Review

Algae Biomass as a Potential Source of Liquid Fuels

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Abstract: 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. The use of wastewater, another liquid waste, or flue gases can reduce the costs of biofuel production while having a measurable environmental effect.

Keywords: 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 gases 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 microalgal 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 Algaladrients 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].

Recently, many research groups work on improvement of microalgae biomass production methods. New technologies are developed such as the use of alternative source of phosphorus in order to control the contamination, for example the use phosphite dehydrogenase (PtXD) that catalyzes the conversion of phosphite in phosphate [14]. Many Chlamydomonas strains have been produced expressing nuclear or chloroplastic PtXD [15,16]. There are many works in literature in which new strategies in tuning photosynthesis are developed. For example, by reducing chlorophyll content of Chlorella vulgaris strains in order to improve the light capture within the inner layers of the mass culture and control the cell-shading [17]. Moreover, strains that are able to resist in light-stress conditions by increasing the amount of carotenoids have also been developed [18,19]. This paper presents the possibility of using algae biomass to produce liquid and gaseous biofuels, including bio-oil, biohydrogen, and biogas.

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 [20,21].

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 [22]. 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 [22].

Photoautotrophic microalgae use light, carbon dioxide, and water to biomass production [23]. This kind of cultivation is usually used for microalgae cultivation in large scale [24]. 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 [20]. On the other hand, the use of the Botryococcus braunii UTEX 572 strain allowed achieving culture yield at 5.5 mg/dm$^3$ × d [24]. 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 [25].

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 effective-
ness 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$_2$ [26]. 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$^3 \times d$. 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) [26]. 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 [27]. An example of such a solution is the Scambiotic 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$^2 \times d$ [28]. In turn, de Morais and Costa (2007) demonstrated that only certain strains, such as *Tetraselmus obliquus*, *Parachlorella kessleri*, and *Arthrospira* sp., were able to grow under conditions of high CO$_2$ concentration approximating 18% [29].

Likewise, bacteria and fungi, selected species of microalgae are capable of heterotrophic development using organic substances [22]. 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 [23]. 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 (Table 1). 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$^3$ [30]. 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 [31]. In the case of *C. vulgaris* ESP-31 strain, the same modification caused an over ten-fold reduction in the biomass concentration [32].

Previous studies have shown that microalgae can assimilate organic carbon from various sources, including acetate, fructose, glucose, lactose, glycerol, sucrose, galactose, and mannose [33]. The possibility of using various organic compounds in the heterotrophic culture was described by De Swaaf (2003), who applied a protocol for acetic acid addition and culture pH control when multiplying the *Crypthecodinium cohnii* strain. This technological solution allowed obtaining very high values of final yield indicators, i.e., the concentration of cell dry matter in the culture at 109 g/dm$^3$ and the final lipid concentration in the culture at 61 g/dm$^3$ [30]. Another research proved that *Auxenochlorella protothecoides* strain grew in a batch reactors on media with technical glycerin as the only carbon source, reaching the final biomass concentration of 23.5 g/dm$^3$ and the bio-oil concentration of 14.6 g/dm$^3$ after 6 days of cultivation. In turn, using the semi-continuous culture strategy permitted growing the oil synthesis rate to 3 g/m$^3 \times d$ [34].

Currently, the possibilities of using cheaper sources of organic carbon to produce algae with a high lipid concentration in the cells, e.g., corn powder hydrolysate instead of glucose, are sought and investigated [31]. Using this organic substrate in a batch culture, Xiong et al. (2008) obtained the average lipid synthesis efficiency at 3.7 g/dm$^3 \times d$ [35].
Other experiments proved that using a relatively expensive raw material, i.e., glucose, and controlling the oxygen transfer coefficient in *Schizochytrium* sp. HX-308 culture increased the production capacity of oily marine fungus, leading to a final biomass concentration of 92.72 g/dm³ and the final DHA concentration of 17.7 g/dm³ [36]. On the other hand, one of the highest reported final concentrations of microalgae cells in a culture (171.5 g/dm³), was obtained by Bailey et al. (2003) during the heterotrophic culture of *Schizochytrium* sp. ATCC20888 [37].

