

Review Article

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Potential of Micro-algae in Future Biofuels

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ABSTRACT

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Overabundance carbon impression is one of the major natural issues of this decade. The major concern is regarding the substitute source of energy as carbon emission is contaminating the environment. Among sustainable power sources, carbon unbiased biofuels from microalgae give off an impression of being a promising move towards manageability and cleaner powers attributable to its presence of high CO<sub>2</sub>-sequestering capacity, high lipid efficiency and being effectively cultivable in an open pond and waste, marine and saline water. Algae are viewed as the most promising asset for the up coming ages as the most sustainable feedstock. There is immense interest for using these algal growth as a potential source of energy by conversion the primary and secondary metabolites into beneficial products. These algal biofuels are considered as third era biofuels. The third era biofuels are the best option to the current circumstance since they have the potential to dispense with a large portion of the biological issues made by the utilization of non-renewable energy sources. In spite of getting consideration during the recent couple of decades, the creation of microalgal biofuels isn't yet adequately practical to compete with conventional fuels based on petroleum as the harvesting and production of microalgae biofuels is financially inadequate. This article emphasizes on different strategies for both biomass reaping and lipid extraction from microalgae accessible up until now which are cost effective. It also elaborates about the varied applications of microalgae in other ways.

Introduction

Marine algae including marine cyanobacteria, marine eukaryotic microalgae, and kelp, are the most senile member from the plant kingdom, that are found from the polar district to tropical zones and from supplement rich beach front oceans to oligotrophic vast sea. These marine algae are accountable for 40%-50% of photosynthesis on Earth (Falkowski *et al.*, 1998). It is assessed that approximately of 50,000 species exist, yet just

a set number, of around 30,000, have been examined and dissected.

Micro algae mostly require three foremost components for growth which consist of sunlight, water, and a carbon source. For the requirements of nutrients they rely on the aquatic habitats, and for the source of carbon they either seize CO<sub>2</sub> from the atmosphere or feed on inorganic compounds and they efficiently produce approximately 50% of the atmospheric oxygen. Since they are

considered to be extremely capable of utilising sunlight and carbon source for making organic compounds. Their extreme capabilities of making high amount of oil yields and satisfactory lipid amount which is required for the biofuel production. Above that they also show high growth rate and have minimum land requirement in comparison to conventional land crop biofuel. Therefore since last decade biofuel production from microalgae have been an area of extensive research as an alternate to conventional fuels like petroleum and diesel so that they can serve as third generation biofuel.

In recent times whole world approximately about 95-97% is dependent upon the direct combustion of biomass. The production of first generation biofuel are manufactured from consumable food materials majorly starch, sugar and oils. But the production of first generation biofuel is an issue of debate due to "food versus fuel" dilemma as in another perspective it has been seen that this kind of biofuel production might hinder the global security of food. Hence another way of generation biofuel has to be devised.

The second generation biofuel were seen as an potential alternative to first generation biofuel. Since these are produced from non edible crops that are primarily derived from lignocellulosic feedstock for instance like wood, sludge, organic wastes etc. These biofuels bring a lot more plus points in comparison with the first generation since they don't rival food supplies and conventionally have better return and lower land necessities. Regardless of potential benefits the constraints of the first-and second generation biofuel is that they are dreadfully deficient to fulfill worldwide needs for biofuels because of, land accessibility and are environment affable. To overcome these demerits there was a shift towards third generation biofuel.

The third generation biofuel have received great attention as they are being generated from algae biomass. The third generation biofuels are viewed as a realistic approach that may vanquish the significant downsides associated with the first and second generation biofuels. Nonetheless, the positive outlook of algae based fuel formation that it in harmony with natural and social viewpoints but it has not been sufficient at present because of the high commercial production costs

The initial studies on microalgae production have been commenced in 1950's under controlled production (Burlew 1953) The first large-scale culture of microalgae started in the early 1960s in Japan by Nihon Chlorella with the culture of Chlorella since then the advancement in microalgae production has been observed. The production of micro algae takes place in several steps

### **Steps of biofuel production**

#### **Cultivation**

The major four classes of cultivation are:

- phototrophic
- heterotrophic
- Mixotrophic
- photoheterotrophic

#### **Photoautotrophic Cultivation**

In this kind of cultivation micro algae uses sunlight as a sole imperativeness source and the sunlight is converted to chemical energy through photosynthesis. In phototrophic procedure, microalgae can be created in open ponds and photobioreactors.

