bio-oil from microalgal cultures can exceed 19 m^3. By comparison, the corresponding values are 6.1 m^3 for oil palm, 4.3 m^3 for sugar cane, 2.4 m^3 for corn, and 0.5 m^3 for soybean [44].

The undeniable strength of the microalgae-based technologies is their well-established high photosynthetic efficiency. The efficiency of the solar-to-chemical energy conversion via algal photosynthesis varies from 4% to 10%, whereas the range for higher plants is 0.5–2.2% [45]. This directly translates to a fast growth rate of microalgae and a high per unit dry matter yield, significantly higher than that of terrestrial plants [43]. Those observations were corroborated by Tredici et al. (2015), who tested the strain *Tetraselmis suecica* in a proprietary photobioreactor design named "Green Wall Panel-II" The research was conducted in Italy (Tuscany), with the final productivity of the culture reaching 36 tons of dry microalgal biomass$\cdot ha^{-1} \cdot year^{-1}$. By contrast, soybean grain yields are only 2.6 tons$\cdot ha^{-1} \cdot year^{-1}$ [46].

Some strains of microalgae can double their mass in just a few hours. This property was described by Maxwell et al. (1994), who tested the growth rate of *Chlorella vulgaris*. The generation time observed for the species was 8.6 h at 27 °C, although cell division extended to 48.5 h at 5 °C [47]. Raslavicius et al. (2014) and Chen et al. (2015) showed that the annual microalgal biomass yields per hectare can range from 4 tons of dry matter to as high as 100 tons of dry matter [38][48]. According to other works, microalgae can double in volume or mass within a few hours, given the right conditions [41][43][45]. The resultant microalgal biomass yields can reach 500 $kg \cdot day^{-1}$ in a 1000 m^2 open pond production system [49].

One indisputable advantage of the microalgae-based solutions is that waste substrates of various properties and characteristics can be used to support rapid biomass growth [50]. Such technologies are most often used for tertiary treatment of urban or industrial waste in maturation or facultative ponds [51]. Such organisms release 1.50–1.92 kg $O_2 \cdot kg^{-1}$ of the produced biomass through photosynthesis, with the oxygenation rate reached during degradation of organic pollutants ranging from 0.48 to 1.85 kg $O_2 \cdot m^{-3} \cdot day^{-1}$ [52][53]. Microalgae absorb a significant portion of the biogenic substances contained in wastewater, as they require large quantities of nitrogen and phosphorus for internal protein synthesis. As such, protein content in the algae dry matter ranges from 20% to 60%, depending on the species. The absorbed biogenic compounds are also used to synthesize nucleic acids and phospholipids [54].

Currently, microalgae-based wastewater treatment processes are often integrated into systems designed to grow algal biomass for biofuel and energy production [55]. Such solutions can be used to remove chemical and biological contaminants from wastewater, while concurrently growing biomass for biofuel production, thus proving to be more viable from the economic and technological standpoint [56]. The use of wastewater as a growth medium directly reduces the costs of supplying water and nutrients necessary for the algae to grow at an efficient rate [57]. Research so far has shown that high CO_2 levels in wastewater promote microalgal growth, thus directly stimulating faster degradation of pollutants [58]. In systems where algae are grown in saltwater, the introduction of wastewater also serves to balance the molecular ratio of carbon, nitrogen, and phosphorus (C:N:P = 106:16:1), known as the Redfield ratio [59].

In light of the widely discussed effects of greenhouse gas emissions, integrated systems capable of reducing gas pollutant levels in the air, while simultaneously harvesting biomass and recovering energy, have attracted much interest [56]. One of the most promising and prospective avenues of evolving such systems lies in using microalgal biomass to remove pollutants from waste gases, mainly CO_2, NO_2, and SO_2 [60][61]. Research to date has shown that intensive microalgae cultivation requires a supply of 1.83 kg CO_2 per 1.0 kg of the grown dry matter, which is why low carbon dioxide concentrations in the growth medium often present a bottleneck that impedes rapid biomass growth [62]. Therefore, additional CO_2 needs to be loaded into the photobioreactor by increasing saturation or enriching the culture with leachate from the digesters [63]. Some promising studies on carbon dioxide fixation in algae cultivation systems indicate that the technology may potentially be used to lower CO_2 emissions [60][63].

One advantage of intensive algae production systems is that microalgal biomass can be grown in both freshwater and saltwater media. Kuei-Ling and Jo-Shu (2012) examined *Chlorella vulgaris* ESP-31 growth in freshwater using a modified Bristol's medium and MBL medium, producing biomass concentrations of 2.0–5.0 g dry matter$\cdot dm^{-3}$ for both media [64]. In another study involving *Nannochloropsis salina* CCAP849 grown in saltwater and F/2 medium, Beacham et al. (2015) obtained a final microalgal cell concentration of 7×10^7 cell$\cdot cm^{-3}$ [65]. Unlike terrestrial plants, microalgae do not require fertile farmland to thrive [45][66] and can live, effectively photosynthesize, and build biomass in various climate conditions [41].

Eutrophic and degraded water bodies can be used as another promising source of microalgal biomass [67][68]. Extracting microalgae from such reservoirs leads to a direct improvement in water quality [43][69]. Microalgae blooms, particularly cyanobacteria blooms, pose a threat to regions attractive to tourists and disrupt the basic processes of natural water bodies [70]. For example, Lake Taihu in China, a source of potable water for over two million people, has been repeatedly struck by cyanobacteria blooms since 2007, impacting water quality and posing a technological challenge concerning water treatment [71]. Some researchers have attempted to use microalgae from Lake Taihu as an organic substrate for biogas production [71][72]. Microalgal blooms, most of which are cyanobacteria blooms, are increasingly occurring in water bodies worldwide. Lake Chaohu and Lake Dianchi are among the reservoirs that regularly experience algal blooms [73].

