Current advances in microalgae harvesting and lipid extraction processes for improved biodiesel production: A review

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Abstract

Microalgae have been considered as a potential feedstock for biodiesel production, since its cultivation uses less land than other traditional oil crops and has a higher growth rate. A great challenge is a choice of an effective approach for microalgae biomass recovery and lipid extraction, since the scheduling of these practices are critical and require an economical and environment friendly route. Flocculation has evolved as an efficient and economic approach for harvesting microalgae biomass. This review discussed the recent progress of chemical flocculants including organic and inorganic, bio-flocculants and nanomaterials-based processes for biomass recovery. In addition, the present review describes modifications made in conventional methods for lipid extraction. Several pre-treatment methods such as mechanical, chemical integrated with various solvents and nanoparticles are vastly investigated for lipid extraction. Use of green solvents namely, ionic liquids, supercritical fluids and switchable solvents are also reviewed, with the focus on cleaner biofuel synthesis. Furthermore, the article discusses policies implemented for the advancement in biofuel production, major challenges and considers future directions in microalgae harvesting and lipid recovery processes. This is the first study that extensively

compares the recent approaches for biomass and lipid recovery. The present work intended to serve a long-term adaptation of the innovative techniques for copious economic benefit. Thus, this review emphasizes on advanced techniques that influence the microalgae biomass separation and cellular disruption for proficient lipid removal from microalgae, which deliberates towards the development of sustainable microalgae biofuel and heighten the bio-economy strategy.

Highlights:

- Approaches for microalgae harvesting and lipid extraction have been outlined in depth
- Flocculation methods could significantly reduce the cost of harvesting microalgae
- Integration of green solvents and ~~physical~~ mechanical methods are effective for cell disruption
- Use of engineered nanomaterials reduces the time and energy for lipid extraction
- Government energy policies for the wide marketing of biofuels are discussed

Keywords: microalgae, cell harvesting, nanomaterials, flocculation, lipid extraction, green solvents, biodiesel

Word Count: ~~10,168~~ 10,568

1. Introduction

Many societies are facing an energy crisis due to rapid industrialization and significant increase in population. Presently, conventional energy sources such as coal, petroleum, and natural gas fulfill 80% of primary energy demand across the world but are consequently depleting rapidly and causing increase in greenhouse gas (GHG) emission [1]. Burning these fuels is also associated with detrimental health problems [2]. The major pollutants are carbon dioxide (CO_2), nitrous oxide (NO_2), and methane (CH_4), which cause changes in climatic conditions. Consequently, there is an urgent need for the evolution of renewable and sustainable energy resources like biofuels. Biofuel derived from plants, food, and non-food crops have been highly criticized by the scientific community and technocrats due to their extensive land usage, leading to food versus fuel dilemma [3].

By considering all the above-mentioned obstacles, researchers have turned their attention to biofuel production from oxygenic eukaryotic photosynthetic phytoplankton-microalgae. Microalgae have innate ability to capture atmospheric CO_2, reducing climate change impact. Microalgae are considered as a source of third- generation biofuel and are among the fastest proliferating photosynthetic biomass on earth, with a high intracellular lipid content categorizing them as a green and sustainable source of fuel [4]. There are various sequential steps that are usually involved in microalgae biodiesel production including cell cultivation, harvesting, lipid extraction, and fatty acid methyl ester (FAME) generation [5] (Fig.1).

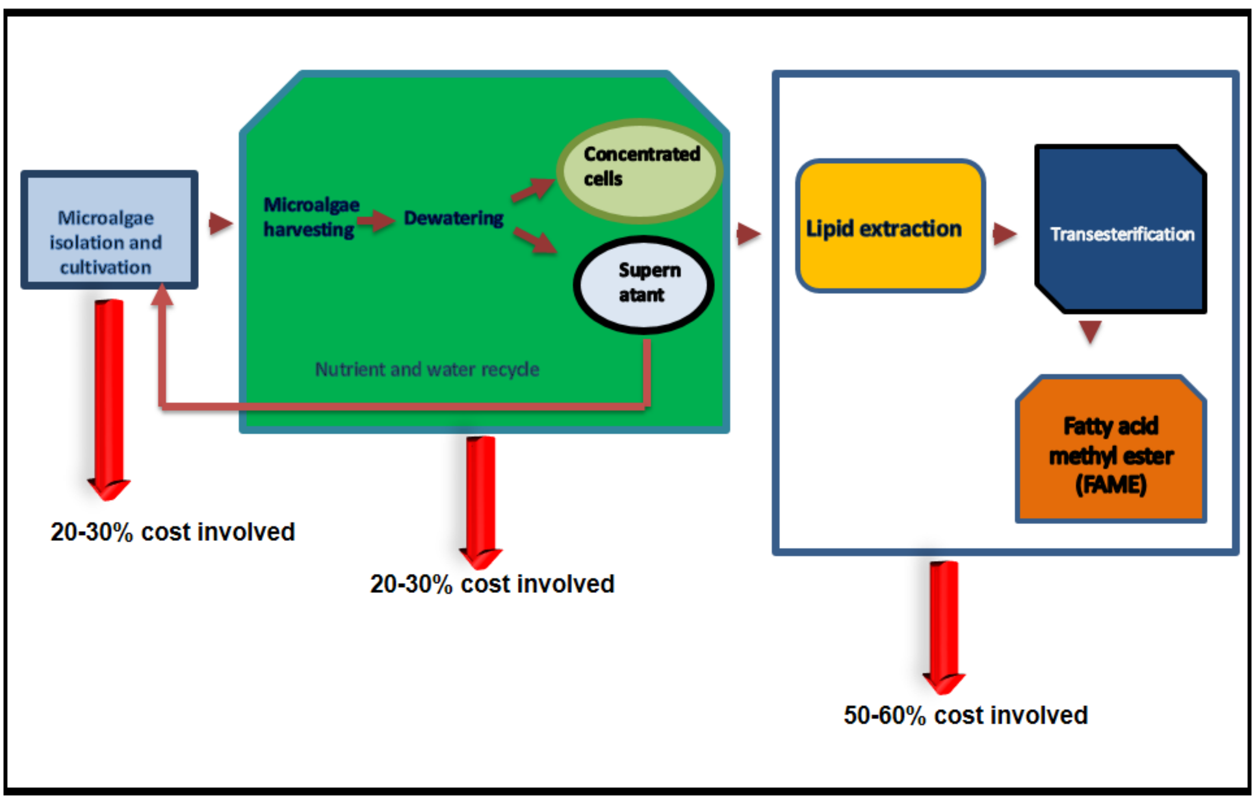

Fig. 1. Schematic diagram showing the various steps involved in microalgae FAME production along with cost contribution by each step [5, 6, 7, 8]

In order to grow, algae require water, CO_2, and micronutrients, which are termed a "culture medium" [9]. Algal culture systems can be categorized into two: open culture and closed culture. Open culture system is exposed to contamination, temperature fluctuations, pH variation and water loss whereas closed culture are subjected to controlled environment, minimum contamination, and less water loss [1]. After cultivation, cells are harvested, which includes three systematic processes: biomass recovery, dewatering, and drying [10]. At this stage, cells contain a large amount of water that must be removed before the conversion of biofuel [11]. Separation of oil from biomass depends on the cell wall disruption, various methods for this comprise: ultrasonication, use of supercritical fluids, and use of organic solvents, etc [4]. The triacylglycerols (TAGs) in the microalgae cells contain oil droplets, which are converted into alcohol esters via transesterification designated as biodiesel [1].

Among all the steps involved in biodiesel production, the cost in harvesting and lipid extraction contributes a maximum of the overall cost that is more than 80% as represented in Fig. 1 .The root cause for the high processing cost is the small size of microalgae (5-7µm) and large growth volume (0.3-5g/L) [12]. In addition, algal cells remain electrostatically stable in the aqueous medium due to the presence of amine (NH) and carboxylic groups (- COO-) on their surface, which cause overall negative charge making harvesting difficult [8]. As previously mentioned, the efficient extraction of lipid is another constraint due to the recalcitrant behavior of the microalgal cell wall. During the process of lipid extraction, organic solvents such as chloroform, methanol, and hexane are used, but the sole solvents may not be enough for the complete extraction of lipids and can dissolve the chlorophyll pigments [13]. A substantial improvement and development of a cost-effective harvesting technique is one of the significant challenges in algal biofuel research [14]. Moreover, finding a suitable greener extraction approach is another requirement to ensure ecofriendly production in the commercialization of microalgae- based biodiesel production.

Scientific and technical knowledge gaps are the obstacle that still exists for commercializing the microalgae biofuel technology. Current technologies for biodiesel production on a large-scale represent an economical and efficient approach for biomass recovery and lipid extraction, but there is a lack of literature which systematically explores these techniques together [15]. Researchers have summarized some of the techniques on harvesting along with lipid extraction approaches involving chemical, and physical processes, however, the mentioned techniques are energy extensive and use toxic chemicals [16,17]. Recently, many attempts have been made like developing plant- based biopolymer linked flocculation methods [16,18]. Advanced nanomaterials are also being investigated to promote effective cell harvesting [19]. In lipid extraction processes, rather than using hazardous organic solvents, some green solvents like eutectic and supercritical fluids are introduced, which accelerate the extraction process. Hence, the current review highlights commercially viable techniques involved in harvesting and lipid extraction.

This review presents a comprehensive overview on the major bottlenecks involved in biodiesel production namely harvesting and lipid extraction, with a critical discussion on their merits and demerits. Detailed studies on conventional modes of cell separation as well as advanced methods including the use of nanomaterials and nanocomposites are investigated. Combination techniques are also reviewed including the use of enzymes, nanomaterials, and green solvents for lipid removal. The main objective of the review is to provide an in-depth understanding of biomass, lipid recovery technologies, their practical implementation and future research pathways. A broad range of literature is investigated for the conventional and unconventional methods involved, and the techniques are critically diagnosed for their potential application in algae biodiesel taking economic consideration into account. The future use of engineered nanomaterials to enhance the capability of microalgae for biodiesel is also considered.

2. Microalgae cell harvesting

Harvesting is generally considered as a sequential process for removing the water content from the culture medium of microalgae by incorporating several downstream techniques in order to concentrate the biomass. An appropriate harvesting technique is selected by considering the overall energy consumption and cost that majorly depends on cell size and density. An ideal cell recovery method must be developed that could be applied for majority of microalgae strains and achieve the maximum biomass recovery along with the moderate operational, energy and maintenance costs at a low environmental impact. The complete overview of cell harvesting methods known for microalgae including conventional and advanced methods is illustrated in Fig. 2.

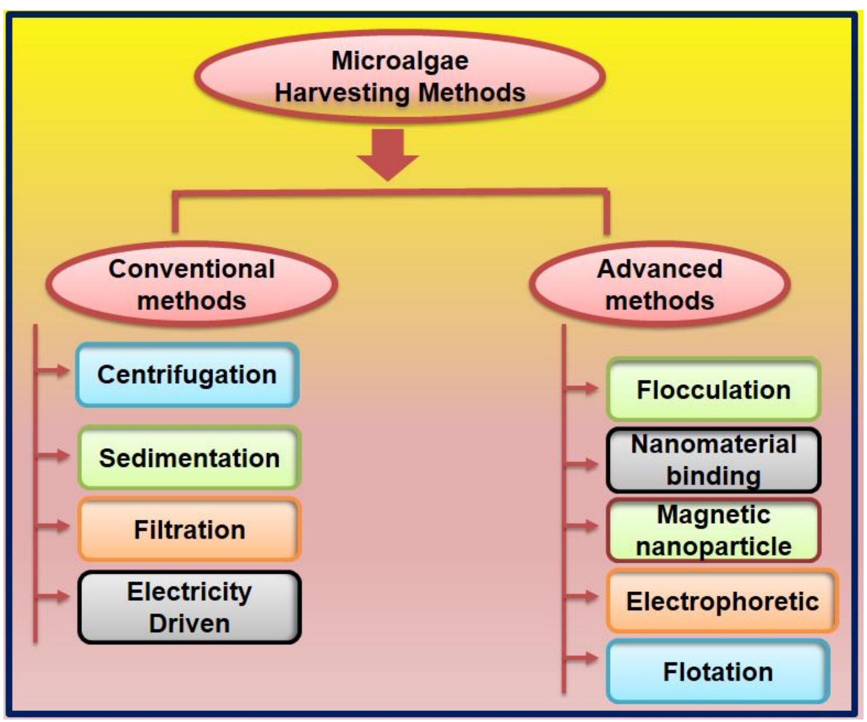

Fig. 2. Techniques of microalgae cell harvesting including conventional and advanced methods [15,16,19]

2.1 Conventional cell harvesting methods

Generally, conventional methods of microalgae harvesting include centrifugation, sedimentation filtration and electrical methods. Centrifugation is the most common method used for harvesting, but the high cost and energy consumption are the main drawback [20]. Sedimentation is simple and cost-effective technique, but it is not suitable for a wide variety of microalgae and consumes much time [21]**.** The method of filtration is effective but has problem of clogging and fouling that can cause low harvesting yields [8]. The electrical method of harvesting includes electro-coagulation and floatation, which requires high energy consumption for supplying and consuming the microbubbles along with high equipment cost, which often makes it unsuitable for harvesting [22]. The cost and energy consumption for harvesting microalgal cells could be reduced by pre-concentrating the microalgae cells through flocculation.

