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Algae-based biofuels: Hope for future energy

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Abstract
A very high oil percentage in algal biomass makes it suitable for use in various conversion processes to produce advanced biofuels. Because of its renewable and ecological qualities algal feedstock has emerged as a promising contender in the research to replace fossil fuels. From this perspective, algae are a particularly promising source of biomass since they can store a large amount of carbon from the atmosphere and industrial emissions. The entire potential for algal applications indicates that this feedstock is currently an underutilized resource and could have a significant positive impact on the global economy because algae are much more prevalent than terrestrial plants. Microalgae will be a strong choice for an alternative energy source for evolution in bioenergy that began with the first generation and continued through the fourth generation. In the current global problems and their negative consequences on the energy and fossil fuel supply chain, the processing of biomass resources, such as algae biomass, into value-added bioproducts and biofuels utilizing innovative technology is highly advantageous. In light of current global events, it is crucial to investigate and advance knowledge on concerns about the potential switch to biomass-oriented products, like products derived from algae. Algae-based biofuels have a lot of potential, but considerable work and obstacles still need to be overcome in this field.

Keywords: Microalgae, biofuels, biodiesel, renewable fuel, sustainability

Introduction

Global energy demand is increasing in correlation with the fast population increase. The three main sources of fossil fuel in the world are crude oil, natural gas, and coal. Our globe will confront an energy shortage because these energy sources are finite and cannot be recycled. On our earth, 1.4 billion people experience daily energy shortages [1]. According to an estimate from [2], the world would need 50% more energy in 2030 than it does now. The main challenge for the global energy system is to achieve complete independence from finite resources without negatively impacting society or the environment. Around 80% of the world's energy needs are satisfied by fossil fuels [3]. Two-thirds of the world's energy consumption comes from liquid fuels derived from petroleum, so when considering energy consumption for transportation, more focus should be placed on renewable energy sources for liquid fuels, such as biofuels made from biomass [4, 5]. Fossil fuels have several advantages, including the production of CO_2, but they also significantly increase atmospheric CO_2 levels, posing serious environmental risks due to global warming. This has been a big problem for both fuel producers and engine makers as CO_2 emissions from the consumption of liquid fuels alone accounted for 36% of world emissions in 2012 [6, 7]. Due to this, it is predicted that global CO_2 emissions would quadruple by 2035 [8] and might surpass 45,000 mega tonnes by 2040 [9]. Due to resource depletion and the accumulation of GHGs in the environment that has already surpassed the dangerously high threshold of 450 ppm CO_2, the usage of fossil fuels is now universally acknowledged to be unsustainable [10].

Algae are a type of unicellular and multicellular organisms that are typically recognized by their morphological and ecological attributes, and they are one of the primary producers in aquatic contexts [11, 12]. Algal photosynthesis produces around half of the oxygen we breathe, and assessing their genomes allows us to reconstruct the evolution of endosymbiosis and horizontal gene transfer (HGT) mechanisms [13, 14]. Some algae are macroscopic and can adhere to rocks or other things (seaweed), developing to lengths of more than 50 m, while others are microscopic and can float in surface waters due to their high lipid content [15, 16]. The fact that algae are a nutrient-rich food has led to their usage in folk medicine, cosmetics, and as a food source. Due to algae's enormous potential, seaweed farming is becoming more widespread, and production of the plant grows by several percent each year.

Farming is a source of profitable trade for the expansion of the economy. The simultaneous operation of two technological processes, namely wastewater treatment and biomass production, demonstrates that algae may grow in nutrient-rich wastewater. Due to health concerns, it is not permissible to use sewage-cultivated algal biomass in food or animal feed, but it is permitted to use it as a raw material for the production of biofuels [17, 18].

According to published statistics, microalgae-derived biofuels perform better than land-based fuels due to several advantages. The cost of producing these biofuels, however, might be a problem. Therefore, methods for producing biomass from algae on a large scale and at a low cost are crucial to the financial viability of this initiative [19]. In the current geopolitical situation, especially in light of the Russian-Ukrainian conflict, the acquisition of new renewable energy sources is evolving into a critical issue. Given the current global issues, particularly the Ukrainian-Russian war and its negative consequences on the fuel and energy supply chain, it is highly advantageous to convert biomass resources, such as algae biomass, into value-added bio-products and biofuels utilizing innovative technology [20]. In light of current global events, it is crucial to investigate and learn more about issues about the potential move to biomass-oriented products, like those derived from algae. The conclusion that can be drawn from all of the above positive attributes of algae is that it is one of the most significant, sustainable, and renewable fuel resources in the world and may also play a critical role in lowering environmental pollution. This article is a study of the literature that considers numerous attributes and characteristics of algae as a potential new ecological feedstock for biofuel generation. This in-depth analysis of the literature is centered primarily on the characteristics of algae, taking into account their accessibility, variables affecting production efficiency, and the difficulties and potential of future algal-based biofuel production. Four-generation biofuels will be briefly described, along with some of their benefits and drawbacks. To overcome these obstacles and advance the development of algal biofuels from a theoretical to a practical stage, we attempt to outline the main obstacles that economically large-scale algae biofuels face in this article.

Biofuels

There is currently a noticeable and very rapid rise in the global human population, which has increased the energy demand. The transition from fossil fuel-based systems to bioenergy and bio-based products is becoming more and more real as a result of human population expansion and other urgent global concerns like climate change, the Russian-Ukrainian war, and the COVID-19 pandemic. The burning of fossil fuels is directly increasing the amount of CO_2 in the atmosphere, which is a significant contributor to the present global warming. Depleting petroleum reserves and greenhouse gas emissions both have a negative influence on the environment. Finding sustainable and environmentally acceptable energy sources for our industrial economies and consumer populations has therefore become increasingly important in recent years. Biofuels, or liquid fuels derived from the biomass of plants, are enticing energy sources since they reduce carbon emissions and the depletion of crude oil reserves. The only way to get out of this obstruction is to find a source of bioenergy that is both economically and sustainably viable. To produce biofuels, which are either liquid or gaseous fuels for the transportation industry, a range of bio-feedstocks are mostly used. According to the reports of Biofuels Annual [21] (2019), bioethanol accounts for 82% of all biofuel production with yearly production volumes of 59.7 billion liters in 2020 and 34.4 billion liters in 2019 [22, 23] respectively, the United States and Brazil are its primary producers. Biodiesel is created by trans esterifying oils or fats, and it is the most frequently used biofuel in Europe [24]. Biofeedstocks or biomass refers to all plant materials that can be produced by photosynthesis. They are biodegradable, continuous, ecological, renewable, carbon neutral over their whole life cycle, and environmentally friendly. They also promote green industries and agriculture. They are suitable for use as motor fuels both with and without slight engine modifications. In the coming decades, it is anticipated that the production of biofuels would increase steadily [25]. According to [26] Dorado *et al*. (2006), biomass energy accounts for 77.4% of all renewable energy sources worldwide or 10.4% of the world's total primary energy supply. The environmental sustainability of biomass production generally determines the spectrum of potential advantages of bioenergy and bio-based products.

