Smart nanomaterials for efficient microalgae harvesting: Bridging laboratory innovation and sustainable biorefinery

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ABSTRACT

The economic feasibility of microalgal biorefineries is largely constrained by downstream processing, particularly biomass harvesting, which contributes approximately 25–30 % of total production costs. Conventional harvesting techniques—including centrifugation, filtration, and chemical flocculation—are often limited by high energy demand, poor scalability, and environmental concerns. In response to these challenges, nanoparticle-assisted harvesting has emerged as a promising alternative, offering rapid separation, high harvesting efficiencies (>95 %), reduced energy consumption, and potential recyclability. This review critically examines recent advances in the use of bare and surface-functionalized nanoparticles, with particular emphasis on magnetic nanoparticles (e.g., Fe_3O_4-based systems functionalized with PEI, chitosan, or urea), as well as selected non-magnetic metallic and organic nanomaterials. The underlying harvesting mechanisms—including electrostatic attraction, bridging flocculation, and Lewis acid–base interactions—are systematically analyzed in relation to key operational parameters such as algal species, growth phase, nanoparticle dosage, pH, salinity, and ionic composition. Distinct from previous reviews, this work provides a mechanistic and integrative recyclability, and downstream compatibility. Special attention is given to multifunctional nanoparticles that enable simultaneous harvesting, cell disruption, and lipid enhancement, thereby facilitating downstream processing. The review further evaluates recyclability, environmental safety, and scalability considerations, positioning nanoparticle-enabled harvesting within a circular biorefinery framework. Overall, this review bridges nanotechnology and microalgal process engineering, offering critical insights and design guidelines to advance sustainable and industrially viable microalgal biorefineries. perspective by linking nanoparticle–microalgae interactions with process performance.

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1. Introduction

Considering the increase in non-renewable energy demand, there is a rapid utilization of the natural stock of fossil fuels. Currently, conventional energy sources, such as coal, petroleum, and natural gas, fulfil approximately 80 % of the world's primary energy demand. However, due to high demand, these resources are being continuously depleted, led to a rapid increase in greenhouse gas emissions (Žarković et al., 2022). Burning these fuels caused numerous health hazards (Kotcher et al., 2019). The main pollutants released by fossil fuels include methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (NO_2). As a result, there is an urgent need to accelerate the development of eco-friendly, non-polluting, and cost-effective alternatives. Additionally, Chen BaiLing et al., (2017) argued against the use of plant-derived biofuels due to extensive land use and the fuel-vs-food dilemma. Considering all such possibilities, researchers have recently shifted their attention toward microorganisms, and more specifically, highlighted microalgae as promising candidates due to their ability to capture biological carbon and reduce greenhouse gas emissions (Choi et al., 2019; Kumar Patel et al., 2021). Apart from this, it helps with the 3rd generation of biofuel feedstocks such as biodiesel, bioethanol, biomethane (Choi et al., 2019; Kumar Patel et al., 2021; Patel et al., 2020) and high-value products like protein, carotenoid, and other bioactive compounds (Patel et al., 2022b). Apart from the biofuels microalgae in recent times also majorly contributed towards nutraceuticals, animal feed, aquaculture feeds, cosmeceuticals, food supplements, and pharmaceuticals, for example, health-promoting fatty acids (FAs): docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), polysaccharides: β-glucan, astaxanthin, lutein,β-carotene are main products (Hudek et al., 2014). Microalgae cultivation exhibited a higher growth rate, required less area than crops, and yielded more oil than other crops (Pratibha et al., 2020). However, the high cost of microalgae cultivation remains a significant challenge for producing low-value products, such as biofuels. To address this, the biorefinery approach has been increasingly adopted worldwide, enabling the cost-effective production of multiple valuable products from a single processing stream. Additionally, alternative cultivation techniques have been adopted to further economize the upstream process by increasing the biomass or lipid content, as well as other high-value products (Patel et al., 2022a). The downstream phase is just as crucial to the bioprocess's economy as the upstream process. Downstreaming advancement in microalgae harvesting can solve the issue of product recovery in a cost-effective manner. oil extraction, biofuel conversion Cultivation, and harvesting, are all sequential steps in the production of microalgal biodiesel. Conventional biofuels cost ~US $2/L in 2025, whereas microalgal biodiesel currently remains higher at US $4–6/L; however, advances in wastewater-based cultivation, nutrient recycling, and process scale-up are expected to reduce costs to ~US $1.5 /L, indicating strong future cost competitiveness (Biswas et al., 2025). Microalgae harvesting alone costs 20–30 % of the total production cost of biodiesel. Cost-effectiveness assessments indicate that surface-modified magnetic nanoparticles (MNPs) remain economically restrictive for large-scale deployment, with reported costs ranging from £ 8.9 to £ 185.7 per kg dry cell weight (DCW), largely due to complex functionalization steps and limited regeneration efficiency. In contrast, unmodified (naked) MNPs substantially reduce harvesting costs (£3–500 per kg DCW), which can be further lowered to £ 0.5–108.0 per kg DCW through ultrasonic or alkaline regeneration. Although these values are still higher than energy-equivalent crude oil costs (£0.037–0.293 per kg DCW biomass), such estimates are primarily derived from laboratory-grade chemical pricing and therefore overestimate real-world expenses (Xu et al., 2017). Importantly, when nanoparticles are integrated across both upstream and downstream operations—enhancing biomass, lipid, and carotenoid productivity during cultivation while simultaneously enabling rapid and energy-efficient harvesting—the overall cost per unit of final product can be significantly reduced compared to conventional cultivation–harvesting schemes. This integrated nanoparticle-assisted strategy highlights a viable pathway toward scalable microalgal biorefineries, while underscoring the need for pilot-scale validation using commercially sourced materials. Although the cost varied depending on the type of harvesting method, microalgae density, and the microalgae species (Kim et al., 2013; Rashid et al., 2014).

In recent times, nanomaterials have received considerable attention due to their unique properties, including high reaction activity and a large specific surface area (Baig et al., 2021; Patel et al., 2022a). The use of metal oxide nanoparticles is frequent in emerging technologies and has a numerous applications, including in the manufacture of commercial products (Zaka et al., 2016). Metal oxide nanoparticles form the basis for the manufacture of new materials used in various applications (Chavali and Nikolova, 2019). In many modern technology applications, enhanced magnetite (Fe_3O_4) nanoparticles are recognized as multifunctional materials, including environmental remediation(Sundaram et al., 2025), food production (Dong et al., 2024), treatment of wastewater (Biswas et al., 2025), removal of pollutants in sediments, soils, and waters (Baragaño et al., 2020; Park et al., 2020; Shi et al., 2023), and medical applications (Ganapathe et al., 2020). Iron nanoparticles, such as zero-valent iron nanoparticles (nZVI), are recognized as remediation agents for in situ treatment of contaminated aquifers and soils. It has also been applied in green technology. As a result of the oxidation of nZVI, reactive oxygen species (ROS) are generated, leading to oxidative stress in treated cells. As a result of ROS interactions with biological macromolecules, lipids are peroxidized, proteins oxidized, and this affects the DNA (Ševců et al., 2011). Titanium is the ninth most abundant element in nature, and the most available form is titanium dioxide (TiO_2). Titanium metals are not found in living things. However, few studies have concluded that a trace amount of titanium may be present in animal cells. TiO_2 NPs, due to their smaller size, tend to enter the cells easily. It is also recognized for a wide range of applications, from everyday products like sunscreens to advanced technologies such as photovoltaic cells, as well as various environmental and biomedical uses, including photocatalytic pollutant degradation, water purification, biosensing, and targeted drug delivery. (Chen and Selloni, 2014).

Nanoparticles (NPs) play an important role in microalgae cultivation due to their large surface area and unique physicochemical properties, including enhanced reactivity, mechanical strength, and electrical behavior compared to bulk materials (Zhang et al., 2013). The application of nanomaterials in microalgal systems has addressed several challenges associated with biodiesel production, particularly through the use of heterogeneous nanocatalysts that improve conversion efficiency and fuel quality (Pattarkine and Pattarkine, 2012; Pugh et al., 2011; Zhang et al., 2013). Iron-based nanoparticles are especially relevant, as iron is an essential

micronutrient for phytoplankton, playing a critical role in photosynthesis, respiration, and ecosystem productivity (Gledhill and Buck, 2012), while also exhibiting catalytic activity through Fenton-type reactions that generate reactive oxygen species (ROS) (LeBel et al., 1992). Although excessive ROS generation can lead to oxidative stress and cellular damage (Erdei, 2002), controlled NP exposure has been reported to enhance biomass and lipid production, with materials such as TiO_2 showing positive effects on microalgal productivity. Beyond cultivation and productivity enhancement, nanoparticles—particularly magnetic nanoparticles—have been widely investigated for microalgae harvesting due to their rapid, energy-efficient, and environmentally friendly separation capabilities, achieving high harvesting efficiencies through flocculation and magnetic recovery (Patel et al., 2022a; Shabatina et al., 2020). Microalgal harvesting using magnetic nanoparticles is governed by multiple interaction mechanisms, including electrostatic attraction, hydrogen bonding, van der Waals forces, and acid–base interactions between negatively charged algal cells and nanoparticle surfaces (Taghizadeh et al., 2020). Surface functionalization of iron oxide nanoparticles with macromolecules such as chitosan, polyethyleneimine (PEI), poly(diallyl dimethylammonium chloride) (PDDA), graphene oxide, and polyphenols has further enhanced harvesting performance by introducing additional functional groups that promote stronger cell–particle interactions (Lim et al., 2012; Toh et al., 2014; Yang et al., 2016). While electrostatic interactions dominate in freshwater systems, marine microalgae harvesting is influenced by high ionic strength, which suppresses electrostatic attraction and necessitates alternative mechanisms such as bridging flocculation and van der Waals interactions to maintain high harvesting efficiency (Kumar et al., 2024a; Roy and Mohanty, 2019).

Although extensive research has focused on microalgal cultivation and metabolite enhancement, downstream bottlenecks, particularly harvesting, continue to limit the commercial success of algal biorefineries. Current bioprocesses are constrained by high operational costs, intensive energy demand, and environmental implications of conventional separation methods. While several reviews have explored general harvesting techniques, few have critically examined nanoparticle-assisted strategies in the context of integrated biorefinery design. The novelty of this review lies in its systematic and critical analysis of nanoparticle–microalgae interactions, encompassing electrostatic, coordination, van der Waals, and bridging mechanisms, along with their synergistic potential for simultaneous cell disruption and enhanced lipid recovery. Unlike existing studies that focus primarily on harvesting efficiency, this review uniquely integrates nanomaterial functionality, interaction mechanisms, process optimization, recyclability, and downstream compatibility within a unified sustainability framework. By bridging fundamental nanotechnology with microalgal process engineering, this work provides actionable insights and design principles to advance scalable, efficient, and environmentally responsible microalgal biorefineries. Furthermore, it advocates techno-economic feasibility, life-cycle considerations, and environmental implications necessary for industrial scaling. The discussion aligns with the Sustainable Development Goals, particularly SDGs 7, 9, 12, and 13, emphasizing clean energy, innovation, sustainable production, and climate resilience.

