

# Encapsulation of brewing yeast in polyvinyl alcohol for continuous beer fermentation

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## Introduction

Immobilized cell systems offer many advantages as compared to traditional fermentation processes such as higher cell densities and cell loads, increased volumetric productivities, possibility for continuous operation, reuse of same biocatalysts for prolonged periods of time and, above all, much higher fermentation rates (Nedović et al., 2005a; Branyik et al., 2005; Willaert and Nedovic, 2006). Systems with immobilized brewer's yeast cells are nowadays successfully applied for beer maturation and industrial production of alcohol-free and low-alcohol beers. However, application of such systems for main beer fermentation is still restricted to laboratory or pilot levels mainly due to mass transfer limitations within cell carriers causing an unbalanced flavor profile of final beers (Willaert and Nedovic, 2006).

After encouraging results of preliminary studies (Nedović et al., 2005b), the aim of this study was to further investigate potentials of polyvinyl alcohol (LentiKats<sup>®</sup>) as yeast carrier for use in batch and continuous main beer fermentations.

## Materials and methods

### *Brewing yeast cultivation conditions*

The cells of brewing yeast (*Saccharomyces uvarum*) were cultivated in sterile, synthetic growth medium at 25°C in shaken flasks. The composition of the synthetic medium was as follows: MgSO<sub>4</sub> · 7H<sub>2</sub>O at 0.25 [g/L], NH<sub>4</sub>Cl at 2.50 [g/L], KH<sub>2</sub>PO<sub>4</sub> at 5.50 [g/L], NaCl at 1 [g/L], CaCl<sub>2</sub> 0.01 [g/L], citric acid at 3[g/L], yeast extract at 2[g/L] and glucose at 100 [g/L]. Cells were harvested in the early exponential phase by centrifugation. Industrial wort with 10.5 – 12% extract was used as a feeding medium both, in batch and continuous fermentations and for carrier storage during the periods between the fermentation runs.

### *Immobilization of yeast cells*

LentiKat<sup>®</sup> Liquid (320 ml) was melted at 90 to 95°C, cooled down to a temperature of 30 to 35°C, and mixed with 80 ml of yeast cell suspension by magnetic stirrer. Tip of wires of LentiKat<sup>®</sup> Printer (GeniaLab, Germany) were dipped into the mixture and lifted afterwards. The droplets were subsequently put on Petri dishes. Petri dishes covered with droplets were put under the sterile, laminar airflow at room temperature. Under these conditions, gelification of the droplets occurred in half an hour with decrease of 75% of the initial mass due to water evaporation. The obtain gel particles in the form of lenses were stabilized and re-swelled in stabilizing solution (GeniaLab, Germany) for two hours. The resulting LentiKat<sup>®</sup> lenses were about 3.5 mm in diameter and 0.3 mm thick with immobilized yeast cells at starting concentration of 1 x 10<sup>7</sup> cell/ml.

### *Gas-Lift Reactor*

Batch and continuous fermentations were performed in internal loop gas-lift bioreactor system with a working volume of 3 L. The reactor system consisted of a glass cylinder (99 mm in internal

diameter, 630 mm in height) and a coaxially positioned draft tube (60 mm in diameter and 300 mm in height). Bioreactor contained 320 g (or 10.67%) of LentiKats<sup>®</sup> and 3 L sterile plant wort of 10,5 to 12 % extract periodically (during the batch fermentations) or continually (during the continuous fermentation) supplied at the side of the column. Nitrogen was introduced at the bottom of the column at the flow rate of 300 mL/min. The initial concentration of immobilized yeast cells was about  $1 \times 10^7$  cells/ml of LentiKats<sup>®</sup>. Fermentation temperature was varied in the range of 13 to 16°C.

### **Analytical assays**

Suspended yeast cell concentration was estimated by Thoma counting chamber.

Immobilized yeast cell concentration was determined in the same way like suspended cells, after carrier dilution. A sample containing approximately 1.0 g of wet LentiKats<sup>®</sup> was taken from the reactor. After short draining on the filter paper, sample was dissolved in 9 ml isotonic solution (0.9 % NaCl) by heating and mixing. 1 ml from this solution was taken and dissolved again in 9 ml isotonic solution. After that, immobilized cells were estimated by using Thoma counting chamber.

Liquid samples from both, batch and continuous fermentations, were collected aseptically from the reactor and analyzed for specific gravity and yeast cell counts (Analytica EBC).

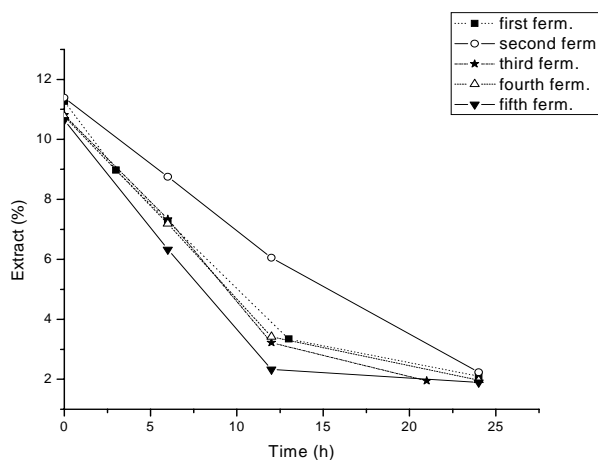
Flavor-active compounds (fusel alcohols and esters) were analyzed by gas chromatograph, Perkin-Elmer 900, equipped with a stainless steel column, 1/8" Carbowax W.A.W. 80/100 mech, with 10 % 1,2,3-trimethylpropane (length 4 m). The injector, column, and flame ionization detector temperatures were maintained at 110, 55, and 200°C, respectively. Nitrogen was used as the carrier gas.

