Disc Stack Centrifugation Separation and Cell Disruption of Microalgae: A Technical Note

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Abstract

A major challenge in the commercialisation of biofuel from microalgae is the reduction of the operational energy required for its production and in particular the energy used in cell harvesting and oil extraction. The use of a disc stack centrifuge to achieve a combined cell harvesting, cell disruption and oil separation process is briefly examined and discussed.

Keywords: Centrifugation, Algae, Biodiesel, Biofuel, Lysis, Cell Disruption, Disc Stack

1. Introduction

The demand for renewable biofuels continues to grow as concerns increase about current fuel costs, dwindling fossil fuel supplies and global warming. The use of first generation biofuels, derived from food crops, such as soya and sugarcane, is controversial due to the influence on world food markets and competition for agricultural land. Alternatives that do not depend on agricultural or forestry ecosystems, known as third-generation biofuels, are proposed as a possible solution.

Algae are a diverse range of aquatic 'plants', ranging from unicellular to multi-cellular forms and generally possess chlorophyll, but are without true stems and roots. They can be divided by size into two groups: macroalgae, commonly known as 'seaweed', and microalgae, microscopic single cell organisms ranging in size from a few micrometres (µm) to a few hundred micrometres. The term microalgae is often used to include the prokaryotic cyanobacteria (blue green algae), although these are no longer formally classified as algae, together with the eukaryotic microalgae such as diatoms and green algae.

Microalgal biomass cultivation for biofuel is receiving a great deal of attention as a potential source of third generation biofuels, for several reasons. They can be cultivated on non-agricultural land, with many species growing in brackish or salt water. Many researchers consider that the productivity of microalgae could be greater than that of terrestrial crops and the lipid content can be high at over 70 % (especially when subject to nutrient stress)(Ferrell & Sarisky-Reed, 2010; Sheehan, Dunahay, Benemann, & Roessler, 1998).

Many types of algal biofuel have been considered, including biogas, bio-hydrogen, algal fuel cells, bioethanol and direct algal biomass combustion (Benemann, 2000; Kruse & Hankamer, 2010; McKendry, 2002; Strik, Terlouw, Hamelers, &Buisman, 2008; Velasquez-Orta, Curtis, & Logan, 2009; Verma, Mehrotra, Shukla, & Mishra, 2010).The demand for liquid fuels, however, coupled with the potential high lipid content of some microalgal species under certain conditions, has resulted in much of the work focusing on the production of biodiesel and other liquid biofuels derived from microalgal lipids.. Unfortunately after some 70 years of research

and with over 50 companies working on algal biofuels there are as yet no commercial-scale quantities or sources of algal biofuel at competitive prices and the process of producing fuel from microalgae would appear to be currently uneconomic (Milledge, 2010a; Pienkos & Darzins, 2009; St John, 2009).

2. Algal harvesting

The process of microalgal fuel production can be divided into three areas; growth, harvesting and energy extraction. The dilute nature of the algal suspension (0.02 to 0.05 %) (Zamalloa, Vulsteke, Albrecht, & Verstraete, 2011) poses considerable challenges and can result in substantial energy being required in all three areas of the process and particularly in harvesting (Figure1). There is no universal harvesting method for microalgae (Mata, Martins, & Caetano, 2010; Shen, Yuan, Pei, Wu, & Mao, 2009) and filtration, sedimentation, flocculation, flotation, and centrifugation or a combination of any of these methods may be used (Brennan & Owende, 2010).

Many types of filters have been used to harvest algae and filtration has been found satisfactory at recovering relatively large algal cells (Molina Grima, Belarbi, Acien-Fernandez, Robles-Medina, & Yusuf, 2003), but can be hampered by low through put and rapid clogging (Mohn, 1988; Oswald, 1988). Ultrafiltration is a possible alternative for recovery, in particular ofvery fragile cells, but has not been generally used for microalgae (Mata, et al., 2010; Molina Grima, et al., 2003): while operating costs are high and maintenance costs very high (Mata, et al., 2010; Purchas, 1981).

