O6-2 **Cider and red wine production with multiple application of immobilized yeast**

Stepanov N.[1,2], Kurbatova A.[3], Scherbakov S.[3], Efremenko E.[1,2] *
[1] The M.V. Lomonosov Moscow State University, Moscow, Russia
[2] N.M. Emanuel Institute of Biochemical physics, RAS, Moscow, Russia
[3] Moscow State University of food production, Moscow, Russia
*E-mail: efremenko@enzyme.chem.msu.ru

INTRODUCTION

The interest to application of immobilized yeast cells in the wine making is high enough. For industrial wine production, it is important to select a suitable carrier and method for cell immobilization. Adsorption of yeast cells on various carriers and entrapment of cells into gel matrices are the most popular methods of immobilization used in the wine making (Kourkoutas 2004). Nevertheless, there are several significant disadvantages typical for known immobilized biocatalysts: the low enough mechanical strength of gel structures under conditions of product production and cell release from biocatalyst volume observed throughout the wine fermentation.
Poly(vinyl alcohol) cryogel (PVA CG) with high chemical and mechanical stability as well as macroporous structure providing good conditions for mass-transfer processes looks very attractive as a carrier for yeast immobilization (Lozinsky 1998).
This work was aimed at investigation of properties of immobilized biocatalyst, developed on the basis of yeast cells entrapped into PVA CG, and analysis of characteristics of alcoholic beverages obtained with immobilized and free cells. Besides, the possible multiple reuse of biocatalyst for production of wines was investigated.

MATERIALS AND METHODS

The *Saccharomyces bayanus* EC 1118 and *S. cerevisiae Challenge Red Fruit* cells were used in the work to accumulate cell biomass for biocatalyst production, the cells were grown up in the following medium (g/L): glucose - 10; yeast extract - 2.0; NaCl - 1.0; $(NH_4)_2SO_4$- 2.0; $MgSO_4x7H_2O$ - 1.0; KH_2PO_4- 13.5. Yeast biomass was immobilized into PVA CG according to previously patented procedure (Efremenko 2008). Medium used for cider production was obtained by mixing dry apple wine (pH 3.9) with liqueur up to the final sugar concentration equal to 30 g/L, which then was filtered through antimicrobial Corning filters (0.20 μm, Corning Inc, Germany) and used for secondary fermentation in 375 mL bottles. The concentrations of free and immobilized cells used for fermentation were similar $3x10^6$ cell/mL. Fermentation was carried out under anaerobic conditions at 12°C for 7 days. The red must was used for red wine production. The initial concentration of sugar was 220 g/L. The fermentation was carried out in 200 mL flasks with flatus tubes at 18°C. The concentration of immobilized cells was controlled using bioluminescent method of determination of intracellular ATP concentration (Efremenko 2006). Concentration of sugar was determined by HPLC. Ethanol was estimated by GC.

RESULTS AND DISCUSSION

The fermentation of apple juice into cider is one of the oldest traditional beverage productions. The immobilized into PVA CG yeast cells *S. bayanus* EC 1118 was tried in this work in fermentation process of apple wine for cider production. To estimate the influence of immobilization on metabolic activity of cells, the fermentation processes catalyzed by free and entrapped yeast cells were concurrently investigated. The concentrations of accumulated ethanol and residual sugar (Table 1) as well as number of free yeast cells were monitored throughout the fermentation process.

Table 1. Characteristics of cider produced with free and immobilized cells

Characteristics	Free cells	Immobilized cells
Residual sugar, g/L	4.0	2.9
Ethanol, % v/v	7.5	7.9
CO_2 pressure, kPa	420	440
Total acidity, g/L	3.8	3.9
Volatile acidity, g/L	0.3	0.3
Esters, mg/L	380	377
Aldehydes, mg/L	70	72
Free cells concentration, cells/mL	$25x10^6$	$0.5x10^6$
pH	3.9	3.9

It was shown that slightly lower concentration of residual sugar and a little higher ethanol concentrations (2.9 g/L and 7.9 % (v/v) and, respectively) were observed in the wines with immobilized cells compared to free ones. This fact testified to a little higher fermentability of immobilized cells as compared to their genius analogues used in a free state. The content of other parameters were actually the same in all samples of wine. It should be noted, that concentration of free cells determined in the samples of cider obtained with immobilized biocatalyst was 50-times lower (5×10^5 cells/mL) as compared to wine obtained with free cells. The investigation of possible multiple reuse of biocatalyst for the apple wine champagniza-

tion in the bottles was carried out. The prepared biocatalyst was used in 5 batch fermentations. Each time the biocatalyst was washed and reused. To reveal the level of cell viability after fermentation process, the concentration of intracellular ATP was determined in the cells. (Table 2).

