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Harvesting microalgae by CTAB-aided foam flotation increases lipid recovery and improves fatty acid methyl ester characteristics

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Foam flotation is an effective and energy efficient method of harvesting microalgae. This study has investigated the influence of growth phase and lipid content on harvesting efficiency. The highest biomass concentration factors were gained during active culture growth. Surprisingly, the quantities of lipid recovered from microalgae harvested by foam flotation using the surfactant cetyl trimethylammonium bromide (CTAB), were significantly higher than from cells harvested by centrifugation. Further, cells harvested by CTAB-aided foam flotation exhibited a lipid profile more suited to biodiesel conversion containing increased levels of saturated and monounsaturated fatty acids. The enhanced lipid recovery was partially explained by the interaction of the cells with the surfactant, CTAB, which adsorbed onto the algae and was carried over into the total lipid extraction process. However, further evidence also suggested that CTAB promoted in situ cell lysis by solubilising the phospholipid bilayer, thus increasing the amount of extractable lipid. This work demonstrates substantial added value of foam flotation as a microalgae harvesting method beyond energy efficient biomass recovery.

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1. Introduction

Microalgae are considered as having great potential as a sustainable, scalable and affordable source of bioproducts including biofuels and high value chemicals [1–3]. With the exception of microalgae grown heterotrophically [4], bulk products derived from algae, such as biofuels, are not yet cost competitive. Two major challenges facing the nascent microalgae industry are: 1) the ability to reliably and affordably produce high biomass yields at scale; and, 2) the harvesting, dewatering, and extraction of biochemicals from large quantities of feedstock [2,5]. Concerning high volume, low value products i.e. algae biodiesel, improvements in production, harvesting, and integrating co-production of higher-value products/processes as part of a biorefinery concept will be critical to reduce algae oil production cost [3,6–8].

In recent years, considerable research efforts have focussed on the application of harvesting technologies to improve biomass recovery; however, the majority of
harvesting methods are not sufficiently cost effective for the economical production of low value outputs [9,10]. Flotation, a separation process originating from the mineral industry, has become an established method to remove algae from suspension [11–16], with potential for application to harvest microalgae for biofuel production [2,5,6,17–19]. Flotation, particularly dissolved air flotation, is the favoured technique for algae removal [5,16,20,21]. During dissolved air flotation an inorganic flocculent such as alum is added to aggregate the cells. Small bubbles formed by supersaturation of the water with air, adhere to and transport suspended biomass to the surface [5,13,16,19,22]. However, despite its effectiveness, dissolved air flotation is energy intensive, consuming up to 7.6 kWh m$^{-3}$ [16]. In contrast, foam flotation, a technique similar to dissolved air flotation, virtually eliminates the need for the energy intensive step of air compression by generating bubbles and foam with the addition of a cationic surfactant e.g. cetyl trimethylammonium bromide (CTAB) and a low pressure sparger or agitator [16,19]; operational energy consumption is reduced to as little as 0.015 kWh m$^{-3}$ [10]. CTAB has proven an advantageous surfactant for algae removal [10,11,13–15,21,23] with reported removal efficiencies approaching 90% [11,13–15]. Additionally, the surfactant, by improving the electrostatic interactions between the bubbles and the microalgal cells, removes the need for a flocculent [21]. There are two main hypotheses as to how surfactants improve electrostatic interactions between bubbles and algae (Fig. 1). The first predicts that cationic surfactants modify the surface properties of the bubble by establishing a positive charge, and therefore electrostatically attracted to the negatively charged algae [20,21,24]. The second hypothesis predicts that cationic surfactant ions adsorb onto the algae making the cell hydrophobic and therefore available for bubble attachment [11,13,15].

Despite the obvious appeal of flotation for harvesting microalgae, there remains limited evidence of its direct application in situations wherein the biomass is a valued product as opposed to a waste/nuisance. It is vital to determine the effects of harvesting on biomass quality and composition, particularly when the biochemical components must achieve defined quality standards for subsequent downstream processing [25–27]. For example, Borges et al. [28] observed that by using a cationic flocculant to harvest the diatom *Thalassiosira weissflogii* higher percentages of C16:0, C16:1 and C20:5 fatty acids were recovered, whereas the yields of C18:0 and C18:1n9c decreased. This situation is further complicated by the fact that relatively few microalgae biochemicals of commercial interest are produced constitutively; rather, the biosynthesis of many biochemicals is induced during specific growth phases or in response to exogenous stimuli such as nitrogen deprivation [29]. To date, the influence of growth phase on the efficiency of settling, tangential flow filtration [30], flocculation [32], and the coagulant dosage required for dissolved air flotation has been investigated [18]. However, there is currently no information available on the possible effects of growth phase on the efficiency of CTAB-

