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Experimental study and thermodynamic modeling for determining the effect of non-polar solvent (hexane)/polar solvent (methanol) ratio and moisture content on the lipid extraction efficiency from *Chlorella vulgaris*

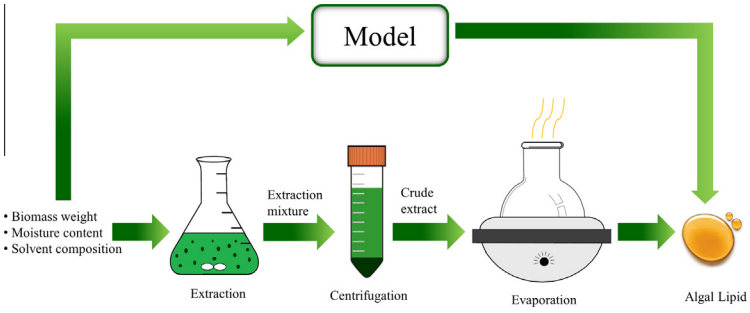
Mohammad Malekzadeh [a], Hamed Abedini Najafabadi [a], Maziar Hakim [a], Mehrzad Feilizadeh [a], Manouchehr Vossoughi [a,b,*], Davood Rashtchian [a]

[a] Department of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, Iran
[b] Institute for Biotechnology and Environment (IBE), Sharif University of Technology, Tehran, Iran

HIGHLIGHTS

- Lipid recovery was decreased for wet biomass when solvent composed mostly of hexane.
- Fatty acid extraction performance was decreased at the presence of water.
- Hexane/methanol (1:1 v/v) is the most efficient solvent mixture for lipid extraction.
- Extraction performance was improved by increase of the temperature.
- The UNIQUAC model can accurately estimate the fatty acid recovery.

GRAPHICAL ABSTRACT



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ABSTRACT

In this research, organic solvent composed of hexane and methanol was used for lipid extraction from dry and wet biomass of *Chlorella vulgaris*. The results indicated that lipid and fatty acid extraction yield was decreased by increasing the moisture content of biomass. However, the maximum extraction efficiency was attained by applying equivolume mixture of hexane and methanol for both dry and wet biomass. Thermodynamic modeling was employed to estimate the effect of hexane/methanol ratio and moisture content on fatty acid extraction yield. Hansen solubility parameter was used in adjusting the interaction parameters of the model, which led to decrease the number of tuning parameters from 6 to 2. The results indicated that the model can accurately estimate the fatty acid recovery with average absolute deviation percentage (AAD%) of 13.90% and 15.00% for the two cases of using 6 and 2 adjustable parameters, respectively.

1. Introduction

In recent decades, reliable alternative energy sources, such as biodiesel have attracted wide spread attention, as a consequence of depletion of available fossil fuel reserves as well as negative environmental effects of fossil fuel consumption (Shahid and Jamal, 2008). Biodiesel enjoys many advantages, of which the most important ones are the biodegradability (Kaercher et al., 2013), feasibility to mix with fossil fuels (Demirbas, 2007), and non-toxicity (Sánchez-Arreola et al., 2015).

Production of biodiesel involves transesterification of triglycerides found in plant and animal lipids (Savaliya and Dholakiya,

* Corresponding author at: Department of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, Iran. Tel.: +98 21 66164140; fax: +98 21 66005417.
E-mail address: vosoughi@sharif.edu (M. Vossoughi).

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2015; Tarakowski et al., 2015). Recently, microalgae have gained much attention as a promising resource for biodiesel production due to substantial amounts of lipids (Mata et al., 2010), high growth rate (Rawat et al., 2013), and cultivation capability in saline water as well as nonagricultural land (Mackay et al., 2015).

Lipid extraction is a critical step in downstream processing of biodiesel production from microalgae (Halim et al., 2012). Organic solvent extraction is one of the most well-known processes used for this purpose (Ramluckan et al., 2014). So far, various organic solvents have been used for extraction of lipids from microalgae. It has been shown by many researchers that the mixture of non-polar and polar organic solvents facilitate the complete extraction of all neutral lipids (Ramluckan et al., 2014; Sheng et al., 2011; Wang et al., 2012). Nonetheless, further investigation is needed on the choice of optimum ratio of non-polar/polar solvent mixture to extract maximum amount of lipids.

To avoid high drying costs, the extraction technique should be applied to wet biomass. However, the effect of the remaining water in wet biomass on the lipid extraction performance is not well known, yet and different scenarios have been proposed in this area, so far. Most of the studies indicated that existence of moisture decreases the mass transfer coefficient and consequently, decreases the lipid extraction efficiency (Kanda et al., 2013; Liu et al., 2013). On the other hand, some researchers reported that moisture content has no effect on the extraction efficiency (Halim et al., 2011) and some of them believe that existence of water in the biomass would increase the extraction efficiency due to the cell inflation (Medina et al., 1998). The effect of moisture depends on several parameters such as algal strain, solvent type, and the extraction temperature. Therefore, more investigation is needed on this topic. Besides, it is also necessary to develop a model to estimate the algal lipid extraction yield. There are some examples for algal oil extraction modeling in literature (Halim et al., 2011; Harrison et al., 2015; Liu et al., 2013). However, the effect of moisture content and solvent composition is ignored in these models.

