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Utilization of residues from agro-forest industries in the production of high value bacterial cellulose

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ABSTRACT

Bacterial cellulose (BC), a very peculiar form of cellulose, is gaining considerable importance due to its unique properties. In this study, several residues, from agro-forestry industries, namely grape skins aqueous extract, cheese whey, crude glycerol and sulfite pulping liquor were evaluated as economic carbon and nutrient sources for the production of BC. The most relevant BC amounts attained with the residues from the wine and pulp industries were 0.6 and 0.3 g/L, respectively, followed by biodiesel crude residue and cheese whey with productions of about, 0.1 g/L after 96 h of incubation. Preliminary results on the addition of other nutrient sources (yeast extract, nitrogen and phosphate) to the residues-based culture media indicated that, in general, these BC productions could be increased by ~200% and ~100% for the crude glycerol and grape skins, respectively, after the addition organic or inorganic nitrogen.

1. Introduction

The massive exploitation of fossil resources during the last century associated to pollution problems raised a considerable number of environmental and economic concerns. Moreover, fossil resources are predictable to be depleted in the near future. All of these reasons are contributing to a progressive transition to an economy based on renewable materials (biomass) as feedstock for the production of chemicals, materials, fuels and energy within the so-called biorefinery concept (Octave and Thomas, 2009). Under this context, polymers from renewable resources, like polysaccharides, proteins and lignin, among others, are gaining considerable and increasing attention. Cellulose is one of the most abundant polysaccharides and is considered as an inexhaustible and unique source of new materials for a wide number of applications (Huber et al., 2006).

Although most of the cellulose available on earth is produced by plants, some microorganisms such as algae, fungi and bacteria are also able to produce an extra-cellular form of cellulose. For instance, some bacteria belonging to the genera *Gluconacetobacter, Sarcina* or *Agrobacterium* are able to produce a particular type of cellulose, designated as bacterial cellulose (BC). BC presents very interesting properties such as high purity, since it is not associated with hemicelluloses and lignin as in plants (Klemm et al., 2001). BC bears also unique physical and mechanical properties that arise from its tridimensional and branched nano and micro-fibrillar structure (Iguchi et al., 2000). Finally, BC shows biocompatibility (Czaja et al., 2006; Helenius et al., 2006). These singular characteristics triggered considerable interest on BC, particularly in the biomedical area. Some examples of applications are as wound healing membranes for substituting natural skin (Czaja et al., 2006), chirurgical implants (Backdahl et al., 2006), but also other high added value applications such as membranes for audio devices (Iguchi, 1988), electronic paper (Shah and Brown, 2005) and optically transparent nanocomposites (Fernandes et al., 2009; Nogi et al., 2005; Nogi and Yano, 2008), among others.

Traditionally, BC is produced from expensive culture media, containing glucose as carbon source and other nutrient sources resulting in very high production costs, which limits the application of this material to very high value added applications. The use of cheap carbon and nutrient sources, such as agro-forestry industrial residues, is an interesting strategy to overcome this limitation and therefore to increase the competitiveness of this unique material. Under this context, some industrial wastes or by-products, namely Konjac powder (Hong and Qiu, 2008), beet molasses (Keshk et al., 2006), sugar-cane molasses and corn steep liquor (El-Saied et al., 2008), several fruit juices, including orange, pineapple, apple, Japanese pear and grape (Kurosumi et al., 2009) and coconut water (Kongruang, 2008) were already successfully used as carbon sources for the production of BC.

However, many other abundant agro-forestry and industrial by-products or residues could potentially be effectively used in this context. Moreover, this would also contribute to the upgrading of such residues with a positive impact in the corresponding industry and in local and national economies. Finally, this utilization of industrial residues for the production of added-value products

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represents an important contribution to the implementation of the biorefinery concept and to the decrease of environmental problems associated with the disposal of industrial wastes.

Winery, milk, pulping and biodiesel industries represent important sectors of the Portuguese economy and generate high amounts of industrial residues, namely, grape skins and wine must, cheese whey, pulping liquors and crude glycerol (the main residue from biodiesel production), respectively. These residues possess high organic loads and are rich in nutrients suitable for microbial growth. In fact, the sugars of spent sulfite pulping and grape skin liquors have already been used for the production of bioethanol (Xavier et al., 2010), cheese whey lactose was used for the production of bioplastics (Guimaraes et al., 2010; Koller et al., 2007) and crude glycerol for the production of polyhydroxybutyrate (Dobroth et al., 2011). However, from the aforementioned residues, only cheese whey was tested as a carbon and nutrient source for the production of BC (Thompson and Hamilton, 2001).

The objective of the present work is to evaluate the possibility of using several relevant residues from Portuguese agro-forest industries, namely the aqueous extract of grape skins, cheese whey, sulfite pulping liquor and crude glycerol, as carbon and nutrient sources for BC production in static condition. The use of these raw materials will result not only in the decrease of the production cost of BC but also in the upgrading of these residues. Consequently, this work intends to contribute for a gradual implementation of the biorefinery concept in aforementioned industries. In this perspective, after isolating a BC producing bacteria, the BC productivity was evaluated in a reference Hestrin and Schramm (HS) culture media (Hestrin and Schramm, 1954) containing glucose, or the main components of the selected residues and finally with the residues in different concentrations, with and without supplementation with N and P sources. The BC samples were then characterized by FTIR, XDR and SEM.

