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The potential use of natural coagulants for microalgae harvesting: a review

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ABSTRACT

Microalgae cultivation has received much interest in foods and biofuel production and provides a significant potential option for cleaning the soil, water, and environment from several contaminants. Accordingly, microalgae harvesting becomes essential to separate the solid–liquid microalgae suspension for other green technologies and sustainable processes. Although several physical, chemical, and physiochemical methods have been widely used for microalgae harvesting, their cost, non-environmental residues, and harvesting efficiencies are still questionable. This review summarized and evaluated the performance of different natural coagulants used for harvesting cultivated microalgae. The operational factors and their effect on harvesting efficiency were discussed. Moreover, the current challenges in utilizing several natural coagulants in microalgae harvesting were considered.

Key words: cultivation, efficiency, harvesting, microalgae, natural coagulants

HIGHLIGHTS

- Microalgae harvesting is a major key to microalgae-based biofuel production.
- Microalgae can be used to separate solid-liquid microalgae suspensrion.
- The optimal removal for microalgae can be obtained at pH ranging between 5 and 9.

1. INTRODUCTION

Fresh water is one of our planet's furthermost essential natural resources, accounting for roughly one-third of its surface area (Wollmann *et al.* 2019); in general, the ocean (salt water) contains more than 96% of all water, whereas the atmosphere, land, and glaciers contain only 3%. However, only 0.5% of the water is suitable for drinking (Abujazar *et al.* 2018a, 2018b).

Rapid urbanization, a growing global population, and various economic activities have exacerbated the world's environmental challenges (Lavrinovičs & Juhna 2017; Ahmad *et al.* 2022). Various organic and inorganic pollutants threaten humans from different wastewater sources, such as industrial and agricultural activities (Koop & van Leeuwen 2017), including sulfur, heavy metals, and high concentrations of nitrates and phosphate (Turner *et al.* 2002; Muñoz *et al.* 2009; Javanbakht *et al.* 2014; Eerkes-Medrano *et al.* 2019; Rezvani *et al.* 2020; Goswami *et al.* 2021). If not handled properly, these cause environmental damage and pose human health problems (Ahmed *et al.* 2022).

This points to the need for effective treatment technologies to reduce the concentrations of organic and inorganic contaminants in wastewater to release into natural bodies (Samhan *et al.* 2017; Ibrahim *et al.* 2020; Abujazar *et al.* 2022). Typical physicochemical methods for wastewater pre-treatment, such as membrane filtering or UV radiation, still suffer from major drawbacks that make them economically unviable and ecologically unsustainable (Cho *et al.* 2011). In order to reduce the nutrient concentration in wastewater, traditional wastewater treatment techniques such as sedimentation, anaerobic digestion, or denitrification and nitrification were used (Kumar & Pal 2015).

In wastewater treatment plants, biological processes or technologies are one of the most critical approaches due to their low cost, environmental friendliness, and long-term efficiency (Crini & Lichtfouse 2019). Biological processes are one of the most important methods due to their low cost, environmental friendliness, and lasting efficiency (Saravanan *et al.* 2021). In biotechnology, microorganisms are used to reduce the amount of (organic or inorganic) toxins in the environment. To remove toxicants from the environment, bioremediation uses a range of microorganisms, including bacteria, fungi, and microalgae (Kouzuma & Watanabe 2015).

Because of its ability to eliminate accessible inorganic chemicals, algae drew much attention (Kube *et al.* 2018). Bacteria consume organic waste and create CO_2 as a byproduct. The microalgae then use this CO_2 to make carbohydrates and O_2 via photosynthesis, resulting in a symbiotic relationship (Molazadeh *et al.* 2019; Aghalari *et al.* 2020), and has been intensively researched in recent decades for its capacity to remove pollution, as well as the biomass created during the process, which may be turned into additional value-added bioproducts (Renuka *et al.* 2013; Shahid *et al.* 2020; Liu *et al.* 2020).

As a result, because it utilizes huge quantities of carbon dioxide (CO_2) in the photosynthesis progression to produce oxygen (O_2) and glucose, microalgae cultivation is a suitable strategy for biofuel production and wastewater purification and treatment for addressing all environmental issues such as global warming and climate change. Algae can adapt to any environment or scenario. As a result, it may be created in open ponds, closed ponds, photobioreactors (PBRs), marine habitats, and wastewater. It is influenced by several variables, such as abiotic and biotic factors and design and operational parameters (Ginzburg 1993).

While producing microalgae is a realistic alternative, extracting microalgae biomass from its growth environment is a costly procedure. Harvesting costs 20–30% of the cost of producing microalgae biomass (Liu *et al.* 2017); hence, optimizing the harvesting process is crucial. Harvesting can be done through different means (physical, chemical, or biological) (Milledge & Heaven 2013; Difusa *et al.* 2015), and the method used should ensure that both biomass and wastewater are gathered for future use. 'Centrifugation, filtration, flotation, bio-flocculation, electrocoagulation, flocculation, and sedimentation' are the most often utilized procedures (Christenson & Sims 2011; Kucmanová & Gerulová 2019; Lam *et al.* 2019). The selected method should aim for low prices, low energy requirements, simple processes, and broad application to a wide range of species (Mata *et al.* 2010).

Coagulation–flocculation, tracked by gravity sedimentation, is the utmost common method for collecting microalgae. Chemical coagulants (aluminum or iron salts) are more widely used and initially less expensive (Papazi *et al.* 2010). However, they are non-biodegradable, can induce cell structure damage, contaminate biomass and growth medium, and limit the utility of associated products (Singh & Patidar 2018). Natural coagulants outperform chemicals as they are nontoxic, produce fewer sludge, do not destroy the biomass with metals, and ensure the effluent generated is safe to use (Fuad *et al.* 2018).

This study outlined the most recent facts on microalgae growing systems and related variables. Natural coagulants' uses for grown microalgae, harvesting, and performance for cultivating microalgae were summarized and assessed. Furthermore, the present obstacles in collecting microalgae using several forms of natural coagulants were explored. Furthermore, the economic and environmental consequences of employing natural coagulants in microalgae harvesting were evaluated.

2. MICROALGAE CULTIVATION SYSTEMS

Microalgae have evolved to scavenge for nutrients in their habitats, store them, and improve their resource use efficiency. Microalgae generally require an appropriate carbon supply and light to carry out photosynthesis to generate biomass (40–50% carbon) (Khan *et al.* 2018).

Different systems exist for the application of algae culture, which may be grouped into open and closed systems. Open ponds, such as high-rate algal ponds (HRAPs), algal turf scrubbers (ATS), and other non-closed cultivation systems, are examples of open systems. Closed systems include any form of PBRs, such as tubes, plates, helical, and plastic bags, which allow for autotrophic culture and fermenters to allow for heterotrophic or mixotrophic cultivation (Sonnleitner *et al.* 2020).