Open lake development are the most established and least complex frameworks generally utilized for augment microalgae

yield. These microalgae developing strategies have been in practice since the 1950s. Open pond development system, which are basically isolated reliant on their size, shape, and material used for advancement, way of unsettling, and tendency.

A prototypical raceway pond involves a closed circle oval channel, 0.25-0.4 m profound, open to the air, and blended in with a paddle wheel to course the water and forestall sedimentation to evade aggregation of poisonous or hindering compounds and to control improvement of microorganisms the ponds water is dislodged at regular intervals usually the water is flushed out in every two months.

Open pond process are less enumerate in plan and are more adaptable; but, they are limited by abiotic growth factors such as temperature, pH, light force, and dissolved oxygen congregation. Above that there are various animals which can corrupt the lake and damage the microalgae. Additionally this method has obstructions in view of moderate cell improvement and low biomass production that incline sparse oil profit-making.

On the other hand photobioreactors require significantly more energy for building and during preparing contrasted with the expansion in profitability that they offer.

However the open pond frameworks appear to be supported for business development of microalgae at present because of their low capital costs but the closed frameworks that is photobioreactors offer better command over defilement, mass exchange, and other development conditions nevertheless in general photobioreactors or closed systems are expensive than open ponds.

## **Heterotrophic Cultivation**

In this type only organic compounds are being used by micro algae as an energy source. The significant favourable circumstances related with heterotrophic development over phototrophic development are the acceptable control on cultivation strategy, termination of sunlight requisite, and ease of biomass harvesting.

Heterotrophic improvement additionally accord better oil profitability. As a result, this approach has gather amazing excitement when set side by side with other advancement techniques. Notwithstanding, heterotrophic microalgae culture is polluted easily that impacts the large scale manufacturing of microalgae.

## **Mixotrophic cultivation**

Carrying out photosynthesis is the essential source of energy, although both organic compound and CO<sub>2</sub> are fundamental or on the other hand it can be explained as that micro algae can live either autotrophically or heterotrophically, dependent upon the availability of organic compounds and sunlight. Hence mixotropic is a type of cultivation of micro algae in which both inorganic and organic carbons source are exploited for microalgae growth mixotrophic and photoheterotrophic production endure from probability of contamination and light prerequisites and therefore require creation of photobioreactor for enhancing that increases the capital and activity costs.

Thereafter, consequently there is a necessity for extra progression of advances with higher microalgae advancement rates, to improve the capacity of open air production and with a strong safe instrument for restricting the tainting chances in the mass scale microalgae production method.

## Harvesting of micro-algae

The financially potent harvesting of micro-algae is viewed as the most troublesome zone of algal biofuel mass production and this is probably the utmost critical factor which is restricting the mercantile usage of micro algae biofuel.

The cost of micro-algae gathering is exorbitant, due to the low mass proportions in the broth medium, while the cells regularly convey negative charge and overabundance algogenic organic matters (AOM) to keep their steadiness in the scattered state. Approximately it takes around 1-10 days to finish off one harvesting cycle. The significant strategies legitimately applied in the gathering of microalgae incorporate:

- centrifugation
- flocculation
- filtration and screening
- gravity sedimentation
- buoyancy
- Electrophoresis techniques.

The inclination of harvesting technique is reliant on the properties of microalgae, for instance density, size, the value of desired product.

### Centrifugation

It has been experimentally observed that all types of produced microalgae could be separated efficiently and with the assistance of centrifugation (mohn 1988)

Further laboratory centrifugation experiments were performed on effluvium obtained from pond cultivation which indicated that around 80–90% mic-roalgae can be recuperated in the time of 2–5 min. Grima *et al.*, in 2003 concluded that centrifugation is a favored

technique for harvesting of microalgal biomass, particularly for creating broadened shell life concentrates for hydroponics.

