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SHORT COMMUNICATION

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## BIOETHANOL PRODUCTION FROM INTER-MEDIATE PRODUCTS OF SUGAR BEET PROCESSING WITH DIFFERENT TYPES OF *Saccharomyces cerevisiae*

*The use of biofuels as an alternative to fossil fuels has expanded in the last few decades. The aim of this study was to examine the application of different strains and forms of Saccharomyces cerevisiae for raw, thin and thick juice fermentation in order to produce bioethanol. According to the obtained results the strain applied in the form of pressed blocks with 70 % w/w moisture, attained higher value of the specific growth rate and lower value of ethanol yield in comparison with strains applied in dried form. In all culture media attained efficiency of sugar utilization was at least from 98-99 % w/w. Maximum productivity was achieved around 30<sup>th</sup> hour of fermentation and amounted  $\approx 1.8 \text{ g l}^{-1} \text{ h}^{-1}$  for all applied yeast strains. Therefore, optimal duration of the process in technical and economic terms should be considered.*

*Key words: Saccharomyces cerevisiae; bioethanol; raw juice; thin juice; thick juice; molasses.*

The basic concern over bioethanol production expansion is depletion of natural resources and demands for environmentally acceptable fuels - biofuels from renewable feedstocks, emitting less carbon dioxide into the atmosphere [1,2]. Less expensive production of sugar from sugarcane indicates that the application of sugar beet for bioethanol production has great potential [3]. Molasses is commonly used feedstock for bioethanol production. In the sugar beet processing raw and thin juices are intermediate products with production costs considerably lower in comparison with molasses, obtained at the end of the process. The only disadvantage of these intermediate products is low storability and easy decomposition by the action of microorganisms. Thick juice is an intermediate product with significantly higher price mostly due to evaporation with storability comparable with molasses [4]. The aim of this study was to examine the application of different strains and forms of *Saccharomyces cerevisiae* for raw, thin and thick juices and molasses fermentation in order to produce bioethanol. These types of *S. cerevisiae* were selected as pro-

duction microorganisms because of their commercial availability at Serbian market and an extensive application in food industry.

### MATERIALS AND METHODS

Raw, thin and thick juices and molasses obtained from a domestic sugar factory were used as fermentation medium. Raw and thin juices were used without dilution with sugar content  $\approx 13 \text{ g/l}$ , resulting from sugarbeet processing technology. Thick juice and molasses were diluted with water to resultant sugar content  $13 \text{ g/l}$ . pH value of culture media was adjusted with 10 % v/v sulphuric acid to the value 5.00. Determination was made using Consort C863 laboratory multiparameter analyzer (Consort, Belgium). The prepared culture media were sterilized by autoclaving at  $121 \text{ }^\circ\text{C}$  and at 1.2 bar overpressure for 30 min. Four different fermentation media were inoculated with five different strains and forms of *Saccharomyces cerevisiae*.

- yeast for strong alcoholic drinks production, dried (SD);
- yeast for wine production, dried (W1);
- yeast for wine production tolerant on enhanced ethanol content, dried (W2);
- bakery yeast, dried (BD);

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– bakery yeast, in the form of pressed blocks (70 % w/w moisture), (BF).

Yeast was suspended in small quantity of culture media and introduced to the rest of it in the rate of 2.7 g of yeast dry solids per 1 l of media. Fermentations of culture media in anaerobic conditions were performed in 2 l Woulff's bottles at  $30 \pm 1$  °C on a rotational shaker with 150 rpm during 60 h. The fermentation course was followed by analyzing the samples in the predetermined time intervals: 0, 4, 8, 12, 24, 30, 36, 48 and 60 h from the moment of inoculation. Yeast cells counts were determined by the direct counting in Neubauer counting chamber using a microscope. The fermentation media samples were centrifuged 15 min at 4000 rpm, after which the fermentable sugars (sucrose, glucose and fructose) content of the supernatant was determined by HPLC [5]. The ethanol content was determined by GS-FID in fermentation media samples. The yeast specific growth rate,  $\mu$  ( $\text{h}^{-1}$ ), was calculated from the slope of the linear dependence of the yeast cell number logarithm ( $\log N$ ) on the fermentation time of culture media (h) during an exponential phase of the growth using the equation:

$$\text{line slope} = \frac{\mu}{2,303} \quad (1)$$

The efficiency of sugar utilization was estimated as percentage of sugars utilized by yeasts (% w/w).

