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Algae, Third-Generation Energy Source: A Comprehensive Review on Methods from Cultivation to Biodiesel Production

Noreen Iftikhar[1], Javaria Ilyas[1], Muhammad Ikram Ramzan[1*], Esha Asghar[1], Areeba Manzoor[1] and Momina Afzal[1]

[1]Center of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan

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***Corresponding Author:**
Muhammad Ikram Ramzan
Center of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan
ikramramzan5288@gmail.com

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ABSTRACT

An increase in population growth has elevated the energy demand, and diminished fossil fuel sources. Their combustion releases carbon dioxide and contributes to environmental pollution. This has initiated intensive research to find alternative sources for economic and environmental sustainability. Amongst all, biodiesel originating from oil crops is a biodegradable, environment-friendly substitute and has properties similar to fossil diesel. Algal sources are promising substrates that require only sunlight and water for oil production. They could fulfil global demand, reduce the use of petroleum-based diesel and have higher oil productivity than other oil-yielding crops. Therefore, the third-generation production of biodiesel through microalgae is the renewable choice to overcome the energy crisis. This review covers algal cultivation methods, including both open and closed systems, lipid-extracting techniques for taking out algal oil or lipids from microalgae, and biodiesel production by the transesterification process. This article aims to assist in selecting appropriate cultivation and extraction methods for biodiesel generation.

INTRODUCTION

Energy demand has been rising steadily over the past ten years, along with adverse environmental effects. Since fossil fuels are the main source of energy for the automobile industry, global warming and a rapid decline in the availability of natural resources are being observed. Previously, researchers have carried out research on first- and second-generation biofuel production methods, and their engine testing showed that the cultivation of the feedstocks used is unsustainable. However, a substantial reduction in the emission of nitrogen oxides was observed [1]. The growing concerns regarding climate change and the depletion of fossil fuel reserves have led to an increased focus on renewable and eco-friendly energy sources to preserve the beauty of the environment and address the depletion of natural resources [2]. One promising avenue for sustainable energy production is biofuels, which are derived from organic matter such as plants and algae. Amongst the various sources of biofuel, third-generation algal biomass has gained significant attention due to its high potential for efficient and environmentally friendly biofuel production [3]. Compared to first-generation (e.g., corn or sugarcane) and second-generation (e.g., lignocellulosic) biofuels, microalgae offer up to 58,700 L/ha/year of oil yield, significantly higher than

