

## **Yeast cell immobilization on wood chips for the purpose of continuous primary beer fermentation**

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Immobilized brewer's yeast technology is nowadays well-established technology for beer maturation and alcohol-free and low-alcohol beer production on industrial level, while its application for primary beer fermentation is still under scrutiny on lab or pilot levels. The main reason for this is reduced cell growth in immobilized conditions coupled with mass transfer restrictions, which cause an unbalanced flavor profile of final beers produced solely by immobilized cells.

In comparison with traditional beer production based on freely suspended cells, immobilized cell systems offer many advantages such as: higher cell densities and cell loads, increased volumetric productivity, possibility for continuous operation, reuse of the same biocatalysts for prolonged periods of time due to constant cell regeneration and, above all, much higher fermentation rates, thereby reducing the overall process time. In the case of cell immobilization by adsorption, one must also take into account that there are no external mass transfer limitations due to the fact that cells are directly exposed to the medium.

Wood chips are very attractive cell carriers for several reasons, mainly because of low price, biocompatibility, large adsorption surfaces enabling high cell concentrations, simple biocatalyst separation, mechanical stability, low mass transfer restrictions, and simple and gentle immobilization technique. It was shown that beer produced by cells immobilized on wood chips had similar chemical composition and sensory characteristics as compared to conventionally produced beers.

The purpose of this study was to develop and standardize the method of yeast cell immobilization on wood chips, to investigate cell activity and viability and, based on those results, to develop and standardize the process of continuous beer fermentation in a packed bed bioreactor. Cell immobilization studies have shown that about 80% of cells were adsorbed on wooden carriers, with the maintenance of practically 100% viability. Most of the cells were attached just after 3 hours and adsorption reached its peak after 24 hours.

During batch fermentations that lasted 9 to 10 hours, attenuations exceeding 85% were achieved, reaching the maximum of 92.6%. Concentrations of immobilized cells and cells suspended in medium increased over time, reaching final overall concentration of about  $1.8 \times 10^9$  cells/ml. Suspended cells comprised 11.6% of the total cell number confirming successful and stable cell adsorption.

High fermentation activities of immobilized cells were confirmed in continuous beer fermentation in a laboratory packed bed bioreactor with recirculation that lasted for 16 days. The system had a total volume of 1000 ml of which the bioreactor comprised 800 ml. Wort was continuously supplied to the system at a rate of 1.8 ml/min, while the recycle to the reactor was 40 ml/min. After four days that took the system to reach steady state, parameters under scrutiny corresponded to those previously attained in batch fermentations.

Collected data reveal great potentials for applications of wood chips in brewing industry. High apparent attenuations were accomplished at high cell concentrations, providing high fermentation activity. It should be also noted that wooden carriers showed good mechanical properties and kept integrity and adsorption properties during repeated sterilizations, cell immobilization and fermentation studies.