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REDUCTION OF WATER CONSUMPTION IN BIOETHANOL PRODUCTION FROM TRITICALE BY RECYCLING THE STILLAGE LIQUID PHASE

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Background. The distillery stillage is a major and arduous byproduct generated during ethanol production in distilleries. The aim of this study was to evaluate the possibility of the stillage recirculation in the mashing process of triticale for non-byproducts production and reducing the fresh water consumption. The number of recirculation cycles which can be applied without disturbances in the ethanol fermentation process was investigated.
Material and methods. Winter triticale BOGO and "Ethanol Red" *Saccharomyces cerevisiae* yeast were used in the experiments. The method of non-pressure cooking was used for gelatinizing the triticale, commercial α-amylase SPEZYME ETHYL and glucoamylase FERMENZYME L-400 were applied for starch liquefaction and saccharification. The process was conducted at 30°C for 72 h, next after distillation the stillage was centrifuged and the liquid fraction was used instead of 75% of process water.
Results. Ethanol yield from triticale fermentations during 40 cycles ranged between 82% and 95% of theoretical yield preserving yeast vitality and quantity on the same level. The obtained distillates were characterized with enhanced volatile compounds (fusel oil, esters, aldehydes, methanol) as well as protein and potassium concentrations.
Conclusions. The liquid part of stillage was proved that can be reused instead of water in bioethanol production from triticale, without disturbing the fermentation process. This investigated solution of distillery byproducts utilization (liquid phase of stillage) constitutes the way which could significantly decrease the bioethanol production costs by reducing the water consumption, as well as wastewater production.

Key words: bioethanol, triticale, liquid part of stillage, recirculation, yeast

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INTRODUCTION

Triticale shows a number of advantages as a raw material for bioethanol production [Pejin et al. 2009 b, Kućerova 2007]. That cereal has a high starch content (about 60%) and as a less expensive crop would provide good economic opportunities and alternatives for the fuel alcohol industry. Triticale does not contain high amount of pentosans like rye, accordingly there are no problems during fermentation connected with high viscosity. It is also significant that triticale requires lower temperatures during the preparation for fermentation what makes the process more energy saving [Mojović et al. 2009].

Nowadays that cereal is very popular, besides rye and corn, in Polish distilleries. In distillery industry major byproduct is stillage. Stillage contains residual oligosaccharides, organic acids and non volatile metabolic byproducts of the fermentation [Kim et al. 2008, Nowak et al. 2008, Szymanowska and Grajek 2009]. Its certain part can be dried together with spent grains to produce dry distiller's grains with solubles (DDGS) and used as ingredient in fodder production. The thin stillage can be concentrated to produce a syrup called condensed distillers' solubles (CDS) or the wet distillers' grains is combined with the syrup giving wet distillers' grains with solubles (WDGS). Often the wet form of stillage is used as animal feed because of energy-consuming drying which makes the process more costly [Mojović et al. 2009, Kim et al. 2008]. However, the stillage application as a fertilizer is possible after formal-legal requirements fulfillment connected with environmental protection. Stillage has to be chemical analysed among others for mineral substances which can be used as fertilizer components [Cibis et al. 2006].

There is high surplus of stillage and that is why a lot of investigations are conducted aiming at creating technology for utilization or reusing this byproduct [Pejin et al. 2009 a]. Reusing the stillage for distillery needs seems to be very economic and easy solution. The whole stillage is usually centrifuged to produce a liquid fraction and a solid fraction (wet cake). Than the liquid fraction can be recycled instead of process water [Czupryński 2004]. The remaining wet cake can be sold as an animal feed.

The aim of this study was to evaluate the possibility of the stillage recirculation in the mashing process of triticale for non-byproducts production and reducing the fresh water consumption. The number of recirculation cycles which can be applied without disturbances in the ethanol fermentation process was investigated.

MATERIAL AND METHODS

Biological material

Winter triticale BOGO was obtained from Poznań University of Life Science Experimental Station in Swadzim. Distillery yeast *Saccharomyces cerevisiae*, preparation "Ethanol Red" by Lasaffre (France) was used for laboratory experiments.

Fermentation process

Non-pressure cooking (100°C, 1 h) was used for gelatinizing the ground triticale according to Stecka et al. [1996]. Commercial α-amylase SPEZYME ETHYL and glucoamylase FERMENZYME L-400 (Genencor International) were applied for starch liquefaction (80°C, 20 min) and saccharification (55-60°C, 100 min). Additionally Optimash VR and GC 106 protease were used together with saccharyfing enzyme.

