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Short communication

Production of polyhydroxyalkanoates using hydrolysate of spent coffee grounds

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ABSTRACT

Spent coffee grounds (SCG) are solid fraction wastes deriving from coffee industries, the disposal of which represents a serious environmental issue. This work aims at the conversion of hydrolysate of SCG (SCGH) into polyhydroxyalkanoates (PHA) by *Burkholderia cepacia*. The bacteria was capable of SCGH utilization and production of copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate [P(HB-co-HV]. Levulinic acid present in SCGH probably served as the precursor of 3HV for the copolymer biosynthesis. To improve the PHA yields, various detoxification methods were tested. The extraction of polyphenols from SCG by ethanol prior to the hydrolysis seems to be the most promising, since, apart from the fact that it enhanced the PHA yields by about 25%, polyphenols extracted from SCG may represent important side products, because they might be used for the production of functional foods and other high value products.

1. Introduction

Polyhydroxyalkanoates (PHA) are polyesters synthesized and stored in bacterial cells in the form of intracellular granules. Bacteria use PHA as carbon, energy and reducing power storage materials. PHA have attracted much attention as biodegradable alternative to traditional petrochemical plastics, their properties and potential applications were extensively reviewed by Philip et al. [1].

Among PHA, the homopolymer of 3-hydroxybutyrate, poly(3-hydroxybutyrate) (PHB), is the most abundant and the best characterized polymer to date. However, PHB possesses several properties, such as high crystallinity, melting point temperature and low flexibility, which complicate its processing and also limit the range of its application. The mechanical properties of this polymer can be significantly improved by the incorporation of 3-hydroxyvalerate (3HV) units into the PHA structure resulting in the formation of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(HB-co-HV)] with better mechanical properties and processability [2].

Since about 45% of the total costs of PHA production are ascribed to carbon sources, such as refined glucose or sucrose [3], cheap wastes or side products of agriculture and food industry are used as inexpensive carbon substrates improving thus the economic feasibility of the PHA production [4]. Moreover, cellulosic and hemicellulosic biomass are considered being very promising renewable sources for the biotechnological production of fuels and chemicals, including PHA [5]. Therefore, the PHA production from *Burkholderia cepacia*, *Burkholderia sacchari* [6] and *Ralstonia eutropha* [7] has been reported by utilizing sugarcane bagase hydrolysate. More recently, Pan and his colleagues studied the PHA production from detoxified sugar maple hemicellulosic hydrolysate by *B. cepacia* [8,9], Zhang et al. utilized oil palm empty fruit bunch for the PHB production using *Bacillus megatrerium* [10] and *B. sacchari* was employed for the PHA production from wheat straw hydrolysate [11].

Coffee is one of the world' most popular beverages and has grown steadily in commercial importance during the last 150 years. Nowadays, coffee is, after petroleum, the second largest traded commodity in the world. In 2010 the worldwide annual production of coffee beans exceeded 8 million tons [12]. During the preparation of coffee beverage or the manufacturing of instant coffee, raw coffee powder is mixed with hot water or steam under the conditions of favoring the release of aroma compounds and other coffee-bean constituents into the liquid, which generates solid residues known

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as spent coffee grounds (SCG). On an average, the manufacturing of one ton of green coffee generates about 650 kg of SCG [12]. Despite the fact that this waste stream of coffee industry has been considered as a raw substrate for the production of various compounds such as polyphenols [13,14], bioethanol [15,16], biodiesel [16,17] or mannooligosaccharides [18], the most of this residue remains unutilized, being discharged to the environment or burned for elimination, which can be considered neither as environmentally friendly nor economically feasible techniques [12].

We have recently investigated the utilization of oil extracted from SCG for the PHA production [19]. Hence, we aimed at the valorization of residual SCG after the oil extraction. To our best knowledge, there is no report on the utilization of cellulosic biomass of SCG for the biosynthesis of polyhydroxyalkanoates. Therefore, in the present work, the ability of *B. cepacia* to produce PHA from SCG was tested. Moreover, various detoxification methods were used to improve the fermentability of SCG based media and thus, the PHA yields obtained on this substrate.

