SUPERCRITICAL CO_2 EXTRACTION OF OIL FROM MICROALGAE FOR BIOFUEL APPLICATION

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Abstract

This study is one part of the Shamash French project whose aim is to produce biofuel from microalgae. Eight academic and industrial partners work together to contribute to the future large scale production of biofuel from microalgae. The main idea is to select and produce microalgae which can accumulate large quantities of oil under nitrogen starvation. Once grown, harvested and dried, microalgae are subjected to extraction with supercritical carbon dioxide, a clean process for recovering oil. Oil can be trans-esterified in methylic or ethylic esters to be used as biodiesel.

This work relates the influence of operating parameters on supercritical extraction yields from dried microalgae. Preliminary results on supercritical CO_2 extraction yields from microalgae AB1 are presented. The influence of operating parameters (pressure, temperature, CO_2 flow rate and extraction duration) upon oil extraction yields has been determined through an experimental design. The experiments were performed under pressures from 28 to 46 MPa, temperatures from 318 to 338 K and CO_2 flow rate from 0.3 to 0.8 kg/h corresponding to CO_2/microalgae mass ratio from 80 to 200. The extraction time was set at 1h30. The studied response to the experimental design was the microalgae mass loss. Depending on experimental conditions the mass loss varied from 4 to 16 %. The latter value corresponds to the total neutral lipid content in the microalgae.

Secondly, the influence of microalgae particle size on extraction yields has also been studied. The experiments were performed under 40 MPa, at 333 K and with a CO_2 flow rate of 0.4 $kg.h^{-1}$. The microalgae tested were *cylindrotheca* and *chlorella.* Microalgae were first crushed and sieved. Two particles sizes were chosen: less than 160 μm and more than 1 mm. Results show the great influence of crushing. As expected, the smaller the particle size, the most rapid extraction kinetics.

The influence of pre-treatment was also studied. The comparison between freeze-drying and drying is proposed for the microalgae *cylindrotheca*. It appears that drying provides more rapid extraction kinetics.

INTRODUCTION

Microalgae are photosynthetic organisms spread over on the globe surface in multitudes of species, in marine environment, fresh water or salt water. Microalgae are ubiquitous in many environments, from polar ices to deserts or other extreme middles. This adaptability and their biological diversity let predict an important potentiality for the extraction of molecules of interest for many applications as human health or energy production. Microalgae are particularly able to accumulate fatty acids up to 80 % of their dry weight when submitted to nitrogen defaults [1]. They are then expected to be a new potential renewable source of biodiesel. Algal bio-oil is traditionally obtained using thermal liquefaction [2-6] or pyrolysis [7-11]. They also may be obtained after an extraction using organic solvents as *n*-hexane [12, 13]. The raw products should be treated to eliminate phospholipids, and trans-esterified with methanol to be transformed into methylic esters of vegetable oil, so called biodiesel. Such methods have the main drawbacks of being energy consuming and/or pollutant. Supercritical CO_2 extraction may be an interesting alternative to these processes. Indeed, this technology is well-known and is considered as a green process. For biofuel applications, supercritical CO_2

is an interesting extraction solvent because it solubilises non polar molecules as triglycerides but not polar molecules as phospholipids avoiding the degumming operation unit.
The aim of this work is to carry out extraction experiments on dried microalgae using supercritical CO_2 and to study the influence of experimental parameters (pressure, temperature, CO_2 flow rate, particle size) on extraction yields.

MATERIALS AND METHODS

Chemicals and microalgae

Analytical grade analysis ethyl alcohol and *n*-hexane were obtained from Carlo Erba (99.8%). Instrument grade carbon dioxide (purity of 99.7%) from Air Liquide Méditerranée (Vitrolles, France) was used.
All microalgae were dried and provided by Alpha Biotech (France). They were crushed and sieved before extractions. Microalgae *AB1* which were used for the preliminary experiments and the experimental design had an average neutral lipid content of 16 %. *Chlorella* were grown under nitrogen starvation and their average contents in neutral lipid were equal to 15 %. *Cylindrotheca* is a marine microalgae. Their average neutral lipid contents were of 12 %. *Cylindrotheca* was pre-treated following two different operations: freeze-drying or drying at low temperatures.

