

**ISOLATION AND SCREENING OF LIPID PRODUCING MICROALGAE AS A
POTENTIAL SOURCE FOR BIOFUEL PRODUCTION: AN OVERVIEW**

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ABSTRACT

Algae are a diverse group of aquatic organisms. It has the capability in alleviating the release carbon dioxide and generate oil with a high productivity due to this it has potential uses to produce biofuel. Algae are the major resource for the further generation as the most appropriate and durable feedstock. Algal biomass producing biofuels are the most suitable alternative fuels for the future as lipid accumulated from microalgal biomass within the cell similar to vegetable oils with a capability to generate 100 times more oil in comparison to other plants. Microalgae, as biomass, are an important source of renewable energy, further they can be converted into energy such as biofuel oil and gas. This study examines the theory involved in lipid extraction from microalgae. It also provides an assessment of totally breakthrough in rapid developing field and reports of microalgae. Lipids are composed to carry the conversion of biodiesel. Methods applied for the extraction of lipid from microalgae are mechanical as well as chemical methods. This paper is a review of different methods used for extracting oils are lipid from microalgal biomass for biodiesel production and also provide an assessment of recent background in this rapidly developing field on suitable of microalgal lipid composition from biodiesel conversation.

**Keywords: Algal biomass, harvesting procedures, lipid quantification, oil extraction,
third generation biofuel**

INTRODUCTION

The heightening costs of the quick effect of its use on nature have prompted exhausting petroleum derivatives and the the quest for elective energizes for diesel

motors [1]. The biofuels that are produced from biomass are considered as promising elective powers and are named solid (bio-char), liquid (ethanol, vegetable oil, and biodiesel), and vaporous (biogas, bio syngas and biohydrogen) fuels. The liquid biofuels are sorted into various ages of biofuels dependent on the sort of feedstock utilized. The original liquid biofuels are gotten from palatable feedstock, for example, corn, soybean, sugarcane, and rapeseed while the second-age biofuels are from non- palatable feedstock, for example, Jatropha, Miscanthus, and Switch grass. Nonetheless, the raising interest for edible feedstock as a nourishment source, combined with the limited accessibility of arable land for the development of eatable and non-palatable feedstock makes the first and second age biofuels unfeasible. In this manner, the third era biofuels, which are gotten from miniaturized scale and large scale green growth have an edge over the past two classifications and the fourth era biofuels are in view of metabolic engineering of photosynthetic life form to produce fuel [2]. Microalgae are tiny photosynthetic living beings that are found in marine and freshwater condition [3, 4] and have a potential of fixing 1.83 huge amounts of environmental CO₂ while creating 1 ton of microalgal biomass [5]. Microalgae are viewed as acceptable contender for biofuel creation as a result of

their higher photosynthetic proficiency, higher biomass production and quicker heightening rate contrasted with other vitality yields, for example, rapeseed and soybean [6]. Also, it is easy to cultivate microalgae with wastewater even in lands that are unsatisfactory for agriculture [7, 8]. The lipid amassing in the cells of microalgae ranges from 25–75% of its dry weight [9]. Henceforth, production of biodiesel from microalgae is a promising step towards finding a reasonable optional fuel for diesel motor. The key procedures associated with the biodiesel reinduction from microalgae are cultivation, harvesting, biomass processing, lipid extraction, and its transesterification. Among these strategies, lipid extraction is a significant and expensive procedure. The pecuniary production of biodiesel from microalgae fundamentally relies on the vitality used for the handling of biomass and the sort of lipid extraction process utilized [10]. A couple of survey papers have clarified the various advances engaged with biodiesel production from microalgae, similar to cultivation, harvesting, lipid extraction, and generation of biodiesel without underlining the different techniques for removing lipids or oils from microalgae.