2. Materials and methods

2.1. Raw material preparation

Spent coffee grounds (SCG) were obtained from a coffee automat machine at the Faculty of Chemistry, Brno University of Technology, Czech Republic. The waste material was firstly dried to the constant weight (80 °C for 24 h). The coffee oil extraction was performed with n-hexane as described by Al-Hamamre et al. [17]. To hydrolyze the hemicelluloses of raw material, 15% (w/v) SCG were firstly treated by 1 vol.% H_2SO_4 for 90 min at 121 °C [16]. In the next step, the enzymatic digestion was used to break up cellulose releasing fermentable saccharides. Therefore, pH of the suspension after the acidic hydrolysis was set to 4.5 (10 M NaOH) and cellulose was treated by 0.5% (vol/vol) of Celluclast 1.5 L (Novozymes A/S, Bagsværd, Denmark) at 50 °C under permanent shaking (150 rpm) for 24 h [16]. At the end of the process the solids obtained after enzyme treatments (representing approx. 30% of SCG after oil extraction) were removed by filtration and the permeate, called spent coffee grounds hydrolyzate (SCGH), was used for the cultivation media preparation and the PHA production.

2.2. Analysis of SCGH composition

The concentration of dry matter was estimated by drying 10 ml of SCGH to constant weight at 105 °C. Ash content was determined as the weight of solids obtained after the incubation of 2 ml of SCG-media at 800 °C for 2 h. Subsequently, the phosphorus content of ash was measured by Ion Chromatography (850 Professional IC, Metrohm, Switzerland) using Metrosep A Supp 7-250/4.0 column. Particular sugars contents (cellobiose, glucose, mannose, galactose and arabinose) were estimated by liquid chromatography (pump LCP 4020, thermostat LCO 101, degasser DG-1210, refractometric detector RIDK 102; Ecom, Czech Republic) with REZEX-ROA column (150 mm × 4.6 mm, 5 μm; Phenomex, USA). The determination of total sugars by Somogyi-Nelson method was employed for routine analysis of sugars. The concentrations of levulinic acid, 5-hydroxymethyfurfural and furfural were determined by gas chromatography equipped with flame ionization detector (GC-FID, Trace GC Ultra, Thermo Scientific) according to Omari et al. [20]. The concentration of nitrogen compounds was determined by Kjehdal method and the overall protein content was calculated using the conversion factor of 6.25. The total phenolics content was estimated using the Folin–Ciocalteu reagent method [21]. All analyses were performed at least in triplicate and the results were evaluated as mean values with SD values.

2.3. Microorganisms and cultivations

B. cepacia CCM 2656 (ATCC 17759) was purchased from the Czech Collection of Microorganisms. The bacterial culture was cultivated in mineral salt medium described by Bertrand et al. [22]. SCGH, salt solutions and microelement solutions were autoclaved separately (115 °C, 20 min) and then aseptically reconstituted at room temperature prior to the inoculation to reach desired portion of SCGH in the medium and final concentration of salts. The cultivations were performed in Erlenmeyer flasks (volume 250 ml) containing 50 ml of the cultivation media. The temperature was set to 30 °C and the agitation to 180 rpm. After 72 h of cultivation, the cells were harvested (centrifugation, 8000 × g, 5 min) and the biomass as well as the PHB yields were determined as described below. All cultivations were performed in triplicate.

Table 1
Composition of SCGH used in this study (150 g SCG per liter).

