



REVIEW

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A Comprehensive Review on the Effect of Climate Change on Algal Biofuel Production

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Abstract

The exponential increase in world population has put enormous increased motorization that led to an overwhelming pressure on the earth's finite supply of fossil fuels. For the benefit of human well-being, renewable and sustainable energy sources must be developed because the lack of fossil fuels is negatively affecting the environment and the economy. Algae with high cellulose/starch/lipid accumulation can be the best substitute for food crops in the case of economic and environmental concerns. These algae can be used to produce bioethanol, a sustainable fuel. Some types of algae can directly contribute to the generation of ethanol by producing it during dark-anaerobic fermentation. Production of algal-based biofuel is an economically effective and environmentally friendly energy source that seems to be a promising alternative for the future generation of biofuel. In the 2030 Agenda for Sustainable Development, sustainable transport is mainstreamed across several SDGs and targets, especially food security, health, energy, economic growth, infrastructure, and cities and human settlements. It aims to make cities and human settlements inclusive and identify ways to develop and implement low-carbon and resilient transport strategies. The current review describes the state of the field of algae biofuel in the past present and future. The likelihood of producing biofuel energy from algae cells in the future can be increased by implementing a sophisticated plan to increase biofuel output. Current theories regarding algal potential for producing biofuel are compiled in this study. It discusses each phase of the process, scientific accomplishments, current issues, and recommendations for future research aims and objectives.

Keywords: Microalgae; Climate change; Biofuel; Fossil fuel; Future generation; Carbon fixation

Introduction

First acknowledged at the 1992 United Nations Earth Summit, the role of transportation in sustainable development was reaffirmed in Agenda 21, the summit's conclusion document. During its nineteenth Special Session in 1997, the UN General Assembly conducted a five-year review of Agenda 21 implementation and added that, over the next two decades, transportation is expected to be the primary driver of the world's increasing energy demand (in fact, it is currently the largest end-use of energy in developed countries and the one that is growing at the fastest rate in the majority of developing countries). Additionally, the Johannesburg Plan of Implementation (JPOI), the final document of the 2002 World Summit on Sustainable Development, once again included the significance of transportation. In the context of infrastructure, public transit systems, commodities delivery networks, affordability, efficiency, and ease of transportation, as well as enhancing urban air quality and health and lowering greenhouse gas emissions, JPOI offered several anchor points for sustainable transportation.

The 21st century widely acknowledges the indefensibility of using fossil fuels due to the ongoing shortage of biofuels, making substitutes for petroleum-derived fuels and chemicals increasingly necessary. Every day, there is more demand. The resources that are now accessible are dwindling quickly and are likely to disappear shortly. In such cases, renewable energy must be given greater consideration. Fossil fuels are widely utilized worldwide but unsustainable since they raise CO₂ levels and build up greenhouse gases that harm the ecosystem. Renewable and ecologically friendly



fuels must be developed to preserve sustainability and keep the environment clean (Schenk, 2008). 20% of the world's total energy consumption is attributable to the transportation industry. Even though they make up only 5% of all bioenergy consumption today and only 3%-4% of all road transport fuel (József Popp et al., 2016). In the long run, next-generation biofuels are likely to require the most capacity growth and financing, despite severe competition from other renewable energy sources. While most of the attention is focused on liquid biofuels for transportation, globally, only a small amount of biomass is now utilized for biofuel production.

There have already been According to (Dufey, 2006), biofuels are liquid fuels derived from the biomass of various crops. Biodiesel, an alternative to petroleum-based diesel, is currently being generated from cellulose, algal lipids, corn, soy, sugar cane, camelina and jatropha, rapeseed, methane, animal fat, and forest products. They are also characterized as the biodegradable fraction of industrial waste. Vegetable oils (Shay, 1993), biobutanol (Dürre, 1997), *Jatropha curcas* (Becker and Makkar, 2008), and algae (Roessler et al., 1994; Sawayama et al., 1995; Dunahay et al., 1996; Sheehan et al., 1998) are all used in the production of biodiesel. The top three countries in the world for producing biodiesel are Brazil, the US, and the EU (Balat, 2007). 35 billion liters of biofuel are anticipated to be produced annually (O European Commission, 2006) However, the widespread cultivation of these crops for the generation of biodiesel is putting the local economy and food supply at risk, raising the cost of food and commodities globally. As a result, efforts are being made to find A feedstock for biodiesel made from non-food, non-terrestrial materials like microalgae.

Their ability to develop without much attention to waste nutrients makes them a superior source of biodiesel production compared to other sources, which generally consist of food-producing plants (Patil et al., 2008; Roberts, 2013). Microalgae are a possible alternative source because they effectively use photosynthesis to transform light energy into chemical energy that is then converted into organic compounds like lipids and carbohydrates. Carbon dioxide (CO₂) found in the atmosphere is used to make these molecules. Diatoms (Bacillariophyceae) are a type of algae that fix a significant portion of the CO₂ in the ocean, ranging between about 41% and 50%. (Field et al., 1998; Williams and Laurens, 2010). In some circumstances, microalgae synthesize secondary metabolites (Mimouni et al., 2012; Gordon and Seckbach, 2012; Bhuyar et al., 2019a; Heydarzadeh et al., 2013; Spolaore et al., 2006). Algal cells contain 30% more lipids than other sources, such as soybean and palm oils (Lam and Lee, 2012; Kligerman and Bouwer, 2015). They may thrive in dry, semiarid, or desert environments and can even grow on non-arable terrain. In comparison to other terrestrial crops, they also need less water for growth and survival (Yeang, 2008). Unlike other crops, which are frequently only harvested once or twice a year, algae may be harvested all year round (Chisti, 2007). Compared to petroleum, algae contain about 80% more energy (Chisti, 2007; 2013). Algae can effectively identify and remove hazardous elements from water, aiding in the clean-up of wastewater.

