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Construction and application of a flocculent (*FLO1*) industrial yeast strain in a repeated-batch system for bio-ethanol production

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Bio-ethanol production is a widely known industrial process, increasingly studied during the last years. Several attempts are constantly being made aiming to obtain the highest ethanol titres and process productivities. The aim of this work is to study the possibility of using a high ethanol-resistant industrial strain, transformed with a flocculation gene (*FLO1*), and use it in a repeated-batch fermentation system with yeast recycling by flocculation.

The host used was *Saccharomyces cerevisiae* strain PE-2, which is widely applied for bio-ethanol production in Brazilian distilleries. In preliminary experiments, we tested the possibility of performing repeated-batch very high gravity (VHG) fermentations, using a medium previously optimized (g/L: 44.3 corn steep liquor; 2.3 urea; 3.8 MgSO₄·7H₂O; 0.03 CuSO₄·5H₂O; >300 glucose) and recycling the yeast biomass by centrifugation. The use of very high inoculum concentrations (85 and 167 g_{FreshYeast}/L) resulted in high ethanol productivities (4.5 - 5.5 g/L/h) during 3 consecutive batches, but the high ethanol titres at the end of each batch (15 - 16% v/v) and the absence of yeast growth resulted in a strong decline in yeast viability (<40% at the end of the third batch). Therefore, we developed a strategy consisting in inoculating the first cycle with 8 g_{FreshYeast}/L and recycling the totality of the yeast biomass produced (50 - 90 g_{FreshYeast}/L) in the next cycles. Periodically, a cycle pitched with only a fraction (ca. 8 g_{FreshYeast}/L) of the yeast recovered was performed in order to allow yeast growth and viability restoring. With this strategy, the ethanol productivity obtained was 3.5 - 4.5 g/L/h with ethanol titres at the end of each batch of 16 - 17% (v/v). The data collected indicates that this system can be operated for at least 15 consecutive batches.

The host strain PE-2 was transformed with a multi-copy plasmid bearing the flocculation gene *FLO1*. Two out of 28 transformants tested showed a strong flocculating phenotype in YPD medium. These two transformants were able to ferment VHG medium with efficiency similar to the host strain, producing 19.40.2% (v/v) ethanol with a corresponding productivity of 2.7 g/L/h. These flocculent strains will be tested in the pre-optimized repeated-batch strategy with biomass recycling by sedimentation, eliminating the centrifugation steps that represent added costs in industrial practice.

Keywords

Bio-ethanol, flocculation, repeated-batch, very high gravity