Prior fermentation *S. cerevisiae* yeast culture was prepared by dispersing active dry yeast in distilled water according to producer recommendation. The fermentation media were incubated at 30°C for 72 h. After distillation the stillage was centrifuged and the liquid fraction was used instead of 75% of process water.

Analytical methods

Dry matter was determined directly drying for ground seeds and two-step for stillage, liquid part of stillage and wet cake to constant weight. Starch was estimated with enzymatic method according to Holm et al. [1986]. Total protein content was analysed with Kjeldahl method and potassium content with dr Lange cuvette test. Ash content was determined by combustion of the sample.

The ethanol concentration was assayed after distillation using areometric method. The composition and purity of the obtained raw distillates were checked on a Hewlett Packard HP gas chromatography, using Supelcowax-10 (60 m × 0.53 mm × 1.0 μm) column and a FID detector.

The acids profile of fermented mashes was measured by HPLC method using Waters Alliance, HPX-87H BIO-RAD column with a RI detector, 30°C, flow speed 0.6 cm^3/min as previously described [Gumienna et al. 2009, Lasik et al. 2008].

Statistical analysis

All experiments were carried out in triplicates. Significances and standard deviation were calculated using the analysis of variance ANOVA, Statistica 6.0 software ($p < 0.05$).

RESULTS AND DISCUSSION

The composition of triticale seeds presented in Table 1 indicated high starch concentration (563 mg/g). The protein content in triticale was determined at 10.21%. According to Aufhammer et al. [1996] substrates for ethanol production should not contain more than 11% of proteins.

Table 1. Chemical composition of winter triticale BOGO

Dry matter %	Starch		Reducing sugars		Ash		Total protein	
	mg/g	% d.m.	mg/g	% d.m.	%	% d.m.	%	% d.m.
89.16	563.08	63.15	8.32	0.93	1.29	1.45	10.21	11.45

The coefficient of variation was below 5% in all cases.

In order to define the ethanol production efficiency in fermentation using 75% of stillage liquid part for recirculation, there were 40 cycles conducted. Starch saccharification in sweet mashes was average 87.45% what demonstrated good starch conversion to reducing sugars for further fermentation (Table 2). Ethanol yield from triticale fermentations during all cycles ranged between 81.56% and 95.78% of theoretical yield (Table 2; differences statistically significant at $p < 0.05$). Similar values Stecka et al. [1996] obtained researching energy saving technology of raw distillate production using non-pressure technology with liquid fraction of stillage recirculation. Stillage liquid part recycled 40 times in 75% instead of process water did not cause ethanol fermentation yield decrease (Table 2). Similar ethanol yield, 91.4% of theoretical, obtained Wang et al. [1999] from triticale but without liquid part of stillage recirculation.

Table 2. The efficiency of ethanol fermentation process from triticale using stillage liquid part recirculation in 75% instead of water

Number of recirculation cycle	Starch saccharification %	pH of the fermented mash	Yeast vitality %	Ethanol		
				l/kg starch	percentage of theoretical yield	l/100 kg triticale
0	85.49	4.09	94.00	64.09	89.14[c]	33.39
1	85.10	4.17	98.14	59.65	82.96[ab]	33.60
8	81.40	4.42	97.62	60.35	83.93[ab]	34.30
16	94.37	4.50	96.73	58.64	81.56[a]	32.76
24	93.58	4.27	99.01	62.20	86.51[bc]	34.95
32	92.76	4.70	97.30	68.87	95.78[d]	35.91
40	89.49	4.72	95.35	64.21	89.31[c]	33.46

The coefficient of variation was below 5% in all cases.
Means within column with different letters are significantly different ($p < 0.05$).

The most important from the presented investigations is that however, the number of recirculation cycles increased to 40 times, the ethanol production efficiency did not decrease. This could be explained by the fact that the liquid fraction enriched the medium with amino acids, vitamins and the products of yeast cells degradation. Pejin et al. [2009 a] researched possibility of liquid part of stillage recirculation in bioethanol production from maize. The authors showed that bioethanol yield was very high (about 98% of theoretical yield) during all of thin stillage recirculation cycles. The recirculation was respected six times with 10, 20 and 30% of the thin fraction instead of water.

Process of thin fraction repeatedly recirculated (till 40 times) did not statistically significant ($\alpha = 0.05$) inhibit the yeast growing. Fermented mashes contained constant level of yeast cells at 10^8 cells/ml and yeast vitality exceeded 94% (Table 2).

