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Utilization of concentrate after membrane filtration of sugar beet thin juice for ethanol production

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HIGHLIGHTS

- Concentrate after ultrafiltration of sugar beet thin juice as ethanol production feed.
- Two yeast strains and three supplementation variants tested for process improvement.
- Fermentation of the concentrate ended with ca. 82% ethanol yield.
- Nutrient supplementation further improved ethanol yield.

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ABSTRACT

The subject of this study was to investigate the feasibility of the concentrate obtained after membrane ultrafiltration of sugar beet thin juice for ethanol production and selection of fermentation conditions (yeast strain and media supplementation). Resulting concentrate was subjected to batch ethanol fermentation using two strains of *Saccharomyces cerevisiae* (Ethanol Red and Safdistill C-70). The effect of different forms of media supplementation (mineral salts: $(NH_4)_2SO_4$, K_2HPO_4, $MgCl_2$; urea + $Mg_3(PO_4)_2$ and yeast extract) on the fermentation course was also studied. It was stated that sugar beet juice concentrate is suitable for ethanol production yielding, depending on the yeast strain, ca. 85–87 g L^{-1} ethanol with ca. 82% practical yield and more than 95% of sugars consumption after 72 h of fermentation. Nutrients enrichment further increased ethanol yield. The best results were obtained for media supplemented with urea + $Mg_3(PO_4)_2$ yielding 91.16–92.06 g L^{-1} ethanol with practical yield ranging 84.78–85.62% and full sugars consumption.

1. Introduction

The application of new technological solutions in industrial environment often causes problems with disposal of novel, unusual waste and by-products formed during processing. The rational waste management is currently of high importance for the industry because of limits in emission of wastes and greenhouse gases. The sugar industry is one of the most energy-demanding branches of industry, which also involves high production of waste and carbon dioxide. One of the ways to reduce energy consumption and CO_2 emission by sugar refineries is application of modern membrane technologies for purification of sugar juices. Membrane filtration could greatly replace conventional liming-carbonation process (Lipnizki et al., 2006). Moreover, many authors proved that membrane filtration of sugar juices leads to higher juice purity (the content of sucrose in dry matter of juice) in comparison to traditional liming-carbonation process (Hakimzadeh et al., 2006; Regiec, 2003, 2004; Shahidi and Razavi, 2006). However, as a result of membrane filtration of sugar beet diffusion juice, except for the purified juice (filtrate, permeate), there always remains the concentrate (retentate) containing impurities and a certain amount of sucrose. During membrane filtration of sugar beet diffusion juices there are used Volume Concentration Factors (VCF) with values of 5 or 6 (Attridge et al., 2001). Unfortunately, the higher VCF value the faster membrane fouling occurs what causes process efficiency reduction. In a sugar beet refinery that processes 10,000 Mg of sugar beet roots per day about 2000 m^3 of the concentrate can be produced (Tyndall, 1999). Sucrose recovery from the concentrate is not profitable due to high amount of insoluble particles but it could be used for ethanol fermentation.

Sugar beets, as well as sugar cane, are well known as efficient raw materials for ethanol production. Their advantage over the other raw materials (starch, lignin–cellulose) is that they can be converted directly to ethanol without hydrolysis of polysaccharides to simple sugars. Moreover reforms in the sugar market in the EU countries and sugar production limits lead to reorganization

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of sugar refineries to beet-based ethanol plants (European commission of agricultural and rural development, 2012). Henke et al. (2006) described the model and simulation of sugar factory with simultaneous ethanol production from intermediates. The model implies raw juice purification by membrane microfiltration, where the retentate flux is led to fermentation process, along with the part of raw juice and molasses. The feasibility of sugar beet processing intermediates and by-products like beet pulp (Balcerek et al., 2011a,b), raw juice (Vučurović et al., 2012), thick juice (Grahovac et al., 2012) and molasses (Tang et al., 2010) for ethanol production have already been the subject of study by other authors. However, so far there have not been made attempts to investigate the suitability of the concentrate remaining after sugar beet juice membrane filtration for ethanol production. It may constitute an efficient and cheap fermentation feed in a modern sugar/bioethanol facility.

The primary aim of this research project was to determine the suitability the concentrate remained after membrane ultrafiltration of sugar beet thin juice for ethanol production by industrial strains of *Saccharomyces cerevisiae* in a batch mode. The secondary aim of this study was to select proper nutrients enrichment of fermentation media and selection of yeast strains ensuring high efficiency of the process.

2. Methods

2.1. Raw material

Sugar beet thin juice (taken after the first liming-carbonation process) was obtained from a local sugar beet refinery in Poland during the harvest season of 2011/2012. It was subjected to membrane filtration process immediately after transportation to the laboratory.