1.1. Conventional harvesting methods

Conventional microalgae harvesting methods are essential techniques used to separate microalgae biomass from the culture medium, crucial for producing biofuels, pharmaceuticals, and food supplements. Key methods include centrifugation, which uses centrifugal force for rapid and efficient separation; filtration, employing membranes to retain algae cells; flocculation, where chemicals or biological agents aggregate cells for easier collection; sedimentation, relying on gravity to settle cells; and flotation, which utilizes air bubbles to lift cells to the surface. Fig. 1 provides an overview of the conventional techniques employed in microalgae harvesting. Each method has its advantages and challenges, with affecting factors such as efficiency, cost, and scalability. The importance of these
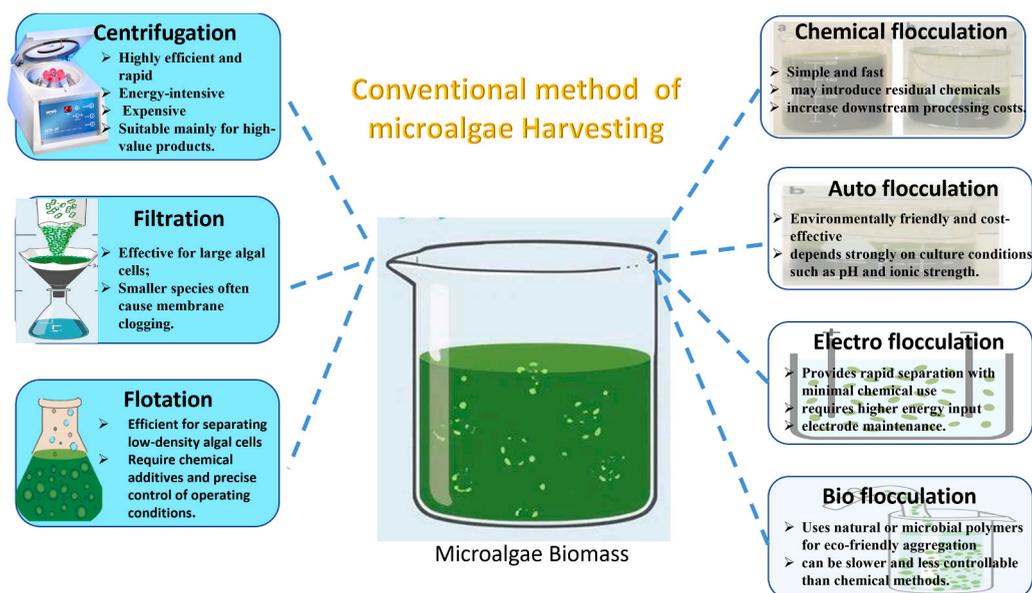

Fig. 1. Comparative summary of traditional microalgae harvesting approaches.

methods lies in their ability to ensure high-quality biomass, reduce production costs, and support large-scale operations, making microalgae-based products economically viable and environmentally sustainable. Efficient harvesting has a direct impact on the profitability and environmental footprint of the microalgae industry, underscoring its critical role in advancing sustainable resource utilization. Harvesting microalgae is a crucial component of microalgae processing. Numerous studies indicate that it accounts for 20–30 % of the overall production costs, largely due to significant energy requirements and high capital expenditures (Barros et al., 2015).

1.2. Filtration

Filtration is a key physical technique for separating solids from liquids, permitting only the fluid to pass through. Various filtration methods, including microfiltration, ultrafiltration, vacuum filtration, and pressure filtration, are widely applied for harvesting microalgal biomass. In these processes, membranes are central to effective biomass recovery; however, membrane fouling remains a major challenge, often leading to reduced flux and increased operational costs. To address this issue, hydrophilic polyvinyl alcohol (PVA) has been applied as a surface-coating material to mitigate fouling and enhance filtration performance. This coating enhanced the membrane's hydrophilic properties and performance, resulting in a 36 % increase in maximum flux and achieving a 100 % recovery rate (T. Hwang et al., 2015). *Coelastrum proboscideum* was processed using pressure filtration with a Netzsch chamber filter, achieving a batch mode in one step with an energy consumption of 0.88 kWh per cubic meter. The concentration factor (CF) reached 245, with a concentration range of 22–27. *Coelastrum proboscideum* was subjected to pressure filtration using a cylindrical sieve rotor by Engelsmann, operating in a continuous mode with a single step and consuming 0.3 kWh per cubic meter of energy. This method yielded a CF of 75, corresponding to a concentration of 7.5 (Molina Grima et al., 2003). In a separate study, Nurra et al. (2014) found that vibrating membrane filtration outperformed traditional crossflow filtration techniques in harvesting cells of *N. gaditana* and *Phaeodactylum tricornutum*.

Zhang et al., (2014) examined how a diatomite dynamic membrane affected *C. pyrenoidosa* filteration effectiveness. They discovered that the membrane disrupted protein, polysaccharides and humic-like substances, as well as certain low-molecular-weight organic compounds. In the membrane filtration process, various types of permeable membranes are utilized to filter microalgal biomass. Previous studies have primarily focused on the effects of microfiltration and ultrafiltration membranes on the harvesting of microalgae. The effectiveness of membrane filtration methods can be enhanced by using membranes with different pore sizes. A significant challenge in membrane filtration is fouling, which occurs due to pore clogging. Consequently, pore size is a key criterion used to classify nano-filtration (0.5–2 nm), ultrafiltration (2–100 nm), and microfiltration (100–10,000 nm) membranes. Generally, microfiltration membranes exhibit a broad range of pore sizes, followed by ultrafiltration have a narrower range, and nanofiltration possess the smallest pore sizes (Muñoz et al., 2021). Moreover, membranes with smaller pore sizes tend to lower the filtration rate. Commonly used membrane materials include, polyethersulfone, polyvinylidene fluoride, polyacrylonitrile polyvinyl chloride, and polytetrafluoroethylene (Ananthi et al., 2021). A nylon 6,6 nanofiber membrane, characterized by a surface porosity of 25.82 %, a pore size of 0.12 μm, and an initial clean water permeance of 1018 $L/m^2h\cdot bar$, experienced a rapid decrease in performance. Its permeance dropped from 1018 to 528 $L/m^2.h\cdot bar$ within the first 15 min, and continued to decline, reaching 300 $L/m^2.h\cdot bar$ by the end of the filtration process (Bilad et al., 2018). In conclusion, filtration is considered an effective and widely used method that is suitable for larger cell sizes and higher biomass concentrations. This technique offers advantages such as simplicity, scalability, and the ability to recover a high percentage of biomass with minimal processing steps. Moreover, the efficiency of filtration can be highly depended on factors like cell size, morphology, and the nature of the algal suspension, necessitating the selection of appropriate filter materials and pore sizes. While filtration is a viable option for many microalgae harvesting applications, challenges such as filter clogging and the energy requirements for large-scale operations should be addressed through optimization and the development of more advanced filtration technologies.

1.3. Flotation

Flotation, also known as "inverted" sedimentation, involves the introduction of gas bubbles into the culture medium to generate the buoyant force needed for particle separation and transport. This approach is widely implemented in wastewater treatment and is generally carried out following coagulation or flocculation processes. Due to the low density and natural buoyancy of microalgae, flotation proves to be more efficient and advantageous for microalgal removal compared to sedimentation. In a study on dissolved air flotation, (Zhang et al., 2012) demonstrated that the HE on *C. zofingiensis* exceeded 90 % with an increase of $Al^{(3+)}$ dosage. A second study found that dispersed air flotation, employing chitosan and saponin, effectively harvested *C. vulgaris* and *Scenedesmus obliquus*. These findings underscore the effectiveness of flotation techniques for harvesting *C. vulgaris* (Kurniawati et al., 2014). Al-Humairi et al. (2023) showed that the HE of *C. vulgaris* increased with higher initial concentrations of cationic trimethylammonium bromide. For *C. sorokiniana*, the dissolved air flotation approach alongside pH adjustment proved to be an efficient way to extract biomass from wastewater. (Leite et al., 2020). The thermal flotation method was employed to harvest *S. obliquus* and *C. vulgaris*, revealing a higher harvesting efficiency for *S. obliquus* (88.16 %) compared to *C. vulgaris* (47.16 %). This difference in efficiency is attributed to the thermal pre-flocculation process, which degrades the proteins, carbohydrates, and lipids on the microalgae cell surfaces (Zou et al., 2019). Ometto et al. (2014) the Ballasted Dissolved Air Flotation (BDAF) technique and concluded it as a superior and environmentally sustainable method for harvesting compared to conventional Dissolved Air Flotation (DAF). They achieved a remarkable 99 % cell recovery rate while reducing energy consumption and coagulant demand by 80 % and 95 %, respectively. Colloidal gas aphrons technology has emerged as a recent approach in flotation processes (Pal et al., 2021). The stable colloidal gas aphrons method was

applied for the removal of *Arthrospira platensis*, *N. oculata*, and *C. vulgaris*. N, N′-bis(cetyldimethyl)-1,4-butane diammonium dibromide (Gemini surfactant) was utilized for *C. vulgaris* harvesting. Compared to the monomeric cetyltrimethylammonium bromide, this novel Gemini surfactant exhibited superior performance in harvesting efficiency (Huang et al., 2019). These methods are particularly advantageous for microalgae due to their low density and natural buoyancy, leading to rapid and effective harvesting. Innovations in flotation technology, including the use of advanced surfactants and colloidal gas aphrons, have further improved harvesting performance and reduced operational costs. Overall, flotation-based harvesting represents a promising solution for large-scale microalgae production and wastewater treatment, offering both high efficiency and potential for sustainable and cost-effective operations.

1.4. Flocculation

In the realm of microalgae harvesting, flocculation stands out for its ability to aggregate cells into larger clusters, aiding in separation from the culture medium. Chemical, biological, and physical methods induce flocculation, optimizing parameters like species, culture medium, and flocculant choice. This method is efficient, especially in biofuel production and wastewater treatment. However, challenges like process optimization and scalability persist. Despite these challenges, flocculation offers a sustainable approach to biomass harvesting, promising both economic viability and environmental benefits in various industrial applications (Jones and Mayfield, 2012).

