Biomass and Bioenergy 115 (2018) 143–150

Contents lists available at ScienceDirect

Biomass and Bioenergy

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

Research paper

Lipid extraction from *Yarrowia lipolytica* biomass using high-pressure homogenization

Lucie Drévillon, Mohamed Koubaa*, Eugène Vorobiev

Laboratoire Transformations Intégrées de la Matière Renouvelable (UTC/ESCOM, EA 4297 TIMR), Centre de Recherche de Royallieu, Université de Technologie de Compiègne, CS 60319, 60203 Compiègne Cedex, France

ARTICLE INFO

Keywords:
Yarrowia lipolytica
High-pressure homogenization
Lipid extraction
Dry biomass
Wet biomass

ABSTRACT

This work aims at investigating the impact of high-pressure homogenization (HPH) for oil recovery from *Yarrowia lipolytica* yeast. First, HPH parameters (pressure and number of passes) were optimized, by measuring the disintegration index and the release of ions, proteins, and nucleic acids during the HPH pre-treatment. Results obtained showed that 1500 bar and 5 passes allowed the maximum cell disruption, and total oil recovery in dry route (lyophilization and *n*-hexane extraction). HPH-treated cells were then extracted in wet route by directly adding *n*-hexane (without drying) and mixing using a high-speed disperser. Different ratios and extraction times were tested in order to optimize the extraction parameters. Maximum oil recovery in wet route was ≈80%, obtained using 40 min extraction, a rotation speed of 10000 rpm, and a HPH-treated suspension:*n*-hexane ratio of 1:2. Microscopy pictures, granulometry, and Peleg's model exploitation supported the results obtained in this work.

1. Introduction

Yarrowia lipolytica is an oleaginous yeast accumulating around 36% oil dry mass basis. The major fatty acids present in the wild type strain are similar to that of higher plants, mainly composed of C16 and C18 fatty acids [1]. *Y. lipolytica* has been considered as a potential oil-producer organism, able to compete with oleaginous plants, as no arable land surfaces are required for its cultivation. This feature is mainly due to the presence of numerous genetic tools [2], complete genome sequence [3], similarity with higher plants' lipid metabolism [4], and the possibility to accumulate modified fatty acids [5,6]. However, single cell oils (SCO) are still facing the high cost required during the upstream and downstream processing steps. To overcome this drawback, research works have been mainly focused in improving the content of oil accumulated in yeast, for instance in *Y. lipolytica* [4,7,8], and in redirecting the fatty acid synthesis toward the production of unusual and high-added value fatty acids (i.e. ω-3 eicosapentaenoic acid (EPA) [9]). It is believed that these achievements could be a solution to compete with plant and animal oils' prices. Besides, SCO production cost, and especially that of *Y. lipolytica*, could be further reduced by improving the downstream processing steps for oil recovery. For instance, either novel extraction processes of oil from wet biomass or the use of emerging technologies [10,11], can reduce considerably the energy required for drying. Generally, the recovery of valuable compounds follows 5 stages: (1) macroscopic pre-treatment, (2) macro- and micro-molecules' separation, (3) extraction, (4) isolation and purification, and (5) product's formation [12,13]. The review of the literature shows that the information on lipid extraction from *Y. lipolytica* is scarce. Only two recent papers were dealing with this purpose studying supercritical carbon dioxide (SC-CO_2) extraction with ethanol as co-solvent [14], and the extraction accelerated by microwave, ultrasound, thermal treatment, and bead milling [15]. Results from these studies showed that oil recovery was more efficient when adding a pre-treatment to the extraction procedure, and none of the techniques tested was efficient to recover all the oil contained in the yeast. Therefore, there is a need to explore and test other cell disruption methods, such as high-pressure homogenization (HPH). This technique is usually used for emulsification processes [16], however, it is the most employed method for the disruption of microbial cells in large scale bioprocesses [17], and its efficiency for oil recovery from algal biomass was demonstrated [18,19]. The mechanism for cell disruption using HPH consists of pumping a cell suspension and forcing its passage through a narrow gap (μm size), where it is accelerated, and undergoes numerous phenomena such as shear stress, cavitation, turbulence and friction [20,21], contributing to cell fragmentation. The review of the literature shows that there is no previous works performed using HPH as pre-treatment for oil recovery from *Y. lipolytica*. Therefore, exploring the efficiency of this cell disruption method on this oleaginous yeast is

