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Intermediate Products from Sugar Beet Processing Used for the Bioesynthesis of Ethanol

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Abstract: The use of liquid biofuels as an alternative to hydrocarbon fossil fuels has expanded in the last few decades. The basic concern over the use of bioethanol as a transport fuel is to reduce carbon dioxide emissions into the atmosphere. The objective of this study was to investigate the possibilities of using raw, thin and thick juices, the intermediate products from sugarbeet processing, for bioethanol production. The obtained results were compared with the results of fermentation with molasses as a traditional feedstock for bioethanol production. The application of cultivation media based on raw, thin and thick juices resulted in fermentation yields of 62.19%, 60.35% and 66.51%, respectively, the values being very close to the yield of 63.09 % achieved by applying the molasses-based cultivation media. After the 12^{th} hour of fermentation, productivity of the process significantly decreased. Therefore, optimal duration of the process in technical and economic terms should be considered.

Keywords: bioethanol, thick juice, thin juice, raw juice, molasses

Introduction

Bioethanol is a modern type of energy and an important substitute for liquid fossil fuels or, in mixtures with gasses, a substitute for natural gas (Ericsson and Nilsson 2006; Reiche 2006; Farrell *et al.*, 2006). 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 (Mojovic *et al.*, 2007). The most significant raw materials in this region are sugar beet molasses and starchy raw materials, such as potato, corn and cereal grains (Tasić *et al.*, 2006; Baras *et al.*, 1996). It is necessary to identify and evaluate the bioethanol production processes or a combination thereof which assures the best results from technological, economic and environmental

points of view. The less expensive production of sugar from sugarcane indicates the great potential of sugar beet application in bioethanol production.

Molasses may be easily stored for long periods of time so its application is not restricted to the marketing season and the advantages of its use also include relatively simple preparation, minor equipment requirement, level of microbial purity, regulation and automatisation etc. (Baras *et al.,* 1996). In view of the fact that molasses is suitable for biotechnological production of a wide variety of products, its cost is relatively high. Furthermore, molasses is a by-product of sugar beet processing obtained at the end of the process and hence its price is considerably higher than that of raw, thin and thick juices. The objective of this study was to investigate the possibilities of using raw, thin and thick juices as imtermediate products from sugarbeet processing in bioethanol production.

Materials and Methods

The raw materials used for the preparation of culture media were analysed according to the Methods of Laboratory Sugarbeet Processing Control (Milic et *al.,* 1992). Raw and thin juices were used as fermentation media, their sugar contents being 12.83% and 13.13%, respectively. The said content was maximal, resulting from sugarbeet processing technology.

Thick juice and molasses were diluted with tap water to obtain final sugar contents in the culture media of 15%. pH-values of the culture media were adjusted to pH 5 by the addition of sulphuric acid solution (10 vol.%). Determinations were made using a Consort C863 laboratory multiparameter analyser (Consort, Belgium). The prepared culture media were autoclave sterilised at 121ºC and at 1.2 bar-pressure for 30 minutes. Fresh baker's yeast *Saccahromyces cerevisiae* (Alltech.Fermin, Senta) was used as production microorganism. Yeast was suspended in a small quantity of culture media and introduced into the remaining part of it at the rate of 10 g/l, corresponding to starting yeast cell concentrations of 1×10^8 cells/ml. Fermentations of culture media in anaerobic conditions were performed in 2l Woulff's bottles at 30ºC on rotational shaker with 150 rpm during 36 hours. Fermentation was discontinued immediately upon CO_2 release. Quantities of the evolved CO_2 were obtained by measuring the weight of fermentation media using automatic technical scale (0.01g). The fermentation course was followed by the analysis of samples at predetermined time intervals: 0, 4, 8, 12, 24, 30 and 36 h after inoculation. Yeast cell counts were determined using the methylene-blue method, in the 1:100 dilution (Vrbaski and Markov 1992). The fermentation medium samples were centrifuged 15 min at 4000 rpm, following which the fermentable sugar (sucrose, glucose, fructose) content of the supernatant and the free nitrogen content were determined by HPLC and Kjeldahl method, respectively (Herlich 1990). The fermentation process productivity was estimated as ethanol content per volume of fermentation medium per unit of time.

