

HARVESTING AND PROCESSING OF MICROALGAE BIOMASS FRACTIONS FOR BIODIESEL PRODUCTION (A REVIEW)

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Abstract

There has been a recent resurgent interest in microalgae as an oil producer for biofuel applications. An adequate supply of nutrients and carbon dioxide enables algae to successfully transform light energy of the sun into energy - rich chemical compounds through photosynthesis. A strain with high lipids, successfully grown and harvested, could provide oil for most of our process by volume, which would then provide the most profitable output. Significant advances have also been made in upstream processing to generate cellular biomass and oil. However, the process of extracting and purifying of oil from algae continues to prove a significant challenge in producing both microalgae bioproducts and biofuel, as the oil extraction from algae is relatively energy-intensive and expensive. The aim of this review is to focus on different harvesting and extraction processes of algae for biodiesel production reported within the last decade.

Keywords: Algae, Biodiesel, Chemical extraction, Mechanical extraction.

Introduction

Microalgae are photosynthetic micro-organisms that convert the light energy, water and carbon dioxide into algal biomass. It has been estimated that about 200,000 to 800,000 species of algae exist, of which about 35,000 species are described. Over 15,000 novel compounds, originating from algal biomass, have been chemically determined. Most of these microalgae species produce unique products, like, carotenoids, anti-oxidants, fatty acids, enzymes, polymers, peptides, toxins and sterols (Cardozo et al., 2007). Algae are accountable for the net primary production of ~52,000,000,000 tonnes of organic carbon per year, which is ~50% of the total organic carbon produced on earth in each year. Average biodiesel production yield from microalgae can be 10 to 20 times higher than the yield obtained from oleaginous seeds and/or vegetable oils (Chisti, 2007).

The principle of cultivation of microalgae is the same for bacteria, yeasts or molds; it is only the media composition and photosynthesis aspect that distinguishes them. Microalgae are

particularly efficient in converting solar energy. Several species of microalgae are rich in oil; the oil content of some microalgae is around 80% of their dry weight in perfect harvest conditions (Wijffels et al., 2010).

Microalgae have the potential to produce 5,000-15,000 gallons of biodiesel per acre per year. However, there are some challenges, including, high yield of algae biomass with high lipid content and an effective technique to harvest the grown algae, to extract the algal oil and transesterify the oil to biodiesel. Harvesting and isolating products from micro algae cultures is one of the most problematic areas of algal biofuel production technology. Oil recovery from harvesting and extraction systems represents a considerable challenge and even using the most productive microalgal species, containing around 50% dry weight as oil, production amounts are very low. How to achieve this recovery process economically is one of the greatest challenges for biofuel production from microalgae. A biorefinery approach to production should thus be considered. Products derived from microalgal

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biomass can include commodity materials destined for a range of chemical products, such as, pharmaceuticals and platform chemicals, including, other fuels by conversion to ethanol and methane (Molina-Grima et al., 2003; Tredici, 2012).

Biomass Harvesting

The choice of harvesting methodology, likely to be a combination of individual methods, depends highly on the biomass type and requirements of the down-stream processing. The key harvesting and dewatering operations, currently used, are sedimentation in gravity field, centrifugation, flotation and filtration. The costs of harvesting microalgal biomass can be a major component of production (Molina-Grima et al., 2003). The first challenge is to concentrate the cells from relatively dilute solutions. It is generally preferable to maintain the system as liquid slurry to facilitate efficient handling for further down stream processing using pumps. Although sedimentation is a simple process but it is very slow (da Costa et al., 2010) and in high-temperature environments, much of the produced biomass will deteriorate during such a harvesting process. In consequence, sedimentation alone is largely dismissed as a viable harvesting method. However, flocculation caused by alkaline adjustment has been used to effectively remove the cell biomass (de-Bashan and Bashan, 2010; Vicente et al., 2007).