1.4.1. Chemical flocculation
Chemical coagulation/flocculation emerges as a primary strategy for economically optimizing microalgal harvesting processes. The necessity for such methods primarily arises from the substantial volumes of microalgal cultures requiring processing and the demand for a versatile approach applicable across diverse species. This pivotal harvesting phase aims to concentrate the suspension, typically achieving concentrations 20–100 times higher. By augmenting the effective particle size prior to dewatering, it substantially diminishes the energy requirements of the process. Consequently, coagulation/flocculation, often succeeded by gravity sedimentation, stands as a preferred approach for cost-effective microalgae harvesting. The efficacy of chemical flocculation in microalgae harvesting has been extensively studied, with various flocculants demonstrating promising results across different species and conditions. For instance, Mg-sericite was effectively employed for harvesting *C. vulgaris*, highlighting the significant influence of pH on harvesting efficiency (Lee and Choi, 2015). *Chlorella sp. KR-1* was harvested using a metal coagulant, mainly $Fe_2(SO_4)_3$, along with sulfuric acid. Additionally, the authors suggested that lipid extraction may be done directly using the biomass of acid-treated *Chlorella sp. KR-1* without requiring any additional catalysts. (Kim et al., 2017). In a batch experiment utilizing chitosan as a flocculant for *Chlorella vulgaris*, the culture medium consisted of CM-BG-11 with a volume (V) of 250 mL. The temperature (T) was maintained at 25°C, and the pH was adjusted to 6.0. A chitosan dosage (CD) of 30 mg/L was applied, followed by a holding period (HP) of 10 min (Rashid et al., 2013). The harvesting mode (HM) was batch, resulting in a recovery efficiency of 92 %. $FeCl_3$ was used as a flocculant in *Nannochloropsis oculata* at 400 mg/L, resulting in a 94 % harvesting rate within 180 min (Surendhiran and Vijay, 2013). Magnesium hydroxide exhibited 90 % harvesting in *Chlorella* sp. within 30 min (Vandamme et al., 2015). Cationic starch is produced via chemical alterations, such as etherification, from starch, a plentiful natural polymer. The extent of functional cationic group incorporation determines its ability to cause flocculation. Due to its affordability and environmental friendliness, cationic starch is frequently applied in wastewater treatment and algae harvesting. Reported concentrations of cationic starch for recovering 1 g of algal biomass were 89 mg for *C. pyrenoidosa* and 119 mg for *Botryococcus braunii*. Machado et al. (2022) examined the impact of various organic and inorganic flocculants on the harvesting efficiency of *C. vulgaris*. Finding that, Zetag 8185 demonstrated the HE, reaching 98.8 % at 50 mg/L and 97.9 % at 100 mg/L. During a flocculation process, the alkaline pH significantly influenced the appearance of harvested cells from *P. tricornutum*, *P. purpureum*, and. *C. vulgaris* Additionally, the inclusion of polyacrylamide resulted in increased harvesting efficiency while retaining more industrially significant chemical components (Kuzhiumparambil et al., 2022).

1.4.2. Autoflocculation
Certain microalgae can undergo natural flocculation, known as auto-flocculation, without the need for additional chemicals answering to environmental stressors, such as variations in dissolved oxygen, nitrogen levels, and pH. This process, triggered at higher pH levels, enables rapid, cost-effective, and straightforward gravity settling. Compared to centrifugation, natural gravity settling is less disruptive to the cells. Pezzolesi et al. (2015) found that flocculated cells were more likely to resuspend in the algal medium at higher pH levels because they were less dense and compact. Additionally, They also found that 95 % of the biomass was harvested in 60 min when the pH was lowered to 4. Christenson and Sims (2012) found that the increase in pH leads to the formation of calcium phosphate precipitates, which may offset the microalgal cell negative charge. Vandamme et al. (2012) raised the pH to 10.8 using $Ca(OH)_2$, NaOH, and KOH, achieving 98 % biomass recovery, while $Mg(OH)_2$ reached a 98 % recovery rate at a pH of 9.7 within 30 min. By harnessing natural pH fluctuations and the consequent formation of precipitates, such as calcium and magnesium hydroxides, this method significantly reduces reliance on external chemical flocculants, thereby lessening environmental concerns. The high efficiency observed in achieving considerable biomass recovery in relatively brief periods underscores its potential. Further research into optimizing pH conditions and exploring the fundamental mechanisms at play could broaden its applicability across various microalgae species and industrial contexts.

1.4.3. Electro-flocculation
Electro-flocculation is an advanced technique for harvesting microalgae from water, which works by applying an electric current through electrodes to induce floc formation and facilitate the separation of microalgal cells from the solution. The electric field induces

the release of metal cations from the electrodes, which react with hydroxide ions present in water to form metal hydroxides. These metal hydroxides eventually act as flocculants, aggregating and binding into larger flocs that settle out due to gravity. The electro-flocculation efficiency depends on various factor, including current density, electrolysis duration, electrode material, pH, and the composition of the microalgae suspension. Among the materials tested, aluminum proved to be more effective than iron (A. K. Lee et al., 2013). Luo et al. (2017) found the application of electrolytic flotation for harvesting microalgae without the need for flocculants. Stainless steel was used as the cathode and carbon as the anode, chosen based on their efficiency in the process. Electro-flocculation with aluminum electrodes was utilized for harvesting *Chlorella vulgaris*. The microalgae were quickly harvested when a higher current density was applied (Shi et al., 2017). One study highlighted the successful integration of alkali-induced flocculation with an electrolysis-based method (salt bridge electro-flocculation) for microalgae biomass recovery. This approach effectively protected microalgal cells from oxidative damage caused by the anodes and ensured that no external contaminants were introduced into the algal biomass (Hou et al., 2022). Despite its benefits, challenges such as electrode consumption, energy requirements, and managing the generated sludge remain areas for ongoing research and development.

1.4.4. Bio-flocculation

Bioflocculation is a natural process in which microorganisms, such as algae, bacteria, or fungi, aggregate and form flocs through biological interactions. This technique is employed in various applications, including wastewater treatment, bio-remediation, and microalgae harvesting (Salim et al., 2011). Unlike chemical flocculation, which relies on synthetic agents to induce particle aggregation, bio-flocculation utilizes naturally occurring biological agents to facilitate the formation of flocs. Extracellular polymeric substances (EPS), which comprise proteins, polysaccharides, and other organic chemicals that encourage particle aggregation, are produced by microorganisms. The EPS acts as a binding matrix, allowing microalgae to form larger aggregates that can be easily separated (Alam et al., 2016). Wang et al., (2022) optimized the harvesting of *C. vulgaris* using chitosan and polyacrylamide. Chitosan exhibited superior flocculation efficiency at 98.10 % with 10 mg/L, whereas polyacrylamide achieved 94.57 % efficiency at 25 mg/L. Chitosan was employed for the harvesting of *C. sorokiniana*, achieving a flocculation efficiency exceeding 99 % at pH levels below 7 (Xu et al., 2013). Prochazkova et al. (2015) demonstrated the effectiveness of spent brewer's yeast for flocculating *C. vulgaris*. After hydrolysis, the yeast was chemically modified with 2-chloro-N,N-diethylethylamine hydrochloride, and the resulting material was assessed for its flocculation efficiency in harvesting *Chlorella vulgaris*. Co-cultivation of *C. vulgaris* and *A. niger* with 2 g/L glucose resulted in a harvesting efficiency greater than 90 %. These findings suggest that the addition of glucose is crucial for enhancing fungal growth and promoting the formation of cell pellets (Gultom et al., 2014).Chu et al. (2021) *Aspergillus oryzae* pellets were employed to harvest *C. vulgaris*, achieving a maximum efficiency of 99.23 % at 30 °C, 130 rpm, and a 1:1 fungus-to-algae ratio. These results suggest

Table 1
Evaluation of microalgae harvesting efficiency using various flocculants.

Microalgae	Flocculants	Recovery (%)	References
Chemical flocculation			
Chlorella sorokiniana	Aluminum chloride (AlCl$_3$)	95.2	(Escapa et al., 2015)
Chlorella vulgaris	Sulfate (Al$_2$(SO$_4$)$_3$ and Fe$_2$(SO$_4$)$_3$) and chloride flocculants (AlCl$_3$ and FeCl$_3$)	98.8	(Machado et al., 2022)
Chlorella sp.	Alum and Ferric chloride	90	(Koley et al., 2017)
Chlorella vulgaris	Aluminum sulfate, Ferric sulfate, Ferric chloride	95–98	(Ma et al., 2023)
Dunaliella salina	Potash alum	99	(Khadim et al., 2018)
Phaedodactylum purpureum	Polyacrylamide, alkaline, and centrifugation	-	(Kuzhiumparambil et al., 2022)
Arthospira maxima	NaOH or CaCl$_2$	90	(Caetano et al., 2020)
Synechocystis sp.	Ferric chloride, Potassium aluminum sulfate, Chitosan	82	(Lal and Das, 2016)
Chlorella sp.	Fe$_2$(SO$_4$)$_3$	98	(Kim et al., 2017)
Chlorella sp.	Fe$_2$(SO$_4$)$_3$	100	(Kim et al., 2015)
Chlorella sp.	Aminoclay-conjugated TiO$_2$	85	(Y.-C. Lee et al., 2014a)
Chlorella sp.	Polyacrylamide and FeCl$_3$	90.5	(Lin et al., 2021)
Scenedesmus sp.	Chitosan	95	(Chen et al., 2013)
Chlorella vulgaris	Chitosan	91.9	(Zhu et al., 2018)
Auto-flocculation			
Chlorella vulgaris	pH-10.6 pH regulations	> 90	(Wu et al., 2012)
Scenedesmus quadricauda	pH-3	78.4	(Aljuboori et al., 2016)
	EPS	86.7	
Phaeodactylum tricornutum	pH-9	90	(Wu et al., 2012)
Chlorella sp.725	Metal ions induction	90	(Yang et al., 2016)
Chaetoceros gracils	pH-10.5	100	(Pérez et al., 2017)
Ettlia sp.	Axenic culture (EPS)	92.54	(Vu et al., 2019)
Bio-flocculation			
Chlorella sp.	Chitosan	98	(Lal and Das, 2016)
Chlorella sp.	Aspergillus niger	90	(Mohd Nasir et al., 2019)
Chlorella prothecoides	Microbial flocculant poly	90	(Zheng et al., 2012)
Nanochloropsis oculata	Cationic cellulose nanocrystals	95	(Verfaillie et al., 2020)
Scenedesmus obliquus	Chitosan, Tanfloc, cationic starch, and *Moringa oleifera*	> 90	(Yang et al., 2021)
Desmodesmus brasiliensis	γ-PGA obtained from Bacillus licheniformis	98	(Ndikubwimana et al., 2016)
Chlorella vulgaris	Walnut protein extract	40	(Xu et al., 2021)
Chlorella sp.	*Pleurotus ostreatus*	64.86	(Luo et al., 2019)

that metabolites released into the medium may contribute to the observed bio-flocculation behavior. Niemi and Gentili (2021) investigated the impact of natural organic flocculants, finding that tannin S5T demonstrated equivalent flocculation efficiency across the tested microalgae species. *Strychnos potatorum* seed powder was used as a bioflocculant to harvest *Chlorella vulgaris*, achieving a maximum efficiency of 99.68 % under optimal conditions of 100 mg/L bioflocculant concentration, 35 °C, 150 rpm, and a 30-minute incubation period (Abdul Razack et al., 2015). Table 1 illustrates the microalgae harvesting using various flocculants.