The research of volatile compounds in raw distillates was made. The percentage of ethanol in the distillates was always higher than 99% of all volatile compounds detected and increased with increasing number of recirculation cycles (Table 3). The most common byproducts found were fusel oil (4.335-6.715 g/l 100% spirit), esters (0.050-0.454 g/l

Table 3. Ethanol and byproducts content of triticale raw distillates from fermentations with stillage liquid part recirculation in 75% instead of water

	Content	Recirculation cycle number						
		0	1	8	16	24	32	40
Aldehydes	g/l 100% spirit	0.147[a]	0.242[c]	0.402[f]	0.344[e]	0.276[d]	0.193[b]	0.345[e]
	% total compounds	0.02	0.03	0.05	0.04	0.03	0.02	0.04
Esters	g/l 100% spirit	0.111[d]	0.207[e]	0.454[g]	0.294	0.050[a]	0.067[b]	0.095[c]
	% total compounds	0.01	0.02	0.06	0.04	0.01	0.01	0.01
Fusel oil	g/l 100% spirit	6.715[g]	6.571[f]	6.317[e]	4.819[d]	4.480[b]	4.552[c]	4.335[a]
	% total compounds	0.84	0.83	0.80	0.61	0.56	0.57	0.55
Methanol	g/l 100% spirit	0.869[g]	0.599[f]	0.432[e]	0.306[d]	0.047[c]	0.042[b]	0.031[a]
	% total compounds	0.11	0.08	0.05	0.04	0.01	0.01	0.01
Ethanol	% total compounds	99.02[a]	99.04[a]	99.04[a]	99.27[b]	99.39[c]	99.39[c]	99.39[c]

The coefficient of variation was below 10% in all cases.
Means within rows with different letters are significantly different ($p < 0.05$).

100% spirit), aldehydes (0.147-0.402 g/l 100% spirit) and methanol (0.047-0.869 g/l 100% spirit) (Table 3). Till the 40th recirculation cycle the quantity of detected volatile byproducts decreased besides aldehydes (differences statistically significant at $p < 0.05$). The distillates obtained after fermentation with thin stillage recirculation were characterized with volatile compounds exceeded values required for raw spirits in Polish distillery industry. The requirements for raw spirits are very important when the spirit is intended for consumption. Taking into account using the spirit for other needs e.g. bioethanol, higher content of volatile byproducts is not of great importance.

HPLC analysis was conducted for organic acid profile of fermented mashes determination. The research showed the increase of acids quantity along with the increased number of thin stillage recirculation cycles (Table 4). Lactic acid content ranged from 0.065 to 0.282 mg/ml, acetic acid from 0.053 to 0.191 mg/ml and propionic acid from 0.035 to 0.163 mg/ml (differences statistically significant at $p < 0.05$). However, higher acid content detected in fermented mashes was not significant for further fermentations yield.

It was observed that dry matter content increased significant ($p < 0.05$) with successive recirculation cycles both for stillage and its liquid part or wet cake (Table 5). Dry matter in stillage increased from 6.50% for the trial with no recirculation to 9.24% for 40th cycle; wet cake was characterised with 15.93% of dry matter to 23.45%, respectively.

The present results showed that the recirculation of liquid fraction increased the protein content (differences statistically significant at $p < 0.05$) in the stillage (1.97 to 2.81%) and wet cake (4.96 to 6.11%; Table 5). Such protein condensation makes the wet cake a good product for fodder production. The thin fraction of stillage was also characterised with higher quantity of protein after 40th recirculation cycle which increased from 0.31% to 0.91% (Table 5).

Table 4. Composition of fermented triticale mashes from fermentations with stillage liquid part recirculation in 75% instead of water, mg/ml

	Recirculation cycle number						
	0	1	8	16	24	32	40
Lactic acid	0.065[a]	0.157[c]	0.282[f]	0.192[d]	0.113[b]	0.277[e]	0.157[c]
Acetic acid	0.053[a]	0.059[b]	0.154[e]	0.127[c]	0.191[f]	0.141[d]	0.059 [b]
Propionic acid	0.035[a]	0.084[b]	0.115[d]	0.148[f]	0.163[g]	0.132[e]	0.102[c]

The coefficient of variation was below 10% in all cases.
Means within rows with different letters are significantly different ($p < 0.05$).

Table 5. Total protein and potassium content in triticale stillage after fermentation (30°C, 72 h) with liquid fraction recirculation in 75% instead of water