2. Methods

2.1. Materials and reagents

Glucose-Glu – (96% purity), galactose-Gal – (97% purity), lactose-Lac – (99% purity), xylose-Xyl – (98% purity) and glycerol-Gly – (99.5% purity) were purchased from Sigma Chemicals. Bacteriological Agar, Yeast Extract, and Bacteriological Peptone were purchased from Himedia. The other chemicals were of analytical grade.

2.2. Isolation of cellulose producing bacteria

The BC producing microorganisms were isolated from a commercial food source, Kombucha tea. One milliliter of the tea was diluted in physiological solution (serial dilution) and spread in Petri dishes with HS medium (20 g/L glucose, 5 g/L peptone, 5 g/L yeast extract, 2.7 g/L Na_2HPO_4, 1.15 g/L citric acid, agar, 15 g/L, pH 5). After 5 days at 30 °C, each grown colony was isolated in a Petri dish containing HS agar and tested for BC production in test tubes containing 10 mL of HS liquid medium. The culture was maintained at 4 °C on HS agar. The bacterial strain was identified (by Nadicom-Gesellschaft für angewandte Mikrobiologie mbH, Germany) as *Gluconacetobacter sacchari*.

2.3. Industrial residues

Industrial hardwood Spent Sulfite Liquor (SSL) from magnesium based acidic sulfite pulping of *Eucalyptus globulus* was supplied by Caima-Industria de Celulose SA (Constância, Portugal) and subsequently treated as previously reported by Xavier et al. (2010).

Grape skins (GS), resulting from white grape bagasse, were kindly donated by Quinta do Serrado, Penalva do Castelo, Portugal. The grape skins liquor was obtained after an aqueous extraction at refluxing temperature.

Bovine whey powder, or cheese whey (Cwh), was purchased from Lactogal, Portugal. Crude whey solution was prepared following the procedure of Ahn et al. (2000).

Crude glycerol residue (Cgly), was supplied by Fábrica Torrejana de Biocombustíveis S.A., Portugal and utilized without pretreatment.

Then all the residues were diluted and autoclaved before use, SSL, GS and Cwh were stored at 4 °C and Cgly at room temperature.

2.4. Determination of carbon sources concentration

Glucose, xylose, glycerol, lactose and galactose were analysed by HPLC using 10 μm Eurokat® (Knauer) ion-exchange column, 300 × 7.5 mm, and a refraction index (RI) detector Gilson 131. The eluent, aqueous sulfuric acid solution (0.01 N), was pumped at a flow rate of 0.4 $ml.min^{-1}$ (25 °C). The injected volume was 20 μL. All samples were centrifuged and filtered off with 0.22 μm filters before the analysis. The absolute calibration was applied for all analysed compounds (Xavier et al., 2010).

2.5. BC production and culture conditions

2.5.1. Pre-inocula preparation

The pre-inocula were prepared by growing the microorganisms at 30 °C during 48 h, in static condition, in HS liquid medium before inoculation (10% v/v) into a 50 mL liquid production medium in 500 mL Erlenmeyer flasks.

2.5.2. BC production using the main pure compounds of each residue

The studies with pure carbon sources were performed with HS medium but, glucose was substituted by the selected compound at 20 g/L, namely xylose, glucose:galactose (1:1), lactose and glycerol, for SSL, GS, Cwh and Cgly, respectively.

The initial pH value of the media was adjusted to 4.5 (Embuscado et al., 1994) and it was not controlled during flask cultivation. All the experiments were carried out in duplicate and under sterile conditions. The flasks were kept at 30 °C, in a static incubator, for 96 h. The pre-inocula were prepared as in Section 2.5.1.

2.5.3. BC production in culture media composed by residues at 20 g/L

The residues, at a concentration of 20 g/L (the same concentration of glucose in HS medium), were used as carbon and nutrient source for BC production without any supplementation. All the experiments were carried out in duplicate and the preparation of the pre-inocula and operational conditions were the same as described in Section 2.5.1 and 2.5.2, respectively.

2.5.4. BC production in culture media composed by diluted residues

In these experiments, the residues were tested for the production of BC in several dilutions, namely: SSL – 1:1, 1:4 and 1:10; GS – 1:1, 1:4, 1:10; Cwh – 1:4, 1:20, 1:50, 1:100; Cgly – 1:10, 1:25, 1:50, 1:100. All the experiments were carried out in duplicate and the preparation of the pre-inocula and operational conditions were the same as described in Section 2.5.1 and 2.5.2, respectively.

For each residue, after the selection of the dilution that led to the highest amount of BC produced, a batch test with the selected value was performed in order to study in more detail the BC production for the determination of substrate conversion ratio, BC production rate and BC production yield.