Centrifugation

Centrifuges can be used to separate and concentrate microalgal cells. It involves in the application of a centrifugal field to a liquid. This force causes relatively dense materials to settle more rapidly than they would under normal gravitational force. Several types of continuous centrifuges are used in industrial processes. Each variation uses a slightly different mechanism to separate dense materials from other materials (Shuler, 2002). The operational variables, such as, centrifugal force, flow rate, biomass settling rate and settling distance, will determine the efficiency of centrifugation (Williams and Laurens, 2010).

If the gravitation field to which the cells are subjected is increased, then cell separation may be achieved more rapidly. In most large-scale centrifuges, a centrifugal force equivalent to

5000-10000g is possible, and this can achieve over 95% removal under the correct operational conditions with large algal cells (Molina-Grima et al., 2003). Centrifugation is suitable for research, final thickening of slurries and recovery of high-value products. However, at a large scale, the use of centrifuges becomes more problematic as the capital costs increase with scale. This, together with specialised materials of construction (high strength, corrosion-free alloys) and high maintenance costs required to operate in saline environments, means that these separations are expensive. Energy costs of about 1kWh m⁻³ have been quoted for centrifuges (Molina-Grima et al., 2003). Membrane filtration technology becomes increasingly attractive for the equivalent duty, because, capital, maintenance and management costs are lower (Wu et al., 2006).

Flotation

Flotation is a process in which a pressurised gas is dissolved into the liquid medium. As the air is released from solution, small bubbles nucleate on particles in the fluid. As the bubbles rise toward the open atmosphere, they bring particles with them. At this point, a concentrated float of particles (e.g., algal biomass) can be skimmed from the top of the solution with a concentration as high as 4% solids (Demirbaş, 2003).

Flotation is commonly used to remove micro algae from reservoir water prior to its use as drinking water. It is a well developed and mature set of processes. Typically, the water is initially ozonated, after which the sensitised cells are then treated with about 10ppm poly electrolyte salts (typically salts of aluminium and iron or formulations of charged organic polymers) prior to being subjected to dissolved air flotation (DAF). DAF involves in the generation of fine bubbles produced by a decompression of pressurised fluid. The fine bubbles less than 10mm adhere to the flocs making them very buoyant and causing them to rise rapidly to the surface of a separation tank. The resultant concentrated cell-foam (7-10% dry weight) is then removed as slurry. These processes work well in fresh water and are capable of dealing with the large volumes required in a commercial scale plant, where additions of ozone and flocculant are made (Srivastava and Prasad, 2000). The main disadvantage of this approach is

the contamination of materials with the flocculant, which may significantly decrease their value (Molina-Grima et al., 2003). Although these methods have also not been proved in saline environments on a large scale, the integration of flotation into the bioreactor has been demonstrated. Using an integrated reactor and foam fractionator under appropriate conditions upto 90% of a *Chaetoceros* sp. could be removed (de-Bashan and Bashan, 2010).

Filtration

Filtration is a mechanical separation method, which usually uses a bed of granular media or a porous membrane. Cloth media can also be used as in the case of a rotary drum filter (Raemy, 2008). The simplest mode of filtration is dead-end filtration. Dead-end filtration of large quantities of dilute algal suspension can only be achieved, using packed bed filters (mixed media or sand). This type of filtration is limited by the rheological properties of the microalgae as these form compressible cakes that easily blind filters. This technique again has been used successfully in the removal of algae from reservoirs, where their concentration is relatively low. The amount of water that can be processed is severely limited by the characteristics of algal materials, e.g., compressible cakes and the presence of extra cellular fouling materials. These processes involve relatively low energy consumption, although the frequency of washing with loading increases energy costs and decreases filter productivity. Pressure or vacuum filtration can be used but concentration of the microalgae is required for these processes to be effective (Molina-Grima et al., 2003).

To avoid problems in dead-end filtration, cross-flow filtration can be used. The advantages of such filtration systems are their ease of scale-up with rapid advances being made in their use and operation. Several laboratory scale studies have shown that these systems are capable of concentrating microalgae and can be used in down stream fractionation (Rossi et al., 2004).

