

## **Continuous beer fermentation with yeast immobilized on spent grains - the effect of operational conditions**

Tomáš BRÁNYIK<sup>1</sup>, António A. VICENTE<sup>1</sup>, José M. M. CRUZ<sup>2</sup>, José A. TEIXEIRA<sup>1</sup>

<sup>1</sup>*Centro de Engenharia Biológica – IBQF, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal, E-mail: jateixeira@deb.uminho.pt*

<sup>2</sup>*UNICER – Bebidas de Portugal, S.G.P.S., S.A.*

The advantages of continuous fermentation process have been already recognized. However, due to increased complexity of operation comparing to batch process, flavor problems, risk of contamination, yeast viability, carrier price and inconvenience of immobilization, the continuous beer fermentation has found few practical applications so far. Since the carrier cost represents a significant part of the equipment cost, the need for a cheap support material easy to regenerate is still relevant. This work deals with continuous primary beer fermentation in an airlift reactor (ALR) containing brewing yeast (supplied by Unicer brewery) immobilized on spent grains, a brewing by-product. The objective of this study was to verify the long-term suitability of this new cellulose-based carrier made from spent grains for brewing yeast immobilization and to study the influence of feed rate, aeration and temperature on bioreactor performance, immobilized biomass load, ethanol production and flavor compounds in green beer.

The carrier is prepared from spent grains by a simple two-step treatment consisting of an acidic hydrolysis of the residual starchy endosperm and a basic partial delignification of the solids remaining after acidic treatment. The preparation procedure gives 10 %wt. yield of carrier, containing ca. 90 %wt. of holocellulose, from spent grains.

The carrier obtained from spent grains met the requirements of high cell load (up to 0,6 g dry cell g<sup>-1</sup> dry carrier), stability, food grade and the possibility to regenerate (washing with caustic) and sterilize (heat). The optimum fermentation performance of the one stage ALR with brewing yeast immobilized on spent grains was in terms of apparent attenuation (70 – 80 %) achieved at residence times between 18 – 25 hours (dilution rate 0,04 – 0,055 h<sup>-1</sup>) and was characterized by a satisfactory ethanol formation in green beer (ca. 4,2 % wt.). The ratio between immobilized and free cells ( $X_{im}/X_{free}$ ) in the reactor was in the range 1,0 – 3,6 reaching the lowest values at high gas flow rates. High gas flow rates (air and/or CO<sub>2</sub>) causing vigorous turbulence turned out to be detrimental to immobilized biomass provoking cell detachment from the carrier. However, the yeast detachment was not irreversible and the system was able to return to the original biomass balance within a few days, depending on the degree of the detachment, as well as the yeast were able to colonize the clean carrier added in the course of the experiment. The influence of aeration rate and temperature on sensorial quality of green beer has also been studied by quantification of higher alcohols, esters and vicinal diketones in the product.

This work clearly demonstrated the technological feasibility of the three-phase ALR with brewing yeast immobilized on spent grains for continuous production of a beer with a balanced flavor profile and can be considered as a promising alternative to existing fermentation systems.

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