2.2 Advanced methods of cell harvesting

Among several harvesting techniques, flocculation is the most effective, convenient and economical process. The technique has undergone various advancements over the years and is accomplished by physical, chemical and biological means. Currently, the use of nanomaterials in the process of flocculation is being considered due to their efficiency and reusability. Microalgae cell harvesting has been reported to be achieved using various flocculants such as metal salts, organic polymers and natural biopolymers [23].

2.2.1 In-depth mechanism of cell flocculation

Microalgae carry negative charge on their surface that prevent them from self-aggregation and hence make them difficult to harvest from the suspension. Flocculation is an advanced technique of cell harvesting that involves organic and inorganic flocculants, which are used to neutralize the negative charge of the microalgae cell surface. Hence, flocculation can increase the particle size of microalgae cells through aggregation and increases the rate of cell settling. Flocculation efficiency is a surface charge phenomenon and can be affected by zeta potential, which is the apparent charge present on the surface of the cells [15]. The mechanism of flocculation is based on three forces: charge neutralization, adsorption and adsorption bridging [24]. The positively charged flocculants are added to the algae culture, absorb negative charge of the cells and subsequently balance the charge. Thus, the electrostatic repulsion between the particles disappear and hence cells coagulate. Adsorption or electrostatic patch mechanism, where cationic polymers bind with the cells of opposite charge and reverse the charge on the cell surface. This results in patch formation over the boundary of the cells, which connect with each other, thus causing flocculation. Adsorption bridging is another process involved in flocculation, where a bridge is formed between two cells with the help of charged polymers that bring the cells together and causes flocculation.

In the recent years, a wide range of approaches have been explored for the flocculation and sedimentation process of microalgae [23]. Various methods have been found to initiate flocculation in microalgae including auto flocculation, chemical flocculation, bio-flocculation and emerging technologies such as cell harvesting mediated by nanoparticles and other advanced nanomaterials.

2.2.2 Auto flocculation

Auto flocculation occurs in microalgae cultures when pH increases above 9 [25]**.** The negative charge present on the surface of microalgae destabilizes with the increase in pH, which causes microalgae to flocculate and settle. This process is cost effective, nontoxic to microalgae and does not require additional downstream steps. Generally, hydroxide salts of monovalent and divalent ions are associated with autoflocculation. Precipitates are formed, which carry the positive surface charges and can induce flocculation by neutralizing the surface charge of microalgae.

Ummalyma et al. [26] reported auto flocculation of *Chloroccocum* sp by adding NaOH. It is documented that self-flocculating behavior in microalgae *Scenedesmus obliquus*, was observed in 30 min [27]. In another study, *Chlorella vulgaris* and *Neochloris oleoabundans* were observed with sedimentation rates of 7% and 15% respectively [28]. *Chlorella vulgaris* JSC-7 demonstrate maximum spontaneous flocculation of 76% as compared with *Chlorella vulgaris* CNW11 (26%) and *Scenesdesmus obliquus* (28%). The results suggested that the hydroxyl and carboxyl groups present in microalgae may have resulted in better flocculation [29]. Perez et al. [30] reported total biomass recovery for *Skeletonema costatum* and *Chaetoceros gracilis* by pH adjustment using hydrochloric acid and sodium hydroxide. The experiment was conducted at different pH values ranging from 8 to 12. *Skeletonema costatum* showed total biomass recovery at pH 11, 11.5 and 12 whereas total recovery for *Chaetoceros gracilis* was obtained at pH value 10.5. Similarly, in the study of Wan et al. [31] 95% of biomass recovery was seen for *Nanochlropsis* sp at pH 10. Regardless of their advantages, these methods are not ~~desired for~~ fully accepted at industrial scale due to uncontrolled flocculation and causes changes in composition of the cells.

2.2.3 Chemical flocculation

Chemical flocculation is a method where inorganic and organic charged species are used for cell aggregation and ~~T~~the process is effective for large scale production. In this method, cells are concentrated and settle due to increased density of the flocculants that can be anionic, cationic or non-ionic polyelectrolytes.

2.2.3.1 Organic Flocculants

In recent times, some advanced materials have been introduced into the process to save time and energy. Flocculants like chitosan and polyelectrolyte are synthetic polymeric compounds having high molecular weight and charge. The process involves the attachment of polymer onto the microalgae surface through electrostatic force. Chitosan has been found to be very effective for cell harvesting of microalgae like *Chlorella vulgaris* and *Chaetoceros muelleri* [32]. In a study by Guldhe et al. [33], chitosan showed a biomass recovery of 55% in 60 min for *Ankistrodesmus falcatus* and in another study, an efficiency of 50-90% was achieved for *Scenedesmus* sp. with a dose of 80 mg/L [34]. Ma et al. [35] reported 96.35 ± 1.96% biomass recovery using chitosan, with 0.12g/L dose, and stirring speed of 150 rpm for 20 min prior to sedimentation. The zeta potential increases with the simultaneous increase in the concentration of chitosan, the charge on microalgae neutralized and the cells flocculate at 0.12g/L of chitosan. In another study [36], chitosan was conjugated with TiO_2 (MNCs) for harvesting of *Chlorella minutissima* at optimal dosage of 0.07g/g. Further, an increase in dose above the optimal range led to a decline in harvesting efficiency due to the electrostatic repulsion of amino groups present on chitosan which destabilized the microalgae cells. The high efficiency can be explained as chitosan has dual characteristics of charge neutralization and bridging, where protonated amine and hydroxyl groups mark chitosan as a good adsorbent. These active sites increase the surface interaction of chitosan with microalgal cells and assist in bridging and sedimentation of the cells [37].

Apart from chitosan, poly y- glutamic acid, moringa oleifera, Cobetia marina L03, Tanin, Tanfloc SL, Zetang and Flopam were also found effective in harvesting of microalgae [38]. Cationic locust bean gum biopolymer (CLBG) is biodegradable, non –toxic organic polymer used for biomass recovery of microalgae. The lower dose of 0.055g/L gave a flocculation efficiency of 96.68 % for

Chlorella sp NCQ [18]. Plant based natural extract obtained from *Moringa oleifera* generated biomass recovery of 75.5 % under 100 min [39]. Cationic polymers are capable of aggregating cells at high rate by reducing the electronegativity of microalgae cells [16]. In a recent study, starch based flocculants synthesized from wheat, potato and corn have been used for microalgae harvesting due to their non-toxicity and low cost [20] (Fig. 3).

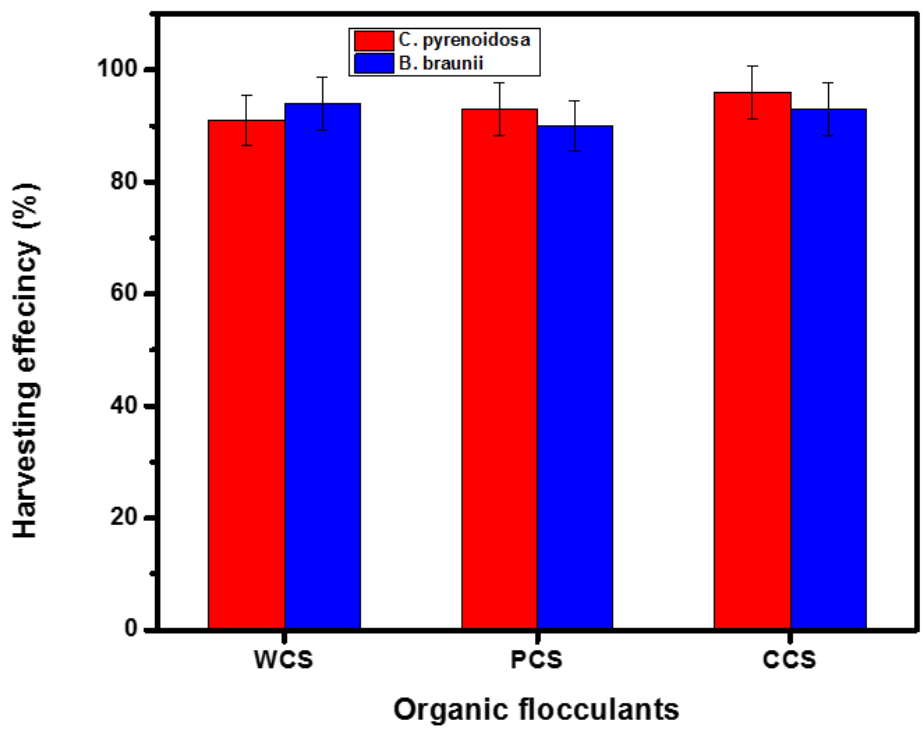

Fig. 3. Organic flocculants: Potato cationic starch (PCS), Corn cationic starch (CCS), and Wheat cationic starch (WCS) used for harvesting of *Chlorella pyrenoidosa and Botryococcus braunii* [20].

Maringa oleifera, was used for biomass recovery of *Chlorella vulgaris* with 89% flocculation efficiency in 120 min, with 1g/L dose [40]. In addition, Zetag (0.01g/L) reported >90% harvesting efficiency for *Chlorella stimataphora* [41]. Organic flocculants are generally bio-friendly;

however, their higher financial cost is main problem. A summary of organic flocculants involved in microalgae harvesting is shown in Table 1.

Table 1

Effect of various organic flocculants their charge, dose (g/L) and time (min) on microalgae biomass recovery (%)

Microalgae	**Organic flocculants**	**Biomass Recovery (%)**	**Charge**	**Medium**	**Dose (g/L)**	**Time (min)**	**Reference**
Chlorella stimataphora	Zetag	>90	Cationic	Fresh	0.01	-	[41]
Chlorella	Magnafloc	8	Anionic	Fresh	0.01	30	[42]
Chlamydomonas Reinhardtii	Magnafloc	24	Anionic	Fresh	0.04	30	
Chlorella sp	Emfloc	48	Cationic	Fresh	0.070	30	
Phaeodactylum tricornutum	Synthofloc	93	Cationic	Marine	0.01 mg	120	[43]
Nanochloropsis salina	Chitosan	98	Cationic	Marine	0.003	60	[44]
Parachlorella	Genfloc	80	Cationic	Fresh water	0.02-0.04	-	[45]
Ankistrodesmus falcatus	Chitosan	55	Cationic	Fresh water	-	60	[33]

Scenedesmus sp	Chitosan	>90	Cationic	Fresh water	0.08	-	[34]
Chlorella vulgaris	Crushed egg shells	98± 0.7	Cationic	Fresh water	0.02-0.04	50	[46]
Nannochloropsis oculata	Tanfloc	97	Cationic	Fresh water	0.05	30	[47]
Chlorella vulgaris		100			0.05	-	
Chlorella vulgaris	Moringa oleifera seed flour	89	Cationic	Fresh water	1	120	[40]
Nanohloropsis sp	Chitosan	90	Cationic	Marine	0.1	60	[48]
Chlorococcum sp	Flopam	84	Anionic	Marine	0.005	-	[49]
Chlorella sp	Ploy separ	95	Cationic	Fresh	0.03	30	[42]
Microcystis	Tannin	97	Cationic	Fresh	0.01	30	[50]
Chlorococcum sp	Magnafloc	84	Anionic	Marine	0.002	30	[44]
Chlorella protothecoides	Poly (y glutamic acid)	98	Cationic	Fresh	0.02	120	[51]
Phaeodactylum	Synthofloc	93	Cationic	Marine	0.001mg/L	120	[43]

Tricornutum							
Chlorella sp	Poly separ	10	Anionic	Fresh	0.02	30	[42]
Isochrysis galbana	Chitosan	90	Cationic	Marine	0.01	30	[41]
Nannochloropsis salina	Zetag	10	Cationic	Marine	0.01	-	[52]
Chlorella pyrenoidosa	WCS PCS CCS	91 93 96	Cationic	Fresh	0.089/g biomass	14	[20]
Botryococcus braunii	WCS PCS CCS	94 90 93	Cationic	Fresh	0.119/g biomass	14	
Chlorella vulgaris	Chitosan	96.35 ± 1.96	Cationic	-	0.12	-	[35]
Chlorella minutissima	Chitosan coated with Fe_3O_4-TiO_2	>98	Cationic	Fresh	0.07g/g	2	[36]
Nannochloropsis oculata	Chitosan	>90	Cationic	Marine	0.075	-	[53]
Chlorella sp NCQ	CLBG	96.68	Cationic	Fresh	0.05	-	[18]

