Utilization of agro-industrial residues as immobilization carriers in lactic acid production

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Lactic acid (LA) is a versatile chemical with a long history of applications in food, pharmaceutical, cosmetic, chemical and textile industries. Recently, the consumption of LA has been increased greatly, mainly due to its role in the production of poly-lactic acid (PLA) polymers which are environmentally friendly alternative to petroleum-based plastics. On a commercial scale, LA is currently produced by fermentation of sugar- and starch-rich edible crops such as sugar beet, sugar cane, corn and cassava (Harmsenet al., 2014). However, since today's society has been facing growing challenge regarding the food and feed insecurity, bioprocessingof alternative feedstocks(e.g. residues from food and agro-industry)into numerous high value bio-based products, including LA, has gained worldwide attention.Combined waste substrate based on potato stillage and sugar beet molasses has beenpreviously found nutritionally suitable for the growth of lactic acid bacteria and LA production(Mladenović et al., 2016). In addition, the use of solid agro-industrial residues as a physical support for microbial immobilization has shown as an attractive option for development of a productive andcost-effective fermentation strategy(Mladenović et al., 2017, Castro et al., 2017). In this way, the lignocellulosic based immobilized systems could provide increased biomass concentration, improved process productivity and the reduction of LA production costs without use of any pretreatment and modification. The material price and abundance, the physical and chemical characteristics, stability in operating conditions and efficiency of the immobilized biocatalyst are crucial factors determining its potential use for cell immobilization.

The aim of this work was to assess three agro-industrial residues: sugar beet pulp (SBP), brewers' spent grain (BSG) and sunflower seed hull (SSH), as carriers for *Lactobacillus paracasei*NRRL B-4564 immobilization in LA production on molasses enriched potato stillage.The carriers were physically characterized in terms of water adsorption index (WAI), critical humidity point (CHP) and porosity-related characteristics. Further, the stability and efficiency of the immobilized biocatalysts were evaluated and compared in repeated batch fermentation.

The study was performed using the combined agro-industrial substrate.The total sugar concentration in thepotato thin stillage was set at approximately 100 g L^{-1} with addition of sugar beet molasses.The biomass of *L. paracasei*attached onto agro-industrialsupport materials was used as an inoculum for LA fermentation.LA fermentations were carried out in repeated batch mode with recirculation of immobilized biomass in orbital shaker (150 rpm), at 41 °C, under microaerophilic conditions. After the depletion of sugar concentration below 20 g L^{-1}, fermentation media was centrifuged, residual lignocellulosic material with immobilized biomass was washed with sterile saline solution and inoculated into the same volume of fresh media for initiation of subsequent batch cycle. During the fermentations, the pH value of fermentation media was maintained at 6.5 in 4 h intervals. The batches were subsequently repeated until the LA volumetric productivity decreased below 1.0 g L^{-1} h^{-1}.

The results have shown that the highest cell number attached on the support surface during the fermentation was detected for material with higher WAI and lower CHP. Among evaluated natural materials, SBP showed preferred WAI and CHP values, as well as superior capacity to immobilize *L. paracasei*cells. Results obtained by porosimetry measurements of the immobilization materials indicate that the porosity was not a key factor for cell attachment on the support surface, while the pore diameter detected for SSH and SBP allowed formation of biofilm mainly on the support surfaces. A strong cell attachment onto agro-industrial supports enabled effective cell

recycling for four times, without much affecting the LA yield and productivity.The superiority in LA concentration and productivity obtained by SBP immobilized biocatalyst was a consequence of higher and more stable immobilized cell concentration on SBP during repeated batchescompared to BSG and SSH. The highest LA process productivity of 1.48 g L^{-1} h^{-1}, with maximal LA concentration of 80.10 g $L^{-1}$and average yield coefficient of 0.97 g g^{-1} were achieved in fermentation of molasses enriched potato stillage using SBP as a supporting material, followed by BSG and SSH.Consequently, the presented immobilization of *L. paracasei*NRRL B-4564 on SBP, BSG and SSH carriers could be an attractive and effective strategy for improvement of LA productivity on agro-industrial substrates.

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