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Review

Bio-Nanoparticles Mediated Transesterification of Algal Biomass for Biodiesel Production

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Abstract: Immense use of fossil fuels leads to various environmental issues, including greenhouse gas emissions, reduced oil reserves, increased energy costs, global climate changes, etc. These challenges can be tackled by using alternative renewable fuels such as biodiesel. Many studies reported that biodiesel production from microalgae biomass is an environment-friendly and energy-efficient approach, with significantly improved fuel quality in terms of density, calorific value and viscosity. Biodiesel is produced using the transesterification process and the most sustainable method is utilizing enzymes for transesterification. Lipase is an enzyme with excellent catalytic activity, specificity, enantio-selectivity, compatibility and stability and hence it is applied in microalgae biodiesel production. But, difficulty in enzymatic recovery, high enzyme cost and minimal reaction rate are some of its drawbacks that have to be addressed. In this aspect, the nanotechnological approach of lipase immobilization in producing microalgae biodiesel is a promising way to increase production yield and it is due to the adsorption efficiency, economic benefit, recyclability, crystallinity, durability, stability, environmental friendliness and catalytic performance of the bio-nanoparticles used. Through increasing post-harvest biomass yield, absorption of CO_2 and photosynthesis in the photobioreactor, the use of nanoparticle immobilized lipase during the generation of biodiesel from microalgae has the potential to also remove feedstock availability constraints. This review article discusses the production of microalgae biodiesel, and effect of nanoparticles and immobilized lipase nanoparticles on biodiesel production. The advantages of using lipase nanoparticles and the challenges in introducing the immobilized lipase on nanoparticles in large-scale microalgae biodiesel production are also discussed. Reducing the water and land use, energy and nutrient footprints of integrated algae-based operations must be the main goal of larger-scale experiments as well as ongoing research and development in order to expedite the adoption of microalgae-based biodiesel production. Also, the cost-effectiveness and large-scale availability of nanoparticles and the impact of lipase nanoparticles on engine performance should be analyzed for commercialization of microalgae biodiesel.

Keywords: biofuel; nanocatalyst; algal biomass; immobilized; renewable; sustainable; lipase

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1. Introduction

Biodiesel generated from microalgae is a potential source of energy [1] and it shows an increased cetane count, a flash point, high lubricant, combustion efficiency, reduced exhaust generation, biodegradability, nitrogen oxides, positive energy balance, prevention of a sulfur shortage, renewable energy infrastructure and domestic origin [2,3]. Using microalgae for biodiesel production is advantageous due to its ease of culturing in waste-piled areas, rivers, ponds, seas, humid wastelands and municipal and industrial waste drainage [4]. Its growth does not overlap or interrupt the animal and human food chains to cause potential food sustainability or safety issues [5,6]. It has the capability of fixing and sequestering carbon content derived from carbon dioxide present in air and exhausted emission [7,8]. Moreover, many species of microalgae have significant oil content; under ideal conditions, some species can have oil content as high as 75% of their dry mass. One of the potential algae genera with significant lipid content is the *Scenedesmus* genus. It also possesses a significant capacity to remove nutrients during wastewater treatment and a resistance to contamination. These qualities make it a desirable input for the production of biodiesel [1].

The most popular and affordable method for producing biodiesel using an alkali, an acid, or an enzyme as a catalyst is transesterification, where the triglycerides and primary alcohol are combined in the presence of a catalyst [9]. Because of high activity under reaction conditions, catalysts like KOH/NaOH are widely utilized for producing biodiesel. However, separating a catalyst can be challenging, and a significant quantity of wastewater is produced [10]. Using enzymes is one of the sustainable techniques and it is due to their biocompatibility, specificity, biodegradability and environmental acceptability. The enzymatic transesterification process is more advantageous with a lower energy requirement and minimal environmental adverse effects [11].

One of the enzymes, lipase, shows enhanced stability, specificity, enantio-selectivity, catalytic effect and region selectivity and hence it is more suitable for the enzymatic transesterification process in biodiesel production [12]. Additional benefits of using lipase include decreased consumption of energy during the reaction due to mild reaction conditions and low temperatures, absence of soap development in the process, minimal or no side reactions, reduced sensitivity to moisture and fatty acid (FA), ease of recovering the fuel produced, compatibility with the environment and reduced alcohol consumption during the esterification reaction [11].

Even while the enzymatic transesterification process and lipase provide numerous advantages for the production of biodiesel, the disadvantages should also be taken into consideration. It should be noted that carrying out enzymatic transesterification is challenging, mostly because of issues in recovery of the enzyme, sustained instability of its functioning, cost and alcohol-induced inactivation of the enzyme. Furthermore, lipases may exhibit improper enzymatic activity. Additionally, the structure and activity of lipase are altered in the aqueous phase. Also, the interfacial area, which is governed by the substrate's moisture content, is what determines lipase's catalytic activity [13]. Enzyme immobilization is developed as a solution to the drawbacks of enzymatic transesterification. It also aimed to increase reaction control, prevent contamination of the product by the enzyme, enhance catalytic stability and allow for the use of different reactor topologies. It can also be used to modify enzyme activity, selectivity and specificity, reduce enzyme inhibitions or couple with purification of enzymes [14]. Also, in comparison with the chemical catalysts, lipase-immobilized nanoparticles are versatile and economical in terms of cost-effectiveness and reusable nature [15].

The IEA World Energy Outlook 2020 reported that biodiesel production volume in 2018 reached 154 billion liters and is predicted to increase by 25% in 2024 [16]. A bar chart representing the production of biodiesel from 2019 to 2025 is shown in Figure 1 [17]. However, despite the increased biofuel production volume, they are still not enough to meet the global energy need, and also developing sustainable low-energy biofuel production technology is a need globally [8,18]. Hence, researchers have utilized nanotechnology to produce microalgae biodiesel using the enzymatic transesterification process with improved yield and quality at a reduced cost.

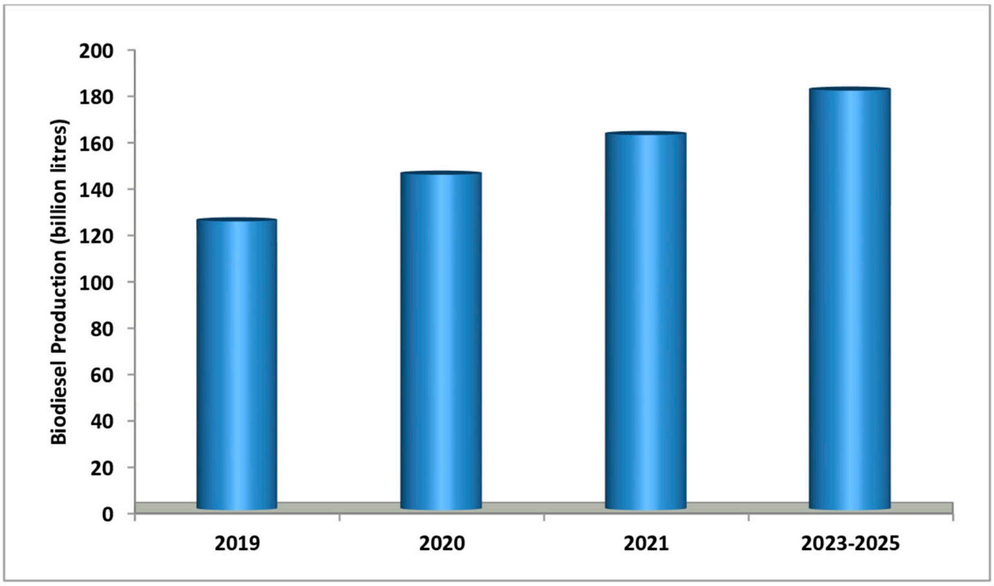


Figure 1. Production of biodiesel from the year 2019 to 2025, as reported by the international energy agency.

