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Dissolved air flotation and centrifugation as methods for oil recovery from ruptured microalgal cells

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Abstract

Solvent-free microalgal lipid recovery is highly desirable for safer, more sustainable and more economical microalgal oil production. Dispersed air flotation and centrifugation were evaluated for the ability to separate oil and debris from a slurry mixture of osmotically fractured *Chaetoceros muelleri* cells with and without utilizing collectors. Microalgal oil partially phase-separated as a top layer and partially formed an oil-in-water emulsion. Although collectors, such as sodium dodecyl sulphate enhanced selective flotation, by just adjusting the pH and cell concentration of the mixture, up to 78% of the lipids were recovered in the froth. Using centrifugation of fractured microalgal slurry resulted in removal of 60% cell debris and up to 68.5% of microalgal oil was present in the supernatant. Both methods, centrifugation and flotation provided options for separation of microalgae oil from *C. muelleri* slurry with similar fatty acid recoveries of 57% and 60%, respectively.

Keywords

Algal oil; dispersed air flotation; microalgal lipid recovery, solvent-free lipid extraction

1. Introduction

Microalgae are considered a promising source for lipids, for example as feedstock for omega-3-rich oil and biodiesel production (Adarme-Vega et al., 2012). Their comparatively high growth rate, high oil content and capability of being harvested more frequently, have made microalgae a potentially strong competitor for traditional oil bearing crops (Schenk et al.,

2008, Lee et al., 2010, Mercer and Armenta, 2011, Balasubramanian et al., 2011). Furthermore, they can utilize non-arable lands and salt/waste water streams for their growth. However, microalgal oil production still is a costly process and requires significant developments to compete more efficiently with other oil-bearing crops or the petrochemical oil industry (Mercer and Armenta, 2011).

An essential pre-requisite of many of the current microalgal lipid extraction/recovery techniques is drying of biomass which imposes high costs on downstream processes for microalgal oil and biofuel production (Yoo et al., 2012). Hence, lipid extraction and recovery from wet biomass plays a central role in removing this existing obstacle. However, wet lipid extraction has main drawbacks of dealing with relatively large volumes and producing an oil-in-water emulsion. This emulsion mainly consists of water, oil droplets and algal cell debris. Organic solvents can be applied for oil recovery from this emulsion, but due to their many disadvantages their application should ideally be minimized or removed. Their disadvantages include environmental and safety issues, high costs and contamination of the product (Lee et al., 2010, Mercer and Armenta, 2011, Balasubramanian et al., 2011, Grigonis et al., 2005, Moser, 2011, Patil et al., 2011, Wahlen et al., 2011). However, solvent-free harvesting and recovery of the lipid bodies from the aforementioned emulsion is not an easy task, as the emulsions are usually hard to be separated due to the small sizes of oil droplets of less than 20 µm (Zhou et al., 2010). Most of the current available techniques in demulsification are more applicable on water-in-oil emulsions or highly concentrated oil-in-water emulsions. For example, electric fields can be utilized to separate the water from water-in-oil emulsions (Cañizares et al., 2007). Membrane separation can be applied on both, oil-in-water and water-in-oil emulsions, but is highly dependent on the oil concentration (Li et al., 2006). Moreover, most of the available techniques have been mainly applied in oily waste water treatments

where the oil recovery is not the main aim or have been utilized by the oil industry to remove small amounts of water from crude oil. In addition, different properties of microalgal oils than those of petrochemicals make demulsification techniques used by the oil industry a non-efficient process for microalgal-derived oil-in-water emulsions. Recently, a new filtration technology has become available based on metal hydroxide hydrate gels that allows efficient and cost-effective removal of oil droplets from water (Schenk and Malekizadeh, 2013). Flotation is an effective separation method which has been mainly applied in mineral processing and which can be used for low concentration oil-in-water emulsions (Matis et al., 1994). To date, its application in removal of algae during water treatment and microalgae harvesting has been investigated in biotechnology (Matis et al., 1994, Edzwald, 1993, Garg et al., 2012). To our knowledge, the present study is the first time that flotation is being investigated for its potential in lipid recovery from wet extracted microalgae.

The principle of flotation is basically utilizing air bubbles to capture fine particles (from less than 10 μm to 500 μm). In this process hydrophobicity plays the main role as the hydrophilic particles are separated from the hydrophobic ones through attachment of the hydrophobic particles to the air bubbles and their flotation in the froth layer (Matis et al., 1994, Garg et al., 2012). For efficient flotation of oil droplets in an emulsion, a good contact between generated air bubbles and oil droplets needs to accomplished through agitation. Then the oil droplets should attach to the air bubbles and keep this contact to subsequently float to the surface of the flotation reactor (El-Kayar et al., 1993). There are different techniques which can be applied in flotation, such as dissolved air, dispersed (induced air) and electroflotation. The bubble diameter in dispersed air flotation (DiAF) ranges from 700 to 1500 μm diameter, while the air bubbles in dissolved air flotation (DAF) are 10 to 100 μm in diameter (El-Kayar et al., 1993). DiAF can be applied on large volumes and has been widely used in the mining

industry at volumes reaching up to 500 m^3 (Garg et al., 2012, El-Kayar et al., 1993). Flotation has introduced many advantages such as low space requirements (e.g. less than 4 m^2 for a Jameson cell), high flexibility (many different applications), rapid operation (typically within several hours) and moderate operational costs (0.8 kWh/m^3 culture) (Angelidou et al., 1977; Garg et al., 2012; Sharma et al., 2013). Collectors can be applied to enhance flotation efficiency. These reagents can render the particles more hydrophobic. They have a hydrophilic polar functional group and a non-polar hydrophobic tail with which they have the ability to attach to both particle and bubble at the same time, hence facilitate the contact of the air bubble and particle. Collectors are usually surface-active compounds and can be classified to thio-compounds, anionics, cationics and nonionics (Matis et al., 1994; Garg et al., 2012).