Based on the comparative data summarized in Table 1, the comparative assessment of chemical, auto-, and bio-flocculation strategies highlights clear trade-offs between recovery efficiency, operational cost, and process sustainability. Chemical flocculation, dominated by aluminum and iron salts ($AlCl_3$, $FeCl_3$, $Fe_2(SO_4)_3$, alum), consistently achieves high biomass recovery (90–100 %) across diverse microalgae, including *Chlorella*, *Dunaliella*, and *Scenedesmus*. However, its effectiveness is often linked to high salt dosages, secondary contamination, and challenges in biomass reuse, particularly for food, feed, and nutraceutical applications. The requirement for pH adjustment and post-treatment sludge management further increases operational and environmental costs.

Auto-flocculation offers a chemical-free alternative, with recovery efficiencies typically ranging from 78 to 100 %, driven by pH elevation, EPS secretion, or metal ion induction. While highly attractive from a sustainability perspective, its strong dependence on species-specific physiology and alkaline pH conditions (often >9–10.5) limits robustness and scalability, especially in continuous or mixed-culture systems where pH control becomes energy-intensive.

Bio-flocculation emerges as a balanced approach, combining relatively high recovery efficiencies (90–98 %) with improved biomass quality and lower ecological impact. Natural polymers (chitosan, γ-PGA) and microbial or fungal flocculants demonstrate broad applicability and moderate pH tolerance. Nevertheless, variability in flocculant performance and production cost remains a constraint.

Overall, although chemical flocculation is an operationally mature and effective method for particle removal, its application is limited by environmental concerns related to the discharge of large quantities of chemicals and the relatively long processing times required. In contrast, bio-flocculation represents a more sustainable and promising alternative for large-scale applications, particularly when combined with recyclable materials and integrated into downstream biorefinery processes.

1.5. Evaluation of Microalgae Harvesting Efficiency

Harvesting efficiency (HE) is a key metric for assessing microalgae recovery and is typically expressed as the percentage of biomass removed from the culture medium. It is commonly calculated using the equation:

$$HE\% = \left(\frac{OD_0 - OD_f}{OD_0} \right) * 100$$

where C_o and C_f represent the initial and final biomass concentrations, respectively. Biomass quantification is generally performed via optical density, dry weight, or direct cell counting. The use of nanoparticles (NPs) or nanocomposites markedly influences HE, as their physicochemical properties—such as surface charge, size, and surface functionalization—govern the nature and strength of interactions with microalgal cells. These interactions, illustrated in Fig. 2, are further modulated by factors such as pH, ionic strength,
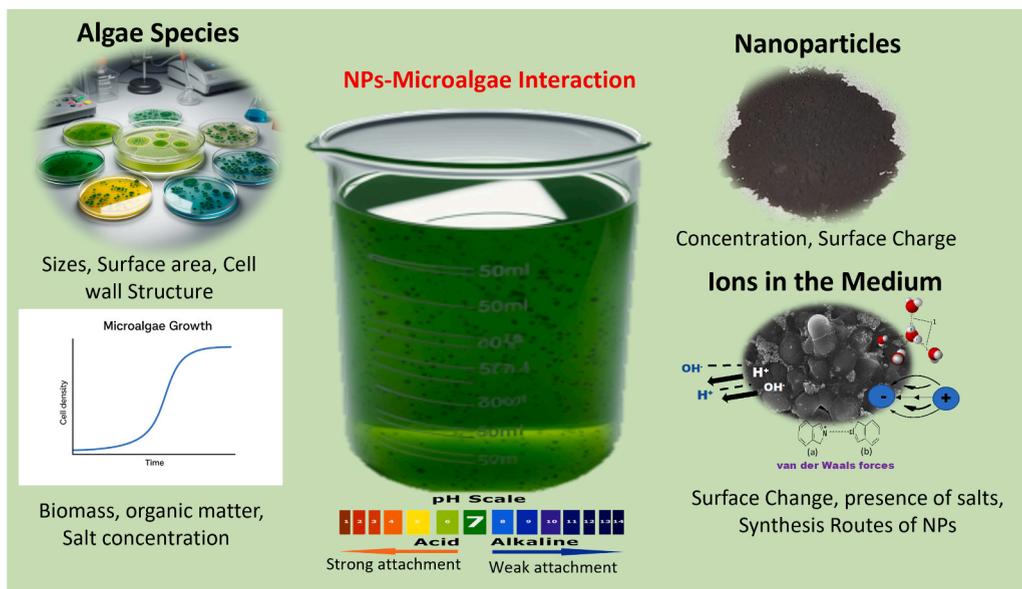

Fig. 2. Schematic representation of major parameters influencing microalgae flocculation and harvesting efficiency in the presence of nanoparticles.

and medium composition, resulting in variability in HE across studies. By summarizing these approaches, this review provides a comprehensive framework for evaluating NP-assisted harvesting and enables direct comparison of harvesting performance across diverse experimental systems.

1.6. Nanoparticle in microalgae harvesting

The rapid development of nanotechnology has led to advancements in downstream processing, owing to its potential to enhance and extract products widely used today (Sumathi et al., 2025).It is essential that microalgae harvesting is conducted without the use of mechanical or electrical force. Various forms of nanomaterials have been explored for harvesting microalgae. Microalgae possess a negative charge on their surface, which helps them bind with positively charged nanoparticles within a specific pH range. Nano-materials with a positive zeta potential can interact with microalgae through physical and chemical interactions involving the plasma membrane, cell wall, and cellular compartments, mainly the cytoplasm and chloroplast, thereby modifying the cellular environment (Wang et al., 2019). NPs can be adsorbed onto the microalgae surface through endocytosis or by causing physical damage. Those with a higher zeta potential exhibit greater harvesting efficiency at low doses. NPs harvest microalgae by attaching to the surface, forming flocs. These flocs can settle using two methods: gravity settling, where larger flocs settle first, followed by smaller ones, and magnetic separation, where magnetic nanoparticles form flocs with microalgae that can be effectively separated under a magnetic field (Patel et al., 2022a).

Recent developments have highlighted the potential of magnetophoretic separation of microalgae over traditional methods. This technique offers advantages such as low cost, ease of operation, rapid processing, and high efficiency. Magnetic particle-induced harvesting involves attaching micro- or nano-sized magnetic particles to the surface of microalgal cells (Wang et al., 2014). The attached particles are then introduced to an external magnetic field to recover microalgae cells from the culture (Cerff et al., 2012). Nanoparticle-based magnetic harvesting is particularly efficient, enabling scalable, automatable, and fast processing with high harvesting efficiency and minimal contamination (Patel et al., 2022a; Saxena and Harish, 2018). It is popular due to its unique physicochemical capabilities, small size, and low production cost (Seo et al., 2017). Two types of magnetic particles are commonly used: naked and surface-functionalized. Negatively charged algal cells are electrostatically attracted to positively charged magnetic particles, resulting in effective harvesting (Prochazkova et al., 2013a). Besides magnetic nanoparticles, other types, such as TiO_2, SiO_2, and chitosan, or various combinations, have also been used for separating microalgae cells. Various types of nanoparticles are utilized in microalgae harvesting, including metallic, magnetic, organic, and inorganic nanoparticles. Magnetic nanoparticles are the most extensively studied and utilized.

1.7. Magnetic nanoparticle

Iron oxide nanoparticles are considered one of the best alternatives for harvesting microalgae because it is inexpensive and easy to use. Successful separation of microalgae depends on various parameters like the species of microalgae, the culture media, pH conditions (Abdelaziz et al., 2013) as well as its features, such as its small size (10 mm), dispersity, and the low concentration of cultures (Wang et al., 2015). Fraga-García et al. (2018) examined the harvesting performance of Fe_3O_4 nanoparticles was evaluated for *Scenedesmus ovalternus* and *Chlorella vulgaris*, achieving up to 95 % harvesting efficiency at pH 4 with a nanoparticle dosage equivalent to 0.5 g of Fe_3O_4 per g of algal biomass and a contact time of 5 min. A harvesting efficiency of 90 % was achieved with a concentration of 10 g/L at pH 6.2 on *Chlorella vulgaris,* which further increases with a decrease in pH (Zhu et al., 2019). The microalgae strain also affects harvesting efficiency. When *Chlorella sp. UKM2* and *Coelastrella sp. UKM4* treated with Fe_3O_4 nanoparticles had a HE of 94 % at 4-min contact times at a dose of 0.5 g/L, using the microalgae *Chlamydomonas sp. UKM6* had a harvesting efficiency of 82 % (Japar et al., 2017). Microalgae *Chlorella sp. KR-1* shows more than 95 % harvesting efficiency on treatment with bare nanoparticles of concentration 12.5 g per g of biomass in less than one min of contact time (K. Lee et al., 2014). *Botryococcus braunii* exhibited a harvesting efficiency of 98 % within 1 min when treated at a biomass-to-nanoparticle ratio of 55.9 mg biomass per mg nanoparticle, whereas *Chlorella ellipsoidea* achieved a comparable harvesting efficiency at a ratio of 5.83 mg biomass per mg nanoparticle using bare nanoparticles. It was further noted that harvesting efficiency improved when the culture was maintained in an acidic medium (Xu et al., 2011). For different concentrations of bare iron nanoparticles, the harvesting efficiency was obtained. A HE of 90 % was achieved for *Chlorella pyrenoidosa* when 500 mg of nanoparticles were applied per gram of microalgal biomass (g /L) for 1 min at pH 3, while *Chlorella minutissima* exhibited an HE of 85 % when treated with 600 mg of bare nanoparticles per gram of biomass under the same pH conditions(Bharte and Desai, 2019). Almomani (2020) demonstrates that the harvesting efficiency of single and mixed algal cultures from real wastewater, treated with a nanoparticle concentration of 25 mg/L, a mixing speed of 120 rpm, a contact time of 7 min, and a separation time of 3 min, and HE was 95 %. They have also studied the harvesting efficiency of individual microalgae cultures, including *Spirulina platensis* and *Chlorella vulgaris*, as well as mixed algal cultures. Patel et al. (2022a) conducted a study on two different strains of microalgae, *C. sorokiniana* Kh12 and *C. sorokiniana* Tu5, treated with 200 mg/L bare nanoparticles at a pH of 3 for a mixing time of 10 min and a contact time of 5 min, achieving a harvesting efficiency of more than 99 %. A similar result was also obtained when nanoparticles were treated with a microalgae strain of *Chlorella vulgaris* (Savvidou et al., 2021). Maghemite (γ-Fe_2O_3) nanoparticles are also used for the harvesting of microalgae. Blooming microalgae were harvested using γ-Fe_2O_3 nanoparticles. It was observed that 82.4 % HE was achieved with a contact time of 48 sec when the reaction mixture was stirred at 310 rpm at 22.5 °C with an NPs dose of 56 mg/L (Duman et al., 2019). Harvesting of *Scenedesmus acuminatus* using Fe_2O_3 nanoparticles was employed in this study, where it was utilized in a membrane filter along with a composite of polyvinyl chloride (PVC), resulting in a 66 % increase in average flux compared to the standard PVC membrane. It was further confirmed that the incorporation of Fe_2O_3 nanoparticles further

enhanced the antifouling performance of the membrane (Q. Liu et al., 2019). nZVI has also been used for harvesting microalgae. Analysis of nZVI along with amino clay in the ratio of (1:1) has shown approx. 100 % harvesting efficiency on *Chlorella* sp. KR-1, when treated with 20 g/L within 3 min of contact time (Y.-C. Lee et al., 2014b).

