Algal Research 15 (2016) 100–109

Contents lists available at ScienceDirect

Algal Research

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

Optimization of oil extraction from *Nannochloropsis salina* biomass paste

Tawan Chatsungnoen, Yusuf Chisti *

School of Engineering, Massey University, Private Bag 11 222, Palmerston North, New Zealand

ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form 31 January 2016
Accepted 10 February 2016
Available online xxxx

Keywords:
Microalgae
Nannochloropsis salina
Oil extraction
Biodiesel
Biofuels

ABSTRACT

A 1-step extraction process is reported for near quantitative (>96%) extraction of the total lipids from the biomass paste (~72% moisture by weight) of the marine microalga *Nannochloropsis salina*. The composition of the solvent mixture of chloroform, methanol and water is first optimized for a near quantitative extraction of the lipids. The optimal solvent mixture is found to be chloroform, methanol and water in the volume ratio of 5.7:3:1. This solvent composition is then used in a 1-step extraction process to optimize the extraction time, temperature and the volume of the solvent mixture required for extraction from 1 g dry equivalent of the biomass paste. The optimal extraction conditions are found to be 25 °C in a 2-h extraction process using 33 mL of the solvent mixture. Compared to the commonly used Bligh and Dyer extraction protocol, the proposed 1-step extraction reduced the mixed solvent use by ~48% and the extraction time by ~78%.

1. Introduction

Oils or lipids sourced from microalgae are potential feedstocks for making biodiesel and other fuels [7,8]. Simple, effective, scalable and low-cost methods are needed for extraction of algal oils from the biomass paste for possible use as fuels [9,25]. This work is focused on oil recovery by solvent extraction of the biomass paste of the marine microalga *Nannochloropsis salina*, a good producer of lipids [5,13,17,22,26].

Although the extraction of lipids from dry algal biomass is relatively easy [3], it requires the biomass to be dried in an energy-intensive and expensive step. Drying is not feasible for producing a cheap fuel oil [9,11,23]. Furthermore, drying adversely impacts the net energy recovery in the algal oil [9] and, therefore, there is no real alternative to direct extraction from the wet biomass paste. The paste biomass typically obtained by filtration or centrifugation of an algal suspension contains more than 70% water by weight.

One of the most common methods of solvent extraction of oils from algal biomass is a modification of the Bligh and Dyer [4] procedure originally developed for extraction of lipids from fish tissue. This method uses a solvent system of chloroform, methanol and water to extract the biomass in 3-steps. The Bligh and Dyer [4] method as used with the microalgal biomass [2,5,16,20,21] affords a near quantitative recovery of the total lipids from the biomass. In laboratory studies, dry algal biomass is generally used for the Bligh and Dyer [4] extraction.

The Bligh and Dyer [4] method and other similar methods (e.g. Folch et al. [14]) for quantitative extraction of the total lipids use a mixture of solvents of different polarities for the extraction. Nonpolar solvents such as hexane and diethyl ether are effective for extracting only the nonpolar lipids from dry biomass. These solvents do not effectively extract many of the polar lipids and they do not readily penetrate the water filled algal cells and other water-rich tissue. Quantitative extraction of total lipids therefore generally requires a mixture of solvents of different polarities. The Bligh and Dyer [4] solvent system has been shown to be highly effective for quantitative extraction of the total lipids. The Bligh and Dyer [4] extraction uses a specific composition of the solvent mixture in multiple extraction steps, but the solvent composition has not been optimized for extraction of algal biomass. Similarly, the conditions of the extraction such as the temperature, the duration and the ratio of the solvent mixture to the mass of the cells being extracted have not been optimized. The present work reports on optimization of the composition of the Bligh and Dyer [4] solvent system and the extraction conditions for a one-step extraction of the total lipids from the biomass paste of the microalga *N. salina*. The optimized method is shown to be superior to the conventional Bligh and Dyer [4] extraction.