In preliminary experiments by the authors, DiAF showed promising results in condensing more than 90% of the microalgal lipids and cell debris of slurry containing osmotically fractured *Chaetoceros muelleri* cells when applied on a much diluted mixture and with utilizing 10 ppm dodecylphosphocholine (DPC). It was observed that the air bubbles in the presence of DPC could carry both algal debris and lipids to the top of the flotation cell, forming a froth, which was subsequently removed manually. While this appears to be a good method to concentrate microalgal slurry, unfortunately it did not separate cell debris from oil droplets. The selective removal of oil droplets from the slurry would provide a good way to recover microalgal lipids, but inversely, the selective removal of cell debris could also be desirable, as oil droplets remaining in the slurry can be subsequently recovered by other techniques. For example recently, a new filtration technology (hydrate filtration) has become available based on metal hydroxide hydrate gels that allows efficient and cost-effective removal of oil droplets from water (Schenk and Malekizadeh, 2013). Hence for the present

study, it was decided to modify parameters to investigate the potential of DiAF to separate oil from cell debris (pellet) of osmotically fractured *C. muelleri* cells to facilitate oil recovery. Application of DiAF with and without utilizing collectors, such as DPC (cationic), SDS (sodium dodecyl sulphate) (anionic), DAH (dodecylamine hydrochloride) (cationic) and diesel (cationic), as well as the frothing agent MIBC (methyl isobutyl carbinol) was investigated on fractured *C. muelleri* slurry with different pH and algal culture densities. All three parameters have previously been shown to alter the flotation efficiency of microalgal cells, in particular pH and the use of collectors can alter the charge of microalgal cells to render them more hydrophobic, a key determining factor for flotation (Garg et al., 2012). It was hypothesized that microalgal cellular lipid bodies which are surrounded by protein may behave in a similar manner when subjected to flotation. These experiments shaped the first part of this study. Then, effects of pH and mixture density on collector-free selective flotation through DiAF were further investigated. Finally, the efficiency of collector-free DiAF was compared with centrifugation through discussing dry weights, chlorophyll contents, lipid fluorescence, total lipids and total fatty acid methyl esters (FAMEs).

2. Material and Methods

2.1. Microalgae cultivation

Chaetoceros muelleri culture was kindly supplied by Queensland Sea Scallops Trading, Bundaberg, Australia. It was cultivated in f/2 medium (AlgaBoostTM F/2 2000x; Ausaqua Pty Ltd, Wallaroo, South Australia, Australia) (Guillard and Ryther, 1962) in artificial sea water (35 g/L of Aqua Sonic sea salt in deionized water) up to a scale of 60 L in clear polyethylene bags and with constant air bubbling under fluorescent light tubes with a light

intensity of 120 μmol photons $m^{-2}s^{-1}$ on a 16 h: 8 h light/dark cycle at 22ºC. Larger amounts were grown in outdoor 180 L vertical cylinder bioreactors in Pinjarra Hills at Brisbane summer time with evaporative cooling.

2.2. Microalgae harvesting and processing

An Avanti centrifuge (HP-20 XPI) (Beckman Coulter) was used for centrifugation of volumes higher than 1 L and a Hitachi himac CT6E was used for small amounts (less than 100 mL). Microalgae were harvested at a cell culture density of 0.6 g/L (Lim et al., 2012). Alternatively, an Interfil IC-45 manual clean centrifuge with a SS316 bowl was used for outdoor-grown *C. muelleri* culture of 180 L.

2.3. Microalgae fracturing

C. muelleri culture (>60 L) was prepared and centrifuged to provide paste as the same starting material for the experiments. Cell fracturing to free up lipid bodies was conducted on *C. muelleri* paste through osmotic shock by adding 10 volumes of deionized water. Then the OD and/or pH of all the systems were adjusted by dilution in water or by adding NaOH or HCl, respectively, before collector addition.

2.4. Nile Red staining

Nile Red (Sigma-Aldrich) was prepared by dissolving 5 mg of Nile red powder in 1 mL DMSO (Dimethyl sulfoxide) and stored in the dark at 4°C as a 15.7 mM stock solution. A total of 1 mL of microalgal culture was stained with 3.5 μL of Nile Red stock solution.

Triacylglyceride (TAG) fluorescence measurement was carried out by utilizing a plate reader at 485/540 nm excitation/emission wavelengths.

2.5. Chlorophyll optical density

Optical density (OD) at 440 nm was used to measure the concentration of algal cell debris pellets in slurry systems or of chlorophyll. This method was used as a rapid alternative to dry weight measurements in optimization experiments shown in Table 1. For ease of these preliminary experiments, it was assumed that OD can represent a measure of dry weight quantities of microalgal cells and debris, and hence was used instead of measuring dry weights. High density samples were diluted enough to give an absorbance in the range of 0.5-1.5 to remain in the linear part of the calibration curve of the chlorophyll optical density.

2.6. Wet lipid extraction and quantification

After cell fracturing, for lipid recovery and subsequent total lipid measurement, 25 mL of the samples were mixed by 15 mL of a solvent mixture of hexane and ethanol (3:1 ratio) (Ghasemi Naghdi et al., 2014) and vortexed. Then phase separation was conducted through centrifugation and the hexane layer (top layer) was transferred to pre-weighted soda glasses. After solvent drying through applying vacuum, total lipids were measured gravimetrically by weighting the soda glasses. Percentages were calculated as follows:

(Tail value * Amount of tail as per 1000 mL of feed * 100)/((Value of tail + Value of concentrated)*1000).

The same formula was also used to calculate percentages of dry weight, chlorophyll OD or lipid fluorescence.

2.7. Dry weight measurements

Although in preliminary experiments chlorophyll OD was used to evaluate the mixture density, for the main experiments dry weights of the samples were also measured to enable more reliable data comparisons. To measure dry weights, 10 mL of the slurry samples were transferred to pre-weighted Falcon tubes and subsequently dried down in a 70ºC oven. Then the Falcon tubes were weighted again to measure the weight of dry material.