Micractinium sp. NCS2	CLBG	96.64	Cationic	Fresh	0.04	-	[18]
Chlorella sp.	Natural extract plant	75.5	-	Fresh	0.008g/ml	100	[39]
Chlorella vulgaris	Starch	99	Cationic	Fresh	0.116g/g	5	[54]

2.2.3.2 Inorganic flocculants

Inorganic flocculants are the most cost effective among all type of flocculants. The process requires low pH to form cationic hydrolysis products [55]. Generally, ferric chloride and ferric sulphate are used for algal harvesting with up to 99% efficiency, when *Chlorella* sp is flocculated with these inorganic flocculants [22]**.** Aluminum chloride is another commonly used inorganic flocculant, which showed 95% harvesting efficiency with *Chaetoceros gracilis* [30]**.** Biomass recovery of 86% was reported for *Ankistrodesmus falcatus* with alum in 60 min [33]. In another study, alum (250 mg/L) resulted in microalgae *Scenedesmus* sp harvesting with an efficiency of 92.39% at pH 7 [34]. ~~The mechanism behind is, w~~When the alum is added to the aqueous medium, aluminum hydroxide is formed, which is cationic in nature. The formation of superficial cationic charge interacts with negatively charged microalgal cells and hence neutralization of charge occurs as a result, cells forms flocs and settle [37]. $FeCl_3$ is trivalent cation, which has been reported with 100 times higher flocculation efficiency than monovalent cations [56]. *Chlorella* sp showed biomass recovery of 98% with $FeSO_4$. [57]**.** Zhu et al. [58] demonstrated that the addition of $Al_2(SO4)_3$ at a concentration of 2.5 g/L increased the harvesting efficiency of *Chlorella vulgaris*. The reusability of media after the biomass recovery was a key advantage of this method. In another report, polydiallyldimethylammonium chloride (PDADMAC) was found to be more effective than chitosan and superfloc. The results suggested that a higher concentration of cells 1.36 $\times 10^8$ produced a high sedimentation rate [59]. $FeCl_3$ processed a biomass recovery of 86% for *Chlorella vulgaris* with a cell concentration of 0.36 g/L. Inorganic flocculants aggregate cells through charge neutralization; the higher charge density of the flocculant causes a better flocculation rate [60]. The hydrolysis of metal is responsible for the metal oxide formation, which precipitates and forms a positive charge ~~which~~ that results in charge neutralization**.** Various inorganic flocculants with biomass recovery are summarized in Table 2.

Table 2

List of inorganic flocculants used for microalgae harvesting, their dosage (g/L), time (min) and effect on biomass recovery (%)

Microalgae	**Inorganic flocculants**	**Biomass Recovery (%)**	**Dose (g/L)**	**Time (min)**	**Reference**
Cheatoceros gracilis	$FeCl_3$	90-95	0.2	60	[30]
	$FeSO_4$	55			
	$AlCl_3$	95			
Scenedesmus sp	$FeCl_3$	97	0.15	2	[61]
	Alum	92	0.25	-	[34]
Ankistrodesmus falcatus	Alum	86	-	60	[33]

Chlorella sp	Methyl esterified clay	99	-	30	[62]
Tetrasalmis sp	$FeSO_4$	85	-	-	[63]
Chlorella zofingiensis	$FeCl_3$	>90	< 0.09	-	[64]
Chlorella sp.	$Fe_2(SO_4)_3$	90	1.06	-	[57]
Aurantiochytrium sp.	$FeCl_3$	98.8	1	-	[22]
Scenedesmus sp	$FeCl_3$	97.2	0.072	10	[65]
Scenedesmus Spinosus	$FeCl_3$	98.4	7	-	[66]
Chlorella zofingiensis	$Mg(OH)_2$	94	0.219	25	[67]
Chlorella vulgaris	$Al_2(SO4)_3$	92.4	2.5	10	[58]
	PDADMAC	90	0.005	60	[59]
	$FeCl_3$	86	0.448/g dry biomass	-	[60]
	$Al_2(SO4)_3$	77	0.504/g dry biomass		

Though the above-mentioned inorganic flocculants are effective in microalgae harvesting, contamination of harvested biomass is a significant concern. A recent study of Perez et al. [30] reported that incorporating organic and inorganic flocculants (chitosan and $FeCl_3$) together, result in effective harvesting of *Cheatoceros gracilis* in less time. Similar results were seen in *Chlorella* sp. with a biomass recovery of 85% by using $FeCl_3$ and sibfloc - a cationic flocculant [68]. Metal salts are used extensively in the flocculation process but, due to the high concentration of metal along with harvested biomass, the downstream processing or eventual application of the microalgae is limited. On the other hand, plant -based biopolymers are nonhazardous, but generally more expensive [24].

2.2.3.3 Bio flocculation

Bio flocculation has recently emerged as a new development in flocculation technology. The process is associated with the use of micro-organisms including bacteria, fungus and their combinations for microalgae biomass recovery. Bio flocculation is caused by secreted biopolymers known as extracellular polymeric substance (EPS). EPS comprises of sugar, polysaccharides and their derivates such as cellulose, glucose, pectins, mannose, uronic acids, xylose and others [8]. The advantage of this technology is that it does not require the addition of chemical flocculants, which makes it a simple, low-cost process.

Plant-based bio flocculants (gaur gam, inulin) are also used for biomass recovery due to its nontoxic and environment friendly behavior [69]. Guar gam has a reported flocculation efficiency of 94% in *Chlamydomonas* sp [70]. Cationic gaur gum was prepared by integrating NH_3 from N-(3-chloro-2-hydroxypropyl) trimethyl ammonium chloride to the backbone of gaur gum, which neutralize the charge on cells. Plant-based flocculants cationic inulin (60 mg/L) was effective for *Botryococcus* sp. harvesting with a recovery rate of 88.61% in 15 min [71]. Another flocculant *Strychnos potatorum* (seed powder) with a dose of 100 mg/L was used for *Chlorella vulgaris* harvesting [72]. The harvesting conditions were optimized using response surface methodology (RSM) and resulted in harvesting of 99.68% in 30 min. Kothari et al. [73] showed that eggshell can be used as a low-cost bioflocculant for harvesting of *Chlorella* sp. The experiment was conducted at different temperatures (0-50ºC) and concentrations (0-100 mg L^{-1}) for *Chlorella* sp

It was found, the highest flocculation efficiency~~,~~ of 99.3% was observed at 50ºC with100mgL^{-1} eggshell powder. Crushed eggshells were also used as natural flocculants and gave biomass recovery of 98% for *Chlorella vulgaris* [46].

Co-cultivating of microalgae and bacteria is another effective technique for biomass harvesting. Bacterial flocculants such as *Bacillus* sp, *Citrobacter freundii* showed excellent flocculating efficiency [74]. Bacteria can be used as flocculants due to their long filamentous branching structures that helps in absorbing or neutralizing the negative charge on microalgal cells, causing flocculation. The process was used for the harvesting of *Chlorella zonfingienis* in presence of *E. coli*, with a biomass recovery of 83% [75]. Poly y-glutamic acid from *Bacillus subtillis* showed more than 90% flocculation efficiency for *Nanochloropsis oculata* LICME 002, *Phaeodactylum tricornutum*, *Chlorella vulgaris* LICME001, *Botryococcus braunii* LICME 003 [51]. When *Chlorella sorokiniana* was cultivated with *lsaria fumosora* gave 97% flocculation efficiency [76]. 90% of flocculation efficiency was achieved with *Chlorella vulgaris* when cultivated with *Sacchromyces pastorianus* [77]**.** Biomass recovery of 75% was seen for *Picochlorum* sp when cultivated with *Saccharomyces.bayanus var.uvarum* [78]. In another study, Guo et al. [79] observed *Pseudomonas sp.* GO_2 to be an efficient bio-flocculant having similar zeta potential and charge as microalgae,~~.~~ where the biomass recovery of 94.7% was reported with a dose of 12.5 mg/L. The phenomenon of sweeping and bridging promoted the aggregation of microalgae. Microbial flocculant actinomycete *Streptomyces* sp. hsn06 was used for harvesting *Chlorella vulgaris*. The dose of 20 mg/L with 5Mm $CaCl_2$ reported the highest flocculation [80].

In a study by Leong et al. [81] microalgae and bacterial symbiotic association was explored to enhanced biomass for biofuel production and treatment of wastewater. Here, activated sludge act as bio-flocculant for increasing the microalgae-bacteria biomass. The bio-flocculation mechanism of algal-bacteria biomass was attributed to the presence of extracellular polymerase substance (EPS) in the medium with positively charged bacterial cells and negatively charged microalgae cells, which promoted the flocculation. In addition, bio-flocculation can be achieved by co-cultivating certain microalgae with each other without the use of any chemical agents [29].*Chlorella .vulgaris* JSC-7 reported a flocculation efficiency of 76.3% by the process of self-flocculation and when *Chlorella vulgaris* JSC -7 and *Chlorella vulgaris* CNW11 were cultivated together, the flocculation efficiency was reported to be 68% [29]**.** The main drawback of bio-flocculation is that when bacteria is used, there is a high chance of microbial contamination [24]. Table 3 summarizes different bio flocculants used for microalgae harvesting.

Table 3

Role of bio flocculants, their origin, dose and incubation time on flocculation efficiency of various microalgae biomass

Bio flocculants	**Mediated**	**Microalgae**	**Flocculation efficiency (%)**	**Dose (g/L)**	**Time (min)**	**Reference**
Cationic Gaur gam	Plant based	*Chlamydomonas* sp	92	0.08	30	[70]
Egg shell	-	*Chlorella* sp	99.3	0.1	30	[73]
Inulin	Plant based	*Botryococcus* sp	88.6	0.06	15	[71]
Bacillus subtillis (poly	Microorganism	*Nanochloropsis oculata* LICME 002	< 90	0.02	-	[51]

y-glutamic acids)	Microorganism	*Phaeodactylum tricornutum* *Chlorella vulgaris* LICME001 *Botryococcus braunii* LICME 003	< 90	0.02	-	
Sacchromyces pastorianus	Fungi	*Chlorella vulgaris*	90	≥0.4mg/g cell biomass	10	[77]
Saccharomyces bayanus var.uvarum	Yeast	*Picochlorum* sp	75	0.01mg/mL	-	[78]
FLC-hsn06	-	*Chlorella vulgaris*	92.7	0.02	5	[80]
γ- PGA	Fungi	*Dorstenia Brasiliensis*	>98	0.5		[82]
Pseudomonas sp. GO2	Microorganism	*Chromochloris zofingiensis*	94.7	0.0125	-	[79]
FLC-xn-1	-	*Chlorella vulgaris*	85.65	0.043	-	[83]
Bacterial cellulose from *Gluconacetobacter xylinus*	Microorganism	*Chlorella vulgaris*	92	2.08×10^5 cells /mL	48	[84]

2.2.4 Nanomaterials mediated cell harvesting

With the recent advances in technology, researchers have shown interest in nanoparticles for cell harvesting. Studies have reported that nanoparticles can be used for microalgae harvesting due to their large surface area, easy to synthesis, stable in nature, easily removed and can be reused [85]. Recently, nanomaterials based magnetic flocculation has been introduced as a fast, simple and inexpensive technique for microalgae harvesting. Magnetic nanoparticles bind with target cells and help in their separation from the liquid culture by movement in response to an external magnetic field [50]. Magnetic nanoparticles can be used singularly, or in hybrid form to increase the efficiency of harvesting. Generally, magnetic nanoparticles are coated with cationic polymers used to improve their interaction with negatively charged microalgae. Fig. 4 describes the binding mechanism for microalgae to cationic polymer.

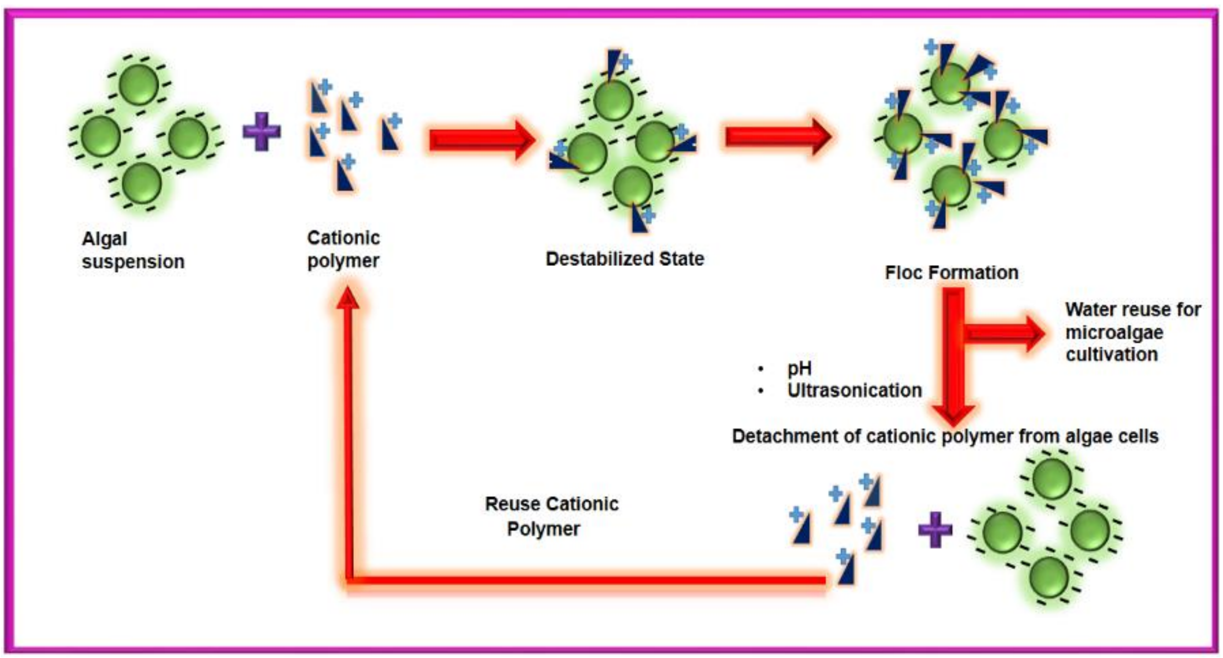

Fig. 4. Schematic illustration showing the mechanism of cationic polymer binding with microalgae, its removal and reuse [38].