During the attachment of microalgae to magnetic particles in aqueous systems, several interactions come into play, including acid–base interactions, electrostatic attractions, and non-covalent Lifshitz–van der Waals forces (Prochazkova et al., 2013a). These interactions can suppress harvesting efficiency; therefore, modifying magnetic nanomaterials is crucial (Liu et al., 2017; Mathimani and Mallick, 2018). Since most microalgae have negatively charged surfaces, cationic polymers are often used as coating agents (Zhao et al., 2015). Numerous studies have been conducted on various coating materials to improve the overall efficiency of the harvesting process.

1.8. Fe$_3$O$_4$-PEI NCs

Polyethyleneimine (PEI) is a synthetic polymer characterized by its high density of amine groups, which confer a strong positive charge, particularly in aqueous solutions. This unique property makes PEI an ideal coating material for magnetic nanoparticles, as it introduces a cationic charge on their surface. The amine-rich groups on the surface of PEI can easily interact with the $-OH$ and $-COOH$ groups on the microalgal cell surface. These interactions facilitate strong binding, enabling amine-functionalized magnetic particles to support efficient microalgal recovery. Microalgae *C. vulgaris, C. ellipsoidea, M. aeruginosa, and A. protothecoides.* When treated with PEI coated with Fe$_3$O$_4$ NPs, it exhibited a 98–99 % harvesting rate at a NCs dose of 10 mg/L at pH 4 (Gerulová et al., 2022). Experimental results showed that *Chlorella pyrenoidosa* (0.5 g/L) achieved a harvesting efficiency of 98.92 ± 0.41 % under optimal conditions using an Fe$_3$O$_4$@PEI NCs dose of 20 mL/L, a flocculation time of 20 min, and a stirring speed of 800 rpm (3 min). Similarly, *Scenedesmus obliquus* (0.4 g/L) reached a harvesting efficiency of 98.45 ± 0.35 % with an Fe$_3$O$_4$@PEI dose of 16 mL/L, a flocculation time of 15 min, and a stirring speed of 730 rpm (3 min)(Y. Liu et al., 2019). *Chlorella ellipsoidea* achieved 97 % harvesting efficiency with a 20 mg/L dose of nanoparticles within just 2 min of harvesting time (Hu et al., 2014). Similarly, other studies have also reported the use of Fe$_3$O$_4$-PEI NCs, as detailed in Table 1.

1.9. Fe$_3$O$_4$-Urea NCs

Urea, also known as carbamide, is an organic compound composed of two amine groups, making it a crucial source of nitrogen. It is widely used as a nitrogen-rich fertilizer, in animal feed, and in the production of plastics and pharmaceuticals. Recently, urea has gained significant attention for its potential in microalgae cultivation. Its solubility, organic nature, abundance, and cost-effectiveness in polar mediums, combined with its role as a nitrogen source, make it an ideal additive for microalgae growth. When used in minimal concentrations over extended periods, urea provides a sustainable and rich source of nutrients for microalgae. (Dolganyuk et al., 2020; Kumar et al., 2024a). Kumar et al. (2024a) demonstrated the use of urea-functionalized Fe$_3$O$_4$ NPs for microalgae harvesting. The study focused on three high lutein-producing microalgae species: *C. sorokiniana* C16, *C. sorokiniana* Kh12, and *Nannochloropsis oculata*. Under optimal conditions, pH 4 with a 15-min contact time, *C. sorokiniana* Kh12 achieved HE of 97.95 %, while *C. sorokiniana* C16 and *Nannochloropsis oculata* reached HE of 98.95 % and 98.62 %, respectively, when treated with a nanoparticle dose of 200 mg/L. The study also included a recycling assessment of the Fe$_3$O$_4$-urea NCs. Remarkably, the nanocomposites maintained a HE of up to 88 % through five consecutive cycles without the addition of fresh nanocomposites to the medium.

1.10. Fe$_3$O$_4$-CTAB NCs

Cetyltrimethylammonium Bromide (CTAB) is a cationic surfactant with a wide range of applications. Surfactants like CTAB are extensively used as biocides, antimicrobial agents, and disinfectants in cell biology and bioprocessing, largely due to their ability to solubilize lipid bilayers in cell membranes. Notably, cationic surfactants with a quaternary ammonium head group, such as CTAB, have been shown to exhibit higher cytotoxicity compared to anionic or nonionic surfactants. Additionally, when combined with magnetic nanoparticles, CTAB plays a crucial role due to its dual functionality: it not only induces flocculation of negatively charged microalgae but also promotes cell disruption through its biocidal activity (T.-L. Hwang et al., 2015; Seo et al., 2016). When treated with CTAB-coated Fe$_3$O$_4$ nanocomposites, *Chlorella sp.* KR-1 achieved a HE of 96.6 % using a NCs dose of 0.46 g per g cell biomass (Seo et al., 2016). Similarly, *Scenedesmus sp.* achieved approx. 82 % HE, when mixed with CTAB-coated Fe$_3$O$_4$ NCs at a 2:1 ratio and subjected to 20 min of mixing followed by 20 min of magnetic separation (Abo Markeb et al., 2019).

Likewise, several cation coatings have been applied to Fe$_3$O$_4$ NPs to date, enhancing the overall harvesting efficiency of microalgae. Arginine is another positively charged amino acid that, when polymerized, retains its strong cationic nature. This property is utilized in microalgae harvesting when polyarginine is functionalized with Fe$_3$O$_4$ nanoparticles. The study selected polyarginine with varying molecular weights to investigate its impact on microalgal harvesting. It was observed that polyarginine with a molecular weight of 15,000–70,000 achieved the best results, with over 85 % harvesting efficiency at pH 4 using a nanocomposite dose of 100 mg/L (Liu et al., 2017). Similarly, due to their cationic properties, polyaluminum chloride (PACl) and polyacrylamide (PAM) were used in combination with Fe$_3$O$_4$ nanoparticles. The results showed a 99 % harvesting efficiency within 30 s when Fe$_3$O$_4$-PACl was used at a dose of 10 g/L, and then 3 mg/L of PAM at in *C. vulgaris* (FACHB-31) with a culture pH of 8.4 (Zhao et al., 2015). cationic polymer diallyl dimethylammonium chloride (PDDA) coated with Magnetic graphene oxide nanocomposite achieved HE of 95.35 % of harvesting efficiency with 5 mines with NCs dose of 70 mg/L ai pH 8 (Liu et al., 2016). Another natural polymer, plant polyphenol, was used in combination with Fe$_3$O$_4$ nanoparticles and demonstrated a harvesting efficiency of 93 % when applied at an NCs dose of 20 g/L

to a *Chlorella vulgaris* culture (1.5 g dry cell weight/L) at pH 9.03 (Wang et al., 2018). Silica-coated Fe_3O_4 nanocomposites achieved harvesting efficiencies exceeding 95 % when applied at dosages of 70 mg and 30 mg of nanoparticles per gram of microalgal biomass for *Phaeodactylum tricornutum* and *Chlamydomonas reinhardtii*, respectively, at pH 12 and pH 8. (Cerff et al., 2012). Similarly, Fe_3O_4-silica coated when functionalized with strong base triazabicyclodecene (TBD) achieved maximum HE within one min of contact time (Chiang et al., 2015). Chitosan is another natural polymer widely used in microalgae harvesting in various forms, including simple chitosan, nanochitosan, and in combination with Fe_3O_4 nanoparticles. The amine functional groups in chitosan provide a net positive charge, allowing it to effectively bind with negatively charged microalgae cells, thereby enhancing the overall harvesting efficiency. Fe_3O_4-Chitosan exhibits 99 % HE when treated with 1.4 g/L of NCs for *Chlorella vulgaris* at culture pH. Upon further increasing the dose of NCs to 3.4 g/L, the HE further decreased to 95 % due to the full occupancy of the adsorption sites present on the microalgae surface (K. Lee et al., 2013). Similarly, *C. pyrenoidosa* and *S. obliquus* exhibit up to 97 % of HE with a Fe_3O_4-Citosan NCs dose of 738 mg/L at pH 9 (Liu et al., 2023). Kumar et al. (2023) conducted a study on harvesting efficiency using Fe_3O_4-Dopamine NCs on *Chlorella sorokiniana* Kh12, achieving over 99 % harvesting efficiency at pH 3 with a NCs dose of 200 mg/L within 5 min of magnetic separation. Similarly, other coating materials used in combination with Fe_3O_4 nanoparticles for microalgae harvesting are also highlighted in Table 1.

1.11. Non-Magnetic nanoparticle

In recent times, non-magnetic nanoparticles have also become a significant factor, as they play a crucial role in the harvesting of microalgae. In general, nanoparticles with cationic surface charges are primarily explored, as they can bind effectively to the surface of microalgae. Metal-based zirconium dioxide (ZrO_2) nanoparticles exhibit 99 % of HE with a low dose of 15 mL/L NCs at pH 10 for microalgae *Chlorococcum sp.* (Khanra et al., 2020). Nickel oxide (NiO) NPs achieved up to 98.75 % of HE within 1 min at pH 7 on *C. vulgaris* with NCs dose of 75 mg/L. Apart from harvesting, NiO NPs also facilitated cell disruption and increased the lipid content when exposed for a longer duration (Huang and Kim, 2016). Nanochitosan achieved a 98 % harvesting efficiency at pH 9 with a dose of 60 mg/L on *Nannochloropsis sp.*, which was an improvement compared to regular chitosan, which showed around 90 % harvesting efficiency at the same pH with a higher dose of 100 mg/L (Farid et al., 2013). Similar studies reported that TiO_2–chitosan nanoparticles achieved up to 97 % harvesting efficiency within 2 min at pH 7 when applied at a dosage of 90 mg of nanoparticles per gram of *Chlorella minutissima* biomass. (Dineshkumar et al., 2017). In another study, *Chlorella sorokiniana* achieved a harvesting efficiency of up to 99.87 % when treated with *Aspergillus pseudonomiae* combined with calcium oxide nanoparticles (AP@CaO) at a dose of 7.5 % v/v after an hour of incubation at pH 5 (Usman et al., 2025). Zinc–aluminum layered double hydroxide (ZnAl-LDH) nanosheets achieved up to 96 % harvesting efficiency for *Chlorella vulgaris* at pH 6 when applied at a dosage of 521 mg of nanocomposite per gram of microalgal biomass. (Elgiddawy et al., 2017). Cellulose nanocrystals (CNCs) derived from cotton wool have been applied for Chlorella vulgaris harvesting using both unmodified CNCs and CNCs modified with Br[PyBnoo]-g and Br[PyNeBnoo]-g. While unmodified CNCs at a dosage of 100 mg/L showed no flocculation activity, both modified CNC variants achieved 100 % flocculation efficiency. (Vandamme et al., 2015). Table 2 presents the different types of nanoparticles used in microalgae harvesting, along with their corresponding pH values and harvesting efficiencies.