![Fig. 1](image-url) — The two proposed hypotheses as to how surfactants improve electrostatic interactions between bubbles and algae. In hypothesis 1, the cationic CTAB causes the bubble to become positively charged facilitating an electrostatic attraction to the negatively charged microalgal cells. In hypothesis 2, CTAB adsorbs onto the microalgae thereby making the cells hydrophobic and promoting bubble attachment (Diagram based on the works of Phoochinda et al. [15], and Zhang et al. [31]).
aided foam flotation. As indicated by Danquah et al. [30] and Zhang et al. [31] the significance of this cannot be discounted when considering the applicability and efficacy of foam flotation. Flotation is reliant upon the electrochemical properties of the algae cell; however, the strength of this charge – measured as the zeta potential – is non-uniform, varying with cell age [31]. The optimal zeta potential may therefore not necessarily be in synchrony with the biosynthetic dynamics of the algae product(s) of interest, potentially resulting in a product-harvest mismatch thus reducing overall operational efficiency. The aims of this study were, therefore, to establish the influence of growth phase and CTAB exposure on foam flotation efficiency with respect to biomass and lipid recovery, and overall fatty acid profile.

## 2. Materials and methods

### 2.1. Cultivation of algae

A non-axenic *Chlorella* sp. obtained from Blades Biological Ltd., Kent, UK, was grown in batch with an initial cell density of 10⁶ cm⁻³ using a proteose peptone medium containing the following (per litre): 0.2 g MgSO₄·7H₂O, 0.2 g KH₂PO₄, 2 g KNO₃ and 1 g proteose peptone (oxoid L85) (Sigma Aldrich). Cultures were grown in 20 L polycarbonate carboys (Nalgene) at 19 ± 2 °C with a 16 L 8 D photoperiod. Lighting was supplied by a combination of warm and cold fluorescent tubes giving a luminance range of between 2200 and 2800 Lux. Mixing and gas transfer was facilitated by an aquarium pump. Growth was determined by cell counts using a haemocytometer every 3 days over a 21 day culture period.

### 2.2. Harvesting experiments

Cells from the same culture were harvested at 3 day intervals between days 3 and 21 using two separate methods: foam flotation and centrifugation. The flotation column was previously described in Coward et al. [10]. During foam flotation 2 L of culture were mixed with 7.5 L of tap water to give an initial dry weight of 126.14 ± 10.06 mg L⁻¹, which is similar to dry weight yields from paddle–wheel open culture raceway systems [33]. All foam flotation harvests were conducted under the following conditions: column height of 0.5 m, air flow rate of 100 L h⁻¹, 30 min batch run time, and 10 mg L⁻¹ of CTAB [10]. CTAB dissolved within 500 cm³ of water was added to the harvest chamber along with the diluted algae to make an initial starting volume of 10 L. A ceramic flat plate sparger with a mean pore diameter of 20.0 μm [34] and dimensions of 2 cm × 11 cm × 11 cm in height, width, and length respectively, was used to generate bubbles with a mean diameter of 860 ± 158 μm. The bubble size was determined using a combination of a high-speed digital video camera (Olympus i-speed 3) and image processing software Image J (National Institutes of Health, Bethesda, Maryland, USA). Each harvest had 4 replicate runs. The concentration factor (CF) of each harvest was calculated as described in Coward et al. [10].

To prepare the cells for freeze drying and to remove any potential CTAB residue the foam flotation harvests were further concentrated by centrifugation (Sigma 3K18C) for 20 min at 8700 × g. The cell pellets were washed in distilled water and centrifuged again. The washing step was repeated once more before the pellet was freeze dried for 48 h at 3 kPa in a Christ Alpha 1-4 LD Plus (SciQuip, UK), with a condenser temperature of −55 °C. To compare harvest methods 2 L of culture were harvested by centrifugation only (20 min at 8700 × g) and then freeze dried, in 4 replicate runs.

To compare the effects of foam flotation and centrifugation on lipid recovery and fatty acid profiles, additional harvests were conducted from the same cultures after 12 days of growth, as this is when the highest concentration factor was gained (see Fig. 2).

