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Microalgae to Biofuel: Cutting-Edge Harvesting and Extraction Methods for Sustainable Energy Solution

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ABSTRACT

The increasing price and demand for fossil fuels are driven by their depletion, greenhouse gas emissions, and industrial air pollution. As a result, the search for renewable alternatives has gained serious attention. Microalgae provide a sustainable alternative for biofuel production, offering high growth rates, significant oil yields and productivity, nontoxic nature, higher photosynthesis efficiencies, and the ability to thrive on nonarable land. *Chlorella vulgaris* and *Scenedesmus dimorphus* strains were chosen for this study to develop effective harvesting and oil extraction methods for sustainable energy. Three types of harvesting methods are used to optimize slurry yields, viz. flocculation, high-speed refrigerated centrifugation, and micro-filtration. Moreover, two oil extraction methods were considered to enhance efficiency: Soxhlet extraction and the direct boiling method. The centrifugation method provides the fastest harvesting rate and highest slurry yields, followed by membrane separation, while flocculation, though slower, is more cost-effective and easier to perform. The direct boiling method optimizes the oil extraction process by effectively rupturing microalgae cell walls. *Chlorella vulgaris* shows a slurry recovery efficiency of 0.76 g per liter of media by centrifuge, consisting of 12%–40% oil content in overall weight; 12.7% of the oil was extracted using Soxhlet extraction, and 18.7% was extracted using the direct boiling method. On the other hand, *Scenedesmus dimorphus* shows better slurry recovery efficiency of 0.81 g per liter media by centrifuge, consisting of 22%–51% oil content in overall weight, and there is 23.8% oil extracted by using soxhlet extraction and 26.4% for every 10 g of the sample by direct boiling method compared to *Chlorella vulgaris*. Future research should focus on cost-effective harvesting and oil extraction methods for microalgae like *Chlorella vulgaris* and *Scenedesmus dimorphus* to reduce production costs, maximize biofuel yields, and tackle the global energy crisis.

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1 | Introduction

The depletion of fossil fuel resources, such as petroleum, natural gas, and coal, poses significant challenges for industries and consumers as demand continues to rise [1]. Burning fossil fuels contributes to air pollution through the emission of greenhouse gases like carbon dioxide, nitrogen oxides, and sulfur oxides, as well as particulate matter and volatile organic compounds [2, 3]. Fossil fuels are becoming more expensive and contribute to rising environmental issues. Sustainable alternatives like solar, wind, geothermal, and hydro energy have been utilized for years to mitigate environmental impact. The increasing cost of fossil fuels, coupled with their environmental impact, has heightened interest in renewable resources to meet energy demands sustainably [4]. Transportation and energy sectors alone account for over 60% of CO₂ emissions, underscoring the urgency of adopting cleaner energy solutions [5–7].

Biomass resources play an important role in major global energy supply because they have the potential to become the most sustainable energy resources [8]. Biomass energy is not a transition fuel as it has often been portrayed but a fuel that will continue to be the prime source of energy for many people for the foreseeable future [9]. Among the most significant advancements in biomass utilization is the production of biofuels, renewable and biodegradable energy sources derived from carbon fixation in living organisms [10]. Biofuels are categorized into various types depending on the type of feedstock employed in their production. Each category has unique characteristics, environmental benefits, and challenges, influencing its feasibility and widespread adoption [11]. First-generation biofuels, derived from edible crops, face inefficiencies and competition with food supply, making them unsustainable in the long term [12]. Second-generation biofuels utilize nonedible biomass, offering environmental benefits but requiring costly pretreatment processes [13, 14]. Third-generation biofuels made from algae have gained considerable attention due to their high productivity, minimal land use, and ability to thrive in a variety of environments [15]. However, their commercialization is still limited by technological and economic constraints, primarily related to the costs of cultivation and lipid extraction. Fourth-generation biofuels aim to further improve upon these limitations by incorporating advanced bioengineering techniques, such as genetic modification and carbon capture technologies, to create biofuels that not only produce energy but also store carbon, enhancing their environmental sustainability [16, 17]. Biofuels not only reduce greenhouse gas emissions and contribute to decarbonizing transportation fuels but also diversify energy sources, promote rural employment, and enhance energy security. Their versatile applications include biogas, hydrogen, ethanol, and plant oil-derived fuels.

Biomass-derived biofuels offer significant environmental benefits as they help mitigate carbon dioxide emissions through photosynthesis, reducing the carbon footprint associated with traditional fossil fuels [18–20]. While biodiesel production from microalgae presents a promising option, challenges related to scalability and cost-effective lipid extraction hinder its widespread economic viability. Despite being slightly less efficient (5%–8%) than fossil diesel, biodiesel provides environmental advantages, including lower CO₂, hydrocarbon, and particulate

matter emissions [21]. A further factor contributing to algae's environmental sustainability is their ability to trap carbon, which is roughly ten times more than that of land-based plants. The capacity of algal biodiesel to reduce resource inputs, protect biodiversity, and produce useful coproducts increases its appeal [22–25].

However, concerns about food-versus-fuel arise, as current biofuel production often competes with agricultural land used for food crops [26–28]. This has led to a growing interest in microalgae, which do not compete with food production and offer a high yield per unit area [29]. Microalgae also contribute to CO₂ sequestration, demonstrating a high photosynthetic rate, rapid reproduction, and adaptability to various environments [30]. With the ability to integrate easily with existing technologies, microalgae have the potential to replace petroleum-based fuels without the ethical concerns of food crop diversion, making them a promising solution for sustainable biofuel production [31, 32].

Microalgae, with their high surface productivity and ability to grow on nonarable land, present a significant opportunity for third-generation biofuels. They mitigate greenhouse gases through photosynthesis and can produce up to 60% oil by weight under stress, making them suitable for biodiesel production [33]. However, challenges such as high cultivation costs, unsuitable species, and limited biomass productivity remain. Despite these issues, algae-based biofuels offer a solution to the “food versus fuel” debate and promise economic and social benefits [34]. Biomass recovery from various microalgae harvesting, flocculants, and their dosage is shown in Table 1. *Chlorella*, a genus of single-cell green algae, is notable for its high protein content and essential nutrients such as omega-3 polyunsaturated fatty acids and astaxanthin, making it a valuable resource for health supplements, nutraceuticals, and cosmetics [36].

Its ability to thrive under various conditions, including freshwater and wastewater, increases its attractiveness by minimizing competition for resources with food crops [23]. The species' ability to tolerate high pH levels, approximately around 7, makes cultivation easier, while its lower nutrient needs help in making the process more cost-effective. *Chlorella vulgaris* is renowned for its adaptability to various growth conditions and easy harvesting, making it a well-established and suitable choice for biodiesel production [37–39]. Advances in genetic manipulation and genome sequencing, including the sequencing of *Chlorella vulgaris* [40]. The lipid composition of *Chlorella vulgaris* and modifications to strains like *C. vulgaris*, *C. ellipsoidea*, and *C. kessleri* have significantly improved its lipid production, stress tolerance, and overall productivity [41]. *Chlorella vulgaris* algae (CVA) demonstrated significant potential in increasing ethanol concentration, reaching a level of 33.2 g/L [42].

This progress supports *Chlorella's* potential for large-scale cultivation and its application in biofuels and other industrial uses. *Scenedesmus dimorphus* has the highest lipid content (16%–40% dw) among the reported species and is considered a strong candidate for biofuel production. This is a green and unicellular

TABLE 1 | Microalgae harvesting, flocculants and their dosage (g/L), time (min), and effect on biomass recovery (%) [35].

