# Algae Biomass as Source of Liquid Fuels

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Algae biomass is perceived as a prospective source of many types of biofuels, including biogas and biomethane produced in the anaerobic digestion process, ethanol from alcoholic fermentation, biodiesel synthesized from lipid reserve substances, and biohydrogen generated in photobiological transformations. Environmental and economic analyses as well as technological considerations indicate that methane fermentation integrated with bio-oil recovery is one of the most justified directions of energy use of microalgae biomass for energy purposes. A promising direction in the development of bioenergy systems based on the use of microalgae is their integration with waste and pollution neutralization technologies.

algae renewable energy source biofuels biogas bio-oil biohydrogen

## 1. Introduction

The development and large-scale implementation of clean, effective, and renewable technologies for energy production is today becoming a challenge for scientists and a priority to energy system operators. The immediate reason for this situation is the need to reduce greenhouse gas emissions, which entails reduced extraction and exploitation of conventional energy carriers, including coal, natural gas, and crude oil.

It is commonly believed that the goals presented above can be partially achieved by stimulating the development of unconventional energy systems based on the use of biomass of various characteristics and origins [1][2]. This common viewpoint has, however, been challenged in a few works. Fargione et al. (2008) and Searchinger et al. (2008) demonstrated that the irrational management of the resources typical of energy crops might, in fact, lead to a negative balance in the volume of gasses released into the atmosphere [3][4]. Research works also suggest that the intensive exploitation of arable lands for the production of plants intended for biofuels can adversely affect the global supply of food and cause a significant increase in food prices [5]. Hence, a strong need emerges to search for alternative biomass sources, the use of which for energy purposes would be justified from the economic and ecological perspective. Given the very high photosynthetic efficiency, the fast rate of biomass growth, resistance to various types of contaminants, and the possibility of management of lands that cannot be used for other purposes, algae seem to offer a perfect alternative to typical energy crops [6].

Most of the research works published so far have focused on bio-oil production technologies based on lipids accumulated in large amounts in algae cells. In the 1980s, the US Department of Energy launched a research program to identify the use of algae for energy production (the Aquatic Species Program). Scientists have analyzed over 3000 microalgae strains, trying to identify species with the highest energy potential [7]. In the following years,

technologies for intensive algae cultivation in photobioreactors and biodiesel production were developed, and commercial biorefineries were launched, including among others, in Turkey and the United States of America [8][9]. Today, many research and implementation programs are in progress across the world, aiming to increase production efficiency of algae biomass and its conversion into biofuels. Several thousand patents related to the technologies of production, separation, and conversion of algae biomass into biofuels are registered annually, proving that this issue is still in the focus of researchers' interest.

There are a few reports in the literature on large-scale studies on the production of biohydrogen or biomethane from algal biomass. Installations for the production of algae biomass dedicated to the production of bio-oil are presented more frequently. For example, research by Muradel Pty Ltd. of Australia aimed at the production of biofuels, oleochemicals, biofertilizers, animal feed, and building materials in a raceway pond [10]. Sea6 Energy, India research was aimed at producing food additives, biofuel, bioplastic, and animal feed in sea water [11]. Production of astaxanthin and DHA in enclosed photobioreactors was carried out by Solix Algadrients Inc., USA [12]. Design and validation of a new integrated "biowaste-to-energy" concept involving algae cultivation and biogas production was carried out by the Technical Research Center of Finland [13].

## 2. Bio-Oil Production

Literature data show that over 19,000 $dm^3$ of bio-oil can be produced annually from one hectare of microalgae cultivation. For comparison, the oil production yield of other plants is much lower, like, e.g., palm oil—6100 $dm^3$/ha/year; sugar cane—4300 $dm^3$/ha/year; maize—2400 $dm^3$/ha/year; or soybeans—500 $dm^3$/ha/year [14][15].

