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APPLICABILITY OF UNCONVENTIONAL ENERGY RAW MATERIALS IN ETHANOL PRODUCTION

Małgorzata Gumienna, Małgorzata Lasik, Zbigniew Czarnecki, Katarzyna Szambelan
Poznań University of Life Sciences

Background. The difficult position of Polish agriculture, including one of its branches, i.e. sugar industry, is conducive of search for solutions aiming at an improvement of the condition of industry. One of the potential solutions in this respect may be to focus on alternative raw materials and search for ways to overcome recession in renewable energy sources. The aim of this work was to evaluate the possibilities of using non-starchy materials – sugar materials, without enzymatic treatment for ethanol production using selected yeast strains.
Material and methods. Sugar beet pulp and thick juice, as a semi product from sugar beet, were fermented. The efficiency of the process was assessed using two *Saccharomyces cerevisiae* preparations – Ethanol Red, Fermiol. Fermentation was run for 72 h at 30°C. Quality of produced raw distillates was evaluated using the GC method.
Results. The research on fermentation processes showed that sugar beet pulp let obtain higher ethanol yield – 87% of theoretical than sugar beet thick juice – 84% of theoretical, both for Ethanol Red and Fermiol yeast preparations. Moreover, it was exhibited that the increase of sugar concentration in the fermentation medium obtained from thick juice, statistically importantly influenced ethanol yield decrease, for both yeast preparations. The distillates' quality analysis showed the influence of raw materials and microorganism used for fermentation on pollution degree. Distillate obtained from thick juice was characterised with the lowest by-products content after fermentation with Ethanol Red.
Conclusions. The results make additional possibilities for sugar beet utilization in distillery industry and new markets using production surpluses both for sugar beet and its semi-product – thick juice.

Key words: bioethanol, sugar beets, fermentation, thick juice

Corresponding author – Adres do korespondencji: Dr inż. Małgorzata Gumienna, Institute of Food Technology of Plant Origin of Poznań University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland, e-mail: gumienna@up.poznan.pl

INTRODUCTION

The difficult position of Polish agriculture, including one of its branches, i.e. sugar industry, is conducive of search for solutions aiming at an improvement of the condition of industry. One of the potential solutions in this respect may be to focus on alternative raw materials and search for ways to overcome recession in renewable energy sources. Annual economic analyses indicate an increased demand for energy, resulting from the dynamics of the population growth and an improving economic standard [Rygielski 2002]. Long-term forecasts predict that the continuous increase in the consumption of natural energy resources will be accompanied by an increasing concentration of CO_2 (the greenhouse effect), thus it is necessary to search for new, alternative sources of energy, including biomass fuels. Replacement of conventional energy carriers with biomass fuels results also from supply safety. To ensure feasibility of plant raw material processing and utilization of mineral raw materials in the production of fuels it is necessary for the energy value of the product to exceed the energy consumption required for its production [Jolly 2007]. The balance of production of ethanol and plant raw materials is dependent on the type of the raw material and the applied processing technology. In the French model thick juice subjected to fermentation is used in bioethanol production, with the further technological line resembling the conventional commercial-scale distillery. In turn, the model used in Italy consists in thickening of raw juice, crystallization of raw sugar and fermentation processing of total run-off syrups into ethanol. Other technologies propose the production of ethanol from thick juice and run-off syrups during the sugar campaign, while during the rest of the year – from other raw materials (potatoes, wheat, maize) [Rygielski 2002]. World economy showed long ago that biofuels may compete with conventional fuels. An advantage of raw materials containing simple sugars and disaccharides, such as sucrose, is their simplified technology of extraction to the water medium, followed by fermentation by microbial strains to ethanol, without the need of additional technological operations connected with chemical or enzymatic hydrolysis, increasing costs of biosynthesis [Szopa and Patelski 2006]. Sugar beets may be processed to spirit using at least two methods: one consisting in the fermentation of juice produced from sugar beets by diffusion, while the other consisting in the fermentation of liquid mass, obtained from whole sugar beet roots boiled under pressure in the water medium.

Although the process of spirit biosynthesis by yeasts is relatively well-known, it is essential to optimize processes of bioethanol production from sugar beet roots, i.e. select adequate yeast strains, develop conditions of optimal sugar extraction and develop fermentation conditions. It is advisable for the applied strains to ferment the medium with a high sugar content and survive higher concentrations of ethanol [Ogbonna et al. 2001, Szopa and Patelski 2006, Grajek et al. 2008, Balcerek and Pielech-Przybylska 2008].

