Bioresource Technology 159 (2014) 36–40

Contents lists available at ScienceDirect

Bioresource Technology

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

Enhanced lipid extraction from algae using free nitrous acid pretreatment

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HIGHLIGHTS

- Free nitrous acid (FNA) pretreatment enhances lipid extraction yield from algae.
- FNA pretreatment boosts mass transfer coefficient of lipid extraction significantly.
- FNA at ppm level can cause algal cell envelope (wall and membrane) disruption.
- Cell disruption is not accompanied by lipid release.
- Algal cell envelope disruption contributes to lipid extraction by organic solvent.

ARTICLE INFO

Article history:
Received 1 November 2013
Received in revised form 29 January 2014
Accepted 31 January 2014
Available online 8 February 2014

Keywords:
Free nitrous acid
Lipid extraction
Algae
Biodiesel
Cell disruption

ABSTRACT

Lipid extraction has been identified as a major bottleneck for large-scale algal biodiesel production. In this work free nitrous acid (FNA) is presented as an effective and low cost pretreatment to enhance lipid recovery from algae. Two batch tests, with a range of FNA additions, were conducted to disrupt algal cells prior to lipid extraction by organic solvents. Total accessible lipid content was quantified by the Bligh and Dyer method, and was found to increase with pretreatment time (up to 48 h) and FNA concentration (up to 2.19 mg HNO_2–N/L). Hexane extraction was used to study industrially accessible lipids. The mass transfer coefficient (k) for lipid extraction using hexane from algae treated with 2.19 mg HNO_2–N/L FNA was found to be dramatically higher than for extraction from untreated algae. Consistent with extraction results, cell disruption analysis indicated the disruption of the cell membrane barrier.

1. Introduction

Microalgae have many advantages compared to other energy crops, including a high growth rate, high biomass production, and minimal arable land use (Amin, 2009). In addition, many algal species can grow in wastewater, brackish water or seawater (Buchanan et al., 2012; Chaumont, 1993; Pittman et al., 2011), thereby avoiding requirement for fresh water, which is a limited resource in many parts of the world. Algal lipids, commonly referred to as crude lipids or total fatty acids of algae, are extracted and then converted by transesterification to algal biodiesel (Chisti, 2007). Considering the whole algal biodiesel production pipeline, lipid extraction, along with the dewatering of the algal biomass, is a relatively energy-intensive process and a major bottle-neck for large-scale production (Chisti, 2007; Halim et al., 2012). This is partly due to the fact that algae possess a cell envelope (cell wall and cell membrane), which is a thick and rigid layer consisting of complex carbohydrates and glycoproteins with high mechanical strength and chemical resistance (Kim et al., 2013).

Solvent extraction is one of the most widely applied methods to recover lipids. Traditional chloroform-based lipid extraction is effective for the lab-scale exercises, but an alternative organic solvent, like hexane, which is easier to handle and more cost effective, is needed for industrial scale activities (Halim et al., 2012). A challenge for solvent extraction methods is the physical algal cell envelope, which prevents direct contact between lipid and solvent. Mechanical, chemical and biological cell disruption techniques are widely studied as a means to overcome this problem (Lee et al., 2010; Prabakaran and Ravindran, 2011). Mechanical methods such as microwave pretreatment have been proven to be effective, but require high energy input. Alternative chemical and biological cell disruption techniques are attracting escalating attention (Jin et al., 2012; Lee et al., 2010; Mendes-Pinto et al.,

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http://dx.doi.org/10.1016/j.biortech.2014.01.133
0960-8524/

2001). But despite the high cell-disruption performance, the potential to scale up these methods is still limited since they require continuous use of expensive chemicals and enzymes (Jin et al., 2012; Mendes-Pinto et al., 2001).

Recent studies showed that free nitrous acid (FNA), which is the protonated form of nitrite, has strong cellular destruction and enzyme interference effects on several microorganisms (Jiang et al., 2011). FNA has been regarded as a biochemical reagent. FNA and its derivatives such as the nitric oxide radical ($NO^{\cdot}$) and nitrous anhydride (N_2O_3) have an effect on protein and polysaccharides degradation (Dedon and Tannenbaum, 2004). Hence FNA has been applied in the water and wastewater industry for sludge treatment and biofilm control. However, there have been no reports on the effect of FNA on algal cells. Our hypothesis is that FNA pretreatment could help disrupt the algal cell envelope, thereby increasing the rate of lipid mass transfer from the algal cells into an organic solvent.

The aim of this study is to determine the efficacy of FNA, a green and renewable chemical that can be produced by nitritation from anaerobic digester liquor (Wang et al., 2013), for pretreatment of algae to increase lipid extraction yield and extraction efficiency. In this study, algal cells were disrupted using different concentrations of FNA prior to lipid extraction by organic solvents. A traditional lab-scale lipid extraction method, Bligh and Dyer method (Bligh and Dyer, 1959), was applied to assess the lipid extraction yields from disrupted cells. Furthermore, hexane extraction was applied to examine the lipid extraction efficiency. We also evaluated the effect of FNA on cell disruption by fluorescence staining, scanning electron microscopy (SEM) and quantification of the release of intracellular compounds.

