

Microalgae: Promising Sources of Biofuels

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Abstract

Microalgae, single-celled photosynthetic organisms, have garnered a lot of interest as a potential source for biofuel synthesis. These microscopic organisms are able to effectively convert sunlight and carbon dioxide into biomass that is high in proteins, carbs, and lipids. Microalgae makes up to 19% starch concentration, providing an eco-friendly and sustainable way to satisfy the growing need for renewable energy sources. Although they can be made from renewable resources and may lower greenhouse gas emissions, biofuels like biobutanol and bioethanol are significant substitutes for conventional fossil fuels. Compared to conventional biofuel feedstocks, microalgae-derived biofuels have a number of benefits, such as increased biomass production, the capacity to grow in saline water and non-arable terrain, and the possibility of simultaneous carbon dioxide sequestration. The potential of microalgae as a sustainable source for the production of biobutanol and bioethanol is examined in this review paper. It talks about the metabolic engineering techniques and biochemical pathways used to increase the generation of these biofuels in microalgae. By controlling the expression of important enzymes involved in industrial processes, genetic engineering approaches can improve efficiency and yield. The review also emphasizes the advantages, both financial and ecological, of employing microalgae as a feedstock for biofuel production. Because they give farmers a source of income and boost regional enterprises, microalgae-based biofuels have the potential to open up new economic opportunities, especially in rural areas. The analysis does, however, highlight the difficulties that come with producing food on a large scale, such as creating economical harvesting methods and improving growing conditions. In conclusion, even with the remaining difficulties, microalgae has excellent potential for producing biofuel and deserves more research. Future studies could produce microalgae-based biofuels that are more competitive and viable, which would help ensure a sustainable energy supply.

Keywords: Microalgae, organic solvents, ethanol, butanol

INTRODUCTION

Numerous sources utilizing biomass from various sectors, including forestry, aquaculture, and agriculture, have been investigated as sources for the production of several biofuels, including biodiesel and bioethanol. Techno-economic studies demonstrated that it was possible to produce biofuels at a fair price. But burning fuels made from biomass that already exists has an environmental impact similar to

burning fossil fuels when it comes to the carbon cycle (carbon balance), or the conversion of permanent carbon into CO₂ [1]. Furthermore, the loss of certain current biomass sources (like wood) without adequate remediation (like replanting) may lead to a significant biomass deficit, which could cause major environmental issues (like deforestation). The efficiency of traditional terrestrial plants in capturing solar energy is low. It has been shown that switchgrass, the fastest-growing crop in agriculture, can convert solar energy to biomass energy at an ideal annual rate of 1 W/m², which is less than 0.5% of the solar energy received at a typical mid-latitude location (200–300

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W/m²) [2, 3]. On the other hand, studies have suggested that microalgae could have a photosynthetic efficiency of up to 10–15% [4, 5]. Additionally, new research found that the additional N₂O entering the atmosphere because of using nitrogen fertilizers to produce crops for biofuels could contribute as much as or more to global warming when measured in ‘CO₂-equivalent’ terms. This contrasts with the quasi-cooling effect of ‘saving’ emissions of CO₂ derived from fossil fuels [6]. For the sake of this review, we define microalgae as any unicellular or simple multicellular photosynthetic micro-organism, regardless of whether they are eukaryotes, another name for microalgae (in a more limited context). Their fundamental architecture enables high rates of photosynthetic efficiency and growth. [7, 8]. Microalgae are considered to have a biomass production 50 times higher than switchgrass, the fastest-growing terrestrial plant [9–13]. The following are advantages of using biofuels made from microalgae: In particular, biodiesel produced by microalgae (open pond production) yields 12,000 hectares compared to 1190 hectares for rapeseed because they grow in aqueous media but require less water than terrestrial crops, lowering the strain on freshwater sources [1]. Because microalgae can be produced year-round, their oil productivity surpasses that of the best oilseed crops [14]. (3) Without compromising the production of food, fodder, and other items generated from them, microalgae may not require land-use change because they can be grown in brackish water on non-arable land, minimizing associated environmental problems. The extraction of microalgae biomass can influence the fixation of waste CO₂ (1 kg of dry algal biomass uses roughly 1.83 kg of CO₂), which may assist in preserving and enhancing air quality. (a) Algae that have the ability to grow rapidly; many species have oil contents between 20 and 50 percent dry weight of biomass [15]. (b) Wastewater contains nutrients, particularly nitrogen and phosphorus, that can be used to cultivate microalgae; as a result, it can be used not only as a growth medium but also to treat organic wastewater from the food and agriculture industries [16].