Experimental set-up

A classical extraction device (Separex, France) was used to perform supercritical CO_2 extraction of lipids from microalgae. The experimental set-up is shown in Figure 1. Experiments were performed in extraction cells of 5, 10 and 20 cm^3 corresponding to 3, 7 and 12 grams of dried microalgae, respectively.

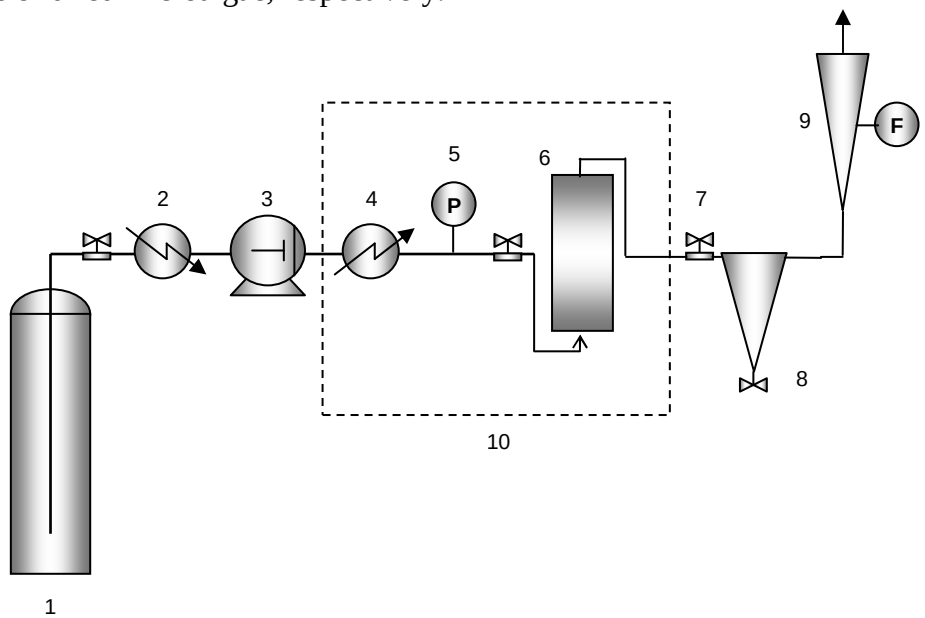


Figure 1: Experimental set-up. 1 – CO_2 cylinder; 2 – Cryogenic bath; 3 – high pressure volumetric pump ; 4 - Heat exchanger; 5 – Manometer ; 6 – Extraction cell; 7 – Expansion valve; 8 – Collector; 9 – Flow meter ; 10 – Thermoregulated area

One extraction experiment is performed as follows:

Liquid CO_2 (1) is condensed in a cryogenic bath (2), filtered and pumped (3) towards the extraction cell (6). Before extraction autoclave, CO_2 is heated (4) until the chosen temperature. The extraction autoclave which contains dried powder is also heated. Pressure is controlled by a pressure gauge. After the extraction cell, CO_2 is released to gas state through an expansion valve (7). The extracted molecules are collected in a collector (8). The CO_2 flow is determined by a flow meter (9) placed at the end of the extraction line.

RESULTS

Preliminary experiments

These preliminary experiments were performed with the microalgae *AB1*. This microalgae have been manually crushed. The reproducibility of extraction experiments was first tested at three different operating conditions. Each experiment was reproduced in duplicate or triplicate for an extraction duration of 180 minutes. Regarding the low mass of raw materials, the extraction yields were obtained through the mass loss (Eq. 1) of the vessel. Table 1 shows that whatever the operating conditions, experiments were reproducible at less than 0.3 %.

$$\text{Mass loss} = \frac{\text{autoclave mass before extraction - autoclave mass after extraction}}{\text{initial microalgae mass}} \times 100 \qquad \text{Eq. 1}$$

Table 1: Reproducibility of extraction experiments.