2. Methods

2.1. Microalgae cultivation and harvest

Tetraselmis striata M8, which is a marine algae with high lipid accumulation ability, was cultured in 40 L airlift photobioreactors at the University of Queensland. F/2 medium was used as the growth media. Culture pH was kept constant at 8.5 ± 0.2 by CO_2 injection with an electronic controller, and the depletion of nutrients (NO_3^- and PO_4^{3-}) was tested using seawater aquaria nutrients kits (DAPI Aquarium Pharmaceuticals for NO_3^- and Nutrafin for PO_4^{3-}). Nitrogen starvation strategy was used to accumulate lipids in the algal cells. After 5 days cultivation for algae growth and 3 days for lipid accumulation, 90 L algal culture was concentrated to a paste by centrifugation in a Beckman Coulter, Allegra™ X-12 at 3750 rpm for 3 min in 800 ml batches. Centrifugation was conducted for a short time to avoid mechanical cell disruption.

2.2. FNA pretreatment

The work plan comprised two batches of four experiments. The experimental conditions are summarised in Table 1. Algal paste was re-suspended with de-ionized water and then the mixed liquor was evenly distributed between four beakers (reactor volume 500 ml) for FNA pretreatment.

Pre-determined amounts of sodium nitrite stock solution (30 g N/L) were added to the batch reactors in different volumes at the beginning of each experiment, which resulted in initial concentrations of nitrite varying between 0 and 1200 mg NO_2–N/L, as summarized in Table 1. Batch 1 was treated for 48 h and batch 2 was treated for 60 h. pH was kept approximately constant during the whole pretreatment at 5.0 ± 0.2 or 6.0 ± 0.2 (according to Table 1) by manually adding 0.5 M HCl. All reactors were well mixed by magnetic stirrers at a constant speed of 350 rpm. The concentration of FNA was varied by controlling the nitrite concentration under pH 5 or 6 as described. The FNA concentration was calculated using the following formula.

$$\mathrm{FNA(mg\ HNO_2 - N/L)} = S_{NO_2^- - N}/(K_a \times 10^{pH}) \quad (1)$$

where $S_{NO_2^- - N}$ is the dissolved NO_2^- concentration (mg N/L) and $K_a = e^{-2{,}300/(273.15+T)}$; temperature T (°C) for this study was 25 °C (Anthonisen et al., 1976).

In each batch test, mixed liquor samples were taken approximately every 12 h using a syringe and immediately filtered through disposable millipore filter units (0.22 μm pore size) for off-line analysis.

Standard microwave was applied as a control to untreated algal biomass using a microwave oven at a high temperature (about 100 °C and 2450 MHz) for 5 min.

2.3. Evaluation of cell disruption

2.3.1. Cell membrane damage assay

Microscopic observation and cell membrane damage assays were performed before and after pretreatment. Untreated algae and algae samples from each reactor after 48 h pretreatment were diluted 10 times before staining. The SYTOX Green fluorescent probe (Invitrogen, Ltd., UK) was supplied as a 5 mM stock solution in DMSO. 0.5 μl of this stock solution was added to 0.5 ml cell suspension giving a final dye concentration of 5 μM, and the mixture was incubated for 5 min at room temperature in the dark. Algal cells in 24 random fields were counted under a fluorescence microscope (BX61, Olympus, Tokyo, Japan) equipped with a double band pass filter set at 473–498 and 548–573 nm for excitation, and at 515–535 and 590–620 nm for emission. Damaged cell to intact cell ratio was then calculated. Images were obtained and post-adjusted with DPC controller 1.2.1 (Olympus optical co. LTD.) and iTEM 5.0 (Olympus Soft Imagining Solutions, Olympus optical co. LTD.) respectively.

2.3.2. Scanning electron microscopy (SEM) observation

SEM analysis was performed as described by Naveena et al. (2005). Briefly, cells were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), dehydrated in ethanol (70%, 90% and absolute), and dried with liquid CO_2. The dried samples were coated with layers of platinum using a SPI module sputter coater. A Phillips XL-30 SEM was used to observe the surface morphology of the algal cells. The SEM images were taken at an acceleration voltage of 10 kV.

2.3.3. Physicochemical characterisation

Total suspended solids (TSS), volatile suspended solids (VSS), total chemical oxygen demand (TCOD) and soluble chemical oxygen demand (SCOD) were measured according to standard methods (American Public Health Association, 2005). TCOD and SCOD were measured using Spectroquant® photometric cell tests (114541 and 114555, Merk, Germany), a Thermoreactor TR 300 (Merck, Germany) and a UV–visible spectrophotometer (Varian Cary®50, Varian, Inc., Australia). NH_4^+-N, NO_3^--N, NO_2^--N, PO_4^{3-}-P, total Kjeldahl phosphorous (TKP), total Kjeldahl nitrogen (TKN) and total soluble Kjeldahl nitrogen (STKN) were measured with a Lachat QuikChem 8000 Flow Injection Analyser (Lachat Instrument, Milwau-kee, USA). The total protein, polysaccharide and lipid content external to the cells and cell debris was quantified by analysing supernatant of centrifuged samples (5000 rpm for 3 min). The protein concentration was measured by the BCA method with BSA as standard (Smith et al., 1985). The polysaccharide concentration was determined by the anthrone method with glucose as standard (Raunkjær et al., 1994). Lipid content of supernatant was measured by an InfraCal TOG/TPH Analyser (Wilks Enterprise, Inc., USA).