Centrifugation being advantageous also has demerits as the micro algal cells are being aquatinted with high gravitational and shear forces by which the cell structure could be disrupted. Additionally centrifugation technique is not cost effective for micro algae separation. It is estimated that approximately the expense of energy, materials, maintenance and the servicing of centrifuge will might cost up to 25% of the overall cost of the production of micro algae.

### Flocculation

Flocculation is a convoluted cycle that is impacted by varied cell properties for instance, cell surface properties, cell concentration, pH of the surrounding, ionic strength, and sort of flocculant (Schenk *et al.*, 2008; Uduman *et al.*, 2010). Inorganic synthetic concoctions are utilised in this approach for example, ferric, aluminium, and zinc salts, micro algae cells gradually sink to the base of the compartment once they get coagulated. The negative charge of micro algae is altered with the help of Cations, like  $Al^{3+}$  or  $Fe^{3+}$ . Subsequently, the cells aggregate and tremendous flocs which are settled in the bottom are segregated from the culture medium. In certain micro algae auto flocculation takes place. Auto Flocculation is a kind of flocculation which can happen naturally in few micro algal growth, and flocculation of micro algae may take place because of environmental pressure; changes in concentration of nitrogen, fluctuation of pH and dissolved oxygen.

### Gravity sedimentation

Sedimentation can be depicted by Stokes' Law which assumes that sedimentation

velocity is proportional to the square of the (Stokes') radius of the cells and the difference in density between the micro algal cells and the medium as demonstrated as follows:

### Settling velocity

$$\frac{2}{9} g \frac{r^2}{\eta} (\rho_s - \rho_l)$$

Here;

R- is a cell radius  $\eta$  is fluid dynamic viscosity

$\rho_s$  and  $\rho_l$  are the solid and liquid densities respectively

Separating a feed suspension into a slurry with higher concentration and an effluent of significantly clear liquid that is in the process of gravity sedimentation solid liquid separation is done (Svarovsky 1979b). Sedimentation velocity is impacted by Density and radius of micro algal cells and the instigate the settling phenomena of suspended solids (Brennan and Owende, 2010) the density of microalgal is vital to ensure the proficiency of this method, nevertheless the reliability of this method is sub-standard. Thus this procedure is not being adopted as the worth of the end product is extremely unfavourable, for example, biofuels. Still gravity sedimentation ought to be chosen for microalgal collecting when the thickness of the system is sustainably high with high moisture.

### Flotation

Flotation is a gravity separation method in which air or gas bubbles are attached to solid particles and afterward convey them to the fluid surface. Chen *et al.*, (1998). This process is being regarded as an more captivating and economic way to harvest microalgae by

utilising their ordinary characteristics of low thickness and self buoyancy. The accomplishment of flotation relies upon the inconstancy of the suspended particles which should be lower than the unsteadiness of the higher air particle contact. Flotation assisted by coagulation can contribute to powerful segregation of micro-algae from the low concentrated medium. Other strong points of flotation are low space prerequisites, moderately short time for harvesting and high adaptability with lower prior apparatus costs.

### Filtration and screening

Filtration has been found acceptable at recovering tolerably tremendous algal cells in this method various sorts of filters have been used to assemble micro-algae and regardless of this it is hampered by low throughput and rapid obstruction.

The process of filtration through these filters are mainly beneficial when there is a requirement to keep the bunches of micro-algae separated, and when expansive washing is required. However the process of filtration can be hindered by both (A) high microalgal concentration - It can bring blockage in the screen, (b) a low microalgal concentration- it can bring about negligent incarceration (Wilde *et al.*, 1991).

Membrane filtration has points of interest that incorporate being ecologically inviting as well as supplying isolated water and supplements that can be reused to develop microalgae; thus it allows for a constant cycle utilizing low energy and additionally is being carried out on a low working expenses in contrasted with alternative cycles, for example, centrifugation and flocculation whereas, Screening incorporates giving the suspension through a screen with a particular pore size there are two screening instruments essentially Microstrainer and vibrating screen channels.

