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Centrifugal recovery of solvent after biphasic wet extraction of lipids from a concentrated slurry of *Nannochloropsis* sp. biomass

Sam Q.K. Law, Binbo Chen, Peter J. Scales, Gregory J.O. Martin*

Algal Processing Group, Department of Chemical and Biomolecular Engineering, The University of Melbourne, Parkville, Victoria 3010, Australia

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ABSTRACT

Wet extraction of lipids from a microalgae slurry requires efficient recovery of the solvent. The use of a non-polar solvent such as hexane allows physical recovery of the solvent by centrifugation, avoiding energy intensive distillation of the aqueous slurry. However, the kinetics and mechanisms of centrifugal separation of solvent from microalgae are yet to be investigated. In this study, hexane was used to perform biphasic extraction of lipids from a concentrated slurry of *ca* 50% ruptured *Nannochloropsis* sp. cells. The hexane droplet size of the resulting emulsion and the viscosity of continuous phase were characterised. The slurry of ruptured cells was highly viscous and shear-thinning. The hexane droplets were < 50 μm in diameter. The rate of separation of hexane during centrifugation at different centrifugal forces was monitored in real time using a centrifuge fitted with optics (LUMiFuge). It was concluded that the rate of hexane recovery was limited by coalescence rather than droplet migration (creaming). A step-change increase in the rate of hexane separation was found to occur at a critical force of approximately 550 g. This was attributed to the initiation and propagation of droplet-droplet coalescence within the emulsion phase above a critical compressive force. Despite the high viscosity of the continuous phase, droplet movement was not limiting, possibly due to collective migration of the hexane droplets. Due to coalescence rather than droplet migration being limiting, as well as the complex rheology of the microalgae slurry, it is not possible to confidently estimate the required capacity of large scale centrifuges from batch experiments performed at a laboratory scale.

1. Introduction

Microalgal biomass is a promising source of triacylglyceride (TAG) lipids that can be used as food or a feedstock for biodiesel. Microalgae are much more productive than conventional vegetable crops such as soybeans or palm, and require considerably less arable land and fresh water [43]. Much effort has been devoted to improving large-scale cultivation of microalgae and the production of TAGs.

In addition to efficient growth of microalgae, large scale TAG production requires energy-efficient and cost-effective means of recovering these intracellular lipids [4]. This is more challenging than for conventional oil seeds where oil can be recovered by pressing and simple solvent extraction from the dry, oil-rich material. In contrast, microalgal lipids are enclosed within small, robust cells that are surrounded by water. Although the cultures can be concentrated into a thick slurry (e.g. 15–25% w/w solids) [27,40], further drying is not energetically feasible [4,20]. This means the lipids must be recovered from a wet, concentrated slurry of microalgal cells.

In the recovery of lipids, polar solvents such as ethanol or isopropyl alcohol can penetrate the cells but these solvents are fully miscible with water and can only be recovered by unfeasibly energy-intensive distillation [30,46]. Non-polar solvents such as hexane are required [13]. Additionally, hexane is selective to neutral lipids including TAGs and is immiscible in water resulting in a biphasic extraction process [16,29]. This requires the cells to be ruptured for the solvent to contact and dissolve the TAG droplets [46]. The TAG-rich hexane (or other non-polar solvent) can then be physically separated from the delipidated biomass and the solvent evaporated from the TAG [16,29].

The effectiveness of this approach has been demonstrated in our laboratory [29]. The process can be performed at low temperature and ambient pressure, using scalable equipment and minimal volumes of solvent. As such, the energy requirements have been shown to be sufficiently low as to allow a positive energy return for biodiesel production [25]. However, the actual recovery of lipid-rich solvent after extraction has yet to be examined in detail. The emulsion that is formed upon mixing the solvent and the slurry of ruptured cells is yet to be characterised. The kinetics and mechanisms of the de-emulsification process have also not been investigated and the associated costs remain

* Corresponding author.
E-mail address: gjmartin@unimelb.edu.au (G.J.O. Martin).

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unknown. A detailed understanding of these issues is required to determine the feasibility of this approach and to understand what opportunities are available for improvement.

To the best of our knowledge, there is no existing study that investigates the centrifugation process of solvent recovery from wet microalgal slurry (15–25% w/w). Past studies investigating the behaviour of concentrated microbial biomass during centrifugation have been limited to unruptured yeast and bacteria in the absence of solvent [9,24,37]. Although there are studies that have used centrifugation to recover solvent after lipid extraction from microalgae, none of these have characterised the resulting emulsion or considered the kinetics or mechanisms of centrifugal solvent recovery in any detail [7,13,15,16,22,29]. The emulsion-forming properties of ruptured microalgae slurry and their role in subsequent separation process are not fully understood [8]. Given the role of emulsification in inhibiting complete solvent recovery [14,23] and preventing an effective solvent recycling process, it is important to understand the mechanism to allow future optimisation. [12]. Complete recovery of solvent is also important as any lipid associated with the retained solvent in the emulsion will also remain unextracted.

In this study we investigated the emulsification and separation behaviour of hexane in concentrated slurries of ruptured *Nannochloropsis* sp., a promising candidate for commercial applications. Cells were weakened, then ruptured by high-pressure homogenisation (HPH), which has been shown to be an effective and scalable method for processing high solid biomass [29,45]. Hexane was chosen as the extraction solvent due to its very low solubility in water, low boiling point, established use in vegetable oil manufacturing and selectivity to neutral lipids suitable for biofuel production [16]. The extraction method was based on the previous work of Olmstead et al. 2013 [29], and key parameters of the resulting emulsion characterized. The kinetics of emulsion separation was investigated using a centrifuge with optics (LUMiFuge) that enabled the biphasic interface to be monitored in real time. This information was used to examine the mechanisms of phase separation. The implications of the results for large scale centrifugal recovery of solvent and lipids from slurries of ruptured microalgae are discussed, and strategies for potential improvements suggested based on the identified mechanisms.