Recirculation cycle number		Dry matter %	Protein		Potassium	
			%	% d.m.	%	% d.m.
0	stillage	6.50[a]	1.97[d]	30.31	0.06[a]	0.89
	liquid part	1.99[a]	0.31[a]	15.58	0.05[a]	2.41
	wet cake	15.93[a]	4.96[e]	31.14	0.08[a]	0.50
1	stillage	5.63[a]	1.52[a]	27.15	0.09[b]	1.53
	liquid part	5.49[b]	0.79[bcd]	14.38	0.07[b]	1.29
	wet cake	16.08[a]	2.83[a]	17.60	0.09[ab]	0.56
8	stillage	8.65[b]	1.67[ab]	19.30	0.11[c]	1.25
	liquid part	5.96[bcd]	0.72[b]	12.08	0.12[cd]	1.98
	wet cake	24.64[e]	3.39[c]	13.76	0.10[bc]	0.41
16	stillage	8.92[b]	1.79[bc]	20.06	0.12[cd]	1.39
	liquid part	6.58[d]	1.07[e]	16.26	0.13[d]	1.97
	wet cake	25.02[e]	3.08[b]	12.31	0.12[d]	0.49
24	stillage	8.93[b]	1.91[cd]	21.39	0.13[d]	1.28
	liquid part	5.73[bc]	0.85[cd]	14.83	0.13[d]	1.97
	wet cake	21.89[b]	3.16[b]	14.44	0.11[cd]	0.52
32	stillage	9.35[b]	2.22[e]	23.74	0.11[c]	1.26
	liquid part	5.76[bc]	0.74[bc]	12.85	0.11[c]	1.94
	wet cake	22.50[c]	4.60[d]	20.44	0.14[e]	0.62
40	stillage	9.24[b]	2.81[f]	30.41	0.11[c]	1.35
	liquid part	6.18[cd]	0.91[d]	14.72	0.11[c]	1.97
	wet cake	23.45[d]	6.11[f]	26.06	0.12[d]	0.51

The coefficient of variation was below 10% in all cases.
Means within columns (among the same trials) with different letters are significantly different ($p < 0.05$).

Potassium is one of the most important macroelements occurred in stillage and its fractions. It was observed an increase in potassium content along with increasing amount of recirculation cycles. The thin fraction of stillage from non recycled trial was characterised with 0.05% potassium and after 40th cycle 0.11% (differences statistically significant at $p < 0.05$; Table 5).

CONCLUSIONS

On the basis of obtained results it can be concluded that the 75% addition of stillage thin fraction to the mashing process instead of process water and 40 recirculation cycles did not negatively affect the ethanol production from triticale. Investigated solution of distillery byproducts utilization (especially liquid part of stillage) constitutes the way which could significantly decrease the bioethanol production costs.

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REDUKCJA ZUŻYCIA WODY PODCZAS PRODUKCJI BIOETANOLU Z PSZENŻYTA POPRZEZ RECYRKULACJĘ PŁYNNEJ CZĘŚCI WYWARU

Wprowadzenie. Wywar gorzelniczy to podstawowy i bardzo uciążliwy produkt uboczny powstający w gorzelniach podczas produkcji etanolu. Celem prezentowanych badań była ocena możliwości redukcji powstawania wywaru oraz zużycia wody poprzez zawracanie płynnej części wywaru do ponownego użycia w procesie zacierania. Analizie poddano liczbę możliwych recyrkulacji bez negatywnego wpływu na wydajność procesu produkcji etanolu.
Materiał i metody. Do procesów fermentacyjnych użyto pszenżyto ozime odmiany BOGO oraz preparat Ethanol Red drożdży gorzelniczych *Saccharomyces cerevisiae.* W doświadczeniu zastosowano metodę bezciśnieniowego uwalniania skrobi oraz enzymy: alfa-amylazę Spezyme Ethyl i glukoamylazę Fermenzyme w celu upłynnienia i scukrzenia zacierów. Proces prowadzono w temp. 30°C przez 72 h, następnie po oddestylowaniu alkoholu uzyskany wywar odwirowywano, a jego płynną część zawracano (tak że stanowiła 75% wody potrzebnej do przygotowania kolejnego zacieru).
Wyniki. Wydajność produkcji etanolu z pszenżyta podczas 40 cykli recyrkulacji zacieru mieściła się od 82% do 95% wydajności teoretycznej bez wpływu na żywotność drożdży. Uzyskane destylaty charakteryzowały się zwiększoną zawartością związków lotnych (oleje fuzlowe, estry, aldehydy, metanol) oraz wyższą koncentracją białka i potasu.
Wnioski. Stwierdzono, że istnieje możliwość kilkukrotnego wykorzystania płynnej części wywarów uzyskiwanych podczas produkcji etanolu z pszenżyta bez istotnych zakłóceń procesu. Prezentowane rozwiązanie utylizacji produktu ubocznego gorzelni (płynnej części wywaru) wskazuje na możliwość bardzo istotnego obniżenia kosztów produkcji bioetanolu poprzez mniejsze zużycie wody oraz redukcję uciążliwych zanieczyszczeń.

Słowa kluczowe: bioetanol, pszenżyto, płynna część wywaru, recyrkulacja, drożdże

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