EFFECT OF DIFFERENT CONDITIONS ON *SACCHAROMYCES CEREVISIAE* IMMOBILIZATION ONTO SUGAR BEET PULP IN ETHANOL PRODUCTION

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ABSTRACT

The use of yeast cells immobilized on sugar beet pulp as support for ethanol production employs a cheap and simple method of retaining high cells densities. The present work describes the effect of yeast concentration and ammount of support on immobilization of *Saccharomyces cerevisiae* onto sugar beet pulp (SBP). Further, the efficiency of immobilized biocatalyst for batch ethanol fermentation of sugar beet thin juice was investigated with goal to examine the optimum conditions of its potential application. The hydratet SBP showed highes cells retention capacity of 0.117 g/g. A maximum sugar conversion of 97.69%, ethanol concentration of 75.66 g/l, ethanol yield per consumed sugar of 0.499 g/g (equal to 97.71% of its theoretical value) was achieved in the batch fermentation of thin juice substrate. This study demonstrates that the efficient ethanol fermentation from sugar beet thin juice using *S. cerevisiae* immobilized by natural adhesion on sugar beet pulp (SBP) is possible even without any nutrient supplementation. The novelty of the approach lies in the effectiveness of exploitation of thin juice and sugar beet pulp with purpose to obtain efficient ethanol production from and to lower high operating cost.

1. INTRODUCTION

Recently, ethanol produced by alcoholic fermentation from sucrose, starch or lignocellulosic biomass has received special attention as the most promising biofuel from renewable resources. Ethanol is widely used as solvent and chemical feedstock in various industries (Sivakumar et al., 2010). Cell immobilization techniques have become increasingly important and are being successfully applied in production of alcohol (ethanol, butanol and isopropanol), organic acids (malic, citric, lactic and gluconic acids), enzymes (cellulase, amylase, lipase and others), and biotransformation of steroids for wastewater treatment, and food applications (beer and wine) (Reddy et al., 2008).Yeast cell immobilization on various plant materials in the ethanol fermentations has many technical and economical advantages compared to free cell system. It is an effective method for improving the efficiency of substrate utilization and productivities of fermentation processes (Kourkoutas et al., 2006). Our previous study of ethanol production by SBP-supported yeast cells showed increased ethanol efficiency in the ethanol production from sugar beet molasses and thick juice (Vučurović and Razmovski, 2012). Molasses is a traditional raw material for distilleries in Serbia, particularly in the Vojvodina province, and about 90% of ethanol production comes from this raw material nowadays. The production of bioethanol from thick juice as an intermediate of sugar beet processing by yeast cells immobilized onto sugar beet pulp gives the benefits of reduced water usage, reduced wastewater purification costs, easier mixing with syrup if used warm, lower use of acids for pH buffering, increased levels of nutrients and higher ethanol efficiency compared to molasses (Vučurović and Razmovski, 2012). However, the ethanol fermentation of sugar beet thin juice by *S. cerevisiae* immobilized onto the hydrated SBP was not investigated so far. The aim of this work was to explore the the effect of yeast concentration and amount of support on immobilization of *S. cerevisiae* onto sugar beet pulp (SBP). Further, the efficiency of immobilized biocatalyst for batch ethanol

fermentation of sugar beet thin juice was investigated with goal to achieve efficient ethanol production.