WHAT IS MICROALGAE

One of the earliest known biological forms is algae [9]. These are primitive plants (thallophytes), meaning they don't have stems, roots, or leaves, and their reproductive organs aren't covered with sterile cells. cells, with chlorophyll serving as their main pigment for photosynthetic processes [10]. The primary function of algae structures is energy conversion; they do not develop beyond cells, and because of this, they can adapt to the conditions of their environment and thrive throughout time. Because prokaryotic cells lack membrane-bound organelles, including plastids, mitochondria, nuclei, Golgi bodies, and flagella, they are more similar to bacteria than algae [9]. Prokaryotic cells are also referred to as cyanobacteria. These organelles, which control the cell's functions and permit survival and reproduction, are present in eukaryotic cells, which comprise a broad range of common algae species. The main factors used to categorize eukaryotes into different classes are their pigmentation, life cycle, and basic functions. The diatoms (*Bacillariophyta*), red algae (*Rhodophyta*), and green algae (*Chlorophyta*) are the three most important classes. It is conceivable for algae to be both autotrophic and heterotrophic; the former just requires inorganic materials like CO₂ and salts [11].

Autotrophic algae depend on photosynthesis, which is the process by which solar radiation and CO₂ received by chloroplasts are transformed into adenosine triphosphate (ATP) and O₂, the cellular energy currency required in respiration to maintain growth. Algae are classified as micro and macro algae; there are more than 3000 species of each kind, and around there are about 200 species that can be used to make biodiesel, medicines, human use, etc. [12]. Microalgae are the oldest living things on Earth, and they can grow quicker, doubling their biomass every day, according to Song et al. With the right growing conditions – temperature, pH, carbon dioxide/oxygen ratio, biomass consistency, etc. – many diverse species can produce biodiesel [13]. Therefore, the consistency of biomass energy—which comes from animal and plant wastes – is crucial for the synthesis of lipids, proteins, and carbohydrates, which are the building blocks of oil. Comparing microalgae to other species, there is a greater production of lipids, which are a component of biomass. It has also been noted that certain microalgae are good hydrogen generators. Hydrogen has a large energy content, and pollution from engines running on pure hydrogen is essentially nonexistent. For this reason, microalgal biomass is regarded as a clean, renewable energy source [12, 13].

DIFFERENT TYPES OF FUEL FROM MICROALGAE

Biodiesel

Biodiesel is created by the mono-alcoholic trans-esterification process, which is catalyzed by alkali, acids, or enzymes and mixes triglycerides and a mono-alcohol (often methanol or ethanol). Its combustion characteristics are comparable to those of diesel, and it has been produced commercially or in backyard facilities for use as vehicle fuel. Significant technical advancements have been made in the transesterification procedure [14]. For instance, Canadian researchers in the Department of Chemical Engineering at the University of Ottawa have developed a novel two-phase membrane reactor that separates reaction products (biodiesel/glycerol) from residual canola oil by using the oil's immiscibility in methanol. In particular, the two-stage membrane reactor helped to produce high-purity biodiesel, shift the reaction equilibrium to the product side, and remove unreacted canola oil from the finished product. However, the high cost of feedstock presents a significant barrier to the manufacture of biodiesel [15]. Currently, biodiesel is produced using both plant and animal fats. This agricultural method will eventually compete with the food industry for available land. For instance, it was determined that in order to produce 5.54 Mtoe (million tons of oil equivalent) of biodiesel, 9.3 Mha of land would need to be used for canola (rapeseed) and sunflower cultivation. This amounts to 1.72 percent of the 321 Mtoe estimated EU-25 (the 25 European Union countries) consumption for transportation fuels in 2003. This is the equivalent of 150% of the area used for these crops in the EU-25 at the moment.