Xu et al. [86] reported harvesting of four microalgae sp**.** using magnetic nanoparticles in combination with $FeCl_2$ and $FeCl_3$ in a ratio of 1:1, 1:2, and 1:4. The result showed the highest harvesting efficiency (94-99%) in all four microalgae at a ratio of 1:4. The advantage of using these nanoparticles is mainly their reactivation after application and it can be used in conjunction with ultrasonic treatment. *Synechocystis, Stigeoclonium, Nanochloropsis, Microytis* showed harvesting efficiencies of 63.1%,71.2%,53.0% and 59.1% after five activations.

Magnetic iron oxide (Fe_3O_4) nanoparticles coated with amino-rich polyamide dendimer (PAMAM), has been used as a flocculant for harvesting of oleaginous microalgae. PAMAM used in the experiment was positively charged, which binds with algae cells and showed a harvesting efficiency of 95% in 2 hours [87]**.** The study used magnetic nanoparticles prepared by depositing Fe_3O_4 nanoparticles onto ZnO_2, which was coated with polyethylene amine. The magnetic nanoparticles were used for *Scenedesmus dimorphus* harvesting with an efficiency of 85% [88]. Graphene oxide-iron oxide nanoparticles (GO- Fe_3O_4) were coated with diallydimethylammonuim chloride (PDDA) and gave a flocculation efficiency of 90% for mixed culture of *Scenedesmus, Spirulina, Chlorella, Tetraedron, Hematococcus* [89]**.** Chiang et al. [90] reported that Fe_3O_4-silica magnetic nanoparticles coated with triazabicyelodecene (TBD) act as a strong base and can be used for microalgae harvesting. $BaFe_{12}O_{19}$ was coated with 3-aminopropyltriethoxysilane (APTES), which is known to have super chemical stability and showed 98.5-99.5% harvesting efficiency for *Oleaginous chlorella* sp. at neutral pH. $BaFe_{12}O_{19}$ was easily detached at pH 12 ~~simply~~ by simple shaking, because of its large size. Recently, magnetic core shell silica coated nanoparticles showed 83.7 % harvesting efficiency for *Chlorella pyrenoidosa* with a four-fold higher lipid extraction in the presence of magnetic nanomaterials [91].

Garcia et al. [92] suggested that Bare Fe_3O_4 significantly enhanced the *Chlorella vulgaris* interaction with nanomaterial and improved harvesting efficiency. As a whole, harvesting efficiency relies on surface composition, morphology, dimension of the cell as well as nanomaterial. Additionally, Bare Fe_3O_4 revealed remarkable separation efficiency by weakening the ionic concentration. Also, the addition of the deionized water has strong impact on the detachment of cells from material. Hena et al. [93] utilized polypyrrole/Fe3O4 nanocomposite for biomass recovery of *Botryococcus braunii*, *Chlorella protothecoides*, and *Chlorella vulgaris*. The highest recovery efficiency of 99% for *Botryococcus braunii* was reached with a dose of 0.02g/L of polypyrrole/Fe3O4 nanocomposite. The electrostatic interaction between cell and polypyrrole/Fe3O4 offered high biomass recovery.

Nanomaterials without magnetic properties were also observed to have high flocculation efficiency. Recently, metal-based nanoparticle (ZrO_2) showed significant role in biomass recovery for *Chlorococcum* sp. A low dose of zirchonium di-oxide ZrO_2 (15 mg/L) gave a harvesting efficiency of 82.44%, due to the positively charged ZrO_2 nanoparticles, which effectively bind with negatively charged microalga cells and create a bridge [94]. Zn Al layered double hydroxide (ZnAl-LDH) nanosheets are used for *Chlorella vulgaris* biomass recovery and showed high flocculation efficiency (90%) due to their inert, stable and biocompatible properties [95]. Cellulose nanocrystals (CNC) isolated from cotton wool can be used for harvesting microalgae *Chlorella vulgaris* with unmodified CNC and modified CNC doped with Br[PyBnoo]- g and Br[PyNeBnoo]-g. Providing a dosage of 100 mg L^{-1} unmodified CNC did not show any flocculation; in contrast, 100% flocculation efficiency was found in both modified CNC [96]**.** Cellulose nanofibrils (CNF) play a significant role in microalgae flocculation and do not require surface modification. CNF is considered to be cost effective, eco-friendly. Flocculation is achieved due to the geometric properties and hydrogen bonding that CNF induces. In addition, the ions and sulphur particles in CNF do not hamper the flocculation process [97].

Electroflocculation is another promising harvesting technique and is regarded as a cost-effective approach with downstream processing to facilitate biomass recovery. The effect of electroflocculation on microalgae *Scenedesmus acuminatus* was observed using magnesium electrodes combined with Aluminium (Al), Zinc (Zn), Copper (Cu), Iron (Fe) and brass. A maximum cell count of 1.86×10^7 cells/mL was achieved with iron and a minimum cell count of 1.23×10^7 cells/mL was obtained by using ~~C~~copper [42]. Various findings of nanomaterials mediated flocculation are shown in Table 4. The major drawback of using this technique is the requirement of high energy input. ~~requirement.~~

Table 4

Effect of various nanomaterials on flocculation efficiency of microalgae

Microalgae	**Sample**	**Dosage (g/L)**	**Size (nm)**	**Time (min)**	**Harvesting efficiency (%)**	**References**
Synechocystis	$FeCl_2$ +$FeCl_3$	0.028g/0.927g cell	10-30	5	94.7	[86]
Stigeoclonium,					94.8	
Nanochloropsis					98.1	
Microytis					98.7	
Chlorella vulgaris	Zn Al layerd double hydroxide	1	-	3	90	[95]
Oleaginous	$FeSO_4$ PAMAM	80mg/L	11.0±1.8	2	95	[87]

Scenedesmus dimorphus	PEI coated nanocomposites	0.075g/g cell	53	-	85	[88]
Oleginous chlorella	Go-Fe_3O_4/PDDA	70 mg/L	-	5	95	[98]
Chlorella vulgaris	CNCBr[PyBnoo]- g	30 mg/L	-	-	100	[96]
	CNCBr[PyMeBnoo]-g.	20mg/L				
Oleaginous chlorella	$BaFe_{12}O_{19}$ APTES	-	0.2 µm	3	98.5-99.5	[99]
Chlorella vulgaris	Bare Fe_3O_4	10	50-100	1	>90	[100]
	$Y_3Fe_5O_{12}$	2.5	<100			
	Bare Fe_3O_4	10g/g cell	13.1± 2.7	5	>95	[92]
	Polypyrrole-Fe_3O_4	0.026	50-100	3-5	90.8	[93]
	NiO	0.075	<50	1	98.75	[101]
Botryococcus braunii	Polypyrrole-Fe_3O_4	0.020	50-100	3-5	99	[93]
Chlorella protothecoides		0.022			92.4	
Chlorella sp. UKM2	Bare Fe_3O_4	0.5	-	5	94	[102]

Chlorococcum sp.	ZrO_2	15mg/L	-	45	82.44	[94]
Mixed algae	MgAC-Cerium aminoclaymixture	1	20-1000	~100	60	[103]
Mixed algae	MgAC Fe_3O_4	4.19-4.72	3.5-7.14	>80	10	[104]
Chlorococcum sp	Ti	15mg/L	-	82.46	45	[105]
Chlorella sp	Fe_3O_4@Arginine	200 mg/L	-	95	30	[106]
Scenedesmus sp	Fe_3O_4	0.14	-	95	27	[107]
Chlorella vulgaris	Fe_3O_4	0.5	50-100	68	-	[15]
	Yttrium(Y-Fe_3O_4)	0.5	<100	83.6	-	[15]

Flotation is another technique introduced for microalgae cell harvesting, in this process bubbles are formed, which attach to the desirable particle size and hence causes cells to upswing to the surface and concentrate [108]**.** *Dunaliella salina* and *Chlorella zofingiensis* reported the harvesting efficiency of 95% and 93% respectively [109]. Reports on economic feasibility, high cost and operational cost are major concern.

Another effective approach to conciliate the issue related to microalgae biomass harvesting is the utilization of fixed support material for microalgae growth. In a study by Li et al [110] porous substrate bioflim photobioreactor were employed to grow microalgae. However, the clogging, inadequate light exposure, limited nutrient diffusion are underlying drawback associated with fixed support material. To overcome these limitations fluidized support material is used due to its freely movable characteristics in the culture medium allowing microalgae cells to get attached to its surface and grow. Fluidized bed bioreactor packed with polyurethane foam material is explored for *Chlorella vulgaris* growth and easy recovery [111]. The underlying mechanism for the attachment of cell to the polyurethane relies on the rise in the interrelate energies and chemisorption arising from the hydrophilic and hydrophobic attraction between the cells and support material [112].

3. Lipid Extraction in microalgae

Pre-treatment of microalgae for cell disruption and extraction of lipids is an energy-intensive step, which limits the sustainability of microalgal biofuel production. Microalgae are made up of highly complex cell walls, polysaccharides intercalated with protein [113]. It is not easy to break the cell wall and extract the lipid completely without the application of a large amount of energy. Usually, prior to lipid extraction, a suitable cell lysis method is performed depending on the algae cell type. Various cell disruption methods for microalgae lipid extraction are illustrated in Fig. 5. The cell disruption methods are broadly divided into mechanical (homogenizer, sonication, microwave, pulse electric field) and non-mechanical or chemical methods (acid, surfactant, enzymes) [114]. Moreover, a distinction prevails between chemical and mechanical methods as chemical methods are easy scalable in contrast with mechanical methods [115]. Cell disruption is followed with lipid extraction where, polar (methanol, chloroform) and non-polar (hexane) solvents are used. Extraction of lipids using suitable environment-friendly solvents is a challenging area of research in biodiesel production. There is an urgent need to find alternative solvents, which can be used for lipid extraction without harming the environment and health. ~~Cell disruption methods for lipid extraction are illustrated in Fig. 5.~~ Single step integration technology via combining cell disruption and lipid extraction techniques can be used in order to achieve high efficiency and better results [116].