Global Scenario of Biofuel

Bioenergy plays a role in the three main energy sectors: Electricity, fuel/heat consumption, and transport energy consumption. Particularly for heat and transport bioenergy/biofuels are the dominant renewable energy type. The main growth of renewable electricity in the past decade has been in wind power, followed by solar power and biomass-based power.

In 2018, only 3.7% of the fuel demand for transport was covered by renewable energy; with most of this being shouldered by biofuels (93%) and the rest provided by renewable electricity [27]. A promising alternative to fossil fuels for sustainable development, biofuels like biodiesel and bioethanol have a tremendous potential to reduce climate change. Environmental pollution policies such as the Paris Agreement and the European Green Deal consider the widespread use of biofuels could importantly contribute to reaching reduction targets of 80–95% for greenhouse gas emissions by 2050 [28]. Many countries, e.g., the USA, Brazil, the EU, and China, have launched biofuel programs to reduce the use of fossil fuels in transport, and it is expected that the global share of biofuels in this sector will reach 17% by 2050 [27]. Renewable primary energy (including biofuels but excluding hydro) increased by around 5.1 EJ in 2021 corresponding to an annual growth rate of 15%, stronger than the previous year's 9%, and higher than that of any other fuel in 2021 [29]. Demand for diesel and gasoil in 2021 was less than 1 Mb/d (3%) below pre-pandemic levels. In contrast, naphtha, and other oil products demand in 2021 were above their pre-pandemic levels [29]. The United States was the largest biofuel producer worldwide in 2021, with more than 40 percent of the global biofuel production. Brazil ranked second, with some 21.5 percent of the world's total production. By comparison, Germany ranked fifth that year, accounting for 3.4 percent of the biofuel production across the globe. Biofuels like bioethanol and biodiesel are currently used in several nations, including Brazil, the United States, Germany, China, Indonesia, and Spain. It is expected that this trend will continue to grow and more countries will use biofuels [30, 31]. Table 1 shows the list of the top 10 biofuel-producing countries.

Table 1: Leading countries based on biofuel production worldwide in 2021 (In petajoules) [31]

S. No.	Countries	Biofuel production (in petajoules)
1.	United States	1,435.8
2.	Brazil	839.5
3.	Indonesia	311.9
4.	China	142.7
5.	Germany	121.2
6.	France	107
7.	Thailand	89.8
8.	Argentina	85.6
9.	Netherlands	84.6
10.	Spain	71.9

Indian Scenario of Biofuels
The demand for petroleum-based products is far more essential and inescapable in a country with a large population like India than it is in other nations. In India, diesel fuel consumption is five times higher than gasoline [32]. According to [33] British Petroleum's statistical review of world energy 2016, India's oil consumption has increased from 180.8 million tons to 195.5 million tons which are 8.1% increase in 2015 when compared to 2014 which is 4.5% of the world's total oil consumption. The availability of large tracts of arable land, rising sugarcane and food grain production that results in excess supply, the technology available to produce ethanol from plant-based sources, and the viability of making vehicles compatible with ethanol-blend gasoline make E20 a national imperative as well as a crucial strategic provision. The cost of ethanol is cheaper than that of gasoline while yet providing equal performance. According to a report by Greenpeace on March 24, 2009 [34], renewable energy can lucratively meet over 35% of power requirements in India by 2030 [35]. India's net import of petroleum was 185 Mt at a cost of US $ 55 billion in 2020-21. The majority of petroleum-based products are utilized in transportation. Hence, a successful E20 program can save the country US $4 billion per annum, i.e. Rs. 30,000 cr. To provide a supportive regulatory and retail environment for the safe and efficient use of ethanol-blended gasoline, various government bodies have acted swiftly. The goal of mixing 20% of the nation's gasoline by 2025 seems achievable and within reach thanks to the recently granted interest subvention incentives for grain-based distilleries. India still has an energy shortage despite its energy generation, which compels the government to work diligently to promote renewable energy sources, which in turn ensure energy security [36].

Table 2: Annual Ethanol Requirement of India in 2025 for E-20 (Billion Liters) [23]

State/Union Territory Requirement	Projected Gasoline Sales	Projected Ethanol
Andhra Pradesh	1.88	0.37
Bihar	1.35	0.27
Delhi	1.47	0.29
Gujarat	2.57	0.51
Haryana	1.56	0.31
Karnataka	3.30	0.66
Kerala	2.44	0.48
Madhya Pradesh	2.16	0.43
Maharashtra	5.29	1.05
Odisha	1.25	0.25
Punjab	1.41	0.28
Rajasthan	2.37	0.47
Tamil Nadu	4.12	0.82
Telangana	1.93	0.38
Uttar Pradesh	5.02	1.00
West Bengal	1.56	0.31
Rest of India	5.24	1.04
Total	45	9.0

First-Generation Biofuels
In addition to reducing CO_2 emissions, "first-generation" biofuels can enhance domestic energy security. However, there are certain issues with the feedstocks' sources, such as the potential effects on biodiversity, land use, and interference with food crops. The oldest source of first-generation fuels are vegetable oils, and raw materials such as trans esterified 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. A "First Generation" biofuel can be blended with petroleum-based fuels, burned in existing internal combustion engines, and distributed through existing infrastructure. It can also be used in existing alternative vehicle technology, such as FFVs (also known as "Flexible Fuel Vehicles") or natural gas vehicles [37]. Almost 50 billion liters are produced yearly in the commercial production of first-generation biofuels [38]. The anaerobic processing of manure and other biomass products yields biogas, another specialized biofuel. However, the volumes of biogas used for transportation are relatively smaller today [38]. But other scientists seem to be dubious about the first generation of biofuels. The main disadvantage of first-generation biofuels is the food-versus-fuel dispute, one of the reasons for rising food prices is due to the increase in the production of these fuels [39]. Therefore, for the abatement of GHG, it is recommended to have more efficient alternatives based on both renewable and conventional technologies. However, questions have been raised about how first-generation biofuels can be produced economically and sustainably.