Another method to describe the various micro-algal culture strategies is as either suspended or immobilized systems (Christenson & Sims 2011). HRAPs and PBRs include suspended cultures. Matrix-immobilized or biofilm systems contain immobilized cultures (e.g., ATS). Due to their high cost, matrix-immobilized systems are unsuitable for low-tech applications. The bioreactor's economic cost is the most critical performance aspect, with examples of micro-algae wastewater treatment systems including (but not limited to) PBR, HRAP, matrix-immobilized micro-algae, and linked micro-algal biofilm systems (Mohsenpour *et al.* 2021). Each of these systems offers various pros and cons as shown in Table 1.

Table 1 | Pros and cons of suspended- and immobilized-cell culture methods in wastewater treatment procedures

Cultivation system	Pros	Cons
Suspended-cell cultivation systems	• Thoroughly researched and improved. • Larger volumes of wastewater can be treated. • Large-scale operations are possible.	• Microalgal harvesting is essential prior to the release of treated wastewater.
Immobilized-cell cultivation systems	• Simpler to harvest microalgae prior to releasing treated wastewater. • Aging cultures are more resistant to photoinhibition. • The immobilization matrix gives cells greater tolerance to adverse conditions, including salinity, metal toxicity, and pH.	• Expensive expenditures related with the polymeric matrix (in the case of cell entrapment). • Requires a large surface area (in the case of microalgal adhesion and biofilm formation). • Light restrictions may arise. • Only applicable for small and pilot-scale activities.

2.1. Suspended-cell cultivation systems

Suspended-cell cultivation systems are a typical strategy for a microalgae cultivation system in traditional microalgal production. Suspended culture systems, one of the most often used algal growth techniques for wastewater treatment, allow cells to move freely in the aqueous phase. Culture of suspended-cell microalgal growth in suspension is commonly accomplished in closed or open ponds (Singh & Sharma 2012; Mathew *et al.* 2022). Figure 1 shows the open and closed ponds for microalgae cultivation.

2.1.1. Opened ponds

Suspended-cell culture systems are often grown in open ponds, which are less expensive to build, maintain, and scale-up than closed systems (Acién Fernández *et al.* 2013). In general, the pond is arranged in a racetrack or track form ('channel length: 25 m; width: 2 m; total surface area: 100 m^2; depth: 0.25 m'), with a paddlewheel that circulates and mixes the algae cells and nutrients (Béchet *et al.* 2017), and mechanical paddles mixing the biomass. Many other designs for open pond systems have arisen, but three primary varieties have succeeded and are currently used on a commercial scale: raceway ponds, circular ponds, and unstirred ponds (Murthy 2011; Costa & de Morais 2013).

Ponds are more susceptible to weather conditions even though they lack control over the temperature of the water, evaporation, and illumination. Furthermore, while they may generate a significant number of microalgae, they take up more land and are more vulnerable to contamination from mostly microalgae or bacteria. Furthermore, because the air contains only 0.03–0.06% of CO_2, mass transfer constraint is likely to slow down microalgae cell development as the low amount of

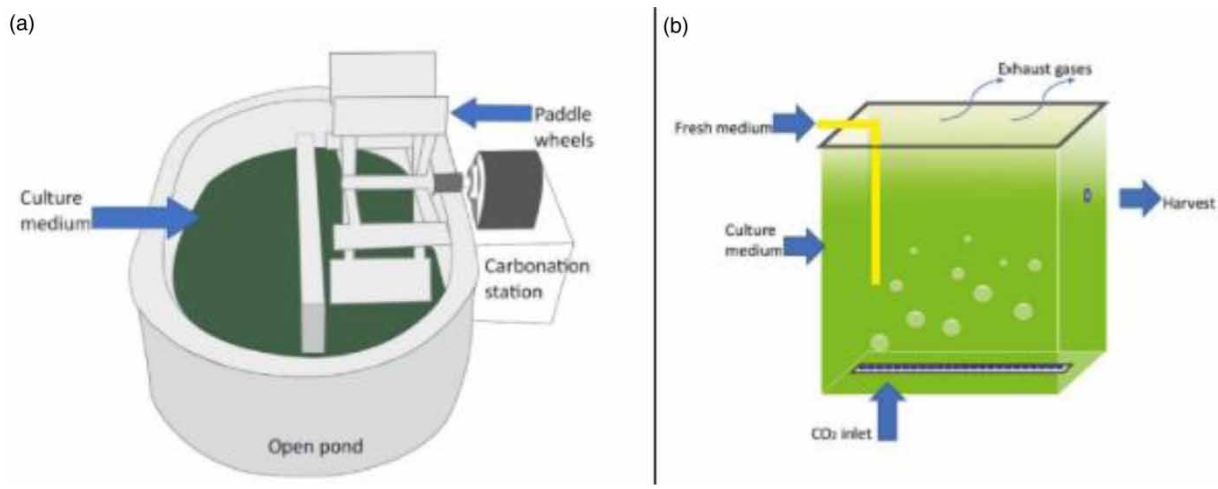

Figure 1 | (a) Open pond and (b) closed pond.

CO_2 can be taken from the air (Mata *et al.* 2010). As a result, the main disadvantages of open ponds include the difficulty of controlling microbial contaminations and the difficulty of maintaining constant microalgal growth, as well as other parameters including temperature, pH, and light; additionally, low productivity (10–20 $g \cdot m^{-2} \cdot d^{-1}$) due to poor gas exchange and the dark zone are the major disadvantages of open bioreactors (Shen *et al.* 2009; Handler *et al.* 2012).

2.1.2. Closed ponds

Microbial cells grown in a tube or flask of liquid medium constitute a closed system (vertical, horizontal, and helical designs are widespread; however, helical designs are regarded as the simplest to scale-up). Closed systems, also known as PBRs, limit direct gas exchange and contamination between the system and the environment. Over the years, the supreme often used PBRs have been flat-plate reactors, bubble-column reactors, and tubular reactors (Paul *et al.* 2021).

Closed PBRs have advantages over open ponds for microalgal growth in terms of culture conditions and growth parameters such as pH, temperature, mixing, CO_2, and O_2 levels, evaporation and contaminations can be easily avoided, and increasing cell intensities with less land area requirement (Perez-Garcia *et al.* 2011; Singh & Sharma 2012). Despite these advantages, PBRs have several disadvantages, including overheating, scaling difficulties, and increasing construction costs (Singh & Sharma 2012). Table 2 compares open and closed large-scale microalgae growing methods.