2.2. Membrane filtration of sugar beet thin juice

Ultrafiltration of sugar beet juice was performed using pilot plant membrane apparatus (Fig. 1). The filtration process was conducted by 'cross flow' method under 0.25 MPa pressure at 50 °C in a closed system. A ceramic, asymmetrical membrane module produced by Fairey Filtration Systems Ltd. (currently Mantec Technical Ceramics, Stoke-on-Trent, England) was used in the ultrafiltration process. The pore diameter of the filter was 0.2 μm and the total surface area ranged 0.24 m^2. The sucrose concentrations and purities of the fractions obtained after the ultrafiltration were as follows: the feed – sucrose 200.70 g L^{-1}, purity 93.02%; the filtrate – sucrose 198.21 g L^{-1}, purity 96.33%. The concentrate was collected and stored at −20 °C until used. Chemical composition of obtained concentrate was as follows: dissolved solids, 19.24 Brix, 203.02 g L^{-1}; sucrose, 199.87 g L^{-1}; α-amino acid nitrogen, 0.039 g 100 g^{-1}; calcium salts, 0.301 g 100 g^{-1}; ash, 0.64 g 100 g^{-1}, pH, 9.2; color, 1157 IU, determinations of all parameters were performed according to standard methods used in the sugar industry (ICUMSA, 2007).

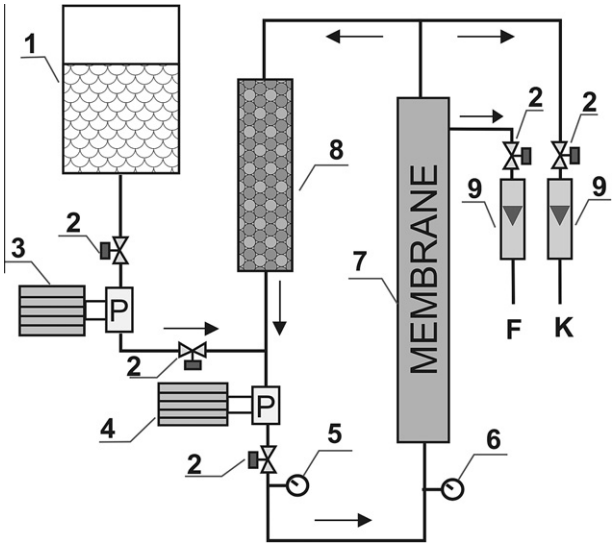

Fig. 1. Schematic diagram of membrane plant used for ultrafiltration of sugar beet thin juice. 1 – feed tank, 2 – valves, 3 – inlet pump, 4 – circulation pump, 5 – temperature sensor, 6 – manometer, 7 – membrane, 8 – heater, 9 – rotameters, F – filtrate flux, K – concentrate flux.

2.3. Microorganisms

Two active dry *S. cerevisiae* yeast strains were used in present study. *S. cerevisiae* Ethanol Red is a strain recommended for ethanol fuel production from various substrates and it is described as thermophilic (up to 40 °C), osmophilic and tolerant to high ethanol concentration (above 18% v/v). *S. cerevisiae* Safdistill C-70 strain is recommended for production of spirits, its optimal temperature is between 25 and 35 °C, and it is reported to be able to produce up to 14% v/v ethanol. Both strains were obtained from Fermentis division of S.I. Lesaffre (Marq en Baroeul, France). Prior to inoculation, both yeasts preparations were rehydrated, and for this purpose 2.5 g (dry matter basis) of active dry yeast were slurred in 20 mL of sterile distilled water and thermostated at 30 °C for 30 min with regular agitation.

2.4. Inoculation and fermentation

The concentrate obtained after ultrafiltration of sugar beet thin juice was subjected to fermentation without removal of residual undissolved solids. Fermentation samples were prepared in the following way: 250 mL aliquots of the concentrate were transferred into 500 mL conical flasks and 2 mL of nutrient supplementation solution was added. There were investigated three variants of nutrient supplementation (and a control sample):

Control – worts without the addition of nutrients;

A- addition of mineral salts (in g L^{-1}): $(NH_4)_2SO_4$, 0.35; $K_2HPO_4 \cdot 12H_2O$, 0.5; $MgCl_2 \cdot 6H_2O$, 0.05;
B- addition of urea (1 g L^{-1}) and $Mg_3(PO_4)_2$ (0.3 g L^{-1});
C- addition of yeast extract Leiber Fermentation E (1 g L^{-1}).

Then fermentation media were sterilized at 117 °C for 15 min using ASL 80 B autoclave (SMS, Warsaw, Poland). After sterilization, pH of the media was adjusted to 5.00 ± 0.05 using 7.11 M H_2SO_4 solution. The pH adjustment was done after autoclaving in order to prevent sucrose from inversion in the acidic environment during sterilization. Sterilized media, with adjusted pH, were cooled to ca. 30 °C and inoculated with 2 mL of yeast slurries, prepared as described in Section 2.3, to obtain initial yeast concentration of 1 g of yeast dry matter per 1 L of medium. The flasks were sealed with silicone stoppers with attached fermentation tubes (filled with 7.11 M sulfuric acid) and sampling ports as described previously (Kim et al., 2011). Batch fermentation was conducted using Type 357 water bath shaker (Elpin+, Lubawa, Poland) at 30 °C with 150 rpm agitation speed for 72 h. All fermentation trials were performed in triplicate.