





# Using Algae for Biofuel Production: A Review

Agata Jabłońska-Trypuć<sup>1,\*</sup>, Elżbieta Wołejko<sup>1</sup>, Mahmudova Dildora Ernazarovna<sup>2</sup>, Aleksandra Głowacka<sup>3</sup>, Gabriela Sokołowska<sup>1</sup> and Urszula Wydro<sup>1</sup>

<sup>1</sup> Department of Chemistry, Biology and Biotechnology, Faculty of Civil Engineering and Environmental Sciences, Białystok University of Technology, Wiejska 45E Street, 15-351 Białystok, Poland

<sup>2</sup> Department of Design, Construction and Operation of Engineering Communications, Tashkent Institute of Architecture and Civil Engineering, Navoi Street, 13, Tashkent 100011, Uzbekistan

<sup>3</sup> Department of Plant Cultivation Technology and Commodity Science, University of Life Sciences in Lublin, 15 Akademicka Street, 20-950 Lublin, Poland

\* Correspondence: a.jablonska@pb.edu.pl

**Abstract:** One of the greatest challenges of the 21st century is to obtain an ecological source of transport fuels. The production of biofuels based on feedstock obtained through the exploitation of arable land translates into an increase in food prices and progressive degradation of the environment. Unlike traditional agricultural raw materials, algae are a neutral alternative in many respects. They can even be obtained as waste from polluted water reservoirs. One of the manifestations of the deterioration of surface waters is the eutrophication of water reservoirs, which leads to an increase in the number of algae. Algae reaching the shores of water reservoirs can be used as a raw material for the production of biofuels, including biogas, bioethanol and biodiesel. However, it should be remembered that water blooms are a periodic phenomenon, appearing in the summer months. Therefore, in order to ensure the continuity of obtaining energy from biomass, it is necessary to conduct algae cultivation in artificial open tanks or photobioreactors. Accordingly, this review first briefly discusses the properties and possible applications of different species of algae in various industrial areas, and then describes the process of eutrophication and the presence of algae in eutrophicated reservoirs. Technologies of algal cultivation in various systems and technologies of algal biomass pretreatment were critically discussed. Various methods of obtaining biomass from algae were also reviewed, and the process conditions were summarized. Biofuels of various generations and raw materials from which they are obtained are presented in order to determine the possible future directions of development in this field. Parameters affecting the selection of algae species for the production of biofuels were also examined and presented. Overall, algal biofuels still face many challenges in replacing traditional fossil fuels. Future work should focus on maximizing the yield and quality of algae-derived biofuels while increasing their economic viability.

**Keywords:** algae; eutrophication; biofuel; bioethanol; biodiesel; biogas



**Citation:** Jabłońska-Trypuć, A.; Wołejko, E.; Ernazarovna, M.D.; Głowacka, A.; Sokołowska, G.; Wydro, U. Using Algae for Biofuel Production: A Review. *Energies* **2023**, *16*, 1758. <https://doi.org/10.3390/en16041758>

Academic Editor: Jlung Kim

Received: 18 January 2023

Revised: 3 February 2023

Accepted: 7 February 2023

Published: 10 February 2023



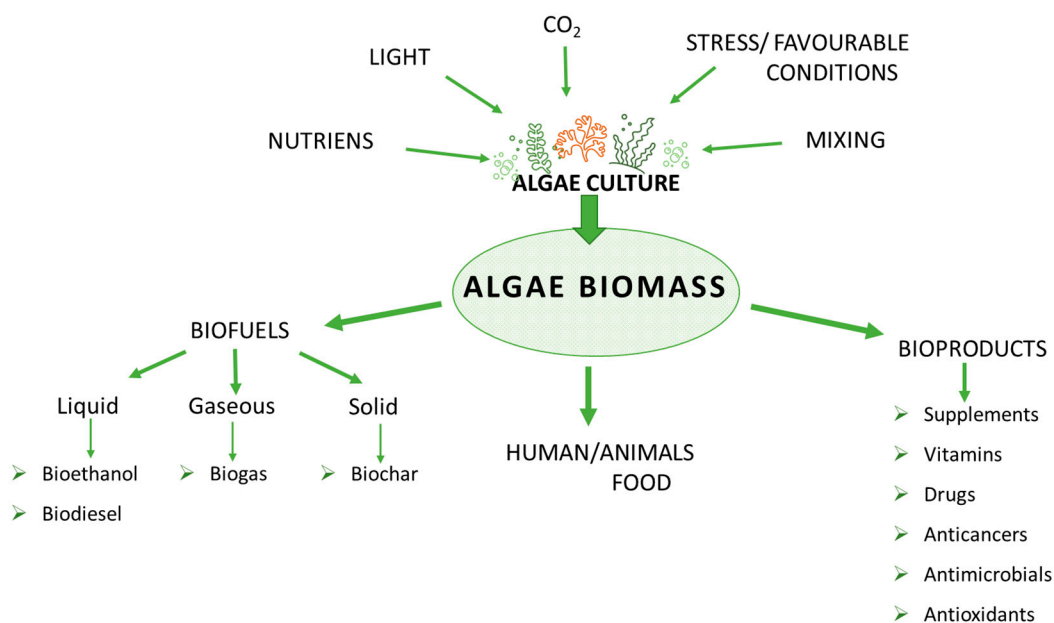
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## 1. Introduction

Algae are a group of unicellular and multicellular organisms defined using morphological and ecological criteria and belong to the key producers in aquatic environments [1,2]. These microscopic or huge thallus-forming organisms are also playing an increasingly important role in human nutrition [3]. Photosynthesis carried out by algae provides about half of the oxygen that humans breathe, and the analysis of their genomes allowed us to trace the history of endosymbiosis and horizontal gene transfer (HGT) processes [4,5]. Algae are characterized by the lack of distinct organs and structures that land plants possess, such as leaves, roots, waxy epidermis and other organs. All algae contain green chlorophyll; however, this can be masked by photosynthetic pigments (blue, red, brown and gold), which give them a distinctive color that is important in their identification. Some algae are microscopic and can float in surface waters (phytoplankton) due to their lipid content,

while others are macroscopic and attach to rocks or other structures (seaweed), reaching more than 50 m in length [6,7]. Some species of green algae of the genera: *Scenedesmus*, *Chlorella*, *Spirogyra*, *Dunaliella*, and *Botryococcus* accumulate lipids (mainly triacylglycerols) in the amount of over 70% of dry matter in laboratory conditions [8].

Algae cover a large part of the world's water bottom. They are a nutritious food source, and they are used in folk medicine cosmetology and as a food source (Figure 1). The cultivation of seaweed is increasing on a global scale, and the production of algae increases by several percent each year due to its diverse potential. Cultivation is a source of profitable business for the economy. In addition, algae have a valuable health and environmental aspect, and they grow very quickly, which allows for harvesting satisfactory crops. Algae are promoted as an important feedstock for the production of biofuels and bio-based products. However, it should be borne in mind that their large-scale production consumes large amounts of water and nutrients. Considering the importance of food security and the high economic value of algal biomass, the use of this biomass in food and feed production should be considered the most important aspect of algae grown using traditional methods and water resources. However, it turns out that algae can also be grown in nutrient-rich wastewater by simultaneously running two technological processes: biomass production and wastewater treatment. For health reasons, it is not allowed to use biomass from algae grown in wastewater in food and feed, but it can be used as a raw material for the production of biofuels [9,10].



**Figure 1.** Physical and chemical conditions influencing algal biomass production and possibilities of the use of an obtained algal biomass in different industry branches.