In sedimentation and flotation gravitational forces cause liquid or solid particles to separate from a liquid of different density, but the process can be extremely slow, especially if density difference or particle size is small. Colonial and filamentous algae could be harvested using low-cost sedimentation methods, but for the majority of microalgae settlement alone is impractical (Nurdogan & Oswald, 1996) and cell recovery and solid concentrations are low (Mata, et al., 2010; Shen, et al., 2009). Increasing the size of particles by the aggregation of algal cells through flocculation can increase the rate of settling or flotation (Mata, et al., 2010). Flocculation may be induced by chemicals or microorganisms, but methods may be algae species-specific and recovery and recycling of the flocculants can be problematic (Mohn, 1988; Molina Grima, et al., 2003; Oswald, 1988; Shen, et al., 2009). The advantages and disadvantages of the different separation systems are summarised in Table 1.

2.1 Disc stack centrifugation

In centrifugation gravity is replaced as the force driving separation by a much greater force, in the case of disc stack centrifuges from 4000 to 14000 times gravitational force (Perry & Chilton, 1973), thus greatly reducing separation time. Disc stack centrifuges are the most common industrial centrifuge (Perry & Chilton, 1973) and are widely used in commercial high value algal product plants and algal biofuel pilot plants (Molina Grima, et al., 2003). Almost all types of microalgae can be separated reliably and without difficulty by centrifugation (Mohn, 1988). A disc stack centrifuge consists of a relatively shallow cylindrical bowl containing a number (stack) of closely spaced metal cones (discs) that rotate. The mixture to be separated is fed to the centre of the stack of discs and under the influence of centrifugal force the dense phase travels outwards on the underside of the discs and the lighter phase is displaced to the centre. Materials of different densities are separated into thin layers and the narrow flow channel of 0.4mm to 3mm between the closely spaced discs means that the distance materials must travel for this separation to occur is small (Mannweiler& Hoare, 1992; Perry & Chilton, 1973). Disc stack centrifuges are ideally suited for separating particles of the size (3 -30μm) and concentration (0.02 to 0.05 %) of algal cells in a growth medium, as shown in Figure 2. They canseparate not only solid / liquid, but also liquid / liquid or liquid / liquid / solid on a continuous basis.

2.2 Energy requirements for disc stack centrifugation

Disc stack centrifuges generally have a high energy consumption (Uduman, Qi, Danquah, Forde, & Hoadley, 2010). As an example, a Westfalia HSB400 disc-bowl centrifuge with intermittent self-cleaning bowl centrifugal clarifier has a maximum capacity of 95 $m^3 h^{-1}$, but is limited to 35 $m^3 h^{-1}$ for algae harvesting (Cawdery, D, GEA Westfalia, personal communication, 2009). The maximum power of the motor is 75 kW, but normal operating demand is probably around 50 kW, giving an energy cost for separation of 1.4 kWh m^{-3} (Cawdery, D, GEA Westfalia, personal communication, 2009). A value of 1 kWh m^{-3} has been reported for concentrating *Scenedesmus* from 0.1 % to 12 % using a Westfalia self-cleaning disk stack centrifuge (Molina Grima, et al., 2003). If a HSB400 centrifuge is fed with a suspension of 0.02 % dry weight of microalgae having an oil content of 20 %, this would yield the equivalent 7 kg of dry algal material per hour and 1.4 kg of algal oil. If 90 % of the algal oil is converted to methyl ester biodiesel then 1.26 kg is produced with a calorific value of 13 kWh, assuming a net calorific value 10.33 kWh kg^{-1} (Defra, 2010). The operating energy for centrifugation is thus approximately four times the energy available in the algal biodiesel. Although this calculation is based on the data from one manufacturer, similar information for Alfa-Laval models (Ord, D., Alfa Laval,

personalcommunication, 2009) also indicates that more energy is used in centrifugation than is available in the biodiesel produced.

This simple calculation together with other studies (Ferrell & Sarisky-Reed, 2010; Molina Grima, et al., 2003) indicates the high energy usage of disc stack centrifuges. The energy return using centrifugation could be improved by: pre-concentration using a combination of separation techniques; use of the entire algal biomass rather than just the lipid fraction for energy production; or the use of the centrifuge to eliminate other energy consuming unit operations in algal biofuel production process.

