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Short Communication

Production of polyhydroxyalkanoates from spent coffee grounds oil obtained by supercritical fluid extraction technology

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HIGHLIGHTS

- Oil from spent coffee grounds was extracted with supercritical carbon dioxide.
- An oil extraction yield of 90% was achieved at a CO_2/SCG mass ratio of 35 kg kg^{-1}.
- *Cupriavidus necator* was cultivated with SCG oil as sole carbon source.
- The cells accumulated up to 78% (w/w) poly-3-hydroxybutyrate.
- The polymer exhibited similar properties to other P(3HB) biopolymers.

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ABSTRACT

Spent coffee grounds (SCG) oil was obtained by supercritical carbon dioxide ($scCO_2$) extraction in a pilot plant apparatus, with an oil extraction yield of 90% at a 35 kg kg^{-1} CO_2/SCG ratio. *Cupriavidus necator* DSM 428 was cultivated in 2 L bioreactor using extracted SCG oil as sole carbon source for production of polyhydroxyalkanoates. The culture reached a cell dry weight of 16.7 g L^{-1} with a polymer content of 78.4% (w/w). The volumetric polymer productivity and oil yield were 4.7 g L^{-1} day^{-1} and 0.77 g g^{-1}, respectively. The polymer produced was a homopolymer of 3-hydroxybutyrate with an average molecular weight of 2.34×10^5 and a polydispersity index of 1.2. The polymer exhibited brittle behaviour, with very low elongation at break (1.3%), tensile strength at break of 16 MPa and Young's Modulus of 1.0 GPa. Results show that SCG can be a bioresource for polyhydroxyalkanoates production with interesting properties.

1. Introduction

After coffee processing and consumption, spent coffee grounds (SCG) are generated in large quantities: 6.0 Mton are estimated to be generated worldwide every year (Tokimoto et al., 2005). SCG are a lignocellulosic material and their chemical composition varies depending on the coffee beans source. They have a lipid content of up to 20% (w/w) (Al-Hamamre et al., 2012; Andrade et al., 2012; Kondamudi et al., 2008).

Conventional oil extraction from SCG involves the use of hazardous organic solvents, such as *n*-hexane. Supercritical fluid extraction (SFE) provides an environmentally friendly alternative whereby extraction/separate recovery of oil and bioactive compounds from biomass can be done without their degradation (Brunner, 1994). Carbon dioxide is the most popular SFE solvent because it is non-flammable, readily available and has a low cost. Through manipulation of temperature and pressure, the density of $scCO_2$ can be adjusted to allow complete separation of oil and bioactive solutes contained in the matrix. Recently, the feasibility of SCG oil extraction by $scCO_2$ has been demonstrated (Couto et al., 2009).

Polyhydroxyalkanoates (PHAs) are biocompatible and biodegradable polyesters that exhibit physical–chemical, thermal and mechanical properties very similar to those of conventional plastics (Akaraonye et al., 2010). Low cost oil-containing wastes/byproducts have been proposed as carbon sources for PHA production, since they exhibit high product to substrate yield (up to 0.8 g g^{-1}) (Akaraonye et al., 2010; Obruca et al., 2010). *Cupriavidus necator* is a well known poly-3-hydroxybutyrate P(3HB) producer, able to reach high cellular content (up to 87%) using oleic substrates, namely jatropha oil (Ng et al., 2010), pure rapeseed and

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waste frying oils (Verlinden et al., 2011), and soybean oil (Park and Kim, 2011). To the best of the authors' knowledge, PHA production from SCG oil extracted with $scCO_2$ was not previously reported.

The main goal of the present work was to evaluate the production of PHA from SCG oil extracted by $scCO_2$. The SFE extraction process was scaled up and optimal conditions and efficiency of oil extraction were determined in a pilot plant. A continuous process for the supercritical extraction of SCG oil was implemented and the resulting oil was used as feedstock for the production of PHA by *C. necator* DSM 428. The final product was purified and characterized.

2. Methods

2.1. SCG oil characterisation

SCG was supplied by NovaDelta (Comércio e Indústria de Cafés, S.A. Portugal). SCG was dried at 105 °C, for 12 h, to remove moisture. The residual moisture content of dry SCG was measured by Karl Fischer titration.

The oil content of dry SCG was determined by Soxhlet extraction using a ratio of 20 g of SCG to 200 mL *n*-hexane, at 69 °C, for 6 h. *n*-hexane was evaporated using a Büchi Rotavapor.

The free fatty acid and unsaponifiable fraction content of SCG oil were determined according to the AOCS Official Methods Cd 3d-63 and Ca 6a-40, respectively. The fatty acid composition of the SCG oil was determined by direct transesterification of the lipids to the corresponding methyl esters, according to a modified Lepage and Roy method (Couto et al., 2009). The mono-, di- and triglycerides content of SCG oil were analysed according to the European norm EN 14105.

2.2. Supercritical oil extraction from SCG

The supercritical extraction of SCG oil was conducted in a semi-continuous high pressure extraction pilot unit equipped with four extractors of 2 L each (internal diameter 6.4 cm; total length 59.6 cm), which can operate in parallel or in series in a countercurrent, continuous mode. The extracted SCG oil was collected from the separators at 10 min intervals, weighed and stored in sterile and light protected flasks, at −20 °C.