### 2.3. Surfactant adsorption

A methyl orange colorimetric test was conducted to assess the extent of CTAB adsorption onto the algae cells. An average culture dry weight of 350 ± 120 mg L⁻¹ was gained by adding two hundred millilitres of Chlorella, which was over 21 days into the culture, to 750 cm³, with three replicates. The volume of each replicate was made up to 1 L by adding 10 mg of CTAB dissolved in 50 cm³ of deionised water. The Chlorella was exposed to the CTAB solution for 1 h during which the mixtures were stirred continuously using a magnetic stirrer. A 1 h exposure was determined as an approximation of the total duration the cells would be exposed to CTAB during the flotation harvesting process. After 1 h, 40 cm³ (V₁) of each algae/surfactant solution was centrifuged for 20 min at 8700 × g in replicates of four. For each replicate the supernatant was collected for analysis and the pellet re-suspended in a known volume of deionised water (V₂). This washing step was repeated for a third time (V₃). For analysis, 10 cm³ of each supernatant sample was treated with 50 cm³ of chloroform and an excess of methyl orange reagent under acidic conditions as described by Wang and Langley [35]. The methyl orange reacted with CTAB forming a chloroform soluble complex. The intensity of colour produced in the chloroform

![Fig. 2 – Growth profile (diamonds) and concentration factors (light grey bars) for Chlorella sp. harvested by foam flotation across a 21 day period. Mean ± standard deviation.](image-url)
layer was directly proportional to the concentration of methyl orange—surfactant complex concentration when measured spectrophotometrically at 415 nm. A calibration curve was created for CTAB in the 2.5–10 mg L\(^{-1}\) range, yielding an R\(^2\) of 99.78% (data not shown).

The percentage of CTAB adsorbed onto the algae (\(A_{\text{CTAB}}\)) was calculated using equation (1); where \(V_a\) is the volume of supernatant removed per centrifugal recovery, and \(MO_i\) is the quantity of CTAB present in the analysed supernatant.

\[
A_{\text{CTAB}} = \left(\frac{V_1MO_1 + V_2MO_2 + V_3MO_3}{V_1}\right) \times 100
\]  

(1)

2.4. Total lipid extraction

A modified version of the Folch method [36] was used for lipid extraction. The freeze dried microalgae was ground to a fine powder using a pestle and mortar. A known quantity of microalgae, 0.095–0.33 g, was homogenised with methanol, followed by chloroform (1:2 v/v). The total homogenate volume was 30 times that of the tissue weight. The homogenates were centrifuged for 20 min at 4500 \(\times\) g to remove cell debris. The lipid fraction was transferred to a clean test tube, using a Pasteur pipette. A 0.88% potassium chloride solution was then added at 25% of the starting volume to wash the lipid fraction and remove any non-lipid contaminants. The final biphasic system was centrifuged for 20 min at 4500 \(\times\) g, and the resulting mixture left to separate into two phases. The lower phase was transferred into a pre-weighed glass tube and evaporated to dryness under a nitrogen stream at 37 °C. The weight of the crude lipid obtained from each sample was determined gravimetrically.

2.5. Solid phase extraction

The total lipid extracts were separated into lipid classes by solid phase extraction following the methods of Kaluzny et al. [37]. Total lipid mixtures in chloroform were applied to EASY® cartridges (Chromabond 3 cm\(^2\), 200 mg, Macherey–Nagel, Germany), which had previously been conditioned with hexane. Solvent mixtures of increasing polarity were used to elute the lipid fraction. The freeze dried microalgae was ground to a fine powder using a pestle and mortar. A known quantity of microalgae, 0.095–0.33 g, was homogenised with methanol, followed by chloroform (1:2 v/v). The total homogenate volume was 30 times that of the tissue weight. The homogenates were centrifuged for 20 min at 4500 \(\times\) g to remove cell debris. The lipid fraction was transferred to a clean test tube, using a Pasteur pipette. A 0.88% potassium chloride solution was then added at 25% of the starting volume to wash the lipid fraction and remove any non-lipid contaminants. The final biphasic system was centrifuged for 20 min at 4500 \(\times\) g, and the resulting mixture left to separate into two phases. The lower phase was transferred into a pre-weighed glass tube and evaporated to dryness under a nitrogen stream at 37 °C. The weight of the crude lipid obtained from each sample was determined gravimetrically.

CTAB has a lipid-like chemical structure; therefore, if CTAB adsorbs onto the algae cell surface it may enter the lipid extraction process where it may inadvertently contribute towards the total pool of recovered lipid. To determine the likelihood of this chemical carry over, known quantities of pure CTAB, 0.05–0.15 g, were put through the same total lipid extraction process as the microalgae and calculated as follows:

The mass of CTAB harvested (\(M_{\text{CTAB}}\)) was calculated using equation (2); where, \(M_c\) is the total quantity of CTAB added prior to harvesting, \(PA\) is the average percentage adsorbance of CTAB onto the algal cell (calculated using method described in Section 2.3), \(N_0\) is the total number of algae cells present in the culture, and \(N_i\) is the total number of algae cells harvested:

\[
M_{\text{CTAB}} = M_cPA\left(\frac{N_i}{N_0}\right)
\]  

(2)