1.12. Nanomaterials and microalgae interaction

Nanoparticles interact with microalgae through complex processes that can significantly impact the behavior and function of both. These interactions include ion release, shading, absorption, adsorption, and cell wall disruption (Fig. 2). Typically, these interactions involve alterations in the nanoparticles that enable attachment to the microalgae, potentially leading to changes in the microalgae's biochemical, physiological, and molecular states (Déniel et al., 2019). However, some significant factors that facilitate interaction between them, which are useful for microalgae harvesting, are discussed.

Based on the comparative data summarized in Table 2, several critical insights emerge regarding nanoparticle (NP) concentration, harvesting efficiency (HE), and pH dependence, which are crucial for assessing the techno-economic feasibility of future microalgae harvesting technologies. A clear trend is observed in which Fe_3O_4-based magnetic nanoparticles consistently deliver high harvesting efficiencies (>95 %) at relatively low dosages, particularly when surface-functionalized. For instance, Fe_3O_4 concentrations in the range of 20–300 mg/L or 0.05–0.5 g/g biomass frequently achieve > 95–99 % HE across multiple algal species, including *Chlorella vulgaris*, *C. sorokiniana*, and *Scenedesmus* sp. In contrast, higher NP loadings ($\geq$1–2 g/L or $\geq$1 g/g) offer only marginal efficiency gains while significantly increasing material cost, sludge generation, and downstream separation burdens, making them less attractive for scale-up.

Surface functionalization emerges as a decisive factor in reducing nanoparticle demand and pH sensitivity. Functionalized magnetic nanoparticles, particularly when coupled with cationic polymers, enhance harvesting efficiency, reduce overall material requirements, and maintain effective performance across a broad pH range. Systems such as Fe_3O_4–PEI, Fe_3O_4–chitosan, Fe_3O_4–dopamine, and Fe_3O_4–urea achieve near-quantitative harvesting efficiencies (97–99.5 %) under neutral to mildly acidic conditions (pH 4–7). This is especially significant because extreme pH adjustment (pH 2–3), while effective for bare Fe_3O_4, introduces additional chemical costs, corrosion risks, and potential deterioration of biomass quality. Consequently, functionalized magnetic nanoparticles that operate efficiently at culture pH or near-neutral conditions offer clear economic and operational advantages.Non-magnetic nanomaterials (*e.g.,* chitosan, ZnAl-LDH, ZrO_2, NiO) can also achieve high HE (>96–99 %), but typically require higher dosages, narrower pH windows, or lack facile recovery, which limits their recyclability and increases life-cycle impacts. In contrast, magnetic recoverability of Fe_3O_4-based systems enables repeated reuse with minimal efficiency loss, directly supporting circular

Table 2

Nanoparticle-based methods for harvesting microalgae and their harvesting efficiencies.

Microalgae	Nano-materials	Concentration	pH	Harvesting Efficiency	Harvesting Method	References
Scenedesmus ovalternus and *Chlorella vulgaris*	Fe_3O_4	0.5 g/g	4	95 %	Magnetic separation	(Fraga-García et al., 2018)
Haematococcus pluvialis, Chlorella zofingiensis	Fe_3O_4	200 mg/L	3–7	99 %	Magnetic separation	(Sumathi et al., 2025)
Chlorella vulgaris	Fe_3O_4	10 g/L	6.2	90 %	Magnetic separation	(Zhu et al., 2019)
Chlorella vulgaris	$Y_3Fe_3O_4$	2.5 g/L	7.3	90 %	Magnetic separation	(Zhu et al., 2019)
Chlorella sp. UKM2 and *Coelastrella sp. UKM4*	IONP	0.5 g/L	Culturemedia	94 %	Magnetic separation	(Japar et al., 2017)
Chlorella sp. KR-1	Fe_3O_4	12.5 g/g	2–3	> 95 %	Magnetic separation	(K. Lee et al., 2014)
Chlorella ellipsoidea	Fe_3O_4	300 mg/L	7.2	98.9 %	Magnetic separation	(Xu et al., 2011)
Chlorella pyrenoidosa	Fe_3O_4	500, 600 mg/g	3	90 %,85 %	Magnetic separation	(Bharte and Desai, 2019)
Chlorella vulgaris	Fe_3O_4	30 mg/L	4,7	97.4 %, 98.6 %	Magnetic separation	(Almomani, 2020)
Chlorella vulgaris	Fe_3O_4- APTES	20 mg/L	7.1	98	Magnetic separation	(Almomani, 2020)
Chlorella sorokiniana Kh12, and *C. sorokiniana Tu5*	Fe_3O_4	200 mg/L	3	99 %	Magnetic separation	(Patel et al., 2022a)
	γ-Fe2O3	56 mg/L		82.4 %	Magnetic separation	(Duman et al., 2019)
Chlorella vulgaris, C. ellipsoidea, M. aeruginosa, A. protothecoides.	Fe_3O_4-PEI	10 mg/L	4	98–99 %	Magnetic separation	(Gerulová et al., 2022)
Chlorella pyrenoidosa, Scenedesmus obliquus	Fe_3O_4-PEI	20,16 mL/L	Culture	98 %	Magnetic separation	(Q. Liu et al., 2019)
Chlorella ellipsoidea	Fe_3O_4-PEI	20 mg/L	9	97 %	Magnetic separation	(Hu et al., 2014)
Scenedesmus dimorphus	Fe_3O_4-PEI	0.075 mL/L	7	91 %	Magnetic separation	(Ge et al., 2015)
Chlorella sorokiniana Kh12	Fe_3O_4-Urea	200 mg/L	4	98 %	Magnetic separation	(Kumar et al., 2024a)
Chlorella sorokiniana C16, *Nannochloropsis oculata*	Fe_3O_4-Urea	200 mg/L	4	99 %	Magnetic separation	(Kumar et al., 2024a)
Chlorella zofingiensis	Fe_3O_4-Urea	800 mg/L	3–4	95 %	Magnetic separation	(Kumar et al., 2024b)
Chlorella sp. KR-1	Fe_3O_4-CTAB	0.46 g/g	Culture	96.6 %	Magnetic separation	(Seo et al., 2016)
Scenedesmus sp.	Fe_3O_4-CTAB	2:1 (M/N)	Culture	82 %	Magnetic separation	(Abo Markeb et al., 2019)
Chlorella sp. HQ	Fe_3O_4-PA	100 mg/L	4	85 %	Magnetic separation	(Liu et al., 2017)
Chlorella vulgaris FACHB-31	Fe_3O_4- PACl-PAM	PACL= 10 g/L; PAM= 3 mg/L	8.4	99 %	Magnetic separation	(Zhao et al., 2015)
Chlorella sp. HQ.	Fe_3O_4-PDDA	70 mg/L	8	95.35 %	Magnetic separation	(Liu et al., 2016)
Botryococcus braunii; Chlorella ellipsoidea	Fe_3O_4-CPAM	25, 120 mg/L	7	> 95 %	Magnetic separation	(Wang et al., 2014)
Chlorella vulgaris	Fe_3O_4-QPP	20 g/L	9.03	93 %	Magnetic separation	(Wang et al., 2018)
Chlamydomonas reinhardtii	Fe_3O_4-Silica	30 mg/g	8	95 %	Magnetic separation	(Cerff et al., 2012)
Chlorella sorokiniana TH01	Fe_3O_4-Silica	600 mg/L	7	99.5 %	Magnetic separation	(Van et al., 2023)
Chlorella vulgaris	Fe_3O_4-Chitosan	1.4 g/L	Culture	99 %	Magnetic separation	(K. Lee et al., 2013)
Chlorella pyrenoidosa and S. obliquus	Fe_3O_4-Chitosan	738 mg/L	9	97 %	Magnetic separation	(Liu et al., 2023)
Chlorella sorokiniana Kh12	Fe_3O_4-Dopamine	200 mg/L	3	99 %	Magnetic separation	(Kumar et al., 2023)
Chlorella vulgaris	Fe_3O_4-tannin	1.5 g/L	8	98 %	Magnetic separation	(Wang et al., 2022)
Chlorella sp. KR-1	Fe_3O_4- APTES-OTES	1.6 g/g	6.8	98.5 %	Magnetic separation	(Lee et al., 2015)

Table 2 (*continued*)

Microalgae	Nano-materials	Concentration	pH	Harvesting Efficiency	Harvesting Method	References
Haematococcus pluvialis	Fe_3O_4- NDs-NSs	700 mg/L	7	95 %	Magnetic separation	(Jeong et al., 2024)
Chlorella minutissima	Fe_3O_4-Chitosan-TiO_2	0.07 g/g	–	98 %	Magnetic separation	(Dineshkumar et al., 2017)
Scenedesmus obliquus	Fe_3O_4-@PAC	55.36 mg/L	6.3	98.6 %	Magnetic separation	(Han et al., 2025)
M. aeruginosa	CGG-Fe_3O_4	20 mg/L	4–10	98.95	precipitation	(Wang et al., 2025)
Chlorella vulgaris	NiO	75 mg/L	7	98.75 %	Bio-flocculation	(Huang and Kim, 2016)
Nannochloropsis sp.	Chitosan	60 mg/L	9	98 %	Sedimentation	(Farid et al., 2013)
Chlorella sorokiniana	AP@CaO	50 mg/L	5	93.04 %	Harvesting Method	(Usman et al., 2025)
Chlorella vulgaris	ZnAl-LDH	0.521 g/g	6	96 %	Magnetic separation	(Elgiddawy et al., 2017)

Abbreviation: NDs: Nanodisks; NSs: Nanospheres; PAC: Poly Aluminium Chloride; CGG: Cationic Guar Gum

biorefinery principles.

From a future technology development perspective, low-dose ($\leq$200 mg/L), surface-engineered Fe_3O_4 nanoparticles operating at pH 6–8 represent the most viable pathway. These systems strike a balance between harvesting efficiency, energy demand, chemical input, and recyclability, while remaining compatible with downstream lipid extraction and biorefinery integration. Future research should therefore prioritize multifunctional magnetic nanomaterials that minimize dosage, eliminate pH correction, and integrate harvesting with cell disruption or biochemical enhancement, thereby translating laboratory success into economically sustainable industrial deployment.

