The Journal of Supercritical Fluids 130 (2017) 261–266

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Supercritical CO_2 extraction of hydrocarbons from *Botryococcus braunii* as a promising bioresource

Ece Yildiz-Ozturk, Ozlem Yesil-Celiktas*

Department of Bioengineering, Faculty of Engineering, Ege University, 35100 Bornova-Izmir, Turkey

ARTICLE INFO

Keywords:
Botryococcus braunii
Microalgae
Supercritical CO_2 extraction
Hydrocarbons
Optimization

ABSTRACT

Microalgae has been a sustainable resource for producing bio-based products and hydrocarbon rich varieties are significant sources for converting into green naphtha and further to ethylene and propylene. The objective of this study was to develop an optimized supercritical CO_2 method for extracting hydrocarbons from *Botryococcus braunii*. A 3-level factorial design was used to evaluate the effects of pressure (125–200 bar) and CO_2 flow rate (5–7 g/min) at a constant temperature of 40 °C to elicit the conditions maximizing total extract and hydrocarbon yields. Maximum total extract and hydrocarbon yields were obtained at a pressure of 200 bar, and a CO_2 flow rate 8.71 g/min, yielding 1.74 g extract and 147.5 mg hydrocarbon/g dry microalgae. Considering the large diversity of microalgae species, the ability to effectively extract oil from cellular biomass is of prime importance and supercritical CO_2 extraction can serve as a green and sustainable alternative.

1. Introduction

Botryococcus braunii is one of the most encouraging microalgae that synthesize and produce high amounts of hydrocarbons and ether lipids at a high rate per unit of dry cell weight. The broad biodiversity of *B. braunii* turns out in the production of three types of hydrocarbons, connected with three chemical races: A (alkadienes, trienes), B (triterpenes) and L (tetraterpene). Besides, hydrocarbon levels and dissociations vary with algal origin [1]. The B race of *B. braunii* accumulates C30–C37 isoprenoid hydrocarbons (botryococcenes) and small amounts of methyl squalenes [2]. The produced hydrocarbons vary between 10 and 40 % dry cell weight in different strains [3]. These chemical compounds are more challenging as biological packaging and biofuels than lipids produced by other microalgae. Most of the hydrocarbons (90–95%) of this microalgae are located in an extracellular matrix (ECM) consisting of a polymer core of aldehydes. According to quick-freeze deep-etch electron microscopy, an outer retaining wall have a role in distinguishing the liquid ECM within the colony by forming a spread between cells. In addition, the retaining wall brings into adjacent contact with the apical cell wall of each cell [4]. The hydrocarbon ECM covers in the gaps between cells. Liquid botryococcenes fill the colony interior and coat the inner surfaces of extended walls and the outer surfaces of nonapical cell walls [5]. The liquid botryococcene hydrocarbon phase is sensitive against fracture and can be widely extracted by hexane, while the polymerized phase has fibrous structure and not extracted by hexane. This polymerized phase serves to stabilize the colony and to facilitate gas exchange in the colony interior. After drying of algae colonies, it should be treated with hexane in order to separate between polymerized and liquid hydrocarbons [6]. Due to the non-polar structure of hydrocarbons, non-polar organic solvents such as n-hexane are good choices for extraction of hydrocarbons from microalgae [7,8]. In the literature, there are wide range of research with respect to hydrocarbon extraction from *Botryoccoccus braunii* by using various solvents such as 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU) and an alcohol (ethanol and octanol) [9], 1,2-dimethoxyethane [10], n-heptane [11], chloroform/methanol (2:1), chloroform, methylene chloride, ethyl acetate, acetone, and toluene [12]. However, different approaches have been applied to reduce the use of toxic and polluting organic solvents to improve the sustainability of the extraction procedures [13–15]. Supercritical fluids (SCF) fulfill these requirements by their low viscosity and surface tension with higher compressibility, gas diffusivity and liquid solubility providing enhanced mass transfer between phases [16–19]. Pressure is considerably significant for enhanced extraction yields and faster extraction kinetics [20]. Another important aspect is the selectivity which increases with density and can be adjusted by tuning the temperature, pressure and co-solvent [16,21]. In addition to these, the extraction efficiency is related to the molecular weight of analytes, their concentration in the sample, type and strength of binding to the matrix and solubility in solvent. Therefore, it is generally advised to work with a high supercritical CO_2/algae mass ratio at supercritical CO_2 extraction [22]. For SC-CO_2 extraction, it is suggested that the water content