Types of microalgae	Recovery of biomass (%)	Dosage (g/L)	Types of flocculants	Time (min)
<i>Cheatoceros gracilis</i>	90–95	0.2	FeCl ₃	60
	55		FeSO ₄	—
	95		AlCl ₃	—
<i>Scenedesmus sp</i>	97	0.15	FeCl ₃	2
	92	0.25	Alum	—
<i>Ankistrodesmus falcatus</i>	86	—	Alum	60
<i>Chlorella sp</i>	99	—	Methyl esterified clay	30
<i>Tetrasalmis sp</i>	85	—	FeSO ₄	—
<i>Chlorella zofingiensis</i>	> 90	< 0.09	FeCl ₃	—
<i>Chlorella sp.</i>	90	1.06	Fe ₂ (SO ₄) ₃	—
<i>Aurantiochytrium sp</i>	98.8	1	FeCl ₃	—
<i>Scenedesmus sp</i>	97.2	0.072	FeCl ₃	10
<i>Scenedesmus Spinosus</i>	98.4	7	FeCl ₃	—
<i>Chlorella zofingiensis</i>	94	0.219	Mg(OH) ₂	25
<i>Chlorella vulgaris</i>	92.4	2.5	Al ₂ (SO ₄) ₃	10
	90	0.005	PDADMAC	60
	86	0.448/g dry biomass	FeCl ₃	—
	77	0.504/g dry biomass	Al ₂ (SO ₄) ₃	—

microalgae species and can be found in colonies of 1, 2, 4, or 8 cells [43]. It has been shown that it can reach a maximum concentration of 1.3 gdw/L in 17 days of culture [44]. Studying *Chlorella vulgaris* and *Scenedesmus dimorphus* for sustainable energy solutions further highlights their advantages. Both strains are rich in proteins, lipids, and essential nutrients, making them ideal for producing biodiesel and bioethanol. *Chlorella vulgaris* is particularly valued for its high protein and omega-3 PUFA content, while *Scenedesmus dimorphus* excels in lipid accumulation [43]. Using these microalgae for landfill leachate (LL) treatment, especially when diluted with recycled harvesting water, shows promise in conserving freshwater and improving biomass and lipid accumulation [45]. Co-cultures of *Chlorella vulgaris* and *Scenedesmus dimorphus* demonstrated superior biomass growth and nutrient utilization efficiency compared to mono-cultures, contributing to more effective waste treatment and biofuel production [46]. Their ability to thrive in diverse environments and adapt to various cultivation systems, coupled with advances in genetic engineering, makes these microalgae a sustainable and economically viable alternative to fossil fuels, advancing both energy security and environmental management.

Selecting the appropriate harvesting method for algae is vital due to its significant impact on the economic viability, time efficiency, and yield optimization of algae-based production systems. Harvesting costs can constitute 20%–30% of total production expenses, making it a critical factor in the overall economics of algae cultivation. Some methods vary in cost-effectiveness and efficiency based on the production scale and characteristics of algae species. A common approach is a two-step process involving thickening and dewatering to concentrate microalgal slurry, reducing costs while

maximizing recovery [47]. For microalgae-based biofuels, despite their advantages over terrestrial crop-based fuels, economic viability remains a challenge. Open-pond systems are cost-effective but prone to contamination and water loss, while photobioreactors, though productive, are costly to construct [48]. Immobilized algae culture systems show promise but require further research due to high material costs and species limitations. Thus, careful selection of harvesting methods is crucial for achieving commercial viability. Harvesting microalgae involves removing water from the culture medium to concentrate the biomass, using techniques chosen based on energy efficiency, cost, cell size, and density. An ideal method should recover maximum biomass with moderate costs and minimal environmental impact. Conventional and advanced harvesting methods are summarized in Figure 1.

Microalgal harvesting techniques have unique advantages and limitations [49]. Flocculation is scalable but may leave residues, while centrifugation is effective but energy-intensive [43]. Filtration works well for algae larger than 70 µm and low-density cells but has high operational costs, frequent fouling, and is suitable only for small volumes [50]. Flotation is low-cost, chemical-free, and flexible but involves high initial and operational costs and is species-specific. Microalgae size is key in filtration, affecting separation efficiency. Smaller algae need finer, costlier filters prone to clogging, while larger algae suit coarser, cost-effective filters. Optimizing pore size ensures efficient, cost-effective harvesting. Gravity sedimentation is affordable but slow and depends on particle density. Electrical processes are highly efficient, exceeding 95%, but can damage cells during prolonged use; they are unsuitable for large volumes and need

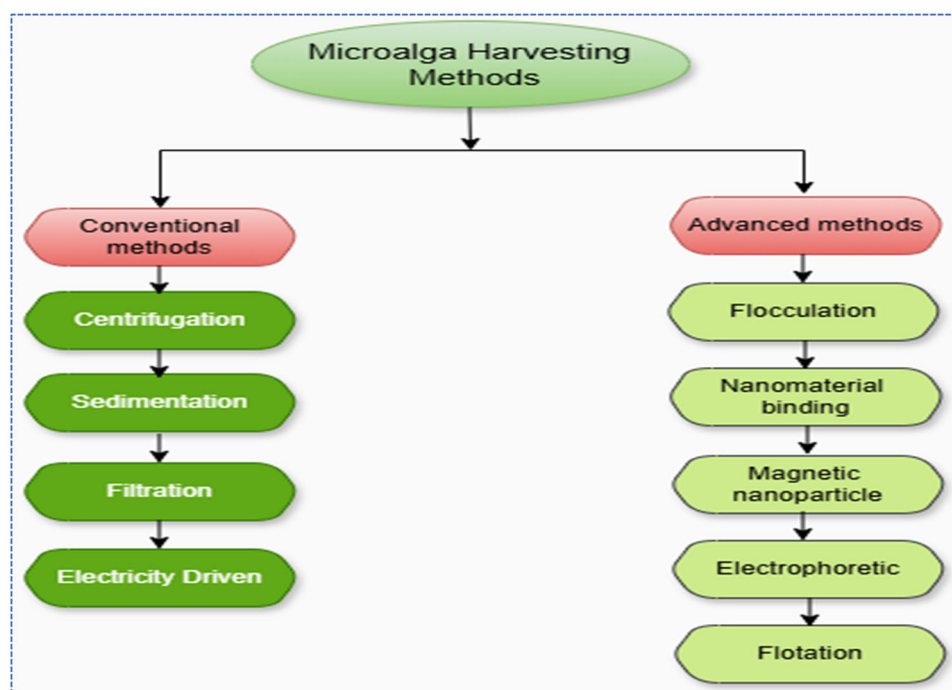


FIGURE 1 | Microalgae cell harvesting techniques encompass both conventional and advanced methods [35].

regular electrode maintenance. Magnetic separation is fast and material-efficient but relies on algal surface charge. Harvesting and lipid extraction account for over 80% of biodiesel production costs due to microalgae's small size (5–7 μm), low biomass concentration (0.3–5 g/L), and electrostatically stable cells with negatively charged surfaces. Efficient lipid extraction is further hindered by the tough cell wall, requiring organic solvents like chloroform, methanol, and hexane, which may be insufficient and dissolve chlorophyll. Developing cost-effective harvesting and eco-friendly extraction methods remains a key challenge for commercializing microalgae-based biodiesel. Compared to methods like sedimentation or flotation, three methods – centrifugation, flocculation, and filtration provide superior recovery, lower costs, and reduced processing time, making them well-suited for both research and commercial applications. Among these, centrifugation offers exceptional efficiency in rapidly recovering biomass, making it ideal for small-scale applications or the production of high-value compounds [51].

Responsibly produced biofuels can reduce CO_2 emissions, but using terrestrial crops risks food chain disruption and demands extensive land use [52]. Microalgae present a promising alternative due to their high growth rate and productivity, needing only sunlight, water, CO_2 , and nutrients to thrive [53]. Unlike other biofuel feedstocks such as rapeseed or soybeans, microalgae require far less land area, up to 49 or 132 times less for the same oil content [54]. Microalgae have a superior ability to fix and mitigate carbon dioxide through photosynthesis, making them an efficient and environmentally friendly source of biofuels [55]. With their potential for producing biodiesel, methane, hydrogen, and ethanol, microalgae offer a sustainable and eco-friendly alternative to fossil fuels [56]. People will also be encouraged to produce biodiesel from microalgae to meet the high demands of burning fuels, reduce the depletion of fossil

fuels, and also to benefit economically and attain environmental sustainability.

As discussed above, harvesting and lipid extraction, accounting for over 80% of biodiesel costs, is challenging due to the small size of microalgae, low biomass, negative charge, and tough cell wall with solvents often ineffective and dissolving chlorophyll. Therefore, advancing cost-effective harvesting techniques and eco-friendly lipid extraction methods is essential for the sustainable commercialization of microalgae-based biodiesel. Scientific and technical knowledge gaps remain a significant barrier to commercializing microalgae-based biofuel technology. While current large-scale biodiesel production methods are economical and efficient for biomass recovery and lipid extraction, there is limited research on the harvesting and extraction techniques specifically for microalgae in biodiesel production.

The novelty of this study lies in its comprehensive approach to optimizing microalgae-based biofuel production through the evaluation of cost-effective and efficient harvesting and oil extraction methods. By focusing on *Chlorella vulgaris* and *Scenedesmus dimorphus*, the study aims to enhance biofuel yield, reduce production costs, and address sustainability challenges associated with fossil fuel depletion. Therefore, this study evaluates three distinct harvesting techniques, such as centrifugation, flocculation and microfiltration, tailored to different production scales and compares two oil extraction methods: Soxhlet extraction and direct boiling. This study stands out by integrating multiple methods to improve both the technical and economic feasibility of large-scale algae-based biofuel production. This study highlights the potential of microalgae to reduce carbon dioxide emissions and air pollution and provides an eco-friendly advanced algae biofuel to meet the global energy crisis without competing with food production.

