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Supercritical carbon dioxide extraction of algal lipids for the biodiesel production

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Abstract

Biodiesel production from algae is a promising technique. Microalgae are able to accumulate fatty acids up to 50% of their dry weight when submitted to nitrogen defaults. They are then expected to be a new potential renewable source of biodiesel. Efficiently extracting algae oil from microalgae plays an important role in the development of microalgae biofuel. Algae bio-oil is traditionally obtained using thermal liquefaction or pyrolysis, and they may be obtained after an extraction using organic solvents as hexane. Such methods have some drawbacks like inherent toxicity, poor selectivity, difficult separation of the contaminants as well as solvents from the desired product, energy consuming and pollutant. Supercritical carbon dioxide extraction is a promising green technology that can potentially displace the use of traditional organic solvents for lipid extraction. The supercritical fluid extraction has several advantages when compared to traditional extraction method (hexane, petroleum ether, chloroform/ethanol) used for obtaining lipids from algae, in which supercritical extraction provide higher selectivities, shorter extraction time and do not use toxic organic solvents. This study examines the performace of supercritical carbon dioxide extraction of lipids from *Botryococcus braunii* for biodiesel production. The experiments were conducted at temperatures of 50 – 80 ºC, pressure from 200 to 250 bar. The evolution of the process was followed by gas chromatography, determining the concentration of the fatty acids at different reaction times. For supercritical carbon dioxide extraction, lipid yield was found to decrease with temperature and to increase with pressure. Relatively high recovery of polyunsaturated fatty acids and essential fatty acids in supercritical fluid extracted algal lipids were observed. The optimum operating conditions for a supercritical extraction were pressure between 220-250 bar and temperature of 50ºC. This research is part of a wider experimental project involving the supercritical carbon dioxide extraction of lipids from microalgae to contribute to the development of algal lipids into a variable energy source by optimizing lipid extraction techniques for efficiency, sustainability, decreased hazard, and selectivity, focusing on the use of supercritical fluids as alternative green solvents.

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1. Introduction

Biodiesel has been considered as one of the substitutes for fossil fuels in recent years, and also a type of renewable energy source due to their biodegradability, negligible SOx emissions and mitigation of global warming [1]. Biodiesel has excellent lubricity and could provide similar energy density to diesel [2-4]. It burns much cleaner than petroleum diesel as it contains oxygen and reduces most emissions (CO_2, CO, particulate, except NOx). Biodiesel does not require new refueling stations, new parts inventories or expensive engine modifications [5-9]. Europe, the US, and many developing countries (*e.g.,* Brazil, India, Indonesia and China), have initiated a large-scale biodiesel production and set up their targets for the national long-term energy planning.

Chemically, biodiesel consists of fatty acid methyl esters (FAMEs) that can be produced from various lipid sources by transesterification reaction with alcohol in the presence of a base, acid, enzyme or solid catalyst [2,4,10,11]. Depending upon the climate and soil conditions, different countries are looking for different types of vegetable oils as substitutes for diesel fuels [12]. However, biodiesel has been strongly criticized because vegetable oils require cultivation of crops like rapeseed, palm, sunflower and soybean, which involves long extension of lands. In this sense biodiesel production encourage competition for the land as well as for the final use of cultivated crops. In addition, deforestation of forests has been in a constant increase (like in Indonesia, Malaysia and Brazil) as a consequence of longer extensions of lands required for biofuels production. In contrast, third generation biodiesel feedstocks, which are derived from microalgae, have emerged as one of the most promising alternative sources of lipid for use in biodiesel production because of their high photosynthetic efficiency to produce biomass and their higher growth rates and productivity compared to conventional crops [13]. In addition to their fast reproduction, they are easier to cultivate than many others types of plants and can produce a higher yield of oil for biodiesel production.

There has been much research on the optimization of algal growth [14-15], harvesting [15-17] and trasesterification [18-20], lipid extraction techniques have not been well studied. Traditionally algal oils have been extracted using noxious organic solvents, (*e.g.* hexane, chloroform, methanol); however, there are high costs, health and safety concerns and significant wastes associated with these processes. While the conventional extraction process is effective for analysis, it is also non-selective, time consuming, wasteful, uses toxic substances and is inefficient [21]. This process requires dry algae starting material, which is time consuming and energy intensive [22]. An alternative to solvent based extraction processes is extraction by supercritical fluid. Supercritical carbon dioxide ($SCCO_2$) is the primary solvent used in the majority of supercritical fluid extractions. Its moderate critical pressure (72.9 bar) allows for a modest compression cost, while its low critical temperature (31.1 °C) enables successful extraction of thermally sensitive lipid fractions without degradation. $SCCO_2$ facilitates a safe extraction due to its low toxicity, low flammability, and lack of reactivity [23-24].

In this study, the performance of $SCCO_2$ extraction and hexane extraction was evaluated based on the yield and the fatty acid composition of the lipids extracted from microalgae under different conditions.

