Bioresource Technology 184 (2015) 280–285

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Energy evaluation of algal cell disruption by high pressure homogenisation

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HIGHLIGHTS

- Energy required for high pressure homogenisation of microalgae was analysed.
- Solids content, TAG content, and homogenisation pressure were considered.
- Processing of concentrated microalgal paste is not only possible but also required.
- Between 6% and 110-times of the energy density of the biodiesel was required.
- HPH is feasible for concentrated pastes of relatively weak microalgae.

ARTICLE INFO

Article history:
Received 31 August 2014
Received in revised form 10 November 2014
Accepted 11 November 2014
Available online 18 November 2014

Keywords:
Cell disruption
High pressure homogenisation
Nannochloropsis sp.
Biodiesel
Energy analysis

ABSTRACT

The energy consumption of high pressure homogenisation (HPH) was analysed to determine the feasibility of rupturing algal cells for biodiesel production. Experimentally, the processing capacity (i.e. flow rate), power draw and cell disruption efficiency of HPH were independent of feed concentration (for *Nannochloropsis* sp. up to 25% w/w solids). Depending on the homogenisation pressure (60–150 MPa), the solids concentration (0.25–25% w/w), and triacylglyceride (TAG) content of the harvested algal biomass (10–30%), the energy consumed by HPH represented between 6% and 110-times the energy density of the resulting biodiesel. Provided the right species (weak cell wall and high TAG content) is selected and the biomass is processed at a sufficiently high solids concentration, HPH can consume a small fraction of the energy content of the biodiesel produced. This study demonstrates the feasibility of process-scale algal cell disruption by HPH based on its energy requirement.

1. Introduction

Despite the vast potential for the commercialisation of algal-derived products ranging from fuel, food and feed, (Becker, 2007; Pulz and Gross, 2004; Scott et al., 2010), accessing the intracellular components in a cost-effective manner remains a major challenge in process-scale operations. Economic analyses of current process designs show that they remain prohibitively expensive despite generally favourable factors such as areal productivity and environmental impact (Sun et al., 2011). Limitations of current dewatering technologies (Uduman et al., 2010) and the prohibitively high cost of thermal drying (Cooney et al., 2011) indicate that recovery and extraction processes need to be focussed on biomass with a high water content of up to 80% w/w (in the form of concentrated paste). In addition, these processes must have the ability to effectively deal with a rigid algal cell wall that encapsulates the components of commercial interest such as lipids and proteins.

The complete disruption of the algal cell wall prior to product recovery has been shown to be critical for successful process-scale lipid extraction from wet algal biomass (Olmstead et al., 2013b), with cell disruption overcoming the mass transfer barriers resulting from the presence of water and the cell wall (Yap et al., 2014). A range of cell rupture techniques including high pressure homogenisation (Halim et al., 2012; Olmstead et al., 2013b; Samarasinghe et al., 2012), ultrasonication (Halim et al., 2012; Keris-Sen et al., 2014; Lee et al., 2010), microwave heating (Balasubramanian et al., 2011; Lee et al., 2010), bead-beating (Halim et al., 2012; Lee et al., 2010), ball-milling (Balasundaram et al., 2012) and osmotic shock (Lee et al., 2010) have been tested on algal suspensions and all have generally been shown to have a positive effect on recovery yield. Even though cell disruption can clearly be achieved by these various techniques, only a limited range of methods are feasible for process-scale implementation

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http://dx.doi.org/10.1016/j.biortech.2014.11.049
0960-8524/

(Middelberg, 1995). Innovative low-energy process routes are still highly sought after as some of the most industrially promising algae such as *Chlorella* sp. and *Nannochloropsis* sp. are highly resistant to mechanical breakage (Spiden et al., 2013).

For a process-scale cell disruption technology to be feasible, it must have the ability to continuously process high solids biomass (i.e. high viscosities), be highly energetically efficient, of short residence time, and cause minimal product degradation. The high pressure homogeniser, a unit operation adapted from the dairy industry, offers the aforementioned advantages and is the most widely used method for process-scale cell disruption for recovering recombinant proteins from *Escherichia coli* and *Saccharomyces cerevisiae* (Diels and Michiels, 2006). Most homogenisers operate under the same basic principle whereby cell suspensions are circulated and forced by a positive displacement pump through an orifice within an assembly of specially designed valves at pressure. The flow velocity increases rapidly and the pressure decreases to atmospheric over a short distance as the suspension exits the unit. The mechanisms of cell disruption through the homogeniser is still not fully understood, but has been attributed variously to fluid shear stress (Clarke et al., 2010), inertial forces (Kleinig and Middelberg, 1998), impingement (Keshavarz Moore et al., 1990), turbulence (Doulah et al., 1975) and cavitation (Shirgaonkar et al., 1998). Even less is understood about the mechanism of rupture when high pressure homogenisation is performed at high solids.