The total dry mass of cells harvested excluding CTAB (\(M_{\text{CX}}\)) was calculated using equation (3); where \(M_{\text{CH}}\) is the total dry mass of the harvest was calculated using equation (3):

\[
M_{\text{CX}} = M_{\text{H}} - M_{\text{CH}}
\]  

(3)

The total mass of CTAB recovered by the lipid extraction process (\(M_{\text{LC}}\)) was calculated using equation (5); Where \(PL_{\text{CTAB}}\) is the average percentage of CTAB able to pass through the lipid extraction process. The average was gained from eight replicates starting with a known range of pure CTAB from 0.05 to 0.15 g to account for the potential increasing concentration of CTAB in the harvested foam:

\[
M_{\text{LC}} = PL_{\text{CTAB}}M_{\text{CH}}
\]  

(4)

The predicted total lipid percentage (\(PL_{\text{pred}}\)) recovered from cells harvested by foam flotation was therefore calculated using equation (6):

\[
PL_{\text{pred}} = \frac{M_{\text{LC}} + M_{\text{CX}}}{M_{\text{H}}}
\]  

(6)

2.6. Fatty acid composition analysis

Fatty acid composition was determined using a Carlo Erba Model Mega 5160 gas chromatograph (Carlo Erba, Milan, Italy). Ten milligrams of the freeze dried cells harvested by foam flotation and centrifugation on day 12 were placed into capped test tubes with heptadecanoic acid (C17:0) as an internal standard. Fatty acid methyl esters (FAMEs) were extracted by the one-step method of Gracés and Mancha [39] (methanol:toluene:2,2 dimethoxypropane (DMP):sulphuric acid; 39:20:5:2, by volume). An injection volume of 1 \(\mu\)L was loaded onto a Supelco column (Sigma Aldrich) at 240 °C (30 m length \(\times\) 0.25 mm ID, 0.25 \(\mu\)m film) with helium as the carrier gas. The temperature was programmed to ramp from 50 °C to 240 °C at 7 K min\(^{-1}\). Fatty acids were identified by comparing the obtained retention times with that of known standards (37 component FAME mix, Supelco™). Fatty acid composition analysis was conducted in triplicate for each harvest method.

2.7. Statistical analysis

Algal growth, concentration factors, total lipid, lipid classes and FAME contents for cells harvested by foam flotation and centrifugation were compared using analysis of variance (ANOVA). All percentage data were arcsine transformed prior
to analysis. An alpha level of 0.05 was used to determine data significance.

3. Results and discussion

The cultures grew linearly until day 15, attaining a peak cell density of \(4.44 \times 10^8 \pm 6.01 \times 10^7 \) cells cm\(^{-3}\) followed by a significant decrease in cell density at day 21 to a cell density of \(2.98 \times 10^7 \pm 3.69 \times 10^6 \) cells cm\(^{-3}\) (\(p = 0.001\)). Fig. 2 illustrates the effect of culture age on the biomass concentration factor. The highest concentration factors – up to 306.89 \(\pm 31.6\) – which occurred on day 12 were significantly higher (\(p < 0.001\)) than the concentration factors gained for all other harvest days. The cultures appeared to enter a brief stationary phase by day 15 with cells beginning to aggregate and drop out of suspension. Danquah et al. [30] reported that microalgae harvested during a period of low growth had increased settling efficiencies compared with algae harvested during a high growth period. This was due to a reduction in the electrochemical stability of the cells within suspension measured as a decline in zeta potential during the low growth rate phase. This may explain why days 15, 18 and 21 yielded lower concentration factors of 111.29 \(\pm 1.7\), 250 \(\pm 45.6\), and 81.96 \(\pm 6\), respectively.

Changes in zeta potential have been linked to changes in the dissolved organic matter (DOM) pool within a culture. DOM concentration generally increases with culture age and can play an important role in promoting or inhibiting flocculation processes [31,40]. Zhang et al. [31] reported that, while the dissolved organic matter (DOM) pool within a culture, the flocculent Al\(^{3+}\) can play an important role in promoting or inhibiting flocculation processes. Nevertheless, a higher zeta potential may also increase the electrostatic interactions between CTAB and the negatively charged cell. CTAB can adsorb onto the surface of negatively charged particles [42], therefore increasing the hydrophobicity of the once hydrophilic solid–liquid interface and allowing efficient separation from the aqueous phase [11,13,15]. CTAB may also create electrostatic interactions between the bubbles and the suspended particles thereby improving flotation [13]. When considering a multi-product operation, i.e. a biorefinery concept, this presents an intriguing challenge of how to select and optimise harvesting technologies and harvesting times to maximise product yield and therefore economic return. Such a situation will require detailed consideration of the technoeconomics of the harvesting process from a biorefining perspective. To our knowledge, this analysis has never been undertaken and further research is required.

