Bacterial cellulose synthesis through circular oriented bioprocesses using confectionary wastes and production of nanocellulose materials

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Bacterial cellulose (BC) constitutes an important extracellular biopolymer produced with high efficiency by acetic acid bacteria of the genus *Komagataeibacter*. The properties of BC are inextricably depended on the high orientation of its three-dimensional network and the micro and macro conditions of the fermentative environment. Increased demand for biocompatibility and biodegradability, are the pillars for further consideration of this polymer in specific and value-added applications including food industry, biomedicine, pharmaceuticals, and chemical industry. The market size of BC is expected to reach USD 539.3 million by 2025 with a CAGR of 12.7% within the forecast period 2020-2025. Nanostructures of BC could be ideal building blocks due to unique structure which gives rise to enhanced mechanical strength, thermal stability, high degree of polymerization and crystallinity. Circular economy aspects could be approached via the holistic valorization of renewable and cost-effective resources for high-performance end-products.

This study evaluated BC production valorizing confectionery waste streams (CWS) as the sole carbon source. Crude enzymes were produced by solid state fermentations (SSF) of CWS combined with wheat milling by-products with the fungal strain *Aspergillus awamori*. The produced enzyme preparations were sequentially applied to biotransform CWS into nutrient-rich fermentation media. Maximum enzyme production was observed at 60% of initial moisture content for glucoamylase, phytase and protease with enzyme activities of 45, 470 and 96 U/g substrate, respectively. Different initial solid concentrations of CWS during hydrolysis at 55°C resulted in similar starch conversion yields of approximately 0.98-0.99 g/g.

Crude enzymatic hydrolysates were used for BC production by the bacterial strain *Komagataeibacter sucrofermentans* DSM 15973. BC concentration was affected at statistically significant levels under various C/FAN ratios and different pH values. Tukey's analysis indicated that in the case of C/FAN ratios, solely the higher ratio of 43.8 showed statistically significant differences compared to the other values (p=2.3 x 10^{-6}). The highest value of BC concentration (5.2 g/L) and yield (0.25 g/g total sugars) was observed under a C/FAN ratio of 19.3 while productivity reached 0.74 g/L/d. When different pH values were applied BC concentration did not demonstrate statistically significant differences between pairs 4.5 to 6.5 and 5.0 to 6.0 (p=2.5 x 10^{-10}). Under different pH values the observed BC concentration ranged between 3.5-5.7 g/L with productivities ranging from 0.32 to 0.63 g/L/d. The highest BC concentration (5.7 g/L) was observed at pH 5.

The production of BC nanostructures was performed by e*x-situ* structural modification of BC applying acid hydrolysis with H_2SO_4.Various H_2SO_4 concentrations and hydrolysis duration were assessed. Physicochemical characterization of the nanostructures demonstrated enhanced thermal stability, high crystallinity and dispersion capacity. The nano-dimensions of length and diameter of structures were confirmed by morphology evaluation via SEM while their good colloidal stability was demonstrated by z-potential values.

Sustainable production of BC employing waste streams of the agro-industrial sector followed by its chemical modification could render this highly promising biopolymer appropriate for targeted applications in domains of the food and biopolymer industry in the form of aerogels, hydrogels, micro- and nanoparticles, nanocomposites, emulsions, and membranes/films nanoparticles.

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