Optimization of sparkling wine production with immobilized biocatalyst

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Introduction

Recently, the volume of sales of bottle-fermented sparkling wines notably increased in the world, especially in Great Britain (by 25%) and USA (by 30%). Last year, the 7%-increase in champagne consumption was registered in France compared to two preceding years. Totally in the world, three billion bottles with sparkling wines are annually consumed [www.rosalcohol.ru]. The competition between the champagne producers is high enough and it stimulates the development of new innovative technologies. Most of them are directed towards the elaboration of new approaches to the production of bottle-fermented sparkling wines allowing to minimize the cost price of target product and to retain its high quality.

Immobilization of champagne yeast cells is one of the most attractive methods enabling considerable simplification of servicing of fermentation process and obtaining of champagne with quality similar to the product prepared with free cells [Fumi, 1987, 1998; Yokotsuka, 1997; Martinenko, 2004].

The use of poly(vinyl alcohol) cryogel (PVA CG) as a carrier for immobilization of yeast cells guaranteed the obtaining of stable biocatalyst with active metabolism and minimal release of cells from polymer matrix [Stepanov, 2005; Efremenko, 2006a]. The macroporous structure of PVA CG provides immobilized cells with good conditions for mass-transfer processes [Lozinsky, 1998] and thereby the high viability of yeast cells is observed.

The productivity of immobilized cells often depends on many factors. In the case of bottle-fermented sparkling wine, the concentration of biocatalyst in the bottle predetermines the rate of fermentation process and the organoleptic characteristics of last product. Since then, this work was aimed at the optimization of sparkling wine production with previously developed immobilized biocatalyst [Efremenko, 2006b] when its concentration was varied in the bottles. Besides, the possible reuse of biocatalyst for production of bottle-fermented sparkling wine was investigated.

Material and methods

The *Saccharomyces cerevisiae* cells were used in the work. The cells were maintained at 4^{o}C on the grape wort with addition of agar (20 g/L). To accumulate cell biomass for biocatalyst production, the cells were cultivated in the malt wart with 10 g/l glucose under aerobic conditions at 26^{o}C for 17 h on a shaker (Lab-therm, Adolf Kühner, Switzerland) with constant agitation (220 rpm). Yeast biomass was separated from the cultivation broth by centrifugation (10,000 g, 10 min, Beckman J2-21 centrifuge, USA), and immobilized into PVA CG according to previously patented procedure [Efremenko, 2006b].

Champagne wine material was prepared by mixing white dry wine (from Risling white grape, pH 3.2) and liqueur up to the final concentration of sugar equal to 24 g/L. Then it was filtered through antimicrobial Corning filters (0.20 μm, Corning Inc, Germany) and used for secondary fermentation in 330 mL bottles. Fermentation was carried out under anaerobic conditions at 15^{o}C for 28 days. The bottles with fermented wine were periodically opened and samples were analyzed for free cell number ethanol and sugar content. All analyses were carried out in triplicate and the average value of each controlled parameter was calculated.

Concentrations of sugar was determined by HPLC using a Shimadzu chromatograph (Japan) with a SCR-101N stainless steel column, an LC-9A pump, a CTO-10A oven at 60°C and a RID-6A

refractive index detector. Milli Q water was used as mobile phase with a flow rate of 0.8 ml/min and butanol-1 was applied as an internal standard. Ethanol was estimated by HPGC (Hewlett Packard HP 4890D, Poropak Q) using nitrogen as carrier gas and hydrogen gas in the flame detectors, both at a flow rate of 32 ml/min. The residual sugar concentration was expressed as (g/L). The number of free yeast cells present in the sparkling wine was calculated by the routine microbiological method [Allen, 1994]. Carbon dioxide pressure accumulated during yeast fermentation was detected in accordance with International methods of wine and musts analysis [OIV, 1990].

Results and Discussion

The following concentrations of biocatalyst were used for the secondary fermentation: 0.45; 0.9; 9; 18 and 36 g/l. For all investigated samples the increase in ethanol concentration by 1.2-1.4 % (v/v) was revealed towards the end of fermentation process (Fig. 1a). The final sugar concentration in all tried samples of wine obtained with various biocatalyst concentrations was less then 0.5 g/l (Fig. 1b). No significant changes in pH of wine samples were observed.