Literature data indicate that biofuels obtained from microalgae are superior in quality as compared to land-based fuels due to several advantages. However, the production costs of such biofuels may be a problem. Therefore, there is an urgent need to develop methods for the inexpensive production of biomass from algae in large quantities in order to make this endeavor economically viable [11]. Algae, like plants grown for energy purposes, accumulate large amounts of reserve substances in their cells, which can be converted into energy in various ways. The high content of reserve substances (starch and lipids) and building materials (protein) in the cells of some species of algae allows for the use of their biomass for energy purposes (production of alcohol, biodiesel and methane) [12]. Moreover, it should be noted that the need for energy consumption is increasing in modern societies. It is essential for the functioning of the global economy in many aspects, from production to the supply of energy needed for lighting, heating

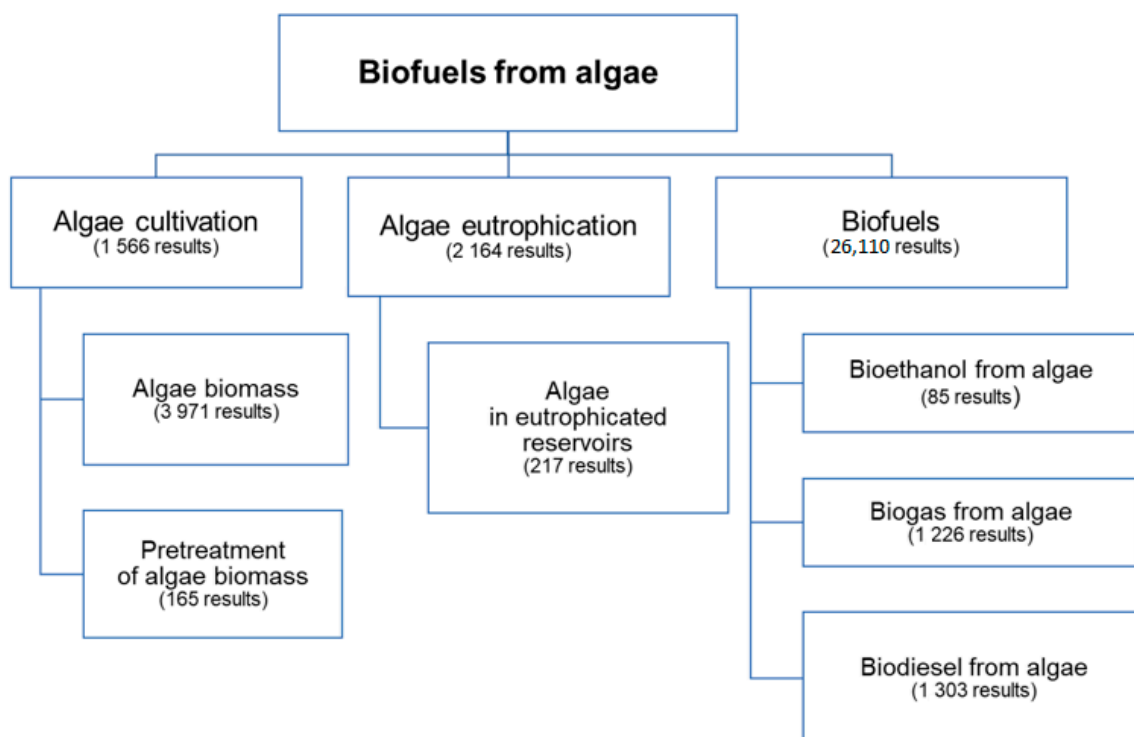
and transport. Fuel consumption is increasing, as is the concentration of CO<sub>2</sub> in the atmosphere, leading to significant climate change mediated by greenhouse gases. Oil is a finite resource that will eventually run out or become too expensive to recover. All these factors contribute to the development of renewable energy sources that can replace fossil fuels and enable the reduction of carbon dioxide emissions into the atmosphere [13]. In the current geopolitical situation, especially in light of the Russian-Ukrainian conflict, the acquisition of new sources of renewable energy is becoming an extremely important issue. The conversion of biomass resources such as algae biomass into value-added bio-products and biofuels using advanced technologies is highly beneficial given the current challenges in the world, especially the Ukrainian-Russian war and its adverse effects on the fuel and energy supply chain [14]. That is why it is so important to explore and expand knowledge on issues related to the possibilities of switching to biomass-oriented products, such as algae-based products, taking into account recent world events.

In this context, biofuels from algae can be a viable alternative to fossil fuels, but in order to be able to compete in the fuel market and be widely used, this technology must overcome a number of obstacles. The problems faced by producers of biofuels from algae are primarily the correct and effective identification of strains and the improvement of productivity, crop protection, allocation and use of nutrients and resources, and the management of by-products and waste. Potential biofuels from algae are very popular, but the whole area still requires much work and overcoming many challenges.

This paper is a literature review, taking into account various aspects and properties of algae as a new ecological feedstock for the production of biofuels. This comprehensive review of the literature is based primarily on the properties of algae and the possibilities of their efficient cultivation as a valuable raw material, taking into account their availability, different cultivation systems (open and closed), extraction of intracellular components (lipids, carbohydrates, etc.), factors affecting production efficiency and the challenges and prospects for the future of biofuel production from an algae source. A brief outline of four-generation biofuels will be discussed, including their advantages and disadvantages. Various algae cultivation systems will be presented, such as open and closed systems (bioreactors), eutrophication and algae harvesting processes from water reservoirs, and various algae pretreatment methods, such as physical, chemical and mechanical methods of cell destruction to facilitate the extraction of intracellular components. In addition, this review will discuss factors affecting production efficiency, challenges and future prospects for biofuels. Although the topic of using algae as a source of raw material for the production of biofuels is widely known, at present, in the era of the energy crisis caused mainly by the Russian-Ukrainian conflict, the priority should be to focus on the search and analysis and improvement of alternative energy sources other than fossil fuels. Therefore, in this article, we try to identify the major challenges facing economical large-scale algae biofuels and increase the focus of the scientific community on these problems to be able to meet these challenges and move the potential of algal biofuels from idea to implementation.

## 2. Methodology

This study aimed at understanding the prospect and challenges faced in the fuel industry trying to use algae for the production of biofuels. The aim of the work is also to provide modern state-of-the-art information on algae as a valuable feedstock for the production of biofuels. For this purpose, a literature review was conducted, focusing on new achievements in the field of algae cultivation, obtaining their biomass, their properties and the possibility of using them as a feedstock for the production of biofuels and four-generation biofuels themselves. More than 160 peer-reviewed research articles, conference proceedings, short communications, and patents published from 1981 to 2022 were analyzed, focusing mainly on articles from the last 10 years, mainly concerning algae cultivation, accumulation of their biomass in eutrophication processes, their chemical and biological properties, types of biofuels and possibilities of using algae as a source of feedstock for biofuel production. Verification of studies results is shown in Figure 2.



**Figure 2.** A diagram for the identification of peer-reviewed papers included in PubMed taking into account different keywords.

### 3. The Problem of Eutrophication in the Aquatic Environment

Surface water pollution, resulting in progressive eutrophication, has been an increased observed phenomenon in recent decades. In turn, progressive eutrophication leads to a drastic increase in biomass production in such water reservoirs. Such biomass, which largely consists of various species of algae, is treated as waste that should be collected and stored, and it can be used for the production of ecological biofuels. It should not be forgotten that eutrophication is a seasonal phenomenon and depends on many factors; therefore, in order to effectively produce biomass, it would be necessary to cultivate algae in tanks specially adapted for this purpose.

In recent years, as a result of the progressive warming of the climate, the depletion of oil resources and the progressive energy crisis, there has been a growing interest in the idea of “sustainable development”, which perfectly fits the possibility of using algae as a source of renewable energy. Algae have proved to be an excellent source of biomass due to the fact that they produce very large amounts of it per unit area compared to terrestrial plants. They are characterized by a very fast pace of growth and development, and they can be grown in a wide variety of conditions, including fresh and saltwater [15,16]. Eutrophication is the overgrowth of plants and algae caused by the increased availability of one or more of the limiting growth factors needed for photosynthesis, such as sunlight, carbon dioxide and nutrients [17]. This process can occur naturally over a very long period of time as lakes age and fill with sediment [18]. However, anthropogenic influences seem to be the key to eutrophication. Human activity has significantly accelerated the rate and extent of eutrophication through both discharges of nitrogen- and phosphorus-rich substances and mixtures into aquatic ecosystems (i.e., cultural eutrophication). This has dramatic consequences for drinking water sources, fishing and recreational water bodies [19]. There are situations where water bodies are deliberately eutrophicated by adding fertilizers to increase primary productivity and to increase the density and biomass of recreationally and economically important fish [20]. However, the vast majority of algal blooms have been linked to nutrient enrichment from anthropogenic activities such as agriculture, industry and wastewater disposal. Known consequences of cultural eutrophication include cyanobacterial blooms,

polluted drinking water sources, degradation of recreational opportunities, and hypoxia. The estimated cost of eutrophication damage in the United States alone is approximately \$2.2 billion annually [21].

