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Revisiting lipid extraction methods for algal biofuel production: a step towards sustainable environment

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ABSTRACT

The algal biofuels are not only receiving researcher's attention now a day but also becoming a quandary situation for industrial scale production due to their high cost. Till date, there is no commercial production report is available on a large scale for algal biofuel as capital investment and harvesting procedure claims maximum among the other input costs. To overcome these bottleneck of this field we should focus on easy and convenient lipid extraction procedures which can make the capital investment minimum. Rather than other lipid in algae, neutral lipid is mainly responsible for biodiesel production. However existing technologies mainly concerns to increase the total lipid content to achieve a better overall target. The current study represents a bird's eye view to exploit different emerging procedure for algal lipid extraction in total. However, it need be manipulated to achieve a possible best protocol which may vary from case to case basis for different algal cells.

Key words : *Microalgae, Algal lipid, Biofuel, Ultrasonic assisted extraction, Photo-bioreactor*

Introduction

Algae are a diverse group of aquatic organisms capable of doing photosynthesis and can be found in oceans, pounds, lakes, rivers, and even wastewater and other places. They have the ability to survive at a wide range of salinity, temperatures and pH. Algae can be broadly classified as Phaeophyta (brown algae), Rhodophyta (red algae), Xanthophyta (Yellow-green algae), Chrysophyta (Golden-brown algae and Diatoms), Euglenophyta (Euglenoids), Pyrrophyta (fire algae), and Chlorophyta (green algae) and according to their size and morphology- as microalgae or macroalgae. Macroalgae are, large-size multicellular algae, visible with naked eye, while microalgae are single cell microscopic eukaryotes which have similarity to cyanobacteria (Chloroxybacteria) (Khan *et al.,* 2018). Microalgae are usually rich sources of carbohydrate, protein and fats and can be utilized for production of biofuels, health supplements, feedstock for cattle as well as cosmetics. Recent findings suggest that they can also be useful in the treatment of wastewater and the mitigation of atmospheric CO_2 (Gautam *et al.,* 2019). Biofuels generated from algae belongs to third generation type of biofuels, which are considered as an alternative energy source for fossil fuels overcoming disadvantages associated with the first and the second generation biofuels. like their negative impacts on food security, water scarcity, global food markets and deforestation. In addition, the second generation biofuels obtained from nonedible oils such as *Jatropha curcasa* and *Simarouba glauca*, lignocellulose biomass, and forest residues require huge areas of land which intimidate the food versus fuel conundrum. Looking at the drawbacks associated with the first and second generation biofuels, microalgae biofuel seems to be a viable alternative energy source to replace or supplement the fossil fuels (Srikanth Reddy Medipally *et al.*, 2015; Kumar and Bera., 2020). Instead of producing greenhouse gases, algae improve the air quality by absorbing atmospheric CO_2, it does not require extra lands and utilizes minimal water. However, disadvantages like low production of biomass, costly harvesting process, low lipid content in the cells etc. are pulling back this new regime of sustainable biofuel industry. Manipulation in metabolic pathways and genetic engineering of algae showing promising results and may improve their potential as a future source of renewable bioenergy field. By this approach, growth rate of algae may be increased along with lipid content (Kumar and Bera, 2020). Microalgae were initially evaluated to be a potential source for biofuel production in the year 1970, but due to economic and technical problems it was temporarily shelved. Subsequent studies from the years 1980 onwards later showed production of biofuel from microalgae to have a high potential (Ramya Ganesan *et al.*, 2020). According to recent studies, microalgae biomass market reached US 608 million/year in 2015, and it is estimated to reach about 1.143 billion by 2024. But there are many limitations that thwart the cultivation of microalgal viz. light availability, temperature and PH, and the amount of essential nutrients (Carbon, Nitrogen, Phosphorous) (Fabris *et al.*, 2020). Hence, the need for innovative technologies for algal cultivation is paramount. However, contamination and other mechanical factors have led to rapid increase in the search for novel and innovative technologies for large scale algal cultivation. Photo-bioreactor which is the oldest method for cultivating microalgae can be tank, tubular, planar, or hybrid photo-bioreactors. It can also be classified as open and closed cultivation, but each has its own advantages and disadvantages. Table 1 shows some existing technologies used for microalga cultivation. According to recent studies, the idea of cultivating microalgae dates by in 1952, at the Carnegie Institute of Washington. And later, there was an experiment performed by Japanese with outdoor culture in an open circulation system. They used shallow open pond with algae suspension, and circulated it using a series of pipes that