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soybean (446 L/ha) or rapeseed (1,190 L/ha) [1]. Moreover, algal cultivation can occur on non-arable land with 95% lower freshwater use and up to 70% reduction in GHG emissions per MJ produced, as shown in recent LCA studies. Techno-economic analyses also suggest competitive production costs with continued optimization and integration of co-products [2, 3]. Algae can grow in diverse environments, including freshwater, seawater, and wastewater. However, this adaptability is strain-dependent—e.g., *Dunaliella salina* tolerates high salinity, while *Chlorella vulgaris* thrives in freshwater. Nutrient needs differ across strains, affecting lipid yields. In wastewater systems, growth is challenged by fluctuating COD/BOD, heavy metals, and microbial contamination, which hinder biomass productivity and require careful pretreatment and monitoring to maintain stable cultures [4-6]. This versatility enables algae production without competing for arable land [7], making it an attractive option for biofuel production without compromising food production. Algal sources are regarded as sustainable feedstocks due to their rapid growth rates and potential for biodiesel production. Moreover, many algal strains contribute to wastewater remediation through mechanisms such as nutrient uptake (e.g., nitrogen, phosphorus), heavy metal sequestration via biosorption, and reduction of chemical and biological oxygen demand (COD/BOD) by assimilating organic pollutants and supporting microbial communities involved in biodegradation [8]. Furthermore, algae have a remarkable ability to photosynthesize and convert sunlight into biomass at an unparalleled rate [9]. They can produce a high yield of biomass per unit area [10] compared to traditional crops, such as corn or soybeans, making algae a highly efficient feedstock for biofuel production. After cultivation, algal biomass is harvested and processed for the extraction of oil. The oil is converted into biodiesel through a chemical process called transesterification [11]. Biodiesel is the most sought-after biofuel due to its high biodegradability and environmentally friendly, non-toxic properties. Algal species are selected depending upon the percentage of the lipid content present in algal cells and the type of oil, hydrocarbons, and lipids to be extracted [10]. Biodiesel can be produced by both macroalgae and microalgae; the common algal species examined for the production of biodiesel are *Thalassiosira pseudonana, Chlorella* sp., *Chlamydomonas reinhardtii, Phaeodactylum tricornutum, Isochrysis* sp., and *Dunaliella salina* [12]. Some species of algae have a high content of lipids as much as 60% of their total weight. Triglycerides (TAGs) are commonly found in lipids that are stored in metabolites, storage products, and components of membranes [10]. The primary storage lipids in microalgae are triglycerides (TAGs) made during times of stressful conditions such as nitrogen starvation. They contain three fatty acids that are esterified to glycerol; consequently, they make the best raw materials for use in the transesterification process as a result of their fidelity in fatty acids and limited polarity. In contrast, TAGs make it possible to achieve a high percentage conversion to biodiesel (>95%) without the formation of undesirable byproducts, as is with phospholipids. Therefore, increased accumulation of TAGs will have a direct positive effect on biodiesel productivity and quality [13]. Studies have been conducted to compare the biodiesel production between macroalgae and microalgae, and it has been found that high biodiesel production is produced by using microalgae as a substrate due to the high growth rate of microalgae, which leads to better yield. Microalgae can produce large amounts of lipids; typically, 30% lipid content is present in algal cells, which increases the quantity of extracted oil that turns into biodiesel. Strain selection of microalgal strains depends on the availability of raw materials, optimization of growth and economic viability. Hossain *et al.* [14] compared the *Oedogonium* and *Spirogyra* algal species and found *Oedogonium* as a good source of biodiesel on one hand; and *Spirogyra* to yield more residual biomass after extraction. This proves that strain-specific lipid productivity and biomass profiles directly affect the amounts of biodiesel as well as process scalability [14]. Besides being environmentally friendly, algal biodiesel production in Pakistan could significantly boost the economy by utilizing 27–28 million acres of saline land, creating rural jobs, supporting energy independence, and generating up to 195 million PKR/year per 1-ton/day plant with a 4-year payback period [15].

This study aimed to discuss different suitable methods of algal cultivation, effective methods of oil extraction from algae using different techniques, possible effects on the economy by using biodiesel in transportation, and future perspectives.

Algal Cultivation System: Open Ponds

An open cultivation system is a method of growing microalgae in an open environment, typically in shallow ponds, raceway ponds, or other open containers. This growth method allows for natural sunlight exposure and atmospheric gas exchange, resulting in the production of algal biomass. The open cultivation system is considered the most traditional and cost-effective approach for large-scale microalga production [1]. Open systems account for approximately 98% of overall biomass production. Due to the high growth rate of microalgae, which reaches 1.5–2.0 grams per liter per day, they can produce 15 to 20 tons of dry biomass per acre per year, with oil comprising 50 to 60% of the dry weight in high-yielding strains; thus, it is economically feasible to produce biodiesel using microalgae [16]. Natural resources like concrete and

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rammed earth can be used for building an open pond system. The main disadvantage of such reactors is the gradual degradation of the light-transmitting walls, among others, because of the deposition of biofilm on the inner surface. Compared to open ponds, closed ponds are made of acrylics and are more expensive [17]. Open pond systems are cost-effective but limited by environmental stress; most algae grow best between 20–30°C and light intensities below 400 μmol m^{-2} s^{-1}, while thermotolerant strains like *Chlorella* sp. GD M4 can withstand up to 49°C and high irradiance, sustaining biomass yield [18]. Accumulation of unwanted contaminants due to fungal growth and algae invasions, uneven distribution of light, and an open pond's inability to hold photosensitive dark zones (as light can only penetrate to a particular depth) are also causes for concern [1]. Phytoremediation using *Chlorella* species has proved to be successful through pilot-scale tests over the recent past. A techno-economic analysis of a tubular photo-bioreactor treating agricultural centrate wastewater with *Chlorella* sp. yielded 34.6 g/m^2/day (TSS) and removal of 70% COD, 61% TKN, and 61% phosphorus [19]. Closed systems overcome numerous disadvantages of open ponds [20]. Most algal biomass is produced in open cultivation systems; such systems are also known as raceway or circular ponds [1]. Although raceway ponds with paddle wheels overcame some limitations of the earlier designs, such as poor scaling of the system and the possibility of contamination [1, 20], mixing and yield were enhanced [17, 21].