The most important, and at the same time, the most visible and burdensome, effect of anthropogenic eutrophication is the formation of dense blooms of often toxic phytoplankton, which reduce the transparency of water and significantly reduce its quality. Algal blooms resulting in the appearance of large amounts of algae in water bodies reduce light penetration, consequently limiting the growth of plants and causing them to die in coastal zones and eliminating aquatic predators that need light to catch prey [22]. An important metabolic-physiological consequence of eutrophication is the extreme increase in the rate of photosynthesis, which in turn can lower the amount of dissolved inorganic carbon and raise the pH to very high levels during the day. Increased pH affects the weakening of the chemosensory abilities of organisms whose survival depends on recognizing chemical substances dissolved in water [23]. The next stage after the death of the mass of algae is microbial decomposition. These processes consume oxygen dissolved in the water and result in “dead zones”, where living organisms are unable to exist. Such areas can be found in many freshwater lakes, including the Great Laurentian Lakes, such as the central basin of Lake Erie, during the summer [24]. Hypoxia is especially common in coastal marine environments surrounding large, nutrient-rich rivers (e.g., Mississippi River and Gulf of Mexico; Susquehanna River and Chesapeake Bay) and has been shown to affect over 245,000 square kilometers in over 400 coastal systems [25]. Hypoxia resulting from eutrophication threatens commercial and recreational fisheries around the world.

#### 4. Algae in Eutrophicated Reservoirs

An observed increase in water pollution changes the physicochemical conditions of the water environment, and it causes disturbances in the biological balance. The progressing eutrophication of water reservoirs causes an increase in the number of algae with a simultaneous decrease in species diversity. This is a very unfavorable and even dangerous phenomenon. At the same time, algae are a good feedstock for the production of biomass, which can be used as an alternative source of energy. Algae, whose growth is caused by excessive eutrophication, are a source of beach pollution and should be recycled, for example, used in the production of biofuels. However, algae washed up by the sea and collected on beaches are raw materials whose availability is not regular. This is because their growth is due to eutrophication and occurs only in the summer months. In order to compensate for the irregular supply of feedstock, additional algae cultivation should be carried out for a specific energy purpose [26]. It is now a fact that climate changes and anthropogenic-derived excess of nutrients are contributing to global eutrophication and increasing the frequency of extensive and dangerous algal blooms around the world. This phenomenon has a name—harmful algal blooms (HAB). It is occurring more and more often, in different places, both in fresh and marine waters, lasts longer and has a number of effects, including significant toxicity [27,28].

#### 5. Algae Cultivation

Depending on the species and growth conditions, a wide range of different macromolecules, such as lipids, carbohydrates and proteins, can be identified in algae. Lipids that build cell membranes of algal cells are molecules with long carbon chains in their structure. An increased lipid content reduces the specific gravity, allowing algae cells to float on the surface of water bodies. A floating algae cell moves towards a source of solar energy. Algae species vary in lipid content from 20 to 80% (dry weight dw) [29–31]. Lipids extracted from microalgae can be used to produce biodiesel. Lipid extraction residues contain mainly carbohydrates and proteins. In contrast, carbohydrates such as starch and other sugars can be fermented to produce ethanol. Ethanol production, as a key step in the transesterification process, can facilitate the oil extraction process for biodiesel production. Ethanol and sodium ethoxide serve as catalysts in the oil transesterification process [32,33].



Various species of microalgae have the ability to synthesize and accumulate a significant part of their dry matter in the form of lipids, as shown in Table 1. Often, as a result of environmental stress, e.g., limiting the amount of nitrogen, phosphorus, silicon or high salinity, the amount of lipids in algae increases. The speed and intensity of lipid synthesis in cells also depend on their growth phase [34–36].

**Table 1.** Lipid content of selected algae species presented as dry weight percentage [37–45].

Algae Species	Lipids (% dw)
<i>Scenedesmus obliquus</i>	11–22/35–55
<i>Scenedesmus dimorphus</i>	6–7/16–40
<i>Botryococcus brauni</i>	25–75
<i>Chlorella</i> sp.	28–32
<i>Chlorella vulgaris</i>	14–40/56
<i>Chlorella protothecoides</i>	23/55
<i>Chlorella emersonii</i>	63
<i>Chlorella minutissima</i>	57
<i>Chlorella sorokiana</i>	22
<i>Spirulina maxima</i>	4–9
<i>Neochloris oleoabundans</i>	35–65
<i>Dunaliella bioculata</i>	8
<i>Dunaliella primolecta</i>	23
<i>Dunaliella salina</i>	14–20
<i>Cryptocodinium cohnii</i>	20
<i>Cylindrotheca</i> sp.	16–37
<i>Isochrysis</i> sp.	25–33
<i>Tetraselmis sueica</i>	15–23
<i>Phaeodactylum tricorutum</i>	20–30
<i>Neochloris oleoabundans</i>	35–54
<i>Nitzschia</i> sp.	45–47
<i>Schizochytrium</i> sp.	50–77

When it comes to carbohydrates, the main group of compounds is polysaccharides, which perform reserve and structural functions in algae cells. Starch and glycogen are stored in plastids as storage components, while cellulose is the main structural component of the cell wall, as are sulfated polysaccharides [46]. The type of stored carbohydrates depends on the species of alga; e.g., cyanobacteria synthesize glycogen, which is a water-soluble  $\alpha$ -polyglucan. In contrast, green microalgae synthesize starch particles with a size of 2 to 100  $\mu\text{m}$ , which consist of 72–82% amylopectin ( $\alpha$ -1,4-glucosidic and  $\alpha$ -1,6-glucosidic bonds) and 18–28% amylose ( $\alpha$ -1,4-glucosidic bonds) [47,48]. Cellulose present in the cell wall of microalgae is also used for the production of bioethanol, which is characterized by a slightly different structure of the cell wall than the lignocellulose of land plants. The lack of lignin in algae means that pretreatment to release biodegradable organic matter does not have to be as intensive as in the case of traditional plant materials [49].

Algae are cultivated in a variety of aquatic systems, from open-air ponds to closed photobioreactors with tightly controlled environments. The range of temperatures needed to support algae growth is specific to the species and strain being cultivated. The optimal temperature for phytoplankton is in the range of 20–30 °C. Temperatures lower than 16 °C slow growth, and temperatures higher than 35 °C are usually fatal to many species [50–53].

Algae can be cultivated in raceway ponds, which can be open ponds and covered ponds, and in photobioreactors. Open ponds are the most economical places to grow algae that consist of natural open ponds that can be both freshwater and saltwater ponds. These ponds can have an area of up to several hectares. The main disadvantages of such open systems are invasions of algae species other than the cultivated one, growth of fungi and contamination of selected species of microalgae [54]. About 98% of the total algae biomass production is obtained using open pond systems, and because the growth rate of microalgae is high, they are able to produce about 15–20 tons of dry biomass per hectare per year. The oil content in the dry matter of algae can reach up to 50–60% for high-yielding varieties. This makes algae cultivation very economical [55]. Literature data describe different types of cultivation of microalgae species in open ponds. For example, *Chlorella pyrenoidosa* grown using secondary wastewater in an open pond removed excess nutrients and produced the highest biomass concentration of 1.71 g/L [56]. Similarly, *Dunaliella salina* and *Nannochloropsis* sp. in open ponds obtained biomass productivity of 0.096 gL/d and 0.208 gL/d, respectively [57]. Therefore, the open pond system can be used to obtain a large amount of biomass for the production of biofuels. The problems that accompany open pond cultures are solved to some extent by the use of covered ponds. Here, the uncontrolled growth of undesirable species of algae and fungi is inhibited to some extent. Another problem, which is evaporation from open ponds, is also eliminated by covering the surface of the pond [58]. The disadvantages of covered ponds should also be mentioned. One of them is certainly an increase in temperature, which can be partly offset by mixing. Various modifications are often used to achieve the greatest possible efficiency in algae cultivation. According to Thomas et al., a microalgae culture was kept in constant motion on an inclined surface by gravity [59]. The microalgae cultures are collected in a tank under the roof and then pumped back to the roof. High evaporation rates on sunny days and volume fluctuations on rainy days are adequately maintained in the buffer tank. Species such as *Scenedesmus* sp., *Dunaliella salina* and *Nannochloropsis* sp. are grown in outdoor systems [59].

