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Microalgal Biomass Production and Oil Extraction for Algae Biodiesel Production – A Review

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Abstract—*Due to awareness about depletion of fossil fuels and the harmful emissions from its use, biofuels are now in high demand. Biodiesel can be used as a substitute for fossils fuels, which is mostly produced from oil synthesized by conventional fuel crops. Microalgae have the potential of accumulating large amount of oil which enables large scale biodiesel production. Microalgae are currently an ideal third generation biofuel feedstock because of their CO_2 fixation ability and as they do not compete with food or feed crops. Moreover they can be easily produced in arable land and a viable means foe economic and environmental sustainability. In this review we present an overview about microalgae biomass production, harvesting and extraction of oil for biodiesel production.*

Keywords*: Microalgae, Biofuel, Biodiesel.*

1. INTRODUCTION

Liquid biofuels (ethanol and biodiesel) can be classified into three generations based on the feedstocks [1]. First generation liquid biofuels were produced from food crops such as corn, sugarcane and vegetable oils [2-3]. Since food crops were used in first generation biofuel production it may cause shortage of food crop and increase in the price of food crop. Second generation liquid biofuels were produced from waste cooking oil, non-edible plant seed oil (crops such as jatropha, castor, neem, karanja, rubber seed) [4], waste vegetable oil and animal fats [2].

Although second generation liquid biofuels were capable to overcome the problems faced by their first generation biofuels, but due to increasing fuel consumption there was a challenge for the supply with consistent feedstock and cost [5]. Also, they conflict with other commercial products such as cosmetics and industrial products [4] this difficulty led to the development of third generation liquid biofuels [2] like microalgae biodiesel [6].

Microalgae are the term assigned to microscopic organisms often living in colonies. It comprises of both prokaryotic and eukaryotic, mono- and multicellular organisms of sizes from several micrometers to over of dozen meters, they can be found in all fresh and salt, cold and warm waters of all geographical zones [7]. Microalgae can be the source of several types of biofuels such as: methane produced during anaerobic digestion of algae biomass [8], straight vegetable oil (SVO), renewable gasoline and jet fuel, hydrogen produced photobiologically in anaerobic conditions and biodiesel derived from lipids accumulated as reserve material in microalgae cells [8].

2. PRODUCTION OF ALGAL BIOMASS

The algae can be grown in both open culture system such as ponds, lakes, raceways etc and in closed culture system such as photobioreactors. The algal biomass requires light, carbon dioxide, water, organic salts and a temperature range of 20-30°C for its growth [9].

2.1 Open culture system

Open culture system is being in use since 1950s [10]. In this open culture system algae is grown in open air in natural water sources like lakes, ponds, and artificial ponds or containers. The most commonly used artificial system is raceway ponds [11]. Open culture systems are the most commonly used growth system and easier to build and inexpensive, more durable and have large production capacity than closed system. They can utilize sunlight and water can be supplied to open systems from nearby land areas, sewage/ water treatment plants, waste water from industries etc. It is one of the cheapest processes for large scale algal biomass production [12]. Open culture system has some technical limitations. Open ponds are exposed to weather conditions due to which it is difficult to maintain water temperature, evaporation , lighting which makes it dependent on the local climatic conditions. Furthermore there are chances of contamination by predators and other fast growing heterotrophs. Limited species of algae can be grown in open system [12]. Only *Dunaliella* which is adaptable to high salinity, Spirulina which is adaptable to high alkalinity and Chlorella (adaptable to nutrient-rich media) have been successfully grown in commercial open pond systems [12].

Raceway ponds are the most commonly used artificial system. These are made up of closed loop, oval shaped recirculation channels having a depth of 0.2 to 0.5m. Mixing and recirculation are required to stabilize algae growth and

productivity. Raceway ponds are built of concrete and compacted earth-lined ponds with white plastic have been also used. In a continuous production cycle algae broth and nutrient are fed in front of the paddle wheel and circulated through the loop to the harvest extraction point. The paddlewheel is continuously operated to prevent sedimentation [13]. The CO_2 requirement is fulfilled from the surface air or submerged aerators may be installed to enhance CO_2 absorption [14].