The nano-immobilized lipase enzyme as a novel biocatalyst is a promising alternative [19]. To date, various studies have reported the increased yield of microalgae biodiesel via nanotechnology. This technology involves the immobilization of the lipase enzyme in the nanoparticles with dimensions of 1–100 nm. The bio-nanoparticles are then used in the fermentation media to promote microalgae growth to maximize the oil accumulation to produce biofuels [1]. The essential features of nanoparticles include their huge surface area relative to their volume, strong adsorption capacity and surface activity, which make them potential candidates to be essential players in the immobilization of lipase enzymes [20] for biodiesel production. This review article presents and overviews the transesterification of microalgae biomass using lipase as an enzyme source and nanoparticles as an immobilization agent for biodiesel production. It also aims to provide insights to future researchers about the challenges in microalgae biodiesel production with the incorporation of nanotechnology, thereby enhancing the large-scale biodiesel production and its commercialization.

2. Microalgae Biodiesel Production

The production of biodiesel from microalgae biomass involves several processes: microalgae strain selection, microalgae cultivation, harvesting, oil extraction and transesterification [21]. Microalgae strains may be marine- or fresh-water-based and marine strains are preferred more due to their wide occurrence. Microalgae such as *Chlorella vulgaris* [22], *Chlorella emersonii* [23], *Chlorella sorokiniana* [24], *Schizochytrium acidic limacinum* [25], *C. protothecoides* [26], *Chlorella vulgaris* ESP-31 [24], *Spirulina*, *Thalassiosira*, *Nannochloropsis*, *Cyclotella, Scenedesmus* [27], etc., are employed for generating biodiesel and they are noted in Table 1. A few characteristics determine the selection of microalgae strains: greater capacity for producing lipids; resilience to stress in photobioreactors and open raceway ponds; competent with the wild type of strains in the event that an open pond system is employed; increased absorption of carbon dioxide; robustness; ability to tolerate limited nutrient supply; tolerance to seasonal variations in temperature; capacity to generate co-products; increased productivity; increased photosynthetic effect; and the distinctive self-flocculation feature. Among the investigated strains, *Schizochytrium* sp., *Nannochloropsis* sp. and *Spirulina* sp. are commonly used to generate biomass to produce biofuels, with *Chlorella vulgaris* as the most suitable strain and it is due to the presence of high lipid content [28].

Table 1. Microalgae cells as renewable feedstock for production of biodiesel.

Sr. No.	Name of Microalgae	Remarks	References
1.	*Spirulina platensis*	Good diesel index, high density	[29]
2.	*Chlorella protothecoides*	High cetane number	[30]
3.	*Chlorella* sp.	High viscosity	[30]
4.	*Botryococcus braunii*	80% lipid accumulation	[31]
7.	*Tetraselmis* sp. *M8*	Lipase catalyzed transesterification	[32]
8.	*Croto megalocarpus*	Immobilized lipase for better production	[33]
9.	*Chlorella sorokiniana*	44.1–87.9% lipid accumulation	[33]
10.	*Scenedesmus* sp.	Lipid accumulation in 20 days	[34]
12.	*Chlorella sorokiniana*	Lipid accumulation	[35]
13.	*Chlorella vulgaris*	95% yield	[35]
16.	*Chlorella* sp. *MJ 11/11*	Improved quality of bio-oil	[36]
17.	*Scenedesmus quadricauda*	High amount of oleic acid	[37]

Microalgae can be cultivated in open systems such as ponds, tanks and raceway ponds, closed systems and hybrid systems that utilize characteristics of both systems. All these systems have their advantages and disadvantages that can be compared based on various parameters. Open ponds have minimal capital and operating costs with low or minimum energy requirements for culture mixing. Open systems have various disadvantages, such as high contamination risk, difficulty in scaling up, more susceptibility to adverse weather conditions, lack of agitation in these systems and low biomass production yield, whereas closed cultivation systems such as photobioreactors are more efficient in terms of quality due to highly controlled conditions during operation [38]. However, bio-fouling and overheating along with high build-up of dissolved oxygen result in growth limitation [39]. In order to overcome the challenges faced in a photobioreactor, a hybrid cultivation method is developed. This method involves two cultivation stages, aiming to combine the advantages of open ponds and photobioreactor systems [40]. In a study, the hybrid cultivation system is developed by combining a baffled reactor with a photobioreactor to cultivate *Chlorella vulgaris*, *Scenedesmus simris* and *Chlorella sorokiniana* in the wastewater system and it produced a biomass with lipid content varying from 44.1 to 87.9% [41].

In one of the comparative studies using marine microalga *Tetraselmis* sp. M8, cultivation is carried out in a photobioreactor, open raceway pond and two-stage hybrid system. The two-stage hybrid system has resulted in exponential biomass in the first tank and separate synchronized lipid stimulation in nutrient-limiting conditions. The comparative account is carried out based on growth, harvesting cycles and maximum lipid accumulation. Three harvesting cycles with 2×10^{-6} cells/mL on the 28th and 29th day are possible in the photobioreactor and open raceway pond whereas six harvesting cycles with 3×10^{-6} cells/mL are achieved with two-stage hybrid systems. The growth rate (μ) is the maximum in the case of the hybrid system (0.17) as compared to the raceway pond (0.10) and photobioreactor (0.11). Thus, maximum biomass productivity and lipid accumulation have been achieved in a hybrid system (14 $\mathrm{g/m^{-2}/D^{-1}}$) and maximum lipid accumulation [32].

Product value, density and size are among the parameters taken into account when choosing the harvesting technique. The harvesting procedure accounts for 20–30% of the total production costs [42]. The physical harvesting method includes gravity sedimentation, centrifugation, membrane filtration and flotation approaches. However, it also has disadvantages such as a higher energy consumption and higher cost of facility implantation [43]. For the flotation process to work, algae cells must be trapped. Although centrifugal sedimentation is a quick method for harvesting algal biomass, it has the drawback of requiring maintenance [44]. Microalgae such as *Spirulina* and *Coelastrum* are ideal for filtration [45]. In the harvesting process, liquids are pumped out, homogenized and dewatered as well. Harvested biomass must be dried or dehydrated since it perishes quickly. Algal biomass can be dried using a variety of techniques, including drum drying, sun drying, fluidized bed drying, spray drying and freeze drying (lyophilization). Sun drying is the least expensive drying technique; however, it has drawbacks, such as a lengthy drying period. According to [46], freeze drying is the most costly drying process when used on a wide scale. Flocculation and coagulation are typical chemical harvesting methods. Chemicals like $FeCl_3$, $Fe_2(SO_4)_3$ or $Al_2(SO_4)_3$ must be added for flocculation. The disadvantages of chemical harvesting methods are sludge formation, biomass toxicity and low lipid content [47]. Autoflocculation and bioflocculation are good examples of biological methods of harvesting microalgae [48,49]. Compared with the above chemical and physical harvesting methods, this approach is cost-efficient and environmentally viable to generate pure harvested biomass [50], but the biological approach requires a high energy input [51].

After harvesting the algal biomass, various chemical and mechanical procedures are employed to extract oil. The methods of extraction include solvent extraction, enzymatic extraction, hot oil extraction, mechanical extraction, ultrasonic aided extraction and supercritical fluid extraction [52]. Although it is useful to use enzymes in the oil extraction process to obtain fractional chemicals in the downstream phase, cost is a disadvantage. The solvent extraction approach is not nearly as effective as the supercritical fluid extraction method. The supercritical fluid extraction technique that involves liquefying CO_2 under pressure yields an extract that is highly pure. The extraction of algae oil employs the stripper column approach [53]. About 60–70% of the oil can be extracted using the solvent extraction method, which uses cyclohexane, acetone, benzene, hexane and chloroform as the solvents. The solvent used to extract oil is very expensive. According to [52], the ultrasonic approach depends on the process of cavitation, which requires very little duration and extracts 76–77% of the oil. These techniques are associated with high energy consumption, significant environmental toxicity and low extraction efficiency [54].