2.8. Fatty acid profiling and quantification

Extracted lipids were re-dissolved 1:1000 in chloroform and 100 µL aliquots were taken and dried down. Then the lipids were hydrolyzed and methyl-esterified with 300 µL of 2% H_2SO_4 in methanol solution at 80°C by shaking (480 rpm) for 2 h on a thermal-mixer. Prior to transesterification, 50 µg of heneicosanoic acid (C21) was added to the pellet in each sample as an internal standard. After esterification, 300 µL of 0.9% (w/v) NaCl and 300 µL of HPLC grade hexane were added and the mixture was vortexed for 20 s. Phase separation was achieved after centrifugation at 16,000 x g for 3 min. The hexane layer was used for FAME profile analysis by gas chromatography-mass spectrometry (GC-MS). GC-MS analyses were carried out on an Agilent 6890 GC coupled to a 5975 MSD using 1 µL injection. A DB-Wax column (Agilent, 122-7032) was used with running conditions as described in Agilent's RTL DBWax method (Application note: 5988-5871EN; David et al., 2002). Identification of FAMEs was based on mass spectral profiles and retention times in the Agilent's RTL DBWax method.

2.9. Statistical analyses

Flotation experiments were carried out in duplicates, while all subsequent experiments such as lipid recovery, lipid fluorescence, chlorophyll optical density and dry weight measurements were performed in triplicate. Quantitative values for all the parameters were determined and expressed as means ± SD. Statistical analysis of the results was carried out either through one-way ANOVA or Student's t-test.

2.10. Separation of microalgal lipids and cell debris pellets by DiAF

Flotation was evaluated as a method to separate microalgal oil from cell debris. The principle of flotation is mainly based on the hydrophobicity of particles which cause the attachment of them to air bubble through hydrophobic force (Garg et al., 2012). Changes of pH in the liquid and the use of collectors were also investigated as they can enhance the hydrophobicity of the particles by neutralising the charge of algal cell debris and/or through the adsorbtion of hydrophilic collector domains to algal cells and the exposure of hydrophobic domains to the surrounding liquid. In selective flotation, which is the main focus of this study, we assessed different collectors, as well as different pH (with and without using collectors) and culture densities to investigate their efficiency on the separation of the oil droplets from the microalgae debris. Figure 1 shows a flow diagram of the experimental setup.

To investigate selective separation of microalgal oil from cell debris pellets, DiAF was applied through utilizing a 1.5 L mechanical device which was equipped with an impeller on its bottom to provide agitation and dispersion of the supplied air. When using collectors the microalgae suspension was first agitated by stirring at 800 rpm for 5 min in the flotation cell. After conditioning, aeration was turned on at a rate of 3 L/min for 5 min. During this time,

the formed froth was continuously wiped and transferred to another container manually. Weights of concentrated (floated) and tail (leftover) fractions were measured gravimetrically as well. Then lipid fluorescence, lipid content, total FAMEs, dry weight and chlorophyll OD were determined.

2.11. Separation of microalgal lipids and cell debris pellet by centrifugation

To evaluate the efficiency of DiAF on selective separation of the microalgal oil and pellet from wet fractured *C. muelleri* slurry, centrifugation was applied on the same batch at 2000 x g. Pellet (paste) and supernatant of the centrifuged slurry, were then separated and their lipid fluorescence, lipid content, total FAMEs, dry weight and chlorophyll OD were measured.

3. Results and Discussion

In an effort to develop a scalable and relatively low-cost method for wet, solvent-free oil extraction, DiAF was tested to remove either oil and cell debris from a slurry mixture of osmotically fractured *C. muelleri* cells. Table 1 provides an overview of preliminary flotation experiments which were conducted with or without utilizing collectors or froth, with fractured microalgal slurry of different pH and cell densities. The results are collectively shown in Figure 2. The percentages of chlorophyll (OD) and lipid fluorescence in the remaining tail provide measures for the separation of cell debris and TAGs, respectively, compared to the feed (original slurry). These data indicate that various percentages of microalgal cell debris could be removed from the slurry and concentrated by DiAF which was collected in the froth.

Considering results shown Figure 2, the best scenarios for removal of oil from slurry can be found in the top left corner, while the best scenarios for the removal of cell debris from slurry are in the bottom right corner. In other words it seems that any of the conditions named F (OD=1, pH=3.3 with 10 ppm SDS) , H (OD=1, pH=5.3 with 10 ppm SDS) , I (OD=2.2, pH=6.2 with 10 ppm DPC), L (OD=1, pH=4.3 without collector), M (OD=1, pH=5.3 without collector), R (OD=0.5, pH=5 without collector) , S (OD=0.9, pH=5 without collector), T(OD=1.4, pH=5 without collector), W (OD=0.9, pH=5 with 10 ppm SDS), CC (OD=2.4, pH=6 with 10 ppm SDS), DD (OD=2.4, pH=7 with 10 ppm SDS), EE (OD=2.4, pH=8 with 10 ppm SDS) and II (OD=1.3, pH=8 with 10 ppm DAH) can introduce a desirable selective separation. The reason is that in these systems most of the cell debris (>50%; measured as chlorophyll OD) ended up in the concentrated (froth), while more than 75% of the lipid fluorescence and hence lipid bodies were left in the tail. Most of these used low cell culture densities with ODs (less than 1.5) and low pH (less than 7) (F, H, L, M, R, S, T, W), while I, CC and DD were the conditions with high OD (OD>1.5) and low pH (pH<7). EE represents the setup that had both high OD and high pH values (OD≥1.5 pH≥7). Although in some of these setups, 10 ppm SDS was utilized as a collector (F, H, W, CC, DD, EE), the experiments also included the conditions which did not involve any collector application (L, M, R, S, T).

3.1. pH and slurry density are the two main parameters affecting selective flotation of cell debris from fractured *C. muelleri* slurry

As mentioned above, although some of the promising results of selective flotation were from the setups in which collectors were used, there were also some conditions that showed similar results without applying any collectors. In other words, even without utilizing any collectors, DiAF was capable of removing most of the algal pigments and likely the algal cell debris

(more than 50%) from remaining slurry containing the algal oil. Hence, the effects of culture density and pH on the selective flotation of *C. muelleri* cell debris without utilizing any collectors were further investigated.

