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Short Communication

Low solvent, low temperature method for extracting biodiesel lipids from concentrated microalgal biomass

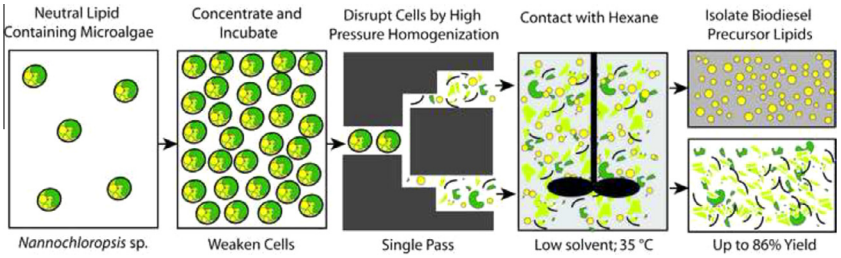
Ian L.D. Olmstead, Sandra E. Kentish, Peter J. Scales, Gregory J.O. Martin *

Department of Chemical & Biomolecular Engineering, The University of Melbourne, Parkville, Victoria 3000, Australia

HIGHLIGHTS

- Biodiesel lipids were extracted from microalgae using scalable equipment.
- Strong *Nannochloropsis* sp. cells were ruptured by incubation and homogenization.
- Lipids were extracted from concentrated pastes using minimal hexane.
- High yields of neutral lipids were obtained at low temperature and pressure.

GRAPHICAL ABSTRACT



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ABSTRACT

An industrially relevant method for disrupting microalgal cells and preferentially extracting neutral lipids for large-scale biodiesel production was demonstrated on pastes (20–25% solids) of *Nannochloropsis* sp. The highly resistant *Nannochloropsis* sp. cells. were disrupted by incubation for 15 h at 37 °C followed by high pressure homogenization at 1200 ± 100 bar. Lipid extraction was performed by twice contacting concentrated algal paste with minimal hexane (solvent:biomass ratios (w/w) of <2:1 and <1.3:1) in a stirred vessel at 35 °C. Cell disruption prior to extraction increased lipid recovery 100-fold, with yields of 30–50% w/w obtained in the first hexane contact, and a further 6.5–20% in the second contact. The hexane preferentially extracted neutral lipids over glyco- and phospholipids, with up to 86% w/w of the neutral lipids recovered. The process was effective on wet concentrated paste, required minimal solvent and moderate temperature, and did not require difficult to recover polar solvents.

1. Introduction

A renewable source of liquid transportation fuel is required and microalgae have promise as a feedstock for oils that can be converted to biodiesel (Brennan and Owende, 2009). While much effort has been devoted to the selection of promising microalgal strains (Griffiths and Harrison, 2009) and the development of efficient large-scale systems for their growth (Rodolfi et al., 2009), relatively little has been reported on the development of scalable methods for processing the biomass to biodiesel (Mata et al., 2010). In particular, a scalable and economic process for extracting lipids from the biomass is yet to be established.

Proposed methods for isolating lipids from microalgal biomass include extraction with organic solvents (Cooney et al., 2009), supercritical CO_2 extraction (Mendes et al., 1995), and in situ transesterification of the lipids to biodiesel (Ehimen et al., 2010). The scalability and economic viability of these microalgal processing methods is yet to be established, however, the use of organic solvents appears most feasible for large-scale implementation. Hexane is currently used commercially to recover lipids from soybeans (Garcia Serrato, 1981) and other oilseeds. When used on conventional vegetable oil feed stocks, hexane is effective because the raw material is a relatively moisture-free and exposed extraction matrix. The presence of water creates a polar barrier between the solvent and lipids, decreasing the mass transfer efficiency (Cooney et al., 2009). This presents a challenge for the economic use of non-polar solvents in microalgal lipid extraction because

* Corresponding author. Tel.: +61 3 83446613; fax: +61 3 83444153.
E-mail address: gjmartin@unimelb.edu.au (G.J.O. Martin).

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the intracellular water cannot be removed by mechanical dewatering and the most effective dewatering techniques typically resulting in a paste comprising about 65–80 wt.% water. Drying beyond this point is energy and capital intensive (Cooney et al., 2011) and therefore only viable for processes focussed on high value products such as omega-3 fatty acids. While the use of solar drying could reduce the energy requirements, relying on this slow and variable drying process prior to extraction is problematic. The cell walls present an additional barrier to solvent extraction of microalgal lipids. There is evidence, however, that disruption of the microalgal cells can improve solvent extraction of lipids while also allowing for harvest of other intracellular products such as carotenoids (Lee et al., 2010; Ulloa et al., 2012). Unfortunately, some of the most promising microalgae for biodiesel production, such as *Nannochloropsis* sp., and *Chlorella* sp., are difficult to rupture using industrially relevant cell disruption methods such as high pressure homogenization (Spiden et al., 2013).