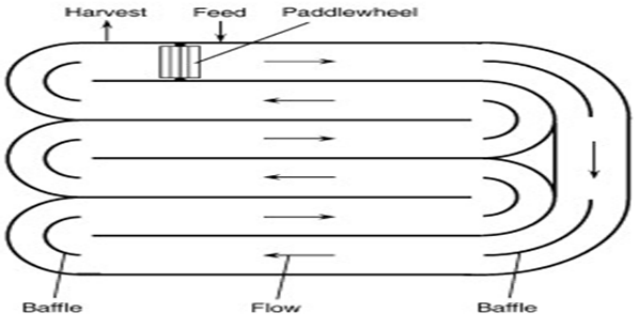

Fig 1: Arial view of a raceway pond [15]

2.2 Closed photobioreactor system (Closed culture system)

Some of the major problems associated with open pond systems can be overcome using closed photobioreactor system. Risks of pollution and contamination can be avoided using closed system. Photobioreactor systems have no CO_2 losses, reproducible cultivation conditions, controllable hydrodynamics and temperature and flexible technical design [13]. Single species of microalgae can be grown for prolonged duration in photobioreactors. Harvesting costs can be reduced because of the higher cell mass productivity. However, the cost of closed systems is higher than open pond systems. Photobioreactors consist of an array of straight glass or plastic tubes. The tubular array captures sunlight. The tubes have diameter of 0.1 m or less [12]. Mechanical pumps or airlift systems are used for re-circulation of algae [16]. Photobioreactors can be categorized into (1) Tubular (ex-helical, manifold, serpentine and α-shaped); (2) Flat (*e.g.* alveolar panels and glass plates); and (3) Column (*e.g.* bubble columns and airlift).

2.2.1 Tubular photobioreactors

Tubular photobioreactor can be horizontal/serpentine [17], near horizontal [18], vertical [19], inclined [20] and conical-shaped [21]. This system has a large illumination surface area, good biomass productivity and relatively cheap. This system also have some disadvantages which are fouling, some degree of wall growth, dissolved oxygen, and CO_2 along the tubes and also pH gradients that lead to the frequent re-carbonation of the cultures, which would consequently increase the cost of algal production. The largest closed PBRs are tubular, e.g. the 25 m^3 plant at Mera Pharmaceuticals, Hawaii, and the 700 m^3 plant in Klotze, Germany. A maximum productivity of 25 g m^{-2} d^{-1} (*Spirulina*) has been achieved in a 10 m^3 serpentine bioreactor with intermitted culture circulation [37].

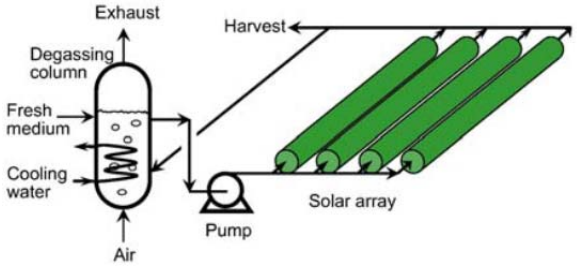

Fig 2: Tubular photobioreactor with parallel run horizontal tubes [15]

2.2.2 Flat photobioreactor

Flat photobioreactors are one of the earliest forms of closed systems. These have large surface area exposed to illumination [22]. In this system a thin layer of very dense algae culture is mixed or flown across a flat transparent panel. Flat photobioreactors are suitable for mass cultures of microalgae because of low accumulation of dissolved oxygen and high photosynthetic efficiency [22]. The panels are illuminated mainly on one side by direct sunlight and have an advantage that it can be positioned vertically or inclined at an optimum angle facing the sun to achieve a better efficiency. But have some drawbacks i.e. difficulty in controlling temperature, some degree of wall growth, many compartments and support materials are required due to scale-up, and possibility of hydrodynamic stress to some algal strains [23].

2.2.3 Column photobioreactors

Column photobioreactors are stirred tank reactors [24], bubble columns [25] or airlifts [26]. Column photobioreactors have the most efficient mixing, the highest volumetric gas transfer rates and the best controllable growth conditions. The columns are placed vertically, aerated from the bottom and illuminated through transparent walls. They are low cost and easy to operate [23].