Second-Generation Biofuels
Since lignocellulosic materials make up the majority of the readily available, inexpensive non-food components from plants, they are mostly used in the production of second-generation biofuels from "plant biomasses." However, due to several technical obstacles that must be removed before their promise can be realized, the manufacturing of such fuels is currently not cost-effective. A prospective source of material for fuels and raw materials, plant biomass is one of the planet's most abundant and untapped biological resources. The simplest form of burning plant biomass is to generate heat and power. However, using plant biomass to make liquid biofuels has a lot of potential. But the generation of biofuel from agricultural waste products may only meet a percentage of the rising demand for liquid fuels. This has generated a great interest in making use of dedicated biomass crops as feedstock for biofuel production [40]. This second generation of biofuels is therefore projected to dramatically reduce CO_2 output, not compete with food crops, and some varieties may even improve engine performance. When commercialized, the cost of second-generation biofuels has the potential to be more comparable with standard petrol, and diesel and would be a cost-effective route to renewable, low-carbon energy for road transport [38].

Third-Generation Biofuels
The emergence of third-generation biofuels necessitated restrictions that first- and second-generation biofuels were subject to, such as complex and expensive manufacturing processes and low economic profitability. These are biofuels made from several kinds of algae, which are themselves raw materials that have many benefits. These benefits range from the ability to be grown and obtained in huge amounts to their low requirements and high environmental tolerance to their

capacity to bind CO_2. They stand out for having a high concentration of high-quality oil and the leftovers can be utilized as fertilizer or as animal feed. Third-generation biofuel has good combustion performance and can reduce atmospheric greenhouse gas emissions. However, compared to petrochemical diesel, the cost of producing it is now relatively high. To ensure that this biofuel is economically feasible to manufacture and compete with diesel on an industrial scale, it is challenging to develop and put into practice strategies to increase the productivity and quality of biomass. Micro biodiesel is the concept of a biofuel that can outperform second-generation. It might arise from industrially processed microalgae or from bacteria that are capable of photosynthesizing. Algal biodiesel is probably going to eventually replace conventional 1G and 2G biodiesel. Multi-stroke diesel engines can operate with 3G biodiesel [41, 42]. This biofuel is ready for the future since it burns efficiently, helps reduce greenhouse gas emissions into the atmosphere, and uses recycled flue gas from industrial settings as a source of inorganic carbon to fuel and hasten cell growth and development. The fatty acid composition of the feedstock, the size and success of the cultivation, the effectiveness of lipid extraction, processing, and harvesting, as well as the transesterification process' operational circumstances, all have a significant impact on the productivity and quality of 3G biodiesels. Microalgae autonomously synthesize and store lipids in chloroplasts and vacuoles regardless of the physiology of their growth and development. Lipid content in the biomass immediately following drying and grinding must be larger than 40.00% to make algal biodiesel economically [43]. Otherwise, industrial agriculture becomes too expensive to be economically viable for the grower, regardless of scale or level of technical sophistication. It should be crucial to either identify or use methods to induce pathways for lipids or screen the fastest-growing, oil-accumulating microalgae to make the use of economically relevant strains in the third generation of biodiesel feasible.

Fourth-generation Biofuels

In fourth-generation biodiesels, photobiological solar energy and electro power are accounted for. Sunlight-based biofuels are produced by converting solar energy into biodiesel using raw materials; this method of conversion is still being researched. The majority of crude materials are simple, plentiful, and affordable. For such a transition, manufactured science is a liberating innovation [44]. An emerging theory for a practical turn of events and fluid fuel stockpiling combines photovoltaic or inorganic water-dividing impetuses with metabolically designed microbial fuel improvement. By combining biofuel production with carbon dioxide capture and storage through oxyfuel burning, or by utilizing genetic engineering or nanotechnology, the fourth generation of biofuel offers more sustainable production alternatives.

Table 3: Comparison of microalgae with other biodiesel feedstocks [45]

Plant source	Seed oil content (% oil by wt in biomass)	Oil yield (L oil/ha year)	Land use (m2 year/kg biodiesel)	Biodiesel productivity (kg biodiesel/ha year)
Corn/Maize (*Zea mays* L.)	44	172	66	152
Hemp (*Cannabis sativa* L.)	33	363	31	321
Soybean (*Glycine max* L.)	18	636	18	562
Jatropha (*Jatropha curcas* L.)	28	741	15	656
Camelina (*Camelina sativa* L.)	42	915	12	809
Canola/Rapeseed (*Brassica napus* L.)	41	974	12	862
Sunflower (*Helianthus annuus* L.)	40	1070	11	946
Castor (*Ricinus communis*)	48	1307	9	1156
Palm oil (*Elaeis guineensis*)	36	5366	2	4747
Microalgae (low oil content)	30	58,700	0.2	51,927
Microalgae (medium oil content)	50	97,800	0.1	86,515
Microalgae (high oil content)	70	136,900	0.1	121,104