One of the reasons that the group of microalgae is put forward as containing ideal organisms for biofuel production is due to the high efficiency of their

photosynthetic reactions in comparison to higher plants. As already noted, up to 10% of solar energy can be fixed into biomass by microalgae, which would equate to 280 tons of dry biomass per hectare per year [11]. To make the cultivation of microalgae more cost effective, it is recommended to use nutrient loaded wastewater as a complex medium in combination with waste CO₂ exhaust, which provides a pathway to remove nutrients from wastewater and CO₂ sequestration [12]. Commercial production of microalgae biofuel using wastewater as media composition is only possible if three main parameters, i.e. high biomass, lipid productivity and high wastewater tolerance, can be fulfilled [13]. Appropriate microalgae strain selection is a step towards successful combination of algae-based biofuel production and wastewater treatment [14]. Hence, this paper is an endeavour to give an outline of the various procedures embraced for the extraction of lipid from microalgae.

Microalgae:

Microalgae are photosynthetic microorganisms present in all prevailing earth biological systems and have ability to produce various helpful products. Also, numerous types of microalgae can be initiated to gather critical amounts of lipids, subsequently adding to a raised oil yield, which is later changed over in to biodiesel

by the procedure called transesterification. Microalgae are important producers that are dominantly found in aquatic conditions. They have been seen as valuable to individuals because of their application in drug improvement and ecological remediation.

Application of microalgae:

- Production of oxygen as ‘by-product’ of photosynthesis: all aerobic heterotrophic organism requires oxygen, example, fungi and animals need oxygen for respiration to stay alive.
- Production of biomass: autotrophic organisms represent the base of food web particularly in aquatic environment.
- They are Primary producers, basis of food webs and “FORESTS/GRASSES OF THE SEA.”
- It produces oxygen and fixing carbon dioxide in aquatic habitat.

Forms of microalgae:

The different types of microalgae which exist are:

- Colonial, Capsoid, Coccoid, Palmelloid, Filamentous, Parenchymatous. The cell dividers of diatoms include polymerized silica known as a frustule. The diatoms frequently oils and chrysolaminarin [15]. The microalgae are especially wealthy in fresh water. They produce starch as significant boss stockpiling compound by the photosynthesis component. Be that as it may, they can likewise produce fat sand

oils. The freshwater microalgae *Haematococcus pluvialis* are a freshwater type of chlorophyte, which is a main source of powerful antioxidant 'astaxanthin', which is very significant in aquaculture, and beauty care products industry. Consequently, it is having high business significance [15].

General attributes of microalgal growth:

microalgae are the eukaryotic living being's forms that have no roots, stems, or leaves however having chlorophyll for the photosynthesis. It tends to be multicellular or unicellular.

Unicellular microalgae happen most often in water, particularly in plankton. Phytoplankton is the number of inhabitants in free-floating microorganisms made basically out of unicellular microalgae. Moreover, microalgae may happen in wet soil or on the surface of sodden rocks and wood.

lipid arrangement of microalgae:

Lipids are natural atoms, which are dissolvable in natural solvents. The fatty acids, which are the primary constituents of both neutral also, polar lipid atoms, are of saturated and unsaturated sorts. Saturated fatty acids don't have double bonds, while unsaturated fatty acid have double bond. The lipid substance of microalgae varies impressively for various species. The constitution and unsaturated fat profile of lipids removed from a specific species

category is influenced by the cultivation conditions, for example, medium configuration, temperature, illumination power, proportion of light/dark cycle and air circulation rate and ranges from 12 to 22 carbons in length [16].

FACTORS ASSOCIATED WITH THE DEVELOPMENT OF MICROALGAE:

1. Development parameters:

There are a few elements which are required to gauge the development of algal biomass. A portion of those variables incorporate light (in the way of power), Carbon source and supplement sources, for example, nitrates, phosphates, sugars and other follow components like manganese, cobalt, zinc, molybdenum and so forth [17]. Different parameters included are the ideal temperature, ideal pH, fine mixing in the photo reactor, expulsion of O₂ and take-up of CO₂ in equivalent proportion [18]. The light, temperature, N, and P have a nearby relationship with development rate and lipid substance of the microalgae [19]. Subsequently these parameters beneficent to be kept up and controlled to be effective in imitating the ideal arrangement of results.