Pre-concentration, by settlement or other low energy methods, to 0.5 % (algal dry weight) could improve the energy balance, but would still require 15 % of the energy in the biodiesel product for centrifugation, based on 175 kg of dry algal material containing 35 kg of algal oil producing 31.5 kg biodiesel having a fuel calorific value of 326 kWh. The financial costs of harvesting are also high and have been estimated at 14 % of the cost of the algal oil (Shen, et al., 2009) to 20 % of the algal biomass (Verma, et al., 2010).

The energetic position of using a centrifuge for the production of biofuel could be improved by the use of the entire algal biomass. A kilogram of dry algal biomass containing 20 % oil would yield around 1.9 kWh of biodiesel, but the calorific value of the entire biomass is around 6 kWh (Milledge, 2010b) and the exploitation of the entire biomass could thus be a key factor in a positive energy balance in the production of biofuel (Heaven, Milledge, & Zhang, 2011; Milledge, 2010a; Sialve, Bernet, & Bernard, 2009; Stephenson et al., 2010).

3. Cell disruption and extraction

In the production of algal biodiesel, lipid must be extracted from the algal cell prior to trans-esterification. Intact cell walls hamper lipid recovery and the most effective methods of recovery are from disrupted algal cells (Greenwell, Laurens, Shields, Lovitt, & Flynn, 2010). Mechanical disruption of the algal cells is generally considered preferable to chemical disruption as it avoids chemical contamination and preserves the functionality of the cell contents (Chisti & Moo Young, 1986). The breaking of cell walls can require large amounts of energy, and cells can be mechanically disrupted by ultrasound, milling, autoclaving or homogenisation (Mata, et al., 2010). Homogenisation can be very efficient, with between 77 % and 96 % of algal cells ruptured per pass (GEA Process Engineering, 2011), but to homogenise 10 l of algal suspension with an algal cell concentrations between 100 and 200 g l^{-1} requires 1.5-2.0 kWh (Greenwell, et al., 2010) or 0.75 to 2 kWh per kilogram of algal cells disrupted. It has been suggested that this cell disruption and subsequent oil extraction represent the largest energy input in the production of biodiesel (Razon & Tan, 2011) and if these processes could be combined with algal harvesting then a considerable reduction could be made in operation energy requirements needed to produce algal biodiesel.

3.1 Micro-eddies and algal cell disruption

Many algae are sensitive to hydrodynamic forces and cells may be damaged in mixing, pumping and gaseous transfer (Garcia Camacho et al., 2011; Hondzo, Kapur, & Lembi, 1997; Joshi, Elias, & Patole, 1996). If the hydro-mechanical forces are sufficient they can fracture cells, but lesser forces may cause reduced growth and cell death without any obvious physical damage. Although information exists on the effect of hydrodynamic forces on a wide range of bacterial, animal and plant cells in defined flow experimental systems, much less is known about the effect of hydrodynamic forces in process equipment (Chisti, 2001). It has been suggested that microalgal cells are damaged when the size of the micro-eddies is of the same order as or smaller than the algal cell (Molina Grima, Fernandez, Acien Fernandez, & AcienFernadez, 2010). Eddies with scales larger than a cell simply carry the cell from place to place, but eddies of similar size or smaller than a cell exert mechanical forces on the cell wall, and if these are greater than cell wall strength the wall is fractured (Doulah, 1977).Disruption of the cells may occur as a result of localised velocity gradients within an eddy or between eddies (Rodriguez, Samo, Hozbor, & Yantorno, 1993), although the exact nature of the micro-eddy hydrodynamic forces acting on the cells (shear, compression, torsion or impact) causing cell disruption isnot fully understood (Clarke, Prescott, Khan, & Olabi, 2010). The size of a micro-eddy may be estimated using Kolomogrof's theory (Davidson, 2004; Molina Grima, et al., 2010).

Equation1.

$$\lambda = \left(\frac{\mu}{\rho}\right)^{\frac{3}{4}} \xi^{-1/4}$$

Where λ is the micro-eddy length, ξ is the energy dissipation per unit mass, μ is the viscosity of the fluid and ρ is the fluid density.

