Alkaline pretreatment of spent coffee grounds for microbial oil production using the oleaginous yeast strain *Lipomyces starkeyi*

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The shift from the fossil-based economy to a more sustainable economy requires the use of renewable resources as sustainable feedstocks for the production of biobased chemicals and polymers. The development of food waste biorefinery from spent coffee grounds (SCGs) depend on the composition and availability of this waste stream. Based on the International Coffee Organization (ICO), total coffee production in 2018 was about 10.2 million t, while global coffee consumption in 2018/19 was about 10 million t, with Europe being the leading continent (ICO, 2019).

The SCGs were obtained from local catering companies. After collection, the residues were air dried at 40°C to achieve a moisture content less than 10%. Coffee oil was initially extracted from SCGs focused on the extraction of the oil fraction with solvents with different environmental impact, such as ethyl acetate and hexane. Following that, the extraction of phenolic compounds was performed by ultrasound-assisted extraction with 70% (v/v) aqueous ethanol solution at different solid-to-solvent ratios. SCGs residues obtained after the extraction of all aforementioned components were subjected to alkaline pretreatment prior to enzymatic hydrolysis. Alkaline pretreatment was carried out at a solid to liquid ration of 1:10 for 1 h under different concentrations of NaOH and temperature using a central composite design (CCD) in order to identify the optimum pre-treatment conditions. After pre-treatment completion, enzymatic hydrolysis was subsequently applied using commercial enzymes cocktail at 50°C under agitation (150 rpm). The proposed biorefinery for SCGs was applied for valorisation of the waste using the oleaginous yeast strain *Lipomyces starkeyi* DSMZ 702096 for microbial oil production.

The SCGs used in this study contained mainly lignin (28.1%, db), hemicellulose (28.9%, db), glucan (10.6%, db), protein (14.8%, db) and oil (12.2%, db). Mannan (17.2%, db) and galactan (8.9%, db) were the major components of the hemicellulose fraction, followed by arabinan and xylan. SCGs treated with hexane resulted in oil recovery of about 97.6%, while an oil recovery of 96.7% was obtained when ethyl acetate was used. The pre-treated SCGs residues were subsequently treated with aqueous ethanol (70%, v/v) in an ultrasonic bath for phenolic compounds extraction. Different solid to liquid ratios (1:5 and 1:10 w/v) were studied for maximum extraction of phenolic compounds. TPC of 0.733 mg caffeic acid equivalents/100 g SCGs and 0.63 mg caffeic acid equivalents/100 g SCGs was recovered utilising solid-to-solvent ratios of 1:10 and 1:5, respectively. Alkaline pre-treatment was subsequently carried out. Increasing NaOH (%, w/v) concentration led to slightly higher overall hemicellulose hydrolysis yield (87.4%). Mannose was the predominant sugar in the hydrolysates.

The oleaginous yeast strain *L. starkeyi* was used for the biotechnological production of microbial oil in a fed-batch bioreactor fermentation under nitrogen limitation. The fed-batch fermentation resulted in the production of total dry weight around 90 g/L with intracellular lipid content of 56% (w/w). Fatty acid methyl esters (FAMEs) were analysed at the early and the late growth stage of the culture. The microbial lipids produced by *L. starkeyi* mainly contained oleic acid ($^{\Delta 9}$C18:1) followed by palmitic acid (C16:0), linoleic acid ($^{\Delta 9,12}$C18:2) and stearic acid (C18:0).

The development of a holistic biorefinery approach is the only way to ensure competitive biotechnological production of microbial oil. Extraction of various value-added fractions could facilitate biorefinery feasibility for the complete valorisation of SCGs.

Reference

http://www.ico.org/prices/new-consumption-table.pdf (Accessed at 05.03.2022)

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