Different amounts of deionized water were added to the paste of *C. muelleri* to implement the osmotic shock and to prepare the different cell culture densities (chlorophyll ODs of 0.8, 1.6, 3.3, and 6.1). Then the pH of all the systems was lowered and adjusted to 6 (the original pH of the osmotically-shocked slurry was in the range of 8.5-9.0) and for the highest OD (6.1), additional samples with pH 2, 4 and 8 were also prepared. However, it was noted that at low pH, chlorophyll OD did not provide a linear relationship to cell debris dry weights, as chlorophyll contents of algal debris collected in the froth appeared higher than at high pH. Therefore, in these experiments dry weights of the samples were also quantified to provide a more direct measure of cell debris for selective flotation results. Figure 3 shows the dry weights of the feed, concentrated (froth) and tail of all setups as well as the percentages of the concentrated dry weights from the feed. From setups A to D the dry weight of the feeds (microalgal slurry) were in accordance with the ODs in an increasing manner. Setup E showed the highest percentage of algal cell debris in the concentrated froth after flotation, indicating that removal of around 50% of the cell debris through flotation is possible from the fractured *C. muelleri* slurry with an OD of 4.8 and pH 2.0. Percentage of concentrated to feed dry weights in systems C, D, F and G were not statistically different, and only maximal 25% of the algal debris was collected in them. Looking at the optical densities (Figure 4), a similar trend could be observed with setup E, again showing the highest percentage of OD in concentrated to feed. In fact, setup E was shown to be able to concentrate more than 90% of the cell debris (based on chlorophyll).

Considering lipid fluorescence results (Figure 5), DiAF setup E showed the lowest percentage of fluorescence of tail to feed (21.6±0.8%) while setup A showed the highest amount of (82.5±3.97%). The lipid fluoresence percentage of tail to feed showed statistically significant different results in setups A, C and E (P<0.05). Table 2 summarizes the DiAF setups as percentages of lipid fluorescence, dry weight and chlorophyll OD of the concentrated and tail from the feed after flotation. Amongst all the conditions evaluated, setups C and E were more efficient in selective flotation. If concentrating the lipids is more desirable, then setup E could be a good candidate. In this system, more than 78% of the lipid fluorescence was detected in the concentrated, while more than 52% of the dry weight still remained in the tail. However, setup E had a very low pH of 2 which makes it not a suitable candidate for large-scale applications. It seems that low pH could affect lipid fluorescence, as the feed lipid fluorescence of setups E and F (with pH 2 and 4) showed low amounts. In this view, setup C can be considered a better candidate as more than 40% of the lipid fluorescence was detected in the concentrated with just 16.1±2.9% of the dry weight. In other words, flotation on microalgal slurry of fractured *C. muelleri* cells with pH 6, OD 3.3 and 0.4% solid matter, resulted in separation of more than 80% of algal debris (around 70% of the chlorophyll) which remained in the tail and more than 40% of the lipids (concentrated in the froth). Further modification and optimisation of DiAF may be necessary to further increase oil recovery rates.

This study focused on developing DiAF for oil separation and recovery from a slurry mixture containing ruptured *C. muelleri* cells. DiAF was a good candidate for the concentration of oil from algal debris and water in the slurry. We showed that slurry density and pH are the two most important factors affecting selective flotation. Although utilizing some collectors can facilitate selective flotation, this study shows that even without their application and by just

changing the acidity and cell culture density of the slurry, selective flotation through DiAF is still a possible option. Amongst the setups for which collectors were utilized, 10 ppm SDS showed the best results. For example, when DiAF was applied on raptured *C. muelleri* slurry with a chlorophyll OD of 0.9 and pH 5 along with applying 10 ppm SDS, more than 70% of the chlorophyll OD was separated from around 90% of the lipid fluorescence (Figure 2, setup W).

Chlorophyll OD has been extensively used to represent the algae growth rate and as a factor to represent microalgal dry weight (Chiu et al., 2008, Hsieh and Wu, 2009, Kamyab et al., 2014, Mikulec et al., 2015). However, when microalgal cells are ruptured, it might not be a precise measure to represent the dry weight, as chlorophyll not necessarily associates with other fractions of the microalgal cell. Most notably at lower pH levels, a higher proportion of chlorophyll seems be collected in the froth that is not representative of the dry weight (Table 2). This might be explained as the effect of acidic environments that can lead to the degradation of pigments (Schanderl et al., 1962). Conversion of chlorophyll to pheophytin by the addition of a weak or dilute acid and subsequent reduction of the solution absorbancy has been previously reported (Lorenzen, 1967, Vernon, 1960). For this reason, chlorophyll OD was only used to initially establish the best conditions for DiAF (Figure 2) and the dry weight was used for all subsequent refinement experiments.

For DiAF experiments at the same pH, the precentage of dry weight concentration increased from 8.3±1.3% in setup A to 23.6±1.7% in setup D by increasing the density of the culture. The same trend was observed in the concentration of the lipids measured by fluorescence, except for setup D, which showed a drop in lipid fluorescence. As mentioned above, setup C seems to offer the best separation through DiAF with the parameters tested, with around 40%

of its lipids and just around 16% of the dry weight concentration in the collected froth (Table 2). In this study we showed the principle that DiAF can be applied for demulsification and oil recovery from a slurry of ruptured microalgal cells. But the OD measurements also demonstrated that it may also be possible to use DiAF for pigment recovery, such as chlorophyll (up to 93%, Table 2).