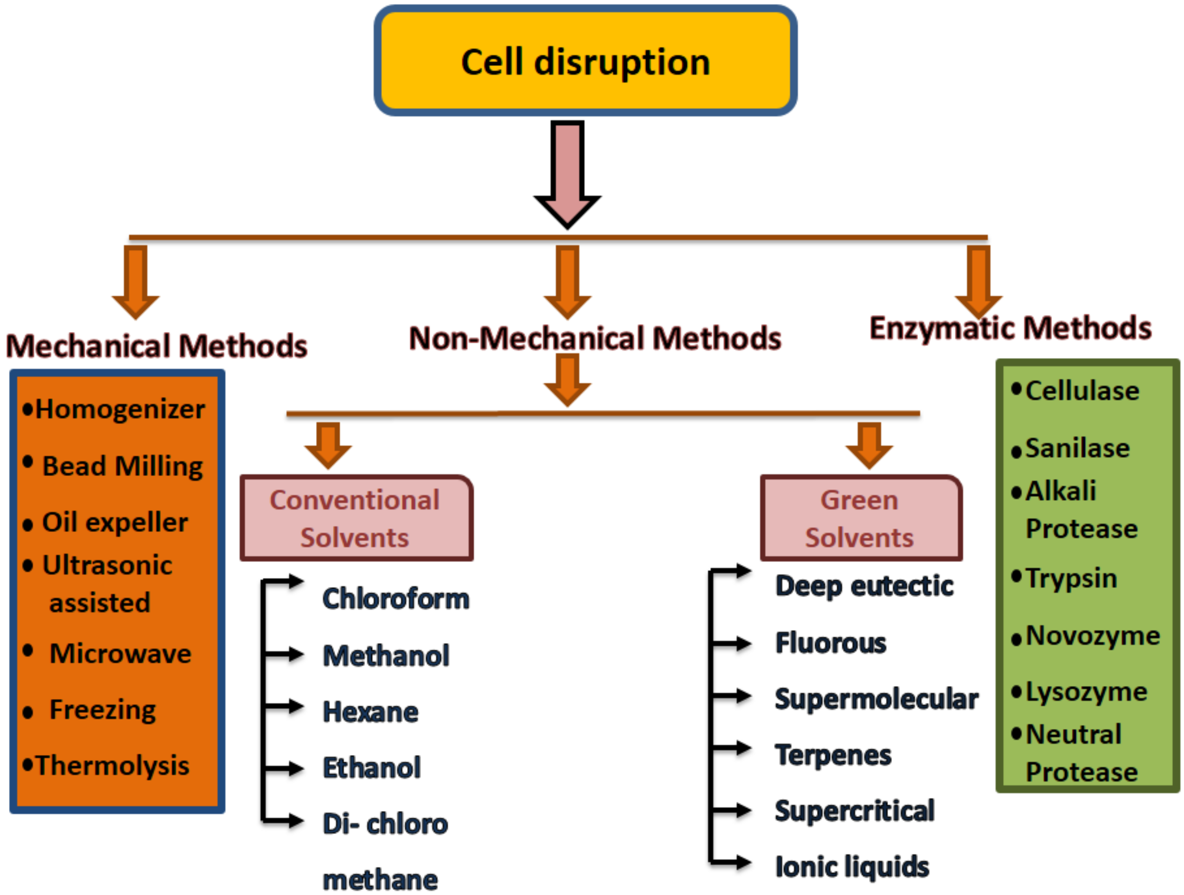

Fig. 5. Detailed representation of cell disruption methods for lipid extraction [13, ~~114~~ 117,~~115~~ 118]

3.1 Solvent mediated cell disruption and lipid extraction techniques: Conventional approach

~~Among them,~~ Effective and efficient lipid extraction is a critical step in order to achieve high yield. Algal lipids are broadly categorized into polar and non-polar lipids. Non-polar lipid include: mono, di and triglycerides, which are valuable for biodiesel production while algal polar lipids such as phospholipids and glycolipids are used for other purposes. Traditionally, lipid extraction from microalgae is carried out using a combination of polar (methanol, chloroform) and non-polar (hexane) solvents by a standard protocol (Fig. 6). The Folch ~~[116]~~[119] and Bligh and Dyer methods ~~[117]~~ [120] have been applied extensively for lipid extraction using chloroform / methanol combination with various ratios of the solvents and the extracted lipids are gravimetrically quantified. Yang et al. ~~[118]~~ [121] proposed a method for lipid extraction from *Picochlorum* sp, using ethanol, where ethanol has a strong affinity to the complex structure of lipids, 33.04% lipid yield was reported. The study has shown that 99.4% lipid extraction can be obtained when ethanol is recycled using a distillation tower to extract the lipid repeatedly from the microalgae biomass.

Several organic solvents have been shown to give feasible lipid extraction, including acetone, benzene, n-hexane, methanol, chloroform, dichloromethane etc. ~~[119]~~[122]. Among these organic solvents, a combination of methanol and chloroform works effectively to release lipids from microalgae cells ~~[120]~~[123].~~ Fig. 6.~~ Here, methanol has the capacity for cell lysis and chloroform is used as an eluting solvent, which facilitates lipid extraction. Table 5 summarizes the studies for organic solvent mediated lipid removal from microalgae.

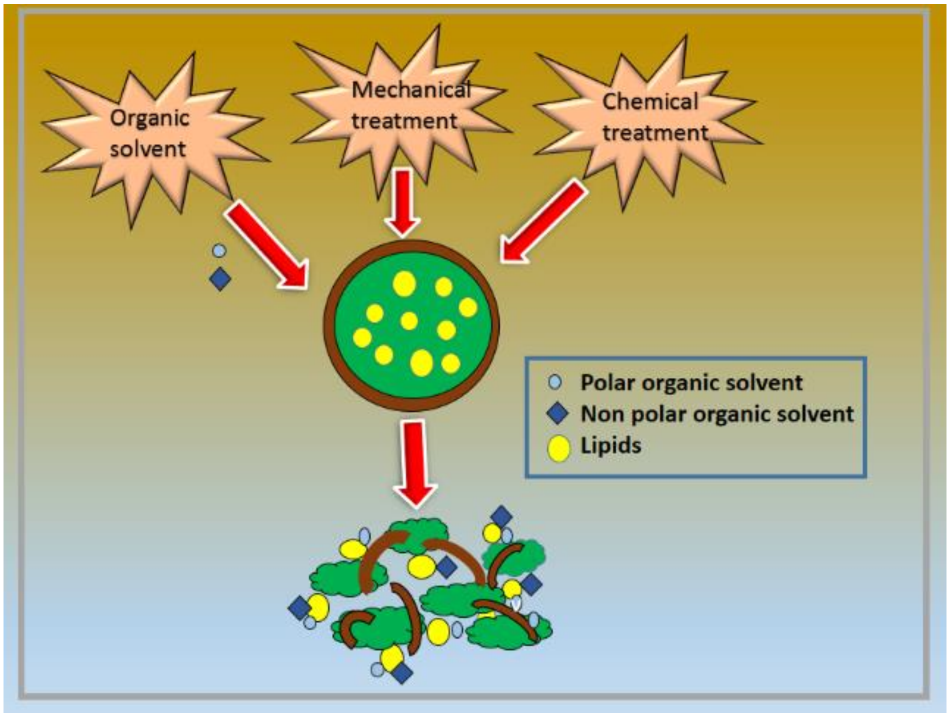

Fig. 6. Diagrammatic representation of different methods known for microalgae cell disruption for lipid extraction ~~[114]~~[117]

~~These well-recognized methods have been used extensively with wet as well as dry algal biomass. Wu et al. [121] reported an enhanced efficiency of lipid removal from wet microalgae cell by using Folch method. Likewise, the Bligh and Dyer method was also successfully implemented for lipid extraction from wet microalgae *Chlorella vulgaris* with a yield estimated at 95%. Lipid extraction from wet biomass extensively reduces the overall cost by skipping the step of dewatering.~~

Table 5

Direct organic solvents for lipid extraction from microalgae

Solvent system	Ratio	Microalgae	Conditions	Phase	Lipid (%)	References

				(Dry/Wet) cell		
Chloroform-methanol	2:1	*Thraustochytrium sp*	50mg/3ml, 15 min	Dry	10.7	~~[122]~~[124]
	1:2	*Botryococcus braunii*	1g/10mL, 300 min	Dry	19.2	~~[123]~~[125]
Hexane-ethanol	1:1	*Tetraselmis* sp	10g/100mL, 120min	Dry	5.16	~~[124]~~[126]
Hexane	-	*Chlorella vulgaris*	10g/200mL, 360 min	Dry	60.2	~~[125]~~[127]
Chloroform – ethanol	2:1	*Nannochloropsis* sp	3g/10ml, 30 min	Wet	90.9	~~[126]~~[128]
Hexane – ethanol	2:1				60.5	
Ethanol (soxlet)	-	*Aceutodesmus obliqus*	20g, 30 min	Dry	9.48	
Hexane (Soxhlet)	-	*Aceutodesmus obliqus*			4	~~[127]~~[129]
Ethanol hexane –	2:1	*Aceutodesmus obliqus*			12.05	

(Soxlet)			
Hexane – ethanol (Soxlet)	1:1	*Aceutodesmus obliqus*	11.76
Ethanol hexane – (Soxlet)	1:2	*Aceutodesmus obliqus*	12.03

The problem associated with using polar solvents is that chlorophyll is also extracted along with lipids. Hexane is unable to cross the membrane, which is made up of phospholipids that bind with proteins [13]. Moreover, the toxic and flammable characteristics of these organic solvents restrict their long-term use. Hence, these solvents are solely not sufficient for the extraction of lipids. Extraction of lipid using suitable or renewable solvents is the challenging area in biodiesel research. Search for some alternative solvents is the major focus in the work of algal lipid extraction without hampering the environment and health.

3.2 Non-conventional approaches for cell lysis and lipid extraction

To alleviate the high cost and toxicity, there are some non-conventional approaches that have been applied to microalgae cells for lipid removal. Several efforts have been adopted to design non-conventional cell disruption and lipid extraction techniques, which are discussed in detail below.

3.2.1 Mechanical methods for cell disruption

A pre-treatment approach, which aims to burst cell membranes, can be facilitated by mechanically and physically assisted techniques, followed by separation involving organic solvents. These mechanical treatments can be beneficial to release intracellular lipids efficiently ~~[128]~~[130]. ~~Mechanically treated cell disruption methods include: bead mill, homogenizer, oil and expeller pressing etc. [129]~~

3.2.1.1 Microwave assisted

Electromagnetic irradiation in the frequency range 0.3-300 GHz is recognized as microwave ~~[114]~~[117]. Microwave radiation can be used in nutraceuticals as well as pharmaceuticals for the release of intracellular compounds. Microwaves are able to interact specifically with polar molecules i.e. water and to generate heat, hence, the algal cell membrane can be damaged, and the lipids can be extracted ~~[120]~~[123]. This process is used for dry and wet algal biomass effectively ~~[119]~~[122]. Moreover, Garoma et al. ~~[130]~~[131] has reported that an enhancement of 28.8% lipid has been achieved from dry cells of *Chlorella vulgaris* using microwave radiation followed by solvent mediated extraction.

3.2.1.2 Ultrasonication

Ultrasonication, is one of the most efficient method of cell disruption and has been extensively used for lipid removal for decades. During this process, sound waves having a frequency of above 20 kHz are applied to the culture medium, which generates an alternative arrangement of compression (high pressure) and rarefaction (low pressure) ~~[131]~~[132]. Micro bubbles can form in the low-pressure region, leading to cytoplasmic disruption and the release of lipids biomolecules. According to Ma et al. ~~[132]~~[133], ultrasonication was one of the best suited method for lipid extraction from *Chlorella* sp. with a maximum amount of lipid content achieved as 11.6 wt%.

3.2.1.3 Pulsed electric field

This method is used for cell disruption by generating short electric pulses with high electric field force to create micropores at the cell membrane~~[133]~~ [134]. Hence, this process is known as electroporation or electro-immobilization. According to Flisar et al. ~~[128]~~ [130], pulsed electric field has been successfully implemented to extract lipid globules from *Chlorella vulgaris*. The results indicated that the higher exposure time increases the lipid yield. However, these non - conventional mechanically assisted cell disruption and extraction techniques are not very feasible at large scale, due to high energy requirements and cost.

3.2.2 ~~Ionic liquids and switchable solvent~~ Non-conventional solvents and chemicals for cell disruption and lipid extraction

Chemical methods imply for the disintegration of cell wall and enables the recovery of intracellular components. Here, we focus on the techniques that are used currently for the lipid recovery. Acid hydrolysis mediated cell disruption and extraction of intracellular lipids is a chemical based method, where strong acids are used for cell lysis. In a study, H_2SO_4 was used for *Spirulina platensis* lysis, and 17.5 % lipid yield was observed ~~[160]~~[135]. However, the use of acid can raise concerns for safety at industrial scale. Recently, various oxidative agents have been explored such as, TiO_2 and $FeSO_4$ for lipid extraction ~~[161162]~~[136,137]. Hua et al. ~~[161]~~[136] observed a 1.5fold increase in lipid production compared to untreated cells, due to oxidation by the T_iO_2 anode. In another study, biomass of *Chlorella vulgaris* was pre-treated with $FeSO_4$ for 3 min and resulted in a 2.4fold increase of lipid production ~~[162]~~[137]. Seo at al. [138] reported another technique for lipid extraction using per sulphate-based oxidation. Initially, $FeCl_3$ was used to concentrate the microalgal cells *Chlorella* sp. KR-1, and then per sulphate catalyst was used for biomass oxidation. The result indicated that the lipid extraction efficacy was 95% at 90ºC. A change in lipid constituents was found when higher oxidation power was used as saturated fatty acid C16:0 and C18:0 seemed to be increased with a subsequent decrease of mono and poly unsaturated fatty acids C18:1 and C18:2. Despite the effectiveness of this approach, cost and energy consumption are high, which makes the process unsuitable for large scale application.

The use of cationic surfactant like Cetyl pyridinium bromide (CFB) is another approach, effective for cell disruption. In this process, a complex is formed between hydrophobic tail of cationic surfactant and phospholipids in microalgae resulting in cell membrane lysis and the release of intracellular lipids ~~[163]~~[139]. Park et al. [140] discussed the effect of cationic surfactant sodium dodecyl benzene sulfonate on free fatty acids (FFA) in *chlorella vulgaris.* Significant increase in lipid recovery (96.7%) was reported in presence of 1% sulfuric acid. In another study, lipid yield was improved with 64.2%, when 200 mg/L of oligomeric surfactant was exposed to *Nannochloropsis* sp ~~[126]~~[128]. Besides, studies exhibited the practice of surfactant method could lower the usage of organic solvents and simultaneously improve the lipid productivity [116,~~126~~128]. Nonetheless, it should be noted that study in this area is still lacking.