Experiment	Mass loss (%)	Average (%)
T = 328 K - P = 37 MPa - Q_{CO2} = 0.56 kg/h		
1	8.8	8.9 ± 0.1
2	8.8	
3	9.0	
T = 318 K - P = 46 MPa - Q_{CO2} = 0.87 kg/h		
1	11.7	11.6 ± 0.3
2	11.3	
3	11.9	
T = 318 K - P = 28 MPa - Q_{CO2} = 0.79 kg/h		
1	6.1	6.2 ± 0.1
2	6.3	

The extraction curves illustrated in Figure 2 show the evolution of extraction yields (Eq. 2) versus extraction duration at 318 K, under two pressures 28 and 46 MPa. 95 % of the oil is extracted with an experimental duration of 90 min. After that duration, time is crippling to extract some more pourcents. For that reason, the extraction duration is set at 90 min.

$$\text{Extracted yields} = \frac{\text{Extracted mass}}{\text{average neutral lipid content}} \times 100 \qquad \text{Eq. 2}$$

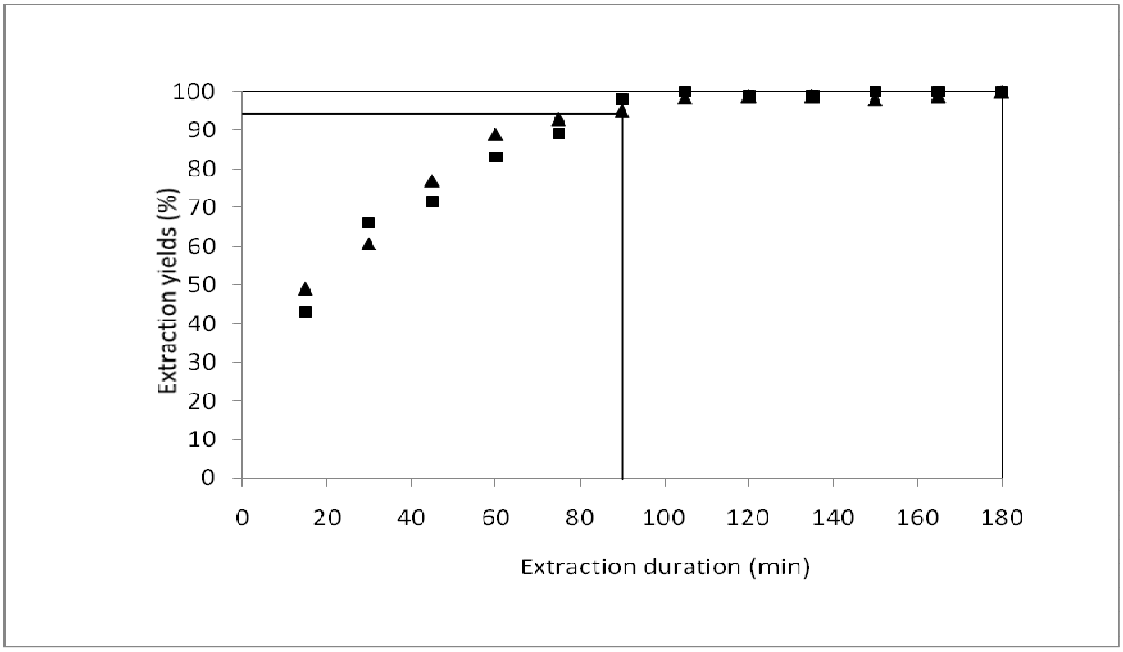


Figure 2: Extraction yields versus time at T = 318 K, at two pressures ▪ 28 and ▲ 46 MPa.

Experimental design - Influence of operating parameters on extraction yields.