Transesterification is an important process where the microalgae biomass's glycerol-based ester is converted to fatty acid methyl ester. Triglycerides are an ester with a higher lipid content that produces a large amount of biodiesel. If there are any ketones, polar lipids or pigments in the algae oil, the transesterification process may be affected. In addition to using methanol as the alcohol, the transesterification process also makes use of a catalyst (KOH/NaOH). Biodiesel yielded from freshwater algal cultivation is around 400 $kg\ kg^{-1}$ [55]. The three variables that impact the transesterification process are inhibitors, humidity and triglycerides. According to [56], a two-step transesterification procedure is advised to prevent saponification reactions. Methanol and sulfuric acid must be used in the pretreatment procedure if the amount of FFA in the algal oil is more than 2% [57]. Transesterification using enzymes is more viable and specifically immobilized enzymes increase the sustainability of biodiesel productivity [33].

3. Effect of Nanoparticles on Microalgae Biodiesel Production

A microalgae culture system is enhanced with nanomaterials that boost microalgal growth and cause lipid accumulation [58,59]. Furthermore, the use of the nanoparticle during biodiesel production from microalgae has the capacity to eliminate the restrictions associated with the availability of feedstock by improving the post-harvest biomass yield and CO_2 absorption and enhancing photosynthesis in the photobioreactor [60]. The technological scheme for the production of biodiesel using bio-nanoparticles [20,45] is depicted in Figure 2 and the different types of nanoparticles used for microalgae biodiesel production [61–64] are depicted in Figure 3. In the sections that follow, the usefulness of nanotechnology in lipid accumulation, transesterification, algal culture and extraction has been evaluated.

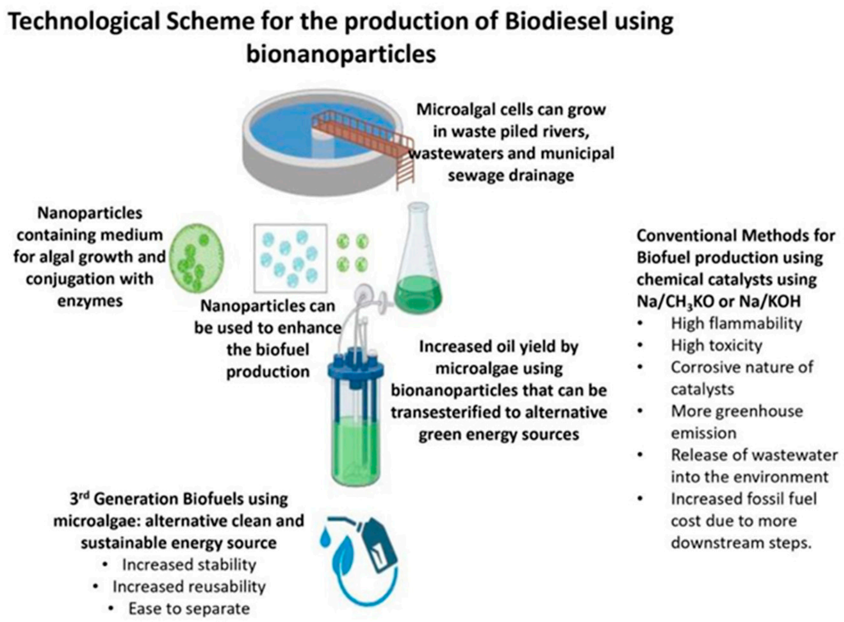
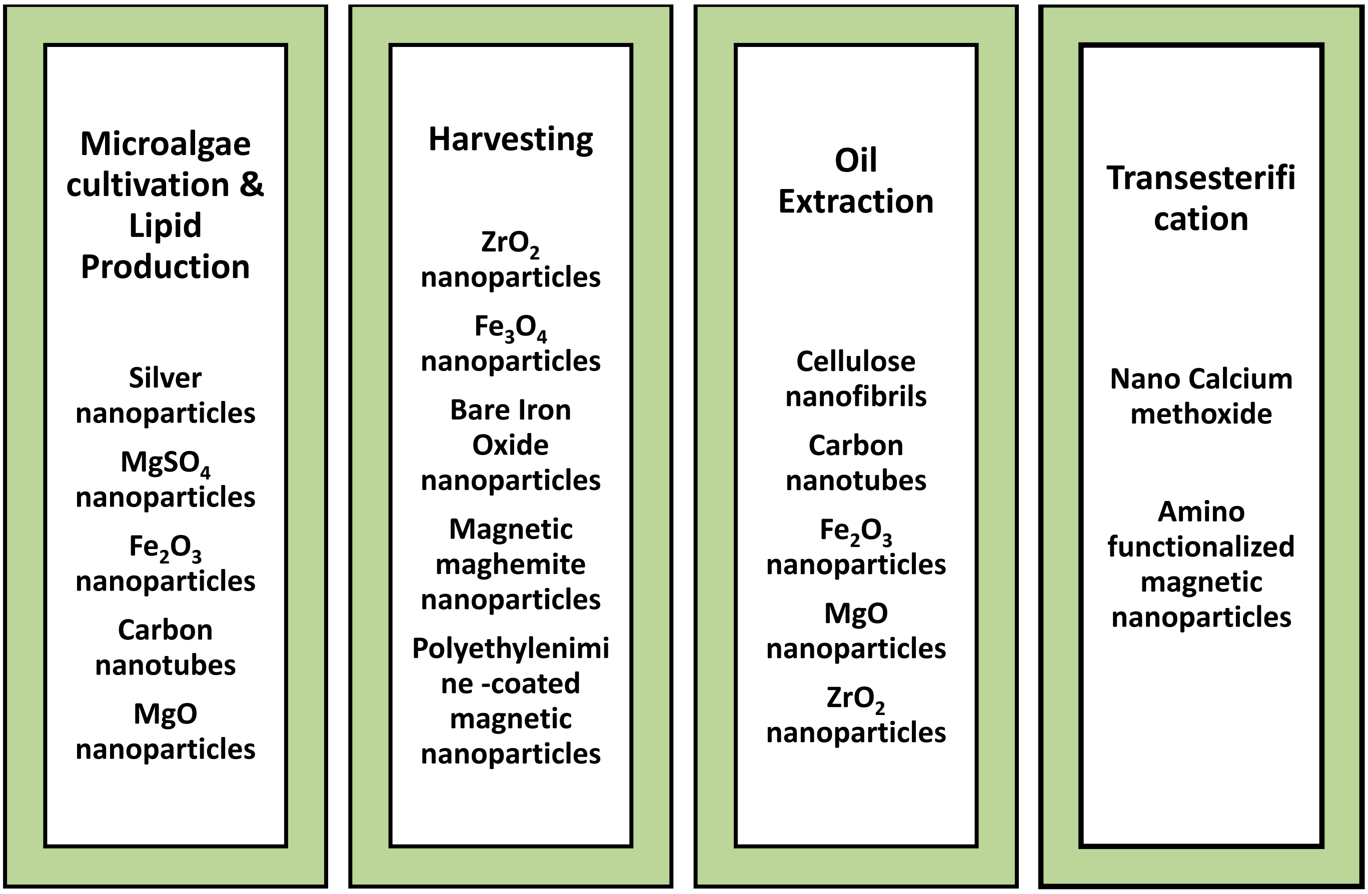


Figure 2. Technological scheme for the production of biodiesel using bio-nanoparticles.