* Corresponding author.
E-mail address: m.koubaa@escom.fr (M. Koubaa).

https://doi.org/10.1016/j.biombioe.2018.04.014
Received 19 February 2018; Received in revised form 12 April 2018; Accepted 19 April 2018

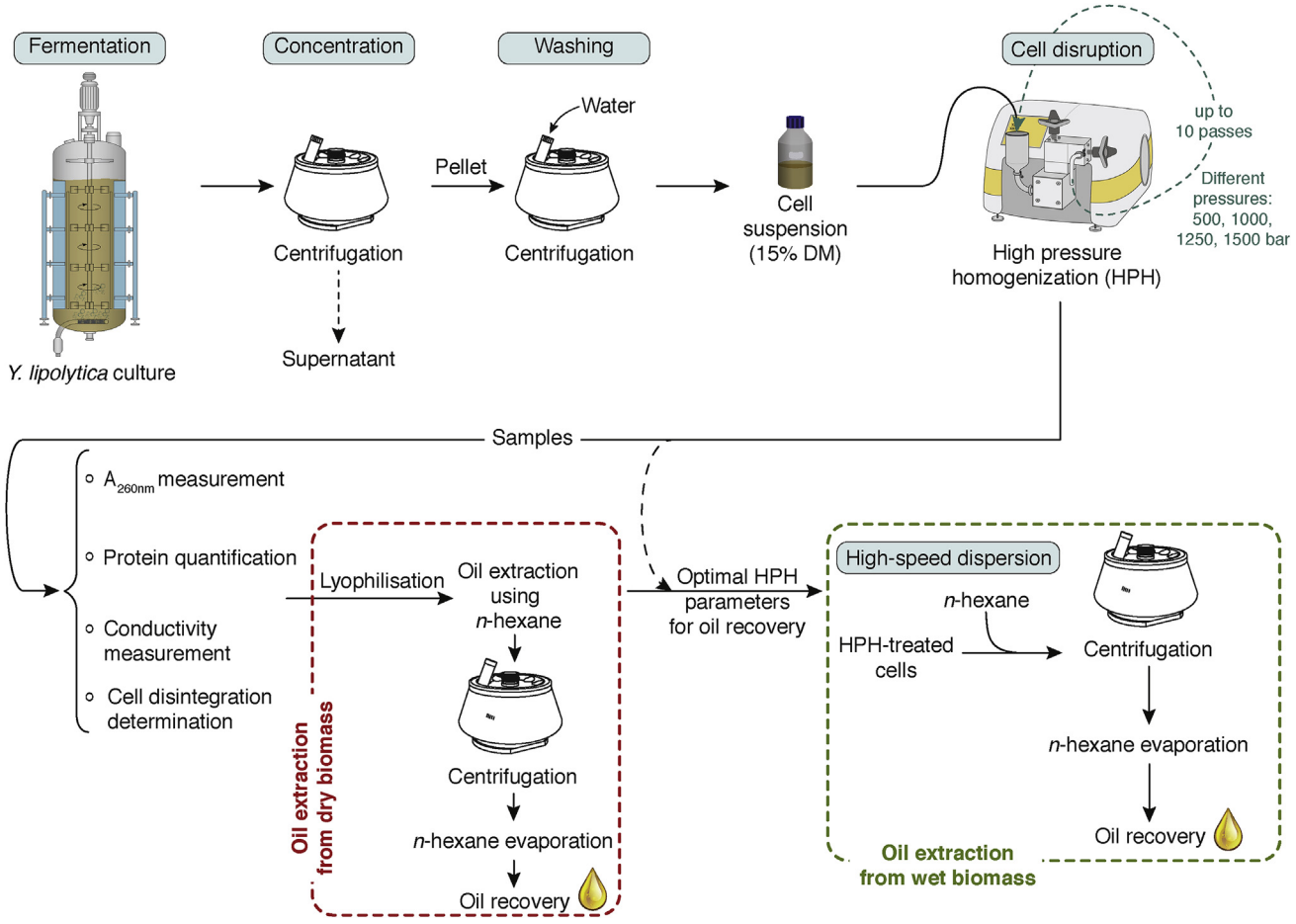

Fig. 1. Experimental set-up for HPH of *Y. lipolytica* cells and oil extraction from dry and wet biomasses.