2. Temperature:

Temperature is considered as a significant factor just as a risky parameter to improve in huge scale outside culture frameworks, for example, the photo bioreactor frameworks and the open lake development

framework. Every day varieties in the temperature can prompt significant decline in the algal lipid efficiency [17]. These microalgae likewise show a decline in cell volume with an expansion in temperature. The ideal growth temperature is large fluctuate in between the 20°C to 30°C [20]. At the point when the light force changes the medium and high temperatures are an ecological viewpoint which at last affects the development of microalgae [21]. Various algal species can withstand the temperatures up to 15°C lesser than their best, with diminished development rates, the temperatures higher than limited degrees can prompt the death of an organism [22]. Be that as it may, low night and low occasional temperatures significantly diminish the biomass productivity [23]. On account of freshwater microalgae, for example, Chlorella, are fit for adjusting to the temperatures in the scope of 5–35°C (perfect temperature range is 25–30°C), which must be brought once more into a perfect temperature go over the span of mass cultivation [19]. On the off chance that the temperature isn't kept up ideally, the biochemical pathways inside the cells may prompt harm and there will be no appropriate accrual of lipids inside the cells [24]. As per study led by Singh *et al* [25]. A few species groups, for example, Chlorella, Nannochloropsis, Neochloris, Scenedesmus, Spirogyra, Chlamydomonas,

Botrycoccus, Haematococcus, Ulva species rare red algae, dark coloured algae and blue algae can develop in a temperature possibility of 20°C-30°C with the light power in the scope of 33–400mmol/m²/s [26].

3. Saltiness, supplements, and pH:

The necessities of different elements like saltiness, supplements, and pH are constantly needy upon the kind of organism selected. For microalgal growth, the superior nourishing requirements are Nitrogen and Phosphorus. Certain diatoms require Silicon [27]. Saltiness likewise affects the development of microalgae. They have their own frameworks in altering the saltiness cluster. In wide-ranging, seawater microalgae are fortified for enduring higher saltiness conditions when contrasted with the freshwater microalgae. A few examinations have demonstrated that microalgae need ideal saltiness for development. For example, when the way of life is furnished with low saltiness development conditions, the circumstance will be steady for the development of algal by the expansion of sodium chloride (NaCl) and sodium sulphate (NaSO₄). Nonetheless, high saltiness (>6g/L) will show the unfriendly effect and furthermore represses the development step of microalgae [28].

The pH assumes a significant job in the development of microalgae. Under basic

conditions, microalgae will effectively catch the CO₂ from the climate and yield extra biomass [29]. The pH continuously raises to essential as the algal development follows and a quick increment in photosynthesis and accumulation of OH⁻ particles happens [30]. Under acidic pH conditions (when the pH is <5), the standard of the disintegrated inorganic carbon (DIC) is CO₂. Then again, change in pH can likewise affect the susceptibility of the microalgal cell and the hydronium types of the inorganic salt, and constantly effect the unification of the inorganic salts [19]. For the development of microalgae, supplements are significant, for example, C, O₂, H₂, N, K, Mg, Ca, Fe, S, P, and other minerals. The key supplements are C, O₂, H₂, N, P, and K. The underlying three, to be specific C, O₂, and H₂ are acquired from water and air and the keep going three, to be specific N, P, K must be taken from the way of culture medium [17]. All through the cultivating, N, and P transform into the prohibitive variables. They together take part in administering the lipid production and growth of microalgae. The growth, reproduction and further functional occasions of microalgae are firmly influenced by the N, which is one among the basic component. The P is one progressively essential constituent focused on the cultivation of microalgae.