A few species of microalgae have the capacity to extract lipids to a significant degree (30–50% dry weight basis), which makes them an attractive alternative source of lipids for biodiesel generation. On 200,000 hectares of land, or less than 0.1% of the climatically suitable territory in the United States or 3.2% of the acreage now used for rapeseed and sunflower growing in the EU-25, microalgal farming has the potential to create a quadrillion BTU of fuel [16–18].

Bio-Oil and Biogas

However, the cost of green solvents is not as cheap as it is for solvents made from fossil fuels [19]. Moreover, a solvent-free osmotic shock pretreatment method was used to extract lipid and then create methane from microalgae D.H. When biomass is handled at a high temperature in the absence of oxygen, three phases are created: the liquid phase, the solid phase, and the vapor phase [20]. The fluid phase is a complex mixture referred to as bio-oil. The types of feedstock and process parameters greatly influence the composition of bio-oils. The main components of the bio-oils made from fast pyrolysis of rice husk are formic acid (7.69%), b-hydroxybutyric acid (2.31%), and toluene. In contrast, the main components of the bio-oils made from a commercial wood biomass feed (lignocel HBS 150-500) that came from beech wood are phenols, alcohols, and carbonyls, with the amounts of these components changing significantly depending on the pyrolysis conditions [21, 22]. However, the cost of green solvents is not as cheap as it is for solvents made from fossil fuels. Moreover, a solvent-free osmotic shock pretreatment method was used to extract lipid and then create methane from microalgae D.H. The use of biomass conversion methods such as vortex reactors, circulating fluidized bed gasifiers, 26–28°C vacuum pyrolysis, and entrained flow reactors has been the subject of numerous investigations [23].

With proper regulation, pyrolysis can result in the creation of an entire cell with energy-to-biomass ratio of up to 95.5%. The two techniques that can be classified are pyrolysis, which produces bio-oils as the primary product, and gasification, producing 'syngas'. Canadian companies have shown world leadership in this field. Ensyn Corp. (EC) (<http://www.ensyn.com/>) [24–26]. Now based in Ottawa, Ontario, has developed one of the most advanced biomass conversion technologies, rapid thermal processing (RTP). Carbon-based raw materials, such as wood biomass or petroleum hydrocarbons, are transformed into valuable chemical and fuel products through a proprietary process known as RTP. Plascoenergy Group, a privately owned company in Ottawa, Ontario, is the owner of the Plasco Conversion System (PCS), which converts carbon-containing materials such as municipal solid waste into energy-rich fuel or syngas and, correspondingly, commercially useful inert solid or 'slag' [23].

It has been shown that bio-oils are ideal for producing power by internal combustion (such as gas turbines and diesel engines) or external combustion (such as steam cycles, organic Rankine cycles, and Stirling engines) or by cofiring with fossil fuels like natural gas or diesel. However, a number of unfavorable characteristics, like a high oxygen content, high viscosity at low temperatures, low heat content, and chemical instability, prevent them from being used as high-quality transportation fuels. Research has been done to improve the bio-oils to high-quality fuels in order to get beyond this restriction. For example, it has been shown that it is technically possible to produce high-grade transportation fuels from biomass through gasification and subsequent. A Chinese group's recent research has shown that steam-reforming bio-oil may reliably yield hydrogen [24, 22].

Bio-Hydrogen

Hydrogen is a significant fuel that finds extensive use in bitumen upgrading, coal liquefaction, and fuel cells. A biological process can create hydrogen by a range of techniques, such as the steam reformation of bio-oils, the photolysis of water facilitated by unique microalgal species, the dark and photo fermentation of organic materials, and more [27, 28].

PRODUCTION OF BIOFUELS FROM MICROALGAE

Development of Upstream

Broadly speaking, there are three primary upstream technologies that are relevant to microalgae. First and foremost, it is preferable to choose a few suitable strains from the wild environment that are hardy, grow quickly, and have a high lipid content.

Secondly, strains with high lipid productivity and a rapid growth rate can be modified by the application of modern genetic engineering. Thirdly, other strategies like metabolic engineering can be employed to promote the buildup of fuel products such as lipids, etc.

Microalgae could be genetically and metabolically engineered to eliminate, for example, photosaturation and photoinhibition. This would significantly boost outdoor culture output and significantly boost the economics of microalgae oil production [29].