Investigating the process of using algae to produce new types of biofuels can help solve this issue. Eukaryotic photosynthetic algae may be found in a range of habitats. Algae are photosynthetic organisms that use carbon dioxide from the environment and solar energy to create their biomass (Demirbas, 2010). They range in size from microscopic to enormous kelps, in number of cells from one to many, and in form from spherical to filamentous. Algae are seen favorably for the manufacture of biodiesel because of several benefits, including high photosynthetic efficacy, rapid growth, and high biomass productivity (Amaro et al., 2011; Demirbas and Demirbas, 2011).

Since microalgae, like diatoms, are among the biological groups most susceptible to hydric stress, they are useful markers of previous hydrological conditions. To improve the direction of stream management initiatives, benthic diatom communities are crucial for the development of predictive models for water quality. With microalgae serving as the main feedstock for biodiesel, there is a lot of potential for applications. Diatoms need high water or at least high humidity (Evans, 1960). Therefore, we require either an extra water supply for the panel or a water-impermeable chamber. Desiccation does not destroy diatoms, and in fact, it enhances the output of oil (Evans, 1958; Evans, 1959). Because cells can survive longer when drying more slowly (Evans, 1959). They are appropriate sources to be cultivated on a large scale due to their remediating and bioindicator roles in wastewater treatment and their high sources of biodiesel (Pittman et al., 2011; Kligerman and Bouwer, 2015).

The finite quantity of fossil fuels on earth has been under tremendous strain due to the exponential rise in energy use and the expanding global population. It is possible to produce energy (biofuel) from algal lipids using a sustainable biological process. Algal lipid formation is a naturally occurring

process; however, water pollution makes it worse. Point-source pollution is defined as pollution that comes from a single source, such as air deposition, runoff from agricultural or industrial stormwater systems, residential and industrial wastewater, and non-point source pollution.

Additionally, anthropogenic sources are those that were produced by humans. Prospects are bright for the production of algal-based biofuel, which is a cost-effective and ecologically responsible energy source. The present state of algal biofuel research is discussed in the current publication. We looked at the number and biovolume of lipid bodies (LBs). In areas with severe metal pollution, eight distinct diatom species exhibited considerably larger numbers and biovolumes of large bodies (LBs) compared to all other studied diatom taxa identified in Khetri and Zawar. Conversely, these diatom species in the less polluted areas showed noticeably reduced LB counts and biovolumes. Under Cu stress, the genera *Navicula* and *Nitzschia* showed considerably higher lipid body induction (in both number and biovolume), a finding in line with earlier research (Pandey and Bergey, 2016; Pandey et al., 2015). Lipid bodies are often stored by diatoms as a reserve food source, and these bodies may become more noticeable in response to certain kinds of stress (Ramachandra et al., 2009), especially deprivation from nitrogen (Jiang et al., 2012). The concentration of CO₂ and Fe⁺³ determined the fatty acid chain's length and degree of unsaturation. The combination of low Fe and 2% CO₂ created the ideal environment for the synthesis of short carbon chain FA and the accumulation of large amounts of SFA. According to (Carpio et al., 2015), This work presented the possibility of adjusting the quantities of CO₂ in aeration and Fe⁺³ in the growth medium to change the lipid content of the freshwater green alga, *C. vulgaris* Beij.

These lipid bodies investigations will also help establish diatoms as a tool for biofuel production. Due to flue gases like CO₂ concentration increasing in the environment, the algal biomass increases. The algae lipid may be converted into energy (biofuel) using the sustainable biological method. Lipid production occurs naturally in algae but it is increased by water pollution and climate change. The purpose of this work is to examine how different aspects of climate change affect algae's capacity to synthesize lipids. The likelihood of producing biofuel energy from algae cells may be increased using a sophisticated technique for boosting biofuel production. This study summarises the most recent theories about the production of biofuels using algae. It details each phase, scientific breakthroughs, current issues, and recommendations for further research aims and objectives.

Potential feedstock for biodiesel production generation by generation

First-generation

Food crops are used directly to make first-generation biofuels, the most commonly used first-generation biofuel feedstock. 1. Most of the corn used to make gasoline-ethanol in the world originates from the United States. About one-fourth of the nation's petrol needs could be met by maize. Rejected – It is good food and it becomes costly by utilizing it as biofuels (Khammee et al., 2020), which leads to famine all over the world and increases the demand for additional pesticides and fertilizers, which is not only expensive but also pollutes the soil and water. Sugar cane the majority of bioethanol is produced in Brazil, which is one of the biggest customers for the product and the country's second-largest producer after the US; however, just 1% of Brazil's fertile land is used for sugar cane farming. However, due to its monocot status need for a certain environment, and lots of sun exposure, sugar cane is not a crop that can be cultivated in most of the world. As a result, most nations are unable to produce sugar cane for bioethanol. Soybeans are grown across most of North America, South America, and Asia, unlike corn and sugar cane. Rejected: According to growing soybeans typically require more energy than can be obtained from their fuel. The amount of farmland utilized worldwide for biofuels is presently between 30 and 35 million hectares or around 2%. The amount of land needed to grow feedstocks is reduced to 1.5% of the total cropland by substituting coproducts for grains and oilseeds (József Popp, et al., 2016). Biofuel is created from agricultural oil crops like soybean and oil palm; however, the yield is only about 200 barrels (30,000 L) of algal oil per hectare of land when oleaginous algae are mass-cultured, which is 100–200 times more than soybean oil.