Table 2 | Comparison between open and closed algal cultivation systems

Culture systems for microalgae	Open systems (raceway ponds)	Closed systems (PBRs)
Contamination control	Hard	Simple
Contamination risk	Exceptionally high	Less
Space required	More	Less
Water losses	Exceptionally large	None
CO_2 losses	More	None
Temperature	Highly variable	Required cooling
Temperature control	Difficult	More uniform temperature
Sterility	None	Achievable
Process control	Difficult	Easy
Mixing	Uniform	Very poor
Operation regime	Batch or semi-continuous	Batch or semi-continuous
Weather dependence	Insignificant, because closed layouts allow productivity even when the weather is severe	Absolute, production impossible during rain
Area/volume ratio	Low (5–10/m)	Large (20–200/m)
Efficiency of treatment process	Low, time-consuming, lower mass per volume	Comparatively higher mass per volume
Gas transfer control	Less	Large
Algal species	Restricted	Flexible
Biomass quality	Variable	Reproducible
Shear	Less	Large
Population density	Less	Large
Harvesting efficiency	Less	Large
Harvesting cost	High	Lower
Light utilization efficiency	Poor	Good
Most costly parameters	Mixing	O_2 and temperature control
Energy requirement	High	Lower
Scale-up	Difficult	Difficult
Capital costs	Low	High
Operation costs	Low	High
Cleaning	None	Required

While open ponds and closed PBRs may produce microalgae using sun radiation or artificial lighting, closed PBRs give better light control than open ones. In lab-scale PBRs, controlling light wavelength and intensity is more accessible than in industrial settings. A lab-scale PBR is employed to establish ideal conditions for large-scale testing models. Correlated light penetration, which reduces exponentially with distance from the light source, is one of the barriers to the scalability of these systems, offering a formidable technical challenge for scaling PBR systems (Bosma *et al.* 2007; Xu *et al.* 2009).

When comparing open and closed systems, the selection between them is strongly dependent on the user's conditions; nonetheless, it should be evident that PBRs are the most practical option for research and improved knowledge of the behavior of a microalgae culture.

2.2. Immobilized-cell cultivation systems

Immobilization culture systems (matrix-immobilized or biofilm systems) have emerged as a viable alternative to suspended-cell cultivation methods for accomplishing both processing goals: metabolic conversion of wastewater components and simple and cost-effective biomass harvesting (Lam & Lee 2012).

An immobilized cell is a biological cell that is stopped from migrating independently from its initial location to all areas of a system's aqueous phase, either passively (naturally) or purposefully (artificially). Except for light transmission, while immobilizing live cells, most immobilization techniques developed for microorganisms, in general, may be used for microalgae. Immobilization techniques are classified as 'passive' or 'active'. Passive or spontaneous immobility occurs due to the inherent inclination of microalgal cells to attach to a specific surface, resulting in biofilm development (Mallick 2002). Natural adsorbent materials (carriers) for passive immobilization are loofa (*Luffa cylindrica*) and sponge materials (carriers). There have been efforts to use loofa biomass as a natural carrier (Akhtar *et al.* 2004).

In contrast, active or artificial immobility therapies include flocculant agents, chemical attachment, and gel encapsulation (Mallick 2002). When removing algae from a liquid medium, flocculant agents such as chitosan were commonly used to reduce the time and money spent on centrifugation (Xu *et al.* 2009). Because chemical contact affects the cellular surface and dramatically lowers cell viability, it is not suggested when living cells are to be immobilized, typically by covalent bonding, cross-linking – using glutaraldehyde, for example – or photocrosslinkable resins. The efficiency of ion attraction is affected by the pH and ionic strength of the surrounding media (Kaparapu 2017; Crini *et al.* 2018).

Gel entrapment is the most often utilized approach for algal immobilization. It can be accomplished using synthetic polymers, proteins, or natural polysaccharides. In wastewater treatment, microalgae are immobilized. In polymeric immobilization systems, like in other biofiltration systems, physical separation occurs between the microbes and the treated effluent (Moreno-Garrido 2008). Because the polymer's holes are smaller than the microbes' pores, they are immobilized (trapped) alive within it as fluid flows through it, feeding their metabolism and eventual development. Immobilization in polymers is critical in wastewater treatment because it eliminates the problem of floating microalgae biomass in wastewater (Kaparapu 2017).

3. FACTORS AFFECTING THE CULTIVATION OF MICROALGAE

A diversity of factors impacts wastewater-based microalgae production. The efficiency of these organisms' development is based on controlling important variables, including abiotic and biotic factors and design and operating parameters. However, determining which element impacts algal development is challenging since all of these factors may influence algal growth concurrently (Mata *et al.* 2010; Gatamaneni *et al.* 2018; Zhuang *et al.* 2018).

3.1. Abiotic factor

Numerous abiotic parameters depending on wastewater treatment might impact microalgae development. Light, pH, nutrient content, temperature, salinity, 'O_2', and 'CO_2' are the essential abiotic elements that promote microalgae development.

3.1.1. Light

The light spectrum and intensity are vital variables that directly affect the enhancement of photoautotrophic microalgae. Light supply from inside 'sunlight', which is preferable in terms of operation cost, and outside 'artificial light'. Because it is the energy source used to convert inorganic carbon, usually CO_2, into organic carbon, it has high-power consumption and running cost. It promotes the photoautotrophic development of microalgae. Photosynthetic activity is proportional to light irradiance below the light saturation limit (Chen *et al.* 2011; González-Camejo *et al.* 2018).

However, high levels of light irradiance, which vary according to the microalgal, because it provides the energy needed to transform inorganic carbon, primarily CO_2, into organic carbon, have high-power consumption and operating cost. It stimulates the photoautotrophic growth of microalgae. Photosynthetic activity is proportional to light irradiance under the light saturation limit. Photosynthesis and hence microalgal growth are inhibited by the photosynthetic receptor system (Chen *et al.* 2011; Cheirsilp & Torpee 2012). Microalgae photoinhibition and saturation irradiance depend not simply on the quantity of light available in their habitats; other environmental characteristics, such as temperature, CO_2 accessibility, and salinity, water, and nutrients, all have a role. The photoinhibition and saturation irradiance levels should be modified before pilot-scale experiments with various microalgal strains (Bohutskyi *et al.* 2016).

Improving light efficacy and boosting microalgal growth at a lesser cost could be a major point toward building a successful microalgae production technology. Solar radiation is the most cost-effective alternative energy source of all current light sources (Kumar *et al.* 2015; Abujazar *et al.* 2018a, 2018b).

Because of enhanced light transmission or CO_2 transfer, microalgae in linked culture systems may be more productive (Gao *et al.* 2015; Huang *et al.* 2016). Because the microalgae cells were well-organized in biofilm rather than scattered in the culture broth, light penetration to the bottom was much higher in an immersed vertically connected microalgal growth system than in a suspended system (Lee *et al.* 2014; Kim *et al.* 2015). Consequently, even at the bottom of the culture system, the microalgae in the connected culture system could obtain ample light for photosynthesis and growth (191–354 mol photon m^2 s).

