

From giant reed to carotenoids: development and optimisation of an innovative cascade process

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Giant reed is a perennial herbaceous plant that can grow in all temperate zones, it does not require pesticides and it needs limited irrigation. Moreover, it is characterised by a high biomass productivity, soil bioremediation capacity and a high polysaccharides content (60 wt%) which can be valorised [1]. On this basis, in this work, this biomass was pretreated with a solvothermal process using an aqueous imidazole solution to remove the lignin fraction, making the solid residue enriched in polysaccharides and more reactive towards the subsequent saccharification step based on the enzymatic catalysis. The pretreated solids were hydrolysed with the commercial enzymatic mixture Cellic[®] CTec 3 HS to obtain glucose and xylose which were converted to carotenoids by the commercial and safe yeast *Rhodospiridium toruloides* [2]. Carotenoids are valuable molecules used in various fields, including the food industry, human health and semiconductor technology [3]. The efficiency of the pretreatment process was affected by several parameters, such as imidazole concentration, reaction time, temperature and initial biomass loading, all of them investigated in this work. Regarding the saccharification step, the effect of enzyme and biomass loadings was studied. Under the optimised pretreatment conditions (22 wt% imidazole solution, 140 °C, 3 h, 9 wt% initial biomass loading), lignin removal of 41.3 wt% was obtained. Lignin was recovered through precipitation by acidifying of the liquid phase, obtaining an average recovery of 95 wt% of pure lignin suitable for subsequent characterisations and exploitation strategies. Under the optimised enzymatic hydrolysis conditions (50 °C, 96 h, 45 FPU/g glucan, 10 wt% initial substrate loading), the glucose yield achieved for the optimised pretreated residue was 99 mol%, whereas the xylose yield was 82 mol%. The monosaccharides fermentation process was also optimised by studying the C/N ratio (20, 40, 60 g/g) in the formulation of the culture medium and the time of the inoculum (48, 72 h). Under the optimised conditions (C/N 60 g/g, inoculum time of 72 h) the cellular carotenoid content of 12.8 mg/g of dry cell weight was obtained from the fermentation of the biomass hydrolysate. Finally, this work also focused on the study of different downstream approaches for the cell lysis and recovery/purification of carotenoids by comparing different methods based on ultrasounds, ball milling or DMSO treatment of cells coupled with hexane or acetone extraction. Separation of carotenoids was performed by silica gel column utilising the mobile phase of hexane/acetone 98.5/1.5 v/v%. The spent yeast obtained from the extraction step contained approximately 5 wt% of total nitrogen, making it suitable for further valorisation as a nitrogen source in fermentation processes or as a feedstock for the production of nitrogen-rich activated carbons by catalytic pyrolysis.

References:

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