LIPID QUANTIFICATION METHODS FOR MICROALGAE:

1. Nile red method:

Nile red lipid visualization technique is increasingly advantageous as the quantity of tests and planning time are significantly diminished. The Nile red (9-diethylamino-5H-benzo[α]phenoxazine-5-one) is a lipid-solvent test that fluoresces at the characterized frequencies relying on the extremity of the encompassing medium. In any case, because of the synthesis and structure of the thick and inflexible cell dividers in some microalgae species, Nile red is kept from entering the phone divider and cytoplasmic film, and in this way, lipids can't give the ideal fluorescence. Consequently, the dimethyl sulfoxide (DMSO) is acquainted with microalgal tests as the stain transporter at a raised temperature [31].

some particular Nile red lipid systems for the assurance of microalgal lipids. Various solvents are joined with Nile red answer for recolor microalgal culture tests, and the examples are weakened if vital. The lipid content assurance is accomplished by contrasting the subsequent fluorescence esteems with a specific standard bend, in which the frequency of excitation and discharge might be extraordinary. All things considered, the lipid substance estimated by this strategy are generally meddled by the natural variables and

different segments in the cell cytoplasm, and the fluorescence power fluctuates between tests. Therefore, the ideal spectra and response conditions ought to be resolved for each sort of test before the fluorescent estimation [32].

2. Sulpho-phospho-vanillin reactions:

The colorimetric SPV method is a speedy another method for lipid quantity because of its quick response in sample handling [33]. Through the SPV reaction method, the lipids are produce a distinct pink colour, and the intensity is measured using spectrophotometric methods; therefore, it is working for direct quantitative test of lipids within wet microalgal culture [34]. Be that as it may, the consequences of SPV examine can be influenced by lots of components, for example, the level of oil immersion, incubation time, heating, and cooling; along these lines the SPV test may give deceptive outcomes [35, 36]. The wide-ranging technique of SPV method contains the sample addition, solvent evaporation, sulfuric acid addition, samples incubation, colour developing by adding phosphovanillin reagent, absorbance reading, and quantity of the lipid content based on the standard curve [37].

Phosphovanillin reagent is set up by dissolving vanillin in absolute ethanol and DI (deionized) water, trailed by the addition of concentrated phosphoric acid. To get ready standard lipid stocks, canola

oil is newly added to chloroform, and afterward different measure of standard lipid stocks is added to the various tubes. From that point onward, these tubes are treated to evaporate the solvent by the addition of water. Consequently, these examples are followed by SPV reaction techniques: (1) suspend tested samples in water and place in a glass tube; (2) add concentrated sulfuric acid followed by heat treatment and ice bath; (3) add freshly prepared phosphovanillin reagent and incubate in incubator shaker; (4) read absorbance at 530 nm and determine the lipid content by comparing the standard curve.

3. Thin-layer chromatography:

TLC is also a hopeful alternative to straight lipids measurement approaches as it needs minimal equipment which is available in most laboratories, and it can also provide extra information about lipid classes, which is important for biofuel production [38]. Among various dissolvable frameworks, the multi-one-dimensionalTLC (MOD-TLC) isolates the lipid classes quickly and reproducibly. The MOD-TLC technique can accomplish the measurement for most of microalgal lipids through alterations in dissolvable blends and lengths of partition times, and the mass of each settled lipid band is controlled by comparing at band intensities of unknown sample (pictured by the lipophilic dye primulin followed by a

robotized laser-fluorescence indicator examining) to dilution curve of reliable principles. Contrasted with thin layer chromatography, MOD-TLC straightforwardly analyse numerous examples on a single TLC plate, while as yet giving great resolution to the measurement of most significant classes of lipid species [39]. Usually, TLC plates must be activated before TLC running, and NLs are separated by certain solvents such as a mixture of chloroform: methanol: acetic acid: water (85 : 12.5 : 12.5 : 3, v/v/v/v). The determination of microalgal lipid content is finally achieved by comparing the resulting fluorescence values with a standard curve.