* Corresponding author.
E-mail addresses: ozlem.yesil.celiktas@ege.edu.tr, ozlemyesil@gmail.com (O. Yesil-Celiktas).

http://dx.doi.org/10.1016/j.supflu.2017.06.019
Received 6 February 2017; Received in revised form 25 June 2017; Accepted 25 June 2017
Available online 27 June 2017

should be less than 20 wt% to achieve the best performance [23]. In this regard, samples with high moisture content should be principally freeze-dried before supercritical CO_2 extraction in order to increase extraction efficiency [24]. Lyophilization could play a role in decreasing cell rigidity while increasing the surface area and pore volume, which can enable more lipids to dissolve in supercritical CO_2 [25,26]. In addition, SCF extraction of biologically active compounds such as phycocyanins, proteins, fatty acids and hydrocarbons from microalgae avoids thermal or chemical degradation and yields solvent-free forms which are of high importance in pharmaceutical applications [27,28]. The objective of this study was to optimize supercritical CO_2 extraction process in order to obtain the highest amounts of total extracts and hydrocarbons and critically analyze performance parameters, also in comparison to the conventional extraction with different solvents.

2. Materials and methods

2.1. Chemicals

CO_2 (99%) was obtained from Habas, Izmir, Turkey. All organic solvents, hexane, ethlyacetate, dichloromethane, chloroform, methanol were purchased from Merck (Darmstadt, Germany). Lyophilized *Botryococcus braunii* (AC761) was supplied from Norsker Investigaciones S.L. (Spain) in the scope of FP7 project, SPLASH.

2.2. Experimental design

Response surface methodology (RSM) was used to study effects of performance parameters elicited as pressure (X1) (120–200 bar) and CO_2 flow rate (X2) (5–9 g/min) on the total extract amount and the extraction yield of total hydrocarbons. In particular, Box-Behnken design was adopted to predict the optimum extraction conditions while 3-level factorial design was used to provide consistency and stability of the responses. Design-Expert 7.0.0 (2005, Stat-Ease, Inc.) was used in order to generate experimental runs, ANOVA variance analysis and numerical optimization. Totally 13 runs were required to cover all points according to the 3-level factorial design with 5 replicates on center point [29]. Models which have regression higher than 95% are acceptable. That means, the difference between experimental and estimated data is less than %5. Another parameter is *p*-value which has been applied for the selected model and coefficients to study the significance of each parameter in the equation. A *p*-value less than 0.05 indicates that model terms are significant. When a factor has a *p*-value smaller than 0.05 it influences the process in a significant way for a confidence level of 0.95. If the *p*-value is greater than 0.05, the possibility of existence of another coefficient in equation is greater than 95 % that means the provided parameter cannot be significant [30].