The aim of this study was to search for new, unconventional raw materials in the production of bioethanol with a simultaneous potential improvement of production output. The study investigated applicability of non-starch raw materials – mainly containing sugars not requiring enzymatic treatment in the production of ethanol. The feasibility of fermentation of sugar beet pulp and a semi-product produced from sugar beets, i.e. thick juice, was assessed; moreover, the effect of applied microorganisms on ethanol yield was investigated.

MATERIAL AND METHODS

The experimental material consisted of sugar beet pulp obtained after grinding of sugar beets, and thick juice – as a semi-product obtained during the technological process of sugar production from sugar beets. Raw material was obtained from the Opalenica sugar factory and came from the 2007/2008 campaign.

Microorganisms used in this study were yeasts *Saccharomyces cerevisiae* – preparations Ethanol Red and Fermiol by Lasaffre (France). Yeasts were used at 0.5 g/kg mash.

Characteristics of used commercial yeast preparations:

– pitching temperature – Ethanol Red (ER – recommended for thick mash) – 32--35°C, Fermiol (F) – 32-35°C, while during fermentation it should not exceed 40°C for ER and 38°C for Fermiol
– tolerated ethanol concentrations: Ethanol Red up to 18% alcohol volume, Fermiol up to 12% alcohol volume.

No additional mineral media for yeast growth were applied during fermentation.

The course of fermentation. The fermentation process was run in Erlenmeyer flasks of 250 ml, in which 150 g fermentation medium were placed, i.e. sugar beet pulp, or 100 ml thick juice corresponding to mash density of 10, 16 and 20°Blg. The medium was inoculated with yeast mother at 10% in relation to the fermenting medium. Fermentation was run for 72 h at 30°C. The course of the process is presented in Figure 1.

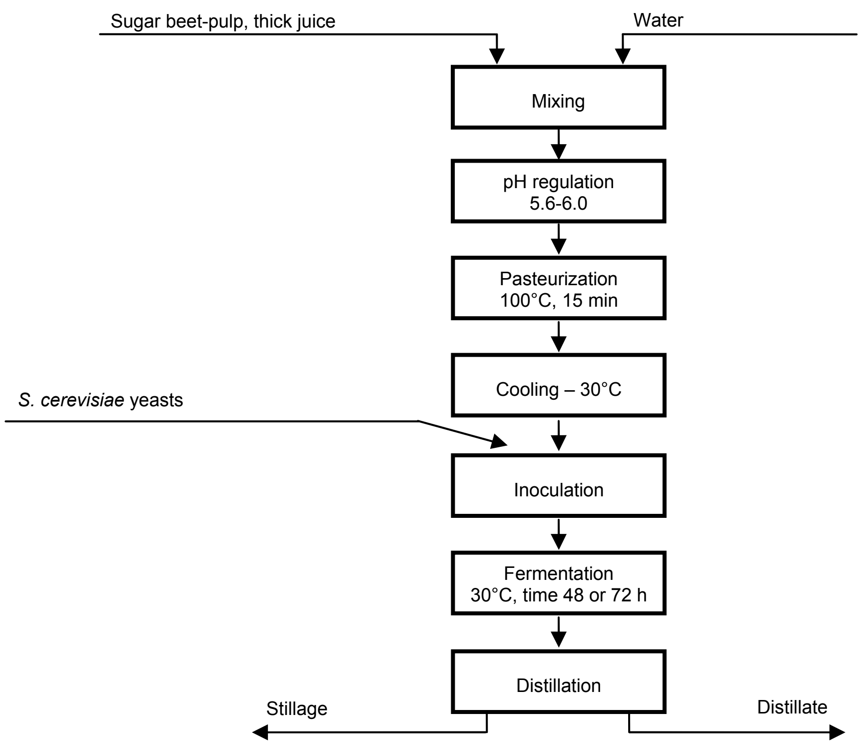

Fig. 1. Ethanol biosynthesis from the beet pulp and thick beet juice

Characteristics of raw material and the evaluation of obtained wash were performed using standard methods and determined total solids of raw material [Kriełowska-Kułas 1993], reducing sugars [Miller 1959], sucrose content by polarimetry [Kriełowska--Kułas 1993] and the viability and population size of yeasts in wash were monitored [Kriełowska-Kułas 1993]. Moreover, ethanol content in wash was determined using the aerometry method and in order to verify collected results by HPLC as modified by the authors [Gumienna et al. 2008]. Quality of produced raw distillates was evaluated using the GC method.