The number of light increases as it goes further in the linked microalgal growth system. Improving light effectiveness and increasing microalgal growth rate at a lower cost would be a significant step toward developing a viable microalgae production system. Solar radiation is the most cost-effective alternative energy source currently available. The upper layer algae in suspended culture systems are light-saturated, whereas the lower layer algae is light-deprived. As a result, higher microalgae output may be attained in the related culture system.

Several studies (Gross *et al.* 2013; Blanken *et al.* 2014; Al-Dahhan *et al.* 2018) have demonstrated that light intensity influences microalgae cell acclimatization in suspended and connected systems. After 18 days of cultivation, algal biomass in the linked system reached 130 g m^2, but only 60 g m^2 in the suspended system (blank control group). In another non-immersed vertical attached microalgae culture approach, *braunii* biomass production was 5.7 g m^2 d^1, showing a 150% increase over the suspended culture strategy (Liu *et al.* 2013; Rani *et al.* 2021).

3.1.2. pH

The pH of a body of water measures its acidity or alkalinity. The neutral to alkaline pH range is appropriate for most algal growth and photosynthesis (Yu *et al.* 2022). Algae can, however, be grown in pH ranging from 7 to 9 (Berberoglu *et al.* 2008). However, certain species may be able to endure severe circumstances in more acidic or basic environments (Mahmoud *et al.* 2016). Algae can, however, be grown in pH ranges from 7 to 9 (Airport & Free 2016). However, certain species may be able to endure severe circumstances in more acidic or basic environments. To avoid the culture breaking down due to the breakdown of cellular activity and low pH, it is vital to keep the pH of the culture at an appropriate range. Increased pH and dissolved O_2 concentrations in microalgae cultures can harm bacterial activity (Posadas *et al.* 2015). Suppose inorganic carbon is not provided at the same rate as consumed. In that case, the assimilation of microalgae can raise the pH of the medium, resulting in an alkaline environment (pH >9) (Mohsenpour *et al.* 2021). High pH in algal ponds also aids pathogen disinfection. The cyanobacterium *Anabaena* variants produced the most at pH 8.2–8.4, somewhat less at pH 7.4–7.8, substantially less above pH 9, and cells could not grow efficiently at pH 9.7–9.9 (Airport & Free 2016).

The pH of the soil influences development in a variety of ways. pH rises during the day owing to photosynthetic CO_2 uptake by algae, then falls at night due to the community's respiratory mechanism (Yousuf 2019). The content of inorganic carbon and the pH of microalgal culture media are related. When photosynthesis occurs, at ideal pH, the algal enzyme carbonic anhydrase converts the bicarbonate in the medium into CO_2, forming hydroxyl ions, which tends to raise the pH. Increasing CO_2 levels can result in more biomass production but also lower pH, which is detrimental to microalgal physiology. Photosynthesis may be inhibited if the pH increases too high due to a shortage of CO_2 (Yousuf 2019).

3.1.3. Nutrients concentration

Algae are photosynthetic organisms that require nitrogen, sulfur, carbon, trace metals, and phosphorus in macronutrients when grown under autotrophic conditions. Carbon fixation is required to create a balanced media for optimal development

(Larsdotter 2006; Al Darmaki *et al.* 2012). Other micronutrients, in addition to nitrogen and phosphorus, are necessary for the development of microalgae. Wastewater contains practically all types of micronutrients; hence, it aids in the growth of microalgae (Airport & Free 2016).

Nitrogen is a necessary macronutrient for algae development. Nitrogen can be obtained from chemical fertilizers or other waste sources such as municipal wastewater. The production of urea and other nitrogen fertilizers requires a lot of energy and natural gas (Murthy 2011).

The mix of nitrogen and phosphorus is essential for lipid formation. According to several studies, a nitrogen-deficient growing media allows algae to produce more lipids. Microalgae can exhibit physiological and morphological abnormalities due to nutritional deficiencies and excess nutrients, which can hamper critical metabolic activities. Recently, scientists have concentrated on a two-phase growth system, in which algae are grown in a nutrient-rich medium in the first phase and then moved to a nutrient-deficient medium where lipid synthesis is increased in the second phase (Gatamaneni *et al.* 2018).

A lack of nutrients results in adverse conditions within the cell (Arguelles *et al.* 2018). Cell development in a culture medium is connected to a high concentration of nutrients in the culture, especially in the early stages of cell growth; hence, a rich media optimizes biomass output. After obtaining the required biomass, the dietary shortage can induce a stressful environment and a rise in lipid synthesis, which is especially noticeable in the late development phases (Praveenkumar *et al.* 2012; Alishah Aratboni *et al.* 2019).

3.1.4. Temperature

The average global temperature is quickly rising due to gaseous imbalances caused by human activity; as a result, the world experiences a greenhouse effect. The global average sea surface temperature is anticipated to rise by 1.40 –5.80 °C (Tait & Schiel 2013). After light, the temperature is the most crucial limiting factor for algal development in closed and open outdoor systems toward the end of the twenty-first century. Temperature is proportional to the quantity of sunlight available and has little effect when light is sparse. When light availability is not a constraint, increasing the temperature can enhance the rate of photosynthesis, resulting in higher growth/doubling rates (Muñoz & Guieysse 2006).

Numerous microalgae could endure temperature up to 150 °C under their ideal; however, temperature increases of 2–40 °C can result in complete colony loss. During warmer days, the temperature within the reactor can exceed 550 °C, which can cause overheating difficulties in closed culture systems. In this situation, evaporative water-cooling devices can be employed to reduce the temperature to roughly 20–300 °C for maximum microalgae development; however, this varies depending on the species (Muñoz & Guieysse 2006; Mata *et al.* 2010).

The influence of temperature variations on other abiotic factors such as pH, wastewater characteristics, gas exchange, and microalgal diversity should not be overlooked (Park *et al.* 2011). Temperature swings have a considerable effect on algal biofilm PBRs. Temperature control is critical for establishing appropriate microbial communities in a wastewater-based algal biofilm system (Muñoz & Guieysse 2006). When adopting an open reactor culture technique, temperature management is complex since seasonal oscillations generate substantial swings in temperature (700–2,500 °C) operated HRAPs.

Consequently, open systems are recommended in places with consistent temperatures throughout the year (Singh & Singh 2015). Because microalgae are prone to photoinhibition at winter light intensities, they are limited in low-temperature open reactors, particularly in cold-weather countries. Temperature regulation is more accessible in a closed system. Closed systems with temperatures ranging from 200 to 300 °C correlate to the temperature range that enhances growth rate and nutrient clearance, making them a desirable option for cold climates (García *et al.* 2000). On warmer days, the temperature within the reactor can exceed 550 °C, producing overheating issues in closed culture systems. Evaporative water-cooling systems can be employed in this scenario to reduce the temperature to roughly 20–260 °C.