**Table.1**

Open ponds	Parameters	Photo bioreactors
More	Space required	Less
Very high	Loss of water in evaporation	Low
Low	Set up cost	High
Low	Maintenance cost	High
Extremely high	Risk of contamination	Low
Less amount required	Energy requirement	large amount required
Difficult	Process control that is control of ph, temperature	Easy
Low	Bio mass quality	High
0.177	Volumetric production (kg m <sup>-1</sup> d <sup>-3</sup> )	1.535
99.4	Oil yield (m <sup>3</sup> ha <sup>-1</sup> ) Based on 70% by wt oil in biomass	136.9

**Fig.1** Various stages of biofuel production

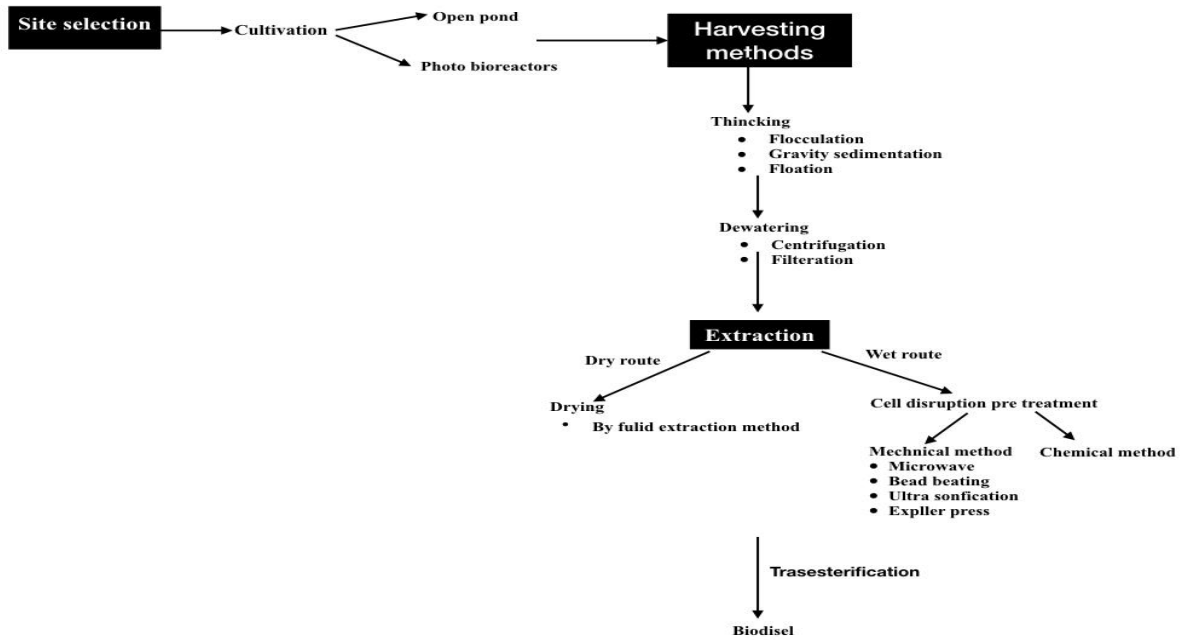


Table.2

Microalage	Marine or fresh	Culture condition	Lipid content (% dry weight biomass)	Biomass productivity	Lipid productivity	Reference
<i>Chlorella vulgaris</i>	Both		5.0–58.0	0.02–0.20	11.2–40.0	
<i>Chlorella vulgaris</i> <i>Beijerinck CCAP 211/11B</i>	Both	Phototrophica	18.0–40.0	0.03–0.04	5.4–14.9	Illman <i>et al.</i> (2000) D
<i>Chlorella protothecoides</i>		Heterotrophic	43.0–46.0	4.0–4.4	1881.3–1840.0	Cheng <i>et al.</i> (2009)
<i>Chlorella protothecoides</i>		Heterotrophic	50.3–57.8	2.2–7.4	1209.6–3701.1	Xiong <i>et al.</i> (2008)
<i>Chlorella protothecoides</i>		Heterotrophic	46.1	2.0	932.0	Xu <i>et al.</i> (2006)
<i>Dunaliellasalina</i>	Marine		6.0–25.0	0.22–0.34	116.0	
<i>Spirulina platensis</i>	Fresh	Phototropic	4–16	0.6–4		
<i>Nannochloropsis sp</i>	Marine	Phototrophica	29.2	0.17	49.7	Rodolfi <i>et al.</i> (2009)
<i>Neochloris</i>	Fresh	Phototrophica,b	15.9–56.0	29–65	10.7–38.8	Gouveia <i>et al.</i> (2009)
<i>Skeletonema sp.</i>	Marine	Phototrophica	31.8	0.09	27.3	Rodolfi <i>et al.</i> (2009)
<i>Dunaliella tertiolecta</i>	Marine	Phototrophicb	16.7	0.12	20.0	Gouveia and Oliveira (2009)
<i>Botryococcus braunii</i>	Fresh	Phototrophica	20.8	0.03	5.5	Yoo <i>et al.</i> (2010)
<i>Isochrysis sp.</i>	Marine	Phototrophica	22.4	0.17	37.7	Rodolfi <i>et al.</i> (2009)
<i>Pavlova salina</i>	Marine	Phototrophic	30.9	0.16	49.4	Rodolfi <i>et al.</i> (2009)
<i>Pavlova luther</i>	Marine	Phototrophic	35.4	0.14	50.2	Rodolfi <i>et al.</i> (2009)
<i>Tetraselmis suecica</i>	Marine	Phototrophic	8.5	0.32	27.0	Rodolfi <i>et al.</i> (2009)
<i>Scenedesmus obliquus</i>	Fresh	Mixotpic	6.6–11.8	0.10–0.51	11.6–58.6	Mandal and Mallick (2009)
<i>Scenedesmus quadricauda</i>	Fresh	Phototrophic	18.4	0.19	35.1	Rodolfi <i>et al.</i> (2009)
<i>Chlorococcum sp.</i>	Fresh	Phototrophic	19.3	0.28	53.7	Rodolfi <i>et al.</i> (2009)

### Extraction of micro-algae

This lipid extraction is considered as one of the most significant approach for separating

biodiesel from micro-algae.

Principally the extraction of lipids from the algal sources has been separated into two