The analysis of variance ANOVA using the Statistica 6.0 software was performed in order to compare the significance of differences between samples (for $\alpha = 0.05$).

RESULTS

In the study sugar beet roots were used as well as their semi-product, i.e. thick juice. The tested raw material was characterized by a high sucrose content, which for sugar beets was 17%, while for thick juice it was 82%. Original parameters of the experimental material are listed in Table 1.

In order to determine the effect of the type of applied non-starch raw material and two commercial yeast preparations, i.e. Fermiol and Ethanol Red, the fermentation process was run (Tables 2 and 3).

As a result of analyses it was found that there is a significant effect ($p \geq 0.05$) of the type of used raw material (fermentation medium) on the yield of ethyl alcohol. The fermentation medium produced from sugar beet pulp exhibited a higher alcoholic fermentation efficiency in relation to media produced from thick juice for both applied yeast preparations.

Table 1. Characteristics of raw material

Material	Dry matter %	pH	Reducing substances $mg \cdot cm^{-3}$	Sucrose %
Sugar beet-pulp	26.52 ±0.17	7.12 ±0.02	4.07 ±0.44	17.27 ±0.03
Thick juice	85.00 ±0.05	8.91 ±0.03	2.34 ±0.30	82.80 ±0.02

Table 2. Ethanol fermentation of sugar beet pulp using two preparations of yeasts

Material	Initial extract °Blg	Initial pH	pH after fermentation	Ethanol yield			Stillage	
				% v/v	$dm^3 \cdot 100\ kg^{-1}$ beet	% of theoret. yield	red. subst. $mg \cdot cm^{-3}$	sucrose %
Sugar beet-pulp	*Saccharomyces cerevisiae* yeasts – preparation Ethanol Red							
	10	5.57 ±0.07	3.17 ±0.05	5.15 ±0.01	10.30 ±0.01	87.54 ±0.01	1.40 ±0.11	0.00 ±0.0
	Saccharomyces cerevisiae yeasts – preparation Fermiol							
	10	5.55 ±0.06	3.43 ±0.09	5.15 ±0.01	10.80 ±0.01	87.54 ±1.01	1.05 ±0.37	0.00 ±0.0

Table 3. Ethanol fermentation of thick beet juice using two preparations of yeasts

Mate-rial	Initial extract °Blg	Initial pH	pH after fermentation	Ethanol yield			Stillage	
				% v/v	$dm^3 \cdot 100\ kg^{-1}$ beet	% of theoret. yield	red. subst. $mg \cdot cm^{-3}$	sucrose %
Thick juice	*Saccharomyces cerevisiae* yeasts – preparation Ethanol Red							
	10	5.50 ±0.11	4.53 ±0.09	5.90 ±0.01	47.97 ±0.01	84.77 ±0.11	1.65 ±0.11	0.00 ±0.0
	16	5.53 ±0.01	4.60 ±0.13	5.90 ±0.01	30.57 ±0.65	54.06 ±0.09	28.55 ±2.23	0.00 ±0.0
	20	5.65 ±0.12	4.74 ±0.08	5.15 ±0.01	26.65 ±0.01	37.63 ±0.21	63.87 ±1.43	0.00 ±0.0
	Saccharomyces cerevisiae yeasts – preparation Fermiol							
	10	5.62 ±0.06	5.24 ±0.25	5.90 ±0.01	48.36 ±0.01	85.38 ±1.79	1.33 ±0.37	0.00 ±0.0
	16	5.50 ±0.50	4.37 ±0.21	7.40 ±0.01	36.60 ±0.04	64.96 ±0.01	14.69 ±3.07	0.00 ±0.0
	20	5.67 ±0.11	4.72 ±0.20	6.65 ±0.01	27.37 ±0.14	65.38 ±0.01	32.03 ±6.76	0.00 ±0.0

The yield of ethyl alcohol produced as a result of sugar beet pulp fermentation reached the highest value of 87.5% in relation to the theoretical yield for a medium with a density of 10°Blg both for Ethanol Red and Fermiol (Table 2). In case of fermentation media, where thick juice was used, the highest yield of approx. 85% in relation to the theoretical yield was also obtained for a density of 10°Blg. At the same time it was found that the type of the applied preparation did not have a significant effect ($p \geq 0.05$) on recorded yield of ethanol (Table 3). However, the application of thick juice as a substrate for the production of ethanol showed a significant reduction ($p \geq 0.05$) of percentage theoretical yield of alcohol with an increase in the concentration of sugar (mash density of 16-20°Blg) in the medium for both applied preparations (Table 3).