2.3. Extraction processes

2.3.1. Supercritical CO_2 extraction

Supercritical CO_2 extraction was carried out at SFE 100 System (Thar Instruments, Inc., UK, 2006) comprised of CO_2 tube, high pressure CO_2 pump, CO_2 flow-meter (Siemens; mass 2100, DI1.5), temperature controllers, high pressure co-solvent pump, Automated Back Pressure Regulator (ABPR), cooler and heater as illustrated in Fig. 1. The extractor volume was 100 ml, thus it was filled with about 10 g of lyophilized *B. braunii*. Liquefied CO_2 was introduced into the sample cartridge through a piston pump with a cooling jacket. Both the pressure and temperature of the cartridge were automatically reached and maintained by a control unit according to settings. After the desired pressure and temperature were reached, the cell was placed in the oven cavity and connected to the manifold and the restrictors. The independent variables were pressure (120, 160, 200 bar) and CO_2 flow rate (5, 7, 9 g/min), whereas temperature was kept constant at 40 °C. These variables were set according to the Box-Behnken (BBD) experimental design (3-level factorial design) which consists of a group of mathematical approaches for the modelling and analysis of the response influenced by the independent variables. When all the desired parameters were reached, the extraction was started at a set flow rate and continued for 60 min under each condition. At the end of the extraction process, valve of the CO_2 tube was sealed off for depressurization. Extracts were collected from the separator outlet (20 °C) after releasing CO_2 from the system and were dissolved at hexane to collect hydrocarbons. Supercritical CO_2 behaves as a nonpolar, lipophilic solvent suitable for the extraction of hydrocarbons. Thus, co-solvent was not used. 3-level factorial design was used to investigate the extraction procedure which provided sufficient data to fit quadratic models and decreased the number of experimental sets.

2.3.2. Solvent extraction

The study was conducted using four solvent systems: (a) hexane, (b) dichloromethane, (c) etlyacetate, (d) chloroform/methanol (2:1). All the chemicals were of analytical grade and purchased from Merck Chemicals. About 1 g of lyophilized *B. braunii* B6 strain from the same culture broth was transferred into a centrifuge tube containing hexane, dichloromethane, etlyacetate and chloroform/methanol (2:1). Extraction of hydrocarbons was carried out by soaking the lyophilized biomass for 1 h with each of the tested organic solvents (30 ml) on the magnetic stirrer. The extraction conditions were temperature (40 °C), time (60 min) and solid/liquid ratio (1:30 g/ml).

2.4. Determination of the amount of hydrocarbons by gas chromatography with flame ionization detection

2.4.1. Sample preparation

All supercritical CO_2 and solvent extracts of lyophilized biomass for *B. braunii* B6 strain were diluted in dichloromethane/methanol (1/1). For preparation of solid samples, 1 g of the sample was dissolved in 5 ml DCM/MeOH (1:1). For preparation of aqueous solutions, the sample was preheated for 10 min at 80 °C, then cooled to ambient temperature. Subsequently, the sample was extracted using hexane and diluted out of the hexane fraction one time using DCM/MeOH (1:1).

2.4.2. GC/MS conditions

Quantitative detection of hydrocarbons in algae extracts was carried out by GC-FID gas chromatograph. Yields were determined as mg squalene equivalent/g DW. Gas chromatography mass spectrometry (GC/MS) (7890A/5975C, Agilent Technologies) equipped with a Phenomenex-7HG-GD10-11 (ZB-5Ms, df = 0.25 μm) (30 m × 0.25 mm fused silica, W.C.O.T.) was employed. The injection volume was 1 μl. Wash liquids and blank were Dichloromethane/Methanol (1/1). Carrier gas was helium and injector (280 °C) was used. The initial oven temperature was 80 °C for 5.0 min and the temperature was programmed to increase from 80 °C to 350 °C for 5 °C/min, final time was 17 min. Inlet temperature was 350 °C and the mode was split 1:10. FID detector was 350 °C. The total GC–FID analysis time was 55 min.

2.4.3. Calibration

Squalene (Sigma: S3627) ($C_{30}H_{50}$, MW = 410 g/mol) was used as a reference standard. For calibration solution, 50, 100, 250 and 500 mg/L of squalene were dissolved in DCM/Methanol (1:1). A blank was run and the squalene calibration solutions were injected before running the sample solutions. Each peak in the sample solution based on the squalene response factor obtained out of the calibration curve to mg/L was calculated at retention time x minutes.