In a rotary drum system, filter material covers the circumference of a drum, which rotates partially submerged in the algae-laden water. The application of a vacuum to the inside of the drum allows submerged sections to collect solid biomass, while sections exposed to air are scraped clean (Shuler, 2002).

Drying of Algal Biomass

The harvested biomass slurry (5-15% dry solids) must be processed rapidly or it can spoil within a few hours in a hot climate. The specific post-harvest processing necessary depends strongly on the desired product. Dehydration or drying of the biomass is commonly used to extend the shelf-life of the biomass, especially, if biomass is the final product. Drying methods that have been used for microalgae are spray drying, drum drying, freeze-drying and sun drying (Molina-Grima et al., 2003; Williams and Laurens, 2010).

Because of the high water content of algal biomass, sun-drying is not a very effective method for algal powder production and spray-drying is not economically feasible for low value products, such as, biofuel or protein (Mata et al., 2010). Spray drying is the method of choice for high-value products but it can cause significant deterioration of some algal components, such as, pigments. The expense of drying can be a significant impediment for producing micro algal biomass powder for use in food and feeds. Freeze-drying or lyophilisation has been widely used for drying micro algae in research laboratories; however, freeze-drying is too expensive for use in large-scale commercial recovery of micro algal products. In some cases, solvent extraction of dry biomass has proved much more effective for recovery of intracellular metabolites than the extraction of wet biomass (Arora, 2012).

Intracellular products, such as, oils, can be difficult for solvent extraction from wet biomass of undisturbed cells but are extracted readily, if the biomass has been freeze-dried (Belarbi et al., 2000). The dried algal biomass, obtained through the above process, can be advantageously used in the production of lipids. Lipids, in turn, can be used in the production of biodiesel or green diesel.

Oil extraction

To efficiently extract materials from the inside of cells, some form of cell disruption is generally required. In most cases, because of the cost and energy involved, these disruption processes are carried out in concentrated cell preparations (50-200kg m⁻³ dry weight). There are many ways to disrupt microalgal cells; however,

the key criterion is the maximisation of the value of the materials obtained from the processes, which means that rapid and precise disruption should be used. In an industrial setting, an appropriate cell disruption technology is selected based on the durability of the cell walls to be disrupted, the size of the process stream, the risk of sub-cellular destruction of important products, the costs of the process and the safety concerns (Huntley and Redalje, 2013; Krisnangkura, 1986).

Some procedures for extracting oil from microalgae are mechanical pressing, homogenisation, milling, solvent extraction, super critical fluid extraction, enzymatic extractions, ultrasonic-assisted extraction and osmotic shock. All of these methods have their individual benefits and drawbacks (Mercer and Armenta, 2011). Extraction can be broadly categorised into two methods:

Mechanical cell disruption

Mechanical disruption includes pressing, bead-milling and homogenisation. These approaches minimise contamination from external factors (Greenwell et al., 2010). In terms of finding an effective and efficient mode of disrupting cells, there are multiple options when it comes to use this kind of technology, some of which include the identification of biological features of the organism that make it possible to weaken the cell wall prior to mechanical disruption, such as, pre-treatments (i.e., acid/alkali and enzymatic action), thus potentially minimising the use of solvents. Mechanical technologies are often used in combination with solvent methods to separate the lipid from the cell biomass. Such methods are energy intensive and better operated at high cell density condition; in addition, pretreatments are necessary to obtain high recovery ratio (Greenwell et al., 2010). Mechanical disruption of cells is chosen in most cases, as this offers an approach that avoids further chemical contamination of the algal preparation while preserving most of the functionality of the material within the cell (Chisti and Moo-Young, 1986).