EXTRACTION OF OIL FROM WET ALGAL BIOMASS:

Drying and extricating oil from microalgae represents 90% of the complete procedure vitality in dry extraction of lipid for algal biodiesel creation [40]. The drying of algal biomass is vitality escalated and 25% decrease in vitality for drying procedure can be achieved by utilizing wet algal biomass [41], in which no drying is required. A couple of producers have detailed extraction of oil from wet microalgae. Kanda *et al.* [42] utilized liquid di-methyl ether (DME) as a dissolvable for extricating hydrocarbons and lipids from wet *B. braunii* indicating a similar yield as got by Soxhlet ex-footing utilizing hexane

as dissolvable with dried algal biomass. They reasoned that with DME, the extraction procedure could spare the energy required for drying and cell interruption of algal biomass. Yoo *et al.* [41] utilized osmotic treatment, which is a novel technique with polar and non-polar solvents to extract lipid from wet *Chlamydomonas reinhardtii* and announced multiple times increase lipid recuperation. A solitary advance supercritical process for synchronous extraction and transesterification of wet algal biomass, *Nannochloropsis sp.* with 90% water content was accounted for by Patil *et al.* [43]. They examined the impact of three factors like wet algal biomass to methanol in weight by volume proportion, response temperature and the reaction time on the transformation of *Nannochloropsis sp.*, biomass to fatty acid methyl esters utilizing reaction surface method (RSM). They got the ideal estimations of the factors, wet algal biomass to methanol (weight by volume) proportion, response temperature, and time as 9:1, 255°C and 25 min individually. Sathish and Sims [44] built up a lipid extraction method that was capable for separating 79% of lipids utilizing acid and base hydrolyses of wet blended society of *Chlorella* and *Scenedesmus sp.* containing 84% moisture. Kanda *et al.* [42] utilized a water miscible dissolvable of 1,2-dimethoxyethane for the extraction of algal

oil from wet cells of *B.braunii* and found that the water substance of algal biomass essentially influenced the lipid extraction effectiveness.

The biofuels are classified into three ages relying upon the source from which they are acquired [45].

- First era biofuels got from plant sources.
- Second era biofuels got from agricultural waste, blunder waste and so on.
- Third era biofuels got from microalgae.

Scientists have turned their enthusiasm towards fuel generation from one of the most established living organism on the earth, microalgae. These are used in delivering fuels as well as in catching the CO₂ from the air which helps in cleaning nature and producing better air to inhale [46, 47]. There are two different classes of algae known as macroalgae and

microalgae. These photosynthetic living beings are mostly found in oceanic environments both freshwater and marine. These are tiny and have extremely astonishing and intriguing structures [48].

The purposes behind algae being the favoured source over plant sources,

(a) The microalgae have a high efficiency for photosynthesis with a flexibility to a wide scope of light and temperature variations [49].

(b) The microalgae can develop in water with different levels of supplements and can capable to the adjustment in the growth attributes and supplement take-up capacity [49].

The methods for the extraction of oil from microalgae is Mechanical method and chemical method. These methods also categorized which is shown in **Figure 1**.