Overcoming the present prohibitions against the release of genetically modified organisms, however, will necessitate sustained research and funding. Therefore, it would be wise to restrict forecasts to what can be accomplished with wild-type strains for the foreseeable future.

Strain Processing

Microalgae are noted for their high lipid content and quick growth rate, but not all of them; although they rely on a specific strain, they are thought to be the greatest lipid producers. In this sense, choosing strains that have the optimal ratio of lipid content to microalgal biomass production in outdoor growth is a fundamental need for microalgae-based biofuel. It would be encouraging, for instance, if more than 40,000 species of microalgae have been identified out of an estimated one to ten strains that could significantly accumulate lipids up to the point of nutritional deficiency [30]. The strains ought to be resilient enough to tolerate shear stress brought on by mixing or interference from other microbes or wild strains [31]. Additionally, they can easily adjust to the modifications in the growing environment's physicochemical characteristics. A micropipette is typically used to separate the strain of freshwater or marine microalgae under a microscope, dilution of cells, and growth on an agar plate or liquid media. It was suggested to use the Winkler test screening methodology, which included a novel and quick technique for mutant strain selection and measurement of algal hydrogen metabolism without the application of nutritional stress, to isolate the *Chlamydomonas reinhardtii* strains that produce hydrogen. Out of thirty microalgal strains, two freshwater and two marine strains were selected for screening, and the resulting four strains had high lipid content and biomass productivity. For instance, under nitrogen shortage, the eustigmatophyte *Nannochloropsis* sp. F&M-M24 was able to attain up to 60% lipid content. Additionally, those strains that are isolated locally are probably acclimated to environments that are highly changeable [32].

Genetic Engineering

The direct use of biotechnology to modify an organism's genes is known as genetic engineering. It has been used to produce microalgae in order to meet the expanding demands and raising the standard of living for people. In order to achieve high microalgal biomass concentration for sustainable industrial applications and to alter the metabolic pathway in order to produce more predicted high-value products, some researchers in the past employed a critical systemic technology. Trans-conjugation, natural transformation and induced transformation, electroporation (or electropermeabilization), biolistic transformation, glass beads, silicon carbon whiskers method, microinjection, artificial transposon method, recombinant eukaryotic algal viruses, and agrobacterium tumefaciens-mediated gene transfer are generally the methods used for transformation in marine algae [33]. Naturally the several techniques, electroporation is a popular one because of its ease of use and high efficiency with a minimal amount of DNA approach for a variety of bacteria and cells for more than 30 years. To enhance the permeability of the cell membrane, an electrical field (e.g., 1–1.5 kV, 250–750 V/cm) can be applied to the cells. Subsequently, chemicals, medications, or DNA can be added to the cells. Prokaryotic cells and eukaryotic algae, such as red, green, and diatoms, can all be treated with this approach. For instance, the marine algae *Nannochloropsis sp.* has been successfully genetically altered to eliminate multiple genes involved in nitrogen metabolism, making it a viable candidate for the production of biofuel. For several diatom strains, direct gene transfer by biolistic transformation – that is, microparticle bombardment – has been shown to be the most effective technique, and it has been extensively used in the nuclear and chloroplast expression systems' transformation. Nonetheless, state that this process is extremely repeatable and requires expensive, specialized equipment to operate. In particular, pressurized helium gas is typically used to coat DNA with gold particles and deliver them to specific locations within the cell.

Using the previously indicated transformation techniques, more than twenty marine microalgal strains have been successfully changed to date. Using the trans-conjugation approach, or natural transformation, five strains of *Synechococcus* (i.e., *Synechococystis*, *Pseudanabaena*) were successfully shown to undergo genetic transformation for marine cyanobacteria [34].