Pressing/Expeller Press

Pressing/Expeller press involves subjecting the microalgal biomass to high-pressure, which ruptures cell walls and releases oil. For this purpose, algae is dried while retaining its oil

content, which then can be pressed out with an oil press. Since different strains of algae vary widely in their physical attributes, various press configurations (screw, expeller, piston, etc.) work better for specific algae types. Many commercial manufacturers of vegetable oil use a combination of mechanical pressing and chemical solvents in extracting oil. A press can extract between 70-75% of the oils out of microalgae. Often, mechanical pressing is used in conjunction with chemical solvents. While simple in design, this is a highly energy intensive and extraction efficiency is low (Popoola and Yangomodou, 2006; Williams, 2007) (Table 1).

Homogenisation

Another method of disruption is called homogenisation. In this process, pumps are used to accelerate the liquid medium to a high velocity. The action of the pump itself or the subsequent collision of the high-velocity impact ring applies shear forces to the liquid, which can destroy cell walls (Krisnangkura, 1986). Although further study is needed to document the shear sensitivity of algae cells (Cooney et al., 2009), existing investigations have shown that it is difficult to achieve high rates of cell wall destruction, using a high shear homogenisation system (Yuan et al., 2011). Due to the high energy requirements of homogenisation of algae, it has been proposed that this process is best suited for the recovery of high-value products rather than biofuel feedstock (Casey and Lubitz, 1963; Doucha and Lívanský, 2008).

Bead milling

A bead mill uses a large number of small high-velocity beads to break cell walls. By exciting the beads, a bead mill produces shear forces large enough to destroy cell walls. Bead milling is traditionally a laboratory-scale process; however, a large-scale type of bead mill, called a dyno-mill, has been used successfully to disrupt microalgae cells. A dyno-mill excites beads using rapidly rotating, notched discs (Krisnangkura, 1986). Although high rates of cell disruption are possible, the process of bead-milling requires a great deal of energy. The degree of disruption mostly depends on contact between biomass and beads and also on the size, shape and composition of the beads and strength of the microalgal cell walls (Doucha and Lívanský, 2008). In spite of

high energy costs, it has been proposed that dynamilling is the most practical method of large-scale, mechanical cell disruption for algae processing (Foglia et al., 1997).

Bead milling is generally used in conjunction with solvents to recover oil and is the most effective and economical when cell concentrations are significant and when extracted products are easily separated after disruption. Typically, this type of cell disruption is the most effective and energy-wise when biomass concentrations of 100 to 200g/L are used (Greenwell et al., 2010).

Ultrasonic-assisted Extraction

Ultrasonic cell disruption is used to apply ultrasound energy to a solution containing a culture of cells (Krisnangkura, 1986). Ultrasonic extraction can greatly accelerate extraction processes. Ultrasonic waves are used to create cavitation bubbles in a solvent material. When these bubbles collapse near cell walls, it creates shock waves and liquid jets that cause those cells walls to break and release their contents into the solvent (Cravotto et al., 2008).

Non-mechanical cell disruption

Algal oil can be extracted, using chemicals. Benzene and ether have been used. Oil can also be separated by hexane extraction, which is widely used in the food industry and is relatively inexpensive. The downsides, using solvents for oil extraction, are the dangers involved in working with the chemicals. Care must be taken to avoid exposure to vapours and direct contact with the skin, either of which can cause serious damage. Benzene is classified as a carcinogen. Chemical solvents also present the danger of being an explosion hazard.

Solvent extraction methods

Solvent extraction is a commonly used method for soybean processing and it is also used to extract lipids from microbial cells. Organic solvents should be insoluble in water, be easy to obtain, have a low boiling point and be reusable. Current industrial solvents for micro-lipids accumulation include hexane, chloroform, acetone, benzene and cyclohexane, which can dissolve lipid without residual cell. The extraction process is significantly affected by operational conditions, such as, temperature and pressure. Accelerated solvent extraction (ASE) is named

when the operation temperature is higher than that of solvent boiling point, which can be used for oil extraction from dry biomass (Cooney et al., 2009). Mixture of chloroform and methanol (Bligh and Hyer method) is the most common organic solvent to extract oil from biomass. This organic mixture can extract oil not only from dry biomass but also from wet biomass. However, the efficiency is different at certain condition (Zhu et al., 2002). The efficiency of oil extraction was not high with wet *Mortierella alpina* biomass. The process generates large amounts of wastewater and solvent often contaminates the final products. Simultaneous extraction and transesterification is more efficient (15-20%) than the separate process (Belarbi et al., 2000); however, the important point of the simultaneous process is to balance the reaction time for the best components of product (Lewis et al., 2000).