2 | Methodology

2.1 | Microalgae Cultivation

Ten liters of *Chlorella vulgaris* are cultivated in the $0.65 \times 0.35 \times 0.35$ m size aquarium, with the aid of 12 h of artificial light emission of $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ intensity and 5% CO_2 supply for 4 weeks before harvesting. The ideal light intensity for microalgae growth ranges from 26 to $400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, with higher intensities stimulating lipid synthesis and requiring 60 to $700 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for maximum lipid production, depending on the species. Four liters of de-chlorinated water and 1 mL of concentrated nutrients, including nitrogen, calcium, phosphorus, magnesium, potassium, microelements, and vitamins, are added to the media every 2 days for a week [57]. To sub-culture 22 L of media for microalgae, prepare and sterilize the media, inoculate it, control light and temperature, monitor growth, and transfer to fresh media after 2 weeks. Eleven liters from the sample are transferred to another $0.65 \times 0.35 \times 0.35$ m size aquarium, which is denoted as a second tank, with 12 L of de-chlorinated water and 5 mL of nutrients added weekly for 2 weeks in each tank. In the 4th week, 15 L of de-chlorinated water and 5 mL of nutrients are added to each tank, and the medium is left for 1 week. The total volume of the media for each tank in a month is approximately 50 L (a small number of media is lost due to evaporation). The culturing temperature is fixed at room temperature of 25°C [58]. The same procedures are applied using *Scenedesmus dimorphus*. The culturing method is repeated once the microalgae have been harvested, as shown in Figure 2.

2.2 | Microalgae Harvesting Methods

Chlorella vulgaris and *Scenedesmus dimorphus* are cultured in the desired volume, approximately 49 L, for 37 days before harvest is carried out. Twenty-five liters of the sample are taken for every harvest (Figure 2). The remaining 24 L of sample are sub-cultured by adding 25 liters of water and 10 mL of nutrients, and the culture is left for a week for the next harvest. The dry mass per liter sample is determined by dividing the dry mass of the 25 L sample by 25 [59].

2.2.1 | Flocculation

Flocculation is a pretreatment procedure that involves the formation of aggregates and is frequently used to naturally, chemically, or physically increase cell density [60]. To produce flocculation, chemical flocculants such as chitosan or starch or inorganic flocculants like Zn^{2+} , Al^{3+} , and Fe^{3+} are typically added [51–54]. The addition of flocculants in algae harvesting can alter the structure of bioactive compounds, depending on the flocculant's type and concentration. To preserve these compounds, using natural or milder flocculants is essential.

Flocculation Harvesting Techniques for Microalgae are shown in Figure 3.

The harvesting process of *Chlorella vulgaris* and *Scenedesmus dimorphus* begins after 30 days of cultivation, during which 25 L of media are collected for each species. For *Chlorella vulgaris*, flocculation is achieved by adding ferric chloride (FeCl_3) solution at a rate of 0.4 g per liter of media. Ferric chloride is chosen as a flocculant due to its effectiveness in aggregating microalgae cells by neutralizing their negative charges, facilitating easier separation. It is cost-effective, widely available, and induces rapid flocculation, improving harvesting efficiency. Its high solubility and compatibility with various algal species and pH conditions make it a versatile and practical choice for many studies [62]. The ferric chloride neutralizes the negative charge of the algae, causing the cells to aggregate into larger particles that eventually settle as sediment after 12 h. This separation creates distinct layers of sediment and water, with the sediment layer being carefully collected by removing the overlying water. The sediment is then filtered using Grade 1 filter paper with a 150 mm outer diameter. In cases where *Chlorella vulgaris* is cultured in saltwater or high-salinity media, sodium hydroxide (NaOH) may be used as an alternative flocculant. The harvested sediment is then dried under sunlight for 6 h to remove any remaining water content, reducing the slurry volume and hardening the biomass. The dried biomass is then crushed into smaller particles to facilitate oil extraction. A mass flocculation process, involving the addition of approximately 10 g of ferric chloride to 25 L of the sample, is conducted over 24 h, with sediment sinking to the bottom of the tank, though some microalgae loss is expected during de-watering. The same procedure is repeated for *Scenedesmus dimorphus*, ensuring efficient biomass recovery for both microalgae species. The algae flocculation process before and after sedimentation and the resulting dry microalgae powder are shown in Figure 4a,b.

2.2.2 | Centrifugation

The centrifugation method is very effective and rapid; it is considered unfeasible in large-scale algal culture systems due to the high capital and operational costs and an energy-intensive process [63]. Nevertheless, to enhance overall efficiency, a centrifuge can be used with other pretreatment methods. For the harvesting of *Chlorella vulgaris* and *Scenedesmus dimorphus*, a centrifugation method is employed using 25 L of media for each species at 7500 rpm with a tube of 172 mm radius and 25°C for 10 min. The centrifuge model used in this study was the Hitachi Himac CR 21 G shown in Figure 5a, known for its robust performance and versatility in various applications. This centrifuge can reach a maximum speed of 21,000 rpm and generate a relative centrifugal force (RCF) of up to $50,000 \times g$, making it highly efficient for a wide range of research needs. Its versatility is further enhanced by a temperature control feature, allowing it to operate within a specific temperature range to suit sensitive applications. After centrifugation, the wet microalgae sample is collected and dried under sunlight for 12 h, split into two sessions of 6 h each, to evaporate the moisture. Alternatively, an oven can be used, setting the temperature to 75°C for 3.5 h. Once dried, the sample is mechanically crushed and pressed into powder, with the dried mass of each species weighed and calculated per liter of media. The centrifugation method is efficient, yielding approximately 0.76 g of *Chlorella*

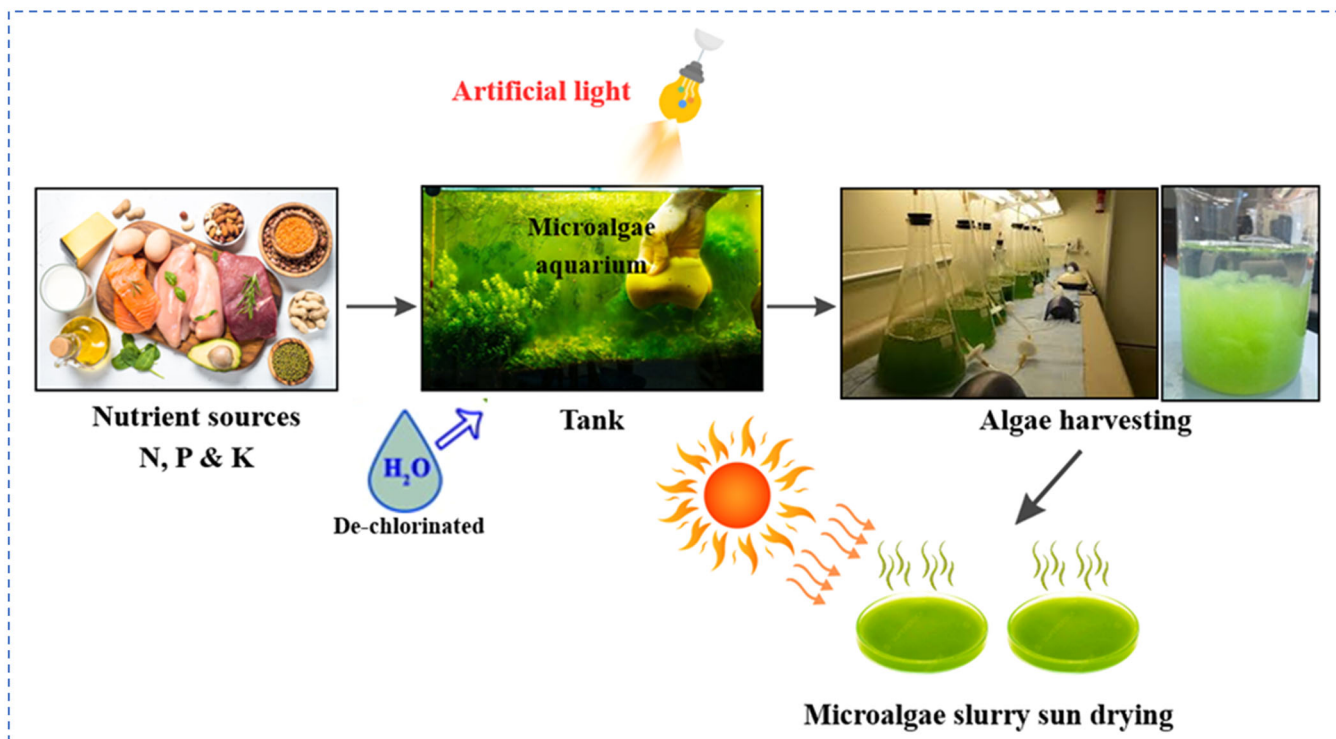


FIGURE 2 | Microalgae cultivation and harvesting system.