Microalgae cultivation & Lipid Production

Silver nanoparticles

$MgSO_4$ nanoparticles

Fe_2O_3 nanoparticles

Carbon nanotubes

MgO nanoparticles

Harvesting

ZrO_2 nanoparticles

Fe_3O_4 nanoparticles

Bare Iron Oxide nanoparticles

Magnetic maghemite nanoparticles

Polyethylenimine -coated magnetic nanoparticles

Oil Extraction

Cellulose nanofibrils

Carbon nanotubes

Fe_2O_3 nanoparticles

MgO nanoparticles

ZrO_2 nanoparticles

Transesterification

Nano Calcium methoxide

Amino functionalized magnetic nanoparticles

Figure 3. Different types of nanoparticles used for microalgae biodiesel production.

3.1. Nanoparticles in Microalgae Cultivation and Lipid Production

The nanotechnological approach of microalgae biofuel production involves the use of different nanoparticles at different stages of biofuel production. The durability, adsorption efficiency, stability, economic advantage, environment-friendly nature, recyclability, catalytic performance, crystallinity and high storage stability are the main factors that make this method a promising approach to improve production yield and biodiesel quality [65]. For culturing algae, one of the requirements is illumination. Low levels of sunshine exposure can lead to improper development in high-density microalgae farms. Thus, employing localized surface plasmon resonance (LSPR) technology in metal nanoparticles has allowed for the resolution of the soft light lighting problem [66]. The basis of LSPR is linked oscillations of electrons (free) at a metal dielectric contact. A specific wavelength of light is specifically absorbed and scattered to cause resonant interactions between surface plasmons and photons. In an experiment with *Cyanothece* 51142 and *Chlamydomonas reinhardtii* in a photobioreactor, strong blue-colored light backscattering from a Ag nanoparticle solution is utilized to measure a 30% improvement in cell growth [67]. In another investigation, adding silver and gold nanorods to *Chlorella vulgaris* significantly increased the amount of carotenoids and chlorophyll. As a result, the algae's ability to absorb light improved [68]. The primary benefit of this technique is its ability to utilize nanoparticle solutions for the development and growth of particular microalgal species. Additionally, by adjusting the size and concentration of the nanoparticles, one can prevent photoinhibition [69]. In one study, the green microalgae *Chlorella vulgaris* is supplemented with $MgSO_4$ nanoparticles and an organic carbon source [50]. Both a significant decrease in the amount of glycerol used and an increase in photosynthetic efficiency are observed [70].

Microalgae produce polyunsaturated fatty acids (14–20 carbons) [71] and are able to accumulate oils up to 20–50%. This oil content can be further increased to up to 80% by manipulating growth conditions and using nanotechnology [65]. Nanoparticles are employed for photosynthetic microalgae in a medium to induce lipid buildup. The usual growing media for microalgae are reported to contain certain nutrients, but not enough for the best possible level of production. The lack of vital nutrients can be compensated by metallic nanoparticles present in some types of wastewater. Since these nanoparticles may boost microbial activity, it is feasible to put them in the medium for enhancing lipid production [72]. The functionalized groups of the hybrid nanoparticles can quicken the CO_2 in the culture medium's uptake and absorption. They supply enough CO_2 for the photosynthetic system of the microalgae. It is observed that nano-Fe_2O_3 promoted cell growth, and via increased concentrations of the CNT, nano-Fe_2O_3 and nano-MgO, it enhanced lipid synthesis and total lipid content. The $MgSO_4$ nanoparticles have the potential to promote flocculation in microalgae, which would limit their access to light and force them to produce more chlorophyll to maintain their photosynthetic activity [73]. This is probably a reaction from a defense mechanism. Green microalgae *Chlorella vulgaris* had grown more quickly when grown in the presence of nano-silica (size range of 38–190 nm) [74].

3.2. Nanoparticles in Harvesting of Microalgae

Microalgae biomass harvesting using the nanotechnology approach involves the use of nanoparticles to reduce the harvesting cost via decreasing energy consumption, enhancing enzyme recyclability and minimizing environmental toxicity. Nowadays, functionalized magnetic nanoparticles are applied in microalgae harvesting and it provides the separation of microalgae from the growth medium and separation of magnetic nanoparticles from microalgae [61]. Different types of nanoparticles used to harvest microalgae and their efficiency are listed in Table 2.

Table 2. Nanotechnology-mediated microalgae harvesting.

S. No.	Microalgae	Nanoparticle Used	Harvesting Efficiency	References
1.	*Chlorella zofingiensis*	Iron nanoparticles	98%	[62]
2.	*Chlorella vulgaris*	Magnetic nanoparticles (MNPs; Fe-MNP-I and Fe-MNP-II)	95%	[63]
3.	*Chlorella* sp.	Fe_3O_4 nanoparticle	95%	[75]
4.	*Chlorella vulgaris*	Naked magnetite (Fe_3O_4)	99%	[76]
5.	*Chlorella pyrenoidosa* and *Chlorella minutissima*	Iron oxide nanoparticles	90%	[64]
6.	*Chlorella vulgaris*	Yttrium iron oxide ($Y_3Fe_5O_{12}$)	90%	[61]
7.	*Chlorella pyrenoidosa*	Magnetic (Fe_3O_4)–silica core–shell nanoparticles	Increased	[77]
8.	*Chlorococcum* sp.	Zirchonium di-oxide (ZrO_2-Np)	Increased	[78]
9.	*Scenedesmus ovalternus* and *Chlorella vulgaris*	Bare iron oxide	95%	[79]
10.	*Scenedesmus* sp.	Magnetite-based nanoparticles (Fe_3O_4 NPs)	95%	[80]
11.	*Nannochloropsis maritime*	Naked magnetic nanoparticles	99.0%	[81]
12.	*Scenedesmus obliquus*	Zn- and Mg-doped ferrite magnetic nanoparticles	99%	[82]
13.	*Microcystis aeruginosa*	Magnetic maghemite (γ-Fe_2O_3)	82.4%	[83]
14.	*Microcystis aeruginosa*	Polyethylenimine-coated magnetic nanoparticles	NA	[84]

Due to the superiority in surface area, magnetism and biocompatibility, magnetic (Fe_3O_4) nanoparticles are the most widely used nanoparticles in the harvesting process. The ability of magnetic nanoparticles to readily attract and adhere to the surface of algae cells, generating a blanket that can be rapidly harvested in the presence of a magnetic force, makes them highly promising for the harvesting of microalgae. For harvesting 85% *Chlorella minutissima* (1 g L^{-1}) in 60 s at pH 5 and 90% *Chlorella pyrenoidosa* (1 g L^{-1}) in 60 s at pH 3, 600 and 500 mg of iron oxide nanoparticles are used, respectively [64]. It indicates the reduced time and energy required in the total cost of production, and the entire scheme of harvesting microalgae using nanoparticles is shown in Figure 4. Various agents like cationic polyacrylamide, polyethyleneimine, poly [diallyldimethylammonium chloride], [3-aminopropyl] triethoxysilane, cetrimonium bromide, polypyrrole, etc., have been applied to magnetic nanoparticles to improve harvesting efficiency [72,85].