significant parts

A -Lipid extraction by chemicals and solvents.

B -Lipid extraction by a mechanical process.

### **Lipid extraction by chemical process**

In chemical solvent lipid extraction here are two broadly utilized conventional solvent extraction techniques, in particular, Folch strategy, and Bligh and Dyer technique.

#### **Folch technique**

Folch method applies the use of chloroform-methanol in the volume ratio of 2:1 separately for the extraction of lipids from the biomass, and this method balances the homogenized cells with one-fourth volume of saline arrangement (Folch *et al.*, 1957). Once the test sample was introduced to the solvent, the entire combination was presented to delicate agitation for around 20–25min thereafter accompanied by centrifugation at 2000 rpm or by filtration with a filter paper to recuperate the fluid phase.

#### **Bligh and Dyer technique**

Bligh and Dyer strategy is driven using 1:2 (volume/volume) chloroform and methanol respectively (Bligh and Dyer, 1959). The Bligh and Dyer extraction technique has been directed in enormous scope measures which empower lipid extraction (Breil *et al.*, 2017).

### **Lipid extraction by mechanical process**

#### **Microwave**

The microwave extraction was first exhibited by Ganzler at. 2450 MHz frequency it is used in microwave. This frequency of microwave is likewise utilized in cell disturbance since,

2450 MHz frequency can turn the dipole of OH bonds in water or alcohol. Microwave radiation is favorable because of its swift entrance into biomass, which brings about instantaneous cell interruption. In spite of various advantages, there are considerable number of disadvantages. As it requires an immense cooling framework because of the high temperature and pressure used, moreover the thermally labile products will exhaust a huge amount of electricity and then will show degradation.

#### **Ultrasonication**

In this method of extraction, microscopic bubbles are formed in the fluid that is exposed to extension and pressure during rarefaction and compression stages individually. Then the Cell leads to disruption with concurrent arrival of gigantic warmth, pressure, and shear stress that takes place just after compression. The major advantage of ultrasonication method is the accomplishment of strong cell disruption dependent on the cavitation effect. Despite there are certain drawbacks. The energy utilization is high because of the high ultrasonic force and substantial cooling, and it is intricate to escalate this cycle since cavitation just happens in small regions close to ultrasonic tests.