Second generation

Scientists developed second-generation biofuel to lessen the issues first-generation biofuel had. Non-food crops are used in second-generation biofuel for example Two categories serve as the major divisions for second-generation biofuels. 1. Homogeneous 2. Non-homogeneous. Whitewood chips and agricultural and forestry waste are examples of homogenous materials. Non-homogeneous materials include low-value feedstock and municipal solid waste, according to (Lee and Lavoie (2013).

- A major reason for the rejection of first and second-generation biofuel sources-
1. The land and water are contaminated by using more pesticides and fertilizers.
 2. The cost of food rises, leading to global food scarcity. Shows in Fig 1.
 3. One of the causes of the hunger crisis is a decrease in agriculture.
 4. Due to the high cost of its growth and harvest, its price is likewise quite high.

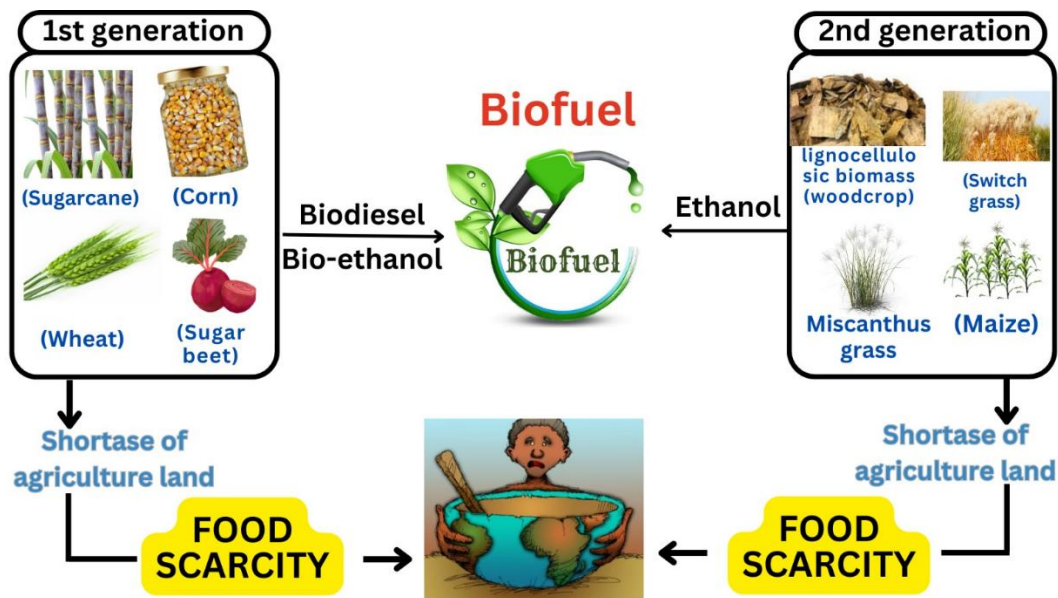


Fig.1. This image shows food scarcity due to the use of first- and second-generation biofuel products

Food versus fuel

Grain reserves are finite, as is the amount of arable land on Earth. Wheat is usually kept in storage for thirty days at a time. Prices decrease and there is a glut when the supply lasts 33 days; at 27 days, prices soar. The limited amount of grain currently being utilized for biofuel mostly oilseeds, sugarcane, and maize has caused a chain reaction that has doubled the price of all grains. There won't be much grain available for emergency food assistance, and this will quickly spread throughout the food chain, doubling the price of all foods. In addition, burning grain to power expensive cars when starving people raises bioethical concerns. The developed world is getting close to the highest yields that are practically possible. By using more fertilizer and pesticides, yields could be somewhat increased, but this would not be very cost-effective and is frequently not ideal for the environment. The developing nations who practice subsistence agriculture and have yields that are below the third world average will need to provide the long-term yield improvements necessary to sustain (strictly speaking) human nutritional needs (which they reduce down).

With grain subsidized from the West and sold below production prices (referred to as "dumped" in economic parlance), these emerging regions should be able to produce at a level of competitiveness thanks to the doubled grain prices. How rapidly this turnaround can occur is an interesting subject. It appears that governments in developing nations should handle this directly, as was recently done in Malawi, rather than relying on international help, which is always accompanied by conditions.

Third generation

However, the renewable source of biodiesel that can supply all of the world's transportation fuel needs in the coming years will be microalgal biodiesel. A wide variety of aquatic conditions, from freshwater to brackish water, support the growth of microalgal species. Microalgae are capable of effectively consuming CO₂ and account for between 35% and 40% of global carbon fixation (Bellou et al., 2014; Ramaraj et al., 2015). Marine-based microalgae are commonly believed to produce CO₂ sequestration. The greatest choice for biofuel among these possibilities is algae. All microalgae can accumulate lipid bodies within their cells that are rich in energy-rich bio-oils (Bhuyar et al., 2019a, b). For example, it has been shown that some *Botryococcus* species retain up to 20% to 45% of the lipid content as long-chain hydrocarbons of their dry mass (Gerken et al., 2013). Several thousand species of algae, including diatoms, have been investigated for their high lipid content. (Guliyev et al., 2001; Sheehan et al., 1998; Sommerfeld et al., 2008; Imahara et al., 2006). Polyunsaturated fatty acids, which account for around 25% of the mass of algae, have been determined to have a lower average melting point than saturated fats over the previous few decades. Historically, it has been believed that a significant portion of third-generation biofuels comes from single-celled algae

known as diatoms. Two frustules and a variable number of girdle bands make up the transparent diatom silica shell, which shields the oil droplets within and absorbs the light required for their formation (Round et al., 1990; Cox et al., 1996). Diatoms are unique in that lipids may be taken from them without causing harm, just like cow milk can be without causing death, due to their hard siliceous cell wall (frustule). (Ramachandra et al., 2009) named this process of removing lipids from diatoms "milking" diatoms. Microscopic cyanobacteria and eukaryotic algae comprise microalgae. Compared to conventional oil seed crops, these algae have the potential to produce significantly more biodiesel while using quite less water and agricultural area. The expectation that biotechnology methods based on microalgae will yield higher productivity than any cultivated agricultural plant per unit surface area of Earth is another reason to be interested in them (Cadoret and Bernard., 2008, Chisti 2007). For example, it is estimated that the production of diatom oil will be two to six hundred times more per unit surface area than oilseed crops (Demirbas, 2009) shown in Fig. 2.

