Bioresource Technology 191 (2015) 469–474

Contents lists available at ScienceDirect

Bioresource Technology

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

An integrated process for microalgae harvesting and cell disruption by the use of ferric ions

Dong-Yeon Kim [a], You-Kwan Oh [b], Ji-Yeon Park [b], Bohwa Kim [b], Sun-A. Choi [b], Jong-In Han [a,*]

[a] Department of Civil and Environmental Engineering, KAIST, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea
[b] Clean Fuel Department, Korea Institute of Energy Research (KIER), 152 Gajeong-ro, Yuseong-gu, Daejeon 305-343, Republic of Korea

HIGHLIGHTS

- Harvesting and cell disruption were simultaneously conducted using ferric ion.
- Ferric sulfate showed lower performance for both processes than ferric chloride.
- Optimal extraction conditions were identified via response surface methodology.

ARTICLE INFO

Article history:
Received 8 January 2015
Received in revised form 2 March 2015
Accepted 4 March 2015
Available online 12 March 2015

Keywords:
Harvesting
Oil extraction
Fenton-like reaction
Cell disruption
Coagulation

ABSTRACT

In this study, a simultaneous process of harvesting biomass and extracting crude bio-oil was attempted from wet microalgae biomass using $FeCl_3$ and $Fe_2(SO_4)_3$ as both coagulant and cell-disrupting agent. A culture solution of *Chlorella* sp. KR-1 was firstly concentrated to 20 g/L and then proceeded for cell disruption with the addition of H_2O_2. Optimal dosage were 560 and 1060 mg/L for $FeCl_3$ and $Fe_2(SO_4)_3$, showing harvesting efficiencies of more than 99%. Optimal extraction conditions were identified via the response surface method (RSM), and the extraction yield was almost the same at 120 °C for both iron salts but FAME compositions after transesterification was found to be quite different. Given iron salts were a reference coagulant in water treatment in general and microalgae harvesting in particular, the present approach of using it for harvesting and oil-extraction in a simultaneous manner can serve as a practical route for the microalgae-derived biodiesel production.

1. Introduction

The typical final cell concentration from mass-cultivation facilities such as racing open-pond is too low to be used for a subsequent step of oil extraction; therefore biomass concentration, namely harvesting, is rather of absolute necessity. Unfortunately, however, harvesting is one of the most energy-intensive steps, consuming around 20% of total biodiesel production cost (Wei et al., 2014). There are a good many harvesting methods available at the moment, such as centrifugation (Schenk et al., 2008), various filtration methods including membrane modification in micro-, ultra-filtration(Hwang et al., 2013; Kim et al., 2014a), and forward osmosis filtration (Buckwalter et al., 2013), flotation (Kwon et al., 2014), electro-floatation (Kim et al., 2012), coagulation/flocculation (Ahmad et al., 2011; de Godos et al., 2011), and simple pH control (Nguyen et al., 2014). Even though each has its own advantages, the practical application has been limited due to its own inherent issues like energy consumption and scaling-up. Lipid extraction is an equally heavy burden to the industrial-scaled production of algal biodiesel: one obvious and critical issue is the prior requirement of biomass drying (Halim et al., 2012). Though laboratory-scale protocols of lipid extraction are well established and routinely practiced for the purpose of lipid analysis, no technology is being implemented at an industrial level (Cheng et al., 2014). Direct extraction of oil from wet biomass omitting a drying step might be a promising or even necessary route of improving overall efficiency in terms of both time and cost (Yoo et al., 2014). In addition, it would become even more competitive if harvesting and oil extraction processes can share energy consumption or chemical use. In the previous study, we developed a new method for cell disruption based on $FeCl_3$ as catalyst and applied it to oil extraction, and achieved noticeable results of high extraction yield and good lipid quality (Kim et al., 2014b). In the present study, an attempt was made to integrate the harvesting step with the cell disruption step, especially in a way that $FeCl_3$ (and also

* Corresponding author. Tel.: +82 42 350 3629; fax: +82 42 350 3610.
E-mail address: jihan@kaist.ac.kr (J.-I. Han).

http://dx.doi.org/10.1016/j.biortech.2015.03.020
0960-8524/

$Fe_2(SO_4)_3$) was used both as a coagulant in harvesting step and as a catalyst for cell disruption in oil extraction step. This combined and simplified process was anticipated to improve the economic feasibility of the algal biodiesel production, by way of consuming less chemicals and energy.

2. Methods

2.1. Microalgae and culture conditions

Chlorella sp. KR-1 (Sung et al., 1998), a freshwater microalgae species, was cultured in a nutrient medium (constituents: KNO_3, 3 mM; KH_2PO4, 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; $CuSO_4$, 0.07 mM; $Al_2(SO_4)_3{\cdot}18H_2O$, 0.01 mM) adjusted to a pH of 6.5. *Chlorella* sp. KR-1 was then cultivated at 30 °C in a Pyrex bubble-column reactor (working volume: 6 L) equipped with 12 fluorescent lamps at the front and right/left sides (light intensity: 80 μmol/m^2/s) and kept in a constant room temperature. The reactor was supplied with 10% (v/v) CO_2 in air at a rate of 0.75 L/min. The lipid compositions and contents of the microalgae species are summarized inTable 1. Dried cells were prepared by centrifugation (4000 rpm, 10 min), washed with deionized water (three times), freeze-dried (FD5512, IlShin BioBase Co., Korea) for 4 days or longer, and finally stored at −20 °C prior to an analysis of their fatty acid contents.