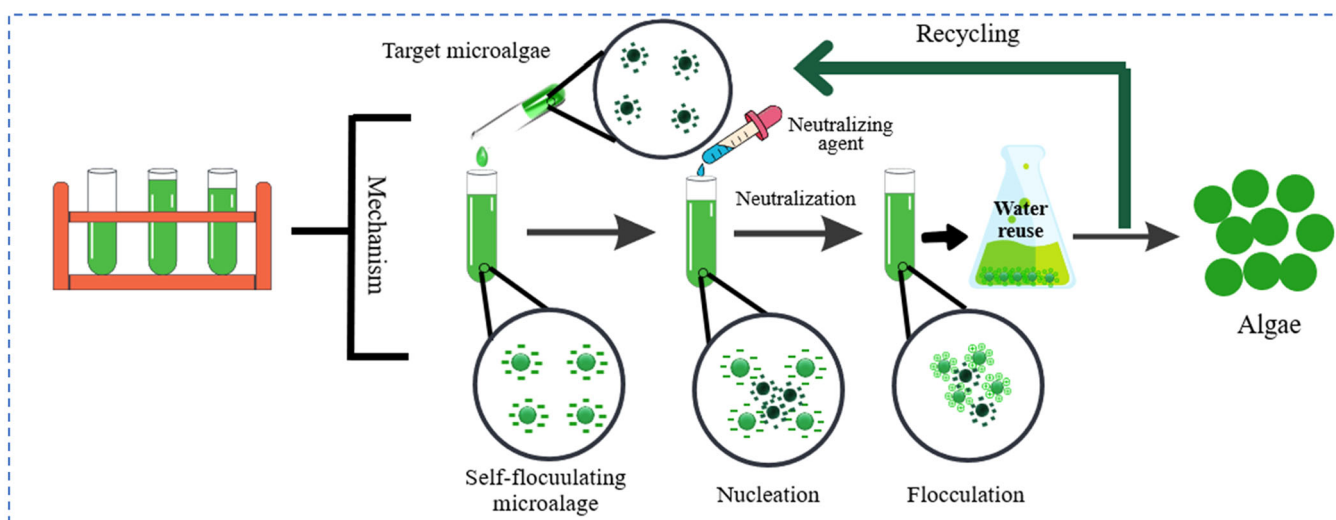


FIGURE 3 | Flocculation harvesting techniques for microalgae [61].

vulgaris and 0.81 g of *Scenedesmus dimorphus* per liter of sample. This method is particularly effective for small-sized microalgae like *Scenedesmus dimorphus*, which settles at the bottom of 250 mL bottles per compartment of the centrifuge bottle, as shown in Figure 5b, and is collected using a spatula before undergoing sunlight drying for 3 h.

2.2.3 | Membrane Separation Unit TR-14

Microalgal biomass can be obtained by filtering the liquid medium through filters that collect the algae as it passes. Based on the filtering methods utilized, such as pressure filtration,

vacuum filtration, tangential flow filtration, etc., the types of filtrations differ [58]. The classification of filtration systems as macro-filtration (pore size > 10 m), microfiltration (pore size 0.1–10 m), ultrafiltration (pore size 0.02–2 m), and reverse osmosis (pore size 0.001 m) depends on the pore size of the filters [59].

For the filtration of 25 L of *Chlorella vulgaris* and *Scenedesmus dimorphus*, a membrane separation unit (TR-14) was utilized, as depicted in Figure 6, using the micro-filtration membrane (the third membrane) for the process. The sample, referred to as the input, was introduced into the feed tank. The operation involved adjusting all necessary valves, starting the thermostat,

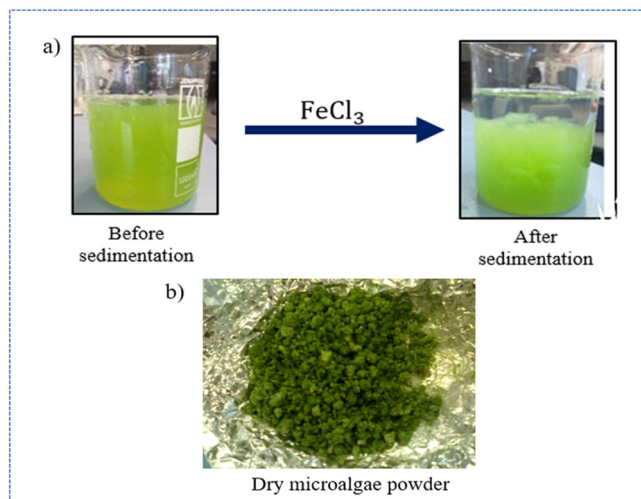


FIGURE 4 | (a) Before and after sedimentation during algae flocculation and (b) the resulting dry microalgae powder.

and activating the pump. The filtering was conducted at room temperature (27°C) with the pump pressure set to 12 bars (1200 KPa). The micro-filtration process was performed over 35 h, divided into 7-h sessions each day for 5 days. Rather than collecting the microalgae slurry in a product tank, it was retained within the membrane. Consequently, the membrane was extracted by disassembling the membrane tank. The microalgae slurry was then collected and dried under sunlight for 12 h, in 6-h intervals each day.

The membrane separation unit TR-14 effectively filtered the sample, resulting in clear water devoid of visible impurities. The microalgae slurry was retrieved by removing the micro-filtration membrane from the storage chamber. The slurry, which was inside the membrane tube, was collected by washing the membrane with 15 mL of water. This washing process was repeated until the slurry was entirely removed from the tube. The collected watery slurry was then placed in a beaker and dried under sunlight for 10 h to eliminate excess water. Experimental results indicated that centrifugation provided superior dry mass collection compared to the membrane filtration method. The high-speed refrigerated centrifuge demonstrated an efficient sedimentation rate, rapidly clumping the microalgae and allowing them to settle at the bottom. Figure 6 presents an image of the membrane filtration test unit (Model: TR 14) utilized in the experimental work.

2.3 | Microalgae Oil Extraction Methods

Oil extraction from microalgae is crucial for sustainable energy production, influencing both yield and quality. Various methods, including mechanical, solvent, Soxhlet, hydro-distillation, and ultrasonic-assisted extraction, each offer distinct benefits and limitations. Mechanical extraction is cost-effective for large-scale operations but has moderate yields. Soxhlet extraction provides high purity and yield but is costly and less scalable. Direct solvent extraction balances cost and effectiveness, making it ideal for large-scale production. Ultrasound-assisted extraction enhances yield with lower operational costs but

requires significant initial investment. Supercritical CO₂ extraction achieves high-purity oil but is economically challenging for large-scale use. The boiling point of solvents impacts efficiency and costs; solvents with lower boiling points facilitate easier recovery and lower energy consumption, whereas Soxhlet extraction involves solvents with specific boiling points, increasing complexity and expense [60]. Combining or developing hybrid methods may further optimize extraction processes and improve commercial viability.

2.3.1 | Soxhlet Extractor

Approximately 10 g of *Chlorella vulgaris* powder is filled into a cellulose timber, which is placed into the Soxhlet extractor main chamber as shown in Figure 7, and 100 mL of n-hexane is filled into the flask at the bottom of the main chamber. The heating scale is adjusted to scale 1, where the temperature of 60°C–65°C is monitored. The extracting process is carried out in 8 h to extract the microalgae oil. At the end of the extraction process, the mixture of n-hexane and microalgae oil is poured into a 500 mL beaker and left in the laboratory to evaporate the excess n-hexane. The volume of the oil obtained is recorded. The process is repeated by using *Scenedesmus dimorphus* powder [61].

2.3.2 | Direct Boiling Method

This method involves refluxing 10 g of *Chlorella vulgaris* powder and 100 mL of hexane at 70°C for 3 h to extract the oil completely. At the end of the extraction, the mixture is poured into another 100 mL beaker and left in the laboratory to evaporate the excess hexane. The volume and weight of the oil obtained are recorded. The process is then repeated by using *Scenedesmus dimorphus* powder [62]. The setup is shown in Figure 8.