2.3. PHA production

Cupriavidus necator DSM 428 was cultivated in a mineral medium with the composition described by Freitas et al. (2009) and supplemented with 20 g L^{-1} of SCG oil.

Inocula for bioreactor cultivations were prepared by inoculation of LB grown cells into mineral medium supplemented with SCG oil (20 g L^{-1}) and incubation for 48 h, at 30 °C and 200 rpm. *C. necator* was cultivated in a 2 L bioreactor (BioStat B-Plus, Sartorius, Germany), with an initial working volume of 1.5 L and a 10% (v/v) inoculum. Samples were periodically withdrawn from the bioreactor for quantification of biomass, PHA and nutrients.

For cell dry weight (CDW), residual oil and PHA quantification, the broth (7 mL) was mixed with *n*-hexane (1:3 v/v) and centrifuged (15 777 *g*, 10 min), resulting in three fractions: a biomass pellet, an aqueous cell-free supernatant and an upper hexane layer containing the residual oil. The biomass pellet was washed twice with deionized water, and lyophilized to gravimetrically quantify the CDW. For quantification of the residual oil, 5 mL of the upper hexane layer containing the residual oil were transferred to pre-weighed tubes and placed in a fume hood at room temperature for 72 h, for solvent evaporation. The residual oil was gravimetrically quantified and its fatty acids composition was analysed as described in Section 2.1. PHA content and composition were determined after methanolysis of dried cells samples (2–3 mg) in 1 mL 20% (v/v) sulphuric acid in methanol and 1 mL heptadecane (internal standard) in chloroform (1 g L^{-1}), at 100 °C during 3.5 h. All analyses were performed in duplicate.

2.4. PHA extraction and characterisation

The polymer was extracted from the lyophilized cells (previously washed as described in Section 2.3), using chloroform as solvent (1 g of dry cells per 50 mL of $CHCl_3$), at 37 °C, 250 rpm, over 24 h. The solution was filtered (Filter-Lab, Ac cellulose, 0.2 μm/47 mm) under vacuum to remove cell debris, and the polymer was precipitated twice by adding the solution drop-by-drop into cold ethanol (1:10), under vigorous stirring, and dried at room temperature.

Polymer composition was determined by GC as described in Section 2.3. The polymer's average molecular weight was determined by Size Exclusion Chromatography (SEC), as described by Serafim et al. (2008). Thermal analysis was performed by differential scanning calorimetry (DSC), as described by Fiorese et al. (2009). Homogeneous films were prepared by solvent casting of P(3HB) solutions (20 g L^{-1}) in chloroform and slow solvent evaporation in a fume hood, at room temperature. Tensile tests of the films were carried out using a TA-Xtplus texture analyser (Stable Micro Systems, Surrey, England). Film strips (20 × 70 mm) attached on tensile grips A/TG were stretched at 0.5 mm s^{-1} in tension mode.

3. Results and discussion

3.1. $ScCO_2$ oil extraction from SCG

In order to evaluate the total oil content in SCG, Soxhlet extraction of SCG with *n*-hexane was performed resulting a maximum oil yield of 14.0% (w/w) on a dry weight basis (g oil per 100 g of dry SCG), which is within the range of values reported in the literature (12–15.3%, w/w) (Andrade et al., 2012; Al-Hamamre et al., 2012).

The feasibility of SCG oil extraction by supercritical CO_2 was demonstrated in a preliminary work (Couto et al., 2009) using a lab-scale high pressure extraction apparatus and a different SCG raw material. It was shown that $scCO_2$ extracted up to 85% of the total amount of SCG oil after 3 h of extraction at the best operating conditions of 50 °C and 25 MPa.

To extract the necessary amounts of SCG oil for PHA production, a scale-up of the supercritical extraction process was required using a pilot plant. The scale-up of the lab scale extraction process to pilot scale introduced variations to the operation parameters. In particular, the $scCO_2$ flow rate affects the SCG oil extraction yield. Thus, different CO_2 flow rates were assessed to determine the optimum CO_2:SCG mass ratio. The $scCO_2$ mass flow rate was varied in the range 7–19 kg h^{-1}, at the same temperature and pressure values of 50 °C and 25 MPa, respectively, as optimised in the lab scale apparatus. In these runs, only one extraction vessel was used, loaded with 0.5 kg of dry SCG.

The effect of the solvent flow rate on the yield of extraction of SCG oil is shown in Fig. 1. Higher CO_2 flow rates increased the extraction rate of SCG oil since more solvent molecules were being fed to the extractor per unit time. Still, CO_2 was able to extract nearly all of the SCG oil irrespective of the solvent flow rate. At an S/F of 35, *ca.* 90% of the total amount of SCG oil available was extracted. For the highest solvent flow rate of 19 kg h^{-1}, maximum concentration of oil was achieved after *ca.* 1 h of extraction, while decreasing the $scCO_2$ flow rate to 7 kg h^{-1} led to extraction times that were twice as high. High solvent flow rates increase the