2.2. Harvesting and lipid extraction

Microalgae culture solutions used for harvesting experiments had a concentration of around 1.7 g/L, and harvesting was carried out by coagulation using iron-based coagulants. Harvesting process was initiated by adding the coagulant with concentrations ranged from 0 to 560 mg/L for $FeCl_3$ and 0–1200 mg/L for $Fe_2(SO_4)_3$. H_2O_2 was then injected to induce a Fenton-like reaction, which is expected to occur together with iron ions coming from the coagulants; in addition to this potent oxidation, a Lewis acid-based reaction, a reaction that is inherently milder and attributed to the nature of the iron-based coagulants, may also take place. For quantification, crude oil was extracted from the treated cells by means of liquid–liquid extraction using hexane for 2 h at 750 rpm. Oil-containing hexane was evaporated using a vacuum evaporator (EZ2 PLUS, Genevac, UK), and the oil was recovered; the weight of remains was used to estimate lipid-extraction yield. Total fatty acid contents converted from freeze-drying cells of 10 mg was assumed to be the intrinsic lipid content accumulated in the biomass and used as a reference throughout (Cho et al., 2011). Experiments were performed in triplicate and then averaged.

Table 1
Fatty acid composition of *Chlorella* sp. KR-1.

(Unit: FAME/cell (mg/g)) *Chlrollela* sp. KR-1			
Myristate	C14:0	0.38	0.40
Pentadecanoate	C15:0	0.00	0.00
Palmitate	C16:0	76.33	79.86
Palmitoleate	C16:1	0.84	0.90
Stearate	C18:0	25.22	26.45
Oleate	C18:1n9c	66.10	68.38
Linoleate	C18:2n6c	69.63	71.91
Gamma-linoleate	C18:3n6	0.91	0.00
Linoleate	C18:3n3c	16.99	17.41
Others		38.48	40.82
	Total	294.87	306.13
	Fame (%)	30.05	

2.3. Experimental design

Response surface methodology (RSM) was taken to obtain an optimal condition of the cell disruption method and to investigate the effect of critical variables including H_2O_2 concentration (A), reaction time (B), and temperature (C) on $FeCl_3$-based extraction yield (Y_1) and $Fe_2(SO_4)_3$-based extraction yield (Y_2). The adopted reaction conditions were as follows: H_2O_2 concentrations of 1–3%, reaction times of 30–90 min and temperatures of 80–120 °C, at the microalgae concentration of 20 g/L. A total of 17 experimental runs of the three variables were designed by Box–Behnken design using the Design-Expert software (Version 8.0, Stat-Ease, Inc., USA).

2.4. Fatty acids content analysis

Fatty acids content was analyzed using a modified transesterification method (Cho et al., 2011). Around 10 mL of extracted crude oil was put inside a 10 mL Pyrex glass tube sealed by a Teflon-covered screw-cap. Lipid extraction reagent [chloroform/methanol, 2/1 (v/v); 2 mL] was added to the tube. The tube was 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 mL of H_2SO_4 were sequentially added to the tube, which was 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 4000 rpm 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) 6 30 m HP-INNOWax capillary column (Agilent Technologies, USA). Helium, as a carrier gas, was injected at 2.2 mL/min. The temperatures of the injector and detector were set at 250 and 275 °C, respectively. Mix RM3, Mix RM5, GLC50, GLC70, heptadecanoic acid, and c-linolenic acid were used as internal standards. All other reagents used were of analytical grade. The other reagents used were of analytical grade. The fatty acids 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. Harvesting microalgae using iron salts

A microalgal culture solution, which was prepared in a photobioreactor and had an initial concentration of 1.7 g/L, was used for coagulation-based harvesting experiments: using either $FeCl_3$ or $Fe_2(SO_4)_3$, the final concentration was reached to approximately 20 g/L. Optimal doses were found to be 560 mg/L for $FeCl_3$ and 960 mg/L for $Fe_2(SO_4)_3$ (Fig. 1). Each of resulting solutions consistently had a pure upper layer without both coagulant and algal particles. After harvesting, pH values of solutions decreased from initial 6.43–3.13 with $FeCl_3$ and 3.04 with $Fe_2(SO_4)_3$. Coagulation, which is generally described as charge neutralization and sweeping floc is bound to be dependent upon colloid concentration and alkalinity level (Şirin et al., 2011). It was therefore natural that our cell suspension with high levels of biomass and alkalinity consumes relatively high doses of coagulant to be harvested. The decreased pHs were caused by the ferric coagulants, as they are