2. Methods

2.1. Microalgae

The microalgae used for the experiment was kindly provided by Solix BioSystems (Solix BioSystems Inc., USA). The microalgae strain used for this study was Botryococcus braunii

2.2. Conventional solvent extraction

Protocols used for solvent extraction were based on those reported by Bligh and Dyer [25]. The solvent used was a mixture of 2:1 chloroform and methanol. Dried algae samples of approximately 5g mass were mixed with 100mL of solvent and soaked overnight. Solids were removed by filtration and the solvent extract was mixed with ~ 50 mL 0.9% NaCl in a separator funnel and allowed to stand overnight. After separation of the organic phase from brine, the solvent was removed by rotary evaporator. Four replicates of each solvent system were evaluated and the results averaged. Lipid percentage was determined by comparing the extracted algal oil mass to initial dried sample mass.

2.3. Supercritical fluid extraction

Carbon dioxide was supplied with a purity of 99.995% from Linde Gases LTDA. Supercritical carbon dioxide extractions were performed at the temperature range 50 – 85ºC and pressures range of 200 – 250 bar. These extraction conditions were chosen taking into account previous work reported for the extraction of lipids from other microalgae [26-28].

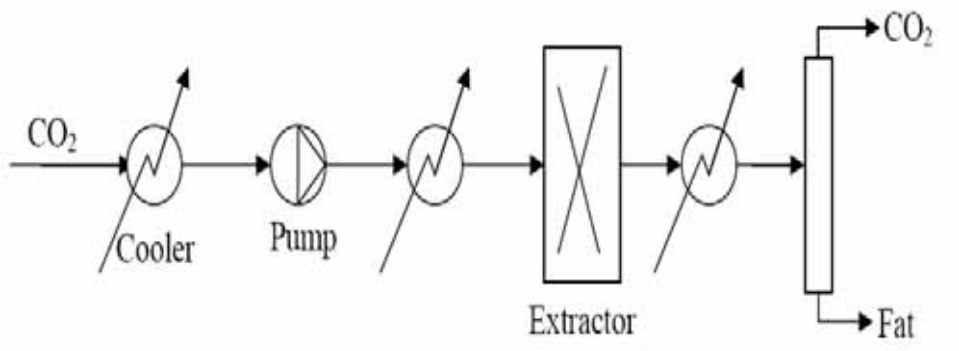

Fig. 1. Scheme of experimental installation

A schematic diagram of the pilot plant, Bench Scale SFE-500 Separex unit, is given in Figure 1. Solvent (CO2), from the cylinder, is delivered through a pipe to the condenser. Liquid CO2 reaches the inlet of the high pressure pump rated up to 200 bar. Compressed fluid is fed to the heater prior to entering the extraction vessel. The unit contains an extraction container of 450 ml, closed with stainless steel porous discs. The extractor is heated by a heating jacket surrounding the outer surface of the vessel. The extraction pressure was controlled by means of a back pressure regulator valve, BPR (Tescom 27-1700) where depressurization of CO_2 flow stream exiting the extraction vessel took place. The extracted substances were precipitated and collected into a glass trap, immersed in an ice bath. To ensure a total recovery of compounds, the gas flow coming out from the first trap passes through a second glass trap.

The extraction pressure is measured at the entrance of the extraction vessel with an accuracy of ±1 bar (Wika, model 881.14.600).

2.4. Fatty acid analysis

Lipid extracts were transmethylated with methanol-acethyl chloride, as described by Lepage and Roy [29], in the presence of an appropriate amount of heptadecanoic acid as an internal standard. The methyl ester were then analyzed on a 7890 Agilent gas chromatograph (GC), equipped with a flame ionization detector. Separation was carried out on 0.32 mm x 30 m fused silica capillary column (0.32 μm film thickness) Sulpelcowax 10 (Supelco, Bellafonte PA, USA) with helium as carrier gas at a flow rate of 3.5 ml/min. The column temperature was programmed at an initial temperature of 200ºC for 8 min, then increased at 4ºC/min to 240ºC and held there for 8 min. Injector and detector temperatures were 250 and 280ºC, respectively and split ratio was 1:50. FAMEs were identified and their response factors calculated by comparing peak areas of known quantities of authentic standards (Supelco) to the internal standard, heptadecanoic acid. Averages of duplicate injections were reported.

3. Results and discussion

The fatty acid concentrations of the algal lipid extracts were determined by GC analysis. Total fatty acids in $SCCO_2$ extraction increased as pressure increased (see Table 1).