Parameter	Concentration/value
Dry solid matter ($g\,l^{-1}$)	98.4 ± 0.1
Ash ($g\,l^{-1}$)	27.6 ± 0.8
Conductivity ($mS\,cm^{-1}$)	23.2
PO_4^{3-} ($g\,l^{-1}$)	0.32 ± 0.03
Total nitrogen (wt.%)	0.162 ± 0.1
Proteins (N × 6.25) ($g\,l^{-1}$)	10.2 ± 0.6
Polyphenols ($g\,l^{-1}$)	3.6 ± 0.1
Levulinic acid ($g\,l^{-1}$)	1.72 ± 0.19
5-Hydroxymethyl furfural ($g\,l^{-1}$)	0.15 ± 0.03
Furfural ($g\,l^{-1}$)	*n.d.*
Total sugars ($g\,l^{-1}$)	50.1 ± 2.2
Cellobiose ($g\,l^{-1}$)	2.7 ± 0.3
Galactose ($g\,l^{-1}$)	17.3 ± 0.3
Mannose ($g\,l^{-1}$)	23.6 ± 0.6
Arabinose ($g\,l^{-1}$)	2.8 ± 0.2
Glucose ($g\,l^{-1}$)	3.9 ± 0.2

Results are in form average ± standard deviation.
n.d. – not detected.

2.4. PHA analysis

The biomass concentration was analyzed gravimetrically, the samples were centrifuged and the cells were washed with 5% (vol/vol) Triton X (10 ml) and distilled water, respectively, and afterwards they were dried (105 °C) to the constant weight. The PHB content of dried cells was analyzed by gas chromatography (Trace GC Ultra, Thermo Scientific, USA) as reported by Brandl et al. [23]. Commercially available P(HB-co-HV) (Sigma–Aldrich, Germany, HV content 12 mol.%) was used as a standard; benzoic acid was used as an internal standard. The molecular weight of PHB was determined by gel permeation chromatography (Agilent 1100 Series; column PLgel Mixed B (300 9 7.5 mm; 10 lm)).

The Differential Scanning Calorimetry (DSC) (204 F1, Netzsch) was used for determination of thermal properties of produced material as described elsewhere [2]. Thermogravimetric apparatus (TGA) (Q500; TA Instruments) was used for evaluation of thermal stability of the PHB.

2.5. Detoxification of SCGH

Several methods which should reduce the amount of toxic substances in SCGH were tested in this study. Polyphenols were extracted from SCG prior to its hydrolysis using various mixtures of distilled water and ethanol (0, 30, 45 and 60 vol.% of ethanol). The polyphenol extraction from 20 g of SCG was performed using 40 ml of particular solvent for 2 h at 50 °C under constant shaking (150 rpm). After the extraction, SCG was filtered, dried and hydrolyzed as described above. The control was prepared identically; however, SCG was not subjected to the extraction.

Further, the overliming was performed with SCGH (after hydrolysis) as described by [8], also the activated charcoal (AC) detoxification was employed; AC was mixed with SCGH in the ratio of 1:20 (w/v) and stirred for 1 h at 60 °C [8].

3. Results and discussion

3.1. Composition of SCGH

To hydrolyze SCG, the combination of diluted acid hydrolysis (1% H_2SO_4) of hemicelluloses and enzymatic execution of cellulose was applied. This approach yielded liquid hydrolysate of SCG (SCGH) the composition of which is shown in Table 1. The total concentration of sugars was determined to be 50.1 $g\,l^{-1}$ (by the hydrolysis of 150 $g\,l^{-1}$ of SCG), the major sugars identified in SCGH are mannose (23.6 $g\,l^{-1}$) and galactose (17.3 $g\,l^{-1}$) followed by glucose (3.9 $g\,l^{-1}$), arabinose (2.8 $g\,l^{-1}$) and cellobiose (2.7 $g\,l^{-1}$). Unlike in other hemicellulose hydrolysates such as wheat straw [13] or maple hemicellulosic hydrolysate [10] containing significant portion of pentoses, SCGH contains only low portion of arabinose. The fact that hexoses are substantially dominating sugars of SCGH may be an important factor positively influencing the PHA production. The metabolic pathways of hexoses utilization generates larger amount of energy, which might, in consequence, enhance the PHB yields. Lopes et al. reported the theoretical ATP/3HB monomer ratio being 3 mol/mol in case of xylose compared to the value of 7 mol ATP/mol 3HB in glucose [24].