To gain insight into which electrochemical interaction and hydrodynamic forces that dominate the foam flotation process, the adsorption of CTAB onto the algae cells was analysed. The adsorption efficiency is influenced by a range of factors including cell age (and therefore zeta potential), cell density, and the surfactant concentration. It was assumed that the surfactant present in the supernatant was representative of that adsorbed onto the air/liquid interface, and that surfactant not present in the supernatant had adsorbed onto the algae cell. From Table 1 it can be seen that only 32.35 \(\pm 5.35\)% of the total surfactant added was recovered from the supernatant after 1 h of exposure (Fig. 1, hypothesis 1). It can therefore be assumed that 32.35% of the CTAB added to the culture assumed a hydrophobic behaviour, sticking the non-polar end of the molecule into the gas bubble [43]. It may therefore be deduced that the bulk of the surfactant (67.65 \(\pm 5.35\)% had been adsorbed onto the cells [43]). It may therefore be deduced that the bulk of the surfactant (67.65 \(\pm 5.35\)% had been adsorbed onto the cells [43]). It may therefore be deduced that the bulk of the surfactant (67.65 \(\pm 5.35\)% had been adsorbed onto the cells [43]). It may therefore be deduced that the bulk of the surfactant (67.65 \(\pm 5.35\)% had been adsorbed onto the cells [43]). It may therefore be deduced that the bulk of the surfactant (67.65 \(\pm 5.35\)% had been adsorbed onto the cells [43]).

From the perspective of a commercial grower, these observations present an inconvenient, yet interesting paradox. If the grower is targeting lipid-derived bioproducts such as biodiesel, the period for optimal harvesting efficiency using foam flotation does not correspond to the period of maximal lipid yield (typically early stationary phase following a period of nitrogen deprivation [1]). However, if the grower is interested in biochemicals synthesised during rapid growth, e.g. phycocyanin [41], then foam flotation would be appropriate. The inverse situation – characterised by a reduction in zeta potential, elevated DOM concentrations and a reduction in major cell surface functional groups (i.e. carboxyl, phosphate, and amine or hydroxyl groups; [31]) – will improve settling efficiency, and may therefore benefit flocculation and subsequent sedimentation. Nevertheless, a higher zeta potential may also increase the electrostatic interactions between CTAB and the non-polar end of the molecule into the gas bubble [43].

Table 1 – The potential percentage adsorption of CTAB onto the algae cells as analysed using a methyl orange colorimetric test. Data displayed is the mean of 4 replicates \(\pm\) standard deviation.

<table>
<thead>
<tr>
<th>Supernatant removal stage</th>
<th>Surfactant mass fraction removed in supernatant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₁</td>
<td>29.05 (\pm) 5.8</td>
</tr>
<tr>
<td>V₂</td>
<td>2.43 (\pm) 0.74</td>
</tr>
<tr>
<td>V₃</td>
<td>0.87 (\pm) 0.47</td>
</tr>
<tr>
<td>Total surfactant removed in supernatant</td>
<td>32.35 (\pm) 5.35</td>
</tr>
<tr>
<td>Predicted surfactant adsorbed onto algae cell</td>
<td>67.65 (\pm) 5.35</td>
</tr>
</tbody>
</table>
electrochemical interaction and hydrodynamic forces that dominate the foam flotation process; however, further work is required to understand how CTAB adsorption changes with cell age and increases in cell density. Interestingly, it was also noted that cells harvested by CTAB-aided foam flotation had improved settling efficiencies over 30 min (14.3%) when compared to control settlement efficiencies (0.9%) (data not shown). This is likely due to algal flocs being generated as a result of charge neutralisation. Therefore, the concentration factor could be further increased post-harvest if a short settlement stage is added; however, further research is required to determine the effect of sedimentation post-harvest.

Lipid yields (mass fraction of dry cell weight) were within a similar range to those reported in other studies [44,45]; however, it should be noted that the cultures in the current study were not deliberately nitrogen starved and therefore lipid biosynthesis was not optimised. The total lipid extracted from the dry biomass harvested by foam flotation varied between a minimum of 14.5 ± 0.47% on day 3, to a maximum of 17.63 ± 0.53% on day 6. For the cells harvested by centrifugation the minimum recovery was 8.3 ± 0.16% on day 3 with a maximum of 11.4 ± 0.47% on day 21 (Fig. 3). Mean lipid yield from all foam flotation harvests was 15.4 ± 0.77% which was significantly higher (p = <0.001) than yields from centrifugation (9.9 ± 0.56%). No significant relationship was observed between total lipid content and growth stage for algae harvested by either method. Although the nutrient levels were not monitored throughout the growth cycle, the lack of any significant increase in lipid content of cells harvested by centrifugation (p = 0.092) suggests the cells were not nitrogen limited. This supports the theory that a reduction in the electrochemical stability of the cells within suspension caused the cells to aggregate and drop out of suspension [30], thereby resulting in the significant decline in cell density at day 21 (Fig. 2), and reducing the harvesting efficiency towards the end of the growth phase at days 15, 18 and 21. Unexpectedly, the results indicated that CTAB-aided foam flotation significantly increased the extractable lipid fraction.