Microalgae: A Feasible Source of Biofuel

According to Chisti (2007) [46], microalgae-based feedstock can displace fossil fuel sources and supply all of the world's oil needs. The environmental benefits of creating biofuel from microalgae include a rapid rate of microalgal growth, a high energy yield, and the need for minimal additional land [47]. Microalgae produce more biomass per unit of area and light needed than other biomass sources [48, 49]. They can use nutrients from sewage and do not require fresh water [50]. According to Raut *et al*. (2015) [51], microalgae-based biofuel production can be grown in closed photobioreactors or open raceway ponds without the need for arable or productive land. From a socioeconomic standpoint, it is possible to avoid a food vs. fuel conflict because the microalgal biofuel production cycle has no direct impact on the human food chain supply system [52]. Additionally, the ability to create microalgal biofuel domestically will help a nation become less reliant on imported oil while also improving the local economy [53]. Microalgae have a substantial advantage over other feedstocks in that they may multiply their biomass concentration every two to five days [54]. In addition, microalgal biomass can be harvested continuously or in batches almost all year long, unlike first- and second-generation feedstocks, which can only be harvested once or twice a year [55, 52]. Thus, compared to conventional feedstocks, the cost of harvesting and transporting microalgal biomass would be substantially offset by the continuous production of microalgal biomass [56, 57]. When compared to palm oil, which is the best plant for oil yield, microalgal oil yield is five times greater in terms of energy yield [58]. Due to reduced oxygen contents, bio-oil from microalgae has a calorific value that is roughly 1.4 times more than that of wood [59]. Due to their low hemicellulose content and lack of lignin, microalgae have higher hydrolysis and fermentation yields [60]. In addition to producing biofuel, microalgae can also be used to create several non-toxic, valued co-products, including soap and glycerine [51].

Microalgae are unicellular algae that can dwell alone, in chains, or in groups [61]. They are one of the earliest living things and make up the foundation of the food chain in rivers and oceans. Only 105 different kinds of microalgae are employed to make biodiesel [62]. According to Meier (1955) [63], they are not only incredibly diverse but also a highly specialized collection of creatures that can adapt to many ecological practices. Over 50 years have passed since the first mention of using algae to produce energy [64]. Research on liquid fuel made from microalgae started in the middle of the 1980s in the 20th century [65].

Microalgae are the world's most effective generator of biological oil and a flexible supply of biomass, making them a great choice as a starting point for the manufacture of biofuels. Due to their high biomass productivity, rapid growth rates compared to taller plants, highest CO_2 fixation and O_2 production, and liquid growing capacity in variable climates and marginal areas unsuitable for agriculture (such as deserts and coastal areas with waters unfit for drinking or even for wastewater treatment), they may soon status among the most significant renewable fuel crops on Earth. Additionally, they don't compete with food crops, consume a lot less water than traditional crops, are not seasonal, and may be collected every day [46, 66]. Because a significant portion of eukaryotic algae's cells are made up of chlorophyll, they are excellent light harvesters. Algae retain chlorophyll and continue their photosynthetic activity all year long, in contrast to deciduous plants that shed their leaves in the winter. Algae don't need to grow roots, leaves, branches, or flowers. They expend all of their energy either reproducing to enhance the cell density of the algal culture or replicating and repairing their photosynthetic system. In comparison to terrestrial plants, algae are consequently more effective at turning sunlight into chemical energy and require less water and space to thrive. Algae cultures grow quite quickly because photosynthesis is so effective. During the exponential growth phase, they can double their biomass in as little as 3.5 hours, and they often do so within 24 hours [67]. In addition to their quick growth, algae also produce a lot of biomasses that are used as fuel. Lipids make up about 30% of algal biomass on average (this percentage can reach 80% in the case of some genetically engineered species), but palm oil only makes up about 5% of biomass [68]. The raw components required to make biofuels like biodiesel, ethanol, and crude oil are produced by cultivating algae. Algae produce up to 6–12 times more energy per acre than corn, making their yield superior to that of many energy crops. High productivity has the benefit of requiring less space for the cultivation of algae. Algae have the potential to be a significant source of biofuels due to their high productivity and high lipid content [69].

Aquatic biomass has a higher rate of growth than land plants, making it a potentially useful alternative. According to estimates by Satputaley *et al*. (2012) [70] the oil yield from algae is between 20,000 and 80,000 pounds per acre per year, which is 7–31 times more than the next best crop, palm oil. The only renewable biofuel that has the potential to replace petroleum-based transportation fuels without compromising the supply of food and other agricultural products appears to be algae-based biodiesel. Algae may produce 30-100 times more energy per hectare than terrestrial crops, giving them a higher yield than second-generation biofuels. According to research by [62] Hu *et al*. (2008), fast-growing microalgae may produce 1800-2000 gallons of oil per acre per year, compared to 50 gallons for soybeans, 130 gallons for rapeseed, and 650 gallons for palm oil. In his essay [62] Sean Milmo makes the point that producing oil from algae on 20-40 million acres of marginal land would completely replace the US's reliance on imported oil, freeing up 450 million acres of fertile land for food production. Microalgae have been the focus of a lot of research up to this point because they can grow in both freshwater and saltwater and have the potential to be exploited for the environmentally and economically sustainable generation of biofuels.