3 | Results and Discussions

Chlorella vulgaris and *Scenedesmus dimorphus* are chosen for research purposes due to their high oil content and availability. However, *Chlorella vulgaris* is more abundant in nature than *Scenedesmus dimorphus*. *Scenedesmus dimorphus* is much more difficult to harvest than *Chlorella vulgaris* due to its small size.

Optimizing the cultivation of microalgae with high oil content, such as *Chlorella vulgaris*, involves a detailed understanding of their optimal growing conditions, nutrient requirements, species characteristics, and metabolic activities. Adjusting these parameters can significantly enhance cultivation output and cost efficiency while minimizing losses. Increasing the culturing tank volume and using larger tanks can improve microalgae slurry production. Adding ionized salts to increase media salinity enhances productivity, while nitrogen depletion during cultivation boosts lipid content. Cultivating microalgae in an open pond system with a paddle wheel for ventilation and circulation prevents sedimentation and reduces operational costs by utilizing naturally emitted carbon dioxide. Covering the

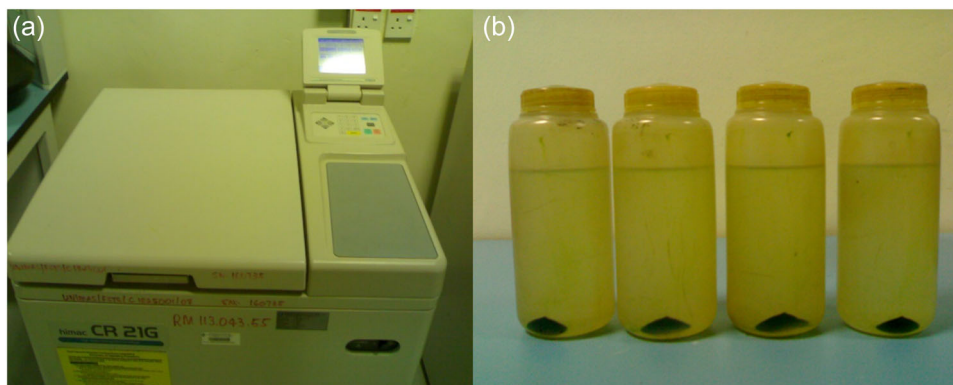


FIGURE 5 | (a) High-speed refrigerated centrifuge and (b) the sediment of *Scenedesmus dimorphus*.



FIGURE 6 | Membrane separation unit TR-14.

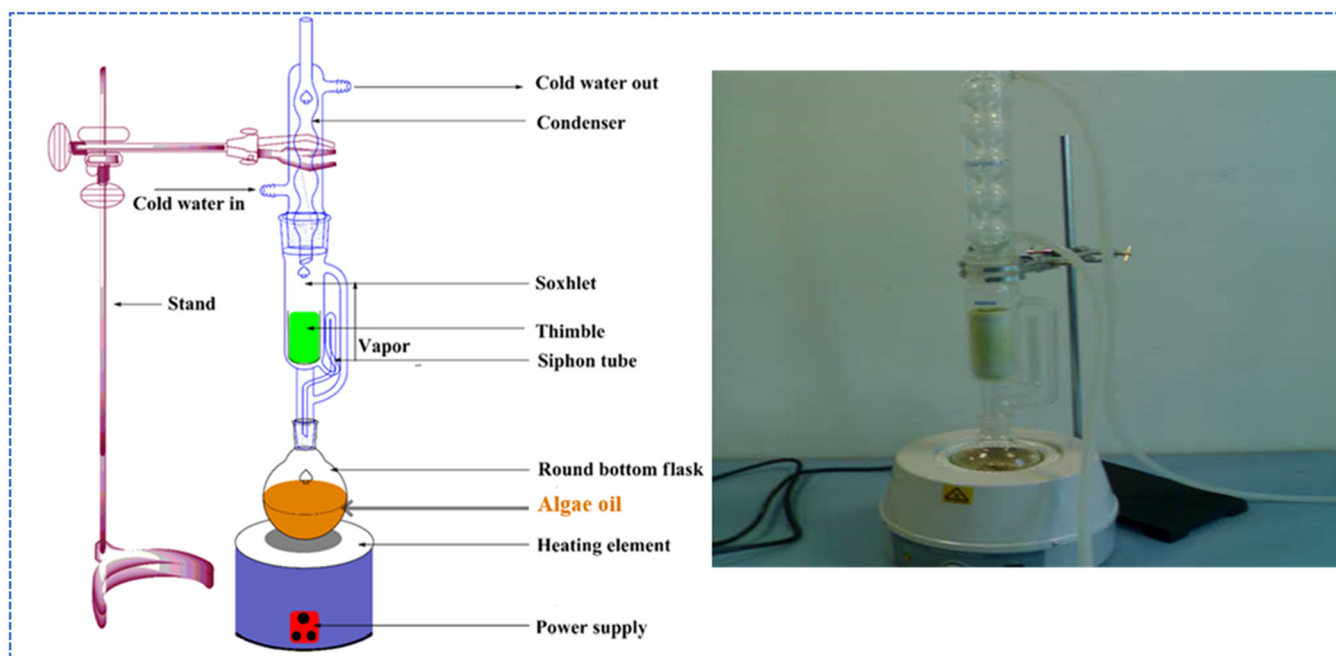


FIGURE 7 | Oil extraction using Soxhlet extractor.



FIGURE 8 | Direct boiling method.

pond with wire gauze helps maintain the culture's purity. These strategies contribute to a more efficient and productive microalgae cultivation process.

3.1 | Microalgae Harvesting Methods

Microalgae harvesting was carried out using three distinct methods: flocculation, centrifugation, and the membrane separation unit TR-14 for micro-filtration. Each technique was evaluated for its effectiveness in separating *Chlorella vulgaris* and *Scenedesmus dimorphus* from the growth medium. Flocculation, which relies on the aggregation of microalgae into larger flocs using chemical agents, demonstrated moderate efficiency. Although it facilitated the initial collection of microalgae, the process often required subsequent steps to fully concentrate on the biomass and remove residual impurities. Flocculation shows the lowest dry mass per liter compared to centrifuge and membrane separation units. Ibrahim A. Matter et al. emphasize the importance of optimizing flocculation processes for microalgae harvesting by considering efficiency, environmental impact, costs, and biomass utilization [61]. It is an economical and low-cost method to harvest microalgae in laboratories and on a large scale. The microalgae are treated with Ferric (III) Chloride to neutralize the negative charges of microalgae cells and to clump together to form large particles to ease the filtration process. It is convenient to flocculate large volumes of samples, but the filtration process is relatively slow. At the end of the filtration and drying process, the solid slurry is removed from the filter paper. However, a small portion of the slurry sticks to the filter paper and cannot be removed. Hence, it is considered as waste and loss. The loss and waste cause the dry mass per liter to be lower than in other harvesting methods. *Scenedesmus dimorphus* shows a lower yield than *Chlorella vulgaris* by flocculation. The small size of *Scenedesmus dimorphus* makes the flocculation and harvesting process difficult and inefficient. It takes a longer time to clump the cells together to form dense particles. Cells that are not neutralized suspend on the surface of the sample and remain unaffected in the media, which is a waste in the water-removing process [63].

Centrifugation proved to be highly effective, particularly with the high-speed refrigerated centrifuge. This method

significantly outperformed flocculation in terms of both time efficiency and biomass recovery. Centrifuge shows the highest dry mass per liter compared to flocculation and membrane separation units. It takes less time to harvest the microalgae. *Scenedesmus dimorphus* shows slightly higher dry mass per liter than *Chlorella vulgaris* in a centrifuge. This is because *Scenedesmus dimorphus* is smaller in size and capable of occupying abundantly in a fixed volume because the centrifuge makes almost complete sediment sink at the bottom of the bottle. However, it is limited to the laboratory scale and is an inconvenience in large-scale production [64].

The membrane separation unit shows a higher yield than flocculation but lower than centrifuge. Micro-filtration is suitable for large-scale harvesting because it completely removes the visible foreign substances from the media and yields clear water at the end of the process. The microalgae slurry is collected in the micro-filtration membrane tube in the membrane's storage chamber. This process can recover large amounts of microalgae slurry and dry mass, but it is time- and electricity-consuming. The micro-filtration is very slow in process. The membrane separation unit consists of a thermostat that monitors the feed temperature and a pump that circulates the flow where these two components consume electricity. Hence, it increases the overall cost and is inefficient with respect to time [65].