This man-made closed system not only reduces contamination during the manufacture of costly metabolites but also prevents evaporation loss, which is a major concern in open systems. Photobioreactors are artificial systems for the continuous cultivation of microalgal strains by recirculation at optimum pH, temperature and light. Light path length is critical, as shorter paths (typically <30 mm) reduce self-shading and improve light utilization efficiency. Effective mixing regimes (e.g., airlift or mechanical agitation) enhance gas-liquid mass transfer and prevent biomass sedimentation. Mass transfer coefficients are equally important to avoid oxygen accumulation and ensure CO_2 availability, directly influencing productivity. A comparative analysis by Carvalho *et al.* highlights how reactor geometry, mixing strategy, and light penetration together impact overall reactor performance [22]. Most studies report biomass productivities in the range of 20–35 g/m^2/day under optimal conditions. However, a huge temperature rise is a major pitfall that can be easily controlled by agitation and modifying the open and closed system organization that upgrades the biomass production [1]. Future modifications of closed PBRs are based on their geometric configurations, like vertical, horizontal, helical and flat panel PBR. As compared to open systems, these PBRs have 5-10 times higher efficiency but are uneconomical [23]. Although PBRs are expensive, they also have many advantages that are given below: (1) Reduce or eliminate external algae, fungi or amoeba contamination, (2) Minimize the evaporation loss to save backup water for open ponds, (3) All the parameters e.g., nutrients and gases levels are supervised and maintained, (4) Biomass can be produced at night by using LED systems which work like natural sunlight. Although artificial lights, especially LEDs, allow biomass production to continue at night by imitating photosynthetically active radiation (PAR), they also make the cost of operation highly energy-dependent. It has been found that artificial lighting may comprise as much as 50–270% of the total energy input in closed photo-bioreactors [22]. Conversely, solar-based systems are more energy-efficient and environmentally viable, especially when evaluated using life cycle analysis [1]. Photobioreactors (PBRs), in the form of transparent glass or acrylic, take the form of tubular geometries and are adapted to sunlight exposure with different modes of operation that enhance algal productivity [23]. The types include vertical (airlift, bubble column) [16, 14], horizontal [16], helical [22], and flat-plate PBRs [22]. Stirring implies the utilization of bubbling/swirling, and PVC/PE PBRs can deteriorate rapidly [24], Figure 1.

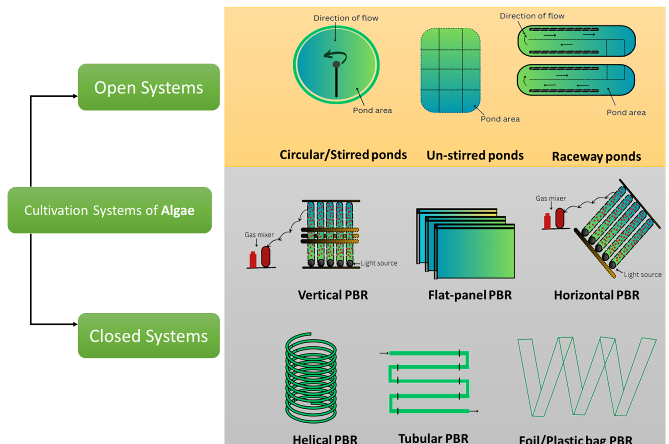

Figure 1: Algae Cultivation Systems: Open and Closed Pond Systems

Pre-processing of Cultivated Microalgae

Microalgal biomass can be wet (70–80 wt.% water), and preprocessing is necessary to dehydrate before lipid extraction because the additional moisture adversely affects the efficiency of solvents [25]. The solvent penetration is improved by the drier, which enlarges the surface area, but the cost of drying biodiesel is increased by 35%; drying with an oven is better than drying in the sun because the drying process can be controlled [17]. Flocculation, sedimentation, centrifugation, membrane filtration, and air flotation have been identified as dewatering processes, and they are determined depending

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on the biomass load [26]. Centrifugation is required in small-scale because of the cost [25], and membrane filtration is motorized by pressure [21]. Flocculation and coagulation are similar phenomena of charge- or coagulant-mediated aggregation, and air flotation relies on bubbles to float particles [27].