The proliferation of microalgae as well as the content and composition of oil in cell dry matter depend on the conditions of their cultivation and the species used [16]. There are many classifications of technologies used in microalgae cultivation for oil. The most important is the one based on the nature of the biochemical processes ensuring intensive biomass growth and the effective production of lipid compounds. Considering this criterion, four main types of culture can be distinguished, namely: photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic [16].

Photoautotrophic microalgae use light, carbon dioxide, and water to biomass production [17]. This kind of cultivation is usually used for microalgae cultivation in large scale [18]. It was proven to result in high variance of the lipid content in the microalgae biomass, ranging from 5% to 68%, depending on the strain used. In the case of the *Chaetoceros calcitrans* CS 178 strain, the lipid synthesis rate was 17.6 mg/$dm^3$ × d and the final lipid concentration was 39.8% of cell dry matter [14]. On the other hand, the use of the *Botryococcus braunii* UTEX 572 strain allowed achieving culture yield at 5.5 mg/$dm^3$ × d [18]. The highest yield was found in studies that verified the effect of high $CO_2$ concentrations on biomass productivity and lipid production in a culture with *Chlorella* sp. strain. In this culture variant, the final bio-oil concentration was at 32–34% of cell dry matter and the maximal rate of lipid production was at 179.8 mg/$dm^3$ × d [19].

The research carried out so far have shown that limiting the source of nitrogen in photoautotrophic cultures increased the lipid content in cell dry matter [9]. The effectiveness of this technological treatment was proved during semi-continuous culture of the *Auxenochlorella pyrenoidosa* strain with a limited amount of nitrogen source in the medium and pH control using $CO_2$ [20]. The cited study confirmed that limiting the nitrogen concentration and adjusting the pH value by dosing $CO_2$ allowed achieving very high parameters of the lipid synthesis efficiency, which amounted to 115 mg/dm$^3$ × d. It was more than three times higher compared to the control culture conducted without this technological treatment.

An appropriate amount of carbon dioxide should be provided to the growing population of microalgae to obtain satisfactory technological effects in photoautotrophic cultures, including biomass production and lipids accumulation. In many cases, $CO_2$ is delivered by simple diffusion from the atmosphere or through an aeration process, as exemplified in a study conducted by Han et al. (2013) [20]. However, given the low concentrations of this gas in the atmospheric air in intensively developing cultures, $CO_2$ may minimize the expected technological effects. Therefore, it is reasonable to locate photoautotrophic systems for microalgae biomass production in the vicinity of the waste source of this gas [21]. An example of such a solution is the Seambiotic pilot installation built in 2006 in Israel, which uses waste $CO_2$ from a coal-fired power plant. In this installation, algae are cultured in open ponds with a total area of 1000 m$^2$, whereas a gas containing about 12% $CO_2$ is directly dispersed into the culture via diffusers. The final yield of this technological solution is 20 g of dry biomass/m$^2$ × d [22]. In turn, de Morais and Costa (2007) demonstrated that only certain strains, such as *Tetradesmus obliquus*, *Parachlorella kessleri*, and *Arthrospira* sp., were able to grow under conditions of high $CO_2$ concentration approximating 18% [23].

Likewise, bacteria and fungi, selected species of microalgae are capable of heterotrophic development using organic substances [16]. The heterotrophic culture eliminates the common problem of photoautotrophic systems related to the overgrowth of the surface of photobioreactors and self-shading of microalgae cells, which directly reduces the access of light imperative for effective photosynthesis, biomass proliferation, and bio-oil production [17]. Heterotrophic algae cultivation systems are characterized by an efficient growth rate and the closing concentrations of biomass and lipids compared to the phototrophic or mixotrophic ones. For example, the heterotrophic cultivation of *Crypthecodinium cohnii* in a medium consisting of glucose, yeast extract, and acetic acid ensured a dry biomass concentration of 109 g/dm$^3$ and a final lipid concentration of 61 g/dm$^3$ [24]. For some microalgae strains, a change in the cultivation conditions from photoautotrophic to heterotrophic increased the lipid concentration of the cell dry matter. For instance, a 40% increase in lipid content was achieved in the culture of *Auxenochlorella protothecoides* after modifying the culture conditions from phototrophic to heterotrophic [25]. In the case of *C. vulgaris* ESP-31 strain, the same modification caused an over ten-fold reduction in the biomass concentration [26].