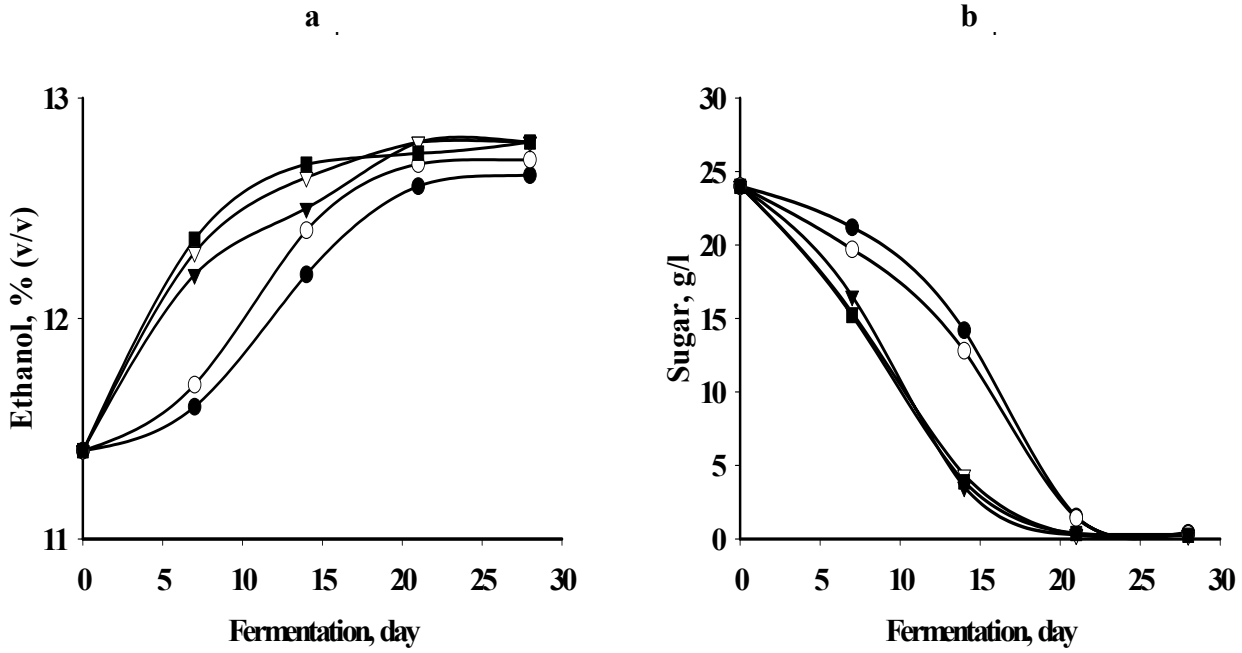

Fig.1. The kinetics of ethanol accumulation (a) and sugar consumption (b) in the bottle-fermentation process with immobilized yeast cells. Symbols ●, ○, ▼, ∇ and ■ correspond to following concentrations of biocatalyst in the wine: 0.45, 0.9, 9, 18 and 36 g/l, respectively

Compared to other analyzed samples, a little lower residual concentrations of sugar and slightly higher ethanol concentrations were observed in the wines with 9-36 g/l of biocatalyst in the bottle. It was shown that CO_2 pressure was actually the same (~500 kPa) in all tested samples (Fig. 2), however it should be noted that this pressure was appeared in the bottles for less than 3 weeks of fermentation when the biocatalyst concentration was varied in the range of 18-36 g/l. In the bottles with low concentrations of biocatalyst same result was registered after 4 weeks.

It should be noted that specific rates of ethanol accumulation in the bottles with 18 and 36 g/l biocatalyst were actually very similar. Thereupon, the lower concentration of biocatalyst between these two was accepted as optimal for the bottle-fermented sparkling wine production.

