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Selection of yeast strains for alcoholic fermentation of sugar beet thick juice and green syrup

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ABSTRACT

Presented work aimed at determination of effect of various strains of yeast *Saccharomyces cerevisiae* and concentration of fermentation worts on dynamics and efficiency of alcoholic fermentation. Fermentation worts contained either thick juice or green syrup.

It was found that yeast strains designated as M_1, M_2 and D-2 most efficiently fermented thick juice worts inoculated with yeast cream at a rate of 2 kg m^{-3} of wort. Fermentation processes lasted for approximately 2 days and ethanol yield approached 92–94% of the theoretical yield. Fermentations of green syrup worts were most efficient (ethanol yield reached 90–92% of the theoretical yield) when these processes were carried out by yeast strains M_1, M_2, D-2 and As4 (inoculum – 2 kg m^{-3} of wort).

S. cerevisiae strains M_1 and M_2 dynamically and efficiently fermented thick juice worts with extract of 200 g kg^{-1} and 250 g kg^{-1} (89–94% of the theoretical yield) while strain D-2 preferred less dense worts (extract of 200 g kg^{-1}) and produced ethanol with the yield of over 92% of the theoretical yield. The optimum green syrup worts extract was 200 g kg^{-1}.

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1. Introduction

In 2006, EU agriculture ministers formally adopted reform of the EU sugar sector (Council Regulation (EC) No 318/2006 on the common organization of the markets in the sugar sector), which came into force on 1 July 2006. EU countries have to obey to regulations of the World Trade Organization (WTO) and must adapt their system of subsidies and supporting of sugar export to obligatory rules of the international trade. Limitation of sugar manufacturing lowered prices of sugar beets and in consequence the area of their cultivation was reduced. This in turn resulted in a gradual decrease in a number of operating sugar factories in EU [1].

Production of ethanol from by-products from sucrose factories such as raw and thick juices, green syrup or molasses is a promising alternative for sugar industry. In farm distilleries these raw materials can be easily converted to fermentation worts through dilution, supplementation with some nutrients and inoculation with yeast cells.

A by-product remaining after ethanol distillation from fermentation wort, known as vinasse can be also utilized. Numerous studies on utilization of vinasse as a component of feedstock [2], a fertilizer [3], substrate for production of biogas [4], fodder yeast, materials for building engineering and an agent slowing down hardening of gypsum [5] were conducted in Poland. Besides, vinasse can be recycled and used for supplementing fermentation worts. This saves technological water and enables concentration of vinasse thereby increasing its nutritive and fertilizing characteristics [6].

Because syrups remained after sucrose crystallization contain yeast inhibitors (hydroxymethylfurfural, hexanol, heptanol [7]) and are deficient in magnesium, potassium and

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nitrogen, determination of fermentation parameters and application of suitable yeast strains being capable of efficient ethanol production are very important [8]. The most reasonable approach consists in fermenting concentrated worts supplemented with missing nutrients. Also the extract of these worts is of importance. Because of natural environment protection reasons the usage of water has to be minimized and only high gravity worts should be fermented. However, in response to stress related to such factors as temperature shock, high osmotic pressure, elevated concentration of salts and acids in worts [9–11], yeast cells can synthesize compounds which decrease ethanol production yield and bring about losses in sugar conversion. One of such compounds is glycerol which can be overproduced because of the following reasons:

- Formation of complex of acetaldehyde and hydrosulfite ions which inhibits synthesis of ethanol and activates synthesis of glycerol;
- The growth of yeast species at pH close to 7 or above;
- Application of osmotolerant yeast strains [12,13].

Fermentation of waste syrups from sucrose manufacturing [6,14,15] ensures production of fuel ethanol. However, this approach has to be competitive to other methods of their utilization and therefore fermentation conditions have to be optimized and yeast strains must be carefully selected. In consequence, both sugar losses and synthesis of by-products will be minimized [10].