#### **Expeller press**

In the method of expeller press the micro algae is initially dried. this technique is considered as one of the most established strategy for extraction. The mechanism of working of expeller press is in the following manner; A screw type machine was used which presses the dried biomass. Then dried biomass is made to entered through one side and the product was exited from the other side of the press. The persistent pressure and friction are required for compressing the algae dried biomass.



### **Bead beating**

This method is also called as bead mill or ball mill is a basic cell interruption procedure that breaks cells by shaking a shut compartment loaded up with the target cells and beads made of quartz or metal. The cells are impeded by collision or friction with the beads. there are varied characteristics that will influence the productivity of bead beating, like the shape of the container, the shaking rate, the size of the bead taken, the number of beads put to use and the types of beads, in addition to this these components will not only impact the cell disruption proficiency but as well as the energy consumption.

### **Electroporation**

Electroporation is defined as the disarrangement of particles on the cell envelope with dipole moments by applying an electromagnetic field (EF) to the biomass. The technique of electroporation is effective to extract larger proportion of lipid from the algal biomass, the absolute lipid that was extricated in a solitary electroporation treatment was around 92% while in the other techniques the absolute lipid gain was observed around just 62%, which is about 20% less then the electroporation technique.

### **Challenges of biofuel production**

The first primary challenge faced in the production of micro algae is its intricate process. As It delve into few stages, beginning from the cultivation, harvesting, cell drying, disruption of cell, in the end lipid extraction for biodiesel production. Moreover there are significant expense related with the machinery, working, and maintenance of cultivation system for oil production.

On top of that development of biofuel requests ideal ecological conditions for the

growth of micro algae in particular, appropriate light, temperature, nutrients and pH. In fact, providing natural stress can might expand biofuel production, but on expense of diminished biomass yield. It is necessary to maintain the harmony between biomass accumulation and biofuel productivity. As per Patil *et al.*, the greatest expense originates from different specialise machines included between the harvesting and transformation of oil into biofuel.

The next key hindrances in utilizing microalgae as a biofuels is using it within the bounds of the commercial cost where the lipid production requires low cost and with a balance of energy. At present, the expense of creating microalgae biofuel ought to be diminished to multiple times so as to contend with the cost of raw petroleum in the worldwide market. Thus, there is a solid need to recognize and create advanced equipped methods for reaping microalgae with a set objective of the cost at less US \$0.013/gallon of gasoline equivalent (GGE) at a production pace of 0.1 to 1 m<sup>3</sup> of micro algae water/hour. At present it is as yet troublesome and difficult for biofuel to be successfully available over non-renewable energy source.

Nonetheless, it ought to be recognized that a billion gallons of microalgae biofuel production would burn-through a lot of water, energy, and nutrients. So if the supplements are not reused back to the soil in forming a billion gallons of microalgae biofuels, it will requires millions tons of nitrogen and phosphorus consistently. Additionally, the major difficulty lies up in building up the ideal methods to choose the best strains of microalgae for cultivating, harvesting and extracting the oil and ultimately processing into fuel. Hence more research is needed for strain improvement in micro-algal species. There is a gigantic difference in the present methodology that is ought to be filled to

assure the progress of sustainable and environment friendly energy from microalgae. Regardless it is vital to investigate the maximum possible potential of microalgae, the efficiency in production and the production cost of different microalgae biofuel systems.

In conclusion as persistently the energy requirements of the world are multiplying, which is a regularly depleting the non renewable sources of energy. That is causing a hazardous impact on environment. To eliminate effect it is crucial to exploit third generation biofuel with utmost commerciality.

Improvising downstream and upstream processes might resolve the cost issues: (1) in upstream processing, profoundly profitable strains should be acquired by strain choice, genetic and metabolic engineering, and (2) downstream processing, in which enhanced biofuels yields are procured by improving the cell lipid content and by innovatory conversion of microalgal biomass to biofuels. It is also recommended that biofuel production might be joined with nitrogen-rich, municipal sludge water and CO<sub>2</sub>-rich fuel gas treatment to make it more feasible and commercial. These changes will help is most effective bio fuel production.

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