<table>
<thead>
<tr>
<th>Microalgae Species</th>
<th>Culture Type</th>
<th>Biomass Production Yield (g_d.m./dm³ × d)</th>
<th>Lipid Production Yield (mg/dm³ × d)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaetoceros muelleri F&amp;M-M43</td>
<td>Phototrophic</td>
<td>0.07</td>
<td>21.8</td>
<td>[20]</td>
</tr>
<tr>
<td>Mychonastes homosphaera UTEX 2341</td>
<td>Phototrophic</td>
<td>0.02–0.03</td>
<td>9.0–10.2</td>
<td>[39]</td>
</tr>
<tr>
<td>Auxenochlorella protothecoides</td>
<td>Heterotrophic</td>
<td>4.0–4.4</td>
<td>1881.3–1840.0</td>
<td>[40]</td>
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<td>Auxenochlorella protothecoides</td>
<td>Heterotrophic</td>
<td>2.0</td>
<td>932.0</td>
<td>[51]</td>
</tr>
<tr>
<td>Chlorella vulgaris #259</td>
<td>Mixotrophic</td>
<td>0.09–0.25</td>
<td>22.0–54.0</td>
<td>[33]</td>
</tr>
<tr>
<td>Tetradesmus obliquus</td>
<td>Mixotrophic</td>
<td>0.10–0.51</td>
<td>11.6–58.6</td>
<td>[8]</td>
</tr>
<tr>
<td>Scenedesmus quadricauda</td>
<td>Phototrophic</td>
<td>0.19</td>
<td>35.1</td>
<td>[20]</td>
</tr>
<tr>
<td>Phaeodactylum tricornutum F&amp;M-M40</td>
<td>Phototrophic</td>
<td>0.24</td>
<td>44.8</td>
<td>[20]</td>
</tr>
<tr>
<td>Scenedesmus sp. DM</td>
<td>Phototrophic</td>
<td>0.26</td>
<td>53.9</td>
<td>[20]</td>
</tr>
<tr>
<td>Scenedesmus sp. F&amp;M-M19</td>
<td>Phototrophic</td>
<td>0.21</td>
<td>40.8</td>
<td>[20]</td>
</tr>
<tr>
<td>Skeletonema costatum CS 181</td>
<td>Phototrophic</td>
<td>0.08</td>
<td>17.4</td>
<td>[20]</td>
</tr>
<tr>
<td>Tetradesmus suecicus F&amp;M-M33</td>
<td>Phototrophic</td>
<td>0.32</td>
<td>27.0</td>
<td>[20]</td>
</tr>
<tr>
<td>Nannochloropsis sp. F&amp;M-M29</td>
<td>Phototrophic</td>
<td>0.17</td>
<td>37.6</td>
<td>[20]</td>
</tr>
<tr>
<td>Chlorella vulgaris CCAP 211/11B</td>
<td>Phototrophic</td>
<td>0.17</td>
<td>32.6</td>
<td>[20]</td>
</tr>
<tr>
<td>Tetradesmus obliquus</td>
<td>Phototrophic</td>
<td>0.06</td>
<td>7.14</td>
<td>[8]</td>
</tr>
<tr>
<td>Rebecca salina CS 49</td>
<td>Phototrophic</td>
<td>0.16</td>
<td>49.4</td>
<td>[20]</td>
</tr>
<tr>
<td>Thalassiosira pseudonana CS 173</td>
<td>Phototrophic</td>
<td>0.08</td>
<td>17.4</td>
<td>[20]</td>
</tr>
</tbody>
</table>

The highest efficiency of lipid biosynthesis obtained from the heterotrophic culture was several times higher than from the phototrophic culture (Table 1). The disadvantages of the heterotrophic culture include frequent contamination with other microorganisms, which diminishes the efficiency of this technological solution and, in some cases, inhibits the biochemical process [31]. This phenomenon was presented by Zhang et al. (2012), who studied the effect of bacteria on biomass and bio-oil production efficiency in a heterotrophic cultivation of *Auxenochlorella pyrenoidosa* with post-process wastewater from soybean processing used as a culture medium. In contrast, bacteria improved the degradation rates of nitrogen and phosphorus compounds and reduced the chemical oxygen demand of the culture, although they decreased the final concentrations of microalgae biomass and lipids [41]. The heterotrophic infections of microalgae cultures can be prevented by the addition of antibiotics, e.g., chloramphenicol, to the culture medium [42].