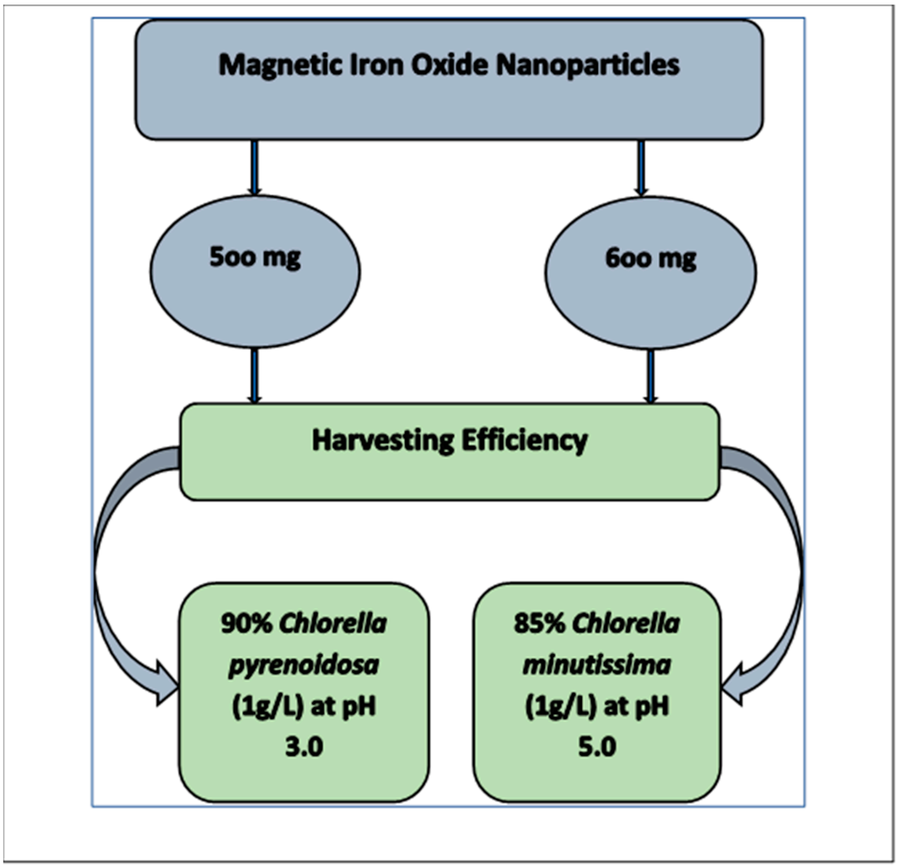


Figure 4. Effect of magnetic iron oxide nanoparticles on harvesting microalgae.

Aside from flocculation, another difficulty that persists in the way of efficient harvesting technology is the collection of smaller algal cells, which fall into the 2–4 µm size range [86]. Magnetic flocculation has proven to be one of the most successful techniques for collecting microalgae in recent times. Harvesting magnetic nanoparticles is energy-efficient and is impacted by an external magnetic field. The microalgal biomass separates from the magnetic nanoflocculant so that it can be recycled. Magnetic iron oxide nanoparticles are being used to clear algae from fishponds and freshwater bodies. Iron oxide and 0.1 mg/mL of cationic polyacrylamide are combined to make the magnetic flocculant with the greatest flocculation capacity, which is used in the algal harvesting process. To assess the efficiency of the harvesting process, *Chlorella* sp. was cultured with Fe_3O_4 nanoparticles covered with CTAB; as a result, 96.6% of the microalgae are collected at a dose of 0.46 g particle/g cell [87].

3.3. Nanoparticles in Oil Extraction

Using nanoparticles can eliminate the costly and time-consuming solvent–lipid separation step from the conventional extraction process [88]. Nanoparticles are used to enhance the oil extraction efficiency from microalgae [54,57]. For example, cetrimonium bromide octyl triethoxysilane-coated magnetic nanoparticles, cellulose nanofibrils, carbon nanotubes and Fe_2O_3, MgO and ZrO_2 nanoparticles have been used to extract the oils from microalgae biomass with high extraction and energy efficiency [89,90]. However, this approach is still at its early stage and its commercial hike is hindered due to an unavailability and increased cost of nanoparticles.

3.4. Nanoparticles in Transesterification Process

The specific surface area of the catalyst influences the biodiesel generation. A catalyst that has an enhanced surface/volume ratio coupled with nanoparticles works better since its surfaces contain greater active regions where reactions can take place. The transesterification is facilitated by an increased surface area of nanoparticles. The esterification process mediated by nanocatalysts has a number of special benefits, such as faster and simpler separation, longer reaction periods and a higher rate of mixing with reactants. By modifying the catalysts' physical and chemical characteristics, the fundamental intrinsic catalytic activity and selectivity can be readily controlled [91].

Amino functionalized magnetic nanoparticles [20] are used in transesterification of microalgae biodiesel production. Nano-calcium methoxide is used for generating biodiesel from *Nannochloropsis* sp. It should be noted that calcium-derived catalysts continue to be the most promising since they are abundant and reasonably priced, exhibit low methanol solubility and are harmless. They are highly catalytically active and affordable, as well as environmentally beneficial. The catalyst's significant activity was attributed to the methoxide species, which made it possible for more methanol to come into contact with the triglyceride's carbonyl group. The substantial pore size and elevated specific surface area facilitate catalyst–substrate interaction, hence increasing transesterification efficiency. Additionally, 99.0% is the greatest conversion attained at 80 °C, and 3 percent by weight of the catalyst. This outcome suggests that nanotechnology has offered a viable and very advantageous method for producing biodiesel in an environmentally friendly manner [92].

4. Enzyme Immobilization and Nanotechnology-Driven Microalgae Biodiesel Production

The process of encapsulating enzymes in supportive materials to maintain their stabilities and reusing capacities is known as enzyme immobilization [93]. This method helps with downstream processing and offers a complete separation of the enzymes from the reaction mixture [94]. Different nanomaterials have attracted a lot of attention as vehicles for enzyme immobilization [95]. One of the many advantages that nanoparticles offer as immobilization carriers is their significantly higher surface areas when compared to conventional materials for enzyme immobilization. The activity, stability and reusability of enzymes can be increased by using nanoimmobilized enzyme systems, which could result in a significant decrease in the expenses related to their use [96]. Nanoparticles are efficient in minimizing the potential steric hindrances and protein structure distortion, while increasing the contact surface area to maximize the enzyme loading capacity and mass transfer rate [97]. In microalgae biodiesel production using enzyme immobilization in nanoparticles, the nanoparticles overcome the challenges in biodiesel production in the absence of nanoparticles and they are shown in Figure 5.

Lipases, which are the triacylglycerol acylhydrolases, function as biocatalysts that exhibit high stability and reactivity when exposed to organic solvents. They can react with a variety of substrates including esters of FAs, synthetic oils, triacylglycerides, lipids, natural oils, etc. *Aspergillus*, *Fusarium*, *Mucor*, *Pseudomonas*, *Rhodotorula*, *Candida*, *Geotrichum*, *Penicillium* and *Rhizopus* are among the species that are frequently listed as good producers and constitute 50% of the commercial volume of lipases [11,98,99]. Different sources of lipases used in microalgae biodiesel production are listed in Table 3. The hydrophobicity of the nanoparticle is a characteristic that helps with both the immobilization of lipase and the synthesis of biodiesel [100]. Because lipases naturally bind to hydrophobic surfaces, their immobilization on nanoparticles is distinct from that of enzymes that adsorb hydrophobically. Moreover, hydrophobic triacylglycerols and fatty acid esters have a strong attraction for hydrophobic supports. It has little affinity for glycerol, which promotes the interaction of lipases with the nanoparticles and greatly inhibits glycerol adsorption [101,102].

