Molecular Yeast Solutions for Whey Bioremediation

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Cheese whey is a by-product of dairy industries, which presents rather high pollutant characteristics (35 g/l<biochemical oxygen demand<60g/l) and is produced in high amounts (world production is around $100x10^6$ tons/year). Whey disposal has been under consideration for several years. Nowadays, after whey powder and demineralised whey powder, the third major products obtained from cheese whey are whey protein concentrates (WPC) (Horton, Whey Processing May:39, 1996). When producing WPC, typically by ultrafiltration, a lactose-rich fraction, the cheese whey permeate, is obtained. Lactose, the largest component in whey (50 gL^{-1}), is the most problematic to dispose of economically. One of the possible solutions to the lactose problem is lactose fermentation. To make this alternative use for lactose attractive, the development of fermentation processes with increased productivity must be considered. It is our belief that by using high-cell-density systems with flocculent yeast cells the productivity of whey permeate fermentation can be improved. The use of flocculent cells with an adequate bioreactor design allows for high biomass concentration inside the bioreactor, making possible continuous operation at higher dilution rates resulting in higher volumetric productivity (Domingues ***et al.*** Biotechnol Bioprocess Eng 5:288-305, 2000). In addition, the downstream processing is greatly facilitated due to cell sedimentation characteristics. Besides lactose alcoholic fermentation, other fermentation products may be obtained using lactose as substrate, namely, recombinant proteins (Domingues ***et al.*** Recent Res Devel Biotech Bioeng 5:65-78, 2003).

Results related with the genetic modification of flocculent ***Saccharomyces cerevisiae*** cells for lactose bioremediation will be presented. Two different approaches have been considered: one based on the cloning of ***Kluyveromyces lactis LAC4*** and ***LAC12*** genes (Domingues ***et al.***, Appl Microbiol Biotechnol 51:621-626, 1999), coding for β-galactosidase and lactose permease respectively, and the other on the cloning of ***Aspergillus niger*** β-galactosidase (Domingues ***et al.*** Appl Microbiol Biotechnol 58:645-650, 2002). With the first approach the aim was to obtain a flocculent yeast strain with the ability of fermenting lactose while in the second approach the aim was to obtain a

good producer of extracellular β-galactosidase, enzyme that hydrolysis lactose into glucose and galactose. In both cases, a flocculent ***S. cerevisiae*** strain was used as host strain to enable continuous high-cell-density lactose fermentations. With the first approach, a high ethanol productivity system was developed, resulting in an ethanol productivity of 10 $gL^{-1}h^{-1}$ when operating with whey permeate as substrate (Domingues ***et al***. Biotechnol Bioeng 72:507-514, 2001). The developed system has been applied in a pilot scale experiment (1 m^3) with encouraging results. With the second approach, the continuous operation for extracellular β-galactosidase production resulted in lost of the recombinant plasmid at high dilution rates (Domingues ***et al***. Process Biochem 40:1151-1154, 2005). Integration of the expression cassette in the genome resulted in a stable producing strain but with low β-galactosidase activity. A multicopy integration strategy was then applied resulting in good extracellular β-galactosidase producers with enhanced long-term genetic stability.

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