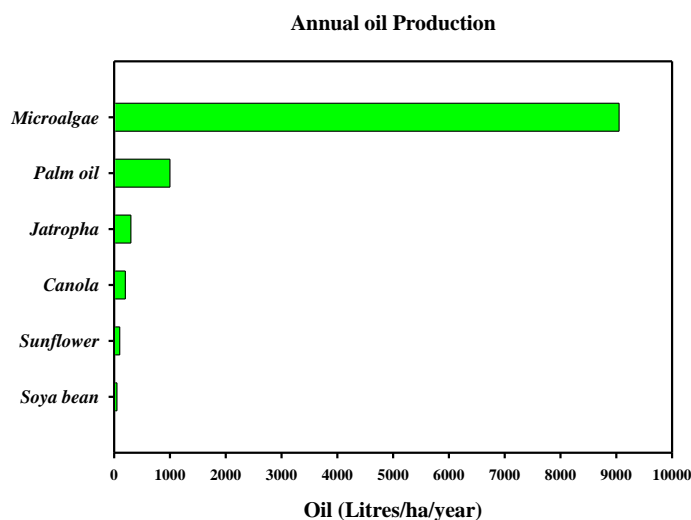


Fig. 2. Potential oil yield per hectare per year (adapted from Emily, 2009)

While algae have been cultivated since the 1950s, mostly for use in pharmaceutical products, their capacity to produce biofuels has only recently come to light. Algae, which produces a significant amount of biofuel, can produce biomass in marine and freshwater environments (József Popp et al., 2016). Algal biofuel doesn't contain any dangerous compounds, therefore after burning, the environment may be maintained clean. However, producing even 1 kg of algae biomass in a lab setting is difficult. We are aware that algae fix 40% of the organic carbon in the planet, and that using algae for biofuel will significantly influence both the world's primary productivity and the quality of its air. If we can solve all of the aforementioned issues, the diatom is a fantastic source for the manufacture of biofuel. But regrettably, the diatom family is declining, there are several reasons for this, the primary ones being water pollution and climate change. Numerous factors can contribute to water pollution. Of all the types of water pollution, metal pollution poses the greatest threat to freshwater diatoms like *Navicula*.

Mechanism behind lipid production in diatoms stress conditions

As with other microalgae, diatoms store lipids in oleosomes (Yatsu et al., 1971). Also referred to as spherosomes, lipid droplets, lipid bodies, oil droplets, etc., diatoms' oleosome count increases under stress as do green algae, as shown by (Davidi, L., Katz, A., and Pick, U. 2012). The endoplasmic reticulum and chloroplasts mediate lipid synthesis in diatoms. As is generally believed by numerous intricately linked events involving the endoplasmic reticulum, it is most likely fatty acids cannot travel directly from chloroplasts to oleosomes, much like in higher plants (Heydarizadeh et al., 2013). The proteome study of isolated oleosomes from the diatom *Fistulifera solaris* JPCC DA0580 has provided the first evidence supporting this theory. This investigation has identified one particular protein that exhibits a domain similar to that of the quinone protein alcohol dehydrogenase (Nojima et al., 2013). It was found that the protein used a fluorescent tag to target the endoplasmic reticulum, where it may play a role in forming oleosomes (Maeda et al., 2014). Exocytosis may be the mechanism by which oleosomes are transported, as seen in the Chlorophyceae alga *Dunaliella salina* (Zhang et al., 1993). It is sufficient to note that the evolution of diatoms has involved multiple endosymbiotic events, such as those involving cyanobacteria and red algae (Moustafa et al., 2009) as well as a chlamydial invasion (Becker et al., 2001), to convince one of this intricacy. Diatoms were able to establish themselves in a variety of ecological settings, such as freshwater, brackish, marine, and hypersaline settings that varied in terms of pH,

temperature, and nutrient availability, thanks to the gene enrichments produced by these events (Armbrust et al., 2004). Some people might be categorized as extremophiles (Kociolek, 2007; Sternburg et al., 2007).

Their capacity for colonization is a reflection of their highly flexible metabolism, which enables them to adjust to a wide range of environmental restrictions (Berth et al., 2001; Nguyen-Deroche et al., 2012; Masmoudi et al., 2013; Rohacek et al., 2014). Metabolic changes, such as the synthesis of secondary metabolites, are frequently a part of long-term adaptation mechanisms (Sharma et al., 2012; Darko et al., 2014). The algae "interpret" stress conditions like salinity (Cheng et al., 2014), nutrient deficiency (Gacheva and Gigova, 2014), temperature, and high light stress (Hasunuma et al., 2014) as "dangerous," leading them to accumulate high-energy molecules like lipids and carotenoids (Lemoine and Schoefs, 2010; Sharma et al., 2012; Cheng et al., 2014; Maeda et al., 2014). For example, it has been demonstrated that diatoms can produce twice or three times as much oil when under stress due to silicon or nitrogen depletion (Burrows et al., 2012; Taguchi, S.; Hirata and Laws, 1987; Zhang et al., 2014). Algae may produce thick mucus sheaths through desiccation, which are frequently discovered to contain oil or starch (Badour and Gergis, 1965). Diatoms can store energy as lipids or as chrysolaminarin (Beattie et al., 1961); therefore, we'll need to figure out how to bias production in favor of oil. While algae with low oil content, like *Dunaliella*, divide more quickly and can be collected every day, High oil content algae (e.g., *Botryococcus*) mature slowly and are harvested only seldom. Because of this, the majority of industrial applications employ algae strains with a lipid concentration of 20% to 40%. To maintain a high rate of division, diatoms can rely on their greater ability to fix CO₂ than other phytoplanktonic groupings (Thomas et al., 1978).