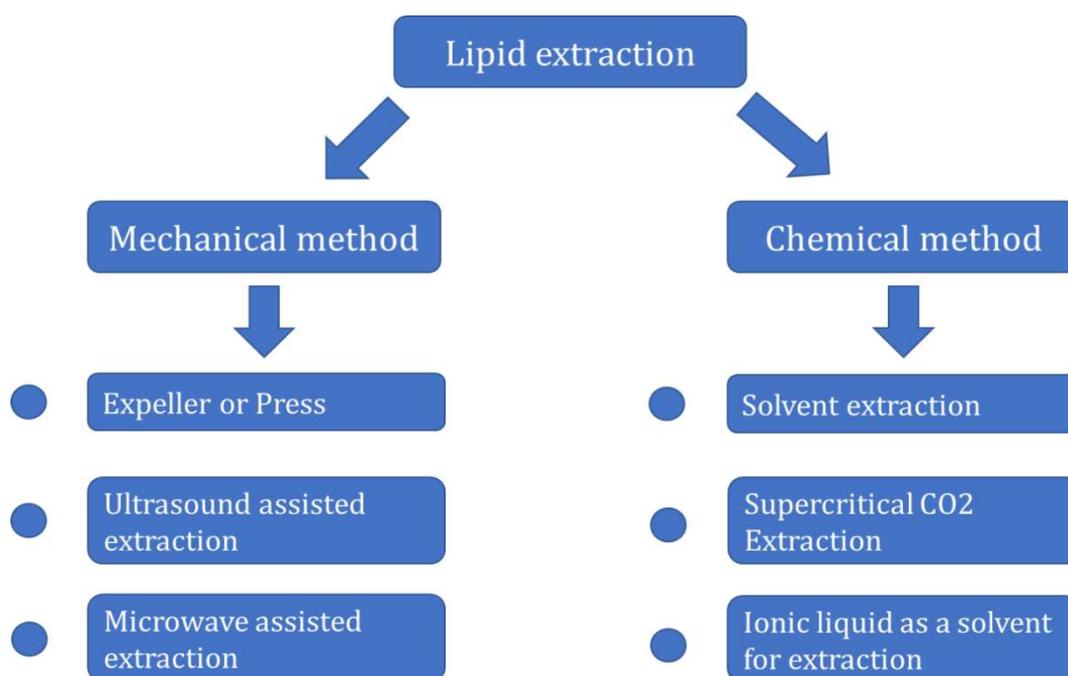


Figure 1: Extraction of lipid from microalgae

1. Mechanical method of extraction of lipid:

1.1 Expeller or press

This method uses a mechanical press to break the cells and compress out the oil from the dry biomass and can extract about 75% of oil. But, this method is quite slow and requires a large quantity of biomass [50]. Niraj *et al.* [51] extracted about 75% of lipid from filamentous algae using a screw expeller press and some amount of lipid present with the slab formed was removed using solvent extraction method.

1.2. Ultrasound assisted extraction

The thick cell dividers of microalgae hinder the arrival of intralipids present inside and the utilization of techniques like dissolvable extraction and mechanical press, yields less lipid [52]. The rule behind ultrasound helped extraction technique is that the serious sonication of fluid generates sound waves that proliferate into the fluid media and results in alter nary high-weight and low-pressure cycles. During high-pressure cycle, the little vacuum bubbles, which are delivered in the low-pressure cycle, breakdown brutally and bring about a marvel called cavitation. The high weight and fast fluid planes structure shearing powers around the green growth cells

during cavitation and break the phone structure precisely and improve material exchange supporting the extraction of lipids. Henceforth, ultrasonic helped extraction system with sound waves having frequencies higher than 20 kHz is utilized and due to this high power, little vacuum bubbles are made in the fluid [52]. This impact bolsters the extraction of lipids from microalgae and an oil yield improvement by 50–500% with 10 overlay decreased extraction time [53] is accomplished. Adam *et al.* [54] proposed another strategy as dissolvable free ultrasound helped strategy to extricate lipids from *Nannochloropsis oculata* biomass. They utilized reaction surface methodology (RSM) for the enhancement of oil recuperation utilizing parameters like ultrasonication power, extraction time and dampness substance of biomass what's more, have contrasted it and the oil yield extricated utilizing Bligh Dyer's technique.