Among its many benefits is the fact that it is the sole reliable technique for repeatedly transforming mitochondria, chloroplasts, and other organelles. It has the ability to deliver foreign DNA into a wide range of plant, animal, microbial, pollen, and other unusual acceptor cells and tissues. Additionally, by using a mature and controlled modification process, diverse endogenous vectors can also be employed in biolistic transformation. There are five different kinds of acceptor cells for brown algae: parthenogenetic sporophytes, juvenile sporophytes, male and female gametophytes, and sporophyte tissue fragments can all undergo transformations through particle bombardment. In the case of diatoms, biolistic transformation has been shown to work in the following instances: the centric diatom *Thalassiosira weissfogii*, *Thalassiosira pseudonana*, *Chaetoceros sp.*, *Cyclotella cryptica*, and the Pinnate diatoms *Navicula saprophila*, *Cylindrotheca fusiformis*, and *Phaeodactylum tricorutum*. However, the lack of refined protoplast preparation and immature regeneration technologies limit the application of this approach in brown algae. Using the previously indicated transformation techniques, more than twenty marine microalgal strains have been successfully changed to date. In the case of marine cyanobacteria, using the trans-conjugation approach, or natural transformation, genetic transformation was effectively demonstrated in five strains of *Synechococcus* [35].

Metabolic Engineering

The process of optimizing an organism's genetic (i.e., adding and removing genes) and regulatory mechanisms in order to change its metabolic activities in a predefined way, such as raising the organism's manufacturing of a certain material. In bioprocess engineering, dietary and environmental regulation typically completes the control of metabolic pathways [36]. Generally speaking, there are three main categories of metabolic reactions: the biosynthesis of large molecules, the biosynthesis of small molecules (such as nucleotides and amino acids), and the breakdown of nutrients [37].

According to a study, overexpressing the acetyl-CoA carboxylase gene (ACCase) during stressful conditions might raise the fatty acid content of diatom *C. cryptica* because ACCase regulates the production of fat. By introducing the heterologous plant fatty acid synthetase enzyme gene, the oil content of diatoms and green algae was also boosted. By inhibiting the pyruvate carboxylase kinase expression of diatom *P. tricorutum* with an antisense cDNA construct, a rise in total neutral lipid of up to 82% was obtained [38].

Under nutrient-replete conditions, overexpression of the endogenous malic enzyme can increase *P. tricorutum*'s lipid accumulation by 2.5 times in addition to pyruvate (Xue et al., 2014). Additionally, certain transgenic techniques are used to boost green algae's lipid accumulation and diatom in nitrogen-starved environments.

Moreover, blocking lipid catabolism might increase lipid buildup. For instance, converting antisense constructs resulted in a 3.3-fold increase in total lipid content of the diatom *T. pseudodonana* compared to the wild-type [39].

Downstream Processing

The following are the primary downstream technologies that can be used with microalgae: large-scale culture of suitable microalgal strains, the creation of efficient cultivation systems and modified cultivation modes to address the relationship between biomass and lipid production, energy-efficient harvesting and dewatering techniques, and effective extraction and conversion methods.

Large Scale Cultivation

The key to commercializing microalgae-derived biofuels is large-scale culture, which is made possible by sufficient biomass. It is generally constrained by such elements as suitable strain, farming, process control, and additional measures.

Stain During High Mass Production

As previously said, choosing potential microalgae species that have a high oil content and can grow quickly in culture is crucial, and it is one of the main keys to generating drop-in fuels, biocrude, and biodiesel while continuing to work on projects that will be profitable. The key factors that drive a successful commercialization of microalgae-based biofuels are five strategies: 'fatter (i.e., algae species with high oil content), faster (i.e., grow more quickly), cheaper (i.e., capital and operating costs), easier (i.e., manipulation at each sub-set of systems), and fractionation marketing approaches (e.g., biomass co-product marketing strategies) [40].

High lipid strains are a promising type of feedstock for biodiesel, and this would form the foundation for productive industrial microalgae farming. A number of local strains were examined in several studies (57 strains) from the shores of *Morocco* and discovered that whereas the lipid content of marine microalgae *Tetraselmis sp.* and *Dunaliella sp.* reached up to 56% and 50% of dry cell weight, respectively, diatoms are often high in triglycerides (TAG) [41].