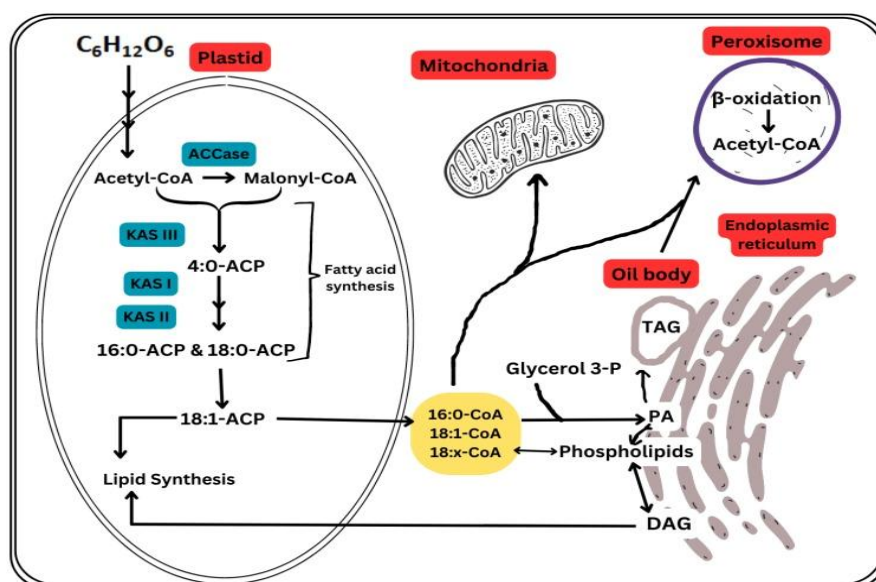


Fig.3. This figure shows how CO₂ fixation product glucose is used for the lipid biosynthesis

One of the main forces behind economic growth is energy. Because fossil fuels are running out and have negative environmental repercussions, humanity needs to develop sustainable and renewable energy sources. Carbon is an essential ingredient that controls microalgae growth and function. Different pathways are used by microalgae cells to assimilate various carbon sources. The CO₂-concentrating mechanisms (CCMs) mostly use inorganic carbon sources, while microalgae primarily absorb organic carbon sources via the Embden-Meyerhof-Pranas (EMP) and pentose phosphate pathways. Thus, microalgae mostly use the Pentose Phosphate Pathway (PPP) and the Embden-Meyerhof-Pranas (EMP) Pathway to absorb the excess carbon input. As a result, the generation of microalgae biomass and lipid accumulation are significantly impacted by the addition of carbon sources (Xiangmeng et al., 2022). The processes of microalgal lipid synthesis and carbon absorption were described in this work; this process is shown in Fig. 3, and the effects of different carbon conditions (forms, quantities, and addition processes) on lipid formation during the production of biodiesel and microalgal biomass were extensively explored.

The potential possibilities for the manufacture of biodiesel are also highlighted in this analysis, along with recent developments in the large-scale commercialization of microalgae lipid culture. Regarding the cost-benefit analysis of producing microalgae biodiesel on a wide scale. Practical solutions are suggested as well as current obstacles. The effectiveness of CO₂ collection depends

on the kind of microalgae, the biochemical makeup of the nutrient medium, and environmental factors including light, humidity, and pH. This efficiency is influenced by the design of the developing system (open system/photobioreactor) (Nath et al., 2023). Microalgae may be cultivated in open or closed environments and need nutrients and carbon dioxide, which can come from burning fossil fuels or sewage. To quickly fix carbon into microalgae, researchers are also interested in CO₂ collection through photosynthesis and how to use CO₂ in different ways for humans. In these reviews, topics like genetic engineering and metabolic changes to improve CO₂ capture are covered, along with photosynthesis, CO₂ fixing, and culture tactics for microalgae.

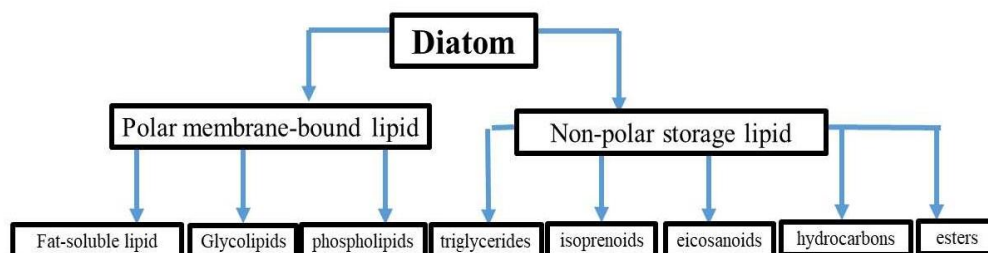


Fig. 4. Classification of diatom lipids

Table 1. Table showing the type of fatty acids found in different diatom species

Type of fatty acid found in different diatom species							
Species	Linolenic	Oleic	Stearic	Palmitic	Linoleic	Palmitoleic	reference
<i>Chlorella vulgaris</i>	21.3	18.7	14.1	5.6	-	5	Velasquez-Orta et al. (2012)
<i>Spirulina platensis</i>	17.79	4.11	1	41.21	12.64	3.39	Nautiyal et al. (2014a,b)
<i>Scenedesmus sp.</i>	8.26	49.64	3.43	18.42	11.3	2.31	Chen et al. (2012a,b,c)
<i>Nannochloropsis salina</i>	0.58	37.52	2.53	13.49	14.49	12.99	Patil et al. (2011)
<i>Caulerpa peltata</i>	-	5.04	4.58	36.82	18.19	5.04	Suganya et al. (2014)
<i>Enteromorpha compressa</i>	-	2.38	2.95	70.26	-	3.71	Suganya et al. (2013)

