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Quantitative evaluation of the ease of rupture of industrially promising microalgae by high pressure homogenization

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HIGHLIGHTS

- Rupture of three promising microalgae by high pressure homogenization was investigated.
- Cell counting was the only reliable method for quantifying rupture of all the algae.
- *Tetraselmis suecica* was highly susceptible to rupture by homogenization.
- *Chlorella* sp. had similar resistance to rupture as the yeast *Saccharomyces cerevisiae*.
- *Nannochloropsis* sp. was highly resistant to rupture.

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ABSTRACT

The susceptibility to rupture of the microalgae *Nannochloropsis* sp., *Chlorella* sp. and *Tetraselmis suecica* by high pressure homogenization was compared quantitatively to the yeast *Saccharomyces cerevisiae*. Methods for quantifying cell rupture were investigated including cell counting, turbidity, metabolite release and particle sizing. Cell counting was the only reliable method for quantitative comparisons of all microalgae, with turbidity complicated by agglomeration of cell debris for *T. suecica*, and measurement of metabolite release affected by degradation occurring for all microalgae after significant rupture. The rupture of all microalgae followed exponential decay as a function of number of passes. The pressure required to achieve rupture of 50% of the cells per pass was determined to be 170, 1070, 1380, and ca. 2000 bar for *Tetraselmis* sp., *Chlorella* sp., *S. cerevisiae*, and *Nannochloropsis* sp., respectively. These results extend the criteria for selecting microalgae for industrial applications beyond consideration of growth and compositional attributes.

1. Introduction

The production of biodiesel from lipids extracted from microalgae has been proposed to address global concerns associated with the unmitigated release of greenhouse gases and the security of future liquid petroleum supplies (Chisti, 2007). While biodiesel from microalgae offers many advantages such as near carbon neutrality and compatibility with existing transport infrastructure (Cooney et al., 2009), significant processing difficulties need to be overcome. These include the challenges of processing large volumes of low concentration feedstock and the selective extraction of the relevant lipids (Molina Grima et al., 2003).

A major research focus for biodiesel production from microalgae is the selection of an optimal strain. The predominant factors usually considered are biomass growth rates and lipid productivity (Griffiths and Harrison, 2009; Mata et al., 2010; Rodolfi et al., 2009). The 'processability' of the strain, for example how easily lipids are recovered from the biomass, is often overlooked. A generalized pathway for biodiesel production from microalgae includes the cultivation of oleaginous microalgae followed by the extraction of the lipids with an appropriate solvent system. The extracted lipids are purified and chemically converted into biodiesel and the residual biomass and aqueous components are recycled or recovered as co-products (Mata et al., 2010).

Rupture of microalgal cell walls prior to solvent extraction has been found to significantly improve lipid recovery (Lee et al., 2010; Prabakaran, 2011) and high pressure thermal hydrolysis of microalgae has been shown to increase the methane yield from subsequent anaerobic digestion (Keymer et al., 2013). Mechanical cell disruption by high pressure homogenization is a particularly promising technique for microalgae, as it is effective in aqueous environments (eliminating the need for energy intensive drying) and can be scaled up to process large volumes (Harrison, 1991; Samarasinghe et al., 2012). When compared to other disruption

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methods it has been shown to have a high efficacy in rupturing the microalga *Chlorococcum* sp. (Halim et al., 2012). However, to date there is little known about the effectiveness of high pressure homogenization on different types of commercially relevant microalgae, and how this compares to traditional industrial microorganisms such as yeast. Literature that has focused on disruption of microalgae for biodiesel production has typically evaluated cell disruption on the basis of improvements to subsequent lipid extraction (Prabakaran, 2011; Wiltshire et al., 2000). As lipid extraction depends on many variables, such as the solvent system, contact time and conditions, lipid extraction is not an ideal indicator of cell rupture (Ryckebosch et al., 2012; Samarasinghe et al., 2012). Subsequently, alternative disruption indicators are required to deconvolute the complexities of solvent extraction optimization from cell disruption and allow direct comparison of the susceptibility of different microalgae to high pressure homogenization.