The ethanol yield,  $Y_{p/s}$ , was calculated from the equation:

$$Y_{p/s} = \frac{P}{S_0 - S} \quad (2)$$

where  $P$  refers to ethanol content at the end of fermentation (g),  $S_0$  to the initial sugar content (g) and  $S$  to the sugar content at the end of fermentation (g). The process productivity was estimated as the ethanol content per volume of fermentation medium per unit of time.

The experiments were carried out with four different cultivation media, each inoculated with five different yeast strains. For each experiment three independent fermentations were carried out and the results shown in this paper represent average values.

## RESULTS AND DISCUSSION

The results of the analyses of raw materials from Table 1 show that the compositions of the applied raw materials are characteristic of sugar beet processing in domestic factories.

Forming of dense foam was evident only during the fermentation of raw juice with all applied yeast

strains (SD, BD, BF, W1 and W2), particularly during the first 24 h, when the metabolism activity of yeast cells was intensive. Hence, a dose of antifoam agents was required. During the industrial production of ethanol, after the distillation these components would remain in draff, increasing the pollution of waste water.

Table 1. Compositions of raw materials

Parameter	Raw juice	Thin juice	Thick juice	Molasses
Dry substance, % w/w	14.70	14.50	58.8	80.80
Sucrose, % w/w	12.85	13.13	53.00	49.20
Coefficient of purity, % w/w	87.41	90.55	90.14	60.89
pH	6.30	9.25	7.27	6.98
Ash, % w/w	0.28	0.34	1.85	9.86
Reducing substances, % w/w	0.07	0.01	0.47	0.86
Total nitrogen, % w/w	0.13	0.13	0.14	1.82

Determination of cell number during the fermentation indicated that there was an intensive and almost linear increase of yeast cell counts during the first 12 h of fermentation with all of the applied yeast strains (data not presented).

The exponential phase of the yeast cell growth was underway, due to the remaining oxygen content of the fermenting media. The total number of yeast cells was almost constant during further fermentation under anaerobic conditions. Figure 1 shows the dependence of the yeast specific growth rate,  $\mu$ , on the applied culture media and yeast strains (according to Eq. (1)). Average values of the specific growth rate of five applied yeast strains amounted 0.0716, 0.0754, 0.0720 and 0.0712  $\text{h}^{-1}$  for raw, thin and thick juices and molasses, respectively. The difference between four applied culture media according to the yeast specific growth rates was evidently insignificant. Average values of the specific growth rate of strains SD, BD, BF, W1 and W2 amounted 0.0748, 0.0765, 0.0853,

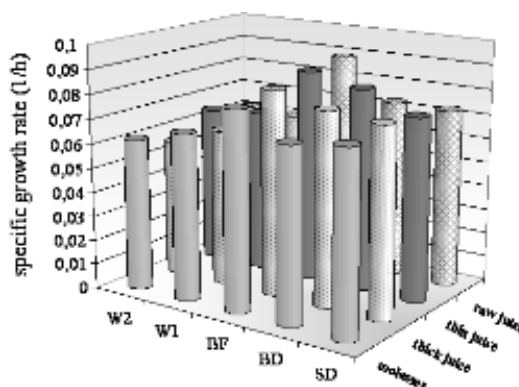


Figure 1. The dependence of the yeast specific growth rate on the applied culture media and yeast strains.

0.0650 and 0.0613 h<sup>-1</sup>, respectively. According to the obtained results the strain BF, applied in the form of pressed blocks with 70 % w/w moisture, attained significantly higher value of the specific growth rate in comparison with strains applied in dried form. It is also evident that strains SD and BD attained higher values of the specific growth rate in comparison with both yeast strains for wine production (W1 and W2).

During the exponential phase of growth, yeast cells incorporated sugar assimilated from the culture media into biomass inducing the intensive cell growth. Simultaneously with this process, ethanol and CO<sub>2</sub> were produced. In second, anaerobic phase, yeast cells used assimilated sugar mostly for ethanol synthesis. Figure 2 illustrates the dependence of the efficiency of sugar utilization on the applied culture media and yeast strains. It is evident that in all culture media the attained efficiency of sugar utilization was at least from 98-99 %. That means that all applied yeast strains were capable of exploiting almost all sugars from fermenting mashes based on raw, thin and thick juices and molasses, applied with the initial sugar content ≈13 g/l, corresponding to the results of Hinkova and Bubnik [4].