1.3 Microwave assisted extraction

Microwaves are electromagnetic radiation of recurrence from 0.3 to 300 GHz. The microwave helped warming uses a non-contact heat source, which can enter into the biomaterials, interface with polar particles like water

in the biomass, and warmth the entire example uniformly. The higher oil yield with unrivalled quality and decreased extraction time are the fundamental favourable circumstances of microwave-helped extraction (MAE). Iqbal and Theegala [55] researched the dissolvable capability of biodiesel (methyl soyate) containing 20% (BD20) and 40% (BD40) biodiesel mixed with ethanol at three unique temperatures of 80°C, 100°C also, 120°C for microalgae lipid extraction utilizing MAE. They analysed the yield with MAE utilizing chloroform methanol and with Soxhlet extraction. The outcomes demonstrated improved lipid extraction yield with BD40 contrasted with other customary lethal solvents like n-hexane and chlorostructure. A resounding ceaseless microwave handling framework was utilized for separating oil from microalgae, *Scenedesmus obliquus* with hexane as dissolvable and 77% of the all-out lipid content was separated at 95°C in 30 min [56].

2. Chemical method for extraction of lipid

2.1. Solvent extraction

The science idea of 'like dissolving like' is the fundamental standard behind lipid extraction from microalgae utilizing solvents. A perfect

dissolvable requires elevated levels of particularity towards lipids particularly acylglycerols furthermore, the dissolvable must be unpredictable enough to guarantee low vitality distillation to isolate the lipid from solvents. The extraction of lipids from algal biomass can utilize non-polar solvents, for example, hexane, benzene, toluene, diethyl ether, chloroform and polar solvents, for example, methanol, (CH₃)₂CO, ethyl acetic acid derivation, and ethanol. The non-polar solvents disturb the hydrophobic connections between non-polar and unbiased lipids accessible in the algal biomass. The solvents utilized for removing lipid from microalgae biomass are n-hexane, ethanol, 1-butanol, DBU (1,8-diazabicyclo[5.4.0]-undec-7-ene), dimethyl ether, and blends of chloroform/methanol, n-hexane/ethanol, n-hexane isopropanol, n-hexane/2-propanol, methanol/1-ethyl-3-methyl imidazolium methyl sulphate, DBU/ethanol, DBU/octanol, methylene chloride/methanol, dichloroethane/methanol, dichloromethane/ethanol, and (CH₃)₂CO/dichloro-methane [52]. Lee *et al.* [57] assessed the presentation of five distinctive solvent frameworks, for example, chloroform/methanol (2:1, v/v); hexane/isopropanol (3:2, v/v);

dichloromethane/methanol (1:1, v/v); dichloro-methane/ethanol (1:1, v/v) and CH₃)₂CO/dichloromethane (1:1, v/v) by means of dab beating of *Botryococcus braunii* biomass. The chloroform/methanol (2:1 v/v) dissolvable framework indicated higher lipid yield of 28.6%. Cheng *et al.* [58] researched the blended dissolvable extraction of ultrasonically pre-treated Pavlov species lipids with dissolvable frameworks, for example, ethyl acetate/methanol toluene/methanol hexane/methanol and demonstrated higher lipid yield of 98% with ethyl acetic acid derivation/methanol dissolvable framework.

The utilization of natural solvents for extracting lipids utilizes vitality intensive refining after extraction for isolating lipid from the solvents [16]. Boyd *et al.* [59] utilized a switchable hydrophilic dissolvable N, N-dimethyl cyclohexylamine for separating lipid from freeze-dried examples of *Botryococcus braunii*. The Liquid Chromatography–Mass Spectrometry (LC–MS) examination of the unrefined lipid demonstrated high centration of long chain mono-, di-and tri-acyl glycerides without any phospholipids. Despite the fact that the dissolvable extraction methods are less expensive

and simple to execute, the utilization of harmful solvents and longer execution times are the primary drawbacks.