An earlier developed 1-step extraction of algal biomass paste with chloroform and methanol (2:1 by vol) was effective, but required nearly 9-times the solvent volume [2] relative to the Bligh and Dyer [4] method. Reducing the solvent volume relative to the amount of biomass being extracted is essential for any large-scale extraction operation.

The efficacy of solvent extraction of intracellular lipids is known to be improved by pretreating the biomass in various ways [10,11,15,19,20,24,28]. Pretreatment methods include mechanical disruption of cells, thermal treatments, the use of lytic chemicals and irradiation with microwaves and ultrasound. All such pretreatments and cotreatments [2,20,24] are expensive. Furthermore, they are energy intensive to the point of being impractical for extraction of oils for fuel applications. A simple extraction with cheap organic solvents without any pretreatment or parallel treatment is apparently the least expensive and most energy

* Corresponding author at: Yusuf Chisti, School of Engineering, Massey University, Private Bag 11 222, Palmerston North, New Zealand.
E-mail address: Y.Chisti@massey.ac.nz (Y. Chisti).

http://dx.doi.org/10.1016/j.algal.2016.02.015
2211-9264/

efficient option for oil recovery. Low-boiling organic solvents are readily recycled for reuse. The use of unconventional extraction solvents such as ionic liquids [24] and supercritical carbon dioxide [15,24] is prohibitively expensive.

A previous supposedly optimal method for extraction of lipids from *Chlorella* sp. and *Scenedesmus* sp. required a pretreatment of the biomass paste with acids and alkalis and a subsequent extraction with hexane at 75 °C [28]. Only about 77% of the transesterifiable lipids could be recovered [28]. The solvent demand was enormous at 60 mL per g (dry basis) of the biomass [28]. Here we develop an optimized extraction protocol using the cheap solvent system of chloroform, methanol and water, for a one-step quantitative extraction of total lipids of the alga *N. salina* at room temperature. No pretreatments or cotreatments are used.

2. Materials and methods

2.1. The microalga and culture conditions

The marine microalgae *N. salina* (CCAP849/3) was purchased from the Culture Collection of Algae and Protozoa (CCAP), Argyll, United Kingdom.

The alga was always maintained and grown in the BG11 medium made with artificial seawater [1]. To prepare the artificial seawater, 40 g of sea salt (natural unrefined Southern Pacific Ocean salt; Pacific Natural Fine Salt; Dominion Salt Ltd., Marlborough, New Zealand) was dissolved in 1 L of distilled water. This solution was filtered using a Whatman GF-C (0.45 μm) 90 mm microfiber filter. The filtrate was the artificial seawater. It had a salinity of approximately 38.5 parts per thousand (ppt) (EcoSense® EC300 conductivity/salinity meter; YSI Inc., Yellow Springs, OH, USA).

The alga was always grown aseptically. The inoculum was prepared by transferring a stock from a liquid or solid (agar) medium to 40 mL of BG11 in a 250 mL Erlenmeyer flask. The flask was held in an incubator shaker (130–140 rpm, 25 °C), under fluorescent light (~30 μmol photons m^{-2} s^{-1}), for 20–30 days. This culture (40 mL) was used to inoculate 360 mL of the fresh medium in a 1 L Duran bottle (borosilicate glass 3.3, LabServ, Biolab, Auckland, New Zealand). This culture was incubated at room temperature (~25 °C) for 7–14 days. This preculture (400 mL) was inoculated into 1.6 L of the fresh medium in a 2 L Duran bottle. The culture was grown for around 7–14 days.

This inoculum was split into 400 mL lots and transferred to 1.6 L of the fresh medium in five 2 L Duran bottles. The Duran bottle cultures were maintained at the above specified temperature under continuous illumination (~219 μmol photons m^{-2} s^{-1}). A bank of six fluorescent tubes (Philips TLD 58w/840, cool white, Thailand) were used for illumination. All Duran bottles were continuously bubbled (0.375 L min^{-1}) with humidified air mixed with carbon dioxide (5% vol/vol). The inlet and the exhaust gases were sterile filtered (0.2 μm Teflon membrane filter, Midisart® 2000; Sartorius AG, Goettingen, Germany). The culture was harvested in the stationary phase (day 52 postinoculation) and kept at 4 °C in the dark. The broth was used within 7-days of harvest.