3.2 Comparison of flotation and centrifugation

Flotation is generally regarded a more cost-effective and scalable process than centrifugation (Sharma et al., 2013, 2014). However, to evaluate and directly compare the efficiency in separation of oil and algal debris, centrifugation and DiAF were both applied on the same batch of microalgal slurry of *C. muelleri* cells pretreated by osmotic shock. A total of 60 L of *C. muelleri* culture was centrifuged to a paste and then osmotically shocked by addition of deionized water to provide a slurry with a chlorophyll OD of around 5 and 0.3% solid matter and a pH of 6.0. Then the slurry mix was subjected to both DiAF and centrifugation. Dry weight, chlorophyll OD, lipid fluorescence, total lipids and FAMEs of the extracted lipids were measured in the concentrated (sediment after centrifugation) and tail (supernatant after centrifugation) as well as the original feeds (Figure 6).

Centrifugation was able to concentrate around 60% of algal debris in the pellet while flotation resulted in around 40% of the algal debris in the froth. On the other hand, more than 80% of the chlorophyll was detected in the concentrated layer after centrifugation while this amount was around 50% in the flotation. Considering the lipid fluorescence, total lipids and total FAMEs, percentages of 73.9±1.1%, 50.1±4.2% and 59.7±6.3% were respectively detected in the tail of the flotation system, while the centrifugation system showed 67.8±0.2%,

68.5±5.2% and 56.9±2.3%, respectively. Percentages of total FAMEs in tail to feed did not show any statistically significant difference between centrifugation and flotation systems. C14 and C16:1 contained more than half of the total FAMEs in all systems used (including the feed, and all flotation and centrifugation results). According to the FAME analyses more than 50% of FAMEs were saturated fatty acids (Table 3). While centrifugation showed around 60% of unsaturated FAMEs in its sediment, this amount was just around 40% in the concentrated of the flotation system.

Previous studies suggested that centrifugation is another technique which can also be applied for demulsification and separation of oil and algal cell debris (Eow and Ghadiri, 2002); although centrifugation is less scalable and has the main drawback of being a costly process (Eow and Ghadiri, 2002, Angelidou et al., 1977, Pal and Masliyah, 1990, Strickland Jr, 1980) compared to DiAF. However, comparison of these two techniques was carried out in this study to give a better evaluation of the flotation efficiency. Centrifugation showed better results for concentrating cell debris than flotation and it was even more efficient for chlorophyll enrichment. Considering the lipid fluorescence, flotation showed a slightly better result, with around 6% more, while total lipids analysis suggested a higher capability of the centrifugation system in separating the lipids in its supernatant. However, both systems showed the same amount of total FAMEs of around 60% left in the tail/supernatant. While 52.2±6.1% of the total FAMEs of the flotation tail were saturated fatty acids, this amount was much higher (68.7±2.5%) in the centrifugation supernatant.

Extraction of oil from wet microalgal biomass is desirable as it avoids the need for complete dewatering and therefore provides a cost advantage to conventional methods that include drying of biomass followed by solvent-based oil extraction (Yoo et al., 2012). In the current

study, both, centrifugation and DiAF were found to be suitable for the partial separation of oil from wet concentrated microalgal biomass when cells were ruptured. A comparison of extraction yields to other methods shows that approx. 60% total FAME could be recovered when using organic solvent mixture as a benchmark which is considered one of the most efficient methods (Ghasemi-Naghdi et al., 2014). A comparison of the energy requirements shows that flotation (0.8 kWh/m^3 culture) fares clearly better than centrifugation (5.5 kWh/m^3 culture; Sharma et al., 2014). Centrifugation would also still be required for conventional organic solvent-based methods with the added cost, safety and environmental disadvantages associated with organic solvent use.

4. Conclusion

Suitability of DiAF and centrifugation for separating microalgal oil and debris from a wet slurry of raptured *C. muelleri* were validated by this study. Effects of slurry density and pH on selective flotation were investigated and confirmed by the results of this study. DiAF can compete with centrifugation for cell debris separation and oil recovery with the added bonus of being less energy intensive and a lower cost process.

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Table 1. Conditions for DiAF experiments on microalgal slurry containing fractured *C. muelleri* cells for separation of cell debris from TAGs. Conditions included High OD/High pH (OD≥1.5 pH≥7), High OD/Low pH (OD≥1.5 pH<7), Low OD/Low pH (OD<1.5 pH<7), and Low OD/High pH (OD<1.5 pH≥7), as well as the use of different collectors at various concentrations.

Legend	Description			Legend	Description		
	OD	pH	Collector/ Concentration		OD	pH	Collector/ Concentration
A	2.2	6.2	DPC/10ppm	Z	0.6	6	DPC/10ppm
B	1.3	6	—	AA	0.6	6	SDS/10ppm
C	1.3	6	Diesel/10ppm	BB	1.2	6	SDS/10ppm
D	1.3	6	Diesel/100ppm	CC	2.4	6	SDS/10ppm
E	1	2.3	SDS/10ppm	DD	2.4	7	SDS/10ppm
F	1	3.3	SDS/10ppm	EE	2.4	8	SDS/10ppm
G	1	4.3	SDS/10ppm	FF	1.3	8	SDS/10ppm
H	1	5.3	SDS/10ppm	GG	1.3	8	DPC/10ppm
I	2.2	6.2	DPC/10ppm	HH	1.3	6	DAH/10ppm
J	1	2.3	—	II	1.3	8	DAH/10ppm
K	1	3.3	—	JJ	1.3	6	MIBC/10ppm
L	1	4.3	—	KK	1.3	8	MIBC/10ppm
M	1	5.3	—	LL	1.3	8	—
N	2.1	6.2		MM	1	5.5	DPC/10ppm
O	2.1	8.6	—	NN	1	6.5	DPC/10ppm
P	2.1	6.2	DPC/10ppm	OO	1	7.4	DPC/10ppm
Q	2.1	8.6	DPC/10ppm	PP	1	8.5	DPC/10ppm
R	0.5	5	—	QQ	1	9.5	DPC/10ppm
S	0.9	5	—	RR	0.45	6	DPC/10ppm
T	1.4	5	—	SS	0.8	6	DPC/10ppm
U	2.5	5	—	TT	1.7	6	DPC/10ppm
V	0.5	5	SDS/10ppm	UU	3.7	6	DPC/10ppm
W	0.9	5	SDS/10ppm	WW	3.5	4	DPC/10ppm
X	1.4	5	SDS/10ppm	XX	4.1	8	DPC/10ppm
Y	2.5	5	SDS/10ppm				

OD = optical density

Table 2. Percentages of lipid fluorescence, dry weight and chlorophyll OD of the concentrated and tail from the feed after flotation

Shown are averages ±SDs of three independent DiAF experiments, each. Values in bold are of particular interest.