Hexane Solvent Method

Algal oil can be extracted using chemicals. Benzene and ether have been used, but a popular chemical for solvent extraction is hexane, which is relatively inexpensive. The downsides, using solvents for oil extraction, are the inherent dangers involved in working with the chemicals. Benzene is classified as a carcinogen. Chemical solvents also present the problem of being an explosion hazard (Rahman, 2008).

Hexane solvent extraction can be used in isolation or it can be used alongwith the oil press/expeller method. After the oil has been extracted using an expeller, the remaining pulp can be mixed with cyclo-hexane to extract the remaining oil content. The oil dissolves in the cyclohexane and the pulp is filtered out from the solution. The oil and cyclohexane are separated by means of distillation. These two stages (cold press and hexane solvent) together will be able to derive more than 95% of the total oil present in the algae.

Soxhlet Extraction

Soxhlet extraction is an extraction method that uses chemical solvents. Oils from the algae are extracted through repeated washing or percolation with an organic solvent, such as, hexane or petroleum ether, under reflux in a special glass ware.

Super-critical Fluid Extraction

Super-critical fluid extraction involves in the use of substances that have properties of both

liquids and gases, when exposed to increased temperatures and pressures. This property allows them to act as an extracting solvent, leaving no residues behind when the system is brought back to atmospheric pressure and RT industries (Mercer and Armenta, 2011). Carbon dioxide is the most commonly used super-critical fluid, sometimes modified by co-solvents, such as, ethanol or methanol. Critical temperature and critical pressure of carbon dioxide is at 31°C and 74 bar, respectively (Cooney et al., 2009). Super-critical fluids produce highly purified extracts without using toxic solvent and the process is fast and safe for thermally sensitive products. Supercritical CO₂ extraction efficiency is affected by four main factors, viz., pressure, temperature, CO₂ flow rate and extraction time. Ethanol (10-15%) co-solvent led to the results similar to Bligh and Hyer method at extracting oil from *Arthrospira maxima* and *Spirulina platensis* (Mendes et al., 2006; Sajilata et al., 2008). The limitation of supercritical fluid extraction is high capital cost and high cost for maintenance.

Enzymatic extraction

Enzymes can also be used to facilitate the hydrolysis of cell walls to release oil into a suitable solvent. The use of enzymes alone or in combination with a physical disruption method, such as, sonication has the potential to make extractions faster and with higher yields (Mercer and Armenta, 2011). The costs of this extraction process are estimated to be greater than hexane extraction.

Physical Treatment

Osmotic shock

Osmotic shock is the sudden reduction in the movement or concentration of water across the algal cell membrane. The stress from the rapid change in movement created by the addition of high concentrations of a solute or other additive (e.g., salt, substrates, neutral polymers, such as, polyethylene glycol, dextran) causes the cells to rupture, releasing the cellular components, including, oil.

Table 1. Some common extraction methods explored in the last decade, with advantages and limitations

Extraction method	Advantages	Limitations	References
Pressing	Easy to use; no solvent involved.	Large amount of sample required	Popoola and Yangomodou, 2006
Solvent extraction	Solvents used a relatively inexpensive; results are reproducible	Most organic solvents are highly flammable and/or toxic, solvent recovery is expensive and energy intensive, large volume of solvent is required	Herrero et al., 2004; Galloway et al., 2004
Super critical fluid extraction	Non-toxic (no organic solvent residue in extracts); green solvent, non-flammable	High energy consumption; expensive/difficult to scale up	Mací'as-Sa'nchez et al., 2005; Pawliszyn, 1993
Ultrasonic assisted	Reduced extraction time; reduced solvent consumption; greater penetration of solvent into cellular materials; improved release of cell contents into bulk medium	High power consumption, difficult to scale up	Luque-García and Castro, 2003; Martin, 1993