2.2. Measurements

2.2.1. Microalgal biomass concentration and growth parameters

A 20 mL sample of the algal broth was vacuum filtered using a preweighed Whatman GF-C (0.45 μm, 90 mm) microfiber disc filter. The cells were washed with 2 × 20 mL of 0.5 M ammonium formate. The filtered biomass samples were dried at 80 °C overnight, cooled in a desiccator and weighed. This data was used to calculate the dry biomass concentration.

A sample of the algal broth with a precisely known dry biomass concentration was serially diluted with the fresh medium and the optical density (A_{680}) was measured at 680 nm using a spectrophotometer [6]. The following linear correlation was established between the measured optical density and the dry biomass concentration of the diluted samples:

$$C_b = \frac{A_{680} \times D}{S}. \quad (1)$$

In Eq. (1), C_b (g/L) is the dry biomass concentration, D is the dilution factor and S (= 5.6773) is the slope of the calibration line. Subsequently, the dry biomass concentration of an appropriately diluted unknown sample was determined from the measured optical density using the dilution factor and the equation of the calibration curve.

The measured cell concentrations were used to calculate the biomass productivity (P_b, g L^{-1} d^{-1}) as follows:

$$P_b = \frac{C_f - C_i}{t} \quad (2)$$

where C_f (g L^{-1}) is the final biomass concentration, C_i (g L^{-1}) is the initial biomass concentration, and t (d) is the time required for growth.

The specific growth rate (μ, d^{-1}) was calculated from the slope of the linear regression line of the semilog plot of the cell concentration versus time during the exponential growth phase [31]; thus,

$$\mu = \frac{\ln(C_2/C_1)}{t_2 - t_1} \quad (3)$$

where C_1 and C_2 are the biomass concentrations (g L^{-1}) at times t_1 and t_2, respectively, within the exponential growth phase.

2.2.2. Total lipids

The total lipids in the biomass were measured using a modification of the Bligh and Dyer [4] method. Microalgal cells were harvested by centrifuging (8370 ×*g*, 4 °C, 10 min) and washed three times with 0.5 M ammonium formate. Each wash volume was the same as the volume of the broth sample used in recovering the biomass. The washed cells were freeze dried (Laboratory Freeze Dryer, CRYODOS-80, Telstar Industrial, S.L., Terrassa, Spain) and then pulverized in a grinder (Breville Model CG2B, China). Total lipids were extracted from 1 g of lyophilized biomass with a solvent mixture of 5 mL of chloroform, 10 mL of methanol and 4 mL of water.

This slurry of the solvents and the cells was homogenized for 3 min and stirred for a further 4 h at 760 rpm with a magnetic stirrer (IKA® KMO 2 Basic IKAMAG™, IKA® Werke GmbH Co. KG, Germany) at room temperature. Chloroform (5 mL) was then added and the mixture was blended for 30 s. Distilled water (5 mL) was added and blending was continued for a further 30 s. The suspension was centrifuged (4000 ×*g*, 4 °C, 10 min) and allowed to separate into three layers. The top methanol/water layer was discarded. The chloroform layer (the bottom layer) was collected. The middle layer was the residual biomass. This was extracted again, as specified above for the fresh biomass and the chloroform layer was recovered. The residual biomass was then extracted a third time by contacting with 5 mL of chloroform for 1 h. The three chloroform extracts were combined. The volume of the combined extract was measured in a graduated cylinder. The total lipids in the extract were determined gravimetrically by evaporating an aliquot of the extract in a preweighed aluminium dish for 12 h (room temperature in the fume hood) and further drying in a desiccator (12 h, room temperature). Using the measured volume of the pooled chloroform, the total lipid concentration in the extract, and the amount of dry biomass used in extraction, the total lipid content was calculated as a weight percent of dry biomass as follows:

$$f\ (\%, \mathrm{w/w}) = \frac{VC_L}{B} \times 100 \quad (4)$$