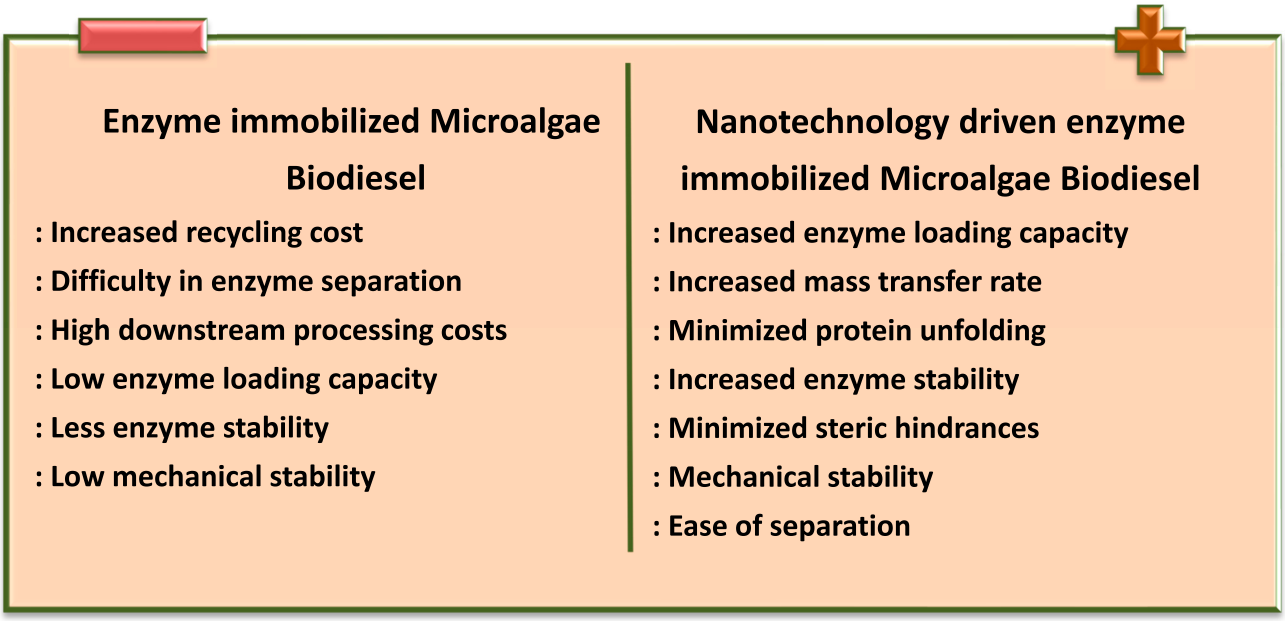


Figure 5. Importance of nanotechnology-driven enzyme-immobilized microalgae biodiesel.

Table 3. Sources of lipase used in microalgae biodiesel production.

Sources of Lipase	Time (h)	Conversion (%)	References
Rhizomucor miehei	24	90%	[103]
Candida antarctica	4	92.6%	[104]
Candida rugosa	4	93%	[105]
Pseudomonas fluorescens	12	95%	[106]
Thermomyces lanuginosus	48	81.64%	[107]

5. Effect of Nanoparticle-Immobilized Lipase Enzyme on Biodiesel Production

Compared with lipases in the original form, the immobilized lipases showed enhanced pH tolerance, functional stability, substrate specificity and thermal stability. This ultimately increases biofuel production yield [59,108]. To date, a wide range of nanoparticles have been investigated for this purpose, including nanosilicones, nano-metal particles, carbon nanotubes, metal–organic frameworks, magnetic nanoparticles, nanoflowers and nanofibers [109]. The nano-immobilized lipases showed the superiority of an increased surface area, enhanced stability and eased separation, compared with the lipase immobilized using conventional supporting materials [88]. Furthermore, a high reactant diffusion rate can be provided to the lipase enzyme's active areas because of the nanomaterials' tiny pore sizes. The factors that render nanoparticles suitable for lipase immobilization in microalgae biofuel production are shown in Figure 6.

Magnetic nanoparticles can offer a great substitute that is practical and affordable due to their enhanced thermostability and ease of recovery from reaction mixtures [110]. *T. lanuginosus* lipase covalently attached to aminofunctionalized magnetic nanoparticles produced 90% biodiesel conversion efficiency in one study [111]. A 100% success rate in turning oil into biodiesel using *P. cepacia* lipase immobilized on Fe_3O_4 magnetic nanoparticles is reported [112]. Lipases immobilized by magnetic nanoparticles are able to be detached from the reaction medium and are reusable, further reducing the biofuel production cost [113]. The conversion efficiency is increased by four times compared to the free lipase [114]. The magnetite-Fe_3O_4 and maghemite-Fe_2O_3 are widely employed for immobilization [115]. The magnetic support modifications are required to enhance the interaction between these nanoparticles and target lipase. In a study, amino silanes are added to the

Fe_3O_4 nanoparticle to improve the lipase loading capacity [116]. The lipase can even be cross-linked with the incorporated amino acids in the nanoparticle using glutaraldehyde to maximize binding [117]. The silica-based nanoparticles are preferred more by the industry due to their biocompatible and non-toxic nature [118]. The silanol group can be attached on silica gel to facilitate further modifications such as the addition of functional groups such as 3–Glycidyloxypropyl trimethoxysilane to improve the immobilization efficiency [119]. In another study, lipase is immobilized on mesostructured onion-like silica via crosslinking using glutaraldehyde to produce biodiesel. This immobilization increased the reusability of the enzyme by 23 times [120]. Additionally, lipase is immobilized on silica aerogels and attained a 93% conversion rate [121]. Metal–organic-framework nanoparticles like zeolitic imidazolate framework-8 significantly improved the stability and reusability of lipase [122,123]. Carbon nanotubes are well-suited for the immobilization of lipase because of their higher mechanical properties [124]. Hybrid nanoflowers are developed to immobilize lipase for biodiesel production. The high production yield results suggested that it had economic feasibility [125]. Organic polymers are a good choice of supporting material for enzyme immobilization since the carboxyl, epoxy, hydroxyl or amino groups facilitate covalent attachment and modification. For example, the polyacrylamide-nanogel-encapsulated lipase has shown remarkable effectiveness in the synthesis of biodiesel. This allowed the lipase that has been rendered immobile to be placed at the oil–water interaction by the amphiphilic polymer shell of the nanogel. In addition, the polymer outer shell's presence increased the lipase stability at high temperatures and methanol concentrations [126]. Different types of lipase-immobilized nanoparticles utilized in microalgae biodiesel production are tabulated in Table 4 and are discussed below:

