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Short Communication

Extraction of lipids from microalgae using CO_2-expanded methanol and liquid CO_2

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HIGHLIGHTS

- Solvents were tested for extraction of lipids from *Botryococcus braunii*.
- Liquid CO_2 only requires 6.8 MPa and is very selective for nonpolar lipids.
- CO_2-expanded methanol extracts more lipid per mL of organic solvent than methanol.

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ABSTRACT

The use of CO_2-expanded methanol (cxMeOH) and liquid carbon dioxide (lCO_2) is proposed to extract lipids from *Botryococcus braunii*. When compressed CO_2 dissolves in methanol, the solvent expands in volume, decreases in polarity and so increases in its selectivity for biodiesel desirable lipids. Solid phase extraction of the algal extract showed that the cxMeOH extracted 21 mg of biodiesel desirable lipids per mL of organic solvent compared to 3 mg/mL using either neat methanol or chloroform/methanol mixture. The non-polar lCO_2 showed a high affinity for non-polar lipids. Using lCO_2, it is possible to extract up to 10% neutral lipids relative to the mass of dry algae. Unlike extractions using conventional solvents, these new methods require little to no volatile, flammable, or chlorinated organic solvents.

1. Introduction

Biofuel from microalgae represents one of the most promising alternative renewable energy sources (Hariskos and Posten, 2014). In contrast, many traditional feedstocks for biofuels, such as corn and sugarcane, require nutrient-rich soils and so may compete with food resources. Microalgae can be grown on marginal land either in open pond systems or in closed photobioreactors with wastewater as a source of nutrients, thereby avoiding the demand for fresh water for algal cultivation (Cooney et al., 2009).

There are a number of limitations preventing the commercialization of algal biofuels. The major challenges of using microalgae as a biofuel feedstock lie in the high demands on certain resources such as CO_2, water and fertilizers, although waste CO_2 and wastewater can be used to mitigate these requirements (Chisti, 2013). Also, better downstream processing technologies for dewatering, extracting and fractionating of the bio-oils will have to be developed to reduce the current high production cost (Halim et al., 2012).

The choice of extraction solvent is crucial to determining the energy consumption in the drying, extraction, and solvent recovery steps. An ideal solvent for lipid extraction should be inexpensive, non-toxic, easily removable, lipid specific to minimize the co-extraction of non-lipid constituents, and be more selective towards desirable neutral (mono-, di-, and tri-acylglycerols) lipid and free fatty acid fractions (Halim et al., 2012). Current extraction methods use flammable and/or chlorinated solvents such as n-hexane (Gouveia and Oliveira, 2009) or a mixture of chloroform/methanol (McNichol et al., 2012), which must be separated from the bio-oil using distillation. Moreover, the simultaneous extraction of undesirable polar lipids (e.g. phospholipids) and pigments interferes with the subsequent transesterification process (Jang et al., 2012), indicating a need for a solvent that is more selective for neutral lipids.

Supercritical carbon dioxide ($scCO_2$) has been investigated as a means of extracting bio-oil from algae (Soha and Zimmerman, 2011). The use of $scCO_2$ has many advantages, including tunable

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solvent properties, rapid mass transfer, facile removal of the solvent, and production of solvent-free crude lipids (Halim et al., 2012). However, the technique requires high temperatures and pressures to obtain yields comparable to conventional solvent extractions.

Gas expanded liquids (GXLs) consist of large amounts of a pressurized compressible gas such as CO_2 dissolved in an organic solvent. The GXLs have the combined properties of a compressed gas and a traditional solvent, resulting in solvent properties that can be adjusted through variations in the pressure (Jessop and Subramaniam, 2007). In CO_2-expanded methanol (cxMeOH), the CO_2 not only reduces the volume of the organic solvent required for the extraction process but also decreases the solvent polarity (Wyatt et al., 2005), which could increase the selectivity of the solvent for neutral lipids and free fatty acids.

Liquid carbon dioxide (lCO_2) offers many of the same benefits as $scCO_2$ but at a lower pressure and temperature. In addition, because of its low polarity compared to most organic solvents, lCO_2 could exhibit higher selectivity towards the neutral lipids, while exhibiting a limited affinity to non-neutral lipids (Moyler and Heath, 1987), but there is a possibility that the overall lipid yield might be lessened by this lower polarity.

In the present study, the use of liquid CO_2 and CO_2-expanded methanol is explored for the extraction of neutral lipids (NL) and free fatty acids (FFA) from microalgae. The extractions were carried out under moderate temperature ($\leqslant$35 °C) and pressure ($\leqslant$7.2 MPa) to reduce the eventual larger scale capital and processing costs. The use of these methods for the extraction of lipids from microalgae could present an advantage to the use of conventional solvents because they require little to no flammable, highly volatile, or chlorinated organic solvents.

