Revista Brasileira de Tecnologia Agroindustrial
ISSN: 1981-3686

https://periodicos.utfpr.edu.br/rbta

Technical feasibility of residual biomass of microalgae *Desmodesmus* sp. after supercritical extraction: evaluation of chemical composition

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ABSTRACT

Supercritical extraction process has been used to obtain bioactive compounds from microalgae biomass that differs in composition because it has protein, esters, carotenoids, chlorophyll, enzymes, antibiotics, vitamins, and hydrocarbons. Due to this, the study of the technical feasibility of supercritical extraction of microalgae and the evaluation of the chemical composition from residual biomass of microalgae *Desmodesmus* sp. is the goal of this work looking for its reutilization in cosmetic, food and pharmaceutical applications. The experimental unit used is formed, basically, of a 42 mL extractor, a high pressure pump, and a micro-metering valve used for sampling. The study showed that it was obtained a higher mass yield at 90 °C and 440 bar of pressure. The residual biomass still contained a high percentage of proteins and carbohydrates, indicating that it could be used in nutraceuticals applications, and as biofertilizers.

KEYWORDS: Microalgae. Supercritical CO_2. Extraction curves. Residual biomass. Green process.

INTRODUCTION

Microalgae have been extensively studied because they can produce compounds with high aggregated value. These microorganisms have been investigated to be used on innumerous kinds of foods, and also on pharmaceutical, cosmetics, and bioenergy applications (Stengel *et al.,* 2011). A controlled biomass of microalgae is a challenge in its use as a source of economical and important products (Skulberg, 2004).

In recent years, the literature has reported the use of residual microalgal biomass, due to the range of products still present after the lipid extraction, but the harvesting methods, cell disruption and extraction of metabolites must be efficient and avoid the degradation of the other fractions to be utilized (Subhadra, 2010; Reis and Gouveia, 2013; Hariskos and Posten, 2014).

Therefore, it is crucial to explore approaches to reduce the costs of biodiesel production from algae by using low-cost raw materials and/or co-producing high value-added products (concentrated oil by supercritical extraction process) at the same time. A few experimental works have been published using microalgae to obtain biofuel and high value-added products, within this concept (Lopes *et al.,* 2013; Hong *et al.,* 2013). Depending on species/strain, environmental conditions and harvesting/processing methods, whole algal biomass and residual 'cake' after oil extraction may be highly attractive sources of essential dietary amino acids, fatty acids, sugars, vitamins, minerals, carotenoids and other health-promoting nutrients well suited as feeds or feed additives for terrestrial livestock and aquatic animals.

The extraction with supercritical CO_2 has advantages as high selectivity, and the carbon dioxide is spontaneously separated from the extract, making the residue completely free of toxic/organic solvent traces. Another advantage, as the ability to directly use the residual biomass for animal feed or as biomass for anaerobic digestion and fertilizer, because of the absence of organic solvents. Moreover, CO_2 can be recycled safely, which represents an economic and environmental benefit (Crampom *et al.,* 2011). Because of these characteristics, this technique has been investigated exploring different kind of raw materials

with the objective to obtain high aggregated value components (Sartori et al., 2017).

Because of it, this work has as goal the study of the technical feasibility of supercritical extraction of microalgae oil, and to evaluate the chemical composition from residual biomass of microalgae *Desmodesmus* sp., looking for its reutilization in cosmetic, food and pharmaceutical applications. Moreover, there are no studies about the residual biomass after the supercritical extraction with the species studied in this work.

MATERIAL AND METHODS

MATERIAL

The solvents used were hexane P.A., mixture of isomers (Isofar, Rio de Janeiro, Brazil) and carbon dioxide (White Martins, Rio de Janeiro, Brazil) with 99.998% of minimum purity.

MICROALGAE

The microalgae biomass (*Desmodesmus* sp.) was obtained from cultures carried out inthe Algae Cultivation Laboratory of the Federal University of Santa Catarina. The cultures were centrifuged, and the biomass dried and maintained under refrigeration until its use.

EXPERIMENTAL METHODOLOGY

Soxhlet Extraction

About 10 g of microalgae were put into a cellulose cartridge fed in the Soxhlet extractor. It was added in the boiling flask 100 mL of solvent, which were heated on a heater plate (Biomixer DB-IVAC), remaining under continuous reflux. After distillation, the solvent was separated from the oil (product) in a rotary evaporator (Fisatom 803), coupled to a vacuum pump (New Pump). The experiments were performed in triplicate, the hexane was used with 1:10 proportion, and the time of extraction was 5 h.