Nanoparticle engineering has enabled new techniques for cell lysis and lipid extraction that has already overcome many challenges in the microalgae biorefinery process. From cultivation to harvesting, lipid extraction and improving the quality of biofuel, nanomaterials have shown encouraging potential. Cetrimonium bromide octyltriethoxysilane (CTAB-OTES) coated magnetic nanoparticle were used for cell harvesting, which resulted in better lipid productivity. A lipid content of 277.2mg lipid/ g cell was reported which is 2-3 times higher than the lipid extracted by using hexane via conventional extraction methods ~~[155]~~[141]. Cellulose nanofibrils (CNF) have been demonstrated as a cost-effective and eco-friendly method to increase lipid removal from microalgal cells. Using nanoparticles can be considered as a substitute for solvent based extraction with low noxiousness, reusability and stability [85]**.** An improvement in lipid productivity by 8.9, 39.6, and 18.5 % was observed when carbon nanotubes (CNTs), Fe_2O_3 and MgO were exposed to *Scenesdesmus* sp at concentration of 5mg/L, 5 mg/L, and 40 mg/L respectively ~~[159]~~[142]. Metal oxide ZrO_2 nanoparticle (15 mg/L) enhanced the lipid release in *Chlorococcum* sp. by 2 folds in presence of chloroform: methanol (2:1) [94]. Similarly, high lipid recovery was achieved in *Chlorococcum* sp. using Ti nanoparticle with the dose of 15 mg/L [105].

Enzymes are very effective in lipid removal from microalgae. Enzymes can bind with some specific molecules present in the cell wall and are able to hydrolyze the bonds, causing membrane rupture, which facilitates lipid extraction ~~[165]~~[143]. Enzyme selection is a key parameter for lipid removal from microalgae, as the efficiency of enzymes differs for the various microalgal strains. Published research illustrated that the cell wall of *Chlorella vulgaris* was burst by using enzymes including chitinases, lysozymes, pectinase, amylase, cellulase etc. whereas, amylase and cellulase had no effect on algal cell disruption ~~[166]~~[144]. A combination of enzymes has also been used for lipid removal with higher efficiency. High cost and highly selective behavior of enzymes are major drawbacks in their application. In a recent study, ozone rich microbubble technique was explored in presence of methanol in 1:2 (v/v) for the lipid recovery from *Dunaliella salina*. The study revealed the increase in hexadecanoic acid and octadecanoic acid with 2.87×10^{-3} and 6.37×10^{-4} g/g dry biomass respectively ~~[N15]~~[145]. The technique was proposed as a low energy consuming process and a replacement of conventional solvent based methods.

Solvents based lipid extraction techniques are very much acceptable, where green and renewable solvents are getting more acceptance than conventional organic solvents. Ionic liquids (ILs) are commonly used for the efficient extraction of lipids, due to their ecofriendly, non-flammable behavior and their capability to maintained in the liquid state at wide variety of temperature (0-140°C). Apart from biphasic systems, the process offers single solvent extraction, which is time saving ~~[115]~~**[**118]**.** To et al. [113] used low-cost choline and amino acid based ILs for efficient lipid extraction from *Chlorella vulgaris* and *Spirulina platensis*. Apart from lipids, these ionic mixtures can be used for the extraction of carbohydrates and other bioproducts. A mixture of ILs was prepared (choline hydroxide and amino acids) and was exposed to heat for 3hours at 70ºC. It was reported that the treatment of ILs over *Chlorella vulgaris* and *Spirulina platensis* yielded 30.6% and 51% lipids and 71% and 26% carbohydrates respectively. These mixtures of ILs have the property to dissolve the lipid and leave carbohydrates behind. Similarly, 1-butyl-3-methylimidazolium methyl sulfate ([BMIM][$MeSO_4$]) with methanol reported the maximum lipid extraction from *Neochloris oleoabundans* [146] (Table 6). Despite the high yields, insufficient knowledge of ionic salts and their present cost, making the process unfavorable for lipid extraction from microalgae at large scale.

Switchable solvents are a new group of solvents known for their reversible properties from hydrophobic to hydrophilic ~~[134]~~[147]**.** In the process of microalgae lipid extraction, low polarity lipids are dissolved in a low polarity form of switchable polarity solvent. When CO_2 is incorporated, the solvent increases its polarity and hence, lipids are separated ~~[135]~~[148]. N-ethylbutylamine was used as a switchable solvent for lipid extraction from *Neochloris oleoabundans* with 17% yield obtained from dry biomass [~~136]~~[149].

Deep eutectic solvents (DESs) have emerged as an environment friendly solvent consisting of two or more components of eutectic solvents with the characteristics of biodegradability, low cost, low volatility, eco-friendliness and renewability. These are innovative substitutes for organic solvents and even ILs. Aqueous deep eutectic solvents (aDES) were found to be suitable to enhance lipid recovery from microalgae, when *Chlorella* sp. was pretreated by three aqueous solvents: choline chloride oxalic acid (aCH- O), choline chloride ethylene glycerol (aCH-EG) and urea acetamide (aU-A). All these solvents were taken in 1:2 ratio and maintained at 40ºC for 48 h. Later, the biomass and aDES were separated by centrifugation and continuously washed with water. Lipid production of treated biomass with (aCH- O), (aCH-EG), (aU-A) was found to yield 80.90%, 66.92% and 75.26% respectively, which is 50% higher than the untreated biomass ~~[137]~~[150]**.**

~~To reduce the operational cost of lipid extraction, wet microalgae biomass is preferred, which skips drying and dewatering steps and reduces around 59% of energy use [13]. DES was found to be exceptional effective in lipid extraction from wet algae biomass *Chlorella* sp and *Chlorococcum* sp [139]. In this process, Choline Acetic acid (Ch-Aa), Choline Oxalic acid (Ch-Oa), Choline Propanedioic acid (Ch-Pa), DESs were found to be efficient for lipid extraction applied in the one step method, compared to two step operation. Among these solvents, Ch-Aa- methanol-H_2SO_4 in a ratio of 60:40:3 showed maximum FAME yield. Huang et al. [101] showed that a biodiesel and methanol mixture can be used instead of chloroform and methanol for the efficient extraction of lipids. The result showed that 68% of total lipid were extracted from wet microalgae biomass where biodiesel penetrates the cells and increases miscibility with lipids.3.2.3 Surfactant and nanoparticle mediated lipid extraction~~

~~Surfactant assisted disruption of *Scenedesmus* wet biomass is a novel approach for cost-effective and sustainable lipid recovery [140]. Among all the surfactants, Myristyltrimethylammonium bromide (MTAB) and Decyldimethylammonio propanesulfonate (3_ DAPS) when mixed with hexane and isopropanol showed the best FAME recovery which was 160-fold higher than the untreated biomass of *Scenedesmus*. Researchers are currently investigating combination techniques for improved lipid extraction from algal cells where physical and chemical methods are combined. Disruption of microalgae cell lining has been reported by applying shear force, microwave radiation, ultrasound, and homogenizer or in combination with organic solvents.~~

~~Derakhshan et al. [141] investigated physical disruption of *Chlorella vulgaris* by using ultrasound for 5 min at 50ºC in presence of chloroform and methanol, the lipid yield was reported to be 221 mg/g cells. 65 % of lipid recovery was seen for *Chlorella vulgaris* when ultrasound (20 KHz, 20-60 min), homogenization (7000 rpm, 20-60 min) and microwave (10min, 120ºC) was used in combination with organic solvents, chloroform and methanol [142].~~

~~Ma et al. [35] studied cell lysis by using ultrasound along with methanol and chloroform (1:2) for lipid reco~~Aiming to the environment and safety concern, supercritical fluids come forth as green solvent in order to replace organic solvents. Carbon dioxide is widely explored supercritical solvent having critical pressure and temperature of 72 bar and 32°C respectively [151]. In another study, supercritical CO_2 gave a lipid recovery of 92% from mixed *Scenedesmus* sp. under industrial plant conditions (12 MPa, 20°C). Amongst the 92% of total lipids, polyunsaturated fatty acids (PUFA) comprised 59% w/w ~~[143]~~[152]. Likewise, the lipid productivity of *Desmodesmus subspicatus* augmented up to 45% when exposed to supercritical CO_2 under 30 Mpa and 60°C [153]. Supercritical CO_2 was considered as economically viable process with the return rate of 10.5 % and net profit was assessed to be $8.31 million [154]. Table 6 summarizes recent studies on chemically assisted lipid extraction process.

~~Microwave assisted extraction provides heat directly to cells, which creates water vapor inside the cell and hence is responsible for breakdown of the cell wall, which enables the removal of intracellular lipids. *Chlorella vulgaris* dried biomass was exposed to ultrasound with 40 KHz frequency with the combination of chloroform: methanol and total triglyceride was reported to yield 55% while only 15 % was obtained with conventional methods (chloroform: methanol) [144]. Wet algae biomass of *Chlorella protothecoides* exposed to ultrasonic radiation in presence~~

~~of chloroform and methanol, gave a lipid content of 42±2.97% whereas the lipid content of untreated sample was 9.34±1.66 % [145]. In a study by Onumaegbu [146], wet algal biomass of *Scenedesmus quadricauda* was pre-treated using microwave for lipid extraction in presence of solvent. In a recent study, *Chlorella vulgaris* was treated with mild pressure and heat shock for high lipid recovery. Additionally, mild pressure triggers the accumulation of neutral lipids in microalgae [147]. An energy efficient process using high shear stress gave a lipid extraction of 83% in 5 mins from *Nannocholoropsis* sp (250 g/L).Solvent screening indicated that the combination of solvent using hexane: ethanol: acid along with mixing provide high mass transfer rate and excellent lipid extraction yield [148]. Table 6 and 7 describe the lipid extraction from wet and dry algal biomass using various emerging techniques respectively.~~

~~**Table 6**~~

~~Lipid extraction techniques applied on wet microalgae~~

~~**Microalgae**~~	~~**Techniques**~~	~~**Lipid %**~~	~~**Conditions**~~	~~**References**~~
~~*Nanochloropsis oculata*~~	~~Photocatalysis TiO_2~~	~~52~~	~~SI: 990W/m^2, SS: 440rpm~~	~~[149]~~
~~*Chlorella* sp~~	~~Deep Eutectic solvents~~	~~30~~	~~60 min, 130°C~~	
~~*Chlorococcum* sp~~		~~35~~	~~60 min, 110°C~~	~~[139]~~
~~*Picochlorum* sp~~	~~Solvent based (methanol) 5ml~~	~~33.04~~	~~30 min~~	~~[118]~~
~~*Chlorella Protothecoides*~~	~~Ultrasound + chloroform :methanol (1:2)~~	~~42.1~~	~~P:500W, F: 20KHz~~	~~[145]~~

~~*Nanochloropsis sp*~~	~~ILs~~	~~59~~	~~1440 min,100°C~~	~~[150]~~
~~Mixed algal cultures~~	~~Acid/ base hydrolysis~~	~~59~~	~~30 min, 90°C~~	~~[151]~~
~~*Chlorella* sp~~	~~Per sulphate based oxidation~~	~~95~~	~~Per sulphate (2Mm), 90°C~~	~~[138]~~
~~*Chlorella* sp.~~	~~C_6DIPA-Im~~	~~123.8 ± 1.8 mg/g~~	~~RT, 12 h, SS: 500 rpm~~	~~[152]~~
	~~C_6DIPA-Pyr~~	~~115.4±1.4 mg/g~~		
	~~C_6DIPA-Tiz~~	~~109.1±1.1 mg/g~~		
~~*Nannochloropsis* sp~~	~~Hydrolytic cellulase enzymes~~	~~88.7~~	~~120 min,70°C~~	~~[153]~~
~~*Scenedesmus quadricauda*~~	~~Microwave + methanol : sulphuric acid (50:1)~~	~~49~~	~~8 min, 600W~~	~~[146]~~
~~*Chlorella vulgaris*~~	~~Heat shock +hexane: isopropanol (3:2)~~	~~94~~	~~1.2.5 Kg/cm^3, 5-15 min, 50-70°C~~	~~[147]~~
~~*Nannochloropsis* sp.~~	~~High sheer mixer+ Hexane/ethanol/acid (9:1:0.4)~~	~~83~~	~~5min, 55°C, 8000rpm~~	~~[148]~~

~~C_6DIPA-Im: Dissopropanolamine -Imidazole, C_6DIPA-Pyr: Dissopropanolamine- Pyrazole, C_6DIPA-Tiz: Dissopropanolamine- 1, 2, 4-Triazole~~