Another type of microalgae culture is photobioreactors, which are artificial culture system that promotes the growth of a selected strain under optimal conditions, such as light, temperature, pH, etc. Photobioreactors are constructed in the form of various structures, including tubular, flat or vertical columns, where algae cultures are pumped and recirculated continuously. Photobioreactors are made of transparent acrylic or glass materials, which allows photosynthesis and algae growth, thanks to access to natural sunlight. They can achieve an efficiency of up to 100 gms/m<sup>2</sup>/h because of the use of light-emitting diodes [54]. The undoubted advantages of these bioreactors as a method of algae cultivation include the possibility of avoiding contamination with foreign species of algae, bacteria and fungi; minimizing evaporation; better dissipation of heat and nutrients; the possibility of continuous monitoring of various parameters; no requirement for natural sunlight. Among the types of bioreactors most commonly used for algae cultivation, the following can be mentioned: tubular photobioreactors, plate photobioreactors, helical photobioreactors, horizontal photobioreactors, foil photobioreactors, tubular bioreactors, flat plate bioreactors and fermenter type bioreactors [60–63].

When considering the cultivation of algae, it is important to remember to maintain basic physical conditions such as adequate lighting, temperature, the composition of the medium and the design of the bioreactor enabling continuous mixing, which determines the free flow of gases and components of the medium (Table 2). Considering the importance of the composition of the algae culture medium, the potential of using liquid anaerobic digestate as a culture medium for microalgae growth was investigated and compared to three different synthetic media in terms of biomass yield, fatty acid composition and nutrient utilization/recovery. The highest nutrient efficiency rates, the highest lipid content (34%) and the highest concentrations of C16:0 (114 mg/L) and C18:0 (60.9 mg/L) were obtained when the mixed culture of microalgae was carried out in anaerobic liquid digestate. An increase in the content of polyunsaturated fatty acids (PUFA) was also observed, which

is a positive and desirable effect of changing the culture medium because the PUFA present in microalgae allows them to adapt more effectively to extreme conditions [64]. Another medium for the growth of algae, already mentioned earlier, can be wastewater. Growing algae using wastewater and even flue gas is taking advantage of their ability and efficiency in converting CO<sub>2</sub> and nutrients into biomass. The addition of CO<sub>2</sub> from the flue gas increases the production of algae biomass, which also extracts nitrogen and phosphorus from the wastewater. Due to the presence of impurities, such biomass cannot be used for the production of food or feed, but it can be used for the production of bioenergy in the form of various biofuels [65,66].

**Table 2.** Different types of algae cultures' cultivation conditions depend on selected factors.

Factor	Cultivation Conditions	Algae Species	References
Culture medium	BG-11 and Chu 13 media with CO <sub>2</sub> supplementation	<i>Botryococcus braunii</i> , <i>Scenedesmus obtusiusculus</i>	[67,68]
	synthetic media with organic carbon sources	<i>Pediastrum</i> sp., <i>Micractinium</i> sp., <i>Ankistrodesmus falcatus</i> , <i>Monoraphidium</i> sp., <i>Desmodesmus</i> sp., <i>Coleastrum</i> sp. <i>Mucidosphaerium</i> sp.	[66,69]
	nitrogen-rich media or nitrogen limiting media	<i>Chlorella vulgaris</i> ESP-31	[70]
Lighting	50–200 µmol·m/s	<i>Chlorella vulgaris</i>	[71,72]
	red light	<i>Arthrospira (Spirulina) platensis</i>	[73]
	High- and low-intensity green LEDs	<i>Brachiomonas submarina</i> , <i>Scenedesmus obliquus</i>	[74–76]
	High-intensity blue and white LED	<i>Rhodella</i> sp., <i>Stauroneis</i> sp. <i>Phaeothamnion</i> sp.	[74,76]
Temperature	20 °C	<i>Enteromorpha</i> sp.	[77]
	25 ± 1 °C	<i>Botryococcus strain SK</i>	[78]
	25 °C	<i>B. braunii</i> 765	[79]
	10–30 °C	<i>Scenedesmus</i> sp.	[80]

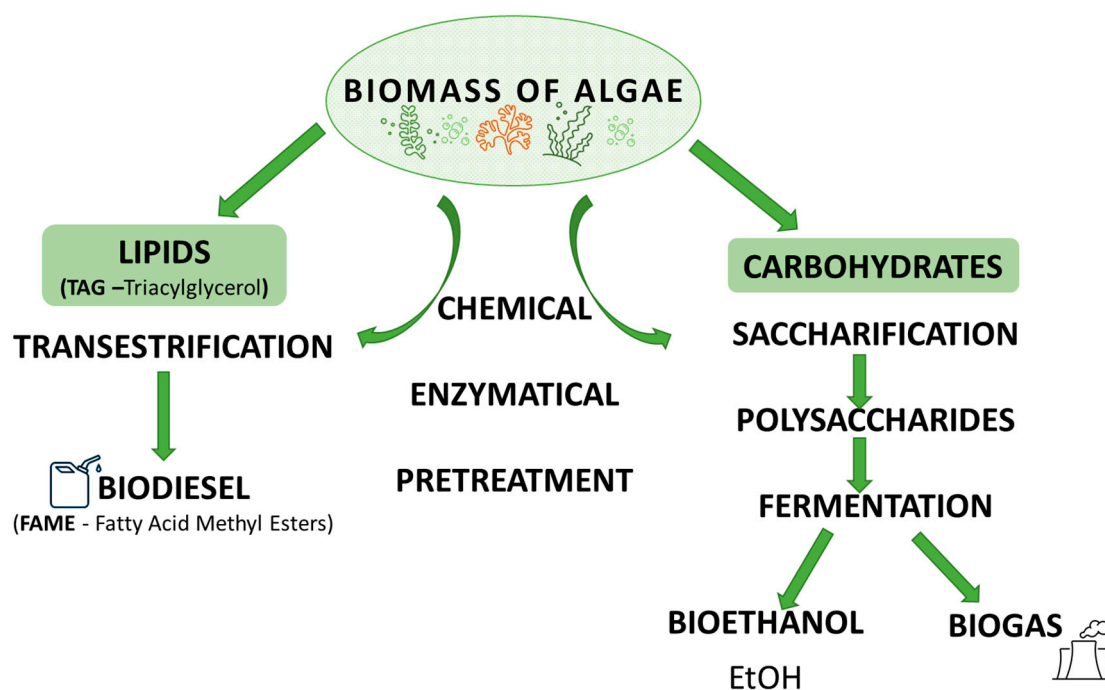
The temperature range in which microalgae can be grown is from 10 to 40 °C. Above this temperature range, cells die, and below this, they do not follow the correct growth curve. Lighting is the most important parameter for the development and growth of algae in bioreactors. Algae can carry out photosynthesis in the range of 400–700 nm [81]. It should also be remembered that excessive sunlight intensity causes photo-oxidation of microalgae, which reduces their productivity. The cell density in the culture is directly proportional to the growth rate of the algae. However, low stocking densities are preferred in open pond systems. On the other hand, in a flat plate or tubular bioreactors, higher culture density is quite well tolerated due to better mass transfer and light distribution. Another key factor affecting algae biomass production is the availability of CO<sub>2</sub> and nutrients. Too much oxygen inhibits the growth of algae, which is why bioreactors carry out continuous biomonitoring of the level of gases and nutrients contained in the culture broth [82]. Continuous mixing of the cultures should also be applied to ensure uniform growth and even distribution of nutrients in the culture broth. This is, of course, impossible for open ponds, but for different types of bioreactors, it is a key factor in the efficiency of algae cultivation. Mixing has a significant effect on light, CO<sub>2</sub> and nutrient availability. Photobioreactors have major advantages over open pond systems in terms of preventing water loss, preventing foreign strain invasion, proximity and nutrients, light and CO<sub>2</sub> [58].