2. Methods

2.1. Materials

All chemicals were used as received from the suppliers. Carbon dioxide (4.0 grade 99.99%) was obtained from Praxair. HPLC grade methanol was used for the expansion and extraction process. A microalgae species of *Botryococcus braunii* race A UTEX 572, cultivated as described by (MacDougall et al., 2011) was obtained from the National Research Council (NRC), Halifax, Canada. Cells were lyophilized to final moisture content of 3% (w/w).

2.2. Methods

The algae used for all the extraction methods were lyophilized (hereinafter called 'dry algae'). All extractions were conducted in triplicate except as indicated, and the data reported include mean values and standard deviations.

2.2.1. Extraction using CO_2-expanded methanol (cxMeOH)

The cxMeOH extraction of lipids from *B braunii* was conducted at 35 °C for 2 h with a series of different mass ratios of dry algae to methanol (Table 1). Dry algae (300 mg) were placed in a 3 mL stainless steel tube vessel. The vessel was packed with glass wool as well as diatomaceous earth to filter any solids from the extract. Methanol (2.7 mL) in a 160 mL high-pressure vessel was heated in a water bath at 35 °C. After the temperature had equilibrated, CO_2 was pumped by an ISCO model 500D pump into the 160 mL vessel for 15 min at 7.2 MPa. The expanded methanol from the vessel was passed through the tube vessel, which was heated at 35 °C, at a constant flow rate of 0.15 mL/min. The compressed CO_2 at 35 °C dissolves into the methanol causing it to volumetrically expand about six fold at 7.2 MPa (Jessop and Subramaniam, 2007). Therefore, the total volume of cxMeOH was assumed to be about 16 mL, which provides a throughput of 2.7 mL methanol and about 13 mL CO_2 mixture over 2 h extraction time. The pressure in the system was maintained at 7.2 MPa by a backpressure regulator (BPR). The extract exiting the BPR was captured in isopropanol in an ice-cooled Erlenmeyer flask. After 2 h of extraction, the remaining CO_2 was vented into the flask. The extract was transferred into a pre-weighed vial and the solvent was removed by evaporation. The extract was further dried in a vacuum oven at 55 °C under reduced pressure for 3 h and weighed to determine the gravimetric lipid yield.

Table 1
The conditions and yields of different solvent extraction methods.

Method	Vol. of solvent (mL)	Vol. of organic solvent (mL)	Algae: organic solvent (w:w)	Temperature (°C)	Pressure (MPa)	Total Yield (wt% of dry algae)	Yield of algae lipid components (wt% of dry algae)		
							NL	FFA	Other
cxMeOH	7.6	7.6	1:20	35	7.2	25 ± 0.5	6.9 ± 0.8	12.8 ± 1	4.3 ± 1
	3.8[a]	3.8	1:10	35	7.2	24 ± 0.4	–	–	–
	2.7	2.7	1:7	35	7.2	24 ± 0.7	6.1 ± 0.4	12.5 ± 0.7	5.2 ± 0.6
	2.7[b]	2.7	1:7	50	7.2	24	5.6	13.1	5.1
	1.9[a]	1.9	1:5	35	7.2	16 ± 2	–	–	–
lCO_2	45.5	0.0	–	25	6.8	19 ± 2	10.0 ± 4	4.5 ± 1	4.5 ± 2
	45.5[b]	0.0	–	25	12.0	20	10.6	4.5	4.3
	45.5	0.0	–	25	17.0	23	10.7	7.2	5.1
	25.0	0.0	–	25	6.8	12 ± 1	6.6 ± 1	4.3 ± 2	1.1 ± 0.6
	12.6	0.0	–	25	6.8	11 ± 1	6.5 ± 4	3.5 ± 1	1.0 ± 0.4
MeOH	12.8	12.8	1:33	35	7.2	23 ± 1	4.2 ± 0.7	11.5 ± 0.6	7.3 ± 1
$CHCl_3$/MeOH	25.0[c]	25	1:104	80	0.0	50 ± 1	14 ± 0.3	9.0 ± 0.9	27 ± 2
	25.0[d]	25	1:104	35	0.0	33	6.5	15.6	10.9

All extractions were performed with 0.3 g of dry algae. The extraction processes were run for 2 h except the chloroform/methanol method. Each value represents the mean ± S.D. (n = 3).

Abbreviations: neutral lipids (NL), free fatty acids (FFA), other undesirable materials extracted (Other).

[a] For these extractions, n = 2 and SPE fractionation was not done.

[b] Only one experiment done.

[c] The process was carried out by Soxhlet extraction for 24 h.

[d] The experiment was performed for 2 h by just soaking dried algae in the solvent.