Table 4. The concentration of contaminations in distillates obtained after fermentation of sugar beet pulp and thick juice, $g \cdot dm^{-3}$ of 100% spirit

Material	Extract °Blg	Aldehyde	Fusel oil	Methanol
Sugar beet-pulp	*Saccharomyces cerevisiae* yeasts – preparation Ethanol Red			
	10	0.062 ±0.011	0.028 ±0.002	0.062 ±0.002
	Saccharomyces cerevisiae yeasts – preparation Fermiol			
	10	0.419 ±0.022	1.433 ±0.041	0.700 ±0.021
Thick juice	*Saccharomyces cerevisiae* yeasts – preparation Ethanol Red			
	10	0.015 ±0.001	0.48 ±0.005	0.051 ±0.002
	16	0.079 ±0.010	0.23 ±0.002	0.013 ±0.001
	20	0.007 ±0.001	0.50 ±0.001	0.040 ±0.001
	Saccharomyces cerevisiae yeasts – preparation Fermiol			
	10	0.035 ±0.002	0.015 ±0.001	0.009 ±0.000
	16	0.135 ±0.061	0.157 ±0.032	0.006 ±0.000
	20	0.189 ±0.032	0.218 ±0.015	0.007 ±0.001

The contaminants in produced distillates were determined using gas chromatography, which made it possible to detect volatile compounds, found next to ethanol, and which constitute its contaminants.

In the produced distillates the following compounds were determined: fusel oils, esters, aldehydes and methanol (Table 4).

The Polish standards require aldehyde content in molasses spirit should not exceed of 0.3 $g \cdot dm^{-3}$ 100% spirit, while methanol content is not regulated; this requirement was met by distillates produced in all variants, apart from distillates obtained after sugar beet pulp fermentation using Fermiol. These distillates are characterised by a 6-fold higher aldehyde content, a 50 times higher content of fusel oils than distillates produced as a result of sugar beet pulp fermentation with a yeast preparation Ethanol Red (Table 4). In turn, in case of thick juice fermentation the lowest amount of contaminants was recorded for Fermiol at mash density of 10°Blg. However, an increase in sugar concentration in the fermentation medium had an effect on an increased aldehyde content and fusel oils for both analysed preparations.

DISCUSSION

Potentially interesting distillery raw materials include semi-products or by-products of sugar industry. The best known raw material in this group is sugar beet molasses, at present not used in ethanol production in Poland [Balcerek and Piech-Przybylska 2008]. In the industrial scale production of bioethanol the raw materials considered most advantageous in terms of production costs include raw juice and concentrated raw juice, i.e. thick juice.

There is a limited body of literature data concerning the yield of ethanol produced from sugar beets. The yield of ethanol from sugar beets may be 3495 $dm^3 \cdot ha^{-1}$ cultivation, assuming that the content of sucrose in the raw material is 14.33%, while the mean yield of sugar beets from 1 hectare is 35.8 ton [Rogulska and Gumieniuk 2006].

As a result of conducted studies the highest ethanol yield was recorded for sugar beets with a sucrose content of 17.27%. Assuming that the mean yield from 1 ha cultivation is 35.8 ton, then 4123.66 dm^3 alcohol/ha cultivation is produced, i.e. this yield is e.g. two times higher than that from rye (1196.6 $dm^3 \cdot ha^{-1}$), while it is similar to the yield which may be obtained from maize (3330 $dm^3 \cdot ha^{-1}$) [Cybis et al. 2006, Grajek et al. 2008]. These data indicate that sugar beets may be efficient raw material for the production of ethanol for fuel purposes; however, the fermentation process needs to be run on a much bigger amount of raw material, thus obviously increasing production costs.

In case of thick juice a study by Balcerek and Pielech-Przybylskiej [2008] showed high efficiency in its fermentation and the yield of ethanol. Depending on the type of strain and the amount of added yeast mother, they yielded from 77 to 96% (vol.) ethanol. Moreover, the authors stated that yeasts recommended for the fermentation of starch mash, particularly strain D_2, are suitable for worts with a lower density, i.e. approximately 20°Blg. Elevated osmotic pressure of worts with a density of 30°Blg inhibits the fermentation activity of yeasts.