In the mixotrophic culture, photosynthesis proceeds in microalgae cells that consume carbon from both organic and inorganic sources [33]. Microalgae assimilate organic compounds, while CO₂ released as a result of respiration is retained and reused as a substrate in the photosynthesis process [9]. Compared to the phototrophic and heterotrophic cultures, the mixotrophic culture is rarely used to produce bio-oil from microalgae (Table 1). This technological solution was used by Bhatnagar et al. (2011), who analyzed the production of *Chlamydomonas globosa*, *Mychonastes homosphaera*, and *Scenedesmus bijugus*. The addition of 1% (w/v) glucose to the cultures of *Chlamydomonas globosa*, *Mychonastes homosphaera*, and *Scenedesmus bijugus* caused a 9.4-, 6.7-, and 5.8-fold increase in the biomass production efficiency in the mixotrophic process compared to phototrophic culture as well as 3.0-, 2.0-, and 4.4-fold increase compared to the heterotrophic cultivation system [43]. Similar research results were obtained by Yu et al. (2009), who proved that the highest rate of *Nostoc flagelliforme* strain biomass growth was obtained in a mixotrophic culture with glucose addition, which proved to be 5.0 and 2.3 times more efficient than the phototrophic and heterotrophic culture, respectively [44].

Although the efficiency of oil production using microalgae largely depends on the strain used, the results presented in Table 1 confirm that the highest final technological
effects, including biomass concentration in the system and lipid content in the cells, can be obtained in the heterotrophic culture. Thus, this culture method is of great interest to enterprise engaged in the implementation of bioenergy systems and investigation groups working on the development of such technologies [37]. The most severe disadvantage of the heterotrophic methods is the possibility of culture impurity with other microorganisms, which causes major problems in the operation of installations exploited on an industrial scale [42]. Moreover, the expense of a pure organic fertilizer makes that this culture type can only be used to produce metabolites with a high market value [45].

Photic autotrophic algal technology are the most frequently used ones. They offer a simple solution for cultivation scale enlargement through the use of open or hybrid bioreactors [46]. These cultures are promising because microalgae can use waste CO₂, e.g., from heat and power plants, breweries, or anaerobic digesters [47]. However, the oil production yield they ensure is usually substantially lower than that achieved under heterotrophic culture conditions (Table 1). This is mainly due to slow cell growth and low biomass production efficiency. However, the lower costs of increasing the scale of this culture still make it very attractive for investors.

A characteristic feature of the photoheterotrophic culture is the use of light to ensure organic carbon fixation and degradation. The main difference between the mixotrophic and photoheterotrophic cultures is that the first uses organic compounds while the latter requires light as an energy source. Therefore, the photoheterotrophic culture requires both sugars and light at the same time [22]. Although the production of specific expensive secondary metabolites can be increased by using the photoheterotrophic culture, this solution is not employed for biodiesel production, as is the case with the mixotrophic microalgae culture [48].

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 [49]. This process runs mainly due to hydrogenase, which catalyzes the oxidation of H₂ and releases gaseous hydrogen by reducing protons [50]. 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, O₂ is produced by PSII, while the electrons generated in this process are used by PSI to reduce CO₂ 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 [51].

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 [52]. 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 [53]. 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% [54]. 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 [55]. 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 [56].

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 [57]. Others argue that a higher density of algae cells directly improves efficiency and prolongs hydrogen production [58]. Ji et al. (2010) achieved hydrogen production at 16 cm³/g biomass with a cell density of 0.5 g/dm³. When the cell density increased to 3.2 g/dm³, they reported hydrogen production over 49 cm³/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 [58].