3.1.5. Salinity

In both open and closed environments, salinity can impact microalgae's growth and cell composition. Every alga has a unique optimal salinity range, which increases in warmer weather due to increased evaporation (Mata *et al.* 2010). The optimal amounts differ depending on the microalgal species. Because of osmotic stress, ion (salt) stress, and changes in membrane permeability to ions, variations in the salinity of the culture medium may affect microalgal growth and composition. Evaporative losses and rainfalls are the principal sources of variations in culture medium salinity (in open systems) (Hu 2013).

Stress from high salt concentrations affects cell development and lipid synthesis. Low salinity boosted biomass output, hydrocarbon, fat, carbohydrate, and carotenoids. Microalgae respond to environmental salinity and osmotic stress by

accumulating small molecule components for osmoregulation (Henry 2004; Rao *et al.* 2007). According to another study, increased salinity induces a modest rise in the overall lipid content of algae. However, excessive salinity is detrimental to development because salt stress encourages microalgae to form colonies during growth, blocks photosynthesis, and lowers the growth rate (Hu 2013).

They determined that *Chlorella ellipsoidea* grows best in low temperatures (15 and 2,000 °C) and low salinity (10 and 20). High temperatures (25 and 3,000 °C) and salinity (30) were, on the other hand, ideal circumstances for *Nannochloris oculata* growth. Temperature and salinity were ideal for *C. ellipsoidea* density at 150 °C and 10, respectively. However, for *N. oculata*, the ideal temperature and salinity parameters were 250 °C and 10 for specific growth rate (SGR), and 250 °C and 30 for maximum density (Cho *et al.* 2007).

3.1.6. Dissolved O_2

Microalgae produce dissolved O_2 during photosynthesis, which bacteria use to break down and oxidize waste. This results in releasing CO_2, phosphorus, nitrogen, and other nutrients algae require. This interaction between bacteria and algae results in wastewater purification and absorption and the storage of nutrients and CO_2 in organic biomass (Sonnleitner *et al.* 2020).

O_2 supersaturation can reach 4–5 times that of air saturation in closed PBRs and open reactor top water layers. Many algal species' development is hampered at O_2 concentrations of more than 20 mg/L, which is toxic or damaging to microalgae activities. O_2 concentrations of 29 mg/L can limit algae photosynthetic capacity by 98%. As a result, one of the key restrictions for closed growth systems is O_2 accumulation in microalgae cultures. The concentration of dissolved O_2 (DO) drops as the mixing intensity and CO_2 supply increase (Posadas *et al.* 2017).

When using impellers or airlifts to mix gas bubbles in closed PBRs, the speed of the bubbles increases their diameter (Ugwu *et al.* 2008) – the bigger the bubbles, the lower the gas–liquid exchanges. Photosynthesis produces a high concentration of O_2, which inhibits microalgal growth. One strategy to offset this adverse effect is to pump gas into closed PBRs with a turbulent labor regime. Excessive turbulence, on the other hand, depending on the microalgal species, might induce cell damage owing to stress and high energy consumption (Pires *et al.* 2012). Low mixing causes hazardous chemicals to accumulate in stagnant regions. Because PBRs are low in height, O_2 has limited solubility and quick outflow in open ponds.

During the day, robust photosynthesis in HRAPs can raise DO levels in the pond or pool water to more than 200% saturation (Molina *et al.* 2001; Park & Craggs 2010). High DO levels above typical air saturation influence algal growth (Molina *et al.* 2001). According to one study, photosynthetic activity was lowered by 17–25% at 200–300% DO saturation when assessed as O_2 generation rate under steady-state algal biomass concentration. More study is needed to discover how high O_2 levels affect algal proliferation in HRAPs used for outdoor wastewater treatment (Molina *et al.* 2001).

3.1.7. Carbon dioxide

Today, burning fossil fuels emits and concentrates greenhouse gases into our atmosphere, accounting for more than 80% of worldwide energy consumption. CO_2 levels in the atmosphere have risen from 260 to 380 ppm in recent decades (Salih 2011; Minillo *et al.* 2013). The increased atmospheric level of CO_2 is now universally accepted as a significant contributor to global warming (Shen 2014). Carbon capture and biological sequestration are considered safe for reducing environmental CO_2. Microalgae perform photosynthesis and release O_2 into the atmosphere using CO_2 and sunlight. Microalgae use CO_2 from their surroundings as a carbon source and exhale O_2 during the photosynthetic process (Shen 2014).

The microalgal species chosen is crucial for constructing successful biological CO_2 systems, and the microalgal species employed is determined by the carbon sequestration approach. CO_2 concentration in the atmosphere substantially influences microalgae development; the higher the CO_2 concentration, the better the growth (Khairy *et al.* 2014). Research that looked at the effect of different CO_2 concentrations on the growth of *Chlorella gracilis* revealed an increase in cell number up to 385 ppm (control, 280, 385, 550, 750, and 1,050 atm), followed by a decline at 550 ppm because the microalga was not CO_2 tolerant above this level (Khairy *et al.* 2014). The development of *Chlorella vulgaris* ARC1 was studied at different CO_2 concentrations ranging from 350 to 200,000 ppm (0.036–20%) (Chinnasamy *et al.* 2009).

3.2. Biotic factors

Diseases such as bacteria, fungi, and virus, as well as competing from several other microalgae, are examples of biotic factors, which may be described as any other living creature that influences microalgae development or changes wastewater ecosystems.

3.2.1. Pathogens 'bacteria, fungi, viruses'

The most prevalent biological contaminants detected are unwanted algae, mould, yeast, fungus, and bacteria. Bacteria and fungi are unavoidable in a microalgal wastewater treatment system. Attempts to cultivate several microalgae species in raceway ponds were unsuccessful due to protozoa predation and contamination by other algal species. Following the removal of the undesirable organism, the closed environment, greater control over growth conditions, and higher cell concentration produced in closed cultivation reactors protect the culture from contamination and allow for the production of a wide range of critical microalgae (Ferrero *et al.* 2012; Posadas *et al.* 2014).

The bulk of wastewater comprises bacteria (both pathogenic and nonpathogenic), viruses, fungi, algae, lichens, parasites (protozoa and helminths), rotifers, and zooplanktons. Microalgae culture and biomass output are directly affected by the symbiotic relationship between microalgae and other microorganisms present in wastewater (Ruiz-Martinez *et al.* 2012; Solovchenko *et al.* 2016; Kube *et al.* 2018). A few microalgal species exhibit antibiotic action, which may impede bacterial growth. Bacteria have an influence on algal development that is both stimulating and inhibitory. Bacteria have a significant impact on algal biomass growth and nutrient removal. Bacteria and algae battle for life due to nutrient limitations (Hancock *et al.* 2010).