		% Tail from Feed			% Concentrated from Feed		
	Description	**Lipid Fluorescence**	**Dry weight**	**Optical Density**	**Lipid Fluorescence**	**Dry weight**	**Optical Density**
A	**pH6 OD0.8**	82.5±4.0	91.7±1.3	76.7±2.6	17.5±4.0	8.3±1.3	23.3±2.6
B	**pH6 OD1.6**	72.5±4.1	89.3±2.0	80.3±3.1	27.5±4.1	10.7±2.0	19.7±3.1
C	**pH6 OD3.3**	58.5±8.2	**83.9±2.9**	67.5±4.4	**41.5±8.2**	**16.1±2.9**	32.5±4.4
D	**pH6 OD6.1**	73.3±1.7	76.4±1.7	66.6±1.8	26.7±1.7	23.6±1.7	33.4±1.8
E	**pH2 OD4.8**	21.6±0.8	52.4±1.3	6.4±0.7	**78.4±0.8**	47.7±1.3	93.6±0.7
F	**pH4 OD5.3**	68.9±2.5	76.7±2.3	51.2±2.3	31.1±2.5	23.4±2.3	48.8±2.3
G	**pH8 OD7.6**	70.5±1.1	74.6±4.7	75.2±0.7	29.5±1.2	25.4±4.7	24.8±0.6

Table 3. Percentages of the total saturated/unsaturated fatty acids from total FAMEs in feed, tail and concentrated from DiAF and centrifugation sediment and supernatant.

	% Total Saturated/ Total FAMEs	% Total Unsaturated/ Total FAMEs
Feed	55.4±1.0	44.6±1.0
DiAF Concentrated	60.7±3.7	39.3±3.7
DiAF Tail	52.2±6.1	47.8±6.1
Centrifugation Sediment	40.8±5.7	59.2±5.7
Centrifugation Supernatant	68.7±2.5	31.4±2.5

Figure legends

Figure 1. Flow diagram of the experimental setup.

Figure 2. DiAF of fractured *C. muelleri* microalgal slurry under various conditions for removal of either microalgal oil or cell debris from the slurry feed. Shown are the percentages of tail lipid fluorescence (a measure of TAGs) and chlorophyll OD (a measure of cell debris) compared to the original feed. Blue symbols refer to High OD/High pH conditions (OD≥1.5 pH≥7), Red symbols refer to High OD/Low pH (OD≥1.5 pH<7), purple symbols refer to Low OD/Low pH (OD<1.5 pH<7), and green symbols refer to Low OD/High pH (OD<1.5 pH≥7) (● with collector, ♦ without collector). Detailed conditions are are provided in Table 1. The best scenarios for removal of oil from slurry can be found in the top left corner, while the best scenarios for the removal of cell debris from slurry are in the bottom right corner.

Figure 3. Dry weight results of the flotation without utilizing any collectors in DiAF systems with different ODs and pHs. A: pH 6/OD 0.8; **B**: pH 6/OD 1.6; **C:** pH 6/OD 3.3; **D:** pH 6/OD 6.1; **E:** pH 2/OD 4.8; **F:** pH 4/OD 5.3; **G:** pH 8/OD 7.6. Different small letters represent significant differences between the dry weight precentages of concentrated to feed from three independently carried out DiAF experiments (P<0.05).

Figure 4. Chlorophyll OD results of the flotation without utilizing any collectors in DiAF systems with different ODs and pHs. A: pH 6/OD 0.8; B: pH 6/OD 1.6; C: pH 6/OD 3.3; D: pH 6/OD 6.1; E: pH 2/OD 4.8; F: pH 4/OD 5.3; G: pH 8/OD 7.6. Different small letters represent significant differences between the dry weight precentages of concentrated to feed from three independently carried out DiAF experiments (P<0.05).

Figure 5. TAG lipid fluorescence of the feed, concentrated and tail and percentages of the tail fluorescence from the feed of the DiAF systems without utilizing any collectors with different ODs and pHs. A: pH 6/OD 0.8; **B**: pH 6/OD 1.6; **C:** pH 6/OD 3.3; **D:** pH 6/OD 6.1; **E:** pH 2/OD 4.8; **F:** pH 4/OD 5.3; **G:** pH 8/OD 7.6. Conditions D-G used the same undiluted culture. Different small letters represent significant differences between the dry weight precentages of concentrated to feed from three independently carried out DiAF experiments ($P<0.05$).

Figure 6. Percentages of cell debris, chlorophyll OD, lipid fluorescence, total lipids and total FAMEs of the tails (supernatant in centrifugation) from the feed in DiAF and centrifugation experiments. Shown are averages ±SDs of three independent DiAF and centrifugation experiments, each using chlorophyll OD of around 5 and 0.3% solid matter and a pH of 6.0. Asterisks represent significant differences between the methods (DiAF and centrifugation) used of each group ($P<0.05$).