Most scientific publications addressing this research issue indicate the high efficiency of H₂ production by unicellular algae, like Chlamydomonas reinhardtii commonly found in soil and saline waters [59]. The H₂ production by this species was reported to reach 90–110 cm³/dm³ [56] and, in some cases, even 80–140 cm³/dm³ [60]. Faraloni et al. (2011) achieved a hydrogen production of 150 cm³/dm³ from the Chlamydomonas reinwardtii algae culture, using waste from olive processing in the algae growth process [61]. 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 noitgama, Chlamydomonas vectensis, Auxenochlorella protothecoides, Oocystis, Desmodesmus subspecificus, and Raphidocelis subcapitata. The highest H₂ production efficiency approximating 140 cm³/dm³ was demonstrated for Chlamydomonas reinhardtii, followed by Chlamydomonas noitgama (80 cm³/dm³) and Chlamydomonas euryale (22 cm³/dm³) [60].

The algae of the genus Chlorella sp. represent a taxon with a significant potency for hydrogen production [62]. This species’ preponderance is due to its eurybiontic character, high adaptability to changing environmental conditions, resistance to pollution, and a fast growth rate [63]. Scientific research have confirmed the effective use of Chlorella sp. biomass in the hydrogen production process at a level comparable to other species of algae widely used in this technology [64]. Zhang et al. (2014) investigated hydrogen production by Auxenochlorella protothecoides algae species as affected by nutrient depletion in the culture medium, and achieved production efficiency at 110.8 cm³/dm³ of culture. Reduced concentrations of two components in the culture medium, namely nitrogen and sulfur, caused the hydrogen production efficiency to increase to the value of 140.4 cm³/dm³ of culture [65]. In turn, Chader et al. (2009) compared three species of algae: Chlorella sorokiniana, Chlorella salina, and Chlorella sp., for their hydrogen production capability and tolerance to oxygen. The research showed the highest hydrogen production by C. sorokiniana reaching 147 cm³ within 220 h of the experiment. However, these algae showed a low tolerance to the oxygen content in the medium, up to the level of 2%. The remaining two microorganisms showed by lower hydrogen productivity and tolerance to oxygen concentrations from 11 to 15.4% [66]. Song et al. (2011) achieved hydrogen production by Chlorella sp. from 260 to 480 cm³/dm³. The highest technological effects ranging from 183 to 238 cm³/dm³ · h were achieved at 37–40 °C, with the initial glucose concentration of 30 mM [64]. It has been proven that genetic modifications of Chlorella sp. algae allowed the hydrogen production process to be carried out without the need to ensure variable aerobic-anaerobic conditions and remove sulfur compounds from the culture medium [67,68]. Amutha and Murugesan (2011) investigated hydrogen production by Chlorella vulgaris MSU 01 algae using various carbon sources in algae growth and hydrogen production processes, including corn stalks. The highest algae biomass growth was achieved using corn stalks as the carbon source at a concentration of 4 g·d.m.·dm⁻³. The proliferated biomass was used for the hydrogen production process in a 0.5 dm³ bioreactor. It ensured production yield at ca. 220 cm³/dm³ of culture after 6 days of the experiment. The average hydrogen production rate was 26 cm³/dm³ · d [62].
Other works have presented the results of research on the use of *Tetraselmis subcordiformis* in biohydrogen production under cyclical light and dark conditions and with an external carbon source, like e.g., acetate, glucose, sucrose, or other simple sugars. The average hydrogen production efficiency ranged from 78 to even 158 cm$^3$/dm$^3$ of the culture [69,70]. Ji et al. (2010) obtained the total production of hydrogen from *Tetraselmis subcordiformis* algae of 236.6 cm$^3$ at a cell density of 3.2 g/dm$^3$. The production efficiency was 49.2 cm$^3$/g · h with the maximum production rate of 7.20 cm$^3$/h [58]. In turn, Ji et al. (2011) presented hydrogen production by *Tetraselmis subcordiformis* depending on the depletion of individual nutrients, such as nitrogen, sulfur, and phosphorus, in the culture medium. The research confirmed that the experimental variant with nitrogen compounds deficiency ensured the highest production efficiency, reaching 55.8 cm$^3$/dm$^3$ of culture. Due to the fast growth rate and ease of use of the systems for its biomass multiplication, this species seems an interesting substrate to produce organic substrate and hydrogen [71]. There is an increasing number of studies on the use of this microalgae species as a direct product in technologies aimed at producing energy carriers [69,70].

The efficiency of biohydrogen production by microalgae, reported in various works, is presented in Table 2 [72].