Despite the harvested cells being washed and freeze dried prior to lipid extraction, a significant amount of surfactant may still have been adsorbed to the cell surface. Therefore, the capacity for CTAB to be carried over into the total lipid extraction procedure was considered. Up to 79.4 ± 4.0% of CTAB by weight was recovered from the total lipid extraction, with an average of 76.6 ± 8.74% recorded. The effect of CTAB on the extractable lipid pool was calculated based on total lipid recovered from flotation and centrifuge harvested cells, CTAB adsorption efficiency, and the proportion of CTAB recoverably from the total lipid extraction process.

Table 2 shows that the predicted values for the total lipid yields from cells harvested by foam flotation (calculated using equations (2)–(6)) were close to those actually measured (p = 0.896), suggesting that the higher yields from foam flotation harvested cells were due, in part, to CTAB adsorption and subsequent carry over into the extraction process.

Although a number of papers have focussed on foam flotation [11,13–15], to our knowledge the effect of surfactant adsorption on lipid content has never been noted. It has however been found that the electrostatic interactions that aid flotation may also compromise the integrity of the cell wall, which may potentially result in cell lysis [46]. It has been suggested that CTAB effects gross membrane damage through protein denaturation, causing the cell membrane to rupture [46,47]. It is feasible that the increased lipid yields from foam flotation harvested cells may also be due in part to an increase in the concentration of phospholipids liberated due to membrane deterioration. To explore this, the lipid fractions of cells harvested by foam flotation and centrifugation were investigated.

The total lipid pool is composed primarily of neutral lipid in the form of energy storage bodies, as well as glyco and phospholipids within structural membranes. Within the literature the total lipid content of microalgae is commonly quoted as an indication of its appropriateness as a biodiesel feedstock [1]. However, not all lipid fractions can be easily esterified into biodiesel; therefore a high quoted total lipid may be misleading. Solid phase extraction was used to determine the crude composition of the total lipid extract from which information on the effect of harvesting technique on the biodiesel production potential and the impact of CTAB on the total lipid recovered could be determined. The extracted lipids from cells harvested by foam flotation and centrifugation consisted mainly of neutral lipids (Table 3), contributing 60.1 ± 12.5% and 58.3 ± 12.2% of the total lipid pool respectively. There was no significant difference between the neutral

<table>
<thead>
<tr>
<th>Culture age</th>
<th>Predicted</th>
<th>Gained</th>
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<tbody>
<tr>
<td>3</td>
<td>16.6</td>
<td>14.5</td>
</tr>
<tr>
<td>6</td>
<td>11.3</td>
<td>17.6</td>
</tr>
<tr>
<td>9</td>
<td>15.7</td>
<td>14.2</td>
</tr>
<tr>
<td>12</td>
<td>18.9</td>
<td>13.4</td>
</tr>
<tr>
<td>15</td>
<td>18.0</td>
<td>17.0</td>
</tr>
<tr>
<td>18</td>
<td>15.6</td>
<td>14.3</td>
</tr>
<tr>
<td>21</td>
<td>16.9</td>
<td>15.9</td>
</tr>
</tbody>
</table>

Fig. 3 – The lipid mass fraction of dry cell weight (% mean ± standard deviation) extracted from cells harvested by centrifugation (grey) and foam flotation (black).
lipid fraction ($p = 0.739$), and the free fatty acid fraction (FFA) ($p = 0.085$) when comparing the lipids recovered from either harvest method. However, a significantly higher phospholipid content of $23.6 \pm 5.4\%$ was extracted from microalgae harvested by foam flotation ($p = 0.008$) compared with centrifugation ($16.1 \pm 12.8\%$). As all harvested cultures were grown under the same environmental conditions it is unlikely that foam flotation harvested cells would have contained a greater titre of phospholipids.

CTAB that had been put through the modified Folch method was also separated by solid phase extraction. The CTAB in chloroform was applied to the separation column under vacuum, which should have left the full mass of CTAB on the column; however, $27.6 \pm 17.1\%$ was removed during the application process. From Table 3 it can be seen that the majority of the CTAB was eluted as a neutral lipid. There was no significant difference in the percentage of neutral lipids when comparing cells harvested by foam flotation and centrifugation; therefore, it is unlikely that CTAB would have affected this cell fraction. Only minor fractions of CTAB were recovered as FFA, and phospholipid, with $25.61 \pm 0.8\%$ of CTAB remaining on the column. This data suggests that the adsorbed CTAB did not significantly affect the composition of the lipid fraction. Therefore, CTAB must have increased the amount of phospholipids available for extraction due to the solubilisation of the phospholipid cell membrane [48]; this may also have contributed to the increased total recovered lipid when harvesting by foam flotation.