2. MATERIAL AND METHODS

Dried sugar beet pulp (SBP) from a sugar factory near the city of Senta in the Vojvodina province, Serbia was kindly provided and used as support for yeast cells. The SBP hydration was carried out by placing an amount of 10 g, 25 g and 50 g of DSBP on dry basis into 1 l Erlenmeyer flasks containing 500 ml of synthetic culture medium consisted of glucose (120 g/l), $(NH_4)_2SO_4$ (1 g/l), KH_2PO_4 (1 g/l), $MgSO_4$ (5 g/l) and yeast extract (4 g/l) at pH of 5.5, and was sterilized by autoclaving at 121°C for 30 min. After the sterilization, flasks were kept at room temperature for 24 h. Working microorganism was a commercial *S. cerevisiae* strain (Alltech-Fermin, Senta, Serbia), commonly used in Serbian baking industry, in form of pressed blocks (70 % w/w moisture). To immobilize cells on hydrated DSBP, the flasks were inoculated with 3 g/l, 4.5 g/l and 6 g/l of yeast on dry basis, and placed on a rotary shaker (120 rpm) in termostate at 30 °C for 24 h. After the immobilization of the yeast, the mass of immobized cells onto the support was quantified gravimetrically according to Santos et al. (2008). Cell retention onto the support (R, g/g) was calculated as the ratio of dry matter of cells immobilized in the support (g) to the support dry mass (g). Carl Zeiss optical microscope connected to a camera Cannon S50 was used to capture yeast cells immobilized onto hydrated SBP. After the immobilization of the cells, the medium was decanted using sterilized gauze. The suport without extra medium, was then dried at 105 °C up to constant weight. The identical procedure was conducted using support particles recovered from the cell-free medium, as a control, in order to avoid any interference in weighing measurements. The selected biocatalyst with highest cells retention (R) was used for the batch fermentation of 500 ml of the sugar beet thin juice in 1 l Erlenmeyer flask. Thin juice was obtained from the mentioned sugar factory. The total sugar content of thin juice was 155,53 g/l, pH was adjusted to 5.5 pH by addition of 10% (v/v) H_2SO_4 and it was sterilized by autoclaving at 121 °C for 30 min. The fermentation kinetics was monitored by measuring the weight loss due to CO_2 release at various time intervals from the beginning of each fermentation batch. Samples of fermented liquids were analyzed for ethanol and sugar. The fermented liquid was centrifuged at 3000 rpm for 15 min. The sample of supernatant was hydrolyzed in 33% HCl at 100 °C for 10 min and neutralized with NaOH solution, and sugars were than determined using the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). The ethanol concentration of distillate was determined based on the density of the alcohol distillate at 20 °C, by pycnometer method (AOAC method 942.06, 2000). Sugar conversion ($S_{u,\%}$) was calculated as the ratio of utilized sugar to the initial and multiplying by 100. The ethanol yield ($Y_{p/s}$, g/g) was calculated as grams of ethanol produced per gram of utilized sugar. Also a percentage of the maximal theoretical ethanol yield ($E_{p/s}$, %) was calculated. The volumetric ethanol productivity (Q_p, g/lh) were calculated as grams of ethanol produced per liter per hour.

3. RESULTS AND DISCUSSION

The immobilization of *S. cerevisiae* on hydrated SBP is a result of natural entrapment into the porous structure of materials, which includes physical adsorption by electrostatic forces between the yeast cell membrane and the support and also the action of capillary forces which pull the cells to approach and keep in close contact with the surface (Vučurović and Razmovski, 2012). Optical microscopic examination of yeast cells immobilized on hydrated SBP (Fig. 1b), confirmed that some of yeast cells were firmly adsorbed onto the specific parts

of surface of the support and also infiltrated into the small pores of the different parts of plant tissue structures, and then multiplied.

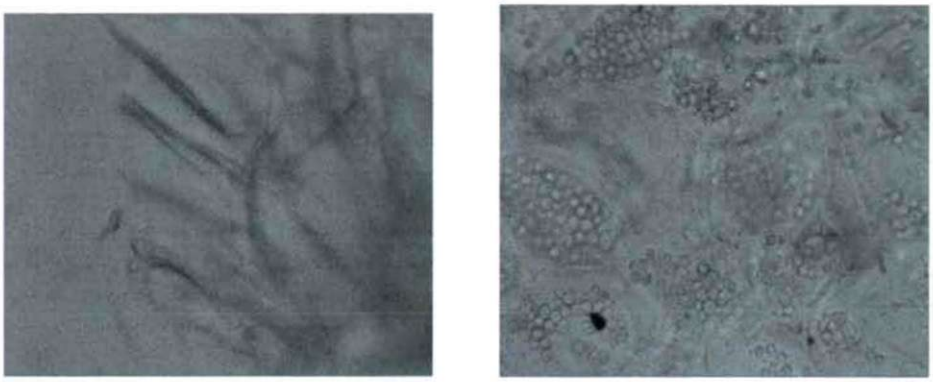

Figure 1. Optical microphotograph of hydrated SBP (a) and Saccharomyces cerevisiae cells (400×) immobilized onto the hydrated SBP (b).

By comparing obtained retention capacities for different yeast and support amounts (Fig. 2) it can be concluded that cells retention increased with the increase of initial yeast concentration from 3 g/l to 6 g/l, while it decreased with the increase of the suport amount from 10 g/l to 50 g/l the optimal yeast. On the basis of yeast immobilization results it can be concluded that the optimal cells retention capacity (R) of 0.117 g/g was achieved for initial yeast concentration of 6 g/l and support amount of 20 g/l. Due to the highest yeast cell retention capacity, immobilized biocatalyst under these optimal conditions was used for thin juice fermentation.

In the ethanol fermentation, the theoretical yield of ethanol and CO_2 is 0.511 g and 0.489 g per g of glucose metabolized, respectively. Hence, in the case of batch fermentation the CO_2 evolution rate could represent fermentation rate instead of ethanol production rate. From time-courses of CO_2 (Fig 3.) CO_2 production indicated normal behavior during the fermentation batch. According to the dynamic of CO_2 production (Fig. 3) very low fermentation time (48 h) was achieved. Fast fermentation times indicated that no period was needed for adaptation of biocatalyst in the fermentation environment. The immobilized yeast showed an important operational and stability without any decrease of its activity.