### Table 2. Comparison of literature data on the efficiency of biohydrogen production by microalgae [62].

<table>
<thead>
<tr>
<th>Microalgae Species</th>
<th>Efficiency of Biohydrogen Production</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetraselmis subcordiformis</td>
<td>157.7 cm$^3$/dm$^3$</td>
<td>[58]</td>
</tr>
<tr>
<td>Tetraselmis subcordiformis</td>
<td>50.0 cm$^3$/dm$^3$</td>
<td>[70]</td>
</tr>
<tr>
<td>Tetraselmis subcordiformis</td>
<td>55.8 cm$^3$/dm$^3$</td>
<td>[71]</td>
</tr>
<tr>
<td>Chlamydomonas reinhardtii</td>
<td>210.9 cm$^3$/dm$^3$</td>
<td>[73]</td>
</tr>
<tr>
<td>Chlamydomonas reinhardtii</td>
<td>120.0 cm$^3$/dm$^3$</td>
<td>[60]</td>
</tr>
<tr>
<td>Chlamydomonas reinhardtii</td>
<td>321.0 cm$^3$/dm$^3$</td>
<td>[55]</td>
</tr>
<tr>
<td>Chlamydomonas reinhardtii</td>
<td>180.0 cm$^3$/dm$^3$</td>
<td>[56]</td>
</tr>
<tr>
<td>Chlorella sp.</td>
<td>7.13 cm$^3$/g o.d.m.</td>
<td>[74]</td>
</tr>
<tr>
<td>Chlorella vulgaris MSU 01</td>
<td>220 cm$^3$/dm$^3$</td>
<td>[62]</td>
</tr>
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</table>

Other biological reaction used by algae to hydrogen synthesis is through the indirect biophotolysis. It proceeds in the organisms of cyanobacteria, which via photosynthesis accumulate carbohydrates resulting from CO$_2$ reduction. In turn, these carbohydrates are degraded by fermentation. The indirect biophotolysis proceeds with the involvement of photosystem I. The proteins it contains transfer electrons to ferredoxin using light energy [75]. A significant role is also played by CO$_2$, which is a carrier of electrons and protons formed during water molecule degradation, and by enzymes, including two NiFe hydrogenases and nitrogenase. The latter catalyzes the reaction of atmospheric nitrogen reduction to ammonia, which is accompanied by proton reduction and hydrogen release, according to Equation (1) [76]:

\[
N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i \tag{1}
\]

Nitrogenase can also reduce protons to molecular hydrogen, according to Equation (2):

\[
2H^+ + 2e^- + 4ATP \rightarrow H_2 + 4ADP + 4P_i \tag{2}
\]

Cyanobacteria represent a very promising taxonomic group that can be used to produce hydrogen. Their advantages include susceptibility to genetic modifications, small environmental requirements, and no need for the delivery of specific nutrients to the technological system [77].

The experiments described by Troshina et al. (2002) [78] are examples of research in which *Cyanobacteria* biomass was used to produce hydrogen. In this particular research, the authors used the population of *Gloeocapsa alpicola* Calu 743 grown under limited access to nitrates, which was expected to promote hydrogen production. An intense H$_2$
production was observed, reaching 25 µL/h per mg dry matter, due to the biodegradation of glycogen accumulated in cells during the photoautotrophic growth period. Similar research was also conducted by Aoyama et al. (1997), who used the filamentous strain of cyanobacteria Arthrospira platensis NIES-46, and reported hydrogen production efficiency approximating 2 µmol/mg dry matter. Apart from hydrogen, the products of the process included ethanol and low molecular weight organic acids, mainly acetic acid [79]. In turn, Khetkorn et al. (2010) analyzed the potential of Cylindrospermum siamense TISTR8012 strain of cyanobacteria for hydrogen production. In this experiment, 0.5% fructose was introduced into the technological system as an exogenous carbon source to intensify biochemical conversion, and continuous access of light was ensured at 200 µE/m²·s. These conditions allowed achieving hydrogen production efficiency at 32 µmol/mg Chl α·h [80]. In another of their works, these authors tested the same species of algae, obtaining a production rate of 29.7 µmol/mg Chlα·h [81].