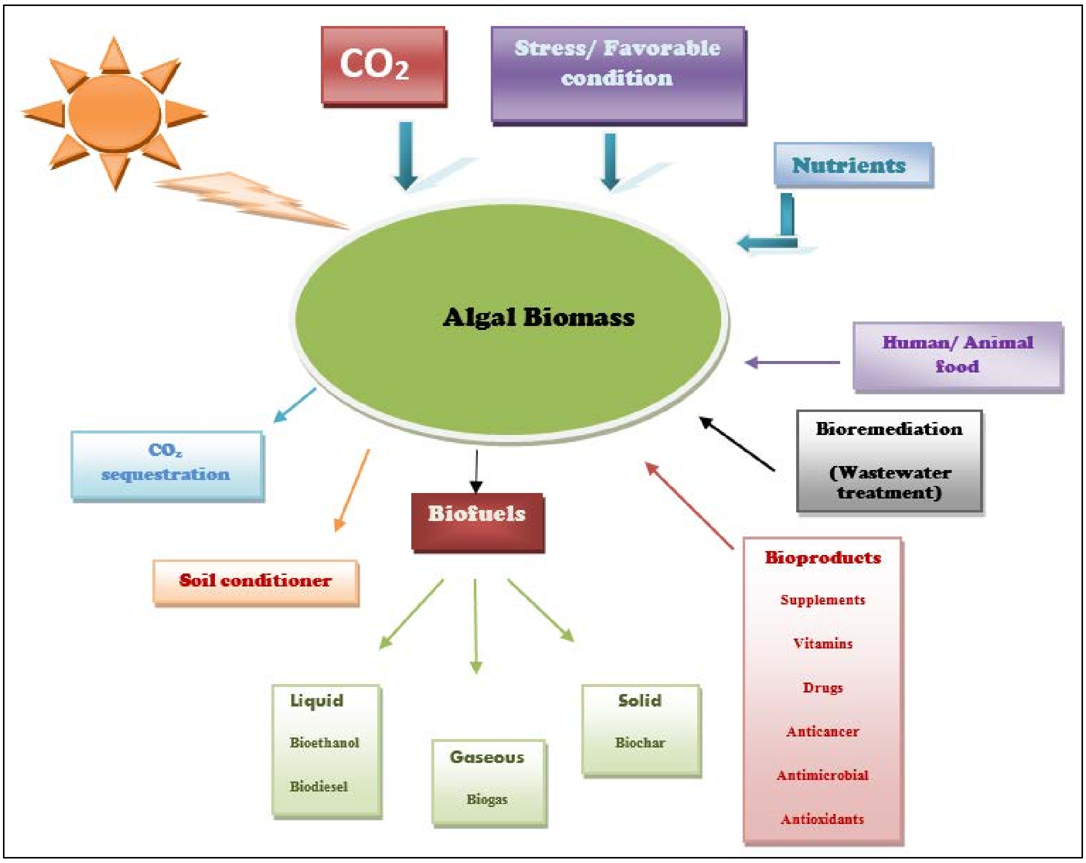

Fig 1: Factors affecting algal biomass production and its industrial applications

Table 4: List of algal species producing high oil content [71, 46, 72, 73]

S. No.	Algae specie	Group	Oil content (% dry weight)
1.	*Botryococcus braunii*	green algae	25-75
2.	*Chlorella vulgaris* CCAP211/11b	green algae	19.2
3.	*Chlorococcum* sp. UMACC112	green algae	19.3
4.	*Chaetocerosmuelleri* F&M	diatom	33.6
5.	*Chaetoceroscalcitrans* CS178	diatom	39.8
6.	*Crypthecodinium cohnii*	dinoflagellates	20
7.	*Cylindrotheca* sp.	diatom	16-37
8.	*Dunaliella primolecta*	Green algae	23
9.	*Isochrysis* sp.	Brown golden algae	25-33
10.	*Monallanthus salina*	Yellow-green algae	> 20
11.	*Nannochloris*sp.	green algae	20-35
12.	*Nannochloropsis* sp.	Yellow-green algae	31-68
13.	*Neochloris oleoabundans*	green algae	35-54
14.	*Nitzschia* sp.	diatoms	45-47
15.	*Phaeodactylum tricornutum*	diatoms	20-30
16.	*Pavlova lutheri* CS182	phytoflagellate	30.9
17.	*Scenedesmus* sp. F&M-M19	green algae	19.6
18.	*Skeletonema*sp. CS252	diatoms	31.8
19.	*Scenedesmus* sp. DM	green algae	21.1
20.	*Tetraselmis suecica*	green algae	15-23
21.	*Scenedesmus obliquus*	green algae	12-14
22.	*Scenedesmus quadricauda*	green algae	1.9
23.	*Scenedesmus dimorphus*	green algae	16-40
24.	*Chlamydomonas rheinhardii*	green algae	21
25.	*Chlorella vulgaris*	green algae	14-22
26.	*Chlorella pyrenoidosa*	green algae	2
27.	*Spirogyra* sp.	green algae	11-21
28.	*Dunaliella bioculata*	green algae	8
29.	*Dunaliella salina*	green algae	6
30.	*Euglena gracilis*	euglenophyceae	14-20
31.	*Prymnesium parvum*	golden algae	22-38
32.	*Tetraselmis maculate*	green algae	3
33.	*Porphyridium cruentum*	red algae	9-14
34.	*Spirulina platensis*	blue-green algae	4-9
35.	*Spirulina maxima*	blue-green algae	6-7
36.	*Synechoccus* sp.	blue-green algae	11
37.	*Anabaena cylindrical*	blue-green algae	4-7
38.	*Schizochytrium*sp.	protist	50-77

Algae-Derived Biofuels

Bioethanol

Bioethanol is one of the biofuels that is gaining popularity due to advancements in production methods. Electrical issues could be resolved by replacing residual oil with bioethanol. It continues to be among the biofuels that are most commonly utilized globally. However, for economic motives, attempts are still being undertaken to boost productivity while lowering manufacturing costs [69]. Microalgae are used to manufacture third-generation bioethanol, which is both secure and safe for the environment. They lower volatility when blended with traditional fossil fuels like gasoline because they raise the fuel's octane rating. The carbohydrate-rich biomass passes through three phases of pretreatment, enzymatic hydrolysis, and yeast fermentation in the conventional production method. The production of hydrogen, acids, and alcohols (such as ethanol) is achieved via a different approach that relies on dark metabolic pathways, in which photosynthesis is diverted during dark fermentation. However, using genetic engineering techniques, typical biochemical processes in microalgae, mainly cyanobacteria, are diverted in a process known as "photo fermentation" to generate bioethanol more effectively. Genetically engineered strains convert carbon dioxide and water into bioethanol in a single step using light as their energy source [69]. Autotrophic prokaryotes known as cyanobacteria (blue-green algae) lack membrane-bound organelles and a cell nucleus. Deng and Coleman (1999) [74] genetically modified them to develop a carbon route leading to the generation of straight ethanol. To transform *Synechococcus* sp. cyanobacteria, the ethanol production gene from the *Zymomonas mobiliz* bacterium was cloned into a vector. Ethanol was immediately produced by the GM cyanobacteria and then diffused out of the cells into the culture medium and the air above it. For this type of GMO cyanobacteria to grow, it needs light, carbon dioxide, and inorganic nutrients like those in sewage [74, 75]. The goal of current research is to improve procedures that are comparable to those employed by Deng and Coleman or to boost the efficiency of ethanol synthesis. Further genetic engineering, changing the growth environment to reduce nutrient concentrations, and developing more effective ethanol capture methods, like sequestration technologies, in a variety of growth mediums may all be necessary to increase the efficiency of producing ethanol from algae [76].