Cultivation System	Advantages	Disadvantages	
Vertical-column photobioreactors	High mass transfer, good mixing with low shear stress, Low energy consumption, High potentials for scalability, Easy to sterilize, readily tempered good for, immobilization of algae, Reduced photoinhibition, and photo-oxidation	Small illumination surface area, their construction, require sophisticated materials, shear stress to algaecultures, decrease of illumination, surface area upon, scale-up,	Ugwu, 2008
Flat-plate photobioreactors	High surface to volume ratio, Low space requirement, High photosynthetic, Efficiency, cheap and economic, and low oxygen build-up	Short light penetration depth, Not scalable requires many components, frequent fouling and clean up issues, and poor temperature regulation	Gupta *et al.*, 2015
Horizontal tubular photobioreactors	Large illumination surface, Area suitable for outdoor cultures, Fairly good biomass, productivities, Relatively cheap	Gradients of pH, Dissolved oxygen and CO_2 along the tubes, fouling, and Some degree of wall growth requires large land space	Yen *et al.*, 2019
Stirred tank	Good heat and mass transfer, Good light dispersion, Lower contamination issues, Simple design, Moderate biomass, and productivity	Low surface to volume ratio, Heating issue due to agitation, Mechanical agitation requires, extra Energy, Expensive, and Not scalable	Gupta *et al.*, 2015

had jets for aeration. Since then, the production of microalgae on a large scale has intensified (Gupta *et al.,* 2015). Using a microalgae cultivation system requires a few considerations for an effective operation. And these considerations or factors include: (1) Effective light source usages (2) the conversion of light efficiency (3) maintaining and control of microalgae biomass for a longer period of time (Yen *et al.,* 2019).

Extraction of algal lipids for biofuel production

To obtain maximum lipid content from microalgae, an efficient lipid extraction technique is required, and an effective cell disruption method with appropriate solvent mixtures to recover maximum microalgal lipid has yet to be established (Kumar *et al.,* 2019; Chauton *et al.,* 2015). Folch procedure or Bligh and Dyer methods have historically been used to remove lipids from microalgae (Kumar *et al.,* 2017). The Folch method involves removing lipids from endogenous cells with chloroform–methanol (2:1 by volume), then equilibrating the homogenized cells with one-fourth volume of saline solution and blending well. The lipids settled in the upper phase of the resulting mixture, which was allowed to separate into two layers (Ranjith Kumar *et al.,* 2015; Folch *et al.,* 1957). The Bligh and Dyer process is somewhat similar to the Folch method, although the solvent/solvent and solvent/tissue ratios are the major variations (Ranjith Kumar *et al.,* 2015). The Floch method's performance is based on the presence of mineral salts in the crude extract and the use of a significant amount of solvent. The bulk of the acidic lipids are washed out during the washing stage in the absence of mineral salts (Kumar *et al.,* 2017; Folch *et al.,* 1957). The bligh and dyer method's performance is based on keeping the proportions of chloroform, methanol, and water compatible with the tissue's water content. Chloroform and methanol are hazardous and flammable compounds that have negative health and environmental effects. These solvents have an effect on product quality because they dissolve undesirable materials (chlorophyll) during the extraction process (Kumar *et al.,* 2017). Many researchers have researched less-toxic but less efficient alternatives for microalgal lipid extraction, such as ethanol, isopropanol, butanol, Methyl-tert-butyl ether (MTBE), acetic acid esters, hexane, and various combinations of solvents (Ranjith Kumar *et al.,* 2015; Sheng *et al.,* 2011). The