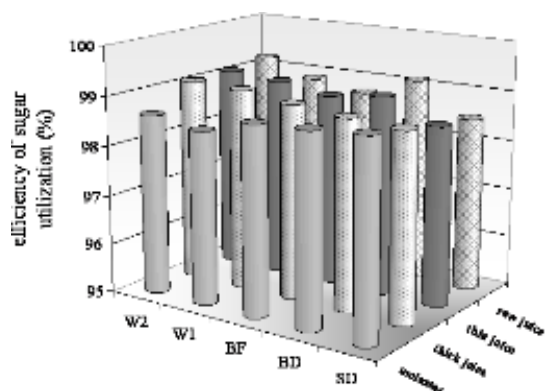


Figure 2. The dependence of the process efficiency on the applied culture media and yeast strains.

Figure 3 illustrates the dependence of the ethanol yield on the applied culture media and yeast strains (according to Eq. (2)). The value of the ethanol yield in applied experimental conditions ranged in the interval of 0.485-0.494 g/g, which is close to the theoretical yield of 0.51 g of ethanol per 1 g of glucose [6]. Average values of the ethanol yield for fermenting mashes prepared from raw, thin and thick juices and molasses were almost uniform. Although sugar utilization by all applied yeast strains was almost total, more significant variations of ethanol yield for different strains were evident and average values amounted to 0.491, 0.490, 0.486, 0.492 and 0.493 g/g for strains

SD, BD, BF, W1 and W2, respectively. Generally, the strain BF gave lower ethanol yields in comparison with the strains applied in dried form. It is also evident that strains SD and BD gave lower ethanol yields in comparison with yeast strains for wine production (W1 and W2). The strain BF, applied in the form of pressed blocks with 70 % w/w moisture, at the moment of inoculation was in a more active physiological state in comparison with strains in dried forms. This fact clarifies a higher yeast growth rate of the strain BF (see Figure 1), accompanied by proportionally higher sugar consumption, leaving a lower sugar content available for the ethanol production. On the other hand, lower achieved yeast cell counts in fermenting mashes with strains in dried forms had no negative effect on the ethanol production, indicating that those values were sufficient for the high ethanol yield.

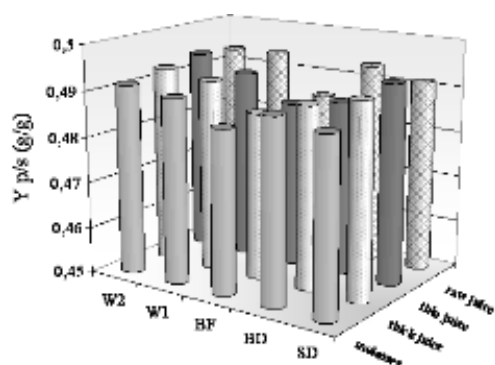


Figure 3. The dependence of the ethanol yield on the applied culture media and yeast strains

Figure 4 shows the dependence of average values of the ethanol productivity for different cultivation media on the fermentation time and applied yeast strains. According to the obtained results maximum productivity was achieved at around 30 h of fermentation and amounted ≈ 1.8 g l<sup>-1</sup> h<sup>-1</sup> for all applied yeast strains (SD, BD, BF, W1 and W2).

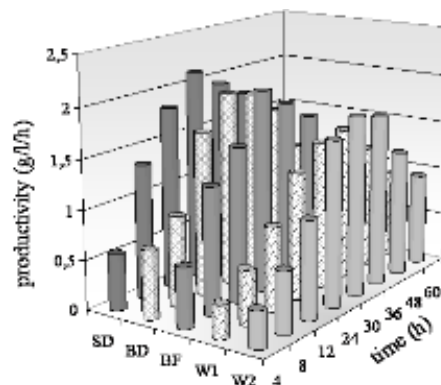


Figure 4. The dependence of the productivity on the fermentation time and applied yeast strains.

## CONCLUSION

The above results demonstrated that the efficient ethanol production from raw, thin and thick juices and molasses is possible with all examined strains of yeast *Saccharomyces cerevisiae* (SD, BD, BF, W1 and W2) without concern of their primary utility in food industry. Maximum productivity was achieved after 30 h of fermentation, and with further prolongation of the fermentation time the ethanol productivity decreased suggesting that optimum duration of the process should be technically and economically considered.

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