Biogas

One of the most potential alternative sources for generating biogas and biofuels is algal biomass. This might also help to address the issue of rivalry between traditional and energy-intensive crops. When comparing the generation of methane from traditional crops and algal biomass, Górka and Cimochowicz-Rybicka Górka *et al*. 2015 [77] showed that the latter might produce up to 20 times as much methane as the former. This is because there is far less lignin in algae than in other plants, making it easier for it to degrade. Because of this, it doesn't need to be pretreated before it can be digested Montingelli ME, *et al*. 2015 [78]. Methane, the most reduced carbon compound, makes up about 65% of the biogas made by algae, and carbon dioxide, the most oxidized compound, makes up about 35%. In addition to these gases, which can be burned directly or utilized as fuel for gas turbines and motors, other gases like hydrogen, nitrogen, ammonia, nitrogen oxide, and hydrogen sulfide are generated as well during this process, though their composition is less than 1% Passos, *et al*. 2014 [79].

The primary processes in the anaerobic digestion process that results in the production of biogas include hydrolysis, acidogenesis, acetogenesis, and methanogenesis Wiley PE, *et al*. 2011 [80]. The little amount of biomass generated during the process and the potential of reusing the digestate to enhance soil qualities are undeniable benefits of algal biogas production. Numerous research has been done thus far in different parts of the world utilizing various kinds of algae to produce compost and biogas Zhao B, *et al*. 2014 [81]. The most popular algae species that have elevated gas yields and methane concentrations in the biogas, according to Demirbas (2005) [82], include *Macrocystis pylifera*, *Sargassum*, *Laminaria*, *Ascophyllum*, *Ulva*, *Cladophora*, *Chaetomorpha*, and *Gracilaria* [82]. A methane content of around 65% was attained in the examined biogas [77]. This was done by using a two-stage anaerobic digestion system on algae species such as *Macrocystis pyrifera* and *Durvillea Antarctica*. On the other hand, it was discovered that biogas production was decreased but the methane concentration was at a comparable level when a 1:1 blend of the examined algae was used. It is indisputable that the methane possibility of algae varies according to the species and is influenced by factors such as the cell wall's composition and the rate at which microorganisms break down the cell wall [81]. Due to the structure of their cell walls, algae like *Scenedesmus* and *Chlorella kessleri* are much more challenging to hydrolyze for anaerobic cultures than are algae like *Chlamydomonas reinhardtii* and *Dunaliella salina*, whose cell walls are abundant in quickly biodegradable proteins. As a result, an additional pretreatment of algae is added to assist in the production of biogas, enabling the walls to dissolve or collapse more quickly. Additionally, the presence of polyphenols (found, for instance, in brown algae), salts, and sulfate polysaccharides, as well as methanogenesis inhibitors and the carbon-to-nitrogen ratio, might hinder biological processes during the formation of biogas [83]. Co-fermenting algal biomass with sewage sludge (or other waste biomass) has the potential to be a practical way to increase the amount of methane produced during the anaerobic digestion of algal biomass. Such algae cultivation on wastewater can have several positive outcomes, including wastewater treatment, nutrient removal, and biomass generation for energy [84].

Biodiesel

Triacylglycerol (TAG) and other lipids can be produced by microalgae, and these lipids can subsequently be Trans esterified into fatty acid methyl esters (FAME), which are precursors to biodiesel [85]. The grading of biodiesel depends on the composition and quality of FAME. Among these, palmitic acid (C16:0), linoleic acid (C18:2), and -linoleic acid (C18:3) are the most prevalent [86]. Many experts are now researching the ability of algae to produce diesel. Alcohols are the acyl chemicals that are now utilized, namely methanol and, to a lesser extent, ethanol. Additionally, a variety of alcohols, including butanol, propanol, tert-butanol, isopropanol, octanol, and branched alcohols, may be employed, but the price is notable. When methanol and ethanol are compared, methanol is more responsive, less expensive, and produces fatty acid methyl esters (FAME) that are more volatile than fatty acid ethyl esters (FAEE). Ethanol, on the other hand, is generated through the fermentation of renewable sources and is less hazardous. Methanol is currently produced primarily from non-renewable fossil fuels, according to studies. They exhibit moderate variances in their preferences as fuels, recognition, and FAEE; for example, FAEE has better viscosities hardly and hardly lessens cloud and pour elements than the comparable FAME [87, 88]. A strong base, such as sodium or potassium hydroxide, is typically required for the reaction, which creates new chemical substances known as methyl esters. Biodiesel is a viscous liquid that is amber-yellow in colour and non-flammable. Because biodiesel is perfectly miscible with mineral diesel and physically and chemically similar to it, it may be used in compression-ignition engines without needing to undergo any significant modifications [89]. The same equipment and procedures that are used to pump, store, and manage traditional diesel fuel can also be used to handle biodiesel. Biodiesel is easier to carry, handle, and store than conventional diesel because it doesn't emit explosive fumes and has a very high flash point (around 15 °C) [90].

According to research by Griffiths, *et al*. and Stephenson, *et al*. [91, 92], nitrogen restriction during microalgae cultivation may boost lipid yield. In contrast, Yao, *et al*. [93] suggest that co-culturing microalgae and some strains of bacteria can improve the efficiency of biodiesel production and retain the symbiotic connections that naturally exist in the environment. Dissolved organic carbon is made accessible to bacteria by microalgae. In return, bacteria offer other elements (vitamin B, siderophores, indole-3-acetic acid) required for the growth of algae. They also remineralize nitrogen, phosphate, and sulfur. Guo and Tong [94] demonstrated that *Pseudomonas* sp. bacteria encouraged *C. vulgaris* development by raising the number of algae cells which also was connected to raising the synthesis of TAG.