The neutral lipids found within microalgae extracts are mainly comprised of triacylglycerols (TAGs) [49]. Although both polar and neutral lipids can be converted to biodiesel, neutral lipids are the desired fraction as TAGs are easily transesterified to biodiesel [50]. FFA can also be converted after esterification [25]; however, Van Gerpen [51] reported that phosphorus containing compounds in the crude lipid oil did not convert into the methyl esters, which may cause problems during conversion and combustion processes [51,52].

However, whilst not necessarily valuable for biodiesel production, there are established and growing markets for certain phospholipids and their by-products [53] that may form important outputs as part of an algae biorefinery. CTAB is used as a food grade chemical for the extraction of pigments from red beet; therefore procedures to ensure that the product is fit for human/animal consumption are established [48]. Phospholipids can also be recycled as sources of nitrogen and phosphorus for microalgae cultivation, which could significantly reduce the operational production costs [54].

The effect of foam flotation on the fatty acid profile was investigated (Table 4). It was important to characterise the fatty acid profile as this can dramatically affect the quality of the biodiesel product [26] and inform the economics of a bio-refinery producing lipid-based high value products. No significant difference was found between the quantity of FAMEs gained from cells harvested by centrifugation or foam flotation ($p = 0.609$), confirming that the adsorbed CTAB did not significantly affect the neutral lipid fraction. The total transesterifiable lipid for cells harvested by centrifugation was $6.4 \pm 1.3\%$ dry weight (DW) and $5.6 \pm 0.3\%$ for cells harvested by foam flotation. However, discernible changes within the FAME profiles were noted. Significantly higher yields of the monounsaturated fatty acid (MUFA) oleic acid (C18:1n9c) were recovered from cells harvested by foam flotation ($5.1 \pm 0.133\%$ DW) compared to cells harvested by centrifugation ($2.17 \pm 1.6\%$ DW) ($p = 0.001$). Significantly greater yields of total MUFA ($9.7 \pm 0.15\%$ DW) ($p = 0.001$) and saturated fatty acids (SFA) ($6.4 \pm 0.05\%$ DW) ($p = 0.006$) were also recovered from foam flotation harvested cells (Table 5). In terms of

<table>
<thead>
<tr>
<th>Properties</th>
<th>Centrifugation</th>
<th>Foam flotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated fatty acids (% of total FAME)</td>
<td>6.0 $\pm$ 0.5</td>
<td>6.4 $\pm$ 0.05</td>
</tr>
<tr>
<td>Monounsaturated fatty acids (% of total FAME)</td>
<td>6.7 $\pm$ 0.09</td>
<td>9.7 $\pm$ 0.15</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids (% of total FAME)</td>
<td>32.5 $\pm$ 0.48</td>
<td>25.3 $\pm$ 0.54</td>
</tr>
<tr>
<td>Dominant biodiesel components (% of total FAME)</td>
<td>24.7 $\pm$ 0.92</td>
<td>23.3 $\pm$ 0.30</td>
</tr>
<tr>
<td>Total FAME content (%CDW)</td>
<td>6.4 $\pm$ 1.27</td>
<td>5.6 $\pm$ 0.26</td>
</tr>
</tbody>
</table>

Table 3  Percentage of lipid class mass fraction with respect to total extracted lipid. Data displayed is the mean of 3 replicates ± standard deviation.

<table>
<thead>
<tr>
<th>Lipid fraction</th>
<th>Foam flotation</th>
<th>Centrifugation</th>
<th>CTAB mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral lipid</td>
<td>60.1 $\pm$ 12.5</td>
<td>58.3 $\pm$ 12.2</td>
<td>37.8 $\pm$ 14.5</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>16.1 $\pm$ 8.9</td>
<td>25.5 $\pm$ 4.6</td>
<td>1.7 $\pm$ 1.6</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>23.6 $\pm$ 5.4</td>
<td>16.1 $\pm$ 12.8</td>
<td>0.4 $\pm$ 0.8</td>
</tr>
</tbody>
</table>

Table 4  Selected fatty acid methyl ester profiles with respect to the dry mass fraction of total fatty acids (%). Data are the mean of 3 replicates. A broader suite of FAMES, including C18, were detected but not presented here. Total C18 series is the sum of all C18 FAMES identified including those not listed in the table.

