

Review Article

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Sustainable Cost-Effective Microalgae Harvesting Strategies for the Production of Biofuel and Oleochemicals

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Abstract

Microalgae have been explored for sustainable production of biofuel and chemicals. Microalgae is promising feed stock for the production of several oleochemicals. It has the ability to utilize a variety of low cost feed stocks, accumulated large quantities of lipids and variety of value added products in their biomass. One of the major obstacles associated with the conversion of algae into value-added products is harvesting. The harvesting of algae is the most problematic area due to its low sedimentation rate, low biomass concentration, and high capital costs. Harvesting of algae is carried out by different physical, chemical, mechanical, biological, and electrolytic methods such as sedimentation, centrifugation, microstraining, dissolved air flotation, electrolytic flotation, chemical flocculation, bioflocculation, autoflocculation, Filtration. This review highlights the various methods of microalgae harvesting with advantages and future perspective of sustainable and cost-effective harvesting of microalgae.

Keywords: Microalgae, Harvesting, Autoflocculation, Sedimentation, Bioflocculation.

Introduction

Algae are simple aquatic photosynthetic microorganisms that live in saline and freshwater environments. Sunlight plays a key role in the harvesting of the algae. They can grow at any place where enough sunlight is found. Apart from sunlight, the chemical composition of algae includes proteins, carbohydrates, lipids, and nucleic acids. Algae are known as third- generation biofuels due to their higher biomass production capacity compared to other cereal- based crops [1]. They are one of the important sources of food, animal feed, medicinal products, and most important oil for fuels [2]. Cost-effective production of microalgae biomass for value added products like biodiesel is usually restricted due to poor efficiency in essential processes like cultivation, harvesting and lipid extraction [3]. Harvesting of microalgae involves two consecutive steps i.e., Bulk harvesting and thickening. Bulk harvesting involves flocculation, floatation, and sedimentation, in which solid biomass is separated from suspensions. Whereas, thickening involves filtration, centrifugation [4]. The present paper foccuses on recent techniques involved in harvesting microalgae and to assess their technical, economical and application potention.

Algal Metabolites

Algae like *Macrocystis*, *Laminaria*, and *Ascophyllum* produce alginic acids. Alginate has the potential to make a highly viscous solution and chelating properties, so they are widely used in the pharma and food industries. Alginates in textile industries are used for sizing cotton yarn [5]. Agar derived from macroalgae is used in making desserts, jellies, candies, frozen foods, etc. due to its semi-solid nature and jellying properties. Agarose, as one of the byproducts of algae, is used significantly in molecular biology and many biomedical fields for the production of tablets, capsules, and anticoagulants [6]. Many of the algae are used for aquaculture feed like *Nannochloropsis*, *Chlorella*, *Tetraselmis*, *Phaeodactylum*, *Pavlova*, etc [7]. For hundreds of years, microalgae have been used as nutrient supplements as it is a rich source of Carbohydrates, protein, enzymes, vitamins, and minerals. There are a variety of pigments produced by microalgae like Chlorophyll, Phycobiliproteins, Carotenoids, β -carotene, Astaxanthin, Lutein, Canthaxanthin [8]. Vitamins like Biotin, pantothenate, riboflavin, folic acid, nicotinic acid are produced by microalgae [9].

Harvesting of Microalgae

Harvesting of algae is a two-step process that includes cultivation and separation of the algae. The separation of the microalgae can be of mechanical, chemical, electrical, and biological methods. Harvesting by using cost-effective procedures is the most difficult phase in algal biofuel production [4]. The techniques applied for microalgae harvesting are filtration, gravity sedimentation, centrifugation, microstraining, floatation, flocculation, etc., [10-11] and sometimes a combination of one or two methods. An ideal harvesting method must be effective for more algal species, which gives a higher biomass concentration with minimal costs, energy, and maintenance.

Centrifugation

Centrifugation is the ideal process for harvesting of the microalgae because of its quickest and effective procedure to remove particles from the algal suspension without any additive agents. During the process of centrifugation, large shear forces are exerted and lyse the algal cells releasing algal oil into the medium. Later to which the oil recovery has been done [2]. *Chlorella vulgaris* shows a very high recovery of biomass and oil yield when subjected to the centrifugation process [12]. Pahl et al. [13] have examined various centrifuges like disk stack centrifuge, tubular centrifuge, nozzle-type centrifuge, decanters, perforated and imperforated basket centrifuges, hydrocyclones for microalgae separation. Disc stack centrifuges are exclusively used in industrial high-value product recovery from algae but recorded poor energy return as more energy is exhausted than produced [14]. Decanter centrifuge is highly efficient but involves high energy consumption, whereas hydrocyclone centrifuges can be used with less

maintenance but due to its high energy and capital cost, restricted its use for large scale purposes [15-16].