Experimental Design

The effect of independent variables, temperature and pressure in the extraction of oil from the microalgae *Desmodesmus* sp., were evaluated using a central composite rotatable design (CCRD).This is a 2^2 including 4 trials in the axial conditions and 3 repetitions in the central point, totalizing 11 tests. Table 1 shows the pressures and temperatures used in this work, as the coded and real levels.

Table 1 - Operational conditions of temperature and pressure used in microalgae oil extraction using supercritical carbon dioxide

	Coded Levels		Real Levels	
Run	Temperature (°C)	Pressure (bar)	Temperature (°C)	Pressure (bar)
1	-1	-1	50	160
2	1	-1	90	160
3	-1	1	50	440
4	1	1	90	440
5	0	0	70	300

Source: Elaborated by the author (2015).

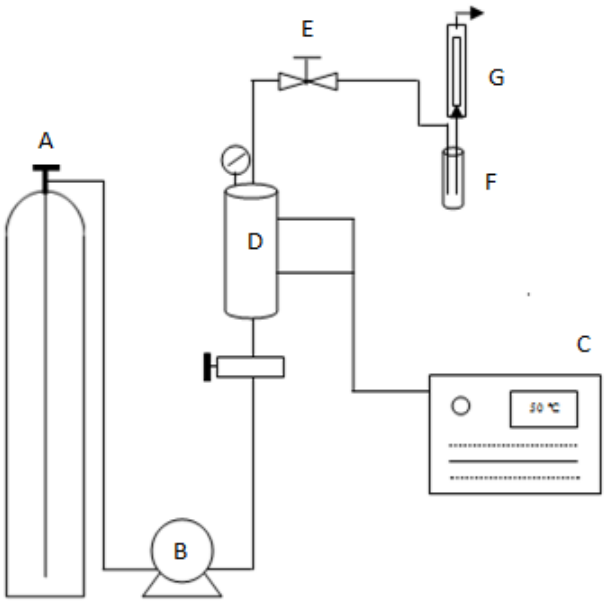
Supercritical CO_2 Extraction

The experimental apparatus, for extraction of bioactive compounds present in the oil of microalgae with supercritical carbon dioxide, is illustrated in Figure 1 and is present at Applied Thermodynamics and Biofuel Laboratory (DCE/UFRRJ). It consists of a 316S stainless steel extractor of 42 mL volume, with stainless steel screens 260 mesh at the top and bottom to prevent the passage of any solid material, preventing the clogging of the line. The temperature of the extractor was controlled by a thermostatic bath (Tecnal) until the desired temperature.

A high pressure pump (Palm model G100), specific for pumping CO_2, was responsible for feeding the solvent. The CO_2 flow rate was approximately 6.04 $mL.min^{-1}$.

The extractor was fed with 10 g of microalgal biomass and the maximum extraction time was 260 min, where it was observed the extraction curve saturation. It was used the depressurization technique through a micro-metering valve to control the flow and make possible the sample collect at certain times.

Figure 1 – Flowchart of the experimental apparatus, where **A**: CO_2 cylinder; **B**: high pressure pump; **C**: Thermostatic bath; **D**: Extractor; **E**: Micro-metering valve; **F**: Sample; **G**: Flowmeter.



Source: Adapted from Mendes et al., 2002.

The sampling was changed according to experimental conditions, due to the fact that the extraction could be slower or faster. For the condition of 160 bar, it was performed at every 20 minutes after the extraction beginning, and then every 10 minutes until exhausted. For the condition of 300 and 440 bar, the extraction was done since the first 10 minutes, thus, the sampling was performed considering this interval.

By reducing the pressure, the sample is recovered on a previously weighed polypropylene tube. After each sampling, the tube was kept inside an ice bath in order to not degrade the extract. The same methodology was adopted in Mendes *et al.,* (2005), Silva *et al.,*(2008) and Vargas *et al.,*(2010).

Chemical composition analysis of residual biomass from microalgal

The biomass, after the extraction process, was characterized in Research Fruits and Vegetables in Nutrition Institute and Control Bromatological Laboratory in Pharmacy Institute, both at Federal University of Rio de Janeiro.

The evaluation of the chemical composition of microalgae biomass was done with the sample in nature to compare to the samples after supercritical extraction.

The following analyzes were performed to characterize the chemical composition of biomass: moisture, protein, lipid, fiber and ash, according to the methodology of the Instituto Adolfo Lutz (2005). Assays were performed in triplicate, except the fibers, which was conducted in duplicate due to the low quantity of sample.