Results and Discussion

The results of the analyses of raw materials from Table 1. show that the compositions of raw, thin and thick juices and molasses are characteristic of sugarbeet processing in domestic factories.

Parameter	Raw juice	Thin juice	Thick juice	Molasses
Dry substance, % (m/m)	14.70	14.50	58.8	80.80
Sucrose, % (m/m)	12.85	13.13	53.00	49.20
Coefficient of purity, % (m/m)	87.41	90.55	90.14	60.89
pH	6.30	9.25	7.27	6.98
Ash content, % (m/m)	0.276	0.342	1.85	9.86
Reducing substances, % (m/m)	0.0695	0.0141	0.469	0.858
Total nitrogen content, % (m/m)	0.127	0.128	0.14	1.82

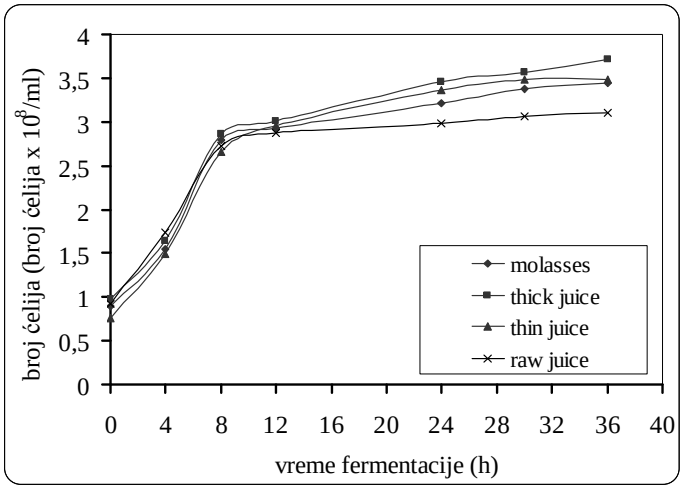

Figure 1. Yeast cell count dependence on the fermentation time of the culture media based on raw, thin and thick juices and molasses

Fig. 1. shows the dependence of yeast cell counts on the fermentation time of the culture media based on raw, thin and thick juices and molasses. During the first 8 hours of fermentation of all four culture media, there was, evidently, a pronounced and almost linear increase in the yeast cell count reaching about 2.75 x 10^8 cells/ml. 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 increased insignificantly during further fermentation under anaerobic conditions.

Fig. 2. illustrates the dependence of the free nitrogen content on the fermentation time of the culture media based on molasses, thick, thin and raw juices. Yeasts assimilate nitrogen-containing compounds during fermentation and use them for metabolism (Pejin 1989). Yeasts assimilate simple compounds, like amino acids and ammonium solids, and create complex ones during fermentation, the resulting total nitrogen content being almost constant.

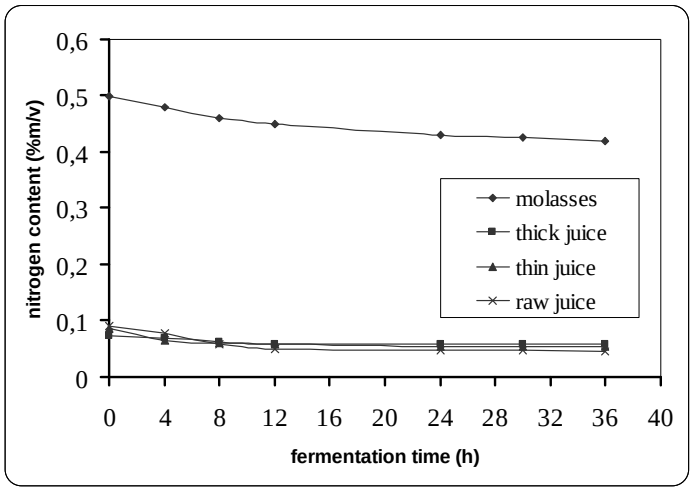

Figure 2. The dependence of free nitrogen content on fermentation time of culture media based on molasses, thick, thin and raw juices

Fig. 2. shows that nitrogen contents of fermenting mashes prepared from thick, thin and raw juices were considerably lower as compared to molasses. The obtained results suggest that during the first 8 hours of fermentation nitrogen contents decreased in all fermenting mashes applied. During that period, the yeast cell growth in aerobic conditions was intensive, assimilated nitrogen being used for primary metabolism and incorporated into biomass (see Fig. 1.). With further prolongation of the fermentation time, the nitrogen content in all fermenting mashes remained almost constant. During this period, under anaerobic conditions, ethanol and CO_2 synthesis was dominant. This was not accompanied by considerable nitrogen consumption.