## 3. Biohydrogen Production

Biohydrogen production carried out by microalgae is based on biophotolysis involving photosynthetic generation of hydrogen from water, in which light energy is necessary for the lysis of the water molecules into oxygen and hydrogen [27]. This process runs mainly due to hydrogenase, which catalyzes the oxidation of $H_2$ and releases gaseous hydrogen by reducing protons [28]. Two transmembrane peptide complexes: photosystem I (PSI) and

photosystem II (PSII), are responsible for hydrogen production by microalgae in the photolysis process. The water molecule breaks down due to the exposure of both complexes to solar radiation. Then, $O_2$ is produced by PSII, while the electrons generated in this process are used by PSI to reduce $CO_2$ and build cellular material (aerobic conditions), or transferred through ferredoxin to hydrogenase and used to produce hydrogen. The simultaneous initiation of hydrogen production and hydrogenase induction can proceed only under anaerobic conditions. In addition, reduced sulfur availability causes reversible inhibition of the PSII photosystem, which entails the simultaneous arrestment of the aerobic activity of photosynthesis. Under such conditions, the oxygen level drops below the value consumed by the respiratory system. However, the PSI photosystem, responsible for electron transfer through reduced ferredoxin to hydrogenase, remains active, enabling hydrogen production [29].

In the presence of organic substrates, the microalgae species capable of producing hydrogen can develop both in the light phase through mixotrophic growth, and in the dark one via heterotrophic transformations [30]. If the inflow of light energy to the cultivation system is limited, the available simple organic compounds are metabolized and used to satisfy cells' needs and synthesize biomass [31]. It has been proved that the most favorable conditions for hydrogen production in cell systems are when the oxygen content in the medium is kept below 0.1% [32]. The deprivation of sulfur compounds in the culture medium is usually achieved via algae culture centrifugation, and then suspending the concentrated and liquid phase-free biomass in the medium, in which sulfur has been replaced with chlorine compounds [33]. The technological treatment based on centrifugation has been proved expensive, time-consuming, and leading to partial damage of the cellular material. An alternative solution is to dilute the culture medium, which directly reduces the sulfur concentration in the technological system. However, this method extends the time needed for sulfur depletion and development of anaerobic conditions [34].

It is also challenging to determine culture time and to identify the onset of hydrogen production. Some authors state that the biomass production process should be carried out to half of the exponential growth phase [35]. Others argue that a higher density of algae cells directly improves efficiency and prolongs hydrogen production [36]. Ji et al. (2010) achieved hydrogen production at 16 $cm^3/g$ biomass with a cell density of 0.5 $g/dm^3$. When the cell density increased to 3.2 $g/dm^3$, they reported hydrogen production over 49 $cm^3/g$ biomass and simultaneous photochemical conversion at 0.3%. The increased substrate density was also accompanied by an almost 10-fold increase in the gas production rate [36].

Most scientific publications addressing this research issue indicate the high efficiency of $H_2$ production by unicellular algae, like *Chlamydomonas reinchardtii* commonly found in soil and saline waters [37]. The $H_2$ production by this species was reported to reach 90–110 $cm^3/dm^3$ [34] and, in some cases, even 80–140 $cm^3/dm^3$ [38]. Faraloni et al. (2011) achieved a hydrogen production of 150 $cm^3/dm^3$ from the *Chlamydomonas reinwardtii* algae culture, using waste from olive processing in the algae growth process [39]. In turn, Skjanes et al. (2008) investigated the possibility of producing hydrogen from 21 species of green algae isolated from an anaerobic environment. They achieved the best production results for: *Chlamydomonas reinhardtii*, *Chlamydomonas euryale*, *Chlamydomonas noctigama*, *Chlamydomonas vectensis*, *Auxenochlorella protothecoides*, *Oocystis*, *Desmodesmus subspicatus*, and *Raphidocelis subcapitata*. The highest $H_2$ production efficiency approximating 140 $cm^3/dm^3$ was demonstrated

for *Chlamydomonas reinhardtii*, followed by *Chlamydomonas noctigama* (80 $cm^3/dm^3$) and *Chlamydomonas euryale* (22 $cm^3/dm^3$) [38].