1.13. Microalgal species

The selection of algal species is an important parameter in determining harvesting efficiency; different adsorption capacities have been reported for different microalgae using the same NPs, as summarized in Table 1. One such reason is the size of the microalgae cells. For instance, a comparative study demonstrated that the adsorption capacity for harvesting *Botryococcus braunii* was approximately ten times greater than that for *Chlorella ellipsoidea*, primarily due to differences in cell morphology. The unicellular *C. ellipsoidea* possesses smaller cells with a significantly higher specific surface area compared to the colonial *B. braunii*. As a result, a higher quantity of magnetic particles was required to achieve comparable recovery efficiency (RE) for *B. braunii*, ultimately resulting in a reduced adsorption capacity.(Xu et al., 2011). A recent study revealed contrasting results when harvesting two microalgae strains, *Chlorella zofingiensis* and *Haematococcus pluvialis*, using Fe_3O_4 nanoparticles. *C. zofingiensis* achieved an optimal harvesting efficiency of 82.9 % at pH 4 with a nanoparticle dose of 800 mg/L, while *H. pluvialis* reached a 99 % efficiency with just 200 mg/L across all pH levels. The significant difference in harvesting efficiency is largely attributed to the size of the microalgae; under a microscope, *H. pluvialis* was observed to be larger, making it easier to flocculate compared to the smaller *C. zofingiensis* (Sumathi et al., 2025). The surface composition of microalgae is the second most important factor influencing the attachment of NPs. Understanding the ultrastructure of algal cell walls is crucial to comprehending their behavior during exposure of NP. Algal cell walls, composed of polysaccharides and a glycoprotein matrix, serve as a defense against environmental factors. The specific composition of these cell walls determines their reactivity with NPs. Cell walls rich in proteins, polysaccharides, and uronic acid exhibit strong adhesive properties to NPs (Chen et al., 2019). Kumar et al. (2024a) explored the presence of aldehydes, acids, and ketones on the cell wall of *Chlorella sorokiniana* Kh12, which plays an important role in binding with the amine group of Fe@urea NCs. Moreover, functional groups on the cell wall impart a negative surface charge, strengthening electrostatic attraction to positively charged nanoparticles (NPs) (Sendra et al., 2017).

1.14. Growth stage of microalgae

Determining the growth stage of microalgae is another crucial factor in optimizing the harvesting process. The growth stage is crucial for achieving maximum biomass, thereby enhancing harvesting efficiency. Hu et al. (2014) demonstrated that when microalgae reached peak value during the growth stage, they achieved maximum harvesting efficiency. The probability of cell-particle collisions with increases in algal biomass. This, in turn, boost the adsorption capacity of the magnetic particles (Zhang et al., 2012). Following peak biomass accumulation, dissolved organic matter released by microalgal cells, as well as that generated through cell autolysis, begins to accumulate. This dissolved organic matter competes with microalgae cells for active surface sites on particles, leading to a decrease in the adsorption capacity of the nanoparticles after the exponential growth stage, which further results in decreased HE of microalgae (Vandamme et al., 2014). The surface charge of microalgal cells varies dynamically during cultivation, influencing their separation efficiency, which is largely governed by electrostatic interactions. Fluctuations in electrophoretic mobility play a crucial role in determining recovery efficiency (RE). For example, during a 14-day cultivation of Chlorella sp., the highest electronegativity was recorded on day 10. This increase is attributed to accelerated growth kinetics and enhanced cell motility, resulting in a strongly electronegative zeta potential. Consequently, day 10 was identified as the optimal point for harvesting. (Lim et al., 2012; Toh et al.,

2014). Another key factor during the cultivation of microalgae is the salt concentration in the culture media. Utilizing salts directly influences HE, as lower salt concentrations reduce interference with nanoparticles, thereby enhancing overall harvesting efficiency.

1.15. Magnetic particle dosage

Recent research articles have shown that as the dose of magnetic nanoparticles increases, the HE of microalgae improves until a maximum HE is reached. Beyond this point, further increases in the nanoparticle dose do not enhance the HE any further (Kumar et al., 2024a, 2023; Patel et al., 2022a). Its mainly due to the distribution of NPs and microalgae cells. At low particle dosages, the magnetic particles are unable to completely cover the cells of microalgae, or do not bind with enough magnetic particles, resulting in incomplete harvesting by the external magnetic field due to insufficient magnetic force. At the optimal dosage, the particle quantity is sufficient to interact with the algal cells, providing a strong magnetic force to fully collect the cells through the external magnetic field, thereby achieving maximum HE. Furthermore, increasing the doses of NPs will not play a role in HE, as the spare NPs in culture will have no influence on HE. It was also observed that when the dose of magnetic nanoparticles (MNPs) exceeded the threshold level, there was a sharp decline in HE (Sumathi et al., 2025). Other researchers have reported similar findings (Liu et al., 2013). Auto-flocculation, a distinct mechanism in the separation process, was the main cause of this decline. In this case, the self-agglomeration of nanoparticles reduced their interaction with microalgae, making the harvesting process more difficult (Matter et al., 2019). In a study by Liu et al., 2013, flocculation was achieved by forming thick flocs through bridging, which enhanced colloidal cohesion with increase in dosage, resulting in a maximum removal of turbidity. Although, once adsorption saturation was reached and overlays formed on the colloids, a second stable phase occurred due to the coagulant overdose, resulting in a decrease in turbidity removal efficiency.

1.16. pH of the medium

pH plays an important role in determining the HE of microalgae cells. The pH of the solution critically affects the ionization state and surface charge of both microalgal cells and nanoparticles, thereby influencing their interaction and separation efficiency. The change in value can be easily determined by zeta potential measuring at different pH levels (Kumar et al., 2024b; Patel et al., 2022a; Xu et al., 2011). Nanoparticles exhibit maximum harvesting efficacy at different pH levels for different algal species. A study on *Chlorella sorokiniana* Kh12 demonstrated that treating the microalgae with magnetic nanoparticles yielded maximum harvesting efficiency at acidic pH levels (Patel et al., 2022a). Similarly, other studies on various *Chlorella* species also demonstrated optimal harvesting efficiency at acidic pH, though with different doses of magnetite nanoparticles (Bharte and Desai, 2019; Fraga-García et al., 2018; K. Lee et al., 2014). When microalgae were harvested using magnetite nanoparticles combined with PEI, maximum HE was achieved at the culture's native pH for *Chlorella pyrenoidosa, Scenedesmus obliquus* (Y. Liu et al., 2019), *Chlorella ellipsoidea* (Hu et al., 2014), and *Scenedesmus dimorphus* (Ge et al., 2015). Similar results were observed when other cationic polymers, such as PDDA (Liu et al., 2016), PACL (Zhao et al., 2015), and QPP (Wang et al., 2018), were attached to magnetite nanoparticles. These variations are mainly attributed to differences in surface ionization properties of the particles. Moreover, change in pH during the separation process further affect multiple functional parameters. The study indicates that at higher pH levels, both the aggregate size of microalgal cells and the amount of released polysaccharides (RPS) significantly influence flocculation efficiency(Wu et al., 2012). Attachment of a cationic polymer onto the magnetite surface can enhance the overall surface charge of NCs, which further facilitates better binding between microalgae cells and NCs at elevated pH. Furthermore, the study also revealed that in acidic or alkaline conditions, CPAM undergoes significant chain deformation, enhancing the interaction between algal cells and the flocculant. This resulted in increased HE when *C. ellipsoidea* and *B. braunii* were separated using the CPAM-Fe_3O_4 magnetic flocculant. However, this case does not apply to all the attachments to Fe_3O_4. This may depend on other factors discussed in this section. Recent studies showed that attaching dopamine and urea to Fe_3O_4 nanoparticles enhanced the overall net positive surface of the synthesized nanocomposites (as indicated by zeta potential analysis). However, this improvement did not translate into increased HE when applied to *Chlorella sorokiniana* Kh12. Maximum HE was achieved at pH 3 for dopamine (Kumar et al., 2023) attachment and pH 4 for urea (Kumar et al., 2024a, 2024b) attachment.

1.17. Ions in the medium

The ion in the medium is another important parameter affecting the harvesting efficiency of microalgae cells. When comparing freshwater and marine microalgae, salinity is a key factor that affects growth, but its impact differs between these groups. For freshwater species, even slight increases above near-zero salinity impose osmotic and ionic stress, reducing growth rates and biomass, although such stress can occasionally enhance lipid or carotenoid production (von Alvensleben et al., 2016). In contrast, marine and halotolerant microalgae generally achieve maximal growth at intermediate, sea-like salinities (~30–40 PSU), with productivity declining only when salinity deviates beyond their tolerance range (Macías-de la Rosa et al., 2024). Highly halophilic or halotolerant taxa, such as *Dunaliella* and *Tetraselmis*, can sustain growth across a broad salinity spectrum, particularly when changes occur gradually, yet extreme salinities still limit biomass accumulation (Parsy et al., 2022). Overall, salinity acts primarily as a growth-limiting stressor for freshwater microalgae, whereas for marine species, it defines an optimal window that supports high growth and biochemical stability, with deviations outside this range progressively reducing productivity. On a lab scale when freshwater microalgae were grown in two different media—IGV and TAP—and subsequently harvested using silica-coated magnetic beads, higher harvesting efficiency (HE) was observed for cultures grown in IGV medium. This improved performance is largely attributed to the elevated concentration of Mg^{2+} ions in the IGV formulation. At higher pH levels, Mg^{2+} ions undergo hydrolysis and form magnesium hydroxide precipitates, which enhance the flocculation and recovery process. These precipitates can bind effectively to the active sites

on microalgae cells, leading to flocculation and charge neutralization (Wu et al., 2012). Comparable findings were reported for marine microalgae, where cultures grown in ASW medium exhibited markedly higher flocculation efficiency than those cultivated in modified Mann–Myers medium. This improvement was largely driven by the elevated levels of Mg^{2+} and Ca^{2+} ions present in the ASW formulation (Tang et al., 2016). The effect of cationic ions was positive, improving HE upon flocculation in various harvesting methods. Ions in the medium also indirectly influence the HE of microalgae. A study demonstrated that the ionic composition of the medium can markedly influence the surface charge of magnetic beads. For example, microwave-synthesized magnetic particles exhibited a shift from a negative to a positive surface charge when KH_2PO_4 was removed, due to the absence of negatively charged phosphate ions. Furthermore, phosphate ions can bind to particle surfaces via electrostatic attraction, blocking functional sites and subsequently reducing the harvesting efficiency (HE) of microalgae (Prochazkova et al., 2013b). Ions in the medium can also affect the attraction between NPs and microalgae cells. Toh et al. (2014) reported that electrostatic interactions serve as the dominant mechanism governing the attachment between bare iron oxide nanoparticles and *Chlorella sp.* in freshwater systems. Conversely, in marine environments, separation of *Nannochloropsis sp.* using surface-functionalized IONPs was primarily driven by Van der Waals forces and Lewis acid–base interactions (Toh et al., 2014). Therefore, ensuring that competing ions are absent from the culture media during flocculation is crucial. Notably, most ions are consumed during the later stages of microalgae cultivation. However, monitoring ion concentrations in the media provides an added advantage when performing microalgae harvesting with nanoparticles.