## 6. Pretreatment of Algae Biomass

The algal biomass pretreatment step involves the degradation/breaking of the biomass to recover and process the carbohydrates and lipids contained therein. On an industrial scale, this step is the most problematic and costly step in the production of biofuels. Literature data provide many different methods of pretreatment of algal biomass, but still, no single, optimal, highly productive and effective method has been presented.

The methods of mechanical degradation of algae cells include ultrasounds, microwaves and churning. These methods are sometimes combined with chemical methods to improve the pretreatment process (Figure 3). They do not pollute the environment because they do not generate large amounts of chemical waste, but they are associated with higher costs due to energy consumption [83,84]. Depending on the purpose of the pretreatment, chemical, biological, thermochemical or thermophysical methods are used [85]. Enzymatic pretreatment of algal biomass is more efficient and beneficial for fermentation processes; however, the high cost of enzymes makes this method extremely expensive for biomass pretreatment on an industrial scale [86]. The right solution is to identify a microorganism capable of overexpressing the genes encoding these enzymes. Mechanical pretreatment methods are used in the production of biodiesel, while enzymatic and chemical methods (including acid hydrolysis) are used in the production of bioethanol, as the fermentative production of bioethanol requires the degradation of polysaccharides (Table 3).



**Figure 3.** Algae biomass pretreatment methods were applied in order to obtain carbohydrates and lipids present in algae cells. Groups of macromolecules undergo further chemical processes to obtain the selected type of biofuel.

The pretreatment of algal biomass is a key process in the production of biofuels necessary for further processing. However, this process is highly dependent on the structure and chemical composition of the algal cells. This can be an energy-intensive production step, significantly increasing the overall cost. Processes that have proven reliability on a laboratory scale, such as sonication and lyophilization, turn out to be too expensive or, in principle, problematic or even impossible to use on an industrial scale. Many of the pretreatments listed in Table 3, such as ultrasound pretreatment, have a positive energy balance. In contrast, classical processes such as high-pressure homogenization and acid hydrolysis are economically competitive, but improvement would still be desirable.

**Table 3.** Different types of algae biomass pretreatment technologies.

Type of Pretreatment	Technology	Comments	References
Mechanical Pretreatment (reduce cell wall particle size, prevent the cells from being contaminated, increase the cell surface area, produce more disruption efficiency)	high-pressure homogenisation	recover lipids during cell rupture	[87,88]
	high-speed homogenisation,	simple but aggressive cell disruption technique, achieves effective results, short operating time, generate lipids and other compounds	[89,90]
	bead milling	good disruption efficiency, easy operating procedures, easily available equipment	[91]
Physical Pretreatment (cost effectiveness, ease of commercialization, and time saving)	Ultrasound Pretreatment	efficient increase in algae biomass	[92]
	Microwave Techniques	increase lipid efficiency and in cell disruption efficiency	[93,94]
Thermal Pretreatment (high biomass yields and low energy requirements)	Steam Explosion	efficiently extract lipids	[95]
	Autoclaving	good biomass yield	[96]
Chemical Pretreatments	alkaline and acidic reagents	corrosive, toxic, produce inhibitory components	[97]
Enzymatic Pretreatment	cellulases and amylases	low energy requirement, effective lipid production, low investment requirements, mild operating conditions, and less energy consumption and represent the best alternative to the aggressive mechanical techniques	[97,98]

## 7. Biofuels

Currently, a very rapid increase in the human population in the world is observed, and thus—an increase in the demand for energy. In addition to human population growth, other pressing global challenges such as global warming and climate change, the Russian-Ukrainian war, and the COVID-19 pandemic make the transition from fossil fuel-based systems to bioenergy and bio-based products increasingly real. Such actions are needed to ensure the implementation of sustainable development plans. In general, the range of potential benefits from bioenergy and bio-based products depends on the environmental sustainability of biomass production. Hence the need to use advanced tools for the assessment of sustainable development, such as technical and economic analysis, life cycle assessment (LCA), energy analysis, exergy analysis and a combination of these techniques, such as exergy-environmental and exergoeconomic analyses. The aforementioned tools are used to determine the overall durability of these systems and to develop solutions to mitigate the impact of environmental hot spots and energy sinks [99]. Currently, the main energy sources are fossil fuels, which provide about 80% of the demand during the year [100]. Although highly industrialized countries such as the USA, Germany, Japan and China still use fossil fuels, because of the development of alternative renewable energy sources, such as geothermal, wind, solar and biomass, their consumption has significantly decreased [101–103]. Due to the fact that the availability of fossil fuels is limited, their consumption causes climate change and greenhouse gas emissions; the production of biofuels from plants (maize, sugar cane, sugar beets) has negative environmental, social and economic effects (consumption of fresh water, impact on biodiversity and soil fertility), clean and renewable marine biomass could be the best available choice of alternative energy sources [104,105]. Among biofuels, the first, second, third and fourth generations are distinguished. Despite the undoubted differences, each of the mentioned generations

of biofuels strives to meet the global demand for energy while minimizing the negative impact on the environment.

Feedstock for the production of first-generation biofuels includes starch, corn, rapeseed, sugar beet, sunflower, wheat, and barley. However, in a situation where a growing demand for food is present, controversial choices such as ‘food versus fuel’ cannot be allowed. An important aspect is also the low profitability of the production of biofuels from first-generation raw materials, which results from the high price of traditional food crops, the dependence of production processes on fossil fuels and the low return on investment. The oldest source of first-generation fuels are vegetable oils, and raw materials such as transesterified vegetable oils such as rapeseed or hemp oil, animal oils/fats, tallow and waste cooking oils that have long carbon chains are the raw material sources for the production of biodiesel. [54].

The reason for starting and continuing research on second-generation biofuels was the need to overcome the limitations associated with the production of first-generation biofuels, such as the use of raw food materials. Hence the interest in raw materials such as tree biomass, agricultural residues, demolition wood, straw, grass, etc., for the production of biofuels. Organic matter obtained from forest litter and wood turned out to be cheap, easily available and rich in carbohydrates, an excellent component for the production of biofuels, which at the same time is not dependent on crops. However, the consequences of reducing biodiversity and increasing the risk of nitrogen emissions due to the high use of nitrogen fertilizers should be mentioned here [106–108].

The emergence of third-generation biofuels required limitations to which first- and second-generation biofuels were subject, such as sophisticated and, at the same time, costly production methods and low profitability from an economic point of view. These are biofuels produced on the basis of various types of algae, which in turn are raw materials with many advantages, ranging from the possibility of cultivation and obtaining them in large quantities, through their low requirements and high environmental tolerance, to the ability to bind CO<sub>2</sub>. They are characterized by a very high content of good-quality oil, and their residues can be used as fodder or fertilizer [109,110].

In order to optimize the production of biofuels, genetically modified algae were introduced as a raw material. In many ways, it is an excellent alternative to fossil fuels, but their potentially dangerous impact on the environment and human life and health raises many concerns and reservations. The main technologies aim at increasing the productivity of selected species of algae by modifying them using genetic engineering methods. However, an important issue to be considered in the production of biofuels from GMO algae is the problem of disposal of residues. By-products from the energy extraction step may contain plasmid or chromosomal DNA, which may pose a risk of horizontal gene transfer, but this has not been confirmed by scientific research [111,112].