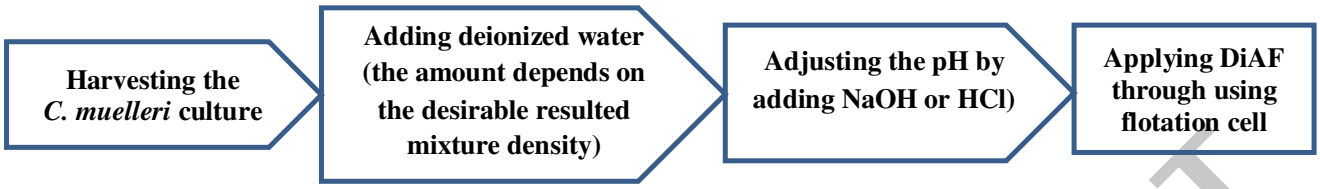
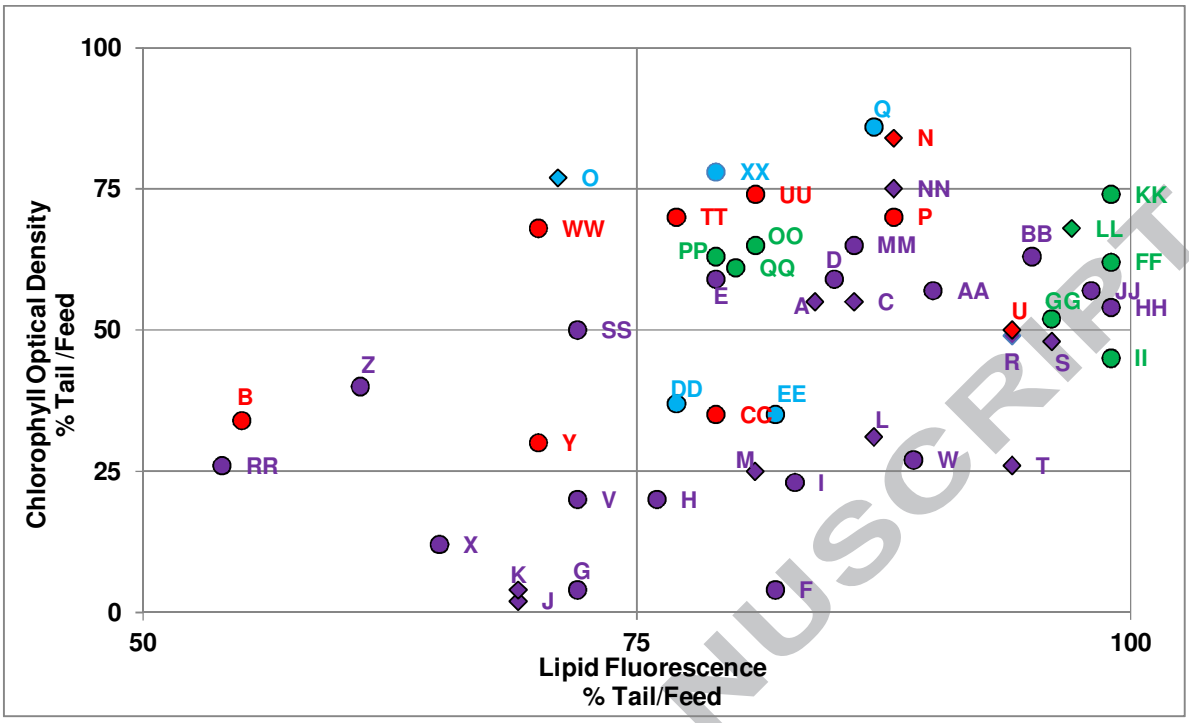


Figure 1. Flow diagram of the experimental setup



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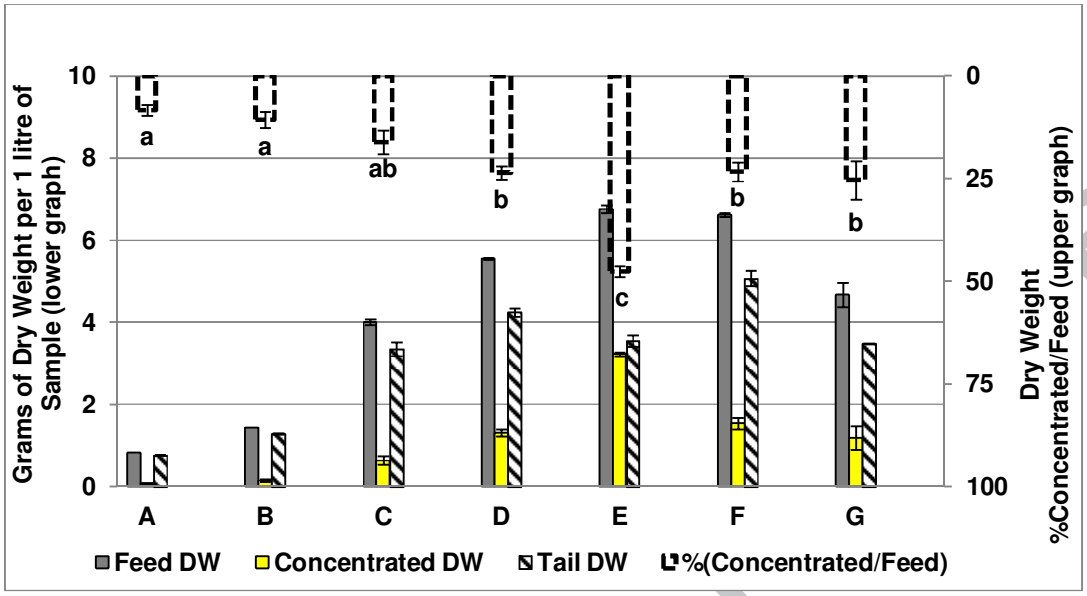