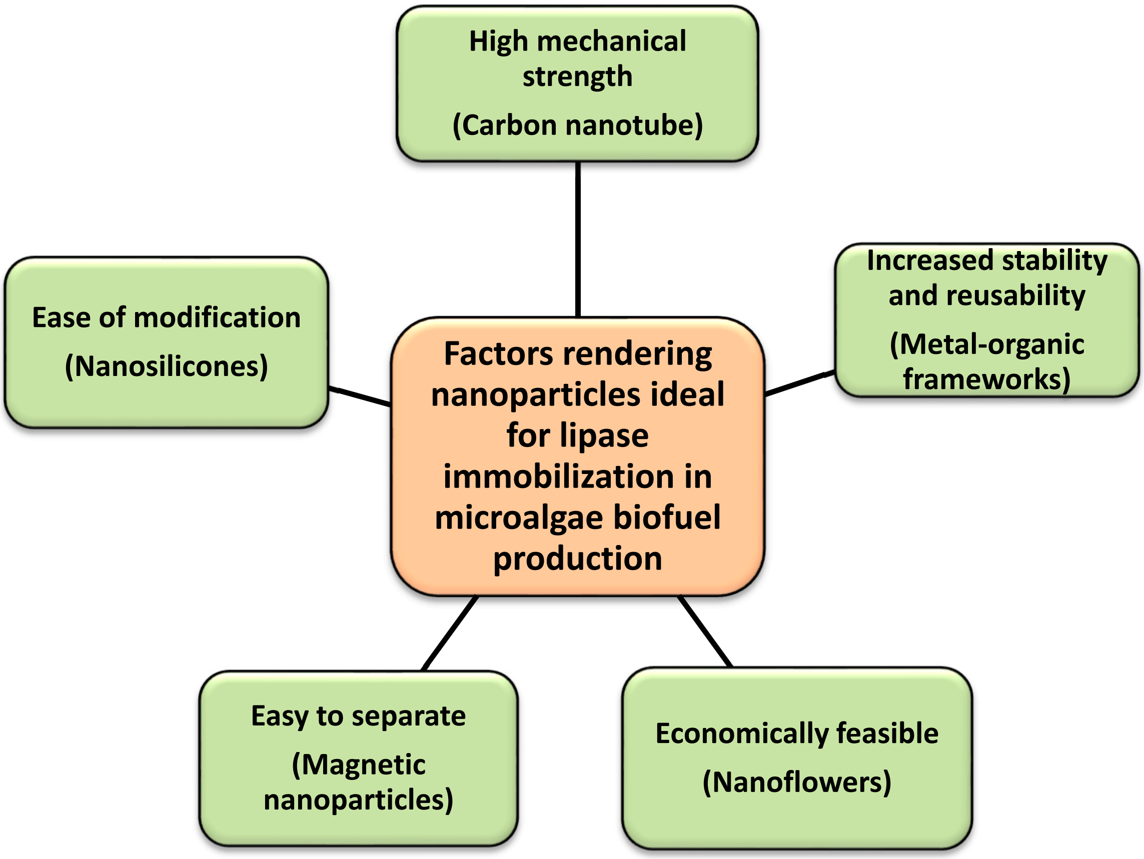


Figure 6. Factors rendering nanoparticles ideal for enzyme immobilization in microalgae biodiesel production.

Table 4. Lipase nanoparticles for microalgae biodiesel production.

Lipase Nanoparticles	Microalga	Catalytic Cycles	Reference
Lipase–Magnetic Biosilica Nanoparticle	*Kamptonema formosum*	-	[127]
Lipase–Magnetic (Fe_3O_4) Nanoparticle	*Chlorella pyrenoidosa*	5 cycles	[128]
Lipase–Superparamagnetic–Graphene Oxide Nanoparticle	*Chlorella vulgaris*	5 cycles	[129]
Lipase-Fe_3O_4 Superparamagnetic Nanoparticle	*Chlorella vulgaris*	5 cycles	[130]
Lipase-Aminated Magnetic Nanoparticle	*Chlorella vulgaris* var L3	10 cycles	[131]
Lipase–Magnetic (Fe_3O_4) Nanoparticle	*Chlorella salina*	10 cycles	[132]
Lipase-Fe_3O_4-SiO_2 Hybrid Nanoparticles	*Chlorella vulgaris* ESP-31	4 cycles	[133]
Lipase–Graphene Oxide–Nickel Ferrite Nanoparticles	*Scenedesmus obliquus*	6 cycles	[1]

5.1. Lipase-Fe_3O_4-SiO_2 Hybrid Nanoparticle

Burkholderia sp. C20 [134] produced lipase and it was immobilized on Fe_3O_4–SiO_2 that had long-chain alkyl groups acting as supports. Two methods are used to convert *Chlorella vulgaris* ESP-31's microalgae oil into biodiesel. Using ultrasonic waves to break the cell walls, the M-I method recovered the microalgae oil using the solvent–chloroform and methanol. After extraction, the oil is used for transesterification. On the other hand, technique M-II employed the ESP-31 strain's cell-wall damaged biomass directly for transesterification. Furthermore, moist microalgae biomass is used in the M-II technique for enzymatic transesterification. It is observed that the lipase could be used for up to six cycles, with at least 65% activity in the last two cycles. It is proved that biodiesel may be effectively produced through transesterification of microalgae biomass. In the M-II technique, the immobilized lipase works with an increased quantity of water and higher methanol–oil ratio and 97.25% transesterification efficiency. Thus, there is a significant chance that the suggested biocatalyst and conversion process (M-II) will be used in the industrial manufacture of microalgae biodiesel [133].

5.2. Lipase–Magnetic (Fe_3O_4) Nanoparticle

Magnetic nanoparticles such as Fe_3O_4 are promising materials to immobilize lipase to produce biofuel. The immobilized lipases can be detached from the system using a strong magnetic force to facilitate their reuse [135,136]. The lipase has been extracted from *Aspergillus niger* (KP001169) and it is immobilized on magnetic ferric and ferrous nanoparticles via the co-precipitation method [128,132]. These immobilized lipases are then used to produce biodiesel via the transesterification of *Chlorella pyrenoidosa* oils. The result suggested that the immobilization significantly increased the biodiesel production yield and the lipase retained its activity even after five production cycles. In another study, Fe_3O_4 superparamagnetic nanoparticles are prepared using the chemical co-precipitation method to immobilize lipase for biodiesel production via transesterification of *Chlorella vulgaris* oils. Similarly, the authors reported that lipase remained efficient after five production cycles [130].

5.3. Lipase–Magnetic Biosilica Nanoparticle

Numerous researchers have employed silica-based materials as a solid basis for the immobilization of enzymes [137]. Among these, diatom-derived biosilica is an inexpensive material with intriguing qualities for its use in a broad range of industrial applications [138]. Diatoms, the large unicellular algae, form unique frustrules and they are the source of the biosilicates. Diatom biosilica is a suitable raw material for its use in the biotechnological field because these biosilicates have nanoporous features with an increased surface area that is easily modifiable, an increased mechanical strength, higher stability, and better biocompatibility properties. Diatomic biosilica particles are superior to other biosilicates in several ways, including being a natural source, non-toxic and inexpensive, and having a large surface area.

The engineering of an immobilized lipase on diatomic biosilica that is magnetically recyclable is the innovative aspect of this study. It is a high-quality, stable, non-toxic particle with strong mechanical and chemical properties. Because paramagnetic nanoparticles are present in the pores of the biosilica, the magnetic biosilica does not aggregate in an aqueous solution. These characteristics enable it to be used in the transesterification procedure for the manufacture of biodiesel and make it an appropriate lipase carrier for enzymatic reactions. Large-scale applications benefit economically from the magnetic characteristic, which makes it easy to retrieve immobilized lipase from a reaction medium using a magnet. This makes biodiesel synthesis a more viable option. A hot spring water algal known as "*Kamptonema formosum*" is grown at three distinct temperatures, and methanol and chloroform are used to extract the algae oil. At 25 °C, the highest amount of algal biomass (1.86 g/L) is collected, and 48.7% oil is extracted. Using a thermal co-precipitation process, diatomic earth (biosilica) can be magnetized and attached with polydopamine (PDA). Schiff's base reaction is applied for the covalent immobilization of lipase with the magnetic biosilica (MBioSi) nanoparticles. After optimizing the immobilization conditions, the immobilization medium's initial lipase concentration was determined to be 3.0 mg/mL, which was found to be the most beneficial. The immobilization yield and the quantity of lipase on the MBioSi@PDA are determined as 67.9% and 81.9 mg/g, respectively, at this lipase concentration. This novel lipase nanoparticle is used as a biocatalyst for biodiesel generation from *Kamptonema formosum* with 91.2% conversion yield [127].

5.4. Lipase–Superparamagnetic Graphene Oxide (GO) Nanoparticle

Using a transesterification reaction and lipase immobilization on superparamagnetic few-layer GO, nano-biocatalysts are used to produce biodiesel. A few-layer graphene oxide (MGO) hybrid is synthesized and 3-aminopropyl triethoxysilane (MGO–AP) and a combination of glutaraldehyde and AP (MGO–AP–GA) are used to functionalize the MGO. Electrostatic interactions and covalent bonding are used to immobilize the lipase of *Rhizopus oryzae* (ROL) on MGO-AP, MGO and MGO-AP-GA. Kinetic characteristics, loading capability, relative action, time-course temperature stability and stability in storage are all evaluated during the nano-biocatalyst assay. The results suggested that the catalytic performance of the enzyme is significantly increased based on this immobilization. Additionally, after five cycles of the transesterification reaction, this nano-biocatalyst maintained 58.77% of its catalytic efficiency, making it the top catalyst in terms of reusability. The maximum biodiesel conversion of 71.19% is attained when ROL/MGO-AP-GA is present. The manufactured nano-biocatalyst based on functionalized and bare magnetic graphene oxide is used to convert microalgae bio-oil to biodiesel. It is compared to bare lipase immobilized on magnetic nanoparticles. The functionalization of MGO enhanced the loading capacity, thermal stability, kinetic characteristics and storage stability, according to the results. In general, the biocatalysts that are made by immobilizing enzymes through covalent bonding exhibited superior qualities [129].