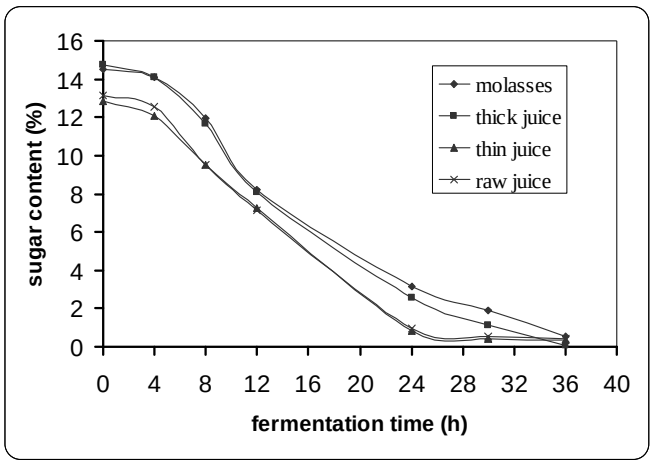

Figure 3. The dependences of fermentable sugar contents on the duration of fermentation for the culture media containing molasses, thick, thin and raw juices

Fig. 3. illustrates the dependences of fermentable sugar contents on the duration of fermentation for the culture media containing molasses, thick, thin and raw juices. The values represent the amounts of sucrose, glucose and fructose detected using the HPLC. The results indicate that the sugar content decreased during the aerobic phase of the process when yeast cell growth was recorded to be intensive. Yeast cells incorporated the assimilated sugar into biomass, inducing intensive cell growth in fermenting mashes. Simultaneously with this process, ethanol and CO_2 were produced. In the second, aerobic phase, yeast cells used assimilated sugars mostly for ethanol synthesis, as confirmed by the results illustrated in Figs. 1, 2 and 3.

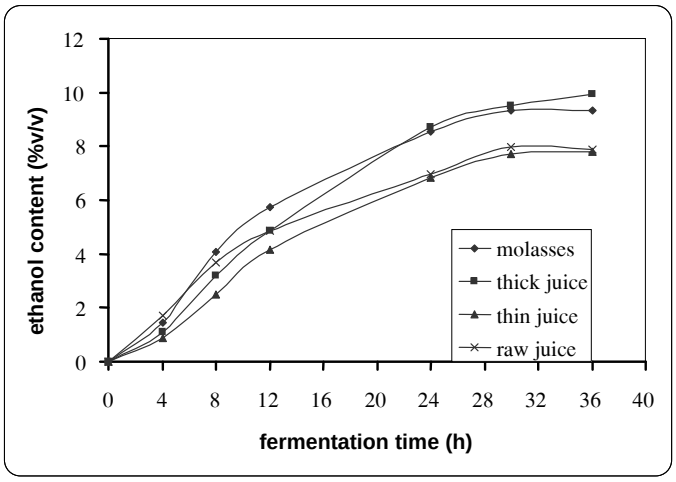

Figure 4. The dependence of ethanol contents on fermentation times for the fermenting mashes containing molasses, thick, thin and raw juices

Fig. 4. shows the dependence of ethanol concentrations on fermentation times for the fermenting mashes prepared from molasses, thick, thin and raw juices. The results indicate that ethanol contents for the fermenting mashes prepared from molasses and thick juice increased intensively and almost linearly during the first 24 hours and insignificantly with further fermentation. After 36 hours of fermentation of the cultivation media based on molasses and thick juice, the ethanol content reached the maximum of 9.35 and 9.93 vol %, respectively. Maximum ethanol content of the fermenting mashes containing thin and raw juices was achieved after 30 hours and it amounted to 7.88 and 7.81 vol%, respectively. Evidently, these differences in ethanol content were due to different initial sugar contents. Fermentation time during which the maximum content of ethanol was achieved corresponded to the detection of the fermentable sugar content which further minimized ethanol synthesis (see Fig. 3.).