## 4. Biogas Production

Research on the use of macroalgae in anaerobic digestion were analyzed by Vergara-Fernàndez [40]. He examined the possibility of using the *Macrocystis pyrifera* and *Durvillaea antarctica* biomass based on the blend of these species. His research showed that the yield of biogas production was similar and shaped on the level about 180.4 ± 1.5 $dm^3/kg_{d.m.}$ × d. The use of the algae mixture directly impacted the lower efficiency of biogas production to 158.3 $dm^3/kg_{d.m.}$ × d. The concentration of $CH_4$ ranged from 60.0% to 70.0% [40].

Singh and Gu [41] and Parmar et al. [42] analyzed the biogas production efficiency with phytobenthos biomass used as an organic matter. They achieved the highest efficiency during fermentation of *Laminaria digitata* belonging to the order Laminariales. In that case, methane generation reached 500 $dm^3CH_4/kg_{o.d.m.}$. The use of *Macrocystis* sp. enabled achieving 390–410 $dm^3CH_4/kg_{o.d.m.}$, whereas upon the use of *Gracilaria* sp. (*Rhodophyta*) and *Laminaria sp.* (*Ochrophyta*, *Phaeophyceae*) $CH_4$ production was for 280–400 $dm^3CH_4/kg_{o.d.m.}$ and 260–280 $dm^3CH_4/kg_{o.d.m.}$. The lowest technological efficiency were observed in the digestion of *Ulva* sp., i.e., barely 200 $dm^3CH_4/kg_{d.m.}$ [41] [42]. Investigation by Dębowski et al. [43] proved that the effects of the anaerobic digestion of macroalgae from the Puck Bay were directly dependent on the organic load rate (OLR) used. The highest $CH_4$ production (240 $dm^3CH_4/kg_{o.d.m.}$) was observed at the OLR from 1.0 kg to 2.0 $kg_{o.d.m.}/m^3$ × d. The higher OLR values had a direct negative effect on anaerobic digestion efficiency [43]. Yuan et al. [44] proved that $CH_4$ generation in the digestion process of blue–green algae was 189.89 $dm^3CH_4/kg_{o.d.m.}$. Zeng et al. [45] analyzed the anaerobic digestion of *Macrocystis* sp. with liquid manure. The $CH_4$ production was 153.66 $dm^3CH_4/kg_{o.d.m.}$. Other research examining the possibility of biogas production were carried out with, among others, *Laminaria* sp., *Macrocystis* sp. [46], Gracilariaceae [47], and *Ulva* sp. [48].

In an investigation conducted by Grala et al. [49], the anaerobic digestion was run with the biomass based on *Pilayella* (90% contribution) and *Ectocarpus* (8% contribution) and sporadically occurring *Ulva*. The biomass was directed to enzymatic hydrolysis with a blend of the enzymes: Celluclast 1.5 L, Novozym 188, and Hemicellulase, and to the process of hydrothermal depolymerization run for 120 min at a temperature of 200 °C under the pressure of 17 Ba. Biogas production was 40 and 54.0 $dm^3/kg$ substrate in the most effective variants. The $CH_4$ concentration was about 73.0%.

The first investigations of anaerobic digestion of microalgae based on *Chlorella* sp. and *Scenedesmus* sp. biomass were conducted by Golueke et al. [50]. They compared the efficiency of the anaerobic digestion of algae and wastewater sludge. The efficiency of the fermentation of sewage sludge reached 1020 $dm^3/kg_{o.d.m.}$, whereas for algae biomass it was at 986 $dm^3/kg_{o.d.m.}$ The concentration of methane was from 61.0% to 63.0% [42].

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