Similar trends were stated in this study for both yeast preparations used in these experiments already for fermentation media with as little as 16°Blg. Also Takeshige and Ouchi [1995], as a result of the analyses concerning fermentation of molasses containing sugar concentration of 30°Blg, they stated inhibition of yeast growth and thus reduced ethanol yield. In turn, studies conducted on raw juice exhibited feasibility of ethanol use for fuel purposes at 0.42 $g \cdot g^{-1}$ sucrose for fermentation media with a density of 16°Blg and the application of yeasts *S. cerevisiae* IR-2 [Ogbonna et al. 2001]. Moreover, raw juice turned out to be an excellent source of minerals, since an additional enrichment of a fermentation medium with a source of nitrogen did not have a significant effect on ethanol yield.

CONCLUSIONS

As a result of the conducted fermentation processes, it was stated that fermentation of sugar beet pulp made it possible to obtain higher ethanol yields (87% theoretical yield) than fermentation of thick juice produced from sugar beets (84% theoretical yield) both for a yeast preparation Ethanol Red and preparation Fermiol.

It was found that an increase in sugar concentration in a fermentation medium produced from thick juice had a statistically significant effect on a reduction of percentage theoretical yield of alcohol in case of both yeast preparations of *Saccharomyces cerevisiae*.

The lowest content of contaminants was recorded for a distillate produced as a result of thick juice fermentation using yeasts *Saccharomyces cerevisiae* – preparation Fermiol. Thus recorded results suggest additional applicability of sugar beets in distillery industry, create new markets for the sale of excess production not only of sugar beets, but also sugar, thanks to the use of its intermediate product, i.e. thick juice.

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MOŻLIWOŚCI WYKORZYSTANIA NIEKONWENCJONALNYCH SUROWCÓW ENERGETYCZNYCH DO PRODUKCJI ETANOLU

Wstęp. Trudna sytuacja polskiego rolnictwa, w tym jednej z jego gałęzi – cukrownictwa, sprzyja poszukiwaniu rozwiązań mających na celu poprawę kondycji przemysłu. Jedną z możliwości jest zwrócenie uwagi na surowce alternatywne oraz poszukiwanie w odnawialnych źródłach energii recepty na wyjście z „recesji". Celem badań było poszukiwanie nowych, niekonwencjonalnych surowców do produkcji bioetanolu z jednoczesną możliwością podniesienia wydajności procesu produkcji.
Materiał i metody. Fermentacji poddano miazgę buraczaną oraz półprodukt otrzymany z buraka cukrowego – sok gęsty, oceniając wydajność procesu z użyciem dwóch preparatów drożdży *Saccharomyces cerevisiae* – Ethanol Red i Fermiol. Fermentację prowadzono przez 72 h w temperaturze 30°C. Ocenę jakości uzyskanego spirytusu surowego przeprowadzono za pomocą metody GC.
Wyniki. Po procesach fermentacyjnych stwierdzono, że fermentacja miazgi pozwoliła na uzyskanie wyższych wydajności etanolu – 87% w stosunku do wydajności teoretycznej niż fermentacja soku gęstego otrzymanego z buraka cukrowego – 84%, zarówno dla preparatu drożdży Etanol Red, jak i preparatu Fermiol. Ponadto okazało się, że wzrost stężenia cukru w podłożu fermentacyjnym otrzymanym z soku gęstego ma statystycznie istotny wpływ na obniżenie procentowej wydajności teoretycznej alkoholu w wypadku obu testowanych preparatów drożdży. Analiza jakościowa otrzymanych destylatów wykazała, że stopień zanieczyszczeń zależy od rodzaju surowca i użytych do fermentacji mikroorganizmów. Najmniejszą zawartością ubocznych produktów fermentacji charakteryzował się destylat otrzymany z soku gęstego z zastosowaniem preparatu drożdży Ethanol Red.
Wnioski. Uzyskane wyniki dają dodatkowe możliwości wykorzystania buraka cukrowego w gorzelnictwie, stwarzają nowe rynki zbytu wykorzystujące nadwyżki produkcyjne nie tylko buraka cukrowego, ale jego produktu pośredniego – soku gęstego.

Słowa kluczowe: bioetanol, burak cukrowy, fermentacja, sok gęsty

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