5.5. Lipase–Graphene Oxide–Nickel Ferrite Nanoparticles

Candida rugosa lipase has been employed for the transesterification of algal lipids after being immobilized on graphene oxides magnetized with nickel ferrite nanoparticles. On hydrophobic supports, interfacial activation led to immobilization [93]. The ideal conditions are found to be 100 µg/mL enzyme concentrations for a 2 h incubation period, resulting in 78% immobilization efficiency. The findings showed that the immobilized enzyme is highly resistant to changes in pH and heat compared to the free enzyme; at 60 °C, it could maintain 70% of its initial activity and only 20% at pH 9. After six cycles of hydrolysis at 37 °C and pH 7, immobilized lipase maintained 47% of activity, showing its resilience and reusable property. The production efficiency of biodiesel is found to be 68% when immobilized lipase is utilized as biocatalysts [1].

6. Reuse and Recovery of Lipase Nanoparticles

The two biggest advantages of using lipase nanoparticles in the biodiesel generation are the recoverability and reusability [131,132]. The lipase nanoparticles are used in repeated cycles to generate biodiesel, and the recovery process is performed at each level. Chemical techniques are often applied for lipase nanoparticle recovery. Heterogeneous catalysts facilitate the rapid and effortless recovery of both primary and secondary products. In this mode of the catalyst, the washing step is not required. The esterification process using nanoparticles has been shown to have benefits such as rapid separation and faster reactant–catalyst mixing [135–137]. Easy enzyme recovery is made possible by an external magnetic field, and prior research has demonstrated that there is multiple-time recovery without significantly lowering the catalytic effect or biodiesel yield [20].

7. Challenges in Large-Scale Commercialization and the Proposed Strategies to Overcome

The challenges for commercialization include both environmental and technological barriers. Environmental barriers can be the usage of land and water, emission of greenhouse gas and loss of biodiversity [139]. Sufficient land and water are required for scaling up of microalgae biodiesel production. The effects of algal biomass production on water stress varied significantly amongst the possible sites because of regional variations in water stress. Proper planning is necessary to increase the water efficiency of large-scale algal biodiesel production. Water stress effects become quite severe when several sites are placed in highly stressed water zones, even though choosing locations according to the yield of biomass ranking could enhance economic feasibility. Water usage can be decreased by ranking sites according to Water Use Efficiency; however, the biomass production will be reduced (~25%). On the other side, the Available Water Remaining-US hotspot avoidance method could greatly lessen the effects of water stress without negatively impacting biomass yield. In contrast to the biomass yield ranking that ignores regional water stress, avoiding locations situated within elevated water stress zones could lower the water scarcity footprint for a long-term biodiesel production aim. AWARE-US might direct the geographical planning of energy system deployment and assess the effects of microalgal biofuel systems on water stress [140]. Future techno-economic assessment and life cycle assessment (LCA) studies should be carried out for envisioned commercial systems in order to confirm earlier findings based on lab- or pilot-scale experiments and to more effectively demonstrate and defend the choice of a specific production process as the best option for a given location.

Even if the use of nanoparticles in microalgae culturing, harvesting and transesterification improved efficiency, there are obstacles to overcome prior to the usage of nanoparticles in the commercial strategy. Particle size, shape, size distribution and clustering of the majority of the nanoparticles from experimental study are not well defined [141]. Thorough research on characterizing nanoparticles well is necessary prior to large-scale implementation. For maximum productivity, consideration should be given to the selection of appropriate nanoparticles, preparation techniques and time for the chosen application. Before being used, the impact of nanocatalyst implementation on engine performance, gas emission and the quality of microalgae-biofuel combustion should be thoroughly investigated and understood. Accordingly, even while nanoparticles are sufficiently available for laboratory use, their large-scale availability may provide a problem for mass use. Another barrier is the cost-effectiveness of nanocatalysts for industrial use, which could hurt business prospects because many of them are highly costly. Thus, as the economic problem is an important factor for large-scale plant installation, a complete techno-economic study is necessary to analyze whether the nanoparticles used on microalgae biodiesel are economically feasible or not. Additionally, these applications will benefit the environment in the near future by generating value-added co-products. Even if the use of nanoparticles has been shown to be environmentally beneficial, a thorough LCA is mandatory to demonstrate the benefits to the environment. Prior to commercialization, a thorough analysis of public safety, the effect on flora and fauna and the potential for biohazards is also required [20].

The impacts of microalgae are not limited to natural resources but also biodiversity. Microalgae cultivation poses a threat to biodiversity and the ecosystem. Large-scale microalgae deployment necessitates effective management and control of the cultivation process [142]. Therefore, dependence on native plant species instead of invading species that could jeopardize biodiversity for the generation of biofuel may be a wiser decision. However, large-scale microalgae farming may result in an overabundance of nutrients in the aquatic environment, endangering biodiversity [139].

8. Conclusions and Future Prospects

Driven by various environmental and economic challenges, there is an urgent demand to produce biodiesel as an alternative energy resource. Among different bioenergy microalgae feed stocks, *Chlorella vulgaris* shows superiority due to the high quality of microalgae-based biodiesel such as a higher calorific value, density, viscosity, etc. Even if this method is environmentally and energy-friendly, researchers are still searching for a breakthrough that will increase the yield of microalgae biofuel from the first stage to the finished product and change the entire process to a fuel that is more affordable. Nowadays, nanocatalysts are investigated to generate biodiesel from the microalgae biomass, with improved selectivity and increased production yield. In addition, the designed nanocatalysts are easily detached from the reaction system and are able to be reused to minimize the production cost. Lipase was used as an effective catalyst and it is immobilized with nanoparticles. The widely used lipase-immobilized nanoparticles are iron oxide nanoparticles, magnetic-biosilica nanoparticles, Fe_3O_4-SiO_2 hybrid nanoparticles, superparamagnetic GO nanoparticles and GO-nickel ferrite nanoparticles.

Large-scale experiments are required to facilitate its commercial applications in the future. For large-scale microalgae biodiesel production, adequate resources of both land and water are needed, and moreover, environmentally friendly production is essential to the industry's long-term sustainability. Careful planning is necessary to increase the water sustainability of large-scale algal biofuel production. Water stress effects are quite higher when several sites are placed in water stress zones, even though choosing sites based on biomass yield ranking would improve economic feasibility.

When several types of nanoparticles were applied at different stages of microalgae development to produce biofuel, the results were good and could lead to a revolutionary improvement in the production of large-scale microalgae biodiesel. The sustainability study of production of microalgae biofuel, however, nevertheless showed a stark requirement for additional study and innovative ideas. These ideas might help in identifying the best nanoparticles, economically speaking, for biodiesel production. Policy formulation and the application of nanoparticles will continue to be the most important factors for commercial production, particularly in poor nations, as nanoparticle application on microalgae is still a relatively new scientific topic. In order to draw in the government and non-government fuel industries, it is therefore necessary to stress management insights on appropriate policy, socioeconomic impact, advantages and constraints for the overall system. The primary obstacles to the manufacturing of biofuels are technological, financial and environmental.

Cultivating algae in an enclosed setting can help to lessen environmental issues, but pretreatment processes' resource and cost-breakeven points still need to be lowered. It is feasible to overcome these obstacles with further study and development, beginning with the generation of biofuel from microalgae and developing a commercially viable solution at a reasonable cost.

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