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Intensification of fermentation process: medium optimization and characterization of robust yeast strains for very high gravity ethanol fermentations

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Efficient ethanol production requires careful selection of the appropriate microorganism, raw materials, nutrient supplements and fermentation process. Ethanolic fermentations with very high sugar concentrations (> 300 g/L) - very high gravity (VHG) fermentations - have many advantages from the industrial point of view potentially resulting in improved overall plant productivity and reduced costs, but these fermentations are often slow and incomplete. Our aim was to develop a low-cost medium (based on industrial substrates/byproducts) and to evaluate the performance of laboratory and industrial *S. cerevisiae* strains in VHG batch fermentations.

Statistical experimental designs were used to develop a medium based on corn steep liquor (CSL) and other inexpensive nutrients. The critical nutrients were initially selected according to a Plackett-Burman design and the optimized medium composition (g/L: 44.3 CSL; 2.3 urea; 3.8 $MgSO_47H_2O$; 0.03 $CuSO_45H_2O$) for maximum ethanol production by strain CEN.PK 113-7D was obtained by response surface methodology, based on a Box-Behnken design. The optimization process resulted in significantly enhanced final ethanol titre, productivity and yeast viability in VHG fermentations, comparing to the yeast fermentation performance in a reference medium (100 g/L CSL as the sole nutrient source) and in 2-fold concentrated YP medium (20 g/L yeast extract and 40 g/L peptone). VHG fermentations with the optimized medium composition by strains PE-2 (isolated from Brazilian bio-ethanol distilleries) and CEN.PK 113-7D resulted in maximum ethanol titres of 19.2 and 17.5 % (v/v), and ethanol productivities of 2.5 and 1.7 g/L/h, respectively. Furthermore, decreasing the fermentation temperature from 30 to 27°C, the robust PE-2 strain was able to consume near 380 g/L glucose and produce an ethanol titre of 22 % (v/v). A deeper physiological characterization of strains PE-2, CA1185 (isolated from 'cachaça' fermentation in Brazil) and CEN.PK 113-7D was undertaken. In 350 g/L glucose fermentations, the industrial strains PE-2 and CA1185 showed higher levels of glycogen (2-fold), sterols (5-fold) and around 1.4-fold higher level of trehalose and intracellular glycerol comparing to the laboratory strain CEN.PK 113-7D.

These results are of practical importance for the development of highly-efficient industrial VHG bio-ethanol fermentation systems.

Keywords
Ethanol, response surface methodology, *S. cerevisiae*, very high gravity fermentation