Table 2. Intracellular ATP concentration in the yeast cells after five working cycle and characteristics of obtained cider

Cycle	ATP, mole/g	Free cells, cells/mL	CO_2 pressure, kPa
1	7.80×10^{-4}	0.8×10^{6}	440
2	2.46×10^{-4}	0.7×10^{6}	410
3	5.38×10^{-4}	0.5×10^{6}	430
4	3.56×10^{-4}	0.7×10^{6}	420
5	4.92×10^{-4}	0.5×10^{6}	420

It was established that ATP concentration in the yeast cells was high $(2.4 – 7.8)\times10^{-4}$ mole/g. The CO_2 pressure in the bottles was more than 400 kPa in all samples after each cycle. The concentration of free cells accumulated in sparkling wine, obtained with immobilized biocatalyst, did not exceed than 8×10^{5} cells/mL. That fact confirmed high metabolic activity of immobilized cells and their possible use for cider production for at least 5 working cycles.

The immobilized yeast cells *S. cerevisiae* Challenge Red Fruit were used in this work for production of natural semi-sweet red wine. The fermentation was carried out in 200 mL flasks with flatus tubes at 18°C. The concentrations of free and immobilized cells used for fermentation were similar 3 x 10^{6} cell/mL. In parallel with application of immobilized yeasts the free cells were used in the experiments as a reference control. The yeast biocatalyst was used in 5 repeated batch fermentations. The sugar consumption (Fig. 1), ethanol and free cells accumulated in wines, fermented by both free and immobilized yeast cells, were analyzed (Table 3).

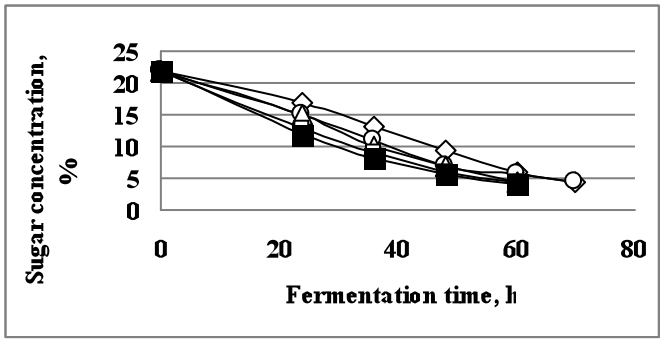

Fig. 1. The dynamics of sugar consumption during red wine fermentation carried out with immobilized yeast biocatalyst. The symbol ◇, O, Δ, □, ■ - number of fermentation cycle from 1 to 5, respectively.

It was shown that characteristics typical for semi-sweet red wines (4.5-5 % sugar) were achieved after 60-70 hours of fermentation. Sugar consumption was slightly lower during the first two repeated batch fermentations. The obtained wine contained alcohol concentration more than 10 % v/v. Total acidity in all testified samples was actually the same 4.8 g/L. Immobilized cells investigated under chosen conditions demonstrated a high enough metabolic activity even after 5 repeated batch fermentations. These facts were confirmed by the high intracellular ATP concentrations in immobilized cells (an average 5×10^{-6} mole/g) analyzed in the biocatalyst after each fermentation cycle (Table 3).

Table 3. Intracellular ATP concentration in the yeast cells analyzed after fermentation process and some characteristics of red wine prepared with free and immobilized yeast cells

Cycle	ATP, mole/g	Ethanol, % v/v	Total acidity, g/L
Free	3.2×10^{-8}	9.4	4.7
1	6.8×10^{-6}	10.2	4.8
2	3.9×10^{-6}	10.5	4.8
3	1.5×10^{-5}	10.4	4.9
4	1.1×10^{-6}	10.0	4.7
5	3.5×10^{-6}	10.2	4.9

CONCLUSION

The use of PVA CG as a carrier for immobilization of yeast cells guaranteed the obtaining of stable biocatalyst providing minimal accumulation of free cells in the wines. The biocatalyst possessed high fermentation activity at least in 5 working cycles. It was shown that application of immobilized biocatalyst based on cells entrapped into PVA CG enabled production of wines with similar or even better characteristics as compared to wines prepared with free cells.

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