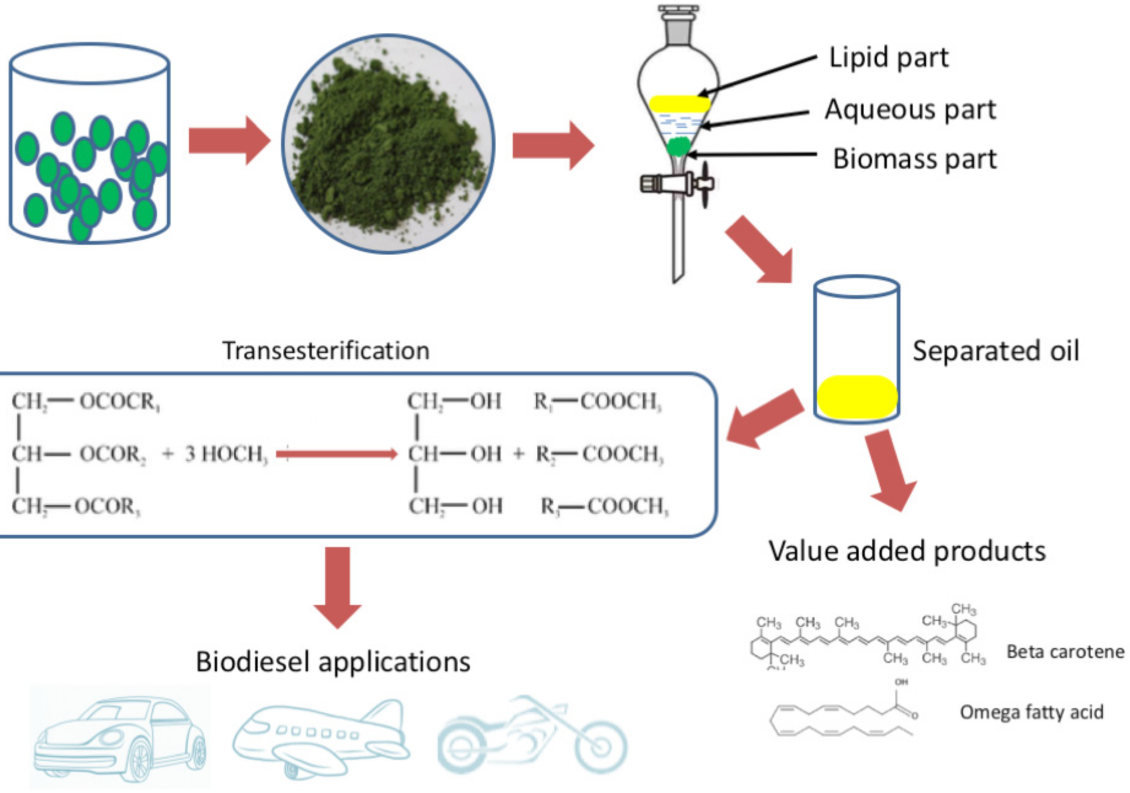

Fig. 1. Extraction of algal lipid and its application

most commonly used organic solvent for large-scale extractions is hexane (Kumar *et al.,* 2019). Mechanical (oil expeller, microwave associated extraction, ultrasound associated extraction, etc.) and non-mechanical methods (Soxhlet extraction, supercritical fluid extraction, solvent extraction etc.) are the two types of extraction methods (Ghasemi Naghdi *et al.,* 2016; Kumar *et al.,* 2019). Figure 1 shows a schematic representation of extraction of algal lipid and its application in various sectors.

Non mechanical methods

Solvent extraction

Several chemicals have been used for different *Chlorella* spp., including *C. minutissima*, *C.protothecoides*, and *C. vulgaris*, including organic solvents, acids, hydrogen peroxide, ozone, and ionic liquid (Kim *et al.,* 2016). Chemical-based lipid removal techniques are normally thought to be low-energy, require little capital expenditure, and are simple to expand. Nevertheless, many management tasks such as chemical cost, bio-toxicity, and lipid degradation should be solved for realistic industrial applications (Kim *et al.,* 2016). Choi *et al.* (2014) used the three solvent systems hexane, hexane/methanol (7:3, v/v), and chloroform/methanol (1:1, v/v) to investigate solvent-based cell disruption/lipid extraction from dried *C. vulgaris* biomass. The chloroform/methanol mixture yielded the highest lipid extraction yield (378 21 mg/g cell), while hexane and the hexane/methanol mixture yielded only low extraction values (185 mg/g cell), suggesting that lipid extraction performance differed by solvent method (Kim *et al.,* 2016; Choi *et al.,* 2014).

Supercritical fluid technology (SCF)

As compared to organic solvents, supercritical technology has higher selectivity, shorter extraction times, and no toxicity. It also does not require a follow-up separation phase like organic solvent-based extraction because $_{SC}$-CO_2 is present in a gaseous state at ambient pressure, allowing for fast recovery of $_{SC}$-CO_2 from reaction streams (Kumar *et al.,* 2017). Furthermore, recycling CO_2 in SCF technology reduces greenhouse gas emissions but SCF technology has the downside of larger capital cost but easy processes; scale-up is simple with SCF technology, which has sparked interest in lipid extraction from microalgae (Kumar *et al.,* 2017).

Ionic liquid (IL)

Green solvents, or green "designer" solvents, are ionic liquids. Ionic liquids are environmentally friendly because they have no visible vapour pressure and hence do not pollute the environment (Kumar *et al.,* 2017). Choi *et al.,* (2014) used a high temperature of 120° C to test twelve ILs and their combinations for lipid extraction from dried C. vulgaris biomass. They discovered that four ILs, namely 1-ethyl-3-methyl imidazolium acetate, 1-ethyl-3-methyl imidazolium diethylphosphate, 1-ethyl-3-methyl imidazolium tetrafluoroborate, and 1-ethyl-3-methyl imidazolium chloride, had higher lipid extraction yields (>200 mg/g cell) than the standard solvent extraction method of hexane (Choi *et al.,* 2014; Kim *et al.,* 2016). Nevertheless, for practical applications, their manufacturing cost and recyclability remain critical factors to be addressed (Kim *et al.,* 2016).