An objective of presented study was determination of the effect of selected strains of yeast *Saccharomyces cerevisiae*, the dose of inoculum and concentration of fermentation worts on dynamics and efficiency of alcoholic fermentation.

The scope of this study embraced:

- Physicochemical analysis of raw materials (thick juice and green juice),
- Preparing and analysis of fermentation worts,
- Alcoholic fermentation and distillation of ethanol,
- Analysis of worts on completion of fermentation,
- Evaluation of fermentation process.

2. Materials and methods

2.1. Raw materials

This study was carried out by using two raw materials derived from sugar factory in Glinojeck, in Poland (British Sugar Overseas Polska, sugar production capacity – over 150 kt y^{-1}), such as: thick juice – concentrated juice obtained after evaporation of thin juice; green syrup – intermediate product in the second stage of sucrose crystallization.

2.2. Yeast

Worts were fermented by yeast *S. cerevisiae*, strains: M_1, M_2, M_3, G-67 and Bc-16 derived from Pure Culture Collection at the Institute of Fermentation Technology and Microbiology of the Technical University of Lodz. Also dried distillery yeast strains: D-2 and As4 (purchased from the yeast factory in Maszewo Lęborskie –Poland) were used in fermentation processes.

Strains: M_1, M_2 and M_3 are commonly used for fermentation of molasses worts while strains: Bc-16, D-2 and As4 are used in distilling industry for fermentation of starch mashes.

2.3. Preparing of fermentation worts

Worts with extract of ca. 200 g kg^{-1}, 250 g kg^{-1} and 300 g kg^{-1} were prepared from either thick juice or green syrup, which were pre-diluted with water (1:1), acidified with 30% H_2SO_4 to pH = 4.9 and supplemented with $(NH_4)_2HPO_4$ (0.3 kg m^{-3} of wort) and $MgSO_4$ (0.06 kg m^{-3} of wort).

2.4. Preparing of yeast cream (inoculum)

Strains M_1, M_2, M_3, G-67 and Bc-16 were two times passaged in malt wort (volume – 30 cm^3; extract – 80 g kg^{-1}; pH = 4.9; 28 °C; 24 h) and used to prepare inoculum. For this purpose they were cultured at 28 °C under agitated conditions in malt wort supplemented with thick juice or green syrup (1:1) (extract – 80 g kg^{-1}; pH = 4.9; 48 h).

On completion of the latter culture, yeast cells were harvested by centrifuging (3200×*g*, 10 min) and washed with sterile distilled water. Suspensions of yeast cells (yeast cream) were analyzed for solid substance. They were added to fermentation media in doses of either 2.0 kg m^{-3} of wort or 3.0 kg m^{-3} of wort (cell number of approximately 4×10^{13} m^{-3} and 6×10^{13} m^{-3} respectively).

Dried yeast (D-2, As4) were re-hydrated prior to inoculation of worts. Portions of yeast biomass were suspended in 20 cm^3 of warm tap water and acidified with 25% sulfuric acid solution to pH close to 2.2–2.5 to eliminate bacterial contaminations. Yeast cream was kept for 15 min and then added to worts (in doses of either 2.0 kg m^{-3} of wort or 3.0 kg m^{-3} of wort – cell number of approximately 4×10^{13} m^{-3} and 6×10^{13} m^{-3} respectively).

2.5. Fermentation processes

Processes of fermentation were carried out in 1 dm^3 flat-bottom flasks, each containing 500 cm^3 of wort supplemented with diammonium phosphate and magnesium sulfate, and inoculated with yeast cream. The flasks were closed with stoppers equipped with fermentation pipes filled with glycerol. Fermentation was conducted at 28–30 °C. It was controlled by gravimetric method (a decrease in mass caused by carbon dioxide evolving was determined – expressed as kg m^{-3} of wort).

2.6. Distillation of ethanol

On completion of fermentation ethanol was collected by distillation in a laboratory system consisting of 500 cm^3 distillation flask, Liebig cooler, 100 cm^3 volumetric flask (used to collect ethanol) and a thermometer.

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