4. Biogas Production

Research on the use of macroalgae in anaerobic digestion were analyzed by Vergara-Fernández [82]. 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 dm³/kg d.m. × d. The use of the algae mixture directly impacted the lower efficiency of biogas production to 158.3 dm³/kg d.m. × d. The concentration of CH₄ ranged from 60.0% to 70.0% [82]. Singh and Gu [83] and Parmar et al. [84] 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 dm³CH₄/kg o.d.m. The use of Macrocystis sp. enabled achieving 390–410 dm³CH₄/kg o.d.m., whereas upon the use of Gracilaria sp. (Rhodophyta) and Laminaria sp. (Ochrophyta, Phaeophyceae) CH₄ production was for 280–400 dm³CH₄/kg o.d.m. and 260–280 dm³CH₄/kg o.d.m. The lowest technological efficiency were observed in the digestion of Ulva sp., i.e., barely 200 dm³CH₄/kg d.m. [83,84]. Investigation by D˛ebowski et al. [85] 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 CH₄ production (240 dm³CH₄/kg o.d.m.) was observed at the OLR from 1.0 kg to 2.0 kg o.d.m./m³ × d. The higher OLR values had a direct negative effect on anaerobic digestion efficiency [85]. Yuan et al. [86] proved that CH₄ generation in the digestion process of blue-green algae was 189.89 dm³CH₄/kg o.d.m. Zeng et al. [87] analyzed the anaerobic digestion of Macrocystis sp. with liquid manure. The CH₄ production was 153.66 dm³CH₄/kg o.d.m. Other research examining the possibility of biogas production were carried out with, among others, Laminaria sp., Macrocystis sp. [88], Gracilariaceae [89], and Ulva sp. [90].

In an investigation conducted by Grala et al. [91], 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 Bar. Biogas production was 40 and 54.0 dm³/kg substrate in the most effective variants. The CH₄ concentration was about 73.0%. The biogas production efficiency with macroalgae used as a substrate in methane fermentation processes is presented in Table 3.
Table 3. Efficiency of biogas production with the use of macroalgae as a substrate in methane fermentation processes.

<table>
<thead>
<tr>
<th>Macroalgae Taxon</th>
<th>Quantity of Biogas/Methane</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Macrocystis pyrifera</em></td>
<td>181.4 ± 52.3 dm³CH₄/kg_ø.d.m. × d</td>
<td>[44]</td>
</tr>
<tr>
<td><em>M. pyrifera</em>+<em>Durvillaea antarctica</em></td>
<td>164.2 ± 54.9 dm³CH₄/kg_ø.d.m. × d</td>
<td>[44]</td>
</tr>
<tr>
<td><em>D. antarctica</em></td>
<td>179.3 ± 80.2 dm³CH₄/kg_ø.d.m. × d</td>
<td>[44]</td>
</tr>
<tr>
<td>Laminaria sp.</td>
<td>260–280 dm³/kg_ø.d.m.</td>
<td>[67,88]</td>
</tr>
<tr>
<td>Gracilaria sp.</td>
<td>280–400 dm³/kg_ø.d.m.</td>
<td>[67,88]</td>
</tr>
<tr>
<td><em>Macroystis</em></td>
<td>390–410 dm³/kg_ø.d.m.</td>
<td>[67,88]</td>
</tr>
<tr>
<td>Laminaria digitata</td>
<td>500 dm³/kg_ø.d.m.</td>
<td>[67,88]</td>
</tr>
<tr>
<td>Ulva sp.</td>
<td>200 dm³/kg_ø.d.m.</td>
<td>[67,88]</td>
</tr>
<tr>
<td><em>Macroystis</em> sp.</td>
<td>189.9 dm³CH₄/kg_ø.d.m.</td>
<td>[45]</td>
</tr>
<tr>
<td>Ulva lactuca</td>
<td>157–271 dm³CH₄/kg_ø.d.m.</td>
<td>[33]</td>
</tr>
<tr>
<td>Pilayella+Ectocarpus+Ulva</td>
<td>40.0–54.0 dm³/kg 29.2–39.4 dm³CH₄/kg</td>
<td>[40]</td>
</tr>
</tbody>
</table>

The first investigations of anaerobic digestion of microalgae based on *Chlorella* sp. and *Scenedesmus* sp. biomass were conducted by Golueke et al. [92]. They compared the efficiency of the anaerobic digestion of algae and wastewater sludge. The efficiency of the fermentation of sewage sludge reached 1020 dm³/kg_ø.d.m., whereas for algae biomass it was at 986 dm³/kg_ø.d.m. The concentration of methane was from 61.0% to 63.0% [84].