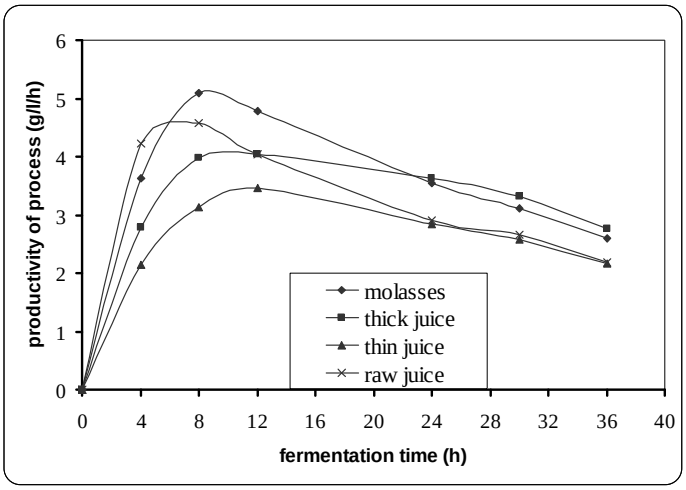

Figure 5. The depedence of the fermentation process productivity for all applied fermenting mashes

Fig. 5. shows the dependence of the fermentation process productivity for all applied fermenting mashes. Maximum productivity was achieved between the 8 and 12^{th} hour of fermentation for all the fermenting mashes applied. With further prolongation of the fermentation time, productivity significantly decreased, suggesting that optimum duration of the process should be technically and economically considered.

Conclusion

The obtained results suggest that raw, thin and thick juices, as intermediate products from sugarbeet processing, could be successfully used for bioethanol production. By applying cultivation media based on raw, thin and thick juices, the achieved yields were 62.19%, 60.35% and 66.51%, respectively. These values are very close to the yield of the process of 63.09% attained by applying the cultivation media based on molasses under the same experimental conditions. The disadvantage of raw and thin juices is the impossibility of their being stored for a long period of

time, implying that their use for bioethanol production has to coindice with the marketing season. On the other hand, their low price is a great advantage. After distillation of ethanol from fermenting mashes based on thick, thin or raw juice there remains marc with considerably lower nitrogen content as compared to the marc obtained from the fermenting mash based on molasses, the sugar content being minimum. The effluent of that composition is certainly a smaller threat to the quality of the environment. The fact that the process productivity significantly decreased after the 12th hour of fermentation suggests that optimum duration of the process should be technically and economically considered.

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MEĐUPROIZVODI PRERADE ŠEĆERNE REPE KAO PODLOGA ZA BIOSINTEZU ETANOLA

- originalni naučni rad -

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Rezime

Poslednjih decenija dolazi do ekspanzije primene tečnih biogoriva kao alternative ugljovodoničnim fosilnim gorivima. Osnovni smisao primene bioetanola kao transportnog goriva ogleda se u smanjenju emisije ugljendioksida u atmosferu u odnosu na fosilna goriva.

U ovom radu je sagledana mogućnost primene gustog, retkog i ekstrakcionog soka, kao međuproizvoda prerade šećerne repe, za proizvodnju bioetanola. Dobijeni rezultati su upoređeni sa rezultatima fermentacije na melasnoj podlozi koja se tradicionalno primenjuje za proizvodnju bioetanola. Primenom ekstrakcionog, retkog i gu-stog soka postiže se prinos procesa fermentacije od 62,19 %, 60,35% i 66,51%, respektivno, što je veoma blisko prinosu procesa na podlozi sa melasom koja iznosi 63,09%. Proizvodnost procesa fermentacije, kod primene sve četiri potencijalne sirovine, znatno opada nakon 12. časa fermentacije.