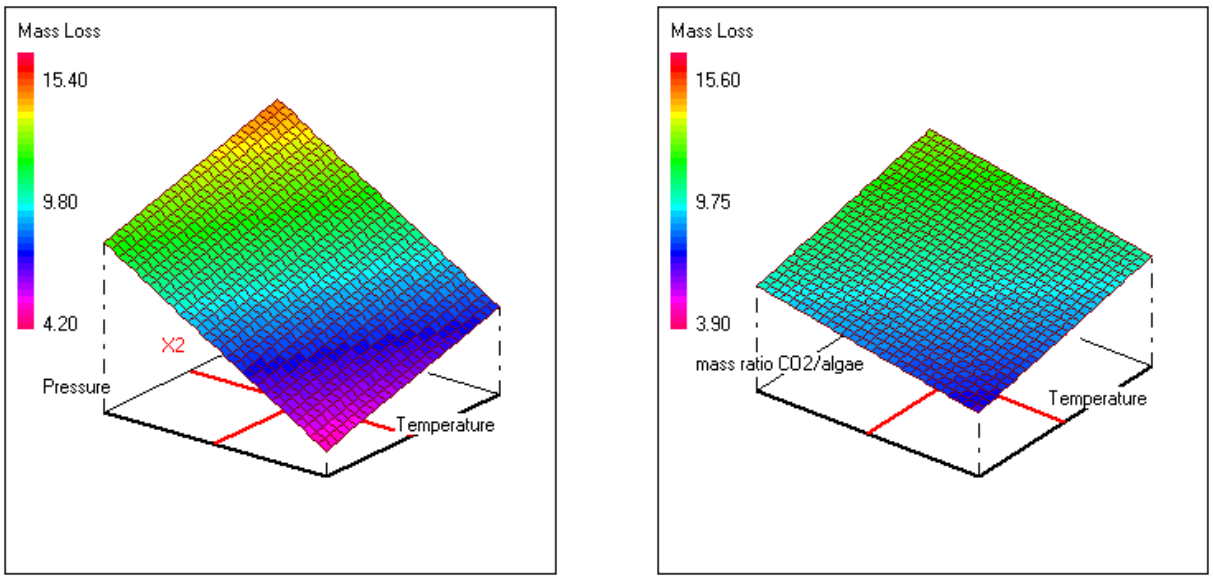
This study has been performed on crushed *AB1* microalgae. Three parameters were studied through the experimental design: pressure, temperature and CO_2/microalgae mass ratio. The extraction experiments were carried out by modifying the following parameters: temperature from 318 to 338 K, pressure from 28 to 46 MPa and CO_2/microalgae mass ratio from 80 to 200. The response of the experimental design was the mass loss in the autoclave. Each experiment was performed during 90 minutes. Table 2 gives the mass loss obtained for each experiment.

Table 2: Mass loss versus operating parameters

Experiment	T (K)	P (MPa)	CO_2/microalgae mass ratio	Mass loss (%)
1	318	28	80	4.3
2	338	28	80	5.5
3	318	28	200	6.1
4	338	28	200	8.2
5	328	37	140	8.9
6	318	46	80	9.9
7	318	46	200	11.7
8	318	46	200	11.9
9	338	46	80	12.6
10	338	46	200	16.3

The mass losses cover a large range of values. The highest ones were reached at 46 MPa, 338 K. Figure 3 shows the surface responses that illustrate the influence of the three studied parameters, pressure (Figure 3.a), temperature (Figure 3.a) and CO_2/microalgae mass ratio (Figure 3.b), on mass loss. It appears that pressure is the most influent parameter. Yields increase significantly with pressure. The temperature also plays an important role. In the pressure range studied, the higher the temperature, the higher the yields. Concerning the

influence of CO_2 flow rate, the higher the flow rate, the higher the yields. The evolution of extracted yields with each parameter is as described in literature [13-23].



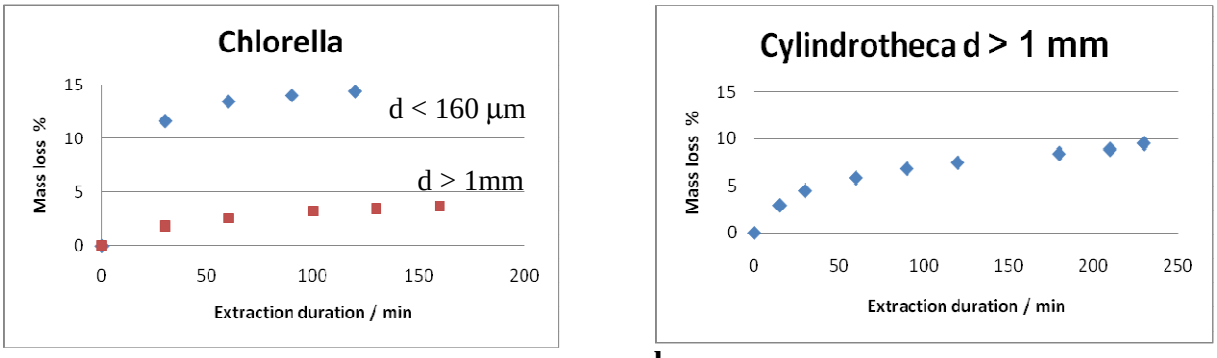
a **b**

Figure 3: Response surfaces for the influence of pressure and temperature (a) and CO_2 flow rate (b)

Influence of particle size on extraction kinetics

This study has been performed on dried microalgae *Chlorella* and *Cylindrotheca*. Microalgae were manually crushed and sieved. Two particle sizes have been tested: more than 1 mm and less than 160 µm. Each experiment was performed under 40 MPa, 338 K and a CO_2 flow rate of 0.4 kg/h.