Gravity Sedimentation

In Gravity sedimentation, the gravitational forces separate the solids and liquids from one another depending on their size and density. The factors affecting sedimentation are particle size and density, pH, temperature, the intensity of light, and aging of the cells [17]. The rate of sedimentation is directly proportional to the difference in densities of the particles. If there is a high difference in densities between solids and liquids, the sedimentation rate is faster and in case of less difference, the sedimentation rate is slower [15; 18]. Sedimentation requires low cost and less energy. Sedimentation is mostly species-specific, slow separation, and low final concentration. The *Chlorella sp.* cultured at pH=10.5 and sedimentation time of 12 h showed effective harvesting from 1.01g/L initial concentration to 3.95g/L [19]. The Centrifugation method is not appropriate for the cost-effective harvesting of microalgae like *Chlorella sp.* due to its small cell size. The application of a combination of various harvesting techniques is necessary for enhanced recovery efficiency. A study suggests that the granulation of the biomass obtained from microalgae (*Chlorella sp.* cells) via gravity sedimentation with filamentous microalgae is a possible solution for effective recovery [20].

Microstraining

In microstraining, drum rotates slowly in the partially submerged condition in the trough of suspended algal cells. A mesh act as a screen that captures large particles of algae, whereas microalgae still pass via mesh and were not able to be harvested [21]. It is a simple cost-effective method, but due to low harvesting efficiency, it is not desired much.

Filtration

The problems associated with the use of the filtration process are the small size of the algal cells, their shape [spherical], and the gelatinous and other extracellular materials of the algal cells. These results in the poor filtration causing plugging of the filters [22]. The particles in the suspension are introduced onto the screen of different sizes, the particles either pass through the filters or retained on the screen depending upon their size [23]. Filtration techniques can be classified into microfiltration, macro-filtration, ultrafiltration, and reverse osmosis (Figure 1).

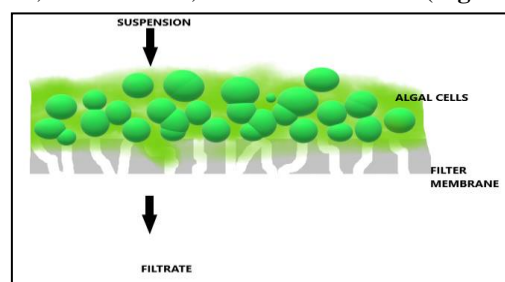


Figure 1: The filtration technique for microalgae harvesting.

Many types of filters and membranes with different sizes are available for easy filtration. This method is suitable for only large algal cells; smaller cells may clog in the filter pores [24]. Filtration is the process of separation of microalgal biomass from the broth medium by passing the suspension through the permeable membrane [25]. Membrane filtration technology for harvesting microalgae comprises osmotic and pressure-driven processes. The pressure-driven membrane process involves hydraulic pressure to force biomass passage *via* the permeable membrane for solids separation from liquid [26].

Common membrane filter designs include microfiltration (0.1-10 μm), macro filtration (10 μm), ultrafiltration (0.01-0.1 μm), nanofiltration (0.001-0.01 μm), tangential flow filtration, vacuum filtration, dead-end and cross-flow filtration based on the size of the filter [27-28]. Osmotic pressure is used to separate the microalgal biomass and liquid medium in osmotically driven membranes; this includes reverse and forward osmosis. However, reverse osmosis has lower water flux, reverse draw solute flux, and fouling process compared to forward osmosis [29-30]. The criteria for the selection of membrane material involve characteristics of species, biomass concentration, hydrophilicity, Hydrophobicity, surface charge, flow parameters, etc. The typical materials used for polymer preparation of membrane are polyethersulfone polyvinylpyrrolidone, polyvinyl chloride, cellulose acetate, polyvinylidene fluoride, polyacrylonitrile, polyethersulfone, polyamide, etc [26; 28].

Recent studies have reported the use of ceramic-based material for membrane in the form of a diatomite dynamic membrane (DDM) [31]. One of the studies has reported that a mixture of sand, starch, and kaoline can be used for the novel ceramic membrane production for microalgae concentration and can overcome difficulties like high shrinkage, low porosity, high thickness, poor flexibility, small pore size, etc [32]. Other membrane developments include electromembrane [33], steel-use-stainless membrane coated with selective polymers, etc [34]. Research on the application of membrane processes for microalgae harvesting is mostly restricted to lab-scale and lab-scale performance does not guarantee the same performance on a large scale too. Hence, a study on a larger scale membrane-based harvesting of microalgae is pre-requisite to focus on the near future [26].

