Continuous ethanol fermentation of lactose by a recombinant flocculating *Saccharomyces cerevisiae* strain

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Cheese whey is a problem of some concern to the cheese industry due to its high BOD (Biochemical Oxygen Demand) content, ranging from 35 to 60 g/L. In Portugal, one million liters of cheese whey are daily produced. The cheese whey is mainly composed by water, protein and lactose. The protein fraction can be removed by ultrafiltration and applied in the food industry while the lactose fraction obtained as permeate (corresponding to a lactose concentration of 50-60 g/L) can be used for ethanol production by fermentation. One of the most promising approaches in whey fermentation has been the development of ethanol as a beverage alcohol or as an alternative energy liquid fuel. The success of this process depends upon the development of a high productivity process for ethanol production. Among several alternatives, a continuous system with flocculating yeast cells is one of the most attractives.

A flocculent *Saccharomyces cerevisiae* strain has been constructed so that it expresses both the *LAC*4 (coding for β-galactosidase) and *LAC*12 (coding for lactose permease) genes of *Kluyveromyces marxianus*. This recombinant strain is not only able of growing on lactose, but also of fermenting this substrate. This is the first time to our knowledge that a recombinant *Saccharomyces cerevisiae* ferments lactose in a way comparable to the existing lactose fermenting yeast strains. Moreover, the flocculating capacity of the strain used in this work presents several advantages concerning the process. On one hand, it allows for the operation in a continuous mode at high cell concentration, thus increasing system overall productivity. On the other hand, biomass concentration in the effluent is reduced, thus decreasing product separation/purification costs.

Data on yeast fermentation and growth on a medium containing lactose as the only carbon source is presented. Continuous experiments were done in a 5,5 L airlift bioreactor. In the range of studied lactose concentrations (up to 100 g/L) total lactose consumption was observed with a conversion yield on ethanol close to the expected theoretical value. For the continuous operating bioreactor, an ethanol productivity of 12,5 $g/L.h^{-1}$ (corresponding to a feed lactose concentration of 50 g/L and a dilution rate of 0,5 h^{-1}) was obtained, which is 7 times larger than the continuous conventional systems. The system stability was confirmed by keeping it in operation for three months.

Its our believe that this recombinant *Saccharomyces cerevisiae* strain is an interesting solution for the valorisation of lactose in cheese whey.

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