ETHANOL PRODUCTION FROM SUGAR BEET THIN JUICE BY IMMOBILIZED *SACCHAROMYCES CEREVISIAE*: CHARACTERIZATION OF VOLATILE COMPOUNDS

Radojka N. Razmovski, Vesna M. Vučurović
Department of Biotechnology and Pharmaceutical engineering, Faculty of Technology, University of Novi Sad, 21000 Novi Sad, Bulevar Cara Lazara 1, Serbia
e-mail: razmovski@tf.ins.ac.rs

ABSTRACT

The potential of by-products and intermediate products of sugar beet processing as raw materials for bioethanol production in Serbia has a big scope in view of the demand of ethanol as an alternative for fossil fuels. The production of bioethanol from sugar beet thin juice by *Saccharomyces cerevisiae* immobilized on sugar beet pulp (SBP) enables one more opportunity for achieving the zero-waste goal, through a rational use of intermediate and by-products of sugar beet processing. The effect of inoculum concentration on volatile compounds content of distillate was examined. The increase of inoculum concentration from 2.0 g/l to 2.6 g/l (dry mass basis) increased ethanol and aldehyde content of the distillate from 9.34 % v/v to 9.60 % v/v and from 238.6 mg/l a.a. to 301.5 mg/l a.a., but decreased methanol, acetic acid and ester content from 650.0 mg/l a.a. to 232.3 mg/l a.a., from 73.4 to 51.3 mg/l a.a., and from 272.0 mg/l a.a. to 220.8 mg/l a.a., respectively. The increase in inoculum concentration of SBP-immobilized yeast indicated improvement of distillate quality.

1. INTRODUCTION

Bio-fuels are attracting growing interest around the world, with some governments announcing commitments to bio-fuel programs as a way to both reduce greenhouse gas emissions and dependence on petroleum-based fuels. Bioethanol is by far the most widely used bio-fuel for transportation worldwide, because it is a renewable, nontoxic, biodegradable resource and it is oxygenated, thereby provides the potential to reduce particulate emissions in compression–ignition engines (Balat et al., 2009). Molasses and other intermediates from sugar beet processing are very good raw materials for ethanol production due to their high content of fermentable sugars, which can be directly used for fermentation without any modification (Rodríguez et al., 2010). In the sugar industry process, sugar beet (*Beta vulgaris*) is processed by extracting sliced beet cossettes with hot water (70°C) to produce the raw sugar juice. The raw juice is purified to get the thin juice with an average sugar content of 16%. The thin juice will be then concentrated in multiple steps resulting in a thick juice with an average sugar content of 67% (Krajnc and Glavić, 2009). Using immobilized cells in ethanol production is advantageous over free cells due to enhanced yield, ease to separate cell mass from the bulk liquid, reduced risk of contamination, better operational stability and cell viability for several cycles of operations (Chandel et al., 2007). As is well known, it is very important for the quality control of final fuel ethanol product, to ensure the absence of volatile compounds. In the present work the effect of inoculum concentration on volatile compounds content of distillate was examined in the case of sugar beet thin juice fermentation by *S. cerevisiae* immobilized onto SBP.

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 25 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 5 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 medium was decanted using sterilized gauze. Different amount of immobilized biocatalyst (2.0 g/l, 2.3 g/l and 2.6 g/l on yeast dry bases) was used for the batch fermentation of 500 ml of thin juice. 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 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). The content of volatile by-products (acetaldehyde, ethylacetate, acetic acid, methanol and furfural) of the distillate was determined by standard AOAC methods 972.08, 950.05, 962.12, 958.04, 960.16, respectively. Volatile by-products concentration were expressed as mg per liter of absolute alcohol (a.a.) in distillate.

3. RESULTS AND DISCUSSION

The ethanol content of distillate is presented in Fig. 1. On the basis of presented results it can be concluded that the ethanol content was increased along with increase of inoculum concentration.

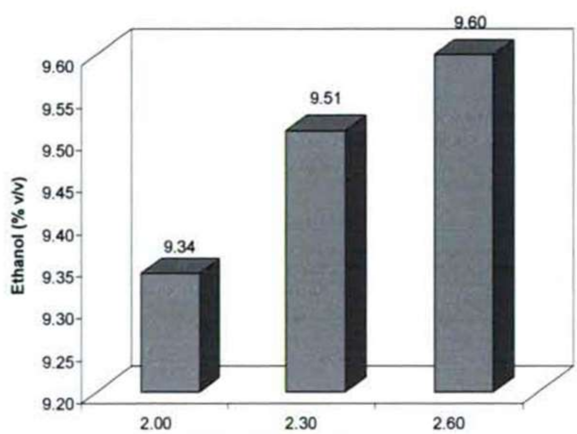

Figure 1. Ethanol content of distillate

The volatile by-products content of ethanol distillate after the fermentation using *S. cerevisae* immobilized onto SBP is shown in Fig. 2-5.

Methanol, which is derived from methylated pectic substances (pectin) by the action of pectic esterases (Reddy at al., 2008), showed a lower concentration in ethanol distillate obtained after fermentation with increased inoculum concentration.