Total nitrogen was determined by Kjeldahl method after acid digestion, ammonium, distillation under steam current, and titration with 0.004 N HCl. Crude protein was calculated multiplying total nitrogen by the conventional conversion factor of 6.25 and total carbohydrates were determined reducing the quantity measured of protein, moisture, ash, lipid, and fiber. The microalgae ash contents were determined by heating the samples to 550 °C for 4 hours using a muffle furnace and the moisture content was determined gravimetrically at 105 °C for 6 hours, respectively, until constant weight. The content of fiber was done adding 50 mL of distilled water in the sample and placed on a heater plate to boil. The material was filtered and acidified with HCl (solution 1:1). The optimum pH is 2.5 after the material was left for 7 days, filtered again and placed in an oven for 24 hours.

Then, the samples was placed in a vacuum desiccator to cool for 30 minutes, weighed and placed in a muffle furnace for 5 h. After, it was placed in a vacuum desiccator for 30 min and weighed.

RESULTS AND DISCUSSION

RESULTS OF SUPERCRITICAL CO_2 EXTRACTION

The oil extraction from *Desmodesmus* sp. biomass with supercritical carbon dioxide was performed under the conditions of 160 bar – 50 °C; 160 bar – 90 °C; 300 bar – 70 °C; 440 bar – 50 °C, and 440 bar – 90 °C. The experimental yield (e %) was calculated according to Equation 1.

$$e\% = \frac{extracted mass}{mass of oil - free dry matter} \quad (1)$$

with extracted mass as the oil extracted mass and mass of oil-free dry matter as the seed mass without oil.

The mass of oil-free dry matter was calculated based on the percentage of oil extracted in the conventional method. From this experiment, it was obtained 16.73 % of the amount of oil extracted by Soxhlet extraction using hexane as solvent.

In Table 2 is possible to observe the yields obtained for all studied experimental conditions. The results indicate that higher pressures led to a higher efficiency in SC-CO_2 extraction of microalgal lipids. Moreover, an increase of temperature, at higher pressures, led to a higher extraction yield. However, at lower pressure the temperature had an opposite behavior.

Table 2 - Experimental results of extraction yield with supercritical CO_2.

Run	Temperature (°C)	Pressure (bar)	Yield (e%)
1	50	160	0.037
2	90	160	0.034
3	50	440	0.414
4	90	440	2.512
5	70	300	0.157

Source: Elaborated by the author (2015).

This behavior is also reported in some literature works such as Mendes *et al.,* (1995), who extracted lipids from microalgae *Chlorella vulgaris* with supercritical CO_2. These authors noticed the same above behavior at 40 and 55 °C and pressures of 200 and 350 bar.