Lipid Extraction Methods

Lipids are extracted after harvesting and lyophilizing the microalgae. The fundamental principles of lipid extraction from microalgal cells include easy scalability and minimal disturbance to lipid components. Common methods are solvent extraction and supercritical fluid extraction [28]. Technological advancements have led to many new methods of extraction that are eco-friendly (Figure 2).

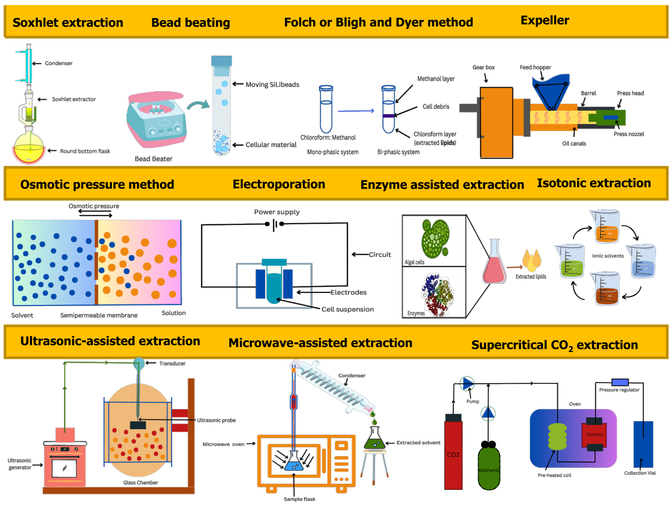

Figure 2: Different Lipid Extraction Techniques

In 1879, the first introduced method of lipid extraction was the Soxhlet method. It was used to measure the total lipid quantity in milk [29], and it has since been steadily driven in the domains of pharmaceuticals, food, and other industries. This method uses a Soxhlet extractor for extraction. In Soxhlet extraction, rather than rupturing the cells to release lipids, the process relies on diffusion across the cell wall. The choice of solvent is critical; various solvents and their combinations have been evaluated, with polarity ranging from 0.1 (petroleum ether and n-hexane) to 5.2 (ethanol) for extracting lipids from microalgae [30]. According to the data, chloroform, n-hexane, and ethanol yield high lipid contents, whereas acetone yields the least [31]. Due to its low cost, simple operations, and high yield, the Soxhlet method remains widely used in research and industry. However, it presents certain limitations, such as the use of hazardous solvents (e.g., carcinogenic benzene, less-toxic hexane), high reagent consumption, and being time-consuming [32]. The Folch and Bligh, and Dyer methods are well applied in the extraction of animal, plant, and microalgal samples. The major differences lie in the ratios of solvents: Folch is 2:1:0.7 and Bligh and Dyer 1:2:0.8 (chloroform: methanol: water) [33]. Both procedures are efficient; however, when it comes to microalgae, rigidity in cell walls is a problem that can create the risk of loss of lipids during the process of disruption [28]. Bligh and Dyer are more cash-efficient and safer. Supercritical CO_2 extraction and greener adjustments are long-established alternatives with better performance [34, 35].

Traditionally, solvent-free expelled pressing involves high-pressure hacks scaling up dried algal biomass to rupture, allowing extraction of oil: 70–75% depending on morphology and strain [36]. Nonetheless, it is expensive because it requires a lot of energy, drying (30% of the production cost), and maintenance of the equipment [26], even though it extracts high-quality oil with minimal oxidation [35]. Laboratory-scale bead beating, where vibratory action rapidly agitates the solution in the presence of small beads, is an efficient cell disruptor by grinding or collision of cells. It is economical and does not dry out, and it also maintains heat-sensitive biomolecules through cooling jackets [35]. Ultrasonication has been proposed to increase both cell lysis and release of lipids due to cavitation and acoustic streaming [37, 38], but its application is energy- and reactor-type-dependent and cell type-dependent [39, 21]. First described in 1986 [36], microwave (MW) extraction applies a variable electric field to the sample, leading to rupture of the cells via internally generated pressures and electroporation, to effect extraction in as little as 15–20 minutes [40]. Despite its effectiveness, it has issues of scalability, heating uniformity, and high maintenance [41]. Electroporation involves using electrical voltages to raise the permeability of the membranes and has advantages such as low energy requirement, reduced contaminants, preservation of membranes, and low energy [36, 25]. Osmotic pressure, as a means of rupturing the cells with hyper- or hypo-osmotic stress due to salt, scales well and has the potential to be a low-equipment process that needs additional species-specific research [21, 25]. There is an opportunity to use ionic liquids, or so-called green solvents and anion-cation pairs, which can be tuned to achieve environmentally friendly extraction [35, 36]. The method can be used instead of toxic solvents, and future studies are required to prove the method. The enzymatic extraction allows the removal of lipid through the rupture of the algal cell walls using special enzymes, which is precise but species-specific and depends on the composition of the lipid and low temperatures (36; 25). Supercritical CO_2 also involves the extraction of selective lipids via pressurized CO_2, but the method requires expensive, complicated hardware [35, 42]. Limitations and applications of the mentioned techniques are discussed (Table 1).