Mechanical method

Various forces such as solid-shear (bead mill and grinding), liquid-shear (high-pressure homogenization), energy transfer (ultrasound and microwave), as well as thermal and pressure-assisted destruct the microalgal cell wall in a non-specific manner regardless of cell status (dried/wet and growth stage) (steam explosion) (Kim *et al.,* 2016).

Microwave assisted extraction (MAE)

Microwaves are electromagnetic radiation with a frequency varying from 0.3 to 300 GHz and act as a non-contact heat source that can penetrate into the biomaterials (Drira *et al.,* 2016). The induced heat promotes the formation of water vapour and an electroporation effect, which disrupts the cell membrane and allows for efficient intracellular metabolite extraction (Kumar *et al.,* 2017). Because of microcracks in the cell wall, MAE research on oil extraction indicate that it has resulted in higher bio-oil yields (Kumar *et al.,* 2017). The MAE method not only eliminates the need for microalgae to be dried, but it also allows the substrate to be specifically transesterified into biodiesel (Kumar *et al.,* 2017). Microwaves are currently the most common alternative due to the economics of the above procedure, and it is expected to be appealing due to short reaction times, low operating costs, and efficient extraction of algal oils (Ranjith Kumar *et al.,* 2015). Microwave heating is two-thirds less costly than conventional heating, and it has the potential to boost micro biodiesel output rates (Drira *et al.,* 2016). The recovery of biodiesel from the reaction mixture in a microwave-assisted process takes about 15–20 minutes, which is much faster than the 6-hour cycle required by the traditional heating system (Ranjith Kumar *et al.,* 2015; Kumar *et al.,* 2017).

Ultrasonic assisted extraction

Ultrasonic-assisted extraction (UAE) is quick, cost-effective, and environmentally sustainable, as it removes the need for waste-water treatment and increases the quality of the final product (Kumar *et al.,* 2017). UAE can rupture cells in the presence of liquid cultures via cavitation, which creates microbubbles across the cell as a result of an ultrasonic wave. The eventual collapse of these bubbles creates a shockwave that shatters the cell wall, allowing the contents of the cell to escape (Ghasemi Naghdi *et al.,* 2016). Furthermore, UAE will benefit MAE because it can be carried out at low temperatures, reducing thermal denaturation of critical biomolecules (Ranjith Kumar *et al.,* 2015; Ghasemi Naghdi *et al.,* 2016). Under hexane/methanol and chloroform/methanol mixture conditions, ultrasound treatments had higher efficiencies (41.4-42.0 wt%) than microwave treatments (17.9-21.4 wt%) in extracting lipid from *C. protothecoides* biomass, according to Piasecka *et al.* (2014) (Kim *et al.,* 2016).

Enzyme assisted extraction

For algal cells, cell disruption using enzymes is an option available to lipid extraction that has received little research. Because of the high selectivity of the reactions, enzymatic treatment results in strong lipid recovery with the added benefit of disrupting cells with minimal damage to the target product. (Demuez *et al.,* 2015; Ghasemi Naghdi *et al.,* 2016). This approach relies on enzymes acting selectively on cellular membranes to assist cell disruption. The process is gentle, precise, eco-friendly, and well-suited to feedstocks with high moisture content, but it takes longer than other methods (Kumar *et al.,* 2017). The unique action of enzymes, on the other hand, has a considerable advantage over other mechanical methods; for example, *Nannochloropsis* is a marine alga with significant industrial potential due to its ability to absorb lipids (Kumar *et al.,* 2017). The lipid class composition and form of microalgae have an impact on this process, and it needs low temperatures and high specificity to be effective (Ranjith Kumar *et al.,* 2015). The cost of enzymes is normally higher than that of chemical and physical cell disruption approaches, and the rate of cell wall degradation is low in either case (Kim *et al.,* 2016).

Conclusion

The US Department of Energy has estimated the price of microalgae and soybean based biodiesel produced on a large scale to be 8 and 4 US dollars per gallon, respectively. Hence it is clear that we need follow a well characterized and balanced roadmap to reach an acceptable position where algal biofuel can replace the existing conventional fossil fuels and may help to solve the energy crisis in future. Low understanding of algal physiology creating a roadblock in the lipid or value added products extraction technologies which have impeded the development of algae based sustainable biofuel. As a result, careful formulation and selection of algal lipid extraction procedure can be a boon for algal biofuel technology in near future.

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