Dan Hu examined the growth of *Chlorella vulgaris* and *Scenedesmus dimorphus* in both mono- and coculture systems to explore their nutrient removal efficiency and growth behavior [37]. *Chlorella vulgaris* is easier to harvest compared to *Scenedesmus dimorphus* by membrane separation unit. Although *Scenedesmus dimorphus* is smaller in size and capable of occupying more abundantly in a fixed volume, the procedure of collecting its slurry from the micro-filtration membrane tube is more difficult. Some portions of its slurry are stuck on the wall of the membrane and cannot be collected; thus, it is considered waste. The measurement of dry weight and extracted oil from the two species of microalgae is shown in Table 2, and the information in the graphic is shown in Figure 9.

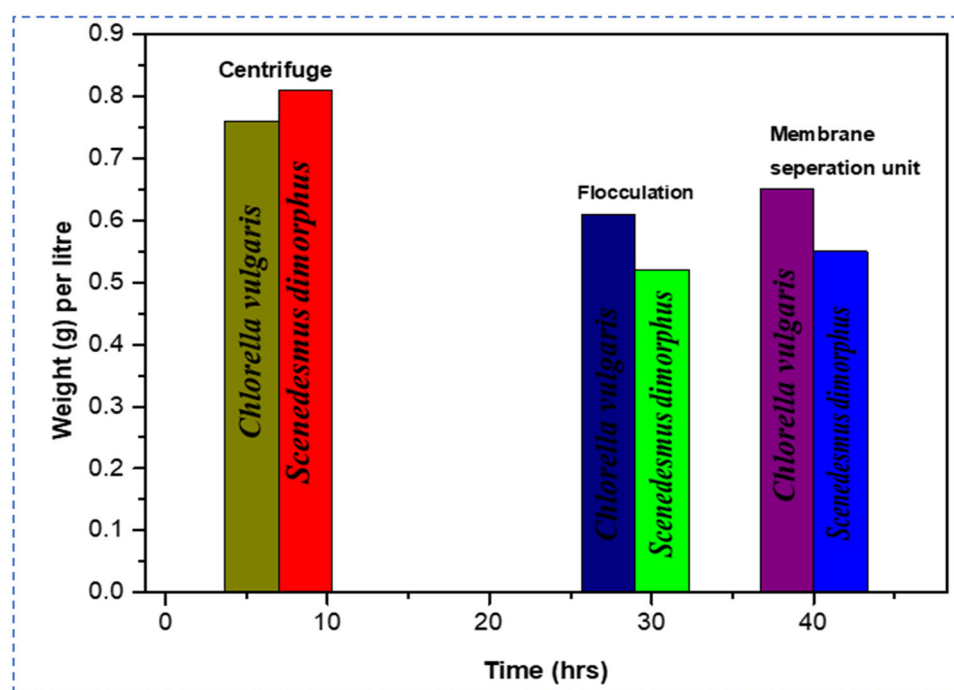
3.2 | Microalgae Oil Extraction Methods

Microalgae oil extraction is a crucial step in the production of biofuels and other valuable bioproducts. Various methods have been developed to efficiently extract oil from microalgae, each with distinct advantages and limitations. The choice of extraction method depends on factors such as the type of microalgae, desired oil yield and purity, economic considerations, and production scale. In this context, the boiling method and Soxhlet extraction were employed. Evaluating these methods for their economic and effective value is essential to determine the optimal approach for maximizing oil yield while considering the operational costs and scalability [66].

The dry biomass of *Chlorella vulgaris* and *Scenedesmus dimorphus* is used to extract the oil. There are two methods of extracting the oil. (1) Soxhlet extraction. Approximately 10 g of dry mass is inserted into a cellulose timber with 100 mL of

TABLE 2 | Measurement of the dry weight of microalgae biomass by various harvesting methods for every 25 L of the sample (9-day culture).

Species	<i>Chlorella vulgaris</i>		<i>Scenedesmus dimorphus</i>	
	Dry weight per liter (g)	Time consumed (hours)	Dry weight per liter (g)	Time consumed (hours)
Flocculation + filtration + drying	0.61	29	0.52	29
Centrifuge + drying	0.76	7	0.81	7
Membrane separation unit + drying	0.65	40	0.55	40

**FIGURE 9** | Comparative harvesting efficiency of *Chlorella vulgaris* and *Scenedesmus dimorphus* using different methods.

hexane solution per extraction. (2) Direct hexane boiling method. Approximately 10 g of dry mass refluxing with 100 mL of hexane solution per extraction; it is boiled for 3 h at 70°C. The oil yield from *Chlorella vulgaris* and *Scenedesmus dimorphus* is recorded. We have thoroughly evaluated both Soxhlet extraction and direct boiling methods for boosting oil yield from biomass. Soxhlet extraction provides a proper oil yield due to continuous solvent circulation but is costlier, making it more suitable for small-scale or lab use. In contrast, the direct boiling method is simpler and more cost-effective, making it better suited for large-scale applications.

Direct boiling has more influence on *Chlorella vulgaris* than on *Scenedesmus dimorphus* due to differences in their cellular structures and compositions. *Chlorella vulgaris* has a relatively simpler and thinner cell wall compared to *Scenedesmus dimorphus*, which often possesses a more robust and multilayered cell wall. The boiling process, which involves applying heat to break down cell walls and release intracellular contents, tends to be more effective on organisms with thinner or less complex cell walls. Consequently, the oil and other bioactive compounds within *Chlorella vulgaris* are more readily extracted through direct boiling, while *Scenedesmus dimorphus* may require more intensive or additional processing to achieve similar yields. This

difference in cell wall complexity makes *Chlorella vulgaris* more susceptible to the effects of direct boiling, resulting in higher oil extraction efficiency compared to *Scenedesmus dimorphus* [37]. The measurement of oil content from Soxhlet extraction and direct hexane boiling method is shown in Table 3.

According to the experimental results obtained, *Scenedesmus dimorphus* yields a higher oil content than *Chlorella vulgaris*. Approximately 2.8 mL from every 10 g of dry *Scenedesmus dimorphus* and 1.5 mL from every 10 g of dry *Chlorella vulgaris* are extracted by using Soxhlet extraction, while approximately 3.2 mL from every 10 g of dry *Scenedesmus dimorphus* and 2.2 mL from every 10 g of dry *Chlorella vulgaris* are extracted by using direct boiling method, as shown in Figure 10. Direct boiling shows better oil extraction efficiency than soxhlet extraction. The Soxhelt method requires more energy than the direct boiling method. In terms of solvent recovery, the Soxhlet method is beneficial as the same solvent is reused throughout the extraction process, while direct boiling requires a larger amount of solvent. Although Soxhlet's solvent recycling feature is good for the environment, long-term heating can cause sensitive chemicals to degrade thermally; therefore, depending on the analyte, direct boiling may be a more environmentally friendly choice. Direct boiling can break the microalgae cell

TABLE 3 | Measurement of oil content per 10 g dry weight using the direct boiling method and Soxhlet extraction for 3 h and from every 25 L of the sample.

Methods Microalgae sample	Direct boiling		Soxhlet extraction	
	<i>Chlorella vulgaris</i>	<i>dimorphus</i>	<i>Chlorella vulgaris</i>	<i>dimorphus</i>
Extracted oil (mL) $\pm$ error	2.2 ± 0.11	3.2 ± 0.16	1.5 ± 0.08	2.8 ± 0.14
Mass of the oil (g) $\pm$ error	1.87 ± 0.09	2.64 ± 0.13	1.27 ± 0.06	2.38 ± 0.12
Percentage oil content (%) $\pm$ error	18.7 ± 0.94	26.4 ± 1.32	12.7 ± 0.64	23.8 ± 1.19

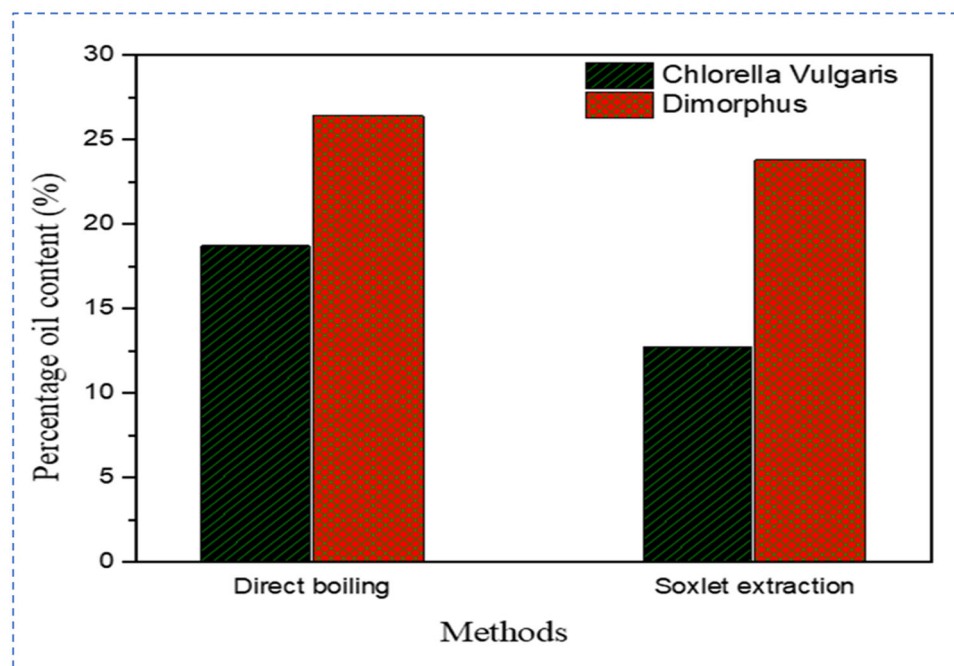


FIGURE 10 | Comparison of oil content in *Chlorella vulgaris* and *dimorphus* using different extraction methods.

wall and dissolve its oil in the cell. Direct boiling provides direct contact of heated hexane with every surface of the microalgae biomass and thus makes the oil extraction more efficient.