Table 5: Average emission impacts of biodiesel blends compared to petroleum diesel [95]

Biodiesel Blends	B20	B40	B60	B80	B100
Unburned hydrocarbons	-20%	-35%	-49%	-59%	-67%
Carbon Monoxide	-12%	-22%	-32%	-40%	-48%
Particulate Matter	-12%	-22%	-32%	-40%	47%
NOx	+2%	+4%	+6%	+8%	+10

Improving lipid production and yield in green microalgae by genetic modification

There is a chance that metabolic engineering could significantly increase the production of algal biodiesel as a result of the development of genome sequences and molecular technologies for algae, for example by enhancing TAG yields or creating new biofuel molecule pathways. Numerous researches on higher plants have investigated the impact of lipid synthesis enzyme overexpression on the synthesis of TAG [96]. The complex regulation of acetyl-CoA carboxylase, the rate-limiting step in fatty acid synthesis, could be the reason that there was little change in oil content in plants with elevated levels of this enzyme. However, overexpression of thioesterases (step (ii)) [97] to promote fatty acid coming out from the chloroplast to the cytosol/ER where TAGs are formed, as well as of TAG production enzymes like acyl-CoA: diacylglycerol acyltransferase (DGAT) [98] proved to be much more effective in increasing the yield of fuel molecules. The last step in the sn-glycerol-3-phosphate pathway leading to TG is catalyzed by the enzyme acyl-CoA: Diacylglycerol acyltransferase (DGAT, EC 2.3.1.20). DGAT1 and DGAT2, two unique membrane-bound polypeptides that have been found in many different organisms, are the main sources of DGAT activity [99].

These findings suggest the significance of knowledge of the associated metabolic pathways and their fundamental regulation, particularly the increasing energy load of any new pathway and, perhaps more crucially, the interconnection of pathways. Although bioinformatics of sequenced algal genomes indicates that the same pathways are likely to operate, there hasn't been much experimental confirmation of putative enzyme activities, and we are still far from fully understanding the detailed molecular biology and regulation of lipid body metabolism in algae [62]. N deficiency has the potential to cause lipid accumulation. An advantageous experimental framework for detecting changes in gene transcript, protein, and metabolic activity during lipid buildup in algae is provided by this inducible mechanism. To evaluate changes in the lipid and protein composition in *C. Reinhardtii* during lipid droplet formation, Moellering and Benning [100] performed N depletion experiments coupled with RNAi suppression. When cultures were switched to N-depleted media, it was confirmed that, compared to the original culture, the total fatty acid content increased on a per-cell basis by 2.4-fold after 72 hours, with 65% of the total fatty acids esterified to TAG in oil bodies [100]. This demonstrated that fatty acid de novo synthesis increases TAG formation. The lipid bodies contained a "major lipid droplet protein" (MLDP), which was found through proteomic research to be extremely prevalent. Following N depletion, a parallel rise in lipid droplets was seen in the abundance of MLDP mRNA transcripts. It appears that the purpose of this protein is to control lipid droplet size because RNAi lines of *C. Reinhardtii* with a 55–60% reduction in the MLDP transcript produced lipid droplets that had a diameter 40% larger than the control lines. Deleting other redundant pathways from the organism is another strategy to increase lipid output because it frees up precursor metabolites for the intended biofuel generation. The *C. Reinhardtii* starchless mutant (sta6), which is lacking in ADP-glucose pyrophosphorylase, a crucial enzyme in the manufacture of starch, has an increase in the number of TAG lipid droplets [101]. After 48 hours of N depletion, wild-type cells exhibited a 15-fold increase in lipid droplet content, but the sta6 starchless mutant algae had a 30-fold increase. After 18 hours of N starvation, the sta6 cells had an average amount of 17 ng TAG (10^3 cells^{-1}), compared to 10 ng (10^3 cells^{-1}) in the wild-type cells. This ultimately led to a greater concentration of TAG per cell [102].

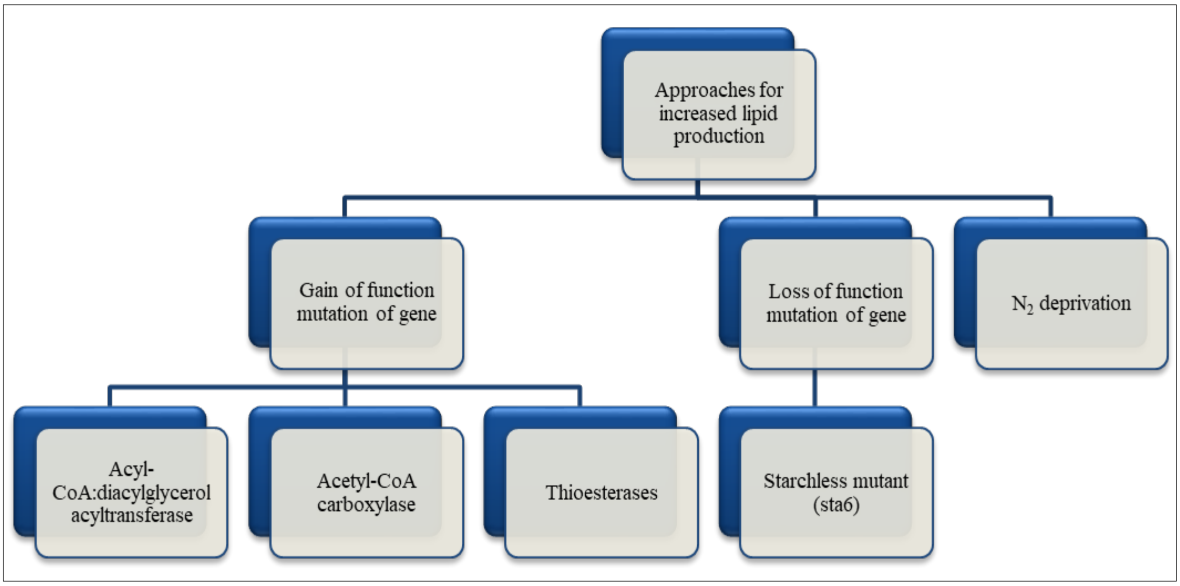

Fig 2: Approaches for increased lipid production in green microalgae

Future strategies for the commercialization of microalgae biofuels

Optimizing stress conditions is important to produce the highest possible yields of oils in the cells. Choosing natural wild species that have already undergone adaptations to the region's growth circumstances is another option. An additional method to improve production efficiency is genetic modification (GM). The process of lipid extraction before esterification needs more study. The cost of biomass pretreatment would be drastically reduced if techniques could be developed that do not include drying or solvent extraction of the algal slurry [103]. A lipid material devoid of both water and free fatty acids is necessary for the utilization of accessible biodiesel synthesis techniques. Due to this, drying the microalgae material results in higher dispensing costs. The drying stage might be eliminated by modifying lipase enzymes using mutational strategies for direct esterification or other extraction techniques. The number of identified freshwater and marine species is enormous, but just a small number of them are currently important for trade. Some of these are *Chlorella*, *Spirulina*, *Dunaliella*, *and Haematococcus*. These are typically grown for the extraction of valuable components like proteins or pigments [104].