Damage to yeast cells has been demonstrated in disc stack centrifuges in the brewing industry (Chlup, Bernard, & Stewart, 2008). If sufficiently high hydrodynamic forces could be generated in a disc stack centrifuge to provide cell disruption, with simultaneous lipid separation through liquid/liquid/solid separations of the type in Figure 3, considerable energy could be saved in the production of algal oil. Areas of high shear stress have been demonstrated in disc centrifuges as shown in Figure 4 (Boychyn et al., 2004). Using Equation 1 with a maximum energy dissipation per unit of $2.00 \times 10^5 W\ kg^{-1}$ from Figure 4, viscosity 9.00×10^{-3} Pa s and density 1115 kg m^{-3}, the minimum size of micro-eddies is estimated at 7 μm which is of the order of the size of many microalgae. This calculation is based on one manufacturer's disc stack centrifuge, but the similarity in the general design of disc centrifuges and similar or higher maximum hydraulic energy dissipation rates occurring in an alternative type of centrifuge (Boychyn, et al., 2004) indicate that damage to algal cells could occur during disc stack centrifugation.

4. Discussion and Conclusion

Disc stack centrifuges, although suited to the separation of the particle sizes and concentrations found in microalgal suspensions, have too high an energy consumption to be suitable for the production of algal biodiesel rather than higher value commercial algal products. The energy balance could be improved by combination with other separation methods and by the exploitation of entire biomass to produce energy.

Disc stack centrifuges have been shown to cause cell damage to yeast, and calculation of micro-eddy sizes indicates that algal cells could also be damaged. If the algal cell fracture was sufficient to liberate oil it is possible that a disc stack centrifuge operating as a liquid/ liquid /solid separator could achieve or be designed to achieve cell destruction, oil separation and algal biomass separation in a single operation. Considerable energy could be saved by eliminating the energy requirement in the process operations of cell fracture and lipid extraction. Although this is an intriguing prospect, it is unlikely that that current disc stack centrifuges will cause sufficient algal cell disruption in a single pass. Work on yeast has shown some cell damage and viability reduction on a single pass through the centrifuge, but 9 passes were required to achieve 92.4 % decrease in cell viability (Chlup, et al., 2008). If cells are fractured the smaller solid particles may also reduce centrifugation efficiency and reduced algal solids recovery as has been shown with both yeast and mammalian cells (Chlup, et al., 2008; Hutchinson, Bingham, Murrell, Farid, & Hoare, 2006).

It would appear that current disc stack centrifuges can cause damage to algal cells, but their use to achieve combined algal cell fracture and oil separation will require some redesign and extensive further research.

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Table 1. Comparison of microalgal harvesting methods (Mohn, 1988; Molina Grima, et al., 2003; Shen, et al., 2009)