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Table 1: Comparative Analysis of Different Oil Extraction Methods by Using Microalgae

Methods	Reaction conditions	Microalgae	Advantages	Limitations	References
Ultrasonic-associated extraction	Intensity of ultrasonic: 40KHz, Ultrasonic Power: 2.68 W/m^2	*Nannochloropsis, Chlorella vulgaris, Trichosporum,*	Enhances extraction rate, Reduce the time of extraction, Fewer solvents, Great penetration into algal cells	Energy loss concerning distance, Expensive approach, Difficult to scale up	[43]
Microwave-assisted extraction	T:120°C Irradiation, Power: 880W	*C.sorokiniana, N.salina, Galdieria sulphuraria*	Efficient heat and mass transfer, Higher extraction yield than conventional methods	Maintenance cost is higher, Scale-up is difficult	[44]
Organic Solvent (chloroform/ methanolextraction method)	T:20°C, Light intensity: 300 µmol m^{-2} s^{-1}	*Chlorella zofingiensis, Isochrysis galbana*	High biodiesel yield, Efficient and reliable, Easy solubility of lipids	Presence of solvent residues after extraction, Some solvents are toxic	[45]
Electroporation	Treatment Intensity: 28kWh/m^3, Appropriate culture conditions	*Nannochloropsis salina, Chlorella vulgaris*	A small amount of energy is required to use short electrical pulses, 90% lipid extraction	Intensity of field, frequency of field and geometry of electric pulses have an impact on the resulting extraction	[30]
Isotonic extraction method	T:0-140°C, Organic and inorganic ions	*Chlorella sorokiniana, Chlamydomonas reinhardtii, Botryococcus braunii*	Ionic liquids enable synthetic flexibility, tailoring the properties of the solvent like polarity, solubility and conductivity	Energy-intensive, High cost of solvents as the solvents used are synthetic, "green"	[46]
Osmotic pressure	T: 20°C, speed: 20-25rpm	*reinhardtii, Chlamydomonas reinhardtii*	Economically feasible, Cost-effective, Consumes low energy	Consumes much time Generates waste salt water	[47]
Supercritical Co_2 extraction	Pressure: 40 MPa, T: 333K, Co_2 flow rate from 0.3 – 0.5 kg h^{-1}	*Nannochloropsis oculata, Cylindrotheca closterium, Chlorella vulgaris*	Non-toxic, solvent-free lipids, consistency supports mass transfer balance.	Expensive equipments needed	[48]
Bead beating	Microscopic beads with high speed	*Nannochloropsis oculata, Chlorella zofingiensis*	Cost-effective, Better disruption of the cell, Extraction with high efficiency	Energy-intensive, Difficult to scale up	[49]
Enzyme-assisted extraction (cellulose, neutral protease, alkaline protease)	T:53°C, pH=4.4	*Nannochloropsis Sp.Chlorella vulgaris, Scenedesmus dimorphus*	Easy extraction of internal lipids, Cell disruption with minimal damage, High lipid recovery	Affected by the composition of lipid class and type, the Type and dosage of enzymes for extraction are high in cost, strongly dependent on pH	[46]
Expeller press	Dried algal biomass, High mechanical pressure to crush and extract oil	*Nannochloropsis oculata, Chlorella zofingiensis, Isochrysis galbana*	Solvent-free extraction, High-quality oil yield, Less oxidation	High cost, Heat generation and possible damage to the compounds	[50]