Fatty Acid Profile

Algal cells contain tightly packed lipid particles called TAGs in their cytoplasm. Saturated fatty acids such as stearic and palmitic acids and unsaturated fatty acids such as palmitoleic, oleic, linoleic, and linolenic acids make up the oil found in algae shown in Fig. 4. Most of the fatty acids in *Scenedesmus obliquus* oil are saturated and monounsaturated, according to Mandal and Mallick (2009), which provides biodiesel and generates a high level of oxidative stability. Table 1 compares the fatty acid content of biodiesel produced from several types of algae. Arachidonic acid, three polyunsaturated acids, docosahexaenoic acid, eicosapentaenoic acid, and algae are known to be generated in large quantities and may provide a problem for the generated biodiesel's stability (Frankel et al., 2002). Because the oil contains more unsaturated fatty acids, it lowers the pour point and cloud point of the biodiesel, improving its cold-temperature properties (Serdari et al., 1999; Stournas et al., 1995).

For biodiesel to function effectively, it needs to contain the right ratio of saturated and unsaturated fatty acids. This is because biodiesel made from highly unsaturated acids (polyunsaturated) loses stability. After all, it oxidizes more quickly than regular diesel and forms insoluble fragments.

The Processes for Biofuel Production Using Algae vs. Crop Plants (First- and Second-Generation Sources)

Algae offer a straightforward way for extracting fatty acids, and the process used to separate biodiesel on a small or experimental scale is mixing. While drying crops and other food-producing plants requires energy, drying algae with sunlight is more cost-effective. In contrast to other plants, the thermochemical drying process in algae is also simple (Banerjee et al., 2002; Tsukahara and Sawayama, 2005), as shown in Fig. 5.

Algal biomass gathering and algal density

Field samples were weighed for their entire dry weight. Microalgae were quantified per unit area/volume using a 1 m x 1 m quadrat and a 10 volume. A quadrat measuring 1 m x 1 m was used

to gather the macroscopic algal biomass from the lakes. At this location, these floating algae were thoroughly cleansed before being taken to the lab for further separation. The samples were carefully cleaned with deionized water following microscopic examination, and they were then concentrated by centrifuging additional lipid extraction. After carefully scraping the pellet with a spatula, it was let to air dry at room temperature. In case they were needed again, the samples were preserved.

Table 2. An overview of how the FA profiles of marine animals have changed after being exposed to pollutants. Free fatty acids are denoted by FFA, saturated fatty acids by SFA, monounsaturated fatty acids by MUFA, and polyunsaturated fatty acids by PUFA. N/A indicates unavailable; N/I indicates little or no change in different diatom species.

Contaminants of exposure (time/concentration)	Contaminant's mode of action	Species name	SFA	MUFA	PUFA	References
Triazine 100 and 150mg/l atrazine	The quality of the photosynthetic activity of diatom cells is impacted by interference with the function of photosynthesis in plants, including some algae.	<i>Seminavisrobusta</i>	N/I	N/I	N/I	De Hoop et al., (2013)
1,2,4-Trichlorobenzene (used in the production of organochlorine pesticides) 0.245mg/l 5days	over prolonged exposure periods, causes the most changes in morphology and fatty acid composition.	<i>Cyclotella meneghiniana</i>	16:0, 15:0, 18:00	16:0	20:05	Sicko-Goad et al. (1989a)
1,3,5-Trichlorobenzene: this chemical is utilized to make pesticides that contain chloroform. 0.245 mg/l five days	generates the highest amount of morphological and fatty acid composition changes in 24 hours. modifies the ability of photosynthetic reaction and reduces cellular lipid stores	<i>Cyclotella meneghiniana</i>	16:00, 18:00, 14:00	18:01	20:05	Sicko-Goad et al. (1989b)
1,2,3-Trichlorobenzene: this chemical is used to make insecticides that contain chloroform. 0.245 mg/l five days	The steady rise in lipid volume, along with a reduction in the so-called "fibrous" vacuole and less notable alterations in the composition of FAs	<i>Cyclotella meneghiniana</i>	18:00	16:01	0.83339 1204	Sicko-Goad et al. (1989c)
Pentachloro benzene, which is a raw material for organochlorine insecticides 0.245 mg/l for five days	causes alterations in diatom Lipid volume in diatoms is increased by FA content and cell shape.	<i>Cyclotella meneghiniana</i>	15:00, 18:00	18:01	20:05	Sicko-Goad et al. (1989d)
Chloroacetamide herbicide: metolachlor 20um added once	Inhibition of long-chain fatty acid biosynthesis	<i>Melosira cf. moniliformis</i>	14:0, 16:0, 18:0	6:1(n-7) 18:1 (n-7)	16:2, 16:3, 16:4 18:4 (5,8,11,14) 18:5 (5,8,11,14,17)	Robert et al. (2007)
PCBs, or polychlorinated biphenyls, N/A	Growth suppression and alterations in the FA profile	<i>Thalassiosira pseudonana</i>	16:00	16:01	N/A	Fisher and Schwarzenbach (1978)

Why are diatoms used as biofuel?