Common methods for quantifying cell rupture are metabolite release (including protein), cell counting and particle sizing (Middelberg, 1995). Each technique has advantages and disadvantages and gives comparable but different measures of rupture (Spiden et al., 2013). Cell counting gives non-ambiguous measures of the proportion of cells which are nominally whole (Spiden et al., 2013). Monitoring the particle size distribution gives details of the homogenate which is relevant to downstream processing optimization but is limited as the process is time consuming and offers restricted insight when clumping occurs pre and post-rupture (Joyce, 2003; Samarasinghe et al., 2012). Measurements of metabolite release (Bradford assays for protein, UV absorbance for general measure of metabolite release) provide quick, indirect measures of the extent to which the cell walls have been compromised. Despite their short processing time and simplicity, metabolite release methods are used with caution at high levels or rupture where the homogenization process may denature the metabolites, resulting in an underestimate of cellular rupture (Van Gaver and Huyghebaert, 1991). Measuring the reduction in suspension turbidity to monitor homogenization is a common industrial practice but not reliable across all microorganisms (Foster, 1995). Previous work has demonstrated that monitoring the decrease in turbidity of *Saccharomyces cerevisiae* with rupture requires very little processing time and closely aligns with cell counting (Spiden et al., 2013) and has previously been used to monitor algal rupture with sonication (Joyce, 2010; Wu, 2011). In this paper a combination of these methods were used to quantify the rupture of the selected microalgae with homogenization. This approach provides the most comprehensive characterization of this process performed to date and allows assessment of the applicability of the various disruption indictors to different types of microalgae.

Three microalgae from different genera were selected for study in this paper, each of which has been identified as industrially promising for the production of biodiesel. *Nannochloropsis* sp., a spherical eustigmatophyte, generally grows to between 2 and 4 μm in diameter. The structural component of the cell wall of *Nannochloropsis* sp. can contain highly resistive biopolymers (Gelin, 1997) and is considered highly impervious to mechanical rupture (Yao et al., 2012). *Nannochloropsis* sp. can accumulate neutral lipids (Converti, 2009; Olmstead et al., 2013; Rodolfi et al., 2009) and is rich in valuable omega-3 fatty acids, photoactive pigments such as carotenoids and a wide range of antioxidants. *Chlorella* is a ubiquitous microalga which can be found in fresh and saline environments. The cell wall of *Chlorella* sp. vary between species but the structural module is generally composed of fibrillar polysaccharides such as glucose, galactose and mannose and can contain glycoproteins (Loos and Meindl, 1982). The *Chorella* sp. strain used in this study is a marine species able to accumulate high quantities of neutral lipids (Olmstead et al., 2013). *Tetraselmis suecica* is an elliptical microalgae of the class Chlorophyceae ranging up to 12 μm in length. The cell wall is composed of coalesced rigid carbohydrate scales (Lee et al., 2013). *T. suecica* has a moderate propensity to accumulate lipids during the exponential and stationary phases and has a high biomass production rate (Montero, 2011).

In this paper the ease of rupture of industrially promising microalgae are compared to each other and to the well-studied and commonly processed *S. cerevisiae*. Within industry and microbiological literature, *S. cerevisiae* is recognized as a microorganism that is difficult to rupture (Hunter, 1988; Wenger, 2008). By comparing the rupturability of industrially promising microalgae to that of the well understood yeast, an appreciation of the degree of difficulty of rupturing each of the microorganisms can be realized. In addition, homogenization models are proposed which can be used in the optimization and economic prediction of the homogenization process, and form a part of the evaluation of the viability of each species for future work. In this work a range of quantification techniques are for the first time used to monitor the rupture of microalgae by the industrially relevant method of high pressure homogenization. The engineering and economic implications of these novel findings are discussed.

2. Methods

2.1. Microalgae

Suspensions of *Chlorella* sp., *T. suecica*, and *Nannochloropsis* sp. were grown in a marine medium with 3% artificial seawater mix and modified "f-medium" nutrients (Olmstead et al., 2013). Growth rates were monitored using optical density at 750 nm (OD_{750}) (Ahn et al., 2012) and all disruption experiments were performed during the exponential growth phase. Prior to experimentation, the biomass was transferred from the growth media to a saline solution at 3% w/w NaCl. Cells were concentrated by centrifugation (9300*g* for 10 min at 20 °C) and re-suspended to give an OD_{750} of *ca.* 1.0 (equivalent to approximately 0.25 mg/mL).

2.2. High pressure homogenization

Homogenization of algal suspensions was performed at pressures between 40 and 1500 bar with a bench-top high pressure homogenizer (GEA Panda 2K NS1001L with Re + valve; GEA Niro Soavi, Parma, Italy) as previously described (Spiden et al., 2013).

2.3. Analysis of cell disruption

All analyses of the samples were completed within a few hours of homogenization. Protein release was determined via the Bradford assay and cell counting, turbidity (optical density of sample at 750 nm) and UV absorbance (optical density of supernatant at 260 nm) were performed as previously described (Spiden et al., 2013). Observations were taken in duplicate. Particle size analysis was performed using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Malvern, Worcestershire, UK) on suspensions diluted to the required concentration with 30 g/L sodium chloride solution. A refractive index of 1.40 was used for yeast (Bryant et al., 1969) and 1.450 for the microalgae (Aas, 1996). Sodium metahexaphosphate was added to the suspension of *T. suecica* at 1% of solids weight (approx. 0.25 mg/L) prior to homogenization.

2.4. Data analysis

Cell counts and turbidity were normalized between the pre-homogenization value, x_i, and a minimum value (corresponding