Table 1: Advantages and limitations of various microalgae culture systems [38]

Culture Systems	Advantages	Limitations
Open systems	Relatively economical Easy to clean up Easy maintenance Utilization of non-agricultural land Low energy inputs	Little control of culture conditions. Poor mixing, light and CO2 utilization. Difficult to grow algal cultures for long periods. Poor productivity Limited to few strains. Cultures are easily contaminated.

Tubular PBR	Relatively cheap Large illumination surface area Suitable for outdoor cultures Good biomass productivities	Gradients of pH, dissolved oxygen and CO2 along the tubes. Fouling Some degree of wall growth. Requires large land space. Photo inhibition.
Flat PBR	Relatively cheap. Easy to clean up. Large illumination surface area. Suitable for outdoor cultures. Low power consumption. Good biomass productivities. Good light path. Readily tempered. Low oxygen build-up. Shortest oxygen path.	Difficult scale-up. Difficult temperature control. Some degree of wall growth. Hydrodynamic stress to some algal strains. Low photosynthetic efficiency.
Column PBR	Low energy consumption. Readily tempered. High mass transfer. Good mixing. Best exposure to light-dark cycles. Low shear stress. Easy to sterilize. Reduced photo inhibition. Reduced photo-oxidation. High photosynthetic efficiency.	Small illumination Surface area. Sophisticated construction materials. Shear stress to algal cultures. Decrease of illumination surface area upon scale-up. Expensive compared to open ponds. Support costs. Modest scalability.

3. HARVESTING OF ALGAL BIOMASS

Harvesting of algal biomass is a major issue in the industrial scale processing of algae for biofuel production. The cost of biomass recovery makes up to 20-30% of the total production cost of biomass. Microalgal biomass harvesting can be achieved in several physical, chemical or biological ways: flocculation, centrifugation, filtration, ultrafiltration, air-flotation, autoflotation, etc [27].

3.1 Flocculation

Flocculation is an initial dewatering step in the bulk harvesting process. It eases the further processing steps. In this stage algal cells are aggregated to increase the effective particle size. Algae carry negative charge which prevents them from self-aggregation. Flocculants are the chemicals which neutralize or reduce the negative surface charge. These chemicals help in coagulation of the algae without affecting the composition and toxicity of the product[27].Commonly used flocculants are Multivalent metal salts like ferric chloride ($FeCl_3$), aluminium sulphate ($Al_2(SO_4)_3$) and ferric sulphate ($Fe_2(SO_4)_3$) [22].

3.2 Flotation

Due to increase in the microalgal lipid content, some algae strains float naturally at surface of water. It is a potential harvesting method [22].

3.3 Centrifugation

In centrifugation process centrifugal forces are applied to separate algal biomass from growth medium. After separation the algae can be separated from the culture by simply draining the excess medium. However the cell structure can be damaged because of high gravitational and shear forces during the centrifugation process. It is also not cost effective due high power consumption [28].

3.4. Filtration

In filtration method broth with algae is run through filters on which algae accumulate and allow the medium to pass through the filter. The broth is continually run through the micro filters till the filter contains a thick algae paste. The different forms of filtration are dead end filtration, microfiltration, ultra filtration, pressure filtration, vacuum filtration and tangential flow filtration (TFF). Filtration appears to be an attractive dewatering option, but they incur extensive running costs and hidden pre-concentration requirements [28].

4. EXTRACTION OF OIL FROM ALGAL BIOMASS FOR BIODIESEL PRODUCTION

The most commonly used methods for algal oil extraction are mechanical extraction using hydraulic or screw, enzymatic extraction , chemical extraction using different organic solvents , ultrasonic extraction and supercritical extraction using carbondioxide.