Floation

In this process, tiny air bubbles are introduced which in turn transport the solid particles suspended in the system to the surface. Later, the floating solid particles from the surface can be removed by skimming or other methods. Dissolved Air Floation: High pressure-recycled water which is saturated with dissolved air is introduced into the chamber. This creates the release of microbubbles, and they get attached to the suspended algal particles causing them to

float on the surface [35]. Electrolytic floation involves the formation of gas bubbles by electrolysis [15]. It is more rapid than the sedimentation method. This method is species-specific and requires high capital and operational costs. Floation involves gravity separation *via* gas bubbled through a microalgal suspension, due to which soil particles get attached to gaseous molecules and can be skimmed off from the surface [36].

Floation processes are classified based on bubble generation methods, these involve dispersed air floation, dissolved air floation, dispersed ozone floation, electrolytic floation, jet floation, etc [37]. Dissolved air floation involves the use of bubbles of mean size 40 μm with range 10 to 100 μm , under high pressure with air dissolved in water and later atmospheric pressure is released in the unit. The air bubbles aggregate are released with the microalgae particles and float on the surface which is later skimmed off [36; 38]. Dissolved Air Floation of *Chlorella sorokiniana* after coagulation with four different coagulants: aluminum sulfate, ferric chloride, tanfloc, and zetag showed the maximum efficiency of 98.4, 94.5, 95.4, and 96.7% at 8 $\text{cm}\cdot\text{min}^{-1}$, respectively [39].

In dispersed air floation method bubbles ranging from 700-1500 μm size are produced by continuous passage of air stream *via* high-velocity mechanical fomentor. This process requires less energy; whereas the equipment cost is relatively quite high. The recovery percentage of microalgal cells has been raised to 90% with surfactants by increasing the hydrophobicity of the microalgae cells and this will facilitate the attachment of bubbles to microalgae cells [37]. Dispersed air floation of *Chlorella saccharophila* with Cationic Trimethyl-Ammonium Bromide (CTAB) added as a surfactant was investigated as an effective harvesting technique for to be used for the production of bio-diesel [40].

In the Electrolytic floation process, the gas bubbles are produced by electrolysis of water. The investigation of electrolytic floation of microalgae *Dunaliella salina* showed similar harvesting efficiency as well as energy consumption while a graphite plate was used as the anode, and different materials and forms of cathodes (stainless steel plate, perforated stainless steel plate and graphite plate) [41]. Dispersed Ozone Floation (DOF) uses ozone gas instead of atmospheric air to produce charged bubbles that promote the floation of microalgal cells on the surface. Ozone oxidizes the organic contents of the effluent and also improves the quality of water by lowering the turbidity [25; 28]. However, the dispersed ozone floation technique is not desired for large scale purposes, due to contamination issues and high operating prices [42].

Flocculation

Flocculation is the aggregation of the microalgal cells to enhance the size of the particle that eases the sedimentation and recovery of the microalgae. The settling

velocity and the concentration factor are the key parameters of the flocculation process. Usually, the negative charge present on the surface of the microalgal cells prevents its aggregation in the suspension [43]. This negative charge can be neutralized by adding flocculating agents to initiate flocculation in the microalgal cells. The flocculating agents should be non-toxic, inexpensive, easily available, and effective at low concentrations [44].

Multivalent metal salts

Ferric chloride [FeCl₃], Aluminium sulfate [Al (SO₄)₃, Alum], and ferric sulfate [Fe (SO₄)₃] are the multivalent metal salts used to initiate flocculation in the microalgal cell suspension. It is the phenomena where positively charged ions are used to neutralize or reduce the negative charge on the surface of the algae to form flocs [45-46]. The addition of these compounds may negatively affect the recycling of the medium and also the quality of the product. The metal salts possess a positive charge to interact with the negative charge present on the algal surface and result in floc formation [43]. The study compared and evaluated harvesting microalgae *Arthrospira maxima* through flocculation by the addition of CaCl₂ as a flocculant and/or pH increase above 10 using NaOH showed an effective harvesting technique [47].

