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BIOETHANOL PRODUCTION FROM MIXED SUGARS USING *Scheffersomyces stipitis*

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Abstract

Bioethanol can be produced from different biomasses, including lignocellulosic feedstocks, which can contain comparable amounts of 5-carbon and 6-carbon sugars. In order to obtain an effective conversion of these biomass carbohydrates, suitable microorganisms are required for the fermentation step. In this paper the ability of *Scheffersomyces stipitis* to ferment mixed syrups has been investigated in pure culture and in co-cultures with other yeasts species. Both the performance of free and entrapped cells were explored. The performance of the *Schef. stipitis* strain NRRLY-11544, was initially tested by using synthetic broths containing different sugars concentrations, with a xylose fraction of 50%. According to the diauxic behavior of *Schef. stipitis,* the sugars consumption occurred sequentially, achieving an average process yield of 75%. To overcome this drawback, the cells immobilization was tested. Silica-hydrogel films and alginate beads were used as immobilization carriers. The sugars uptake in the entrapped cells occurred simultaneously. In all tests, xylose consumption was almost completed when the process time was prolonged. As common trend the ethanol level decreased at increasing the xylose fraction in all the systems investigated. This phenomenon appeared less evident at higher sugars level, probably due to a favored fermentative metabolism according to the Crabtree effect. However, the fermentation with both free cells and immobilized cells yielded comparable results in terms of xylose consumption and ethanol yields, but the immobilization offered the advantage of enabling the fermentation of subsequent batches of sugars with similar performances over several weeks. The use of *Schef. stipitis* and *S. cerevisiae* in free co-cultures ensured faster processes. However, the rapid production of ethanol by *S. cerevisiae*, inhibiting *Schef. stipitis*, caused a stuck of the process, resulting in a lower ethanol yield than pure culture fermentation.
Finally, co-cultures of inhibitors-adapted *Schef. stipitis* and *S. cerevisiae,* co-immobilized in alginate gel beads, have been used in order to explore their co-fermentation ability of an un-detoxified enzymatic hydrolyzates, obtained from a steam pretreated corn stover. The results indicated that, in the enzymatic hydrolyzates, adaptation increases the xylose consumption from *Schef. stipitis* by 62% and improves the ethanol yield by 17%, respect to wild type yeast.
Overall the use of immobilized and adapted co-cultures, could be a feasible strategy for the bioethanol production from lignocellulosics. Nevertheless the ethanol inhibition on *Schef. stipitis* metabolism is still a problem. Further investigations on fermentation strategy by co-cultures are in progress in order to develop a process providing the online sequestration of ethanol.