Transesterification and biodiesel production

After extraction from biomass, triglyceride lipids can be converted into fuel compounds by transesterification (Fig. 1). Transesterification can be performed continuously or by using a batch process. The main by-products of

transesterification are fatty acids ethyl ester (FAEE) and glycerol (Lang et al., 2011). An acid catalyst, such as, sulfuric acid, is used when the oil has high acid value. When the oil has low acid value, an alkali catalyst may prove to be more effective. The principal alkali reactant is a short

chain alcohol; methanol, CH₃OH. When methanol is deprotonated in solution and exposed to a triglyceride, it cleaves the glycerol group off of the triglyceride, resulting in one molecule of glycerol and three molecules of methylated fatty acids. Glycerol is periodically or continuously removed from the reaction solution in order to drive the equilibrium reaction toward completion.

The presence of methanol, the cosolvent that keeps glycerol and soap suspended in the oil, is known to cause engine failure. To prevent this, centrifugation washes biofuels from the soap (and glycerol). Dry bubbling, a longer process (2-3 days) that promotes the evaporation of methanol, can expedite by products separation and settling, ridding the biofuel of soap (Smith et al., 2009).

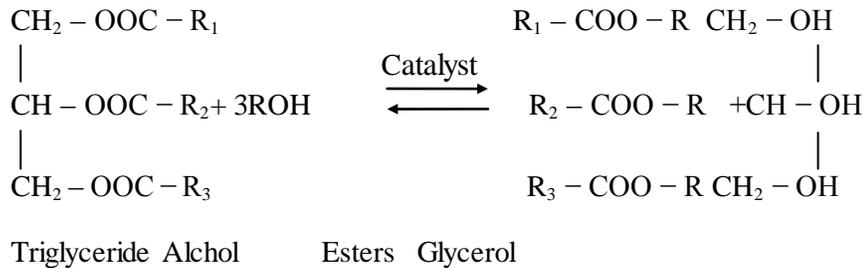


Fig.1. Transesterification reaction for biodiesel production

After transmethylation is carried out in a commercial biodiesel production process, the product must be isolated and purified. Glycerol is denser than biodiesel and can be drained out of a reactor. Impurities are removed by washing the product with water. Residual methanol is removed by distillation (Smith et al., 2009).

Challenges associated with oil extraction

- Microscopic algae suspended in water are virtually indestructible.
- Cell wall has a high elasticity modulus.
- Before extracting the algae oil, high moisture present in algae biomass must be removed by means of drying. The temperature used in microalgae drying is crucial factor for separating the oil from dried algae biomass. Higher drying temperature decreases both concentration of triacylglycerides and lipid yield (Widjaja et al., 2009).
- Even when free water has been removed, wet biomass retains sufficient interstitial water to act as lubricant.
- Rupture of cell wall through mechanical friction and steam explosion is only possible when it is dry.
- The alkaline catalyst based biodiesel production is not suitable for algae oil with high FFA content (Um and Kim, 2009).

Conclusion

Algae are an economical choice for biodiesel production because of its availability and low cost. The microalgae oil has the potential to replace the conventional diesel fuel but the harvesting and isolation of products from micro algae cultures is one of the most problematic areas of algal biofuel production technology. There are large knowledge gaps needed to develop extraction/fractionation processes, such as, cell wall composition and chemistry, the impact of high water content and chemistry on the extracted materials, understanding the effect of cultivation and strain selection on the production of carbohydrates and lipids. Additionally, the need for demonstration facilities to provide standardised materials, develop new tools and methods, is critical to accelerate progress towards the goal for biofuel production from microalgae.

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