Zamalloa et al. [93] investigated the possibility of fermenting *Tetraselmis obliquus*, *Phaeodactylum tricornutum*, and *Arthospira platensis*. After 30 days of incubation, they achieved 210 ± 3.0 dm³CH₄/kg_ø.d.m. in the case of *T. obliquus* and 350 ± 3.0 dm³CH₄/kg_ø.d.m. in the variant with *P. tricornutum* biomass. By comparison, in the fermentation process of *S. platensis*, the methane production yield reached 280 ± 0.8 dm³CH₄/kg_ø.d.m. In turn, Mussgnug et al. [94] tested six species of phytoplankton, i.e.: *Chlamydomonas reinhardtii*, *Dunaliella salina*, and *T. obliquus*, *Parachlorella kessleri*, *Euglena gracilis* and blue-green algae *Arthospira platensis*. The anaerobic digestion of *C. reinhardtii* resulted at 587 ± 8.8 dm³/kg_ø.d.m., *D. salina* at 505 ± 24.8 dm³/kg_ø.d.m. of biogas. The biogas production in anaerobic degradation used to *A. platensis* and *E. gracilis* resulted at 481 ± 13.8 dm³/kg_ø.d.m. and 485 ± 3.0 dm³/kg_ø.d.m., respectively. Biogas production from the biomass of *P. kessleri* and *T. obliquus* algae was 335 ± 7.8 dm³/kg_ø.d.m. and 287 ± 10.1 dm³/kg_ø.d.m., respectively [94]. Authors state that anaerobic digestion efficiency was not dependent on the taxonomic group of algae. The main determination of the biogas amount and CH₄ concentration was feasible upon individual verification of experiments for each of the analyzed species.

Literature present a correlation between the structure of cells of the microalgae biomass and susceptibility to degradation in anaerobic reactors and efficiency of biogas production. The high biogas production was observed when algae with no cell wall, as in the case of *D. salina* [95], or their cell wall did not contain cellulose and hemicellulose components and was made of protein substances, as in the case of *C. reinhardtii* [96], *A. platensis* [97], and *E. gracilis* [98]. Contrary to the aforementioned species, *P. kessleri* and *T. obliquus* have cell walls built of hemicellulose [99,100]. The cell wall of *T. obliquus* is described in the literature as especially difficult to break owing to the presence of a sporopollenin biopolymer [101]. Even more complex is the silica structure of the cell wall of Bacillariophyceae [102].

5. Conclusions

Although very prospective, biofuels’ production from algae biomass is characterized by many limitations that must be verified in installations operating in a technical scale. Unfortunately, most of the research works had been carried out under laboratory conditions, rarely on a fractional-technical scale. This significantly curbs the possibility of obtaining reliable data for a comprehensive evaluation of the technological, environmental, and economic efficiency of these technological solutions. Their analysis is also made difficult because authors present contradictory opinions on the efficiency of microalgae biomass production and the actual yields of such solutions.
Among all the directions of using algae biomass for biofuel purposes, the conversion of algae biomass to biogas is indicated as a highly profitable and economically justified technological solution. Next to biogas, it results in the production of post-digestion sludge, which can be used as a fertilizer for arable crops or, after processing, returned to the algae cultivation photobioreactors as a medium component. Many researchers claim that using the methane fermentation process carried out under appropriate conditions as the most important method for algae biomass conversion determines a higher economic effect compared to the integrated system of lipid extraction and anaerobic processing of post-extraction residues. Other results suggest that the balance of unit operations carried out in the methane fermentation process is the most effective both in terms of economic analysis and emission of pollutants to elements of the natural environment. The research results prove that methane fermentation may be the most practical way of converting algae biomass into energy. At the same time, it was found that energy inputs and environmental effects were highly diverse, depending on the technological solution used in the methane fermentation process. Therefore, for a complete and objective assessment, it is necessary to carry out an environmental life cycle assessment (LCA) in each case.

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**References**