Soxhlet extraction applies the same mechanism as the reflux method. The microalgae biomass does not submerge in the hexane solution in the flask. The microalgae are placed in the cellulose timber, which is then placed into the main chamber of the Soxhlet extractor. The biomass is separated from the product (the mixture of hexane and oil). However, the temperature of the main chamber is lower than that of the flask where heating is applied. This causes longer breaking of the microalgae cell wall and makes it less efficient to extract the oil in a short time. However, this method produces a clean product without mixing with microalgae powder. The microalgae oils are shown in Figure 11a,b.

An earlier study showed that the highest lipid content is 10%–67% in *Chlorella* and *Scenedesmus* species [64, 65]. There is 12.7% oil extracted by using soxhlet extraction and 18.7% by direct boiling method from *Chlorella vulgaris*. A study conducted by Vishnu Priya et al. observed that 6.23% of the oil yield from *Chlorella vulgaris* by leaching with hexane [66]. On the other hand, 23.8% of the oil was extracted by using soxhlet extraction and 26.4% by direct boiling method from *Scenedesmus dimorphus*. The highest lipid concentrations reported by

Nirupa et al. in *Scenedesmus dimorphus* were 35% and 34% [67]. The oils extracted from *Chlorella vulgaris* and *Scenedesmus dimorphus* were analyzed for their physical and chemical properties using a variety of chromatographic and spectroscopic techniques, including FT-IR, ^1H NMR, GC/MS, and column chromatography. In Figure 11, the lipid oils extracted from *Chlorella vulgaris* and *Scenedesmus dimorphus* exhibit the following characteristics:

Both oils display a yellowish-green color, which is typical for microalgae-derived oils due to the retention of pigments such as chlorophyll. The visual appearance of the oils suggests they are sticky and moderately viscous, which is common for lipid oils extracted from microalgae. These oils generally exhibit moderate viscosity, typically ranging from 20 to 35 cP at 40°C, depending on the ratio of unsaturated to saturated fatty acids. The density of the oils falls within the typical range for microalgal lipids, around 0.86–0.92 g/cm³, although this may vary slightly depending on the extraction and purification processes involved. Lipid yields from *Chlorella vulgaris* are typically around 12%–40% of the dry biomass, while *Scenedesmus dimorphus* yields slightly higher lipid content, ranging from 22% to 51%. These advanced analytical methods provided detailed insights into the composition and characteristics of the extracted oils.

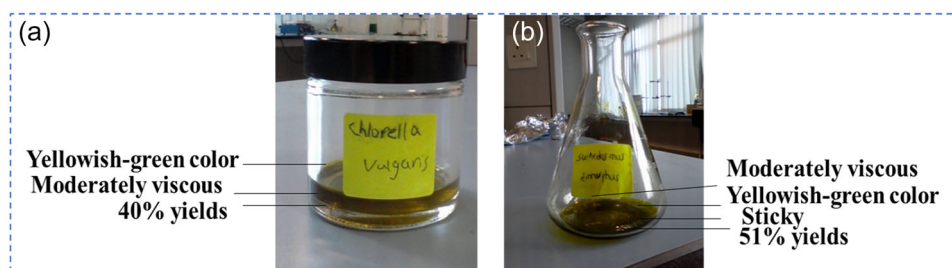


FIGURE 11 | (a) *Chlorella vulgaris* oil and (b) *Scenedesmus dimorphus* oil.

Fourier Transform Infrared Spectroscopy (FTIR) was used due to its rapid, non-destructive analysis of functional groups, making it ideal for routine characterization. FTIR effectively identifies functional groups like -OH, -CH, and -CO, providing a broad overview of the oil's composition. Additionally, FTIR is more cost-effective and accessible, with simpler sample preparation. While GC-MS and GC-FID offer detailed compound identification, FTIR aligns with our focus on general composition, making it a practical choice for this study. The Fourier Transform Infrared Spectroscopy spectra are shown in Figure 12 [67].

The FTIR spectra reveal the presence of key functional groups associated with carbonaceous materials. Broad peaks observed in the 3500–3200 cm^{-1} range correspond to hydroxyl (-OH) and amine (-NH) stretching vibrations, indicating surface hydroxyl groups or hydrogen-bonded water molecules. The C-H stretching vibrations, detected between 3000 and 2800 cm^{-1} , suggest the presence of aliphatic or aromatic hydrocarbons. The distinct absorption bands in the 1750–1650 cm^{-1} region correspond to carbonyl (C=O) groups, including carboxyl (-COOH) and ketone/aldehyde functionalities, while peaks in the 1300–1000 cm^{-1} range are attributed to C-O stretching from ester, ether, or alcohol groups. Additionally, the skeletal vibrations of aromatic rings are identified between 1600 and 1400 cm^{-1} . A comparative analysis of the spectra indicates that the left spectrum (blue) exhibits higher transmittance values, suggesting fewer surface functional groups than the right spectrum (red). The more pronounced O-H peak and broader C-O stretching region in the right spectrum imply a greater presence of oxygenated surface groups, likely resulting from differences in activation methods, precursor materials, or treatment conditions. Overall, the presence of oxygen-containing functional groups, such as carboxyl (-COOH), hydroxyl (-OH), and C-O groups, suggests that both materials have undergone oxidation or activation processes, which influence their adsorption properties.

The FTIR spectra (Figure 12) provide detailed insights into the biochemical composition and structural features of microalgae-derived oil and biodiesel. In the microalgae oil, characteristic absorption peaks confirm the presence of functional groups relevant to biofuel production. A prominent C-H stretching vibration peak, associated with the alkane group, is observed at 2862.35 cm^{-1} , while a carbonyl group (C=O) peak is identified at 1734.01 cm^{-1} , indicative of triglycerides and fatty acids. Similarly, in the biodiesel spectrum, the strong absorption peak at 1734.01 cm^{-1} corresponds to the stretching vibration of ester groups, which form during the transesterification process. This

similarity suggests that microalgae oil shares properties with conventional diesel and biodiesel, further validating its potential as a sustainable biofuel precursor [67, 68].

The spectra also highlight the presence of diverse biochemical components, including proteins, lipids, carbohydrates, and nucleic acids, as observed in the algal species *Chlorella vulgaris* and *Scenedesmus dimorphus*. Key peaks, such as the broad O-H stretching vibrations at approximately 3400 cm^{-1} represent hydroxyl groups, while C-H vibrations at ~2900 cm^{-1} are attributed to alkanes. Additionally, C-O stretching vibrations near 1100–1300 cm^{-1} confirm the presence of esters and other oxygenated compounds, further supporting the rich lipid content in these species. The biochemical composition underscores the versatility of these microalgae species. The substantial lipid content positions *Chlorella vulgaris* and *Scenedesmus dimorphus* as ideal candidates for biodiesel production through catalytic transesterification. Furthermore, their high protein content enhances their value for nutritional and biotechnological applications. FTIR analysis thus confirms the structural properties and functional groups essential for transforming microalgae oil into biodiesel, paving the way for its use in sustainable energy production [69].