of great importance, and this work is devoted to investigate this effect. The pressure applied for HPH as well as the number of passes were first optimized, then a comparison between dry and wet route extractions of oil from *Y. lipolytica* were discussed.

2. Materials and methods

2.1. Yarrowia lipolytica strain and culture

Y. lipolytica strain (JMY 5289) was kindly provided by the laboratory "Biologie Intégrative du Métabolisme Lipidique", INRA, UMR1319, Jouy-en-Josas, France. Strain cultures were performed and provided by the BIOGIS Center (SAS PIVERT, Venette, France). Cell recovery was performed by culture broth centrifugation for 10 min at 3076 *g* (4000 rpm) using a Sigma 3-16P instrument (Fisher Bioblock Scientific). To remove medium traces, two washing steps were performed by resuspending the pellet in one volume of distilled water followed by 40 min centrifugation at 3076*g* (4000 rpm). The final biomass concentration was adjusted with distilled water to get 15% dry matter cell (DM) suspension. Dry matter was determined using an infrared desiccator (Sartorius, France).

2.2. Oil quantification in Y. lipolytica

Oil content in *Y. lipolytica* was quantified after bead milling pretreatment. First, wet biomass obtained after the washes was lyophilized using Alpha 1–4 LD Plus equipment (Martin Christ, Germany). Then, 100 mg of lyophilized samples were introduced in triplicate into screw tubes, in presence of a ceramic bead, and disrupted using a Precellys-24 homogenizer/grinder (Ozyme, France) for 3 × 30 s at 6000 counts per minute (cpm). Oil extraction was then performed by adding 1 mL of chloroform/methanol mixture (2:1, v:v) followed by rigorous shaking for 3 × 30 s at 6000 cpm using the same homogenizer/grinder. After a centrifugation step during 3 min at 6000 *g*, recovered supernatant from each tube was transferred to a new tube and the extraction was repeated under the same conditions. Non-lipidic compounds in the recovered supernatants (2 mL) were removed by adding 2 mL of KCl (1 M)/methanol solution (4:1, v:v, with 0.034% of $MgCl_2$), and shaking rigorously for 2 min. All tubes were then stand until the separation of phases. The lower phase containing lipids was recovered and evaporated under nitrogen flow at 50 °C. The recovered oil was then quantified by gas chromatography – flame ionization detector (GC-FID). For this purpose, 100 µL of 5 mg/mL C12:0, used as internal standard for oil quantification, and 750 µL of methanolic HCl (4%) were added. The reaction was conducted at 80 °C during 1 h to allow the formation of fatty acid methyl esters (FAMEs), which were then extracted by adding *n*-heptane (1.5 mL), vortexing rigorously, and centrifuging during 2 min at 100 *g* (1000 rpm). FAMEs extracted in the upper phases were then transferred into new tubes, and the extraction was repeated under the same conditions. The pooled supernatants were first evaporated under nitrogen flow, and FAMEs were then resuspended in *n*-heptane and analyzed using a Shimadzu GC-FID equipment as previously demonstrated [22], with slight modifications. The GC oven, equipped with a BPX-70 capillary column (30 m length, 0.25 mm diameter, and 0.25 mm film thickness) was set first at 120 °C and held for 2 min, then increased to 250 °C at 10 °C/min and held for 2 min. The carrier gas used was H_2 and was set at a flow rate of 1.2 mL/min. The injection volume was 1 µL with a split of 10, and standard molecules of FAMEs were injected for fatty acid identification. Oil content in *Y. lipolytica* was determined based on the quantified C12:0 (internal standard) and