Table1. Fatty acid content of lipid extracted from *Botryococcus braunii* using $SCCO_2$ and the Bligh and Dyer extraction method under different pressures and temperature

Fatty Acid (w/w%)	200 bar/ 50ºC	220 bar/ 50ºC	250 bar/ 50ºC	200 bar/ 80ºC	220 bar/ 80ºC	250 bar/ 80ºC	Bligh and Dyer Method
C12:0	9.2	5.1	6.8	4.4	2.6	4.1	3.1
C14:0	28.5	21.5	27.8	19.9	21.3	17.3	12.9
C16:0	29.9	27.3	30.1	26.9	22.4	21.9	23.4
C16:1ω9	0.4	0.5	0.2	0.5	0.6	0.3	0.3
C18:0	1.5	1.9	1.7	1.6	1.3	1.8	2.4
C18:1ω9	6.2	7.4	6.9	7.6	7.3	6.8	7.1
C22:5ω3	0.3	0.7	0.5	0.4	0.5	0.4	0.7
C22:6ω3	18.9	29.5	24.8	32.9	38.4	36.4	39.5
total fatty acid	9.8	10.4	17.6	13.6	7.4	14.1	18.2

Both methods presented a higher content of 14:0, 16:0 and 22:6ω6. No particular differences were found in the fatty acid profiles of extracts when different $SCCO_2$ extraction were performed, also in comparison with the traditional Bligh and Dyer extraction method.

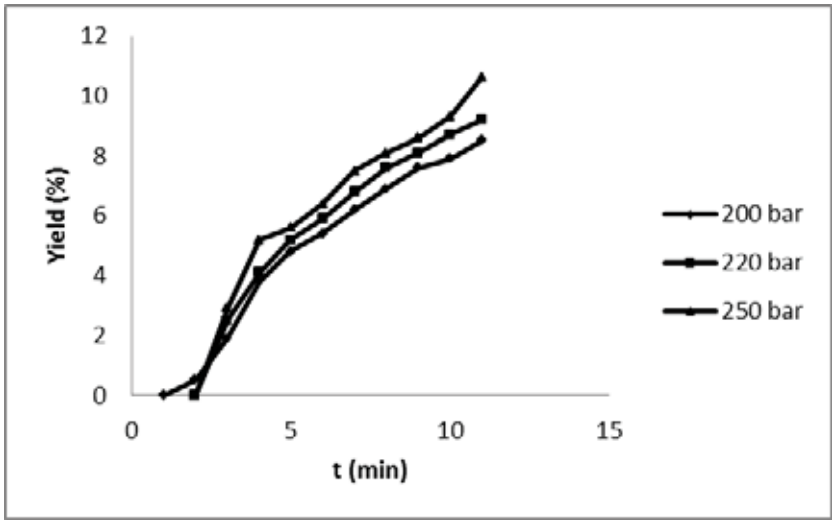

Fig. 2. $SCCO_2$ extraction from Botryococcus *braunii*. Lipids extracted as a function of the run time at 50ºC

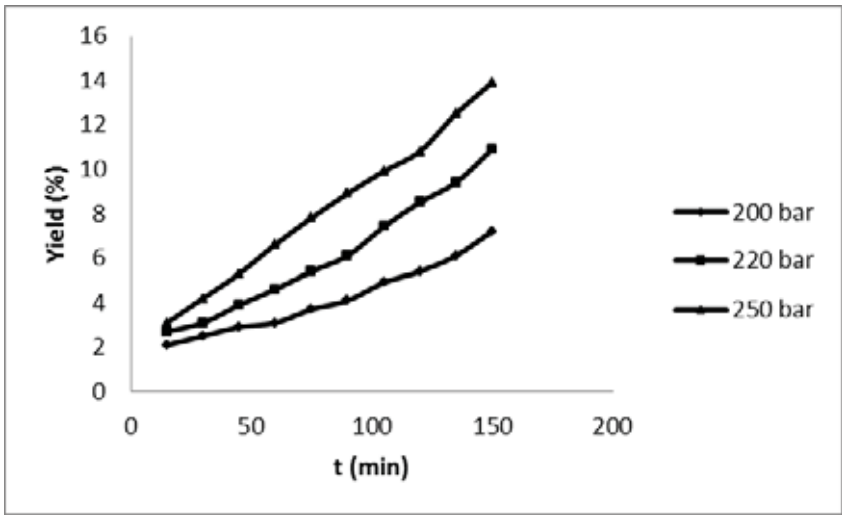

Fig. 3. $SCCO_2$ extraction from Botryococcus *braunii*. Lipids extracted as a function of the run time at 80ºC

Figure 1 and 2 report the experimental points of the lipids extracted from *Botryococcus braunii* as function of the run time and working conditions (temperature and pressure) adopted. The rate of extraction rapidly falls at the given operating conditions. This fact indicates that $SCCO_2$ extraction is mainly controlled by the diffusion kinetics of the lipids within the microalgae since the beginning of the extraction.

4. Conclusion

$SCCO_2$ extraction and Bligh and Dyer method conditions affected the fatty acid content. The supercritical extraction at lower pressure, more saturated fatty acids was extracted. As pressures and densities of fluid increased, the amount of unsaturated compounds and degree of unsaturation increased. This indicated that as pressure, triglycerides containing more unsaturated fatty acids were soluble at higher densities. The optimum conditions for $SCCO_2$ extraction from *Botryococcus braunii* studied seemed to be at 250 bar and 50ºC.

Both methods have been shown to be comparable on the theoretical process yield and the fatty acid composition of the extracts, the conventional extraction process showed to be slightly faster. Although both solvents gave process yields lower than expected, probably as a result of some losses in the most polar lipid fractions.

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