Transesterification Process

Transesterification is commonly adopted as a process of converting algal oil into biodiesel. It is a reversible combination of triglycerides and surplus methanol (at a 3:1 molar proportion), yielding glycerol and methyl derivatives. The stepwise reaction occurs in three phases: triglycerides transform into diglycerides and then monoglycerides, and then into methyl esters and glycerol, with the highest yield of 98% [1]. Acids, bases (e.g., NaOH, KOH), biocatalysts (lipases), and alkoxides such as sodium methoxide are catalysts used. The catalytic reaction is four thousand times quicker in bases compared to acidic conditions, and the temperature is usually maintained at 60°C under 1 atmospheric pressure, with a time of 90 minutes [51, 16]. Methanol and oil have to be dry to prevent the formation of soap. Nevertheless, the fact that methanol does not mix easily with oil causes mass transfer problems; thus, intense mixing is necessary. Biodiesel and glycerol cannot be easily purified after the reaction because these two components separate into different phases. An essential recovery of methanol, which is beneficial in terms of costs and the environment, is obtained through flash evaporation or vacuum distillation [46, 52] (Figure 3).

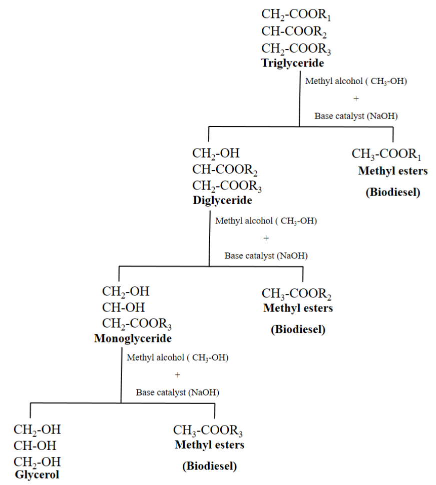

Figure 3: Transesterification Reaction for Conversion of Lipids into Biodiesel

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Economic Challenges and Future Perspectives

The microalgae are bioactive products that contain several bioactive compounds, such as lipids and carbohydrates, which can be processed through processing by enzymatic and mechanical processes to produce biodiesel efficiently. This practice is dubbed green and commercially feasible as well as time-effective. Microalgae are a third-generation feedstock and, as such, can provide an environmentally friendly alternative to fossil fuels as well as other biofuels of the first and second generation. Nevertheless, commercialization, particularly the high costs involved in the process, is its major challenge, as far as cultivation, harvesting, and the extraction of lipids are concerned. Physical and biological factors light, temperature and pH, also influence large-scale production. In the base-catalyzed transesterification process, which is most often employed, the separation and purification of biodiesel and glycerol are both complex and require vigorous mixing and repetition of the washing procedures. Vacuum distillation plays an important role in the quality of the products and the sustainability of the environment in recovering methanol [52]. Although the existing extraction methods have presently not yet been perfected, multidisciplinary studies coupled with algal genomics have opened the portals to optimal lipid synthesis and an enhanced yield in biofuels of numerous strains of algae [53]. CRISPR/Cas9 and Cas12a systems have improved editing precision and multiplexing capacity for transcriptional modulation and metabolic rerouting[53].

CONCLUSION

Gradually increasing energy demand globally cannot be met with the usual biofuel production methods. The constant use of these sources of biofuel also changes our global carbon cycle. Algae, as an autotrophic organism, are utilized as a prospective mass production source for biofuel production. Biomolecules of algae cells, like lipids and carbohydrates, can be exploited for bioethanol and biodiesel production. In this review article, the prospects of algae as an emerging source for biofuel production for biofuel are thoroughly narrated. For research purposes, the cultivation of algae in various methods, like open and closed (photo-bioreactors) pond systems with their drawbacks, is comprehensively discussed. Solvent extraction and supercritical fluid extraction were seen as the most common methods of extraction. However, although every method has its advantages and limitation, new technological advancements lead to many new methods that will be eco-friendly, will have high efficiency, and require low maintenance costs. Still, a lot of research and development work is required for an efficient biofuel production system from algae.

Authors Contribution

Conceptualization: NI
Methodology: NI, JI, MIR, EA, MA
Formal analysis: NI, EA, AM
Writing review and editing: NI, AM, MA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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