The use of microalgae as a raw material for the production of biofuels can be an excellent alternative to other sources of energy, as algae are the most efficient biological oil producer on the planet and a versatile source of biomass. They may soon become one of the most important renewable fuel crops on earth due to their high photosynthesis efficiency, high biomass productivity, faster growth rates than taller plants, highest CO<sub>2</sub> fixation and O<sub>2</sub> production, liquid growing capability in variable climates and in marginal areas unsuitable for agricultural purposes (e.g., deserts and coastal areas) with waters unfit for drinking or even for wastewater treatment. In addition, they use much less water than traditional crops and do not displace food crops; their production is not seasonal, and they can be harvested daily [113,114]. Eukaryotic algae harvest light efficiently because a large percentage of their cells consist of chlorophyll. Unlike deciduous plants, which lose their leaves in the winter, algae retain chlorophyll and remain photosynthetically active throughout the year. Algae do not have to sprout roots, leaves, shoots or flowers. All of their energy is devoted to the replication and repair of their photosynthetic apparatus or to reproductive efforts that increase the cell density of the algae culture. Algae are, therefore, more efficient at converting sunlight into chemical energy than terrestrial plants

and require a smaller geographic range and less water to grow. Due to the high efficiency of photosynthesis, algae cultures develop extremely quickly. They usually double their biomass within 24 h and are capable of doubling in as little as 3.5 h during the exponential growth phase [115]. Not only do algae grow rapidly, but much of their biomass is used as fuel. On average, lipids account for about 30% of algae biomass (this value can be as high as 80% in the case of some genetically modified species), compared to 5% of the biomass in the case of palm oil [116,117]. Algae can produce more biomass per unit of time and more biomass per unit area than any other plant [118].

Algae are cultivated in order to produce the raw materials needed for the production of biofuels, such as biodiesel, ethanol and crude oil. In fact, the yield of algae is higher than that of many energy crops (up to 6–12 times the energy production of corn). The advantage of high productivity is that algae cultivation requires less land. High productivity and high lipid content make algae a potentially important source of biofuels [113,119–121].

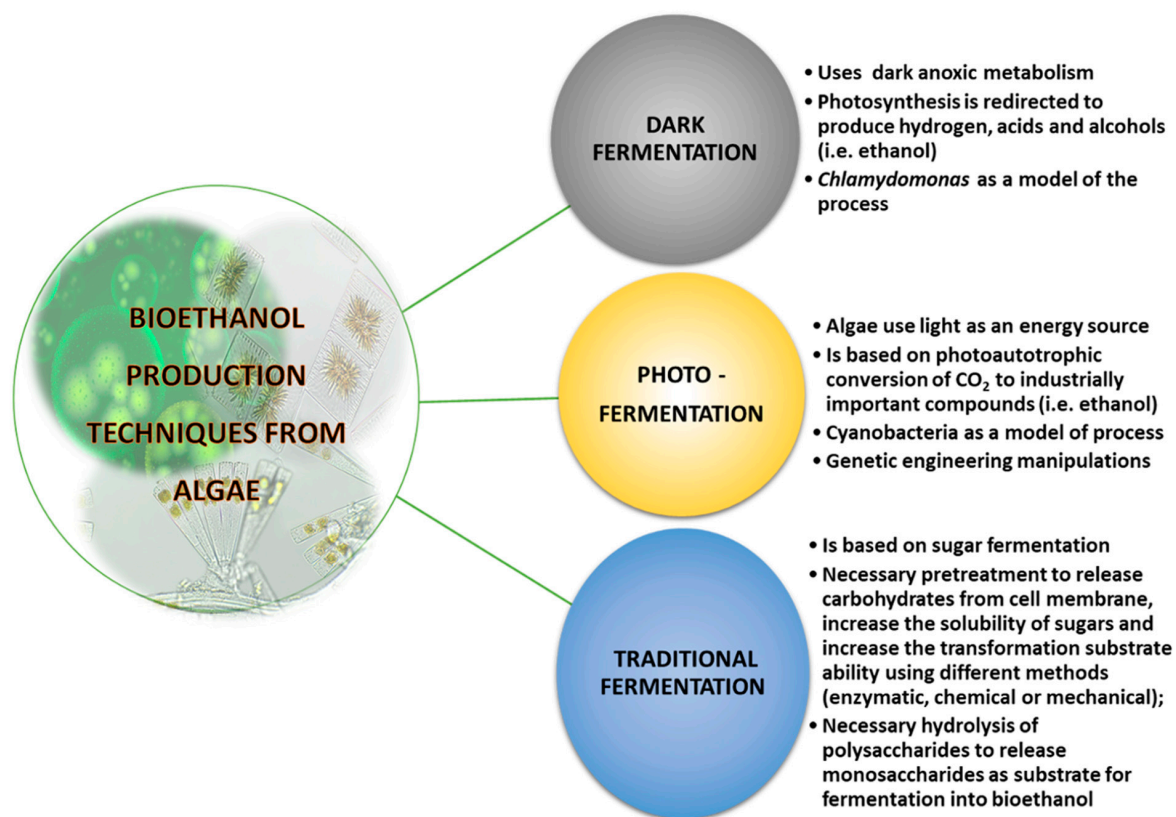
## 8. Algae-Derived Biofuels

### 8.1. Bioethanol

Among biofuels, bioethanol is becoming more and more popular due to advances in its production technology. It is still one of the most widely used biofuels in the world. However, for economic reasons, efforts are still being made to increase efficiency while reducing the cost of its production [122–124]. Third-generation bioethanol produced from microalgae is an environmentally friendly and safe solution. Compared to first and second-generation biofuels produced from higher plants, it definitely has more advantages [125]. Algae relatively quickly create a large amount of biomass due to the speed of cell multiplication, which means that they have a short harvest cycle (1–10 d). Their cultivation does not require arable land, only external ponds of open or closed photobioreactors (PBR) [126]. They can be cultured in both fresh and marine water, as well as in municipal wastewater, allowing for simultaneous water treatment and nutrient utilization [127].

Bioalcohols can be produced by simply fermenting sugars, starches or cellulosic biomass from food crops using yeast or other alcohol-forming microorganisms that can be used as alcoholic beverages as well as engine fuels [128]. If used as a blended fuel, they increase the octane rating of the fuel when mixed with conventional fossil fuels such as gasoline, which reduces volatility. Literature data indicate three basic ways of processing microalgae biomass for the production of bioethanol (Figure 4). In the traditional production method, the carbohydrate-rich biomass undergoes the stages of pretreatment, enzymatic hydrolysis and yeast fermentation. Another method is based on dark metabolic pathways where during dark fermentation, photosynthesis is redirected to produce hydrogen, acids and alcohols (such as ethanol). On the other hand, in a process called ‘photofermentation’, regular biochemical pathways in microalgae, preferably cyanobacteria, are redirected using genetic engineering techniques to produce bioethanol more efficiently. Genetically modified strains use light as an energy source to produce bioethanol from carbon dioxide and water in a single step [97,129–131].

Cyanobacteria (blue-green algae) are autotrophic prokaryotes that lack a cell nucleus and membrane-bound organelles. These were genetically engineered by Deng and Coleman (1999) in order to create a carbon pathway leading to direct ethanol production. The ethanol synthesis code from the *Zymomonas mobilis* bacterium was cloned into a vector and then used to transform *Synechococcus* sp. cyanobacteria. The GM cyanobacteria directly synthesized ethanol that diffused from the cells into the culture medium and the air space above it. Growth requirements for this form of GMO cyanobacteria are light, CO<sub>2</sub>, and inorganic nutrients like those found in sewage [132,133]. Currently, research is aimed at increasing the efficiency of ethanol synthesis or optimizing processes similar to those used by Deng and Coleman. Improving the efficiency of ethanol production from algae may involve further genetic modification, manipulation of growth conditions to lower nutrient concentrations, and the development of more efficient ethanol capture techniques, such as sequestration technologies, in a mixture of growth mediums [134].



**Figure 4.** Basic techniques (both traditional and based on genetic engineering) are applied in order to produce bioethanol from algae biomass.

## 8.2. Biogas

The obtained algal biomass represents one of the most promising alternative sources for the production of biogas while producing fertilizers and biofuels. This may also contribute to solving the problem of competition between conventional and energy crops. According to Górká and Cimochoicz-Rybicka [135], comparing methane production from an algal biomass or conventional crops, it has been found that methane production from algal biomass can exceed that from conventional crops by a factor of 2 to 20. This is due to the small or negligible amount of lignin present in algae, which is more easily degradable as compared to conventional plants. Therefore no pretreatment is required before it can be digested [136].