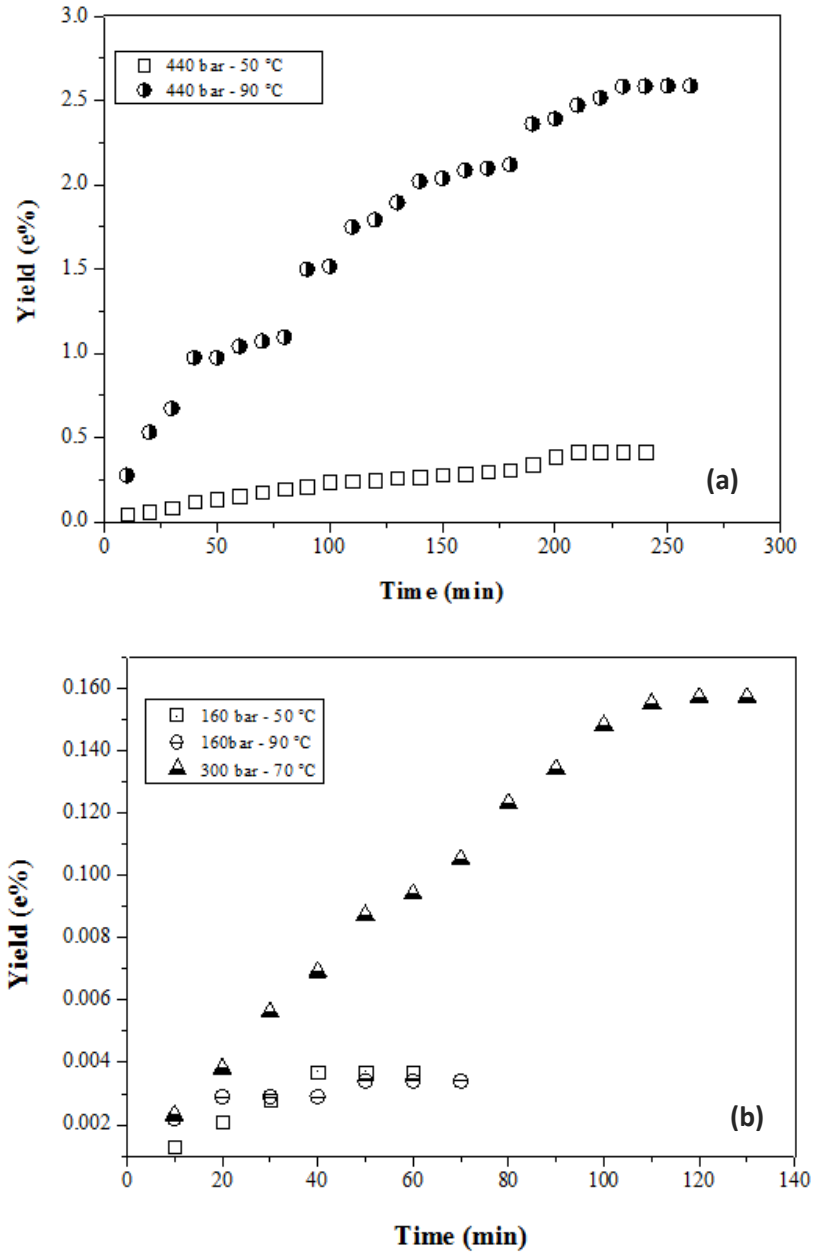
Mendes *et al.,* (2003) reported that, at constant temperature, the extraction oil of the microalgae *Dunaliella salina* increases in yield at higher pressures. However, the effect of the temperature is not very significant for some pressure ranges but, at 200 bar, the temperature increasing corresponds to a decrease in the yield of carotenoids extracted.

As Palavra *et al.* (2011) mentioned, in some microalgae extractions with supercritical CO_2 performed at 39 °C and pressures of 125, 200 and 300 bar, it was observed that the extracellular content, for example hydrocarbons, is quickly extracted, while the intracellular, such as lipids, is more difficult to extract.

Extraction curves of *Desmodesmus* sp. oil for each pressure condition (160, 300 and 440 bar) and temperature (50, 70 and 90 °C) can be observed in Figure 2

(a) and (b). It shows that the highest extraction yield occurred on the condition of 440 bar with 90 °C.

Figure 2 - Extraction Curves of *Desmodesmus* sp. biomass: (a) Conditions of 440 bar and 50 °C and 90 °C; (b) Conditions of 160 - 300 bar and 50 °C, 70 °C and 90 °C.



Source: Elaborated by the author (2015).

Each microalgae contains an oil with different fatty acids composition, but this oil is general composed of fatty acids known as C14:0-C20:0, mainly concentrated with C16:0, C18:1 (Ω-9), C20:4 (Ω-6), and C20:5 (Ω-3). However, due to the supercritical CO_2 selectivity, it is possible to slightly modify the extracted oil composition.

Cheung *et al.* (1998) showed that the best yields of fatty acid omega-3 occurred at low temperature and high pressures. Meanwhile, Cheung (1999) showed that saturated fatty acids are more extracted at low pressures and, when the pressure increases, the percentage of unsaturated fatty acids increases at the extraction phase.

CHEMICAL COMPOSITION ANALYSIS OF RESIDUAL BIOMASS FROM MICROALGAE

The analysis results of the chemical composition after extraction with supercritical CO_2 are shown in Table 3.

Table 3 - Results of the chemical composition, in percentage, of biomass after supercritical CO_2.

Samples	Moisture	Ash	Lipids	Proteins	Fibers	Carboydrates
"in natura"	10.70	16.04	16.73	42.44	0.011	14.08
160 bar 50 °C	8.02	6.50	16.69	41.05	0.007	27.73
160 bar 90 °C	7.73	6.53	16.69	39.75	0.002	29.29
300 bar 70 °C	6.40	6.68	16.57	37.41	0.003	32.93
440 bar 50°	10.65	6.07	16.31	38.70	0.006	28.25
440 bar 90 °C	7.63	6.11	14.14	34.76	0.003	37.36

Source: Elaborated by the author (2015).