While the isolation of lipids from microalgae has been well studied (Lewis et al., 2000; Mercer and Armenta, 2011), most information relates to analytical methods for lipid characterisation which typically require dried biomass or expensive (and harmful) solvents such as chloroform. Very few studies have reported extraction methods suitable for concentrated wet microalgal biomass which appear scalable or composed of conventional process units which would enable techno-economic evaluation of large-scale systems required for commercial oil extraction and biodiesel production. The first study to report successful extraction of lipids from concentrated microalgal paste used 1-butanol, ethanol, or hexane/2-propanol to isolate lipids from a 15% solids paste of the diatom *Chaetoceros muelleri* (Nagle and Lemke, 1990). Yields of over 90% w/w of the available lipids were recovered; however, a high ratio of solvent to biomass (20:1 w/w) was employed. More recently, Chen et al. have shown that lipids can be effectively extracted from concentrated pastes of *Nannochloropsis* sp. (Chen et al., 2012), a species which has been shown to be highly promising for the production of both biodiesel and omega-3 fatty acids (Olmstead et al., 2013). In the study by Chen et al., hexane/ethanol mixtures were contacted with 35% w/w solids paste at a solvent:biomass mass ratio of between about 3:1–12:1 and a temperature of 75–135 °C to recover up to approximately 90% of the available lipids. A different study showed it is possible to recover lipids from wet pastes of *Nannochloropsis* sp. using isopropyl alcohol (IPA) at 80 °C (Yao et al., 2012). Very high solvent:biomass ratios (16:1–114:1) were used, and the miscibility of IPA with water would make solvent recovery difficult. In another recent work, lipids from wet algal biomass were extracted using terpenes at comparable yields to those obtained using conventional solvents such as hexane and chloroform (Dejoye Tanzi et al., 2013). However, the solvent:biomass ratio was again high (*ca* 35:1) and high temperatures were used to initially evaporate water and to also aid the extraction process. While these results show promise for solvent extraction from wet microalgal paste, it is highly desirable to develop a lower temperature and pressure process, which does not require polar solvents and which uses a low solvent:biomass ratio.

In this work, the effectiveness of a novel and scalable method for lowering the energy required for cell disruption and lipid extraction from concentrated pastes of *Nannochloropsis* sp. is presented. The ability of this method to achieve significant and selective lipid recovery using a single, non-polar solvent at low solvent:ratios and under low temperature, low pressure extraction conditions is investigated using four batches (300–600 g) of paste (20–25% w/w solids) harvested from outdoor cultures. A quantitative analysis of lipids recovered is included to evaluate the effect of lipid composition on biodiesel production.

2. Methods

2.1. Algal strains and cultivation

A wild type strain of *Nannochloropsis* sp. from the University of Melbourne Culture Collection was kindly provided by Dr. Rick Wetherbee. The culture was maintained as previously described (Olmstead et al., 2013). Algal biomass was generated from monocultures of *Nannochloropsis* sp. grown at ambient temperature in outdoor photobioreactors during autumn 2012 at The University of Melbourne Parkville campus (Parkville, Victoria, Australia; latitude: 37° 47′ 54″ S; longitude: 144° 57′ 40″ E). The photobioreactors consisted of four separate 100 L polyethylene bags attached to a frame and sparged with air to provide agitation and the ambient CO_2 providing a source of carbon for growth. Shading and external evaporative cooling used to prevent overheating during hot days (>30 °C). Cultures were grown under nutrient replete conditions until a target cell density of approximately (OD_{750} = 2.8, or g/L = ~1.0) was reached, at which point 50% of the culture volume was removed and replaced with fresh medium with or without nitrate. The nitrate deprived bags were left for approximately 10 days to allow the algae to accumulate neutral lipids. Algae from these bags was concentrated (to approximately 30% w/w solids) by centrifugation using a type OTC 3-02 Westfalia Separator (GEA Westfalia Separator Australia Pty. Ltd., Thomastown, VIC, Australia). Batches of algal paste were harvested from the concentrator and stored at 4 °C for up to 14 days before processing.

2.2. Preparation of algal paste

Four separate batches (A–D) of *Nannochloropsis* sp. paste were processed with final dry biomass concentrations in the range of 20–25% w/w dry solids (see Table 1). Each batch was diluted with tap water from a more concentrated paste (*ca* 30% w/w) to a viscosity of *ca* 1 Pa s at 100 s^{-1} which is the upper operating limit of the high pressure homogenizer. The viscosity of the paste was measured using an AR-G2 rheometer (TA Instruments, New Castle, DE, USA). The final concentration of biomass in each batch was determined by mass loss from drying overnight at 60 °C and adjusted to account for ash content.

2.3. Biomass incubation

After adjustment of biomass concentration, 300–600 g batches of algal paste were incubated for 5–26 h at 37 °C in a temperature-controlled 316 stainless steel agitated vessel with a total working volume of 2.4 L, an internal diameter of 95.5 mm and a height of 386 mm. The vessel was airtight and fitted with a pressure relief valve. Temperature was controlled by recirculation of water through an external jacket. Agitation was supplied via a two-blade anchor-type impeller rotated at 60 rpm by an external overhead stirrer (Heidolph RZR 2102, Heidolph Instruments, Schwaback, Germany).

2.4. High pressure homogenization

Following pre-conditioning of the biomass, the algal paste was passed through a GEA Panda 2K NS1001L high pressure homogenizer (GEA Niro Soavi, Parma, Italy) fitted with a manufacturer supplied cell disruption valve (Re+ valve). The homogenization