The optimal algal species must be picked to remediate wastewater. The algae species were mainly chosen for their capacity to extract nitrogen and phosphorus from wastewater and their tolerance to various wastewater. With a few exceptions, such as *C. vulgaris*, which has a negative impact on starvation, to improve the rate of nutrient ejection, before being exposed to wastewater, algal cells are starved. Bacteria have an influence on algal proliferation that is both stimulating and inhibitory (Ruiz-Martinez *et al.* 2012). Bacteria play an essential role in algal biomass production and nutrient reduction. Bacteria and algae compete for life due to the restricted supply of nutrients. Some bacteria release enzymes such as glucosidases, chitinases, and cellulases that break down algae cell walls, resulting in algal lysis. Following algal cell lysis, bacteria utilize the intracellular chemicals of algae (Hancock *et al.* 2010).

Viruses are microorganisms that can only multiply in live hosts; they are significantly smaller in size (20–300 nm) than bacteria. Viruses replicating in bacterial hosts are called bacteriophages, but viruses replicating in algae and fungi are called cyanophages and mycophages, respectively. Pathogenic virus strains discovered in wastewater are highly infectious, antibiotic-resistant, and exceedingly dangerous to human health (Gómez *et al.* 2006). The majority of wastewater contains enteric viruses originating from feces. When these viruses are present, they may inhibit algal growth in ponds. Viruses do this by fundamentally altering cell structure, after a few days of infection, algal growth plummets. Unlike bacterial pathogens, viruses cannot be identified and quantified using culture-based methods (Rani *et al.* 2021).

3.3. Operation parameter factors

Operating variables like depth, hydraulic residence duration, and mixing can all impact microalgae development (Kumar *et al.* 2010; Gonçalves *et al.* 2017).

3.3.1. Depth

Algal pools are commonly built to maximize the extent of light available to the cultures. Depths ranging from 15 to 50 cm are commonly advised. On the other hand, depths smaller than 20 cm should not be utilized to account for the reduced incident light strength in the winter. For example, the depth of algal pools or ponds is precisely controlled to provide optimal light intensity for algae formation. Mixing allows suspended algal cells from crossing the pond depth more often and minimizes the amount of time, and the individual cell must spend suffering from light inhibition on the pond surface or a lack of light at the pond bottom (Sutherland *et al.* 2014).

Sequence, every cell in the pond has a better light profile. Filamentous algae have a different light intensity profile than microalgae because they are multicellular and can form floating or connected mats rather than being evenly suspended. In macroalgae systems, the distribution of cells and the self-shading regime can vary substantially depending on their physical behavior. As a result, the sensitivity of filamentous algae to these circumstances is crucial for outdoor cultivation and wastewater treatment (Sutherland *et al.* 2014).

As a result, all cells in the pond have a superior light profile. Because filamentous algae are multicellular and can form floating or linked mats rather than being equally suspended, their light intensity profile may differ from microalgae. The distribution of cells and the self-shading regime in macroalgae systems can vary greatly depending on their physical behavior. As

a result, the sensitivity of filamentous algae to these factors is crucial for outdoor culture and wastewater treatment (Larsdotter 2006; Liu *et al.* 2020).

3.3.2. Hydraulic residence time and harvesting frequency

Hydrostatic residence time (HRT) and biomass concentration can also impact algal production and nitrogen removal effectiveness (Sutherland *et al.* 2014). The biomass must be gathered after the microalgae have matured. There are two types of microalgae harvesting methods, namely thickening (centrifugation and filtering) and bulk harvesting (flocculation, flotation/gravity sedimentation) (Brennan & Owende 2010). In wastewater treatment systems incorporating secondary settling tanks, gravity thickening, filtration, and a secondary clarifier for separating sludge and water, an efficient, low-cost method must be used to gather biomass while producing microalgae (Kumar *et al.* 2011).

An appropriate harvesting process may consist of one or more phases. It can be carried out using a range of physical, chemical, or biological methods to produce the necessary solid–liquid separation to remove significant amounts of water and treat huge algal biomass volumes. While there is no uniform harvesting method, experience has demonstrated that this is still an active field of study, with the possibility of designing an appropriate and cost-effective harvesting technology for each algae species (Molina Grima *et al.* 2003).

Sedimentation, centrifugation, filtering, and ultrafiltration are the most popular harvesting methods, frequently with an additional flocculation stage or a combination of flocculation and flocculation. Flocculation is a method of aggregating microalgal cells to increase effective particle size and hence help sedimentation, centrifugation recovery, and filtering (Molina Grima *et al.* 2003).

3.3.3. Mixing

Mixing is a crucial growth characteristic because it homogenizes cell distribution, heat, and metabolites and enables gas movement. Furthermore, mixing is important for the system's gas balance and pH. Turbulence reduces the presence of gradients in microalgae cells, which can impede cell activity. As a result, mixing reduces the culture's nutritional gradient to avoid system cell sedimentation. It is critical for preventing algal deposition and transferring algae between the bright and dark sections of the pond/reactor. Without forceful mixing, algae on the surface absorb all available light and may become photo-inhibited, while algae deeper in the medium are deprived of light (Costa *et al.* 2019).

Whatever mixing mechanism is used, the provided energy has a cost that must be minimized. Furthermore, excessive mixing may cause cell damage, resulting in a decrease in culture growth. If microalgae are susceptible to hydrodynamic and mechanical shear stresses, aeration and agitation by pneumatic and mechanical devices may induce cell damage, reducing culture efficacy (Barbosa *et al.* 2004). The presence of fragile flagellate determines shear sensitivity, the composition and thickness of the cell wall, the strength and character of the shear stress, and the adequacy of the culture conditions to which the cells are subjected (pH, temperature, and irradiance) (Alías *et al.* 2004). Shear rates in single-phase and multi-phase flow have been extensively studied (Deb *et al.* 2012).

A study that examined the impact of mixing on *Spirulina platensis* in three different ways (using a magnetic agitator within the column, bubbling air into the column, and recirculating through a pump) discovered that utilizing a bubble column resulted in the most microalga formation. However, highly similar values for stirring and mixing (0.0122, 0.009, and 0.010/h) were found (Ravelonandro *et al.* 2011). A study of the impact of mixing (that used a shaker) on *Desmosomes communis* discovered that mixing significantly increased the microalga's production and yield (Vanags *et al.* 2015).

Sánchez *et al.* (2013) discussed the daily growth *of Isochrysis galbana* microalgae culture in raceway culture systems stirred by paddles was tripled compared to the system without stirring (8.8 105 versus 4.0 105 cells $mL^{-1}\,d^{-1}$); this highlights the need of stirring the culture medium during industrial microalgae production operations.

4. APPLICATION OF NATURAL COAGULANTS FOR MICROALGAE HARVESTING

The use of natural coagulants for harvesting microalgae establishes their potential to complement conventional flocculants under optimal conditions. In this section, the application of natural coagulants for microalgae harvesting are presented.