~~S.I: Solar intensity~~

~~S.S: Stirring speed~~

~~P: Power~~

~~F: Frequency~~

~~RT: Room Temperature~~

~~Table 7~~

~~Lipid extraction techniques applied on dry microalgae~~

~~**Microalgae**~~	~~**Techniques**~~	~~**Lipid %**~~	~~**Conditions**~~	~~**References**~~
~~*Chlorella* sp~~	~~ILs~~	~~30.6~~	~~180 min, 70°C~~	~~[113]~~
~~*Spirulina* sp~~	~~(Choline amino acid based)~~	~~51~~		
~~*Chlorella* sp~~	~~Deep eutectic solvent choline chloride-oxalic acid (aCh-O) + ethyl acetate: ethanol (1:1)~~	~~80.90~~	~~120 min, 50°C~~	~~[137]~~
~~*Chlorella vulgaris*~~	~~Ultrasound + ethanol~~	~~22.1~~	~~8min, 50°C~~	~~[141]~~
	~~Enzyme assisted (Sanilase)~~	~~34~~		~~[154]~~
	~~Enzyme assisted (Trypsin)~~	~~34~~	~~Cocn:8%~~	
~~*Chlorella* sp~~	~~Cetrimonium bromide (CTAB)~~	~~71.2~~	~~0.8mM, 291 mg/L~~	~~[155]~~
~~*Chaetoceros gracilis*~~	~~Liquefied dimethyl ether~~	~~22.0~~	~~25°C, 0.59MPa~~	~~[156]~~
~~*Pleurochrysis carterae*~~		~~11.6~~		

~~*Chlorella vulgaris*~~	~~Aluminum sulfate + hexane: ether (1:1)~~	~~28.3~~	~~2.5g/L, 60 min~~	~~[157]~~
	~~Aluminum potassium sulfate + hexane :ether (1:1)~~	~~29.4~~		
	~~Ferrous sulfate + hexane: ether (1:1)~~	~~26.8~~		
~~*Chlorella vulgaris*~~	~~Ultrasound assisted+ methanol: chloroform (1:2)~~	~~24.45 ± 1.67~~	~~10 min, 60°C~~	~~[35]~~
~~*Schizochytrium* sp.~~	~~Ultrasonic assisted + soxhlet ethanol~~	~~93.76 ± 0.48~~	~~150 W, 30 min, 50°C~~	~~[158]~~
~~Mixed *Scenedesmus* sp.~~	~~Supercritical CO_2~~	~~92~~	~~12MPa, 20°C~~	~~[143]~~

~~Conc: Concentration~~

~~3.2.4 Chemically assisted lipid extraction from microalgae~~

~~Chemical treatments for lipid removal include: acid hydrolysis, oxidation and surfactant mediated techniques [114]. Table 8 summarizes recent research on chemically assisted lipid extraction.~~

~~**Table 8**~~

~~Pre-treatment of algae for lipid extraction by chemical methods~~

~~**Chemical treatment**~~	~~**Microalgae strains**~~	~~**Organic solvents**~~	~~**Condition**~~	~~**Lipid yield (%)**~~	~~**References**~~
~~Acid hydrolysis~~	~~*Spirulina platensis*,~~	~~n-hexane~~	~~H_2SO_4,60 min, 100°C~~	~~17.5~~	~~[160]~~

	~~*Chlorella vulgaris*~~	~~Hexane~~	~~H_2SO_4, 60 min, 120°C~~	~~33.74~~	~~[164]~~
~~Oxidation~~	~~*Scenedesmus dimorphus*~~	~~Chloroform : methanol~~	~~Anodic oxidation, Ti_4O, 20V~~	~~23.4~~	~~[161]~~
	~~*Chlorella vulgaris*~~	~~Ethanol (2:1)~~	~~Iron oxidation, $FeSO_4$, 3min~~	~~17.2~~	~~[162]~~
~~Surfactant~~	~~*Scenedesmus obliquus*~~	~~Chloroform: ethyl acetate~~	~~CPB, 2880 min, 45° C,~~	~~31.4~~	~~[163]~~

~~3.2.5 Biologically mediated lipid extraction~~

Table 6

Chemical based treatment of microalgae for lipid extraction

Chemical treatment	Microalgae strains	Organic solvents	Condition	Lipid (%)	References
Photocatalysis TiO_2	*Nanochloropsis oculata*	-	SI: 990W/m^2, SS: 440rpm	52	~~[149]~~[155]
	Chlorella sp	Choline chloride-Acetic acid (Ch-Aa)	60 min, 130°C	30	
	Chlorococcum sp		60 min, 110°C	35	~~[139]~~[156]

Deep Eutectic solvent	*Chlorella* sp	oxalic acid (aCh-O) + ethyl acetate: ethanol (1:1)	120 min, 50°C	80.90	~~[137]~~[150]
Organic Solvent	*Picochlorum* sp	Methanol	30 min	33.04	~~[118]~~[121]
	Chaetoceros gracilis	Liquefied dimethyl ether	25°C, 0.59MPa	22.0	~~[156]~~[157]
	Pleurochrysis carterae			11.6	
Ionic Liquids	*Nanochloropsis* sp	Hydrated phosphonium	1440 min,100°C	12.8	~~[150]~~[158]
	Chlorella sp	Choline amino acid based	180 min, 70°C	30.6	[113]
	Spirulina sp			51	
	Neochloris oleoabundans	[BMIM][$MeSO_4$]	120 min, 70°C	17	[146]
	Chlorella sp	C_6DIPA-Im C_6DIPA-Pyr C_6DIPA-Tiz	RT, 12 h, SS: 500 rpm	123.8 ± 1.8 mg/g 115.4±1.4 mg/g 109.1±1.1 mg/g	[159]

Metal Sulfates	*Chlorella vulgaris*	Aluminum sulfate + hexane: ether	D: 2.5g/L, 60 min	28.3	~~[157]~~[160]
		Aluminum potassium sulfate + hexane :ether		29.4	
		Ferrous sulfate + hexane: ether (1:1)		26.3	
Enzymes	*Nannochloropsis* sp	Hydrolytic cellulase	120 min,70°C	88.7	~~[153]~~[161]
	Chlorella vulgaris	Sanilase Trypsin	Cocn:8%	34	~~[154]~~[162]
Supercritical CO_2	Mixed *Scenedesmus* sp	-	12MPa, 20°C	92	~~[143]~~[152]
Nanomaterials	*Chlorococcum* sp	ZrO_2	Chloroform: methanol D: 15 mg/L	78.52	[94]
		Ti	Chloroform: methanol, D: 15 mg/L	74.29	[105]
	Chlorella sp	CTAB-decorated Fe_3O_4	Hexane, 0.8mM, 291 mg/L	71.2	~~[155]~~[1421]

Acid hydrolysis	*Spirulina platensis*	n-hexane	H_2SO_4, 60 min, 100°C	17.5	~~[160]~~[135]
	Chlorella vulgaris	Hexane	H_2SO_4, 60 min, 120°C	33.74	[163]
	Mixed algal cultures	Methanol	H_2SO_4, 30 min, 90°C	59	~~[151]~~[164]
Oxidation	*Scenedesmus dimorphus*	Chloroform : methanol	Anodic oxidation, Ti_4O, 20V	23.4	~~[161]~~[136]
	Chlorella vulgaris	Ethanol (2:1)	Iron oxidation, $FeSO_4$, 3min	17.2	~~[162]~~[137]
	Chlorella sp	Per sulphate	(2Mm), 90°C	95	[138]
Surfactant	*Scenedesmus obliquus*	Chloroform: ethyl acetate	CPB, 2880 min, 45^o C	31.4	~~[163]~~[139]

C_6DIPA-Im: Dissopropanolamine- Imidazole, C_6DIPA-Pyr: Dissopropanolamine- Pyrazole, C_6DIPA-Tiz: Dissopropanolamine- 1, 2, 4-Triazole
CTAB: Cationic Cetrimonium bromide
S.I: Solar intensity
S.S: Stirring speed
P: Power
D: Dose
RT: Room Temperature

Researchers are currently investigating combination techniques for improved lipid extraction from algal cells where physical and chemical methods are combined. Disruption of microalgae cell lining has been reported by applying shear force, microwave radiation, ultrasound, and homogenizer or in combination with organic solvents. Derakhshan et al. ~~[141]~~[165] investigated physical disruption of *Chlorella vulgaris* by using ultrasound for 5 min at 50ºC in presence of chloroform and methanol, the lipid yield was reported to be 221 mg/g cells. 65 % of lipid recovery was seen for *Chlorella vulgaris* when ultrasound (20 KHz, 20-60 min), homogenization (7000 rpm, 20-60 min) and microwave (10min, 120ºC) were used in combination with organic solvents, chloroform and methanol ~~[142]~~[166].

Ma et al. [35] studied cell lysis by using ultrasound along with methanol and chloroform (1:2) for lipid recovery. Microwave assisted extraction provides heat directly to cells, which creates water vapor inside the cell and hence is responsible for breakdown of the cell wall, which enables the removal of intracellular lipids. *Chlorella vulgaris* dried biomass was exposed to ultrasound with 40 KHz frequency with the combination of chloroform: methanol and total triglyceride was reported to yield 55% while only 15 % was obtained with conventional methods (chloroform: methanol) ~~[144]~~[167]. In a recent study, *Chlorella vulgaris* was treated with mild pressure and heat shock for high lipid recovery. Additionally, mild pressure triggers the accumulation of neutral lipids in microalgae ~~[147]~~[168]. An energy efficient process using high shear stress gave a lipid extraction of 83% in 5 min from *Nannocholoropsis* sp (250 g/L). Solvent screening indicated that the combination of solvent using hexane: ethanol: acid along with mixing provide high mass transfer rate and excellent lipid extraction yield ~~[148]~~[169]. Lipid recovery via hazardous organic volatile solvents like, methanol, chloroform, hexane is strictly governing by European Directives including REACH 2006/1907/EC address the restriction of using these solvents in order to protect human health and environment [170]. Recently, the bio-based solvents are used in combination with other techniques and has the potential to replace the toxic solvents. The combined use of ILs as green solvents with microwave treatment has emerged as novel process for direct biodiesel production [171]. In this study1-ethyl-3-methylimmidazolium methyl sulphate [EMIM][MeSO4]

ILs along with microwave treatment at 65°C was able to achieve biodiesel yield of 36.79% after 15 min of reaction in *Nannochloropsis* sp. In another study of Krishnan et al. [172] imidazolium ILs are used for microwave assisted lipid extraction from *Chlorella vulgaris*.1-octyl-3-methylimidazolium acetate [Omim][OAc] at 2.5% augmented the lipid content (19.2%) in *Chlorella vulgaris*. Similarly, Tommassi et al. [173] studied combination approach using DEs with promising mechanical process microwave and ultrasound. Lipid recovery of 19% was achieved in *Phaeodactylum tricornutum* when exposed to ChCl/OA DEs in combination with microwave at 100°C for 60 min. Microwave coupled DEs is effective method for lipid extraction due to its polar behavior. This combination delivers heating effect and ensures the homogeneous temperature in the process. In order to replace chloroform with less toxic solvent, Hara and Radin [174] suggested lipid recovery using hexane: isoproponal in a ratio of 3:2 (v/v), 108.66±4.78mg/g biomass total lipid was extracted in the process. Furthermore, the above method was modified by Jesus et al [175], where green solvent cyclopentyl methyl ether (CPME) and 2-methyltetrahydrofuran (2-MeTHF) were used for lipid extraction in *Chlorella pyrenoidosa*. The results revealed high lipid recovery of 71.11 ±5.55 mg/g biomass in presence of 2-MeTHF: isoproponal (3:2 v/v) compared to CPME: isoproponal (61.06 ± 2.32mg/g biomass).

Considering the economic aspect and to reduce the operational cost of lipid extraction, wet microalgae biomass is preferred over dry biomass, which skips drying and dewatering steps and reduces around 59% of energy use [13]**.** Wu et al. [176] reported an enhanced efficiency of lipid removal from wet microalgae cell by using Folch method. Likewise, the Bligh and Dyer method was also successfully implemented for lipid extraction from wet microalgae *Chlorella vulgaris* with a yield estimated at 95%. Table 7 summarizes the combination approach applied for cell disruption and lipid extraction from wet and dry algal biomass. Surfactant assisted disruption of

Scenedesmus wet biomass is a novel approach for cost-effective and sustainable lipid recovery [177]**.** Among all the surfactants, Myristyltrimethylammonium bromide (MTAB) and Decyldimethylammonio propanesulfonate (3_ DAPS) when mixed with hexane and isopropanol showed the best FAME recovery, which was 160-fold higher than the untreated biomass of *Scenedesmus*. DESs was found to be exceptionally effective in lipid extraction from wet algae biomass *Chlorella* sp and *Chlorococcum* sp [156]. In this process, Choline Acetic acid (Ch-Aa), Choline Oxalic acid (Ch-Oa), Choline Propanedioic acid (Ch-Pa), DESs were found to be efficient for lipid extraction applied in the one step method, compared to two step operation. Among these solvents, Ch-Aa- methanol-H_2SO_4 in a ratio of 60:40:3 showed maximum FAME yield. Huang et al. [101] showed that a biodiesel and methanol mixture can be used instead of chloroform and methanol for the efficient extraction of lipids. The result showed that 68% of total lipids were extracted from wet microalgae biomass where biodiesel penetrates the cells and increases miscibility with lipids. Wet algae biomass of *Chlorella protothecoides* exposed to ultrasonic radiation in presence of chloroform and methanol, gave a lipid content of 42±2.97%, whereas the lipid content of untreated sample was 9.34±1.66 % [178]. In a study by Onumaegbu [179], wet algal biomass of *Scenedesmus quadricauda* was pre-treated using microwave for lipid extraction in presence of solvents methanol and sulphuric acid.