When we make biofuel from crops, the crop is harvested only once and gets destroyed; if we produce biofuel from algae, it can be reused. Ramachandra et al. (2009) mentioned in their review that just as a cow does not have to die to extract milk from it, similarly, lipid extraction can be done from algae (diatoms) without killing them. Ramachandra et al. (2009) compared the process of

extracting dairy milk to crushing cows and is accepted as inevitable. For example, many organisms, including photosynthetic microalgae, employ the same inputs. But unlike cows, you cannot milk them. It would help if you destroyed them (Lane, 2015).

The demand for nitrogen/phosphorus fertilizers would be decreased or eliminated if only hydrocarbon high-value molecules (HVM) were milked (Rickman et al., 2013). The idea behind milking is that the cells shouldn't be killed during extraction. Because of this, milking eliminates the requirement for periodic culture and reestablishing of the entire algae stock, which usually takes a few hours to several weeks.

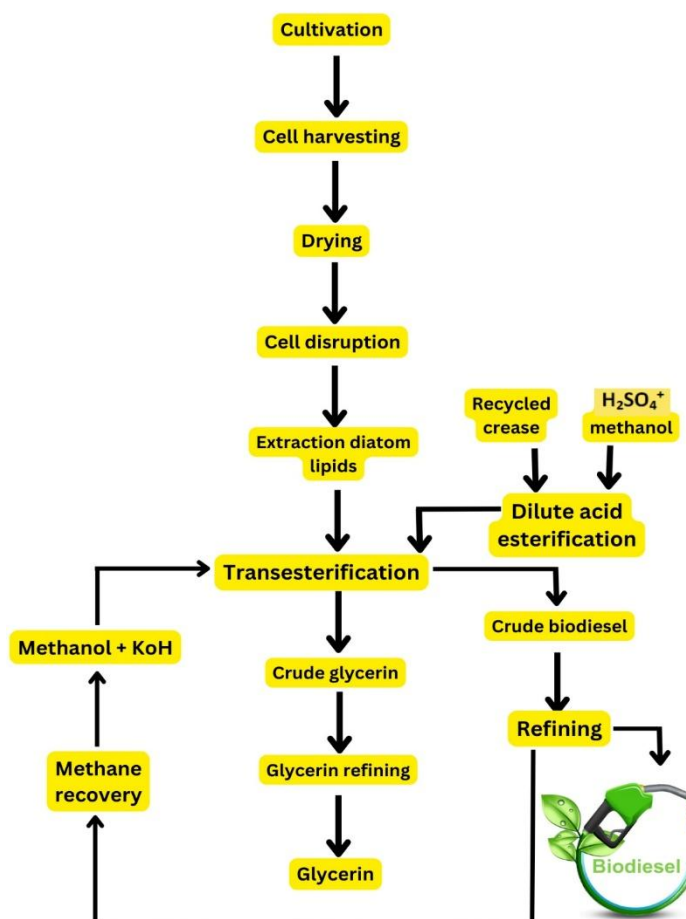


Fig.5. Process of extraction of biodiesel from algal lipid

What we now refer to as extraction was referred to as "milking" in some of the literature because the distinction between extraction and milking was not made (Zhang et al., 2011). Likewise, secretion and extraction were formerly included in the category of "milking" (Yadugiri et al., 2009). We believe that rigorous differentiation between these approaches will facilitate more fruitful discourse.

When provides some food for thought regarding the progress of microalgae milking, specifically concerning diatoms, in the article. The main lines of reasoning that we have outlined to reach our goal are (a) the creation of substitute methods for harvesting and extracting HVM; (b) the construction and management of photobioreactors; (c) biochemistry; and (d) diatom (stress) physiology. In this contribution, they are addressed individually and, if feasible, accompanied by unique findings on the accumulation of lipids by diatoms. We call these algae "oleaginous diatoms." While most of the topics included in this contribution have been covered in recent reviews (Hildebrand et al., 2012; Hasunuma et al., 2014), none of them specifically addressed diatoms and milking.

Algal oil extraction

The secret of milking is to extract HVM without destroying the cells that produce it. In the case of higher plants, this idea was initially used in the milking of rubber 2000 years ago (Ciesielski 1999); maple syrup both historically (Svanberg et al., 2012) and prehistorically (Nearing and Nearing, 2000;

Munson 1989); turpentine dating back to Hippocrates (Haller, 1984); and more recently, halophilic bacteria (Saver and Galinski 1998; Van-Thuoc et al., 2010); and microalgae. Biocompatible solvents were employed in the initial applications with algae to extract HVM (Hejazi et al., 2004). Using organic solvents. (Gillet, 2015) recently computed the plasma membrane's molecular dynamics during milking. This section's goal is to offer a thorough examination of these options within the context of a milking strategy. Although heating and microwaving can cause damage in moderation, we do not think about them (Ghasemi Naghdi et al., 2014). However, they can also cause milking.

Pulsed Electric Field

Diatoms and other microalgae have been electroporated, albeit mostly to enable genetic change. (Dunahay et al., 1992; Coll, 2006; Leon and Fernandez, 2007; Miyahara et al., 2013; Gao, 2014; Zhang, 2014). On the other hand, high-value compounds can also be released from cells more favorably by applying trains of electric pulses. Using this technique, electric pulses pierce cell membranes, causing the constituent parts of the cell to leak out. The technique was developed initially with yeast (Ganeva et al., 2003; Stirke et al., 2014) and more recently with photosynthetic organisms such as microalgae. (Coustets et al., 2013; Coustets et al., 2015) and cyanobacteria (Sheng et al., 2011). The "punctured" cells subsequently recover and continue to be alive, allowing the same batch of algae to be used again to extract more HVM (Reep and Green, 2012). Electric pulses can cause irreversible electroporation in bacteria (Joubert et al., 2013) algae (Antezana Zbinder et al., 2013) and mammal cells (Deipolyi et al., 2014). Therefore, choosing the electric pulses' length, intensity, polarity, repetition frequency, and other parameters requires striking a balance between milking efficiency and algal survival.

