**Biorefinery development using spent coffee grounds for the products of bacterial cellulose and value-added products**

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**Highlights**

* Phenolic-rich extracts, oil and protein can be extracted from spent coffee grounds
* Hemicellulose hydrolysate has been used for bacterial cellulose production
* Spent coffee grounds is a promising resource for biorefinery development

**1. Introduction**

The development and implementation of biorefineries dealing with renewable resources strongly depends on the heterogeneity and availability of the feedstock in combination with the profitability of the whole process. Spent coffee grounds (SCGs) are generated as waste streams through the production of instant coffee and coffee brewing and they amount to approximately 6 million t annually [1]. SCGs are rich in polysaccharides and protein [2]. The high carbohydrate content renders SCGs a promising substrate for bioprocesses including carotenoids, poly-(hydroxyalkanoates), enzymes and bioethanol [3]. The recovery of natural antioxidants (mainly caffeine), proteins and lipids contained in SCGs, could enhance the viability and sustainability of a biorefinery development. Under this concept, the main objectives of this study were the fractionation of SCGs for the recovery of value-added products and the production of bacterial cellulose from hemicellulosic hydrolysates derived after diluted acid or enzymatic hydrolysis of hemicellulose.

**2. Methods**

The fractionation of SCGs included the extraction of phenolic compounds, oil, protein and carbohydrates. Initially, phenolic compounds were extracted and evaluated in terms of total phenolic content (TPC) (via Folin-Ciocalteu method) and antioxidant activity index (AAI) (via 2,2-diphenyl-1-picrylhydrazyl-free radical scavenging method) using ethanol as solvent under different solid to liquid ratios and extraction time. The main phenolic compounds of the extract were identified and quantified employing HPLC-DAD. The phenolic-free solids were subsequently used for oil extraction. The SCGs to solvent ratio and the extraction time were evaluated. The remaining SCG solids were used for protein recovery. The extraction process included alkaline treatment followed by acidic precipitation at the isoelectric point of the corresponding proteins. At a final step, the remaining lignocellulosic-rich solids were pretreated by mild chemical and enzymatic processes to the respective monomeric sugars. The galactose and mannose rich hydrolysate obtained was utilized for bacterial cellulose production by *Komagataeibacter sucrofermentans* DSM 15973 under batch mode. The physiochemical properties of the produced bacterial cellulose were determined and evaluated.

**3. Results and discussion**

Phenolic compounds from SCGs were extracted applying an environmental friendly and cost-effective process using aqueous ethanol under mild temperature conditions. SCGs exhibited the highest TPC (15 mg gallic acid equivalents-GAE/ g dry sample) with a liquid-to-solid ratio of 10:1 after 20 minutes of extraction. According to the obtained AAI, extracts were classified as strong antioxidants. Extraction of oil with hexane and n-propanol resulted in high oil recovery (higher than 95%). Protein extraction reached a recovery yield of 92% with high purity.

The hemicellulosic hydrolysate rich in mannose and galactose was utilized as the sole carbon source for bacterial cellulose production with the bacterial strain *K. sucrofermentans*. Bacterial cellulose concentration of 2.4 g/L was achieved after 6 days of fermentation. Properties determination of the produced bacterial cellulose showed enhanced water holding capacity and degree of polymerization. The IR spectrum of bacterial cellulose indicated that the bacterial strain *K. sucrofermentans* synthesizes both Iα and Iβ cellulose types, with Iα cellulose being the dominant type.

**4. Conclusions**

SCGs were effectively utilized as renewable resource for the development of an integrated bio-refinery leading to extraction and production of high added value products. This approach enables the exploitation of SCGs adding value to this abundant and low-cost feedstock. Further study should focus on practical and novel concepts for potential applications of the obtained products in the food and oleochemical industry.

**References**

1. R. Campos-Vega, G. Loarca-Pina, H.A. Vergara-Castañeda, and B.D. Oomah, Trends Food Sci. Technol. 45 (2015) 24-36.
2. P. S Murthy, & M. M. Naidu, Food and Bioprocess Technology (2012) 5(3), 897-903.
3. T.M. Mata, A.A. Martins and N.S. Caetano, Bioresour. Technol. (2018) 1077-1084.