Algal Research 6 (2014) 86–90

Contents lists available at ScienceDirect

Algal Research

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

The effects of microalgal cell disruption via $FeCl_3$-based synergistic effect between Fenton-like and Lewis acid reaction for lipid extraction

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ARTICLE INFO

Article history:
Received 19 April 2014
Received in revised form 17 August 2014
Accepted 30 August 2014
Available online xxxx

Keywords:
Oil extraction
Fenton-like reaction
Lewis acid
Biodiesel production
Cell disruption
Coagulation

ABSTRACT

In this study, crude oil was extracted from *Nannochloropsis salina* for biodiesel production using $FeCl_3$ as a catalyst. Scanning electron microscopy (SEM) analysis revealed that microalgae cells became wrinkled or burst after reaction. An optimal extraction condition, via the response surface methodology (RSM), was evaluated with respect to $FeCl_3$ concentration (1–3 mM), reaction time (30–90 min) and temperature (60–100 °C). At a condition of $FeCl_3$ of 2 mM, 90 min, and 87 °C, a maximum extraction yield of 213 mg/g biomass and a FAME conversion rate of more than 80% were achieved. In light of $FeCl_3$ being a reference coagulant, even for microalgae harvesting, its use for lipid extraction is expected to conceivably reduce the overall cost of microalgae-derived biodiesel production.

1. Introduction

The idea of using microalgae biomass as a feedstock of biofuel is not new, but has attracted intense public attention lately. The high and unstable price of petroleum is one reason for this interest, and greater still is an emerging concern about global warming raised by increased carbon dioxide emissions [1–3]. In addition to the proven carbon neutrality and potential economic viability, microalgae have advantages of being not a major food source and also growing exceedingly fast [4,5]. In spite of all this, the production, especially to a commercial level, of the microalgae-derived fuel (e.g., biodiesel) has a number of issues that are not easily solvable; this is true over the entire production process. Oil extraction is known as a cost-determining step [6,7], partly because it requires harsh treatment for cell disruption for efficient lipid recovery. This pretreatment can be broadly categorized into two types: 1) mechanical disintegration such as expression/expeller press [8,9] and ultrasonication [10,11], and 2) chemical means such as hexane solvent [12], soxhlet [13,14], and supercritical fluid extraction [15,16]. However, each of these preexisting methods, though effective, has its own critical drawbacks of requiring massive energy consumption (mechanical disruption), long operating time (hexane solvent extraction), high amount of chemicals consumed and the associated safety issues (soxhlet extraction), and energy-intensive equipment (supercritical extraction). The Fenton (or collectively Fenton-like) reaction that makes use of one of the strongest oxidant hydroxyl radical may offer an alternative route [17–20]. The hydroxyl radical is potent enough to disrupt the microalgal cell and make it apt for the subsequent oil extraction. In view of its potency, the Fenton-based method is anticipated to be more efficient than those with acid catalysts like H_2SO_4 and HCl. Its inherent caveat of potentially high cost can be adequately dealt with by way of integrating several steps, either with pre- or post-processes [21,22]. Moreover, there is a possibility that used ferric ion (Fe^{3+}) is regenerated after cell disruption via precipitation or simple pH control. In the present study, therefore, oil extraction from oleaginous microalgae biomass based on the Fenton-like reaction was systematically investigated by means response surface methodology (RSM).

2. Methods

2.1. Microalgae and culture conditions

A marine microalgae species *Nannochloropsis salina*, which has average diameter of 2.4 ± 0.5 μm, was locally isolated and cultured in a nutrient medium (constituents: KNO_3, 3 mM; KH_2PO_4, 5.44 mM; Na_2HPO_4, 1.83 mM; $MgSO_4 \cdot 7H_2O$, 0.20 mM; $CaCl_2$, 0.12 mM; FeNaEDTA, 0.03 mM; $ZnSO_4 \cdot 7H_2O$, 0.01 mM; $MnCl_2 \cdot 4H_2O$, 0.07 mM; CuSO4, 0.07 mM; $Al_2(SO_4)3 \cdot 18H_2O$, 0.01 mM) adjusted to a pH of 6.5. It was then cultivated at 25 °C in an open pond with 10 tons of working volume. Mature algal solution reaching the stationary phase was then kept at a constant room temperature. The open pond was supplied

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http://dx.doi.org/10.1016/j.algal.2014.08.009
2211-9264/

with 10% (v/v) CO_2 in air at a rate of 0.75 L min. The lipid compositions and contents of the *N. salina* are summarized in Table 1.

2.2. Preparation of microalgae sample and cell disruption by Fenton-like reaction and Lewis acid reaction

A dried microalgae sample was prepared by ultrafiltration, centrifugation (1500 ×*g*, 10 min), and freeze-drying for 4 days, or longer at −80 °C and stored at −20 °C. Firstly, lipid was extracted from 10 mg of cells, then converted to fatty acid methyl esters using a modified transesterification method, and the result was assumed to be the intrinsic lipid content accumulated in the biomass and used as a reference throughout [23]. The concentration of the microalgae solution used was adjusted to 20 g/L using the freeze dried cells with deionized water, and 50 mL was used for each experiment. Cell disruption was carried out in a stainless steel reactor (Φ26 mm × 132 mm with 70 mL of volume) coated with Teflon on the inside. For cell disruption, pH was first adjusted to 3.0 using sulfuric acid, and subsequently $FeCl_3$ (1 mM to 3 mM) and H_2O_2 (1%, 290 mM) were both injected. As a preliminary test, reaction temperature was varied from 20 °C to 100 °C at the consistent condition of $FeCl_3$ concentration and reaction time.