Diacetyl was analyzed by spectrophotometer on the  $\lambda=335$  nm (Analytica EBC).

## **Results**

### **Repeated batch fermentations**

The course of repeated batch fermentations, given as extract's change vs. time, is presented in figure 1. High operational stability of the immobilized bioreactor system is obvious. Three fermentation runs had practically the same fermentation pathways. Differences that can be noticed in other two fermentation runs are consequence of the slight variations in temperature, which fell in the range 13 - 19°C. Moreover, fifth fermentation showed that process could be realized in term of only 12 hours, which is very important fact for possible industrial application.



**Figure 1. The course of repeated batch primary beer fermentations in gas-lift bioreactor**

Cell number in LentiKats<sup>®</sup> was measured in time intervals. Initial concentration was  $1 \times 10^7$  cell/g of carrier. After the first fermentation run number of cells was increased to  $1.91 \times 10^9$  cell/g of carrier, and at the end of the fifth fermentation run that number was  $2.97 \times 10^{10}$  cell/g of carrier. This concentration is higher than the one measured in previous studies carried out at similar conditions in the gas-lift bioreactor (Nedović et al., 2001).

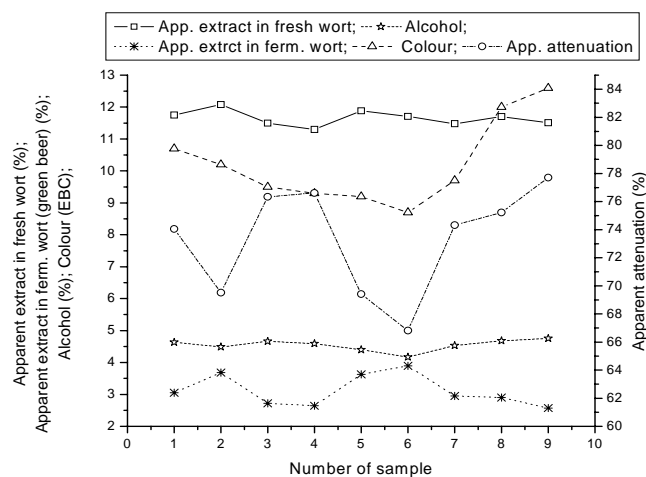
The concentrations of beer flavor-active compounds are presented in table 1. It can be noticed that the concentration levels of the most compounds were in the range that is typical for conventionally produced beers.

Flavour compound	4 <sup>th</sup> ferm. run	5 <sup>th</sup> ferm. run	Typical concentrations in lager beers (Kronlof, 1994; Enari, 1995)
Diethyl-sulfide (mg/L)	0	0	0.06-0.09
Acetaldehyde (mg/L)	6.5	11.9	1.2-24.4
Methyl acetate (mg/L)	0.18	0.16	0-1.5
Ethyl acetate (mg/L)	21	18.5	8-47.6
Isobutanol (mg/L)	17.8	20.1	7-33
Isoamylacetate (mg/L)	0.4	0.57	0.2-1.5
Izoamylalcohol (mg/L)	79.9	77.3	40-70

**Table 1. Measured and typical concentrations of beer flavour-active (volatile) compounds**

### *Continuous fermentation*

Continuous fermentation was conducted 20 days at dilution rate  $D = 0.042 \text{ h}^{-1}$ . Temperature was maintained at around  $15^\circ\text{C}$ . Some of the obtained results are presented in figure 2. According to the alcohol and extract content the fermentation was very stable during the mentioned period – some small variations were the result of variations in temperature. In addition, content of alcohol and volatile compounds (data not shown) was in the same range as in batch experiments. Preliminary taste tests indicated that the beer produced by the cells immobilized in polyvinyl alcohol had acceptable aroma profile.



**Figure 2. Variations of process' parameters during continuous beer fermentation in a gas lift bioreactor**

## Conclusions

Polyvinyl alcohol showed good mechanical characteristics and negligible resistance to the nutrient diffusion. The overall cell concentration in LentiKats<sup>®</sup> carriers reached very high value of  $2.97 \times 10^{10}$  cell/mL, while the concentration of suspended cells was about  $5 \times 10^7$  cell/mL.

During batch fermentations, apparent attenuation around 80 % was achieved in some experimental runs for only 12 – 13 hours. This result indicates high fermentation activity of immobilized brewing yeast cells. Besides, concentrations of alcohol and volatile compounds were in the range typical for the lager beers.

During continuous fermentation, stability of operation was achieved very fast and maintained during the experimental run of 20 days. The quality of the obtained beer was comparable to the quality of beer produced by traditional fermentation with suspended cells. In addition, immobilized cells retained their activity over a long period of time (over a six months of investigation) while lenticular particles retained their shape and firmness. These data confirm a great potential for application of gas-lift bioreactors with immobilized cells in brewing industry. One prospect of future studies with LentiKats<sup>®</sup> carrier in developing novel brewing processes is to increase the carrier hold-up in the bioreactor, which would probably result in even faster fermentation rates.

## Acknowledgment

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