The biogas produced from algae consists mainly of methane, which is the most reduced carbon and accounts for approximately 65% and carbon dioxide, which is the most oxidized and varies at approximately 35%. Other gases such as hydrogen, nitrogen, ammonia, nitrogen oxides and hydrogen sulphide are also produced during this process, but their content is less than 1% [137], and they can be burned directly or used as fuel for engines and gas turbines.

The anaerobic digestion process leading to biogas production consists of several main steps, which include hydrolysis, acidogenesis, acetogenesis and methanogenesis [138]. Undeniable advantages of algal biogas production are the low amount of biomass produced during the process and the possibility of reusing the digestate to improve soil properties. So far, a large number of studies have been conducted worldwide using different species of algae for composting and biogas production [139]. According to Demirbas (2005), the most commonly used algal species for biogas production with high gas yields and methane concentrations in the biogas are *Macrocystis pyrifera*, *Sargassum*, *Laminaria*, *Ascophyllum*, *Ulva*, *Cladophora*, *Chaetomorpha*, *Gracilaria* [140]. Górká and Cimochoicz-Rybicka [135] reported that when a two-stage anaerobic digestion system was applied to algal species such as



*Macrocystis pyrifera* and *Durvillea Antarctica*, a methane concentration of approximately 65% was achieved in the studied biogas. In contrast, when a 1:1 mixture of the analyzed algae was prepared, it was found that biogas production was lower, but the methane content was at a similar level. It cannot be ignored that the methane potential is species-specific for the algae and depends on the structure of the cell wall and the rate at which it is decayed by microorganisms [139]. In the case of algae such as *Scenedesmus* and *Chlorella kessleri*, due to the construction of their cell wall, hydrolysis for anaerobic cultures is much more difficult than in the case of algae such as *Chlamydomonas reinhardtii* and *Dunaliella salina*, whose cell walls are rich in easily biodegradable proteins. Therefore, in order to facilitate the biogas production process, an additional pretreatment of algae is introduced, which allows for faster dissolution of the walls or their breakage. Moreover, biological processes during biogas production can also be inhibited by the presence of polyphenols (e.g., in brown algae), salts and sulphate polysaccharides, as well as the presence of methanogenesis inhibitors and the carbon-to-nitrogen ratio [141]. According to González-Fernández et al. (2012) [142], the methane yield obtained from algae in practice is always lower than the values reported theoretically. Sialve et al. (2009) [143] report that the theoretical calculation of algae methane yield was estimated to be between 0.48 and 0.80 L CH<sub>4</sub>/g volatile solids (VS). During the course of the study, it was expected that the yield of methane from *Phaeodactylum tricornutum* would be higher than the theoretically calculated result. However, the obtained value was 0.27 L CH<sub>4</sub>/g VS.

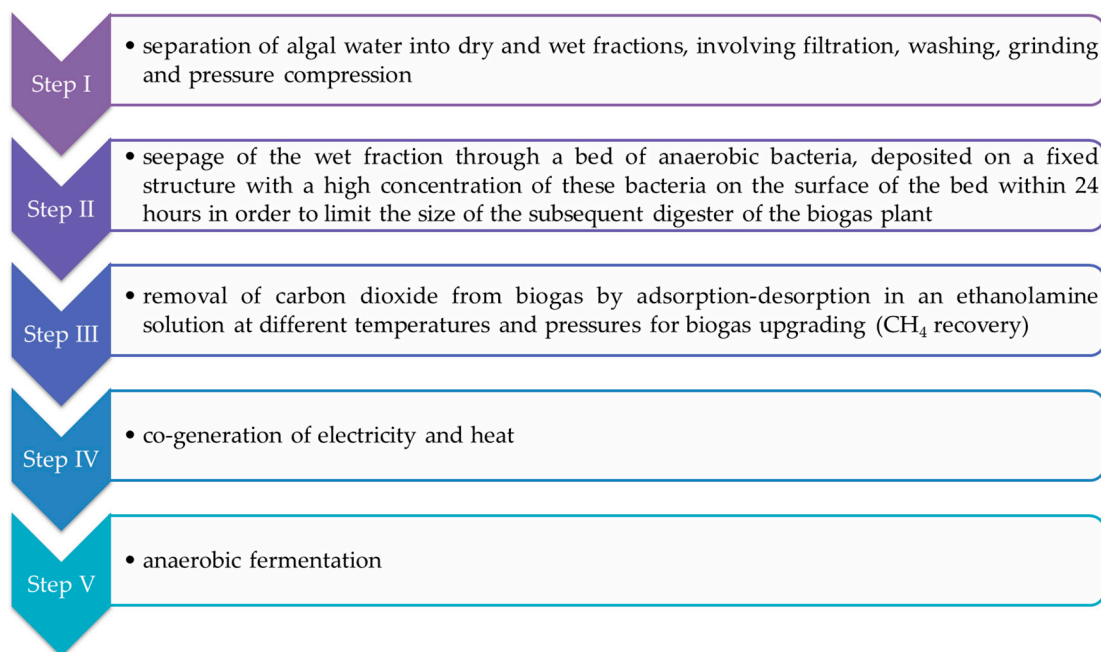
However, as reported by some scientists, in many regions of the world, the production of biogas from algae is related to the seasonality of the supply of raw material, and thus in such regions, it may not be economically viable [144]. This is confirmed by a study by Mosier et al. (2005), [145] in which point out that in order to reduce algal transport costs, the biogas plant should be located as close as possible to the source of the algae, in some cases can be a major problem for building a biogas plant on a beach. In addition, the direction of the winds would need to be traced so that possible odor emissions do not pose a problem for inhabited areas. In addition, for biogas production to be profitable, it requires a continuous supply of fresh macroalgae, so there is a need for more efficient machines that could collect biomass in water for further processing (Figure 5). A means would also need to be found to remove free-floating algae from ports, where the layer is sometimes so thick that recreational boats have difficulty approaching the jetties [141].

Additional attention should be paid to the co-fermentation of algal biomass with sewage sludge (or other waste biomass) as a potentially cost-effective method to improve methane yields from the anaerobic digestion of algal biomass. Such cultivation of algae on wastewater can lead to several beneficial effects: wastewater treatment, nutrient removal from wastewater and biomass production for energy [146]. Furthermore, such culture affects the ability of algae to improve the methane fermentation efficiency of sewage sludge under mesophilic conditions, while under thermophilic conditions, biogas production may be reduced by 30% to 40%. The beneficial effect of biogas production from algae cultivated on sewage sludge under mesophilic conditions is confirmed by a study by Górká and Cimochoicz-Rybicka (2015) [135] in which the amount of biogas produced ranged from 0.46 to 0.66 m<sup>3</sup>/kg vs. (volatile solids) and the concentration of methane in the biogas was between 60–70%. Therefore, as noted by Montingelli et al. 2015 [136], the use of algae as a co-substrate in methanogenesis can increase the efficiency of the process and the volume of biogas produced from sewage sludge [136,147].

### 8.3. Biodiesel

Microalgae are capable of producing triacylglycerol (TAG) and other lipids, which can then be transesterified into fatty acid methyl esters (FAME), which are biodiesel precursors [148]. The composition and quality of FAME determine the rating of biodiesel. The most common are palmitic acid (C16:0), linoleic acid (C18:2) and  $\gamma$ -linoleic acid (C18:3) [149]. The production of diesel from algae is currently of great interest and is the subject of research by many scientists. The use of algae as a substrate for the production of biodiesel is sup-

ported by their properties, such as higher efficiency of biomass accumulation compared to land plants or the ability to accumulate large amounts of TGA by some species. In addition, algae, unlike terrestrial plants, do not require high-quality agricultural land for cultivation. In addition, microalgae are able to fix organic and inorganic carbon (CO<sub>2</sub>) [150,151]. Brine, salt water or waste CO<sub>2</sub> streams and minerals from sewage can be used for their cultivation [152].