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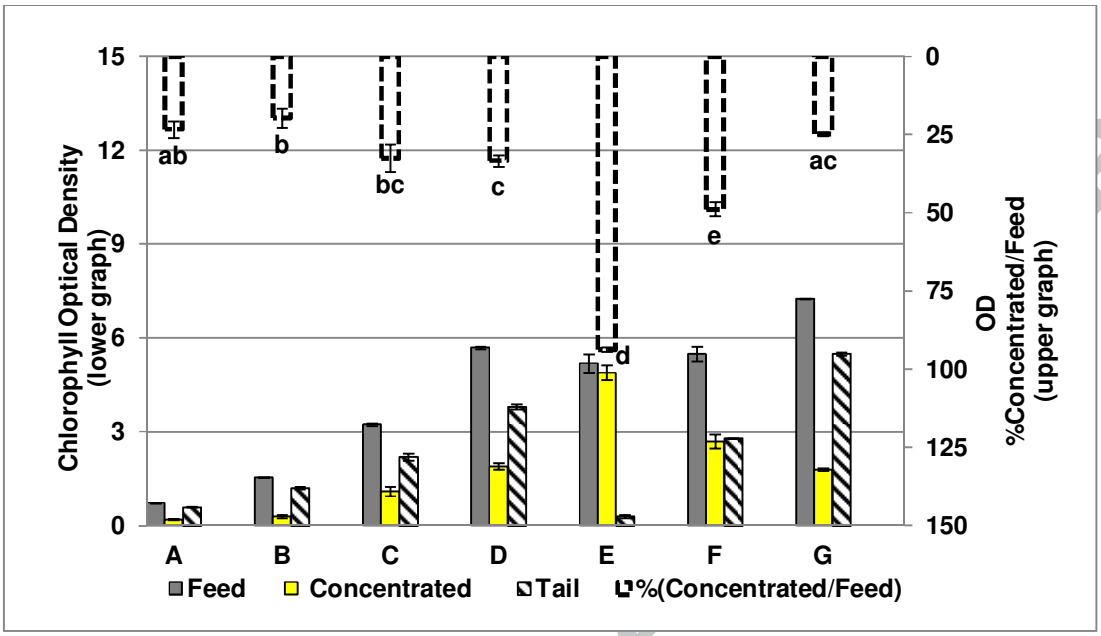

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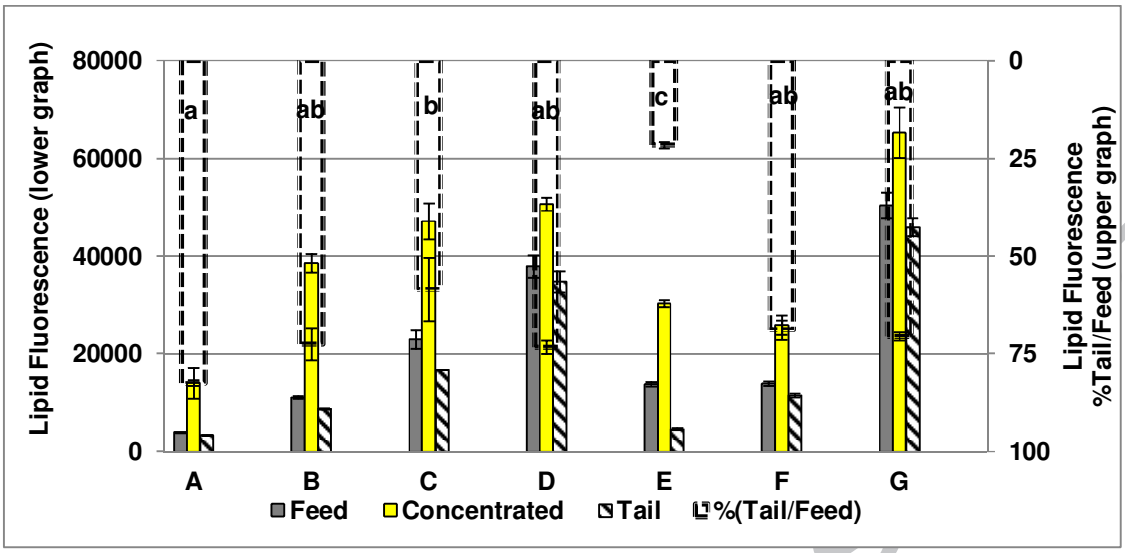

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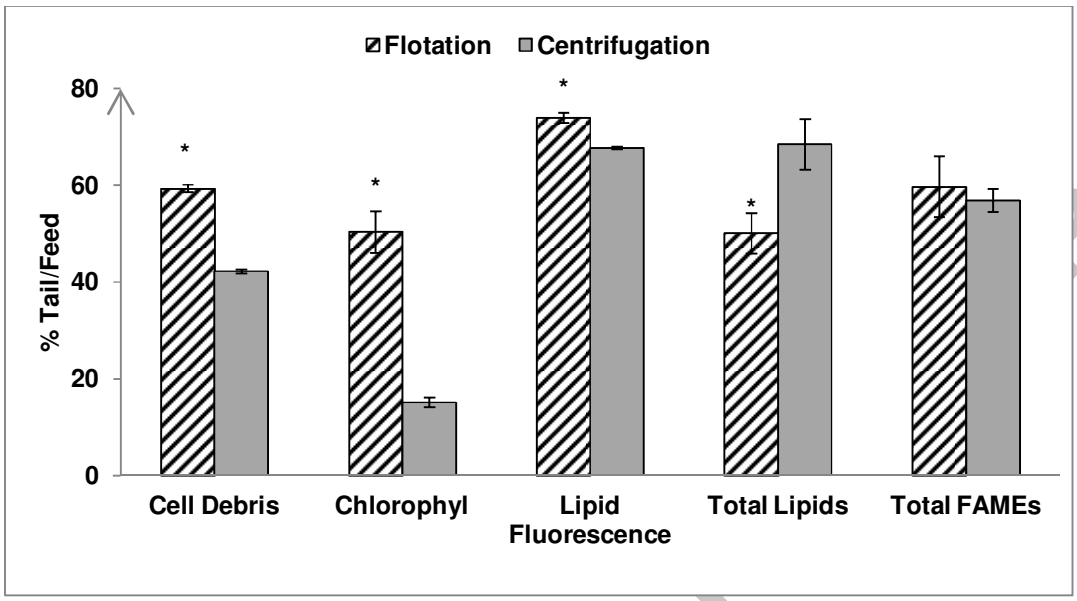

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- DiAF was developed for solvent-free lipid recovery from microalgal slurry
- Microalgal slurry pH and culture density were the main contributing factors
- DiAF could compete with centrifugation for cell debris separation and oil recovery
- The process may contribute towards more economical microalgal oil production