4.1 Mechanical extraction

In mechanical extraction method , techniques such as mechanical pressing, bead milling, and homogenization are used and accounts for large scale of cell dsruptions. Mechaning pressing puts high pressure on the cells being extracted, ruptures the cell wall , allowing the intracellular lipids to be extracted and collected [29]. Homogenization ruptures the cell wall by forcing the cells through a small orifice at high pressures. When the cell reaches the opening there is a sudden drop in the pressure along with a strong liquid shear force cause the cell to break open allowing the lipids to be extracted [30]. At laboratory and industrial scales, bead milling, or bead beating, has been used for size reduction of particles and the disruption of cells [31]. In the presence of beads, this technique works by agitating the algal biomass, which results in pulverization of the algal cells and breaking them apart by mechanical force and providing a means to extract the lipids [29].

4.2 Enzymatic extraction

In enzymatic extraction process water is used as solvent with the cell wall degrading enzymes to fractionate oil, proteins and hulls. The oil is found inside plant cells along with proteins and carbohydrates. The cell is surrounded by thick walls which have to be opened to release oil and protein. By using enzymatic extraction it is possible to fractionate the

components to a degree which cannot be reached using the conventional technique like mechanical pressing. But the cost of enzymatic extraction process is much higher than the most popularly used solvent based extraction processes and this high cost is the limitation factor for large scale use of this process [32].

4.3 Chemical extraction

Solvents are used for oil extraction. Soxhlet method is the most commonly used solvent extraction method. In this method oil and fat from the solid algal biomass is extracted by repeated washing with organic solvents under reflux in a special glass apparatus called Soxhlet extractor. Petroleum ether and n-hexane are most commonly used solvents. By using this method large amount of extraction can be done using limited solvent and is cost effective so it can be more economical if used at large scale. Poor extraction of lipids, long time required for extraction and hazards of boiling solvent are its limitations [32]. When hexane was used as a solvent (solvent extraction method) it can recover almost all the oil to leave behind only 0.5% to 0.7% residual oil in the raw material [33].Addition of solvents such as n-hexane or chloroform to the in situ reaction system could lead to a higher biodiesel yield [34]. The use of an additional solvent such as hexane or chloroform helps the easy extraction of oils within microalgae cells and enhances the contact of its oil with the esterification reagent [35].

4.4 Ultrasonic extraction

In ultrasonic extraction method intense sonication of liquid is done which generates sound waves that propagate into the liquid media which causes alternating high-pressure and low-pressure cycles. Ultrasonic waves support the diffusion of solvents into the cell structure during high pressure cycle. As the cell wall breaks mechanically by the cavitations shear forces, the lipids get transferred from the cell into the solvent. The oil gets dissolved into the solvent; the tissues are then filtered out. The oil is separated from the solvent by distillation. This method improves the extraction of oil from algae as well as helps in conversion into biodiesel. This method is not feasible with large scale applications as it is not cost effective with the amount of oil production [32].

4.5 Supercritical extraction

In this process CO_2 is liquefied under pressure and heated to the point such that it has properties of both liquid and gas. This liquefied fluid is then used as solvent for oil extraction. This process is more efficient and can extract almost 100% of oil and provide high purity and product concentration [36].

5. CONCLUSION

Different processes of microalgal biomass production, harvesting of microalgal biomass and extraction of oil for production of algae biodiesel has been discussed. Open Culture system is more durable, has large production capacity and is cheaper than closed system. But this system is exposed to weather conditions therefore it is difficult to maintain favorable conditions for algae production. This problem can be overcome by using closed photobioreactor system. But its cost is higher. Therefore there is requirement to develop a safe, reliable, low cost algae production system for low cost and efficient biodiesel production. Harvesting of algae biomass contributes to 20-30% of total production cost of biomass. Therefore we need to develop a cheap, efficient and easy harvesting system. The most commonly used methods for algal oil extraction are mechanical extraction using hydraulic or screw, enzymatic extraction , chemical extraction using different organic solvents , ultrasonic extraction and supercritical extraction using carbondioxide. Each of these methods has drawbacks: The mechanical press generally requires drying of the algae, which is an energy intensive step; the use of chemical solvents poses safety and health issues; however, solvent extraction is usually applied to get high oil yields from algae; supercritical extraction requires high pressure equipment that is both expensive and energy intensive. The cost of production of algae is higher which makes it expensive than petroleum fuels, the cost of production of algal oil must be reduced to compete it with petrodiesel. Extensive efforts are required to be taken to achieve commercial-scale production of cheap microalgal biodiesel.

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