Cell morphology (Pamboukian and Facciotti, 2005) and viability tests are being performed.

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68.

Avoiding proteolysis during fermentation by using high gradient magnetic fishing

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Proteolysis during a fermentation process may have a severe impact on the yield and quality of a secreted product. However, in the work presented here the use of high gradient magnetic fishing (HGMF) for direct in situ removal of proteases during a fermentation process is shown to efficiently stabilise the protein of interest. Micron-sized non-porous magnetic particles, composed of a silanized magnetite base matrix coated with polyglutaraldehyde and subsequently derivatized with bacitracin via a divinylsulphone linker were used. The magnetic adsorbents were sterilized then added during a *Bacillus licheniformis* fermentation and incubated for 5 min. The fermentation broth was then pumped through a high gradient magnetic separator and the protease-loaded magnetic adsorbents were removed from the liquid. The broth was subsequently returned to the fermentor and the cultivation process continued. The magnetic adsorbent addition and subsequent separation was carried out twice with a 12 h interval between. SDS-PAGE results clearly showed that the stability of the model protein, bovine serum albumin, which was spiked into the fermentation, was extended by more than 18 h compared to the case where HGMF was not employed. Furthermore, no changes to the physiology of the cells were observed.

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69.

Use of castor oil for aroma production by the yeast *Yarrowia lipolytica*: Optimization of operating conditions

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The yeast *Yarrowia lipolytica* is one of the more intensively studied non-conventional yeast species. This microorganism is not only of interest for fundamental research, but also for biotechnological applications (Barth and Gaillardin, 1997). As it is considered as non-pathogenic, this yeast has been used in several industrial applications considered as GRAS by the American Food and Drug Administration, like production of single cell protein, peach flavour and citric acid (Tsugawa et al., 1969; Beckerich et al., 1998). The range of substrates used by *Y. lipolytica* include alkanes, fatty acids, organic acids, proteins and some sugars, which also contributes for the interest in this yeast (Fickers et al., 2005).

Y. lipolytica is able to carry out the biotransformation of ricinoleic acid into gamma-decalactone, a peach aroma compound of industrial interest (Aguedo et al., 2004). Ricinoleic acid (12-hidroxioctadec-9-enoic acid) is a hydroxylated C_{18} fatty acid that in its sterified form is the major constituent (about 80%) of castor oil, which makes it an abundant compound, being the precursor most usually used in the production of this aroma.

Investigation in our laboratory has been made in order to optimize this production and to better understand the all process. Previous studies indicate that oxygen availability in the medium used for this biotransformation may be a determining factor in the process, intervening in the control of the peroxisomal beta-oxidation pathway, which leads to gamma-decalactone formation (Aguedo et al., 2005). So, results concerning this aspect will be presented, such as results concerning the use of different substrates (castor oil and methyl ricinoleate) at different concentrations.

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