Biodegradable organic flocculation

Biopolymers like guar gum, starch, alginic acid, and chitosan are used as organic flocculants for microalgae harvesting. These biopolymers may not contaminate the algal suspensions [48-49]. Chitosan has been shown to be an effective biopolymer for harvesting microalgae without any toxic effect on the harvested algae.

Bioflocculation

Bioflocculation is the harvesting of microalgae without any addition of flocculating agents. Bioflocculation involves other microorganisms having the capability of flocculation in the medium, as shown in **Figure 2**.

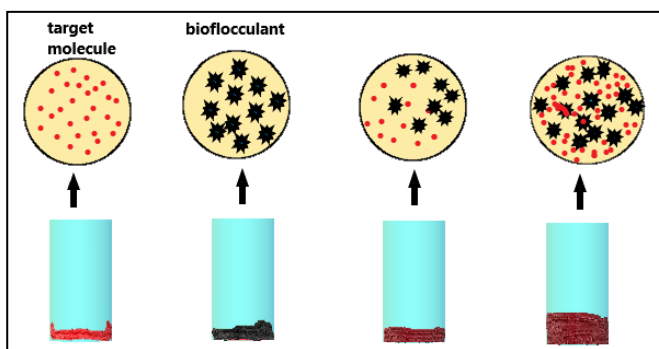


Figure 2: Bioflocculation process in microalgae harvesting.

The flocculating fungi or bacteria or algae are harvested along with the non-flocculating microalgae in the medium. Extracellular polymeric substances secreted by organisms such as bacteria or fungi or algae act as bioflocculants. These bioflocculating agents can increase the

growth rate of the algae in the system [44; 50]. The flocculating microorganisms do not require any special cultivation conditions and their presence don't interfere with the downstream process of algal lipids. The study showed that the co-flocculation of *Citrobacter freundii* and *Chlorella pyrenoidosa* (bacteria: microalgae ratio was 1.6:1) showed the maximum flocculation efficiency of 97.45% and *Mucor circinelloides* and *Chlorella pyrenoidosa* (microalgae: fungi ratio was 333:1) showed the maximum flocculation efficiency of 92.08% [51].

Autoflocculation

Few autoflocculating microalgae can form flocs without any addition of flocculating agents. Autoflocculation can be defined as the interaction between the surface molecules of the algae and the surrounding medium or interaction between the microalgae among themselves. The algal cells aggregate and form into larger flocs. The flocs induce the sedimentation of the algae [52]. The microalgae produce extracellular polymeric substances [EPS], which plays a key role in the autoflocculation. When this EPS production reaches its maximum level, the microalgae tends to undergo flocculation by settling down itself. Autoflocculation is largely dependent on algal species and pH manipulation [53]. *Chlorococcum sp.* at pH 12 has been reported with a harvesting efficiency of 94% [50]. In the case of *Scenedesmus obliquus*, autoflocculation efficiency was improved from 10.4 to 33.2%, when the pH increased from 7 to 10 [54]. A study on auto-flocculation of *Ettlia sp.* driven by polysaccharides had a positive impact on harvesting efficiency of 91 ± 2.7%; whereas, auto-flocculation of *Chlorella sp.* driven by polysaccharides had a negative impact on harvesting efficiency of 51 ± 1.3%. This study revealed that autoflocculation is species-dependent and for each species harvesting protocol will vary [55].

Electrolytic flocculation

In electrolytic flocculation, electrodes are placed in the culture, and the current is supplied to run across the electrodes. The negatively charged algal cells migrate toward the positive charge of the electrode causing aggregation [23]. This method is more effective than chemical flocculation [56]. The study evaluated the harvesting of *Scenedesmus sp.* using Electro-Coagulation-Flocculation (ECF) showed the effective harvesting efficiency (>99%) under optimal conditions [57]. Thus, this technique could be well-suited harvesting technique for biofuel production.

Polymeric flocculation

In polymeric flocculation, long-chain polymers are used to bind the algal cells by bridging the gaps between the cells, causing aggregation and settling. Polymers with a positive charge like polyethylene amine and polyacrylamides bind with negatively charged cells of

microalgae in the suspension and result in floc formation [18]. The harvesting of freshwater microalgae viz., *Chlorella vulgaris* and marine algae viz., *Phaeodactylum tricorutum* using a cationic polyacrylamide flocculant, showed 100% and 90% biomass recovery, respectively. The optimal flocculant concentration for *Chlorella* and *Phaeodactylum* was 18.9 and 13.7 mg/g of dry algal biomass, respectively [58].