The proteins make it a potential ingredient in food and feed products, while the lipids are ideal for biodiesel synthesis. The oil characterized by FTIR in our study is lipid oil extracted from *Chlorella vulgaris*, with functional groups, such as -OH, -CH, and -CO identified, indicative of fatty acids and triglycerides commonly found in lipid oils. These groups correspond to the characteristic bonds in the lipids extracted from the algae, primarily consisting of unsaturated and saturated fatty acids. The abundant carbohydrates and nucleic acids further enhance their utility in biotechnological processes, including bioethanol production and genetic engineering. In contrast, *Scenedesmus dimorphus* was found to have high levels of lipids and carbohydrates, making it an excellent candidate for biofuel production, particularly biodiesel and bioethanol. The prominent lipid content in *Scenedesmus dimorphus* is especially advantageous for efficient biofuel conversion, while the carbohydrate reserves support its role in renewable energy production. These findings highlight the distinct yet complementary potential of these two microalgae species in various industrial applications. The fatty acid profile, rich in C16 and C18 fatty acids, makes this lipid oil an ideal precursor for conversion into biodiesel, a widely recognized form of alternative energy. Therefore, the microalgal lipid oil characterized in this study is suitable for use as a sustainable biofuel feedstock.

The factor that contributes to the difference from the theoretical value of oil content is the oil loss to the environment. The

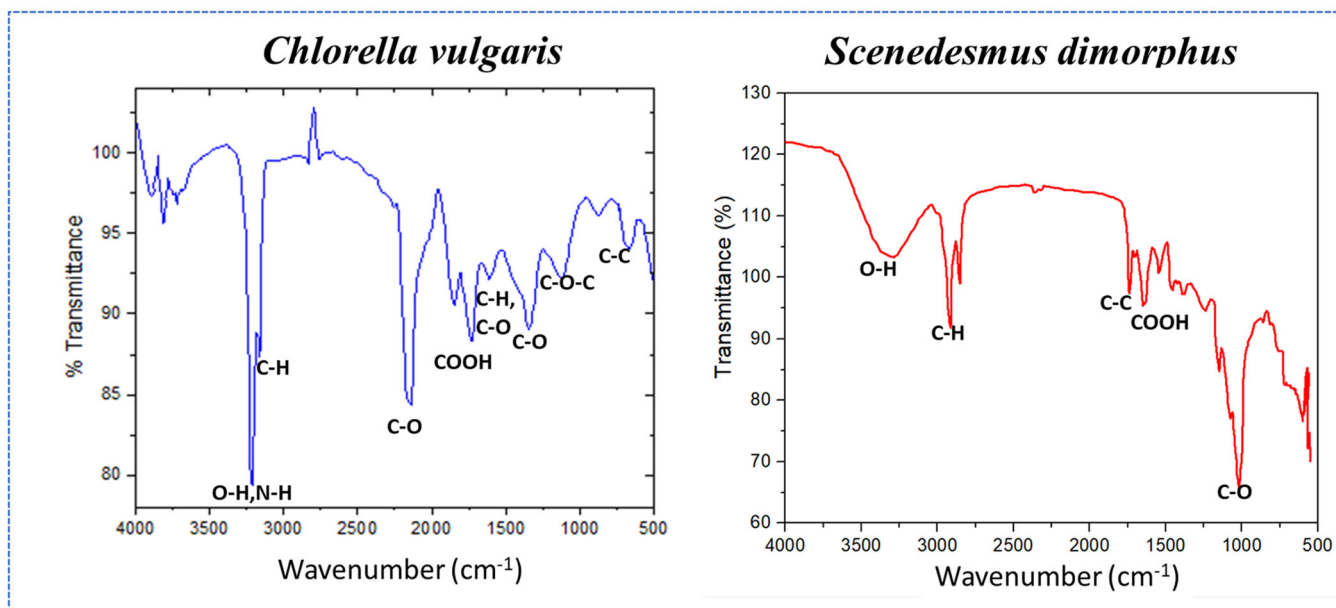


FIGURE 12 | Fourier transform infrared spectroscopy spectra of microalgae oil.

process of converting microalgae slurry to dry biomass is time-consuming. Microalgae are made up of unicellular structures. They can easily exchange nutrients with the environment. The slurry collected must proceed to oil extraction immediately to prevent oil loss. Hence, the oil may evaporate into the atmosphere in the harvesting process. On the other hand, the oil is potentially evaporated during the drying process. These cause the oil extracted to be less than the theoretical value. Besides, the microalgae biomass is not completely crushed into powder form. This leads to inefficiency of oil extraction as the cell walls of the microalgae are not completely disrupted. Hence, the hexane solution cannot dissolve the oil in the cell, which is protected by a cell wall. Another factor that leads to the inefficiency of oil extraction is extraction time. The experiment is conducted approximately in 3 h. The time used to conduct the experiment may not be able to fully extract and dissolve the microalgae oil. This is because the hexane solution must be heated for a period of time to disrupt the microalgae cell wall. However, the excess time used to extract the oil may lead to cost-effectiveness and waste.

Scenedesmus dimorphus is categorized as diatom and small in nature. Diatoms are usually high in oil content as they are capable of accumulating oils in their unicellular structure. The unicellular structure enables them to absorb nutrients from the environment easily and boosts the growth rate at optimum situations. Hence, this factor promotes better lipid production throughout their life cycle. *Scenedesmus dimorphus* has longer life span than *Chlorella vulgaris*. This is proven by chlorophyll loss indication where *Chlorella vulgaris* lose their chlorophyll faster than *Scenedesmus dimorphus* at control volume, temperature, carbon dioxide, nutrient contents and light intensity. Hence, *Scenedesmus dimorphus* has a better capability to accumulate lipids in their cells with respect to time.

The oils that can be used as precursors for preparing alternative energy from *Chlorella vulgaris* and *Scenedesmus dimorphus* are primarily triacylglycerols (TAGs) [70]. These microalgae

produce lipids rich in fatty acids, including palmitic acid, oleic acid, linoleic acid, and linolenic acid, which are ideal for biodiesel production. The process involves transesterification, where the triglycerides react with alcohol, usually methanol, in the presence of a catalyst to produce biodiesel and glycerol. In addition to TAGs, these microalgae also contain free fatty acids (FFAs) and polar lipids like phospholipids and glycolipids, which can contribute to the overall lipid profile. While FFAs can be directly converted into biodiesel through esterification, polar lipids may require further processing. The lipid content of *Chlorella vulgaris* and *Scenedesmus dimorphus* can be optimized through specific cultivation conditions, such as nutrient limitation, to enhance oil yield. These oils, particularly triglycerides, serve as promising precursors for sustainable energy solutions, making these microalgae valuable candidates in the production of biofuels. Notably, the fatty acids produced by these microalgae were predominantly C16 and C18 types, with linolenic and palmitic acids being the most prevalent. The biodiesel properties of oil derived from *Scenedesmus dimorphus* meet American (ASTM D6751) and European (EN 14214) biodiesel standards, indicating its significant potential for biodiesel production. Moreover, its productivity can be enhanced through mixotrophic cultivation. These findings highlight the distinct yet complementary industrial applications of these two microalgae species, particularly in the production of biofuels and other biotechnological processes [71].

4 | Conclusions

This study successfully identified and evaluated various harvesting and oil extraction methods for *Chlorella vulgaris* and *Scenedesmus dimorphus* providing key insights into optimizing biofuel production from these microalgae strains. The findings underscore the potential of *Scenedesmus dimorphus* as a superior candidate for large-scale biofuel production due to its higher growth rate and biomass yield. While centrifugation

emerged as the most efficient harvesting method, its high cost suggests the need for alternative approaches, such as flocculation and membrane separation, particularly for large-scale operations. Direct boiling proved to be the most effective oil extraction technique, highlighting its potential for industrial application. Looking forward, future research should focus on refining these methods to improve efficiency and scalability. Incorporating genetic engineering to boost lipid production, optimizing harvesting and extraction techniques, and integrating microalgae biofuel production with other renewable energy systems will be crucial steps toward advancing sustainable biofuel production. These efforts will enhance the commercial viability of microalgae-based biofuels, contributing significantly to the development of a sustainable energy future. The conclusion effectively summarizes key findings while also providing specific recommendations for future research. It emphasizes the need to refine harvesting and extraction techniques to enhance efficiency and scalability, explore genetic engineering to increase lipid production and integrate microalgae biofuel production with other renewable energy systems. These recommendations offer clear directions for further studies, addressing both technical improvements and commercial viability. To further strengthen this aspect, we will explicitly discuss the economic and operational challenges associated with scaling these methods, including cost considerations, energy demands, and potential integration with existing biofuel production frameworks. Additionally, we will highlight future research avenues aimed at improving scalability, particularly through advancements in cost-effective harvesting techniques, such as flocculation and membrane separation. We appreciate your valuable feedback and will incorporate necessary revisions to ensure a more comprehensive discussion on these critical aspects.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available on request.

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