Even though high pressure homogenisation can rupture even the most mechanically resilient algae such as *Nannochloropsis* sp. (Spiden et al., 2013) and can be used to process pre-treated concentrated paste (Olmstead et al., 2013b), it can only be practically applied to large scale processing of algal biomass provided the energy requirements are sufficiently low. A number of recent studies have evaluated the performance of high pressure homogenisation in the context of algal processing, with largely negative conclusions (Coons et al., 2014). However, a major limitation of these studies is that they have all been performed on the basis of processing dilute algal suspensions of <5% w/w solids, which as we will show, greatly overestimates the associated energy consumption. As an example, the studies by Lee et al. (2012), Halim et al. (2012) and Balasundaram and Pandit (2001) all concluded that current cell disruption technologies including high pressure homogenisation consume significantly more energy than the potential energy output of algal-derived biodiesel. However, these conclusions were all based on studies using dilute suspensions (<1% w/w solids) of algae (Halim et al., 2012; Lee et al., 2012) or yeast (Balasundaram and Pandit, 2001). To properly evaluate the potential of high pressure homogenisation for algal processing, analysis of the associate energy requirements and processing effectiveness must be performed across a range of solids concentrations.

In this study, a detailed examination of the energy requirement of rupturing algal cells by high pressure homogenisation is undertaken. *Nannochloropsis* sp., one of the most industrially promising yet difficult to mechanically rupture species of algae, was chosen as the target organism. It is a fast growing and highly robust species with a fatty acid profile suitable for biodiesel and omega-3 production, having an abundance of saturated, monounsaturated, and eicosapentaeoic fatty acids (Olmstead et al., 2013a). The energy consumption and rupture performance of a bench-scale homogeniser were investigated as a function of solids concentration (0.25–25% w/w). Subsequently, an analysis to identify key processing criteria to ensure feasibility upon scale-up was undertaken according to the equipment manufacturer specifications and realistic process assumptions. Commentary is also provided for issues concerning scale-up in an algal biodiesel production facility.

2. Methods

2.1. Algal biomass

Biomass slurries of *Nannochloropsis* sp. (20–25% w/w solids) were sourced from an outdoor growth facility in Karratha, Western Australia. Total solids in the biomass were determined by a method developed by the National Renewable Energy Laboratory NREL (Sluiter et al., 2008). Biomass was stored in a freezer at −20 °C and thawed prior to experimentation, which was conducted 2–4 months later. Prior to commencing experimental work using freeze–thawed paste, it was confirmed that the extent of cell rupture resulting from high pressure homogenisation (as measured by cell counting) was minimally affected by the freeze–thawing process. Freshly harvested and freeze–thawed biomass samples were homogenised at the same processing conditions (solids concentration, homogenisation pressure, number of passes and feed temperature) and subsequently quantified by cell counting. A deviation of less than 10% was observed over five homogenisation passes.

2.2. High pressure homogeniser

A GEA Panda2K NS1001L bench top high pressure homogeniser (GEA Niro Soavi, Parma, Italy) with a nominal flow rate of 10 L h^{-1} and equipped with a cell disruption valve (RE+ valve) was used in this study. A pressurised feeding hopper assembly was fitted to the homogeniser to process high solids biomass. Power consumption was measured by a three-phase power metre (ABB Australia). Slurries of different concentrations were passed through the homogeniser at pressures ranging from 30 to 150 MPa. Homogenates were recovered and subsequent analyses were performed within 3 h of homogenisation.

2.3. Quantification of cell disruption

Cell rupture was quantified by cell counting, due to its accuracy and reproducibility (Spiden et al., 2013). The number of intact cells remaining after homogenisation was determined by microscopic observation using a Neubauer improved haemocytometer (Laboroptik Ltd., Lancing, United Kingdom) with a 100 μm chamber depth. ImageJ, a public-domain image processing and analysis software (Rasband, 2008), was used to ensure consistency in cell counting by minimising variability in analysis between samples. Measurements of the total area occupied by cells were used to verify the intact cell counts. This was particularly useful for samples with cell clumping, typically observed in samples that had been homogenised at higher pressures. All imaging was performed using an Olympus BX51 light microscope with a DP72 digital camera attachment (Olympus, Mt. Waverley, VIC, Australia). Cell counts were normalised between the control sample (unhomogenised cell count) and zero.

3. Results and discussion

3.1. High pressure homogenisation as a function of feed concentration

Process-scale dewatering techniques, typically consisting of a pre-concentration flocculation step followed by centrifugation (Vandamme et al., 2013), produce algal pastes of up to 25% w/w solids. As the capital and operating costs associated with downstream processing of algal biomass are expected to be reduced as a function of increasing solids volume fraction, the ability to directly feed dewatered algal paste (at *ca.* 25% w/w solids) into the high pressure homogeniser would appear highly desirable.