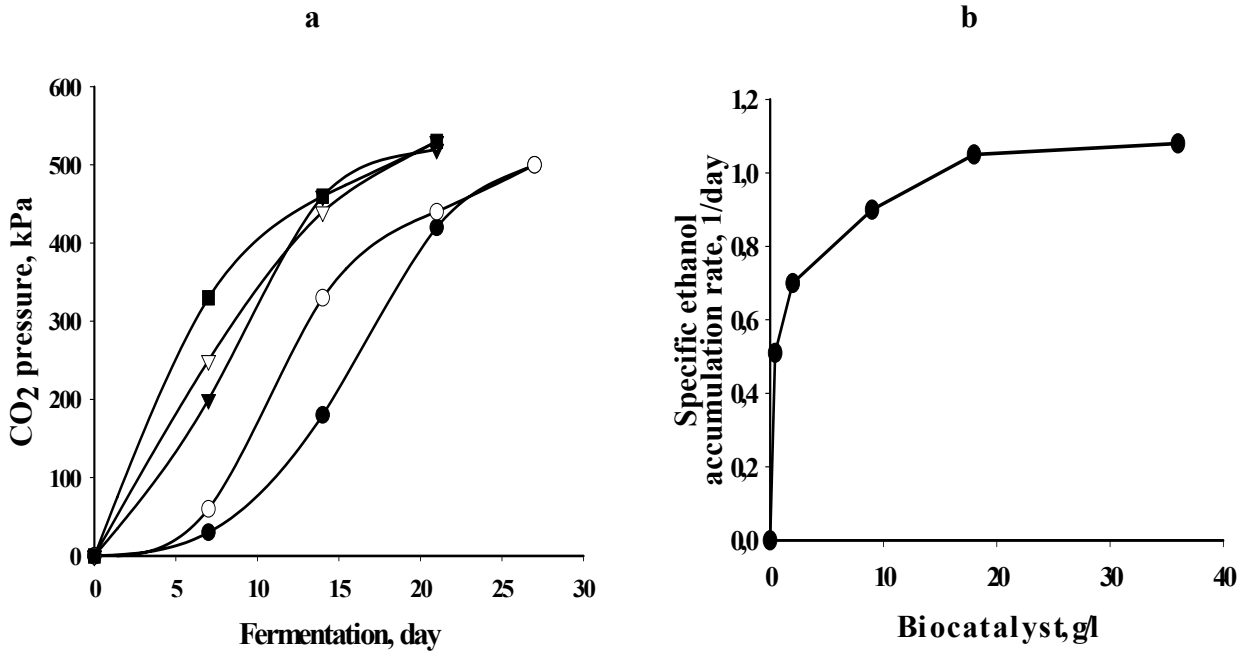

Fig. 2. A. The kinetics of CO_2 accumulation in the bottles with biocatalyst. Symbols ●, ○, ▼, ∇ and ■ correspond to following concentrations of biocatalyst in the wine: 0.45; 0.9; 9; 18 and 36 g/l, respectively. B. The dependence of specific rate of ethanol accumulation on the concentration of biocatalyst in the fermented medium.

The number of free yeast cells was analyzed throughout the fermentation process. The concentration of free cells determined in the samples of sparkling wine obtained with immobilized biocatalyst was lower than 5×10^3 cells/ml in all cases.

The investigation of possibility of multiple use of biocatalyst for the wine champagnization in the bottles was carried out with optimal concentration (18 g/l). The fermentation activity of immobilized yeast was controlled by CO_2 pressure measuring (Fig 3).

As it follows from obtained data, the CO_2 pressure was over 500 kPa in tried samples even after 100 days of biocatalyst application.

That fact testified to the high fermentability of immobilized cells being an active part of tested biocatalyst. The approximation of analytical data allowed to establish that the half-life time of the biocatalyst is 150 days in the processes of bottle-fermented sparkling wine production.

The taste evaluation of samples of sparkling wines obtained with biocatalyst was conducted at the end of fermentation process.

All tested samples of champagne were characterized by clear, light straw-color. The absence of yeast tone was noted. The tested marks of the samples obtained with different concentration of biocatalyst varied in the range of 7.95-8 points, when the 8 points composed the maximal value.

Conclusions

It was shown that certain concentration of biocatalyst should be chosen for the production of sparkling wine (18 g/l). Sparkling wine with perfect organoleptic characteristics was obtained with the immobilized biocatalyst. The multiple use of the biocatalyst in the sparkling wine production was demonstrated.

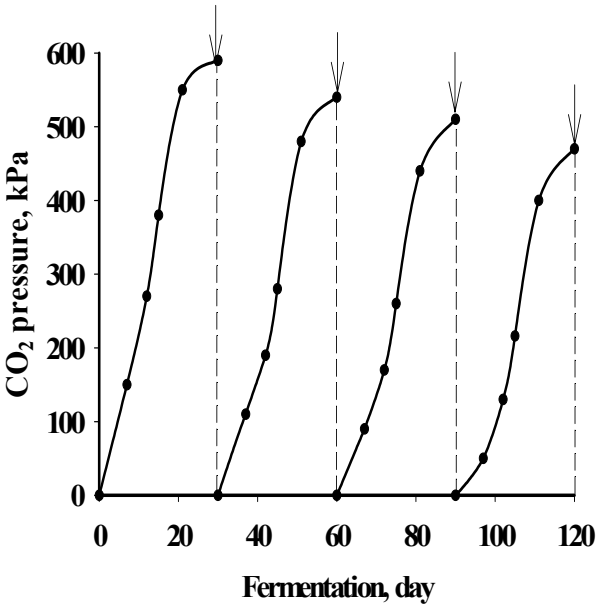

Fig. 3. The accumulation of CO_2 in the bottles during the multiple use of biocatalyst (18 g/l) for the sparkling wine production. Arrows mark the replacement of fermented medium by the fresh one.

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