1.18. Scale-Up and integration with biorefinery & circular bioeconomy concepts

Scaling nanoparticle-enabled harvesting from laboratory to industrial scale presents both significant opportunities and complex challenges within the context of integrated microalgal biorefineries. At the lab level, magnetic nanoparticles (*e.g.,* Fe_3O_4 functionalized with urea or chitosan) have demonstrated harvesting efficiencies exceeding 95 %, rapid separation, and capacity for nanoparticle reuse, aligning with circular bioeconomy principles (Kumar et al., 2024a). However, when integrated into a full biorefinery chain: cultivation, harvesting, extraction, and valorization of lipids and carotenoids, the transition to pilot or commercial scale necessitates addressing multiple factors. Firstly, reactor design and operation must accommodate high-density cultures and efficient application of magnetic separation in continuous or semi-continuous flow. Incorporating nanoparticle separation into the upstream stage allows for efficient biomass recovery with minimal downtime, but the downstream extraction of high-value products (lipids, astaxanthin) must be tightly coupled to the harvesting step to maintain yield and purity. Secondly, material reuse and nanoparticle recovery are critical: biorefineries must implement onsite magnetic regeneration units, ensure minimal nanoparticle loss, and establish closed-loop processes to avoid environmental release (Wang et al., 2019). Thirdly, integration with other valorization streams (*e.g.,* lipid extraction for biofuel, pigment recovery for nutraceuticals) enhances economic viability. By embedding nanoparticle harvesting within a cascade biorefinery architecture, the system supports multiple product streams and maximizes resource efficiency. Lastly, circular bioeconomy alignment implies that the residual biomass after pigment/lipid extraction can be utilized for feed, fertilizer, or anaerobic digestion, thereby closing the loop on waste and valorizing all fractions (Kumar et al., 2023) The primary scale-up bottlenecks include ensuring uniform nanoparticle distribution in large reactors, optimizing magnet placement for efficient recovery, managing nanoparticle lifecycle, and integrating the process into continuous industrial workflows. Addressing these challenges will determine how effectively lab-scale innovation can be transitioned into a commercially viable and sustainable biorefinery platform.

1.19. Techno-economic and life cycle assessments

A rigorous techno-economic analysis (TEA) and life cycle assessment (LCA) are crucial for evaluating the viability of nanoparticle-enhanced microalgal harvesting in a biorefinery context. Recent works highlight that downstream harvesting alone can account for 25–30 % of total production costs, offering a clear opportunity for cost reduction. For example, Vázquez-Romero et al. (2022) demonstrated that harvesting strategy significantly impacts minimum fuel selling price (MFSP) in algal biofuel scenarios. Meanwhile, global LCA studies have emphasized that microalgal systems often incur high energy and material inputs unless integrated co-product valorization and resource recovery are implemented (Ubando et al., 2022). In the context of nanoparticle-based harvesting, TEA models must account for nanoparticle synthesis costs, functionalization, reuse cycles, energy input to the separation unit, and any disposal or regeneration burdens. For instance, the overall reduction in harvesting costs must outweigh the additional capital or operating expenses associated with nanoparticle regeneration, magnetic separation infrastructure, and potential safety or remediation measures. LCA modeling must incorporate the entire life cycle of the nanocomposites—raw material sourcing, synthesis, functionalization, operational use, reuse cycles, eventual disposal or recycling and compare with baseline harvesting technologies. Key metrics include energy return on investment (EROI), global warming potential (GWP), cumulative energy demand (CED), and unit cost per kilogram biomass or per gram product. A study has shown that algal conversion pathways can achieve fuel GHG reductions of over 50 % when coproducts are valorized, highlighting the need for harvesting innovations to be considered within full-system modeling (Wiatrowski et al., 2022). Sensitivity analysis must identify cost drivers, such as the nanoparticle reuse cycle number, magnet separation efficiency, energy consumption per kg biomass, and the downstream product value (nutraceutical vs. biofuel). Crucially, for commercialization, the nanoparticle-enabled harvest must reduce cost per kg biomass and per unit product, improve energy efficiency, and lower environmental footprint compared to conventional methods. Without integration into the full lifecycle and economic model, the benefits may be overstated. Therefore, future studies should develop standardized TEA/LCA frameworks specific to nano-harvesting in algal biorefineries, enabling robust comparison across configurations and informing design decisions that support the circular bioeconomy and industrial scaling.

1.20. Toxicity, environmental safety, and regulatory concerns

The application of NPs for microalgal harvesting introduces critical concerns regarding toxicity and ecosystem impacts. Recent studies have shown that exposure of microalgae to manufactured nanomaterials (MNMs) can alter gene expression, photosynthesis, nitrogen fixation, and growth rate through mechanisms such as oxidative stress, ion release, membrane disruption, and light-shielding effects (Huang et al., 2022). For instance, the review by Nguyen et al. showed that although many nanoparticles facilitate harvesting, almost all commonly used NPs exhibit algal toxicity under varying conditions. Specific parameters, such as particle size, surface charge, concentration, and the ionic strength of the cultivation media, dictate toxicity outcomes (Nguyen et al., 2020). A critical yet often overlooked factor in techno-economic and life cycle assessments (TE-LCA) of nanoparticle-assisted microalgae harvesting is the long-term stability of organic surface coatings (e.g., chitosan, PEI, PDDA) on magnetic nanocomposites during repeated reuse cycles. Most current studies assume near-complete regeneration efficiency over multiple cycles without validating the durability of functionalization. However, for systems aiming to align with circular economy principles, the degradation or leaching of organic modifiers after 5 or more cycles can significantly increase operational costs, reduce harvesting efficiency, and alter environmental impact profiles due to the need for re-functionalization or disposal. Existing literature on nanocomposite aging suggests that coating integrity often declines after 3–5 cycles, yet this reality is rarely incorporated into TE-LCA models. To ensure realistic sustainability claims and accurate cost projections, future assessments must integrate empirical or literature-based data on coating stability over extended reuse scenarios. In terms of environmental release, nanocomposites used in large-scale biorefineries may leach metal ions or persist in water systems, leading to unintended ecological effects. Consequently, rigorous life cycle and ecotoxicological assessments are imperative. Regulatory frameworks are currently underdeveloped for the use of nanoparticles in algal bioprocessing, raising questions about the standardization of recovery, safe disposal, and reuse protocols. Adoption of biodegradable or green-synthesized nanoparticles (*e.g.,* chitosan, alginate carriers) is proposed to mitigate risk (Garlapati et al., 2025). Without such measures, the sustainability claims of nanoparticle-enabled harvesting remain incomplete.

1.21. Future perspectives

As microalgal biorefineries transition toward commercial maturity, nanoparticle-enabled harvesting emerges as a key innovation pathway; however, its full potential remains contingent on overcoming several interlinked challenges. First, future research must prioritize the development of biodegradable and green-synthesized nanocomposite materials (*e.g.,* chitosan-magnetite, alginate-iron) that maintain high harvesting efficiency while minimizing environmental risk. This aligns with sustainable material design and supports life-cycle circularity. Next, scaling efforts must move beyond static laboratory experiments to continuous-flow or semi-continuous reactor systems where nanoparticle dosing, magnetic separation, and biomass throughput are optimized in real-world conditions. Digital process control and predictive analytics can enhance system stability and productivity. Third, material reuse and recovery cycles must be quantified: achieving ≥ 5 reuse cycles without performance loss will reduce cost per kg biomass and validate nanoparticle economics. Fourth, regulatory frameworks and standardized protocols for nanoparticle release, regeneration, and ecotoxicity testing must be established; collaborative efforts between engineers, ecotoxicologists, and policymakers will be essential. Fifth, integrated biorefinery configurations that combine harvesting with downstream processing (lipid extraction, pigment recovery, co-product valorization, waste valorization) must be demonstrated at pilot scale to validate techno-economic and environmental performance across the value chain. Finally, leveraging circular bioeconomy models means incorporating residual biomass into feed, fertilizer, or bioenergy streams, thereby maximizing revenue and minimizing waste. Real-world implementation should also quantify the carbon-capture potential of the platform, linking microalgal systems with CO_2 streams (*e.g.,* flue gas), and aligning with climate-mitigation goals under SDG 13. With concerted focus on materials innovation, system integration, ecological safety, and process economics, nanoparticle-assisted harvesting can evolve from laboratory promise to industrial reality, enabling large-scale, sustainable microalgal biorefineries that deliver both high-value bioproducts and meaningful environmental benefits.

2. Conclusions

Nanoparticle-assisted harvesting represents a transformative strategy to overcome the economic and technical limitations associated with microalgal biomass recovery. Magnetic nanoparticles functionalized with cationic polymers (e.g., PEI, chitosan, urea, and dopamine) demonstrate exceptionally high harvesting efficiencies (>95–99 %) through electrostatic attraction and bridging interactions, enabling rapid, energy-efficient, and scalable separation processes. Many of these nanomaterials exhibit excellent reusability with minimal performance degradation, significantly enhancing process sustainability. Certain nanoparticles (e.g., NiO and nZVI) further contribute to cell disruption and lipid enhancement, offering opportunities to simplify downstream processing. The uniqueness of this review lies in its integrated and critical synthesis of nanoparticle design, interaction mechanisms, reusability, and downstream compatibility, providing a unified framework that connects harvesting efficiency with biorefinery performance rather than treating them as isolated processes. Despite these advances, challenges remain regarding nanoparticle ecotoxicity, long-term stability, and large-scale integration. Accordingly, future research should prioritize life-cycle assessment, techno-economic analysis, and environmental safety evaluations under realistic operational conditions. This work uniquely highlights the need to transition from proof-of-concept studies toward system-level validation for commercial feasibility. The development of green-synthesized or biodegradable nanocomposites—such as chitosan-, cellulose-, or alginate-based carriers—offers a promising route to mitigate ecological risks while maintaining functional performance. Furthermore, the review introduces hybrid magnetic–photocatalytic nanomaterials as an emerging and underexplored platform capable of integrating harvesting, lipid regulation, and process intensification. Embedding

these advanced nanotechnologies within a circular biorefinery framework can enhance resource efficiency, carbon capture, and economic viability, thereby accelerating the sustainable commercialization of third-generation biofuels.

CRediT authorship contribution statement

Reeta Rani Singhania: Visualization, Validation, Methodology, Conceptualization. **Prashant Kumar:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Zoltán Kónya:** Writing – review & editing, Supervision, Software, Resources, Project administration, Funding acquisition, Formal analysis. **Alfonz Kedves:** Writing – review & editing, Data curation, Conceptualization. **Anil Kumar Patel:** Writing – review & editing, Visualization, Supervision, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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