2.3. Lipid (oil) extraction

Crude oil was extracted from 1 g of the damaged cells by means of liquid–liquid extraction adding 40 mL hexane for 2 h at 750 rpm (Wisestir MSH-5DA, Witeg, Germany) [21]. Oil-containing hexane was evaporated using a vacuum evaporator (EZ2 PLUS, Genevac, UK), and the oil was recovered; the weight of the remains was used to estimate oil-extraction yield. The experiments were performed in triplicate and then averaged. In order to confirm degradation of microalgae cell wall, the morphologies of microalgae before and after cell disruption were inspected under optical microscopy (Leica, model DM2500). Also, the surface morphologies of sample after lipid extraction were revealed using a field emission scanning electron microscope (Fe-SEM, SIRION-100, FEI, USA).

2.4. Experimental design

Response surface methodology (RSM) was taken to obtain an optimal condition of the Fenton-like method and to investigate the effect of critical variables including $FeCl_3$ concentration (A), reaction time (B), and temperature (C) on extraction yield (Y). The adopted reaction conditions were as follows: $FeCl_3$ concentrations of 1–3 mM, reaction times of 30–90 min, and temperatures of 60–100 °C, at a microalgae concentration of 20 g/L. A total of 15 experimental runs of the three variables were designed by Box–Behnken design (Box and Behnken, 1960) using the Design-Expert software (Version 8.0, Stat-Ease, Inc., USA).

Table 1
Fatty acid composition of *Nannochloropsis salina*.

Fatty acid	*Carbon length*	*Content (mg/g)*
Laurate	C12:0	0.56 ± 0.03
Myristate	C14:0	12.38 ± 0.25
Palmitate	C16:0	63.55 ± 1.22
Palmitoleate	C16:1	78.44 ± 1.68
Oleate	C18:1	22.44 ± 0.60
Linoleate	C18:2	5.34 ± 0.15
Arachidonate	C20:4	15.90 ± 0.44
Behenate	C20:5n3	44.25 ± 1.18
Others		29.93 ± 1.12
Total		272.78 ± 6.42
Fame (%)		27.28 ± 0.10

2.5. Fatty acid content analysis

Fatty acid content was analyzed by using a modified transesterification method [23]. Around 10 mg of extracted crude oil was put inside a 10 mL Pyrex glass tube sealed by Teflon-covered screw-caps. Lipid extraction reagent [chloroform/methanol, 2/1 (v/v); 2 mL] was added to the tube. This mixture was then vortex-mixed (Vortex Genius 3; Ika, Italy) for 10 min at room temperature. Then, 1 mL of chloroform (including heptadecanoic acid as an internal standard), 1 mL of methanol, and 300 μL of H_2SO_4 (0.0015 N) were sequentially added and vortex-mixed for 5 min. The tube was reacted in a 100 °C water bath for 10 min, after which it was cooled to room temperature, supplemented with 1 mL of deionized water, and intensely mixed for 5 min. Subsequently, the mixture was centrifugally layer-separated at 4800 ×*g* and 4 °C for 10 min. The lower layer (organic phase) was extracted using a disposable plastic syringe (Norm-ject, Henke Sass, Wolf GmBH, Germany) and filtered with a disposable 0.22 mm PVDF syringe filter (Millex-GV; Millipore, USA). Methyl esters of fatty acids were analyzed using a gas chromatograph equipped with a flame ionization detector and a 0.32 mm (ID) 630 m HP-INNOWax capillary column (Agilent Technologies, USA). The GC oven was kept at 140 °C and helium, as a carrier gas, was injected at 2.2 mL/min. Temperatures of the injector and detector were set at 250 °C and 275 °C, respectively. Mix RM3, Mix RM5, GLC50, GLC70, heptadecanoic acid, and c-linolenic acid were used as the standards [21]. The other reagents used were of analytical grade. The fatty acid content of the extracted oil was analyzed following the modified direct transesterification method described above. The experiments were performed and then averaged.

3. Results and discussion

3.1. Lipid extraction

The lipid content of *N. salina* measured by a modified transesterification method was about 27.3% (Table 1), and based on this value, extraction yields after cell disruption were evaluated and compared with each other. As a preliminary test, dried algae samples were suspended to an equal concentration of 20 g/L, and lipids in the cell body were extracted by the Fenton-like reaction at conditions of predetermined $FeCl_3$ and H_2O_2 concentrations for 1 h at varied temperatures (Table 2). An increase in temperature led to better extraction, even without H_2O_2. Since $FeCl_3$ is a strong catalyst on the oxidation process and in fact is known to be able to attack cellulose [24], which is a main composition of the microalgal cell wall, it may play a role in the hydrolysis of the microalgal cell. Curiously, the highest extraction yield of 19.3% was achieved with H_2O_2 at 80 °C, rather than 100 °C. It is possible that above a certain temperature the combination of acid and hydroxyl radicals is overly powerful to damage even relatively inert lipids. It was clearly seen that the microalgae cells were flocculated by $FeCl_3$ via forming algae flocs (Fig. 1); the harvested cells, though somewhat vaguely, appeared to have wrinkled and even disrupted surfaces after extraction (Fig. 2).

Table 2
The effect of $FeCl_3$ concentration, reaction time, temperature and H_2O_2 use on extraction yield.

Experiment	Parameter				
	Fe (mM)	Time (minute)	Temp (°C)	H_2O_2 (1%)	Extraction (%)
1	2 mM	60	100	0	9.4 ± 0.3
2	2 mM	60	20	1	4.2 ± 0.1
3	2 mM	60	40	1	4.2 ± 0.2
4	2 mM	60	60	1	7.5 ± 0.2
5	2 mM	60	80	1	19.3 ± 0.2
6	2 mM	60	100	1	17.4 ± 0.1