Scaling Production Up

According to Quinn et al. (2012), large-scale microalgae culture is necessary to achieve high biomass concentrations and lipid contents for the commercialization of microalgae-based biofuels. However, extensive farming confronts challenging hurdles, and the main obstacle is the high cost of production. This is because complicated algae cultivation processes, such as gas exchange, illumination, aeration, mixing, sterilization, and others take a lot of energy. For instance, it is difficult and expensive to carry out procedures like sterilizing a large number of cultures and maintaining sterility across the entire cultivation system, as well as deoxygenation and efficient lighting techniques, among other actions for improved process control [42]. Therefore, it's critical to reduce the expense of microalgal cultivation, which may be brought on by the aforementioned processes, while also achieving high lipid production

and biomass concentration. Co-producing value-added goods, streamlining the processes involved in algae growing, and reducing cultivation expenses by using wastewater or fuel gas as a source of carbon and nutrients are the key strategies for offsetting overall costs. A few successful cases of large-scale microalgae farming have been adopted in businesses in recent years, thanks to the ongoing efforts to improve and develop technologies. As an example, Sapphire Energy was established in 2007 and has been working with other firms on research projects involving algae for a number of years [43]. The goal has been to co-develop algae-to-fuel production systems on a commercial scale in the last few years. The Green Crude Farm is a commercial demonstration algae-to-energy facility that was announced by the company in 2012. The farm is made up of two ponds measuring 100 acres each and a 300-acre facility that will house processing and mechanical equipment used in processes like harvesting, extracting, and recycling algae [42].

Algenol is a renowned global team equipped with cutting-edge facilities and patented techniques for growing algae. The business uses VIPER and photobioreactors, which have production yields 2-3 times higher than those of open ponds. manufacturing (i.e., the patented photobioreactors made by Algenol) at a 40,000-square-foot facility. Algenol not only manufactures high-quality products like protein, natural colorants, Spirulina, ingredients for personal care products, biofertilizers, and biostimulants, but it also specializes in producing biofuels like bioethanol and green crude oil using a patented vapor compression steam stripping unit (VCSS) for ethanol purification and hydrothermal liquefaction (HTL) technology for crude oil production [44].

An Appropriate Culture System for the Growth of Microalgae

Microalgae can flourish in artificial systems such as open ponds and photobioreactors, as well as in natural ecosystems like oceans, rivers, lakes, and ponds. Open ponds can be artificial ponds like raceway ponds, circular ponds, shallow huge ponds and tanks, and container-based systems like hanging plastic bags. They can also be natural waters like lakes, shallow lagoons, and ponds. Open ponds typically have the benefits of being less expensive, simpler, and easier to maintain. But they also have some significant drawbacks [45]. For instance, they need a lot of land, evaporate more water more easily, produce less biomass, use little light and CO₂, mix poorly, and are more easily contaminated. In contrast, closed systems such as photobioreactors can be classified as membrane photobioreactors, spectrum shifting, internally illuminated photobioreactors, tubular photobioreactors, vertical column photobioreactors, flat panel photobioreactors, and plastic bag photobioreactors, according to [42]. They can provide effective control over several crucial operational factors for microalgal production, such as temperature, light path length, pH, species management, and others. In comparison to open ponds, photobioreactors offer a greater chance of attaining significantly higher growth rates, microalgal cell densities, and volumetric biomass productivity. Complex regulation, lighting, cooling, mixing, deoxygenation, and other operational needs lead to expensive capital and operating costs. In the current situation, creating an appropriate cultivation method is essential, especially for large-scale microalgal farming. Considering the benefits and drawbacks to both open ponds and photobioreactors, some businesses would rather use photobioreactors than open pond systems or natural formations; however, open pond microalgae cultivation is an appealing option in areas with sufficient land that is not in competition with arable land. According to reports, in raceway ponds with a 30% oil content, the majority of microalgae systems today can produce between 2500 and 5000 gallons of oil per surface acre [46].

Methods of Cultivation to Resolve Conflicts Between the Production of Lipids and Biomass

Environmental stressors such as salinity, temperature, nutrition, and pH typically trigger lipid production. Of these, nitrogen limitation is thought to be the most effective method for promoting microalgal lipid accumulation, albeit at the expense of cell proliferation. Studies have shown that it is feasible to enhance strains from low or medium starting lipid content to extremely high levels; under saline stress, *Dunaliella tertiolecta*'s lipid content increased significantly from 21 to up to 70%. In an attempt to overcome the conflict between biomass accumulation and cell expansion, strains with rapid growth rates that are genetically selected or screened locally elevated lipid content [45]. Simplified cultivation, which includes medium composition optimization, process control and optimization for