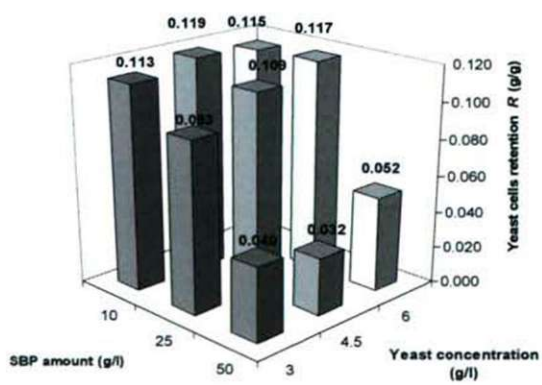

Figure 2. Rettention of S.cerevisiae cells onto hydrated SBP.

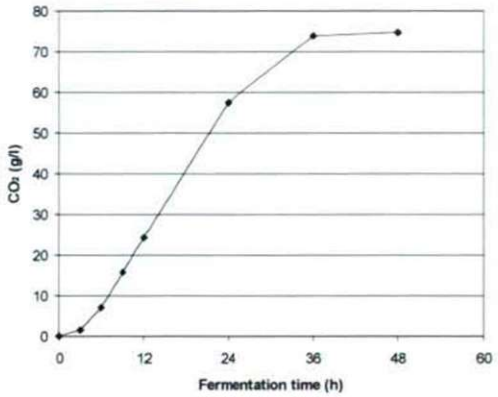

Figure 3. CO_2 production during the fermentation of sugar beet thin juice by S. cerevisiae immobilized on SBP

Table 1 summarizes the fermentation parameters such as sugar utilization, ethanol volumetric productivity, ethanol yield and percentage of theoretical yield obtained at the end of the fermentation batch of thin juice by *S. cerevisiae* immobilized onto hydrated SBP.

Table 1. Parameters of sugar beet thin juice fermentation by S. cerevisiae immobilized on hydrated SBP

Parameter	Value
Initial sugar, S_0 (g/l)	155.53
Utilized sugar, S_u (g/l)	151.95
Sugar conversion, S_u (%)	97.69
Ethanol concentration, P (g/l)	75.66
Ethanol productivity, Q_p (g/lh)	1.58
Ethanol yield, $Y_{p/s}$ (g/g)	0.499
Percetage of the theoretical yield, $E_{p/s}$ (%)	97.71

A maximum sugar conversion of 97.69%, indicated that immobilized cells onto SBP utilized almost all available sugar from thin juice sugesting that thin juice is very good raw material for ethanol production. Final ethanol concentration of 75.66 g/l and ethanol productivity of 1.58 g/lh were achieved at the end of thin juice fermentation batch. Ethanol yield per consumed sugar of 0.499 g/g was achieved, equal to 97.71% of its theoretical value, indicating that almost all utilized sugar was converted to ethanol during the fermentation process.

By comparing results obtained in the present work, with other biocatalysts prepared by yeast immobilization on natural, food grade materials that have been extensively studied, such as dignified cellulosic materials, gluten pellets, pieces of fruit etc. (Table 2) it can be concluded that the present biocatalyst was equally efficient for alcoholic fermentation.

Table 2. Fermentation parameters (average values) obtained in batch fermentations with Saccharomyces cerevisiae cells immobilized on various supports, at 30°C

Support material	Medium	Initial sugar (g/l)	Ferm. Time (h)	Residual sugar (g/l)	Ethanol P (g/l)	Ethanol productivity Q_p (g/ld)	Sugar conversion S_u (%)
Delignified cellulosic materials (Iconomou et al., 1996)	Molasses /sucrose	172	36	10.2	104	69.3	94
	Glucose	350	67	68	144	51.5	80.5
Gluten pellets (Bardi et al.,1996)	Glucose	119	15	12.5	39.5	63.2	89.5
	Grape must	206	17	18.9	83.7	118	90.1
Dried figs (Brkatorou et al., 2002)	Wort	129	18	0	47.4	64	100
	Glucose	120	45	1.4	45	24	98
Quince pieces (Kourkoutas et al., 2003)	Grape must	185	28	0.1	84	72	99.9
Apple pieces (Kourkoutas et al., 2001)	Grape must	206	80	30.8	85	26	85
	Glucose	125	9	4	51.4	128.3	96.8
	Molasses /sucrose	128	14	2	58.9	100.1	98.4
Orange peel (Plessas et al., 2007)	Raisin extract	124	12	2.3	55.3	110.4	98.1
Watermelon rind pieces (Reddy et al., 2008)	Grape must	202	64	tr	87.0	45.8	100
Sugar beet pulp (present study)	Thin juice /sucrose	150	48	3.6	75.66	1.58	97.7

4. CONCLUSION

The results demonstrated that efficient fermentation system was achieved by using *S. cerevisiae* immobilized onto the SBP as biocatalyst for alcoholic fermentation of sugar beet thin juice. Further investigation on specific food applications using this biocatalyst would be interesting.

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