Table 7

Pre-treatment approach by employing combination techniques for lipid extraction

Microalgae	Techniques	Lipid %	Phase	Conditions	References
Scenedesmus quadricauda	Microwave + methanol : sulphuric acid (50:1)	49	Wet	8 min, 600W	~~[146]~~[179]

Chlorella vulgaris	Heat shock +hexane: isopropanol (3:2)	94	Wet	1.2.5 Kg/cm^3, 5-15 min, 50-70°C	~~[147]~~[168]
Nannochloropsis sp.	High sheer mixer+ Hexane/ethanol/acid (9:1:0.4)	83	Wet	5min, 55°C, 8000rpm	~~[148]~~[169]
Chlorella vulgaris	Ultrasound + ethanol	22.1	Dry	8min, 50°C	~~[141]~~[165]
Chlorella vulgaris	Ultrasound assisted+ methanol: chloroform (1:2)	24.45 ± 1.67	Dry	10 min, 60°C	[35]
Schizochytrium sp.	Ultrasonic assisted + soxhlet ethanol	93.76 ± 0.48	Dry	150 W, 30 min, 50°C	~~[158]~~[180]
Chlorella vulgaris	[Omim][OAc] ILs + Microwave	19.2	Dry	Conc: 2.5%, 700W, 5 min, 60°C	[172]

Conc: Concentration

The selection of methodology highly influences the design of the technology to be employed. The integration approach in regards with technologies offers energy and chemical savings. The use of bio-based solvents in combination process following the wet route for lipid extraction in microalgae can be the eco-efficient process.

4.Techno –economic analysis

Techno-economic evaluation of microalgae derived lipid production is a key factor for cost analysis, which may lead to commercially viable biodiesel development ~~[167]~~[181]. Despite achieving eco-friendly biodiesel production from microalgae at lab-scale, the higher operational and productivity costs are major hurdles for commercialization ~~[168]~~[182]. Therefore, to explore the financial viability of lipid production, a number of strategies have been implemented including harvesting, dewatering, and pre-treatment methods that aimed to reduce the overall production cost ~~[167]~~[181]. Microalgae harvesting accounts for 20-30% of the total cost production, which includes thickening and dewatering ~~[169]~~[183]. Additionally, life cycle assessment (LCA) analysis revealed that the integration of harvesting with lipid production accounts for ~ 90% of total energy. Flocculation has been considered as a low operational and energy cost in last few years. In this regard, the operational costing and energy required for closed cultivation lies in the range of 0.1 to 0.6 €/kg and 0.1-0.7 kWh/kg biomass respectively [7]. Bio-flocculation prior to centrifugation reduces the operational energy from 13.8 MJ kg/DW to 1.83 MJ kg/DW [28]. Chen et al. ~~[170]~~[184] harvested *Chlorella* sp biomass through bio-flocculation assisted with fungal pellet and fungal spore method and reported the cost of fungal pellet to be $0.825, which is much lower than the fungal spore $1.65. Cost effectiveness analysis revealed that to harvest 1metric tonne (MT) of wet algae biomass using $Al_2(SO_4)_3$ would cost $0. 28. However, to harvest the same amount of biomass by other techniques would cost $ 9.02. It is worthy to note that recovery of 1MT biomass with natural coagulant costs $0.037 [16]. A brief economic analysis revealed that flocculation is an economical approach for microalgae cell harvesting. Economic cost associated with pre-treatments methods for lipid extraction are too high and comprise 50-60% of the total cost. High capital cost of solvents is the major limiting factor in economical biodiesel production. 813 kg of hexane on 95.77 kg wet biomass is required to produce 1 kg of the fatty acid, which costs ~$1034 ~~[171]~~[175]. In comparison, when using green solvent pass, 581 kg of solvent is required to produce the same amount of fatty acids at high cost of $28345. Integrating of two solvent can reduce the economic cost as when Bligh and Dyer combined with 2-MeTHF (2:1 v/v) the cost corresponds to $7133. In regard with cost effective utilization of solvent, isopropanol is more economical as hexane and isopropanol in ratio 3:2 (v/v) on wet biomass (43.16 kg) accounts for a cost of $167.22 ~~[171]~~[175]. According to the economical aspect, alone green solvents are uncompetitive when compared with other solvents however, an integrated approach can significantly reduce the cost and energy (Table 8).

Table 8

Economic cost analysis in lipid extraction processes

Extraction method	**Biomass weight**	**Extractable components**	**Solvent cost ($)**	**References**
2-MeTHF	53.17 kg wet	1 kg fatty acid	28345	[175]
CPME	66.08 kg, wet	1 kg fatty acid	5947	[175]
2-MeTHF: methyl alcohol	78.73 kg, wet	1 kg fatty acid	7133	[175]
Soxhlet (Hexane)	95.77 kg, wet	1 kg fatty acid	1034.43	[175]
	2g, Dry	43% fatty acid	0.84/kg	[185]
Hexane: propanol	43.16 kg, wet	1 kg fatty acid	167	[174]
Sonication + solvent (Chloroform: methanol: water)	1.56g, dry	240mg/L lipid yield	1.56/Kg (only solvent)	[186]

Microwave + ILs	0.2 g, dry	5.461 mg/g fatty acid	50/Kg	[172], [114]
2-MeTHF: Isomyl alcohol	35.23, wet	1kg fatty acid	6385	[175]

5. Practical implications, key challenges and future directions

Microalgae are being eagerly explored for commercial and industrial applications. The use of microalgae biomass for the production of pigments, antioxidants, proteins, natural colorants in food, wastewater treatment have been successfully reported ~~[172]~~[187]. Microalgae has emerged as feasible source in major application of life sciences such as bio-hydrogen, bio-fertilizers, bioelectricity, food supplements and biofuels ~~[173]~~[188]. In the field of sustainable energy sources, biofuels- liquids fuels from numerous biological resources have achieved great momentum due to lower emission levels than petrol ~~[174]~~[189]. The extensive research is underway on biofuel synthesis from microalgae to address the current energy crises. During the oil crises in 1970s, numerous renewable energy programs were implemented involving microalgae biofuels by NREL formerly known as US Aquatic Species Program ~~[175]~~[190]. Until now, microalgae biofuel is less completive compared to conventional fossil fuels. In order to make microalgae bio-refineries a commercial possibility, major obstacles associated with energy consumption and cost need to be tackled. For long-term economic and environmental sustainability, certain polices are needed. With regards to the biofuel policies among various countries, the importance of biofuel was initially acknowledged by Brazil ~~[176]~~[191]. Brazilian biofuel programs were successfully supported by legal mandates and implementations. Now, several countries have taken the initiative to promote microalgae biodiesel. For instance, in China, the government is determined to implement the principle: “no competing with people for food, no competing with grain for land” ~~[177]~~[192]. The National Development and Reform Commission in China released the five-year

(2016-2020) plan for biomass energy development ~~[177]~~[192]. In this plan, the target biofuel consumption is 2 million tons by 2020 with the investment of 18 billion Chinese Yuan. The Europe Union set a goal of lowering the greenhouse gas (GHG) emission by 40% in order to promote renewable energy by 2020 ~~[178]~~[193]. In US, the Environment Protection Agency (EPA) and Renewable Fuel Standard (RSF2) have developed the Energy Independence and Society Act (EISA) based on biofuels. Under this Act, the US should hold a minimum 36 billion gallons of renewable fuels by 2020, and moreover, 21 billion gallons of transport fuels should be generated from cellulose, non-corn and other biomass ~~[176]~~[191]. Moreover, EISA in US provided $550 million for Research and Development in advanced biofuel plants ~~[179]~~[194]. Indonesia has set a target to blend ethanol and 20% diesel in biofuel by 2025. In India, National Policy on Biofuel was endorsed in 2008 for the effective and transparent marketing of biofuels with standard legal guidelines ~~[176]~~[191]. Further, the Ministry of Petroleum and Natural Gas (MoPNG) mandate the 5% blending of ethanol with petrol ~~[176]~~[191]. These energy policies are strictly necessary in order to achieve ambitious biofuel targets.

The existence of numerous harvesting technologies offers immense choice of application to the researchers. However, determining the most effective methods for harvesting and oil extraction is not straightforward [14]. In microalgae harvesting, a large volume of water must be eliminated to obtain the concentrated biomass, which covers one third of the total biomass production cost. As emphasized in this review, flocculation using nanomaterials, organic and inorganic polymers seem to be a suitable approach for microalgae harvesting. Concentration of reagents, dimension and nature of the material are important parameters that influence flocculation efficiency. Low concentration of microalgae cells forms smaller flocs as few cells remain in contact with flocculant, which may hamper settling [16]. Therefore, harvesting of microalgae aided by flocculation should consider the optimum cell concentration for maximum separation efficiency. In addition, the recovery of the material used for flocculation is another concern, which should be taken into consideration to achieve complete cell harvesting [14]. Lastly, the harvesting approach should be scalable, so that the techniques can dewater the large portion of the biomass without hampering its effectiveness for economical biofuel production. Selection of solvent for the extraction of lipid is the prime concern for developing processes for sustainable biofuel synthesis. In this paper, the most recent studies on cell disruption and lipid extraction have been summarized. It is believed that a combination approach using more than one technique together is the most effective method for high lipid extraction. Furthermore, the use of nanomaterials and green solvents are efficient and can potentially improve the process [13]. Regarding nanotechnology, the major issues that still need to be addressed are production cost, yield and environment safety ~~[180]~~[195]. Emerging green solvents such as deep eutectic, switchable solvents, show considerable promise to replace organic solvents. Nevertheless, technological aspects regarding application in lipid extraction are required to be studied.

Future directions:

Microalgae biofuels are emerging as a potential solution to the energy crisis, failure of the eco-system and various other complex issues. Nonetheless, there is a need for more research to make this technique suitable for large scale industrial application. From the aspect of microalgae harvesting, an efficient harvesting material should be compatible with the cell and able to be recycled. In order to resolve the challenge in biomass separation, non-toxic, ecofriendly additives should be explored. In this regard, plant-based flocculants and microalgae lipid free residue derived materials represent an ideal choice. The use of lipid free microalgae residue offers nonhazardous material and supports a zero-waste approach. Additionally, the surface of the flocculants can be modified using a polymer matrix, for effective harvesting.

From the aspect of lipid extraction, the microalgae rely on numerous parameters including, microalgae species, nature of cell wall and the category of lipids as polar or non-polar. Researchers need to focus on the techniques that reduce energy and time for lipid extraction. More emphasis must be given to green solvents and their compatibility with microalgae. Green solvents can be combined with other solvents or mechanical processes in order to increase the lipid yield.

Environment and cost consideration are also important topics for further study. The cost of cultivation can be reduced by growing microalgae in rural areas, where land prices are comparatively lower than urban areas. Furthermore, microalgae cultivation with fish or shrimps uplift the productivity of animals and improves the quality of water. Microalgae can be grown in wastewater effluents in order to replace synthetic media, as a cost-effective approach. Using nanomaterials synthesized from microalgae can be an alternative way to improve water quality as they act as contaminant absorbers. Additionally, the practical implementation of polices should be adopted for successful and broad promotion of microalgal biofuels.

6. Conclusion

In the recent years, the expansion in the field of microalgae biodiesel production is mainly due to the increasing pressure to find fossil fuel alternatives. The present review focused on covering the major challenges in microalgae technology, including biomass harvesting and lipid extraction. As emphasizes in this review, flocculation using organic, inorganic polymer and nanomaterials could be the immediate solution for efficient and cost -effective method for biomass recovery. Organic polymer such as chitosan, tanin, flopam could minimize pollution and improve the harvesting process. Subsequently this review discussed various advanced chemicals and nanomaterials, which lead to significant improvements in lipid recovery and biofuel production. On the other hand, the combination of two processes like green solvents with mechanical methods found more effective for lipid removal. New generation solvents like deep eutectic and switchable solvents have the potential to become technologically and economically viable for lipid extraction with minimal environmental impact. Government policies and biodiesel programs have encouraged the agency of biofuel.

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