light utilization, oxygen accumulation mitigation, contamination prevention, and cultivation system improvement, can result in high biomass concentrations of microalgal cultures. At this time, it has been shown that a two-stage process is a win-win method for achieving high cell density and biomass concentration (i.e., hydrogen, lipids, or carbohydrates) at lower operating as well as investment fees. (During the procedure, the biomass is transferred to a heterotrophic reactor where the cells consume organic matter after first being grown in photoautotrophic conditions). Carbon to create lipids and starch. In order to increase the treatment of organic-rich wastewater and the yields of microalgal lipids, a hetero-photoautotrophic microalgal growth model was also investigated. This provided a sustainable method for the production of microalgae-based bioenergy and byproducts [42].

Energy Saving Approaches for Harvesting and Dewatering

Due to the energy-intensive nature of harvesting and dewatering microalgal biomass, these processes are thought to be a major barrier to the production of microalgae-based biofuels. Therefore, it is critical to identify energy-saving strategies in order to offset the production cost of microalgae-based biofuels. Generally speaking, microalgae can be harvested in two steps: bulk harvesting and thickening/dewatering [46].

Cutting-edge techniques, such as combining hydrothermal liquefaction (HTL) with pulsed electric field (PEF), have been suggested as a viable and appropriate pretreatment for the wet extraction of residual biomass from microalgae. A greater biocrude yield of 6 weight percent was predicted by the claim that PEF may speed up the production and extraction efficiency of amino acids by up to 150% in 60 minutes. Additionally, other techniques are used, such as supercritical fluid extraction at high-temperature and pressure, high energy ultrasonic or microwave-assisted extraction, and ionic liquids with extremely low melting points—typically below 100°C – to disrupt cell walls [47].

The efficiency and cost of harvesting are directly impacted by the technology chosen, and each of the aforementioned technologies has pros and cons of its own. Coagulation and flocculation, for instance, are possible for small sizes of microalgae (such as less than 5 µm) and allow them to form larger flocks (between 1 and 5 mm). While filtration is costly and prone to fouling, other technologies, like centrifugation [48], have advantages including low energy consumption, high capital and operating costs, and the ability to cause shear stress on algae cells. ‘ZOBİ Harvester,’ created by Global Algae Innovations, however, the cost of green solvents is not as cheap as it is for solvents made from fossil fuels. Moreover, a solvent-free osmotic shock pretreatment method was used to extract lipid and then create methane from microalgae D. It is well-known for its automated membrane filtration system, which results in a 100% harvest efficiency and eliminates the need for additional dewatering when harvesting and dewatering simultaneously. This system can produce 20,000 L/h, achieving a 4-fold decrease in water flow and a 30-fold reduction in harvest and dewatering energy (EnergyGovOffices, 2016). The automated harvesting system is easy to use and expandable, with a size range of 5–200,000 gpm. It harvests microalgae at room pressure without being subjected to strong centrifugal or strain pressures [49].

Extraction Process

Microalgal fatty acids can be extracted from the dehydrated microalgae biomass through the use of mechanical or non-mechanical methods such as autoclaves, cell homogenizers, and ultrasonography, the use of bases; enzymatic procedures; osmotic shock; polar and nonpolar solvent bead mills; spray drying; freezing, and supercritical carbon dioxide (SC CO₂) [47]. Nonetheless, due to their complexity and bulk, the majority of extraction techniques are difficult to apply on a broad scale. They do, however, function well in the laboratory. For instance, a few earlier studies revealed that the biggest cost involved in green techniques for lipid extraction from wet microalgae culture is the enormous amounts of solvent consumed. Solvents like cyclopentyl methyl ether and 2-methyltetrahydrofuran. Furthermore, they contrasted various methods of lipid extraction and demonstrated that the Harada and radiant method [46], which extracted 1 kg of fatty acids from *Chlorella pyrenoidosa* (at 65.71% moisture) using

hexane/isopropanol (3:2 v/v), was the most effective. However, the cost of green solvents is not as cheap as it is for solvents made from fossil fuels. Moreover, a solvent-free osmotic shock pretreatment method was used to extract lipid and then create methane from microalgae *D.H. salina* and *Chaetoceros muelleri*; lipid recovery efficiencies of 21% and 72% were achieved. Additionally, a few more modern techniques, including supercritical fluid extraction, ionic liquids, microwave-assisted extraction, and adjustable hydrophilic properties liquids, are suggested for affordable extraction of lipids [49].