Physical flocculation

Sometimes the flocculating agents may contaminate the algal suspension; to avoid such problems physical forces were induced. One such example is sonication. In this process, high-frequency sound waves are introduced into the algal suspension the cells charge negative and will move to the anode where they get neutralized and form into aggregates. Always high-frequency sound waves should be used, as low-frequency sound waves may cause rupture of algal cells [59].

Magnetic separation using nanoparticles

Magnetic separation using nanoparticles has recently gained attention for harvesting microalgae due to its potential over traditional harvesting techniques [60]. In this technique, magnetic nanoparticles are tagged to the suspended microalgal cells and are recovered from the culture broth *via* external magnetic field [42]. Microalgal harvesting is carried out by two types of magnetic nanoparticles i.e., naked and surface functionalized. In naked magnetic nanoparticles, a wide range of microalgal strains is recovered based on its specific surface area, biocompatibility, super-paramagnetism, etc [61-62]. In the case of surface-functionalized magnetite, there are two strategies for tagging polyelectrolyte, "attached-to" and

"immobilized" strategy. In the "attached-to" approach, the surface of microalgal cells is coated with a polymer binder that helps attach with the magnetic particles. In the case of "immobilized-on" strategy surface of the uncoated magnetic particles is functionalized with a polyelectrolyte that aids the binding with the algal cells [63].

The harvesting efficiency of microalgae can be improved by surface coating of magnetic nanoparticles with various polymers, surfactants, etc [64]. A recent study has confirmed that the harvesting efficiency of *Scenedesmus* sp is improved when different Fe₃O₄-based nanoparticles are used as adsorbents. Cetyl-trimethyl-ammonium bromide-coated Fe₃O₄ nanoparticles, polyethyleneimine coated Fe₃O₄ nanoparticles, amino-propyl-tri-ethoxy-silane functionalized Fe₃O₄ nanoparticles. All the synthesized magnetite-based nanoparticles showed high potential efficiency for microalgae harvesting [60].

Concluding Remarks

Various methods for microalgae harvesting were reviewed. Centrifugation is a rapid and effective method that required high capital and operational costs. Other methods like floatation, filtration techniques, and other electrochemical techniques require high capital cost. Autoflocculation and bioflocculation are found to be inexpensive and effective dewatering techniques for algal harvesting. Autoflocculation has a high sedimentation rate without any addition of the flocculants. The autoflocculation can be enhanced by a high aeration rate, CO₂ concentration, and nitrogen levels. Bioflocculation is also an efficient, eco-friendly, and cost-effective algal harvesting method (**Table 1**).

Table 1: Various microalgal harvesting technique advantages and disadvantages.

Sl. No.	Harvesting Method	Advantages	Disadvantages	References
1	Centrifugation	<ul style="list-style-type: none"> • Rapid and efficient cell harvesting • Handles most of the microalgae cells 	<ul style="list-style-type: none"> • High operational cost 	[2]
2	Sedimentation	<ul style="list-style-type: none"> • Reduces cost and energy 	<ul style="list-style-type: none"> • Mostly used for non-motile algal cells • Species specific • Low separation rate 	[15, 17, 18]
3	Filtration	<ul style="list-style-type: none"> • Filters and membranes of different sizes are available which makes the process of harvesting easy and rapid 	<ul style="list-style-type: none"> • Species dependent • Mostly suitable for large algal cells • Fowling and clogging becomes the main problem when using filtration as the harvesting method 	[22-24]
4	Floatation	<ul style="list-style-type: none"> • Rapid when compared with sedimentation 	<ul style="list-style-type: none"> • Species specific • High capital and operational costs 	[15, 35]
5	Chemical flocculation	<ul style="list-style-type: none"> • Low cost • Simple and rapid harvesting • no energy requirements are needed 	<ul style="list-style-type: none"> • Removal of chemical flocculants • Sometimes the chemicals are toxic to the algae 	[43, 45, 46]
4	Bioflocculation	<ul style="list-style-type: none"> • eco-friendly and cost effective algal dewatering technique. • No energy inputs are required 	<ul style="list-style-type: none"> • The bioflocculants [bacteria/fungi/algae] used in the flocculation sometimes causes contamination 	[44, 50]
5	Autoflocculation	<ul style="list-style-type: none"> • Effective • High sedimentation rate with no energy inputs 	<ul style="list-style-type: none"> • Changes in cellular composition 	[44, 52]
6	Magnetic Separation	<ul style="list-style-type: none"> • Short time period for microalgal harvesting. • Reusability of the culture medium 	<ul style="list-style-type: none"> • Nanoparticles are costly. • require special recycling equipment 	[63]

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