Figure 4 shows the extraction curves obtained for each microalgae and for two granulometries for chlorella.



a **b**

Figure 4: Influence of particle size on extraction curves. *Chlorella* (a); *Cylindrotheca* (b)

The extraction curves illustrate the important role played by crushing. At a fixed extraction duration it appears that mass loss are higher with a smaller particle size. For instance, with *Chlorella* 12 % of neutral lipids were extracted in 15 minutes with the smallest particle size while less than 5% were extracted with the biggest one. Moreover, for *Cylindrotheca* after an extraction duration of 4 hours, the biggest particle size did not allow the extraction of more than 10% of neutral lipids while the average neutral lipid content is of 12%.

The different extraction curves show that the mass transfer is the limiting factor from the beginning of the extraction.

Influence of pre-treatment

After the harvest and before proceeding to the supercritical fluid extraction, microalgae must be concentrated and dried to eliminate water. Two pre-treatments are mentioned in literature: freeze-drying [14-23,27-29,32-34] and drying under low temperature [2,3,24-26,30,31] but none data are given concerning the influence of such pre-treatment on oil recovery.

The extractions have been performed on *Cylindrotheca* under 40 MPa, 333 K, a flow rate of 0.4 kg/h and a particule size less than 160 µm.

The dried microalgae extraction curve is more rapid than for the freeze-dried one. For instance, with the dried microalgae a mass loss of 7.7 % is observed after 15 minutes extraction while it only reached 4 % with freeze-dried microalgae (Figure 5).

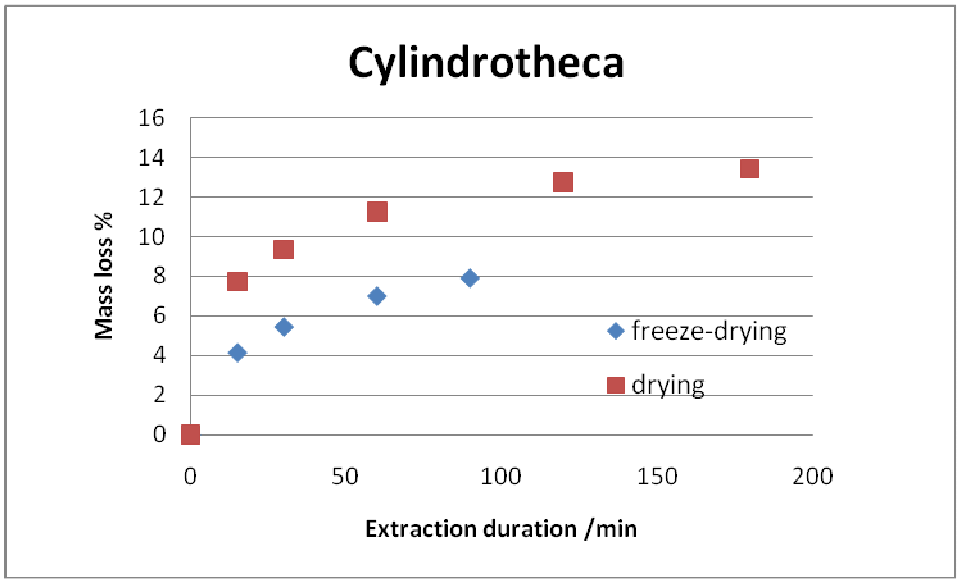


Figure 5: Influence of pre-treatment

Influence of salt

Cylindrotheca is a marine microalgae. Through the maximum yields obtained corresponding to the neutral lipid content in the microalgae, the presence of salt in the microalgae is not *a priori* an issue for supercritical CO_2 extraction.

CONCLUSION

From the experiments carried out at lab-scale, it was shown that pressure is the most influencing parameter on extraction yields. The particle size also plays an important role for the mass transfer kinetics. The smaller the particle size, the higher the yields. Concerning pre-treatment, drying under low temperature seems to be the most efficient one.

Acknowledgments

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