2.2 Supercritical CO₂ extraction

Supercritical liquids are seen as appropriate as an extraction dissolvable since the dissolvable intensity of a supercritical liquid, being an element of thickness, can be differed by changing the extraction weight and temperature, and is equipped for delivering dissolvable free unrefined lipids [60]. Superbasic CO₂ (SCCO₂) is the essential dissolvable generally utilized for the dominant part of supercritical liquid extractions because of its moderate basic pressure (7.4 MPa) and low basic temperature (31.1°C) [61]. Crampon *et al.* [62] utilized SCCO₂ strategy for extraction from *N. oculata* with wind stream drying and freeze drying as pre-treatment techniques. The wind current drying at a temperature of 35°C was the most sufficient pre-treatment strategy for removing 90% by weight of triglycerides with no phospholipids. Halim *et al.* [16] assessed the presentation of SCCO₂ exfooting and hexane extraction dependent on the yield and unsaturated fat composition of lipid extricated from *Chlorococcum* sp. for biodiesel creation. The lipid yield diminished with increment in

temperature and weight utilizing SCCO₂ extraction. They additionally announced that Soxhlet extraction utilizing hexane took 5.6 occasions the time required for SCCO₂ extraction for accomplishing a similar lipid yield of 0.058 g lipid/g dried microalgae also, utilizing co-solvents like ethanol with CO₂ could build the lipid exfooting yield in SCCO₂ extraction. Nobre *et al.* [63] removed 33% of lipid from *Nannochloropsis* sp. at the best working states of 40°C and 30 MPa with a CO₂ stream pace of 0.62 g/min.

2.3 Ionic liquid as a solvent

Ionic fluids are reasonable for the extraction of lipids from green growth due to their non-instability, warm soundness, and manufactured adaptability. Kim *et al.* [64] utilized a blend of ionic fluids like [Bmim][CF₃SO₃] and methanol, which could remove 19% of lipids from *C. vulgaris* utilizing Bligh and Dyer's strategy. This restricted the respect 12.5% of lipids and described the unsaturated fat profiles of extricated lipids utilizing gas chromatograph. Kim *et al.* [65] utilized ionic fluids like [MeSO₄].[Bmim] [MeSO₄] with ultrasonication and detailed the aggregate sum of lipid extricated from *C. vulgaris* by Soxhlet's and Bligh and Dyer's strategies are 21 and 29 mg/g

dry cell weight, individually contrasted with 47 mg/g dry cell weight utilizing ionic fluids with ultrasonication. The lipid extricated with ionic solvents was 1.6 occasions higher than Soxhlet's and Bligh and Dyer's techniques. The unsaturated fatsprofiles of lipids extricated indicated similarity with those lipids got by Bligh and Dyer's strategy. The liquefaction of microalgae, *Spirulina* with Fe (CO)₅-S impetus at 350°C for 60 min in tetralin under 5 MPa of hydrogen indicated an expanded oil yield from 52.3 to 66.9 wt.% [66].

CONCLUSION:

Microalgae have shown to be one of the most promising feedstocks for the creation of third time biofuels that are both fiscally conceivable and normally reasonable. Quick, exact, conservative and monetarily discerning methods for the lipid extraction and estimations are major for the sensible usage of microalgae-based biofuel creation. Gravimetric procedure is most comprehensively used at this point requires numerous estimations of tests; Nile red lipid measurement method is speedy as the amount of tests and arranging periods are tremendously diminished while a relationship Among fluorescence and lipid levels must be as of late settled as the phone recolouring changes among different microalgae; the eventual outcomes of the

SPV test can be affected by numerous components, for example, the degree of oil immersion, hatching time, warming and cooling; along these lines the SPV test give misleading outcomes; in the expansion to the quantitative estimation of microalgal lipids, TLC can in like manner give additional information about lipid classes which is huge for biofuel creation. Diverse lipid assessment techniques could be considered relies on the circumstance, yet logically fruitful for microalgal cell interference and extraction are up 'til now required to intensify lipid yields while avoiding the issue of noxious quality, combustibility and time utilization for extraction.

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