Thermodynamic modeling is a suitable method to study the behavior of equilibrium systems, such as extraction, and therefore the effect of various parameters on the process can be analyzed by using this method (Abedini Najafabadi et al., 2012; Hakim et al., 2013; Nautiyal et al., 2014). In our previous work, we have used this technique to model the purification process of extracted lipids from microalgae (Abedini Najafabadi et al., 2015a).

In the present study, mixture of hexane (non-polar solvent) and methanol (polar solvent) was used for one step oil extraction from *Chlorella vulgaris*. The effect of various hexane/methanol ratios on the recovery of lipids and fatty acids was examined. In order to investigate the influence of biomass moisture content on the lipid and fatty acid recovery yield, the extraction was also performed on the wet biomass with moisture content of 70% and 85%. Furthermore, the extraction was carried out at five different temperatures between 25 and 65 °C. Finally, a thermodynamic model based on solid–liquid equilibrium was employed to estimate the fatty acid recovery in the extraction process.

2. Methods

2.1. Materials

The solvents used in this work included hexane (99.9%, Merck) and methanol (99.5%, Merck). Sulfuric acid (95–98%, Merck) was used as catalyst for transesterification reaction. All nutrients used for culture media were laboratory or ACS grade. Methyl heptadecanoate (99%, Sigma–Aldrich) was used as an internal standard for fatty acid methyl ester quantification.

2.2. Microalgae strain and cultivation conditions strains

The *C. vulgaris* CCAP (211/19) was purchased from the Culture Collection of Algae and Protozoa, Ambleside, Cumbria, UK. Microalgae was cultivated in a 5 l (ID, 16 cm; height, 25 cm) photobioreactor containing sterilized Bold's Basal Media (BBM) (Stein, 1979) for about two weeks, followed by one week of stressing in nitrogen-starvation medium (N-BBM) as described by Abedini Najafabadi et al. (2015b). The BBM media includes mostly phosphates such as K_2HPO_4 (75 mg/L) and KH_2PO_4 (175 mg/L), nitrates such as $NaNO_3$ (250 mg/L), sulfates such as $MgSO_4{\cdot}7H_2O$ (75 mg/L), $FeSO_4{\cdot}7H_2O$ (4.98 mg/L), $ZnSO_4{\cdot}7H_2O$ (17.76 mg/L), and $CuSO_4{\cdot}5H_2O$ (3.14 mg/L), as well as chlorides such as $CaCl_2{\cdot}2H_2O$ (25 mg/L), NaCl (25 mg/L), $MnCl_2{\cdot}4H_2O$ (2.88 mg/L), and $CoCl_2{\cdot}6H_2O$ (0.8 mg/L). Other materials such as KOH (31 mg/L), Na_2EDTA (50 mg/L), H_3BO_3 (11.42 mg/L), and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (1.74 mg/L) are also used in this medium. 0.1 g/l/d of sodium acetate was added to the medium to provide carbon source. The culture was grown under 300–400 μmol photon $m^{-2}\,s^{-1}$ of artificial light (continuous fluorescence illumination) and its temperature was adjusted to 25 °C. Rate of aeration was 1 vvm under an injection/non-injection cycle of 1/3 min.

2.3. Harvesting and dewatering

For primary dehydration, the biomass was centrifuged at 4000 rpm for 10 min. A sample of the centrifuged algal biomass was collected and lyophilized completely using freeze drier at −80 °C. Weight of the dried sample was used to determine the moisture content of the centrifuged biomass according to the following equation:

$$M\ (\%) = \frac{W_{wet} - W_{dry}}{W_{wet}} \times 100 \quad (1)$$

where W_{wet} and W_{dry} are weight of wet and dried microalgae, respectively.

Other wet biomass samples with different moisture contents were prepared by adding specific amount of distilled water to the centrifuged sample.

2.4. Extraction of microalgal crude oil

0.5 g of lyophilized algal biomass was mixed with 15 ml of solvent in a flask. The sample was sonicated in an ice-bath at 38 kHz for 20 min. The lipid extraction was performed by agitating the mixture with a magnetic stirrer at 180 rpm and 25 °C for 24 h. This long time of extraction ensured the chemical equilibrium between the solvent and algal biomass. For the oil extraction from the wet microalgae, a given amount of wet biomass (equal to 0.5 g dry biomass) was weighted, and the same procedure used for lyophilized biomass was repeated. The resulting mixture was centrifuged at 4000 rpm for 10 min and the supernatant was collected.

2.5. Lipid and fatty acid determination

To determine crude lipids amount, the solvent was removed using a rotary evaporator at the temperature of 50 °C and the vacuum of 200 mmHg. After complete vaporization of the solvent, the remaining lipids in the flask were weighed. The crude lipid recovery from the algal biomass was calculated as follows:

$$R_{Lipid}\ (\%) = \frac{W_{Lipid}}{W_{dry}} \times 100 \quad (2)$$

where the R_{Lipid} and W_{Lipid} are the recovery and the weight of crude lipid extracts, respectively.