2. Materials and methods

2.1. Microalgal biomass

A proprietary strain of the marine alga *Nannochloropsis* sp. was grown in an outdoor facility in Karratha, Australia under nitrogen-replete conditions. The microalgae biomass was harvested via chitosan-assisted flocculation and further concentrated into a high concentration slurry (paste) via centrifugation. The concentrated slurry was stored at − 20 °C and kept refrigerated at 4 °C upon thawing. All experiments were conducted within 3 weeks of thawing. The dry weight (w/w) of the biomass slurry was determined to be 19.0 ± 0.1% w/w by oven drying at 60 °C until a consistent weight was obtained [29]. The total lipid content of the *Nannochloropsis* sp. slurry was determined to be 23 ± 1% (w/w) by a modified Bligh & Dyer protocol [29]. The lipid extract comprised 62 ± 2% neutral lipids, 28 ± 2% glycolipids, and 10 ± 1% phospholipids as determined by solid phase extraction (SPE) [28]. The protein composition of the biomass was 50–53% (w/w) with a conversion factor of 6.25 as determined previously using the Kjeldahl nitrogen method [13]. The remainder of the dry mass includes the carbohydrates, ash content, and the flocculants that remain with the biomass [3]. The density of the slurry was determined using a 5.0 ml density bottle (Blaubrand, BRAND Gmbh, Wertheim, Germany) calibrated at room temperature (22 °C).

2.2. Mechanical cell rupture

The cell weakening and mechanical rupture methods of Olmstead et al. [29] were modified to accommodate smaller experimental batches. Batches of thawed biomass (100 ± 20 g) were incubated at 37 °C in a rotator-mixer (LabQuake, Barnstead International, Iowa, USA) and subsequently subjected to a single-pass through a high pressure homogenizer (Panda 2K NS1001L, GEA Niro Soavi, Parma, Italy) at 1050 ± 100 bar. The extent of cell rupture was approximately 50% as determined by cell-counting using a Neubauer-improved haemocytometer [46]. All the experiments, including cell rupture, lipid extraction, and centrifugation analysis, were performed in at least duplicate.

2.3. Lipid extraction using hexane

Lipids were extracted from the homogenised biomass by contacting with hexane. 4.0 ± 0.1 g of homogenised biomass and 1.3 ± 0.05*g* of n-hexane (95% AR, Ajax Finechem, Victoria, Australia) were added into air-tight 14 mL screw-cap glass vials (20 mm cylindrical diameter × 72 mm height). The vials were hand-shaken 5–10 times until the mixtures formed a single phase. The contents were then mixed by rotating the vials at 8 rpm (Labquake, Barnstead International, Iowa, USA) for 2 h at 37 °C. The hexane-biomass mixtures (emulsions) were cooled to 25 °C in a water bath before loading into LUMiFuge centrifuge tubes.

2.4. LUMiFuge centrifugation analysis

The kinetics of phase separation was investigated using a centrifuge instrument with an integrated photo-analyzer (LUMiFuge®, L.U.M. GmbH, Berlin, Germany). Aliquots (1.3 ± 0.1*g*) of the emulsion mixtures were loaded into the LUMiFuge tubes (10 × 8 mm diameter rectangular synthetic polyamide cells, 10 mm optical path length, part no. 110-135XX, L.U.M. GmbH, Berlin, Germany). Centrifugation was conducted between 36*g* to 2330*g* (radius = 130 mm) for a pre-determined time. The LUMiFuge instrument and the samples were allowed to equilibrate to the target temperature separately before initiating centrifugation. Duplicate samples were analysed for each of the repeated experiments. The representative data is the result of quadruplicate experiments.

During centrifugation, the emulsions (mixtures of hexane, ruptured biomass and salt water) were separated into distinct hexane, emulsion, aqueous and biomass layers [13]. The positions of the hexane-emulsion and emulsion-water interfaces were tracked via transmission of near-infrared light along the length of the centrifuge tube during the centrifugation. Based on the interface heights and the cross-sectional area of the LUMifuge tube (72.1 mm^2), and the mass and composition of the sample, the percent recovery of hexane and water were calculated according to Eqs. 1 and 2. The hexane interface heights obtained by the LUMiFuge light transmission profile were verified to be in good agreement with physical measurements using a Vernier calliper. The sample masses were recorded throughout the experiment to verify that the loss of hexane by evaporation was minimal (< 1 wt %). The hexane layers separated from LUMifuge centrifugation were subsequently collected and dried under nitrogen to obtain the gravimetric measurements of the extracted dry total lipids.

$$\text{Hexane recovery (\%)} = \frac{\text{Upper interface height} \times \text{area of centrifuge tube}}{\text{Sample mass} \times \text{hexane: biomass ratio} \times \text{hexane density}} \times 100\% \quad (1)$$

$$\text{Water recovery (\%)} = \frac{\text{Lower interface height} \times \text{area of centrifuge tube}}{\text{Sample mass} \times \text{water content of sample} \times \text{water density}} \times 100\% \quad (2)$$