Physical parameter adjustment is important since the strength of the electric therapy is directly proportional to the size of the cell, meaning that a smaller cell will have a greater effect. (Coustets et al., 2013; Sixou and Teissie, 1990; Bellard and Teissie, 2009). Diatoms, or microalgae, are beneficial for biofuel generation because of this.

Spontaneous oozing

There are now confidential reports indicating that certain bacteria (Tsukagoshi et al., 1983; Raetz, 2001; Wald, 2015), green algae (Frenz et al., 1989), and cyanobacteria that have undergone genetic engineering (Liu et al., 2011; Joule, 2012; Robertson et al., 2011; Liu et al., 2011; Reppas and Ridley, 2010) can secrete lipids (Ladd and Venter, 2010) from their cytoplasm into the surrounding environment. According to Vinayak et al. (2014), the mechanics underlying oozing are still unknown. The droplets gather in the cytoplasm (oleosomes) or the chloroplasts (plastoglobules).

Mechanical Pressure

One may infer that applying HVM leakage from algal cells could be caused by mechanical pressure, such as ultrasonic or tactile stimulation. that don't have a built-in oozing mechanism. Ultrasound has been employed in procedures to enhance lipid extraction of *Chlorella vulgaris* (Araujo et al., 2013), like electric pulse treatment (Rosello-Soto et al., 2015).

The ultrasonic treatment's parameters should be selected in a way that maintains the cells' viability and qualifies them for use in the milking process. For example, when comparing the yield attained without treatment, Araujo et al. (2013) reported that *Chlorella vulgaris* treated with ultrasound significantly improved in terms of lipid recovery. However, since only a slight improvement was noted, the treatment's effectiveness depends on the strength of the cell wall. Diatom cells are special in that they have a cell wall made of hydrated silicon dioxide, known as the frustule. The forms and embellishments of frustules vary greatly (Sterrenburg et al., 2007; Round et al., 1990).

Additionally, one valve can fit inside the boundary of the other since one frustule is somewhat larger than the other. each diatom possesses defective bilateral symmetry. This, along with the frustule's resilience, makes mechanical approaches potentially one of the most potent ways to encourage the release of HVM. Testing the oozing capacity of more diatoms with an apical pore field (Kocielek and Stoermer, 1988) would be intriguing. We can presume that the force needed to shatter an isolated diatom valve is significantly less than the force needed to exert oil on a live diatom, even if this force has not yet been measured. The mechanical properties of several diatoms under tensile and compressive loads were published by (Hamm et al., 2003). Additionally, they mentioned that an isolated diatom valve had a breaking force of 750 μN .

Centrifugation

We still don't know if the centrifugal force that kills them is less than the one that releases oil. In that case, centrifugation might be used as a method of milking. Algal separation is now accomplished by centrifugation (Abodeely et al., 2013). Lipids ascend to the centripetal end of sea urchin eggs centrifuged at 9000x g and exert sufficient buoyancy to split the egg in two (Anderson, 1970).

Fatty acid composition using GC–MS

The component of fatty acids was assessed using mass spectrometry (Agilent Technologies 5975C insert MSD with triple-axis detector) and a gas chromatograph (Agilent Technologies 7890C, GC System). The injection and detector temperatures were maintained at 250°C and 280°C, respectively, in accordance with ASTM D 2800. A 1 ml injection of sample was made into the column, which had a starting temperature maintained at 40°C. The oven temperature was raised at a rate of 10 degrees Celsius per minute after one minute. Afterward, the oven was heated to 230°C at a rate of 3°C per minute, and then, at a rate of 10°C per minute, to 300°C, where it remained for two minutes. A silica column was loaded with the methylated sample utilizing split-free helium gas as the carrier. 47.667 minutes was the calculated run time. By comparing the obtained retention period to that of established standards, fatty acids were identified.

Lipid extraction and biodiesel production

After ethanol manufacturing, the wasted solids underwent the Soxhlet extraction procedure to separate the lipid for biodiesel synthesis. Less than 2% of the lipid recovered from algal biomass contained free fatty acids, it was discovered. To create a sodium methoxide solution, 50 ml of methanol was mixed with around 0.5 g of NaOH pellets to create biodiesel from the obtained lipid. Algal oil was next carefully incorporated with the sodium methoxide solution.

Conclusion

Microalgae are one of the most effective third-generation generating organisms at turning solar energy into chemical power, which is then utilized to absorb and transform atmospheric carbon dioxide into biomass. The principal source for biodiesel, microalgae, has demonstrated excellent application potential. The upstream cost limits lipid output from microalgae, which prevents large-scale biofuel production from being realized. The focus of attempts to boost microalgae's lipid content and productivity over the past few decades has been on altering lipid-rich microalgae cells. Fatty acids may be extracted from algae using a straightforward process, and biodiesel can be separated on a small or experimental scale by mixing. While drying crops and plants that produce food requires energy, drying algae with sunlight is more cost-effective. In contrast to other plants, the process of thermochemical drying is also simple in algae. This work aligns with the Sustainable Development Goals (SDGs) 2030. Producing biofuel from microalgae (diatoms) instead of crops allows agricultural land to be used for food cultivation. This supports SDG Goal 2 of the 2030 Agenda, which aims to end hunger, ensure food security and improved nutrition, and promote sustainable agriculture.

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Author Contributions

S, APS, VKS, LKP and JNM conceived the concept, wrote and approved the manuscript.

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