Controlled cultivation of microalgae in eutrophic sea waters has been shown to directly lower biogenic compound concentration in the water and reduce the likelihood of marine life loss. Thus, it can be viewed as a method of revegetation used to improve reservoir condition [57]. Some of the associated issues were addressed in a research program launched by the present authors, which in large part aimed to assess the potential of incorporating microalgal biomass sourced from the Lagoon of Wisła and microalgae sourced from the Puck Bay into methane fermentation processes [74][75][76]. The analysis of the microalgae sourced from the Lagoon of Wisła showed a taxonomically differentiated biomass undergoing season-to-season changes. Bacillariophyceae species prevailed in the spring months from April to May and in the autumn months from October to November. From June to September, the Cyanoprokaryota division species were the most populous, with Chlorophyta and Dinophyceae as the subdominant groups *[70]*. It was shown that the time of microalgae extraction from Lagoon of Wisła waters had a significant effect on the organic compound concentration in phytoplankton dry matter. The lowest concentrations were recorded for the Bacillariophyceae-dominant period, whereas the highest ones were correlated with Cyanoprokaryota and Chlorophyta presence [262]. Respirometric analyses showed that the technological performance of the methane fermentation process was the highest in the variants utilizing algal biomass extracted between June and September (i.e., rich in Cyanoprokaryota and, to a lesser extent, Chlorophyta) loaded into model digesters. Biogas yields within this period ranged between 389.07 ± 8.21 and 420.95 ± 0.95 $cm^3 \cdot g$ dry $matter^{-1}$ [70].

Microalgae can be grown in water sourced from natural reservoirs (with a high content of biogenic substances), as well as in liquid waste and wastewater of various compositions. The use of such culture media not only leads to increased biomass productivity but can also deliver positive environmental outcomes. Microalgae employed in a photobioreactor with a scrubber allowed for a 60–90% reduction in nitrogen content and 70–100% reduction in phosphorus content in an effluent from manure condensation [77]. Microalgae-based technological systems also offer the advantage of pesticide-free cultivation, which significantly reduces the risk of secondary environmental pollution[41].

The reservations and controversies surrounding microalgae production/utilization technology mostly relate to the identified investment, technological, and operational barriers to implementation. Such barriers directly impact the costs of biomass cultivation, thickening, and separation. Another dissuading factor is the financial burden connected with converting the biomass into valuable end products [41][42]. The investment and operating costs intrinsic to microalgal cultivation are several times higher (more than tenfold in some cases) than the costs of extracting lignocellulosic biomass [49][78]. As such, the priority task of commercial enterprises and research groups is to increase the cost-effectiveness of such systems [39]. Furthermore, operating microalgae production installations and converting biomass into other products are still subject to many technological hurdles [66]. Gouveia (2011) noted the multiple deficiencies of algae cultivation methods, pointing to the recurring problems with growing microalgae in photobioreactors, i.e., biofilm build-up on photobioreactor walls, blockage of light sources by the growing culture, high oxygen concentrations, and accumulation of compounds toxic to microalgae cells [79]. Other authors also highlighted the importance of these technological problems [80][81].

In order to obtain economically profitable, pure cultures and metabolites of microalgae, it is necessary to employ complex substrate compositions, containing nitrogen, phosphorus, iron, silicon, vitamins, and microelements [43][66]. Operators of intensive microalgae production systems face the major technological challenge of ensuring proper composition of the growth medium and monitoring its quality throughout cultivation. The choice of growth medium depends on the tested microalgae species, as well as on the desired product of cultivation. For example, Nannochloropsis oceanica cultivated for biofuel production is grown on BG-11 medium, at 2% CO2 (v/v), with an artificial light intensity of 80–100 μmol photons·m−2·s−1 and a temperature of 25 °C [82]. In contrast, Crypthecodinium cohnii microalgae grown to produce omega-3 acids need glucose as a source of carbon, yeast extract as a source of nitrogen, a temperature of 27 °C, dark conditions, and oxygen levels of more than 30% [83].

Improper operation of microalgal biomass production systems may lead to problematic environmental pollution with undigested nutrients. This phenomenon causes adverse changes in the functioning and structure of aquatic ecosystems, leading to accelerated eutrophication. The problem stems in large part from bioreactors being fed with an imbalanced nutrient load. The discharge of effluent rich in excess nutrients into natural reservoirs may result in acidification and water pollution, which in turn lead to ecotoxicity, eutrophication, and degradation [69][84].

Other disadvantages of microalgal biomass technologies relate to the potential competition of algae with food crops and industrial crops, land use and the change thereof, and negative effects on biodiversity [66]. Researchers also pointed to potential disruption of natural aquatic ecosystems [85], ozone depletion [86], and structural restrictions on the market's operation [66]. Additionally, genetically modified microalgae used for cultivation may proliferate in the wild and produce various mutations, including ones detrimental to the environment [69]. The lack of legislative/legal measures and incentives, such as subsidies and tax credits, also presents a barrier to the widespread take-up of microalgae-based technologies, including those relevant to biofuel production [85].

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