Conversion Process

Microalgal biomass can be processed using a variety of techniques to produce sustainable fuels (such as electricity, heat, and fuels) and energy sources, such as (1) transesterification, (2) thermochemical processes like combustion, pyrolysis, gasification, and thermochemical liquefaction, and (3) biochemical/biological conversion, including the process of fermentation, anaerobic metabolism, and synthesis of hydrogen [50–52].

By burning microalgal biomass directly, hot gases can be created that can power a turbine and a generator to produce electricity. The condition of the furnace, boiler, steam turbine, or oxygen approximately. Direct-firing biomass in a boiler to produce high-pressure steam, which is then fed into a steam turbine and passes over several turbine blades in order to cause the turbine and electric motor to rotate and produce electric power, is a method to use biomass for heat and power. Triglyceride + methanol + transesterification is the chemical equation that generates methyl esters (FAME), and it can be done directly (one stage) or conventionally (two stages). Serving as an initiator for the production of glycerol and methyl esters. On the other hand, the process of turning microalgal oil into jet fuel through hydro treatment or gasification entails converting fatty acids and esters using a number of steps, such as pyrolysis, combustion, gasification, as well as the water-gas shift reaction.

The process of breaking down biomass by heat in the absence of oxygen is called pyrolysis. It can be categorized as fast, conventional, or flash pyrolysis, and it produces solid fuel (biochar), gas fuel products (H₂, CH₄), and liquid fuel (bio-oil) [51]. Both traditional (two-stage) and direct (single-stage) transesterification can yield methyl esters (FAM). According to the chemical equation, glycerol and methyl esters are produced when triglyceride and methanol react with one another as a catalyst. On the other hand, the process of turning microalgal oil into jet fuel through hydro treatment or gasification entails converting fatty acids and esters using a number of procedures, such as pyrolysis, combustion, gasification, and/or water-gas shift reactions. Pyrolysis is the process of breaking down biomass thermally without the presence of oxygen. It can be done in three different ways: conventionally, quickly, or to generate solid fuel, liquid fuel (bio-oil), and gaseous fuel products [50].

The process of anaerobic digestion involves progressively going through the phases of hydrolysis, fermentation, acetogenesis, and methanogenesis to extract methane from delipidized algae material that contains carbon and nitrogen. Utilizing this technique, organic biomass is transformed into biogas (about 60% CH₄ and roughly 40% CO₂), which is then used in numerous emerging economies, including China, India, Nepal, Brazil, South Korea, and Thailand [50]. High biomass output and lipid production are both possible with mutually beneficial techniques like two-stage cultivation. To guarantee high production of microalgal biomass, suitable strains of microalgae have been selected or modified content or other energy sources. Microalgae-based fuels are additionally more promising due to the utilization of cutting-edge technologies in the harvesting and biomass-to-fuels conversion processes. The scaling up of microalgal farming systems still faces some significant obstacles, though, and these issues need to be resolved in the future [53–55].

CONCLUSION

It is evident that the decline of fossil fuel sources and rise in CO₂ concentration in the atmosphere need the quick development of green, carbon-neutral substitutes. Microalgae's CO₂ fixation offers a viable substitute for reducing CO₂ emissions and can be used as feedstock, pharmaceutical by products,

etc. With this method, wastewater can be treated concurrently. Thus, this could provide a sustainable approach by integrating the CO₂ capture, wastewater treatment, and biofuel-generating processes. Continuous large-scale microalgae-based CO₂ collection is quite uncommon. Nonetheless, research conducted in labs and pilot plants indicates that using microalgae to absorb CO₂ could be a practical approach to reduce CO₂ emissions from human sources. Despite the apparent benefits of algal oil, production costs and inadequate technology have caused a delay in the commercial production stages. However, until we finish producing algal oil, we still have the option to rely on other renewable energy sources rather than microalgae. According to economic analyses, in the coming decades, the cost of manufacturing biofuel from microalgae and carbon capture could match the cost of producing fuel derived from petroleum. Thus, one of the primary biofuel products will be microalgae biofuels.

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