<table>
<thead>
<tr>
<th>Fatty acid methyl ester</th>
<th>Centrifugation</th>
<th>Foam flotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caprylic (C8:0)</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Capric (C10:0)</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Lauric (C12:0)</td>
<td>0.24</td>
<td>0.21</td>
</tr>
<tr>
<td>Myristic (C14:0)</td>
<td>1.27</td>
<td>1.77</td>
</tr>
<tr>
<td>Pentadecanoic (C15:0)</td>
<td>0.12</td>
<td>0.18</td>
</tr>
<tr>
<td>Palmitic (C16:0)</td>
<td>1.76</td>
<td>1.52</td>
</tr>
<tr>
<td>Stearic (C18:0)</td>
<td>0.30</td>
<td>0.69</td>
</tr>
<tr>
<td>Elaidic (C18:1n9t)</td>
<td>4.54</td>
<td>4.53</td>
</tr>
<tr>
<td>Oleic (C18:1n9c)</td>
<td>2.17</td>
<td>5.16</td>
</tr>
<tr>
<td>Linoleic acid (C18:2n6c)</td>
<td>22.30</td>
<td>17.48</td>
</tr>
<tr>
<td>Linolenic acid (C18:3n3)</td>
<td>10.20</td>
<td>8.14</td>
</tr>
<tr>
<td>Arachidic (C20:2)</td>
<td>2.23</td>
<td>1.68</td>
</tr>
<tr>
<td>cis-11-Eicosenoic (C20:1)</td>
<td>0.12</td>
<td>0.10</td>
</tr>
<tr>
<td>Behenic (C22:0)</td>
<td>0.10</td>
<td>0.24</td>
</tr>
<tr>
<td>Total C18 series</td>
<td>41.12</td>
<td>38.85</td>
</tr>
</tbody>
</table>

Table 5  Composition and mass fraction of FAME (%) harvested by centrifugation and foam flotation. Data expressed as means of 3 replicates ± standard deviation. Saturated fatty acids = C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0, C20:0, C22:0; Monounsaturated fatty acids = C18:1n9t, C18:1n9c; Polyunsaturated fatty acids = C18:2n6c, C18:3n3; Dominant biodiesel components = C18:0, C18:1n9c, C18:2n6c [25].
biodiesel quality, higher proportions of MUFA and SFA are desirable as they increase the fuels’ energy yield, cetane number, and also improve the oxidative stability [44]. Surprisingly, cells harvested by centrifugation had higher yields of total polyunsaturated fatty acids (PUFA) at 32.5 ± 0.48% DW compared to 25.3 ± 0.54% DW for cells harvested by foam flotation (p = 0.001); including higher yields of linoleic (C18:2n6c) (p = 0.001) and linolenic acids (C18:3n3) (p = 0.001) (Table 4). There was no significant difference between the C18 series between either harvest method (p = 0.084). Knothe [55] stated that palmitic, stearic, oleic, and linolenic acids are the most common fatty acids present in biodiesel. These components equate to 24.7 ± 0.46% for centrifugation and 23.3 ± 0.30% for foam flotation; there was no significant difference between the harvesting methods (p = 0.091) (Table 5).

Lee et al. [32] tested the effect of three different flocculating methods: pH adjustment, treatment with aluminium sulphate, and treatment with Pestan (a microbial flocculant), on the lipid content of Botryococcus braunii. It was found that the total lipid content was unaffected by the harvest method; however, no investigation into the fatty acid profile was carried out. Borges et al. [28] also found no significant difference for the total microalgae lipid content with respect to harvest method when comparing anionic and cationic polyacrylamide for the total microalgae lipid content with respect to harvest methods: pH adjustment, treatment with aluminium sulphate; however, the fatty acid profile differed significantly between either harvest method (p = 0.11–36).

4. Conclusion

Harvesting of Chlorella sp. by foam flotation is most effective during phases of active culture growth, suggesting that foam flotation may prove particularly advantageous for species that synthesise desirable biochemicals during active growth, but not as beneficial necessarily for species cultured specifically for biodiesel production. A greater quantity of lipid was recovered when biomass was harvested by foam flotation as opposed to centrifugation. This study is the first to investigate the effect of CTAB-aided foam flotation harvesting on lipid content and fatty acid profiles. The improved lipid recovery occurred due to a combination of an increase in the total extractable lipid caused by the solubilisation of the phospholipid bilayer by the surfactant CTAB, and also a proportion of the CTAB dose becoming adsorbed onto the cell and entering the lipid extraction process. Foam flotation resulted in a predominance of saturated and monounsaturated fatty acids within the fatty acid profile, which provide many favourable features for biodiesel production. Foam flotation is an advantageous microalgae harvesting technique and a full techno-economic analysis in relation to microalgae bio refining is greatly needed.

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