Microalgae have gained attention in recent years for producing valuable molecules ranging from therapeutic proteins to biofuels. This is due to their outstanding distinctiveness in that they combine the renewable energy-capturing ability of photosynthesis with the elevated yields of controlled microalgae cultivation, potentially making them precious organisms for cost-effective, industrial-scale production processes in the coming decade [45]. It is also necessary to compare a variety of large-scale systems based on their fundamental characteristics, such as their efficiency in utilizing light, ability to control temperature, ability to maintain the axenic culture, and ability to progress from R&D scale to industrial scale. The system's final preference is more or less always a collaboration, which connects all of these factors to produce a product that is both affordable and satisfying [105]. To meet the demands of a rapidly expanding market, microalgal technology's long-term goal is to increase the production of these organisms [106].

The main goal for generating microalgal biofuel is to choose algal strains with high lipid contents, ease of cultivation, and rapid development. Certain algae species, including *Botryococcus braunii*, produce considerable quantities of lipids when cultured. Fast-growing algae with high lipid content can lower production costs. The economics of the method determines how much algae biofuel can be produced. The amount of biofuel produced ought to be decreased by developing new technology and organizing each stage of the procedure. The microalgae production system comprises several associated systems (cultivation, harvesting, product recovery, and drying systems), and the system's notably low cost is due to the elimination of unnecessary processes in the processing of algal fuel. A fundamental financial dilemma for algae producers is the search for low-cost lipid extraction and harvesting techniques. The coproduction and commercialization of several other important components are also necessary for the viability of algae-based biofuels. After oil extraction, protein-rich biomass that is recovered can be fed to livestock, poultry, and aquaculture systems. Thus, it can be claimed that the adoption of a hybrid refinery concept for microalgae-based biofuels is feasible. Algae-based biofuels may be a good substitute for fossil fuels in this situation, but they face several challenges before they can be competitive and widely adopted. The main challenges for generators of algae-based biofuels are accurate and efficient strain recognition, yield increase, crop protection, distribution and use of nutrients and resources, and byproducts and waste handling. Algae-based biofuels have a lot of potential, but considerable work and obstacles still need to be overcome in this field.

Table 6: Comparison of properties of biodiesel from microalgal oil and diesel fuel and ASTM biodiesel standard [65]

Properties	Biodiesel from microalgal oil	Diesel fuel	ASTM biodiesel standard
Density (kg L^{-1})	0.864	0.838	0.86–0.90
Viscosity (mm^2 s^{-1}, CST at 40 °C)	5.2	1.9–4.1	3.5–5.0
Flashpoint (°C)	115	75	Min 100
Solidifying point (°C)	−12	−50–10	–
Cold filter plugging point (°C)	−11	−3.0 (max −6.7)	Summer max 0 Winter max <−15
Acid value (mg $KOHg^{-1}$)	0.374	Max 0.5	Max 0.5
Heating value (MJ kg^{-1})	41	40–45	–
H/C ratio	1.81	1.81	–

Conclusion and Future Prospective

Recently, there has been an increase in interest worldwide in finding alternate energy sources to substitute petroleum. Algae-based biofuel is a strong contender to eventually replace petroleum-based fuel due to its high amount of oil, significant production, minimal need for land, and other reasons. Microalgae, which are mostly made of lipids with significant amounts of proteins, carbohydrates, and other nutrients in the remaining portions, are the only biofuel source that might be grown without clashing with farmland. This makes the waste from oil extraction attractive for use in animal feed or other goods with additional value. The price difference between biomass and petrochemical fuel is the main disadvantage of the 3G biodiesel generation process. The capacity to produce this biofuel and make it strong competitor with diesel on a commercial basis should depend critically on the ability to grow microalgae quickly enough to extract oil. Another feasible and reliable low-cost solution to this problem is the stimulation of lipids or the development of synergistic algae consortiums. It is necessary to do comprehensive research to ascertain whether the idea of adding the fastest-growing, oil-accumulating microalgae is economically viable. Research on the evaluation, manufacturing, operation, and harvesting of biomass feedstock is required to support the commercialization of this promising and cutting-edge technology and remove the current technological and financial barriers preventing the production of bioenergy from microalgae. Similar to plants grown for energy, algae store enormous amounts of reserve molecules in their cells that can be converted into energy in several different ways. Due to the high concentration of cellular building blocks (protein) and reserve constituents (starch and lipids) in their cells, certain species of algae can be utilized to make alcohol, biodiesel, and methane by using their biomass as fuel. The fact that modern civilizations have an increasing requirement for energy utilization should also be addressed. In several aspects, including the production and supply of energy needed for heating, lighting, and transport, it is essential for the efficient functioning of the global economy. A significant change in the climate is being brought on by greenhouse gases as a result of increasing fuel consumption and CO_2 levels in the atmosphere. All of these factors contribute to the development of clean energy options that can replace fossil fuels and reduce global carbon dioxide concentrations. In the current geopolitical climate, especially in the context of the conflict between Russians and Ukrainians, acquiring new renewable energy sources is evolving into a critical issue. Given the current global problems, particularly the Ukrainian-Russian battle and its negative consequences on the energy and fossil fuel supply chain, the processing of biomass resources, such as algae biomass, into value-added bioproducts and biofuels utilizing innovative technology is highly advantageous. In light of current global events, it is crucial to investigate and advance knowledge on concerns about the potential switch to biomass-oriented products, like products derived from algae.

Conflict of interest

The authors declare no conflict of interest.

Author’s contribution

The idea of this review by Sanjay Singh, data curation by Khushaboo Soni; writing, and draft preparation by Khushaboo Soni; writing review and editing by Sanjay Singh and Preeti Maurya. All authors have read and agreed to the published version of the manuscript.

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