Repeated-batch fermentation of sugarcane bagasse hemicellulosic hydrolysate to ethanol using two xylose-fermenting yeasts

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Bioethanol production from sugarcane bagasse is one of the best alternatives to replace fossil fuels and should be further explored. In Brazil more than 166 million tons of bagasse are generated annually from cane milling to produce sugar and/or ethanol (first-generation ethanol – 1G). Sugarcane bagasse has a structural matrix constituted mainly of 42% cellulose (C6), 28% hemicellulose (C5) and 22% lignin. Part of this bagasse is used in the mills to energy generation and another part has been used to produce cellulosic ethanol (second-generation ethanol – 2G) in industry or in academic research. Not only the cellulose fraction can be used, but also hemicellulosic fraction. However, to make sugars available for bioconversion by microorganisms, it is necessary to deconstruct the cell wall of this material with the removal of the lignin. In this study, it was evaluated the sugarcane bagasse (SB) pre-treatment to optimize the xylose concentration in the hemicellulosic hydrolysate. Secondly, a repeated batch process was carried out to ferment the hemicellulosic hydrolysate of sugarcane bagasse by two xylose fermenting yeasts, *S. stipitis* and *S. shehatae*, comparing the efficiency of ethanol production as a strategy to be applied to the large-scale commercial ethanol production. The pre-treatment stage was evaluated according to a 2^3 face-centered full factorial design (FCCD). The fermentations were performed in a 2.4 L Bioengineering KLF 2000 bioreactor (Bioengineering Inc, CA, USA). For the repeated-batch fermentation without recycling the cells, after the 72h batch fermentation, 2/3 (800 mL) of fermented broth was removed and the same quantity was added of fresh supplemented hydrolysate. Batch fermentation was repeated sequentially for 3 cycles. The optimal pre-treatment condition was: 127ºC, 150 mg H_2SO_4/g dry SB for 10 min, 1:10 solid/liquid ratio, and it was possible obtain 14.06 g L^{-1} of xylose with 73.49% of efficiency. Fermentative parameters through the repeated-batch were significantly increased and the ability of yeasts were maintained during two-cycle repeated-batch. *S. shehatae* was 85% more efficient in xylose to ethanol conversion than *S. stipitis*, with 21.5 g L^{-1} ethanol, yield - $Y_{P/S}$ = 0.436 g g^{-1}, productivity - Q_P = 0.241 g $L^{-1}h^{-1}$. The results shown *S. shehatae* has great potential for industrial bioethanol production from lignocellulosic hydrolysate in repeated-batch operation. From the results found in this study it was possible observe two different aspects about production of ethanol by *S. stipitis* and *S. shehatae* from repeated-batch fermentation of sugarcane bagasse hemicellulosic hydrolysate. The first is that for both yeasts, ethanol production decreased after two-cycle repeated batch. The second is that *S. shehatae* presented better ability to convert sugars in ethanol than *S. stipitis*. Therefore, despite preliminaries, the results showed that the combination of repeated-batch operation and *S. shehatae* yeast have great potential for the industrial production of bioethanol from lignocellulosic hydrolysate.

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