**Figure 5.** Steps involved in the technical processes for recovery of the energy from algae.

Although it is possible to obtain biodiesel from algae, there are many aspects that need to be investigated and improved. They mainly concern increasing the productivity of the culture, developing cheap and efficient ways of collecting and dehydrating algal cells, reducing the cost and energy consumption of the processes used during microalgae cultivation (mixing, pumping or gas compression) or preventing predation and pollution by other species of algae [152]. Table 4 presents examples of microalgae species identified as potential substrates for the production of biodiesel, taking into account various processes affecting the efficiency of lipid production, including TAG. *Chlorella vulgaris* is one of the species characterized by high production of TAG and, at the same time, represents a great potential for the production of biodiesel. Research by Griffiths et al. and Stephenson et al. indicate that nitrogen restriction during microalgae culture may increase lipid productivity [152,153]. In turn, Yao et al. [154] indicate that better efficiency of biodiesel production can be achieved by co-culture of microalgae and some strains of bacteria, which allows for maintaining symbiotic relationships naturally occurring in the environment. Microalgae make dissolved organic carbon available to bacteria. In turn, bacteria remineralize nitrogen, phosphorus and sulfur and provide other components (vitamin B, siderophores, indole-3-acetic acid) necessary for the growth of algae. Guo and Tong [155] proved that *Pseudomonas* sp. bacteria promoted the growth of *C. vulgaris* by increasing the concentration of algae cells, which was also associated with increasing the production of TAG. In turn, the cultivation of *Auxenochlorella protothecoides* with *Escherichia coli* resulted in an increase in algal biomass up to 6 times and a doubling of lipid concentration [156]. The marine microalga *Tetraselmis striata* is a potential candidate for biodiesel production due to its rapid growth and high lipid content. Co-culture of *T. striata* with *Pelagibaca bermudensis* resulted in a two-fold increase in algal biomass productivity and an increase in lipid accumulation [157].

Another element that needs to be optimized during algae production is algae harvesting. The small size of the cells (less than 10  $\mu\text{m}$  in diameter) and density close to the density of water require energy-intensive and expensive processes such as microfiltration or disc-stack centrifugation in large-scale fuel production. The future seems to be the use of magnetic nanoparticles for effective algae harvesting [158]. However, studies by Liu et al. (2018) indicate that the use of magnetic Fe-nanomaterials to harvest *C. vulgaris* may cause a slight decrease in the TAG content and the relative content of FAME in algae cells compared to algal culture without nanoparticles. In turn, the research of Seo et al. [159] showed that the trifunctional (magnetic, cationic and lipophilic) carbon microparticles used to harvest *Chlorella* sp. KR-1 did not show significant changes in the composition of fatty acids in algae cells, which is necessary for the production of biodiesel. Ho et al. [160] developed a *Chlorella* sp. MTF-7 harvesting strategy using ferrofluids with magnetic flocculation. The solution allowed increasing harvest efficiency, while the lipid content and FAME composition were similar to those in the culture without ferrofluids. Kandimalla et al. [151] studied the effectiveness of the mixotrophic culture of *Scenedesmus quadricauda*, *Chlorella vulgaris* and *Botryococcus braunii* with the input of organic and inorganic carbon of various origins, the source of which was sewage, glucose and exhaust gases. It was shown that the highest biomass and FAME production were obtained in a glucose-enriched medium in *S. quadricauda* culture. Santana et al. [161] conducted research on the use of vermicompost extract to increase the growth efficiency of *Graesiella emersonii* algae and lipids. In addition, the mixture improved the qualitative composition of FAME. In addition, in the production of biodiesel from microalgae (e.g., *C. vulgaris*), ultrasound has been successfully used to extract lipids, which increases the reaction speed, shortens the reaction time and is potentially cost-intensive [162]. Research is also conducted using a mixture of microalgae with butanol and nanoparticles (titanium oxide) as an alternative, more environmentally friendly fuel [163].

In order to improve the efficiency of biodiesel production from algae, genetic manipulations are also carried out. They are based on various strategies that aim to hyperaccumulate fatty substances, including TAG. The microalgae most commonly used in genetic engineering are *Phaeodactylum tricornutum*, *Thalassiosira pseudonana* or *Nannochloropsis oceanica*. Modification approaches are based, among others, on the overexpression of selected genes, endogenous expression, in situ mutagenesis or knockdown strategies of genes responsible for lipid biosynthesis [164]. Research by Wang et al. [165] showed that overexpression of the GPAT2 gene in *P. tricornutum* causes TAG hyperaccumulation. The increase in lipid content in *Neochloris oleoabundans* was obtained as a result of the co-expression of plastid NeoLPAAT1 and NeoDGAT2 located in the endoplasmic reticulum [166].

**Table 4.** Examples of the efficiency of biodiesel production by selected microalgae using different optimization strategies.

Microalgae Species	Biodiesel <sup>1</sup> Content (Productivity)	Comments	References
<i>Chlorella vulgaris</i>	46% dw <sup>2</sup>	growth under nitrogen limitation	[153]
<i>Chlorella vulgaris</i>	57% dw	growth under nitrogen limitation	[152]
<i>Auxenochlorella protothecoides</i>	1.8–30.9%	Coculturing with <i>E. coli</i>	[156]
<i>Tetraselmis striata</i>	18–23% dw	Coculturing with <i>P. bermudensis</i>	[157]
<i>Chlorella</i> sp. MTF-7	Up to 39.3% dw	harvest with ferrofluids	[160]
<i>Graesiella emersonii</i>	3.18 mg/L/d	cultivation with vermicompost extract	[161]
<i>Scenedesmus quadricauda</i>	0.3 g/L	cultivation in the presence of sewage sludge, glucose or flue gases	[151]

<sup>1</sup> lipid, TAG or FAME concentration, <sup>2</sup> dw—dry weight of biomass.

## 9. Conclusions and Future Research Directions

The current requirements of the global fuel market are too high to be met by biofuels of the 1st and 2nd generation. However, it should not be forgotten that the high dependence on these raw materials affects the global carbon cycle. With this in mind, algae are living photosynthetic organisms that are excellent potential feedstock for biofuel production. Lipids and carbohydrates from algae can be converted to bioethanol and biodiesel, respectively, using appropriate methodologies described in the literature. Algae have great potential in the production of both biodiesel and bioethanol, and biogas. They can be grown in a variety of systems, both open and closed, each with both advantages and disadvantages, and different algae pretreatment processes are just one of the factors affecting production efficiency. The commercialization of algae-derived biofuel production within the entire biofuel industry will have a huge social impact in the future. Waste products that are currently discharged into the environment as pollutants will be used to produce much-needed renewable energy sources. There is a need to develop a methodology for the use of algae that will allow the development of knowledge and assessment tools to understand the potential implications best.

The choice of algae species for cultivation should be based on the purpose for which the algae are cultivated, i.e., what end product is to be obtained. In turn, the cultivation conditions, the selection of medium and ingredients, and the method of harvesting algae should be adapted to the species of algae, the size of their cells and other characteristics of a given species. Efforts should also be made to improve not only cultivating methods but also the algae species themselves in order to effectively use the potential of algae in the production of biofuels. Future-proof solutions such as nanotechnology, genetic engineering and genetic manipulation come in handy here, which by modifying or introducing selected genes, can increase the production of biofuels.

Considering the fact that currently, the biggest problem with introducing biofuels to common use is their price, the use of algae for production could have a very positive impact on the entire fuel industry sector. It seems crucial to develop such methods of algae cultivation and obtain the necessary raw materials from them to maintain a relatively high production efficiency while reducing costs. If a method of economically viable production and purification of fuel from algae can be developed, this technology may turn out to be a quantum leap in the field of biofuel production. This would be a great prospect because there are vast sea and ocean areas in the world that could be used as water farms. The problems faced by the commercialization of biofuels from algae are primarily: low efficiency caused by limited access to light and poor crop dynamics, as well as high costs of harvesting the raw material. If these challenges can be overcome in the future, it will be possible to produce cost-effective algae biofuels that will reduce carbon emissions, mitigate climate change and reduce humanity's dependence on oil.

**Author Contributions:** Conceptualization A.J.-T.; visualization, methodology, writing—original draft, and writing—review and editing, A.J.-T., E.W. and U.W.; writing—review and editing, A.J.-T., E.W., U.W., G.S., M.D.E. and A.G. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was financially supported by the Ministry of Education and Science, Poland, under the research project number WZ/WB-IIŚ/6/2022.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

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