The results show that after extraction the biomass remains with high levels of protein and carbohydrates, yet having a potential for nutraceuticals, animal feed and bio-fertilizers applications. As the supercritical extraction is a clean extraction process, because the biomass is free of organic solvents, it becomes an efficient method to be applied in the case of reutilization of microalgae residue.

Some authors have studies the best destination for residual biomass, in general, after the extraction of oil for biodiesel production, to biorefineries concepts. Hernández *et al*., (2014) and Ferreira *et al*., (2013) evaluated the reuse of microalgal biomass after supercritical fluid extraction, which can be applied in studies about pharmaceuticals and for the production of other types of biofuels.

Verdugo, Lim and Rubilar (2014) reported that there are many studies using the residual biomass from microalgae, which is rich in protein and carbohydrates. The mainly used are the low-value animal feeds and focused on their studies exploring the utilization to protein concentrate from *B. braunii*.

Lee *et al*., (2013) investigated after photoautotrophic culture of LB999 *D. tertiolecta*, the harvested and concentrated cells. The compositions of

carbohydrate, lipid, protein, ash, and moisture in *D. tertiolecta* biomass were 37.8, 20.6, 25.5, 9.6 and 6.5% (w/w), respectively. The authors suggest that lipids residual biomass was thoroughly extracted with chloroform and methanol, and the percentage of the total carbohydrates of the residual biomass after lipid extraction was 51.9% approximately (w/w). It was also studied the use of this residual biomass for bioethanol producing from the enzymatic saccharification products by *S. cerevisiae* YPH500, and the fermentation yield was 0.14g ethanol/ g residual biomass and 82.0% of the theoretical fermentation yield.

Ramos-Suárez *et al*., (2014) investigated amino-acid extracted from microalgae *Scenedesmus sp*. It was evaluated the residues that are the substrates for anaerobic digestion. They assert that nutrient recovery is supposed to be able to extract 59% of the total amino acids in proteins.

It can be seen that research in recent years have been on the rise in considerable numbers in order to reuse the residue of microalgal biomass in a sustainable way, as it has great potential in many areas of industry.

CONCLUSIONS

In this work the technical feasibility of supercritical extraction of microalgae *Desmodesmus* sp. and the potential of residual biomass after extraction was investigated. The supercritical fluid extraction was efficient, with the best result (2.586% of yield) obtained at 440 bar and 90 °C. Biomass after the supercritical extraction did not changes their characteristics as composition of lipids, carbohydrates and proteins values, showing a potential to be reused in different areas of study. Further studies are necessary to be conducted with the residual biomass in order to properly allocate this residue.

ACKNOWLEDGMENTS

The authors would like to thank the financial support received from CAPES and FAPERJ.

Viabilidade técnica da biomassa residual da microalga *Desmodesmus* sp. após extração supercrítica: Avaliação da composição química

RESUMO

O processo de extração supercrítica tem sido utilizado para a obtenção de compostos bioativos de biomassa de microalgas que diferem na sua composição por possuírem proteínas, ésteres, carotenoides, clorofilas, enzimas, antibióticos, vitaminas e carboidratos. Devido a isso, o estudo da viabilidade técnica da extração supercrítica de microalgas e a avaliação da composição química a partir da biomassa residual de microalgas *Desmodesmus* sp. foi o objetivo deste trabalho buscando sua reutilização em aplicações de cosméticos, alimentos e farmacêuticos. A unidade experimental utilizada é formada, basicamente, de um extrator de 42 mL, uma bomba de alta pressão e uma válvula micrométrica usada para amostragem. O estudo mostrou que foi obtido um maior rendimento em massa (2,58%) a 90 °C e 440 bar de pressão. A extração com fluido supercrítico não alterou a porcentagem de proteínas e carboidratos, que continuou alta, indicando que poderia ser utilizada em aplicações nutracêuticas e como biofertilizantes.

PALAVRAS-CHAVE: Microalgas. CO_2 supercrítico. Curvas de extração. Biomassa residual. Processo verde.

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Recebido:23 jan. 2017.
Aprovado:15 mar. 2018.
Publicado: 04 jun. 2018.
DOI:10.3895/rbta.v12n1.5189
Como citar:
SOUZA, A. L. B. Technical feasibility of residual biomass of microalgae *Desmodesmus sp*. after supercritical extraction: evaluation of chemical composition. **R. bras. Tecnol. Agroindustr.,**Ponta Grossa, v. 12, n. 1, p. 2578-2591, jan./jun. 2018. Disponível em: <https://periodicos.utfpr.edu.br/rbta>. Acesso em: XXX.
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