4.1. Tannin-based natural coagulant

Tannins have recently gained popularity as a natural coagulant in water and wastewater treatment due to their effectiveness in removing organic, inorganic, and heavy metals (Banch *et al.* 2019). The polymeric structure of tannin has been taken from various species, including *Acacia*, *Castanea*, and *Schinopsis* (Acosta-Ferreira *et al.* 2020). Roux *et al.* investigate the chemical

and physical characteristics of tannins extracted spontaneously. Adding aliphatic hydroxyl groups and substituting phenolic building blocks improved the reactivity of natural tannin (Arbenz & Avérous 2015; Tondi 2017; Acosta-Ferreira *et al.* 2020). Its natural polyelectrolytes were examined for their ability to enhance flocculation efficiency in removing suspended particles and heavy metals from contaminated water (Özacar & Şengil 2000).

Wang *et al.* (2013) reported 90% removal efficiency for *Microcystis aeruginosa* from water by utilizing a tannin dosage of 20 mg/L. Mezzari *et al.* (2014) utilized tannin for *C. vulgaris* harvesting, which grew within a PBR of wastewater digestate. In this study, the performance of natural organic and modified tannin with polyacrylamide to harvest *C. vulgaris* biomass was evaluated and reported with 95% using 165 mg/L of tannin at pH 5. Several applications of tannin used for microalgae harvesting are summarized in Table 3. Tannin reported significant efficiencies (90–99%) for microalgae harvesting in different types of cultivation with different pH levels. Because of the neutral electric charge, the particles' adsorption capabilities will be strong at pH levels ranging from 7 to 9 (Xia *et al.* 2008). The variation in the removal efficiencies may be attributed to several factors such as the type and concentration of microalgae, tannin dosage, the volume of the cultivation reactor, the physiochemical characteristics, and the element contents in the cultivation medium. However, the effect of these factors on microalgae harvesting using tannin was not well explained in the literature. By interacting with tannin particles, cations in a cultured medium may promote coagulation by neutralizing and destabilizing the negative charges of the coagulant functional group residue (Zhang *et al.* 2013). Monovalent and multivalent cation concentrations, such as Mg^{2+}, Ca^{2+}, Na^{+}, and Fe^{2+}, stimulated and promoted flocculation activities (Wang *et al.* 2010; Okaiyeto *et al.* 2013).

4.2. Chitosan-based natural coagulant

Chitosan has proven to be quite successful in cleaning water and protecting the environment, but it also demonstrates intriguing qualities in the removal of both fresh water and marine algae. Adsorption and charge neutralization are two mechanisms that are believed to be involved in this coagulation. Because of its high charge density, chitosan has a net positive charge. Because the total charge of these cells is negative, the negatively charged microalgae cells are safely adsorbing the positively charged chitosan. As a result, the majority of the charged groups are concentrated near the cell surface (Ruthven 1997), thus destabilizing the microalgae (Ruthven 1997) thus destabilizing microalgae (Wu *et al.* 2007). Chitosan reduces interparticulate repulsion by first neutralizing charges on microalgae cells and decreasing electrostatic attraction. This phenomenon is known as charge neutralization (Divakaran & Pillai 2002). Chitosan might successfully flocculate algal species at levels ranging from 5 to 200 mg/L. Chitosan concentrations of 5 mg/L efficiently reduced 90% of turbidity (Ahmad *et al.* 2011). *Chlorella* and *Skeletonema costatum* (Morales *et al.* 1985) reported >95% microalgae removal using 20 mg/L from chitosan. Chitosan must be used sparingly in fresh water; however, its flocculating ability is diminished in saline water. Acosta-Ferreira *et al.* (2020) evaluated the effectiveness of low-molecular-weight chitosan produced from shrimp shells for wiled microalgae consortia harvesting in a separate investigation. At 20 mg/L chitosan dose, the removal effectiveness of four microalgae strains, namely *Chlorella* sp., *Scenedesmus* sp., *Chlamydomonas* sp., and *Schroderia* sp.,

Table 3 | Tannin applications for microalgae harvesting

Type of cultivation	Tannin dosage	Experimental conditions	Microalgae removal (%)	References
Microalgae cultivated at the wastewater treatment plant	100 mg/L	Sedimentation time 5 min; pH 7.2	90	Teixeira *et al.* (2022)
Cultivated microalgae used for wastewater treatment	20–50 mg/L	Sedimentation time 10–20 min	>90	Xia *et al.* (2008)
Lab-scale cultivation tank	35 mg/L	Fast mixing rate (550rpm)	99	Ruggeri *et al.* (2021)
Cultivated microalgae in anaerobic domestic experimental sewage treatment plant	100 mg/L	Sedimentation time 5 min; pH 9	95.6	Cassini *et al.* (2017)
Cultivated microalgae in PBR of wastewater digestate	165 mg/L	pH 5–7	95	Mezzari *et al.* (2014)
Cultivated microalgae used for wastewater treatment	20 mg/L	pH 9	90	Wang *et al.* (2013)

was greater than 92.9%. Mohd Yunos *et al.* (2017) used chitosan for *Chlorella* sp. harvesting from the aquaculture system. The removal efficiency of microalgae was 98% at 30 mg/L coagulant dosage. The author reported that chitosan can be a sustainable alternative for microalgae harvesting. In a recent study, Chua *et al.* (2020) applied chitosan-based flocculant to harvest *Nannochloropsis* biomass. The PBR and jar test results indicated that up to 95% of microalgae biomass was successfully harvested, whereas 2,000 L raceway pond results demonstrated that the proper mixing should be applied for effective *Nannochloropsis* harvesting. Table 4 presents the applications of Chitosan for microalgae harvesting.

4.3. *Moringa oleifera*-based natural coagulant

Several studies have demonstrated that *Moringa oleifera* (MO) benefits water and wastewater treatment (Blockx *et al.* 2018; Chua *et al.* 2019; Moniem *et al.* 2021; Islam *et al.* 2022). The MO is used for hardness, turbidity, and heavy metal removal (Elemile *et al.* 2021; Ojewumi *et al.* 2021). Compared to a chemical-based coagulant, MO is readily available, cost-effective, eco-friendly, biodegradable, has no harmful byproducts, and produces low sludge volume (Singh & Patidar 2020; Ogunmodede *et al.* 2021; Alazaiza *et al.* 2022). MO unique properties include low toxicity, low cost, high coagulation properties, and affordability, making MO a promising alternative for microalgae harvesting from aqueous media (Abdul Hamid *et al.* 2016). Much experimental research in India, Malaysia, and Mexico documented the use of MO for microalgae biomass collection. *Chlorella* sp. and *Scenedesmus obliquus* are the most studied microalgae strains (Hasan *et al.* 2021). Abdul Hamid *et al.* (2016) investigated the performance of MO for harvesting microalgae; the authors also studied the effect of zeta potential on the coagulation–flocculation process. The harvesting efficiency reached up to 97% at 10 mg/L dosages. The author reported that alum harvested 30% of microalgae biomass at the exact dosage, indicating the superiority of MO over chemical coagulant. In another study it was found that more than 89% of microalgae can be harvested at pH7: this pH significantly affected the performance of MO harvesting, whereas ionic strength did not affect the process'.