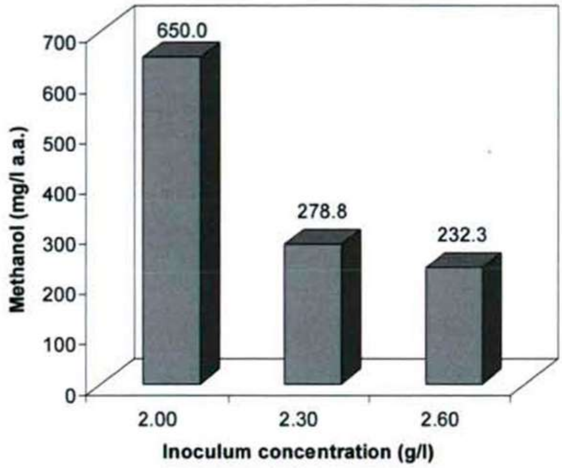

Figure 2. Methanol content of distillate

The levels of volatile acidity (expressed as acetic acid concentration) presented in Fig. 3. were significantly higher for lower inoculum concentration. This reduction of volatile acid content more pronounced in immobilized cells system with higher inoculum concentration, may be due to the very specific immobilization onto SBP as new biocatalyst. Besides, low volatile acidity was recorded indicating a high quality product.

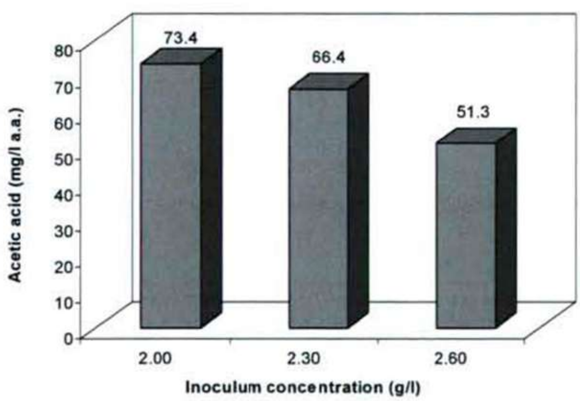

Figure 3. Acetic acid content of distillate

Esters (expressed as ethyl acetate), formed from the organic acids and alcohols during fermentation and secondary fermentation, play an important role in the formation of the sensory features. Their formation is catalyzed mostly by the yeast enzymes belonging to the group of esterases (Klosowski and Czupryński, 2006). The ester content of the distillate (Fig. 4) decreased along with increase of inoculum concentration.

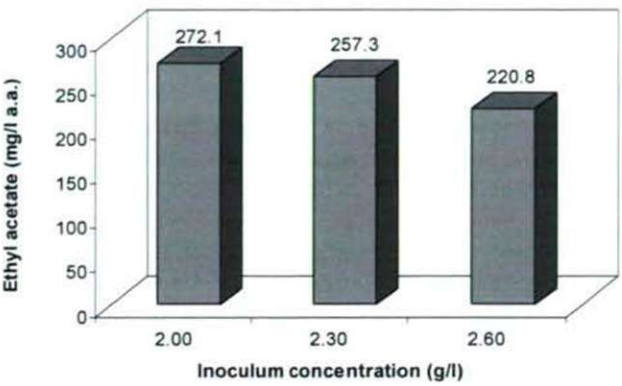

Figure 4. Ethylacetate content of distillate

Acetaldehyde, precursor of the acetates and ethanol, is formed from pyruvate by the glycolytic pathway enzyme pyruvate decarboxylase (Regodón et al., 2006). In contrast to other volatile compounds, the increase of acetaldehyde content (Fig. 5) was obtained along with increase of inoculum concentration. In this study the amount of aldehydes was significantly higher when inoculum was increased. Furfural content has not been determined in any sample of distillate.

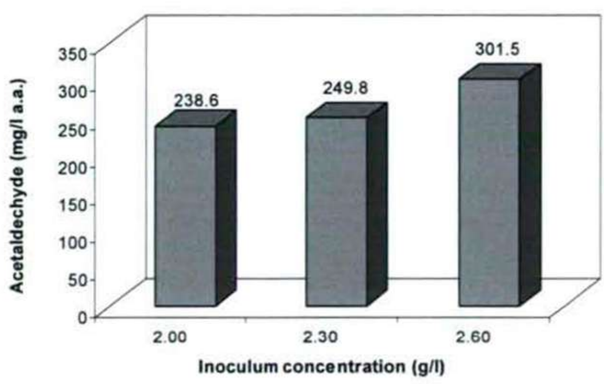

Figure 5. Acetaldehyde content of distillate

4. CONCLUSION

The increase of inoculum concentration from 2.0 g/l to 2.6 g/l (dry mass basis) increased ethanol and aldechyde content of the distillate from 9.34 % v/v to 9.60 % v/v and from 238.6 mg/l a.a. to 301.5 mg/l a.a., but decreased methanol, acetic acid and ester content from 650.0 mg/l a.a. to 232.3 mg/l a.a., from 73.4 to 51.3 mg/l a.a., and from 272.0 mg/l a.a. to 220.8 mg/l a.a., respectively. The increase in inoculum concentration of SBP-immobilized yeast indicated improvement of distillate quality due to the increase of ethanol content and descrease of methanol, acetic acid and ethylacetate content.

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