|	Advantages	Disadvantages	Dry Solids Output Conc'
Centrifugation	Can handle most algal types with rapid efficient cell harvesting.	High capital and operational costs.	10-22%
Filtration	Wide variety of filter and membrane types available.	Highly dependent on algal species; best suited to large algal cells. Clogging or fouling an issue.	2-27%
Ultrafiltration	Can handle delicate cells.	High capital and operational costs	1.5-4%
Sedimentation	Low cost, potential for use as a first stage to reduce energy input and cost of subsequent stages.	Algal species specific, best suited to dense non-motile cells. Separation can be slow. Low final concentration.	0.5-3%
Chemical Flocculation	Wide range of flocculants available, price varies although can be low cost.	Removal of flocculants, chemical contamination.	3-8%
Flotation	Can be more rapid than sedimentation. Possibility to combine with gaseous transfer.	Algal species specific. High capital and operational cost.	7%
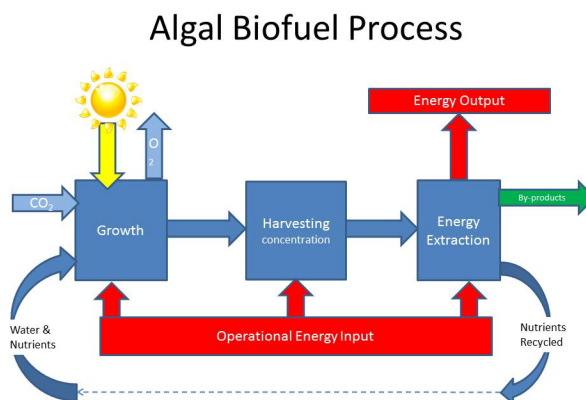
Figure 1. Algal Biofuel Process
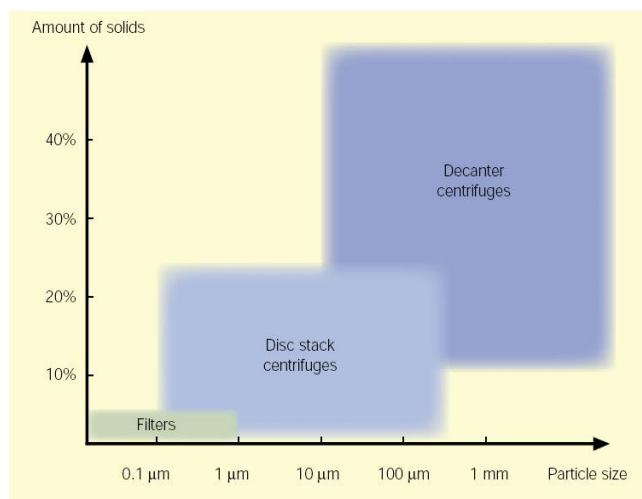
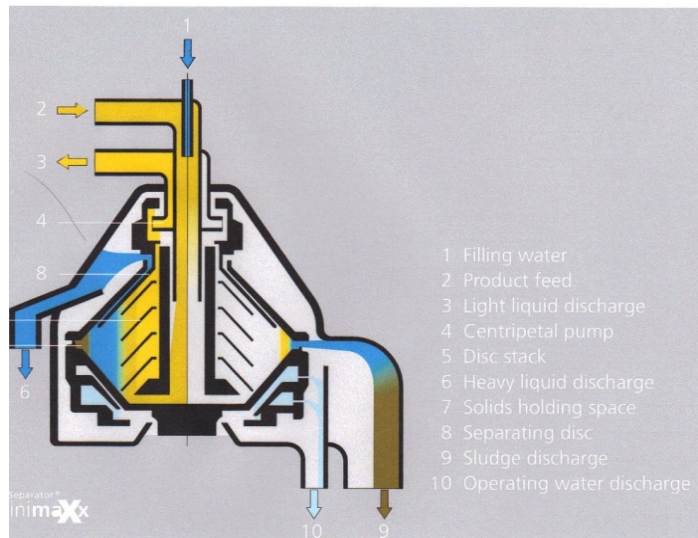Figure 2. Centrifuge Application Graph. Courtesy Alfa Laval

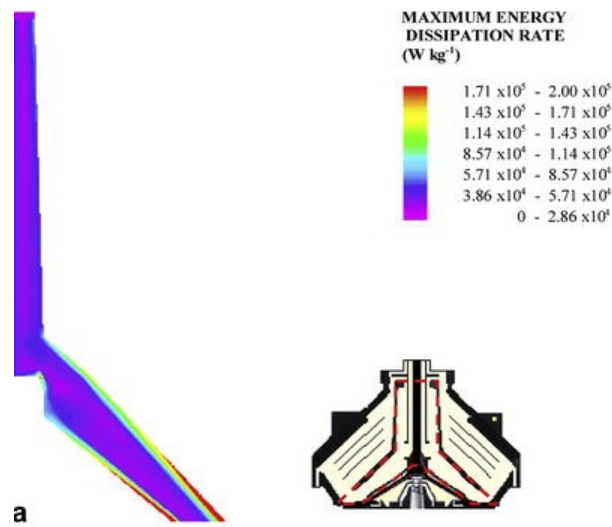Figure 3. Liquid/Liquid/Solid Separation Disc Stack Centrifuge (Courtesy GEA Westfalia)

Figure 4. CFD analysis of the feed zone of a pilot disc-stack centrifuge (Boychyn, et al., 2004). Greatest energy dissipation rates are indicated in red, while the lowest ones are in purple.