Moreover, they found that the surface character of MO was responsible for the enhancement of harvesting efficiency as it can shift from hydrophilic to hydrophobic. Mohamed *et al.* (2017) examined MO and alum performance for microalgae harvesting were compared. Regarding *Scenedesmus* sp. harvesting, the recovery efficiency using alum was 94.87% at 50 mg/L doses, but the recovery efficiency using MO was high (96.5%) at a low dosage (10 mg/L). This result revealed the superiority of MO in terms of microalgae harvesting. Interestingly, Yang *et al.* (2021) examined the performance of four natural coagulants (chitosan, cationic starch, tanfloc, and MO) for microalgae harvesting. The results showed that investigated natural coagulants enhanced microalgae harvesting by two mechanisms: bridging and electrostatic binding. In addition, the authors reported that pH did not affect the performance of MO, while it affected other natural coagulants.

Table 4 | Chitosan applications for microalgae harvesting

Type of cultivation	Chitosan dosage	Experimental conditions	Microalgae removal (%)	References
Jar test microalgae reactor	15 mg/L	Chlorophyll-a concentrations 80–800 mg m^3, pH 7	90	Divakaran & Pillai (2002)
PBR microalgal–bacterial culture broth for fish wastewater treatment	214 mg/L	Flocculation speed 131 rpm	92	Riaño *et al.* (2012)
Lab-scale microalgae reactor	10 mg/g of algae	pH 7	99	Xu *et al.* (2013)
Anaerobic domestic sewage treatment reactor	20 mg/L	pH 9.9	90	Şirin *et al.* (2012)
Marin microalga (*Nannochloropsis oculata*)	75 mg/L	pH (8–9)	90	Acosta-Ferreira *et al.* (2020)
Fresh water microalgae (*Nannochloropsis* sp.)	22 mg/L	Initial pH 6, final pH 10	97–99	Chua *et al.* (2020)
Microalgae cultivated in domestic wastewater using PBR	17–26 mg/L	pH 7 COD 150 mg/L NH_4-N 31 mg/L NO_3-N 50 mg/L	95	Mohd Yunos *et al.* (2017)

5. ECONOMIC AND ENVIRONMENTAL ASSESSMENT OF NATURAL COAGULANT FOR MICROALGAE HARVESTING

Natural coagulants remove trapped *Escherichia coli*, suspended solids, turbidity, heavy metals, organic matter, dyes, and harvest microalgae. However, one of the primary drawbacks of using natural coagulants in microalgae harvesting is the lack of full-scale use (Yin 2010; Muhammad *et al.* 2021). Table 5 demonstrates that biocoagulation technology is a low energy-intensive and ecologically friendly alternative to physical separation techniques or chemical flocculation for extracting microalgal biomass. Even though using natural coagulants for microalgae harvesting is cheaper than chemical one (as depicted in Table 5), the affordability of natural coagulants depends on plant cultivating, harvesting, and extraction of active coagulants, which highly influence by spatial and temporal characteristics (Behera & Balasubramanian 2019). Studies examining the technological efficacy and economics of various harvesting techniques have revealed that plant-based coagulants have the lowest cost of biomass recovery. Since greenhouse gases like CO_2 are mostly to blame for ozone layer depletion and global warming, large-scale biomass recovery systems must be ecologically benign and enable sustained biomass production. Many investigations were conducted to evaluate the unsuitability of using natural coagulants. The results demonstrate that using chitosan has higher energy consumption and high greenhouse gas emissions than alum (Behera & Balasubramanian 2019).

In contrast, the intensive production method was assumed to be the primary cause of alum and chitosan's high energy and carbon emission levels. Similarly, iron(III) chloride negatively influences the environment and is expensive (Udom *et al.* 2013; Ogbonna & Nwoba 2021). One notable advantage of employing nontoxic, biodegradable plant-based biopolymers as flocculants are recycling previous microalgal growth medium. By permitting the utilization of nutrient leftovers during

Table 5 | *Moringa olefra* applications for microalgae harvesting

Type of cultivation	*Moringa olefra* dosage	Experimental conditions	Microalgae removal (%)	References
PBR (*Chlorella* sp. and *Scenedesmus* sp.)	Coagulant dosages (10, 25, 40, 55, and 70) mg/L	pH 8 Stirring device speed (20, 60, 100) rpm Mixing period (10, 30, 50 min)	85% at 60 mg/L dosage	Hasan *et al.* (2021)
Pond culture (mix)	15 mg/L	pH 8 Mixing time 5 min Mixing rate 30 rpm Settling time 20 min	93%	Moniem *et al.* (2021)
Anaerobically digested black water (AnBW)	475 mg/L	Sedimentation time 45 min pH 7	95%	Quesada *et al.* (2019)
Wastewater, PBR	70 mg/L	Mixing rate 20 rpm Mixing time 10 min	83%	Kapse & Samadder (2021)
Fresh water microalgae	10 mg/L	Sedimentation time 20 min pH (6.9–7.5)	95%	Santos *et al.* (2016)

Table 6 | Flocculant cost study for harvesting microalgae biomass

Coagulant/flocculant	Microalgae	Coagulant/flocculant cost/ton of biomass harvested (US$/ton)	References
$AlCl_3$	*N. oculata*	40	Garzon-Sanabria *et al.* (2013)
$Al_2(SO_4)_3$	*C. vulgaris*	28	Vandamme *et al.* (2012)
Chitosan	*N. oculata*	44	Garzon-Sanabria *et al.* (2013)
MO	*Microalgae consortium*	12.7	Behera & Balasubramanian (2019)

biomass recovery, reusing the old medium can lower the cost of fertilizers and the water footprint (Jethani & Hebbar 2021). Table 6 summarizes the flocculant cost study for harvesting microalgae biomass.

6. CONCLUSION

The work summarized different cultivation systems that can be used for several sustainable and environmental applications, including sustainable wastewater treatment technologies and biofuel generation. Light, temperature, pH, nutrients, and CO_2 concentration are the main abiotic factors that may affect the biomass of microalgae during their cultivation. Tannin, chitosan, and MO are the most often utilized microalgae harvesting processes due to their high efficacy for microalgae removals in various production methods and their economic and environmental benefits. The three coagulants reported their optimal removal for microalgae between 90 and 99% at pH ranging between 5 and 9.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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