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SHORT COMMUNICATION

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THE BIOETHANOL PRODUCTION WITH THE THIN STILLAGE RECIRCULATION

In this paper, the bioethanol production with the thin stillage recirculation in mashing was investigated. The mashing was performed with recirculation of: 0, 10, 20 and 30 % of the thin stillage. The thin stillage recirculation was repeated six times. In the experiment without the thin stillage, the recirculation bioethanol yield (compared to the theoretical yield) was 97.96 %, which implicates that the experiment conditions were chosen and performed well. With the addition of the thin stillage, the bioethanol yield increased and was above 100 %. Higher bioethanol yield than 100 % can be explained by the fact that the thin stillage contains carbohydrates, amino acids and yeast cells degradation products. The bioethanol yield increased with the increased number of thin stillage recirculation cycles. Dry matter content in fermenting slurry increased with the increased thin stillage quantity and the number of the thin stillage recirculation cycles (8.04 % for the first and 9.40 % for the sixth cycle). Dry matter content in thin stillage increased with the increased thin stillage quantity and the number of thin stillage recirculation cycles. Based on the obtained results it can be concluded that thin stillage recirculation increased the bioethanol yield. The highest bioethanol yields were obtained with recirculation of 10% thin stillage.

Key words: bioethanol; maize; stillage; recirculation.

The world and European production of bioethanol for fuel is constantly increasing, tending to double till 2012. The reason for this is the fact that the addition of bioethanol in a conventional motor fossil fuel enhances the fuel combustion characteristics, increases its octane number and decreases the negative ecological effects, primarily the emission of carbon dioxide, carbon monoxide, benzol and sulphur [1]. However, the bioethanol production also generates considerable amounts of by-products, *i.e.* waste products. The major by-products of the bioethanol production are carbon dioxide and stillage [2]. In the majority of industrial facilities in Serbia, the bioethanol by-products have not been utilized posing therefore a hardly solvable environmental problem [3-6].

The average stillage amount produced in the bioethanol process is approximately 13 hL per hL of bioethanol [7]. Physical, chemical and nutritive characteristics of stillage are highly variable and dependent on the raw materials and various aspects of the ethanol production process [8]. It has around 7-10 % of dry matter that originate from the grains used as a raw material except for the part of carbohydrates and sugars that were converted to ethanol, carbon dioxide and other volatile products. Besides non-converted substances of the raw materials used, stillage also contains all products of yeast fermentation such as the complex of B vitamins and growth supporting compounds. The major constituents of stillage are proteins and of that, 15-20 % of nitrogen is in a dissolved form (expressed on total nitrogen content in raw material) whereas 85-90 % of nitrogen is in a non-dissolved form [9]. The complex composition of stillage causes high BOD5 values which range from 15 to 340 g/L. Such high concentrations of organic and inorganic substances disable its disposal to water flows without previous treatments to lower the BOD5.

The aim of the paper was to investigate the possibility of the thin stillage recirculation in the mashing process in order to decrease the amount of stillage and water used in the process. The effects of the stillage recirculation on various process parameters such as a fermentation rate, a bioethanol yield, and the content of solids in stillage after distillation were evaluated.

MATERIALS AND METHODS

Materials

Maize was used as a starch raw material. Enzy-

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mes were used for hydrolysis and yeast was a producing microorganism.

Maize. The maize grits provided from the Panon ethanol processing plant, Crvenka, Serbia was used. It had a moisture content of 14.12 % and a starch content of 61.95 % dry matter.

Enzymes. The enzymes used for the present work were acquired from the company Novozymes, Denamrk. Two enzymes were used: Termamyl 120L type S and SAN Super 240L.

Producing microorganism. Dry instant active yeast *Saccharomyces cerevisiae* (Alltech Fermin, Senta, Serbia) was used for the fermentation. The yeast was suspended in 0.1 % sterile peptone at 38 °C. The yeast suspension was incubated for 20 min at 38 °C. The number of yeast cells was determined by the method of cell counting in the Neuberg counting chamber. An aliquot taken from the suspension was inoculated. The inoculums contained 30×10^6-35×10^6 cells/mL [10].

Stillage. The composition of thin stillage used in our experiments and in literature [7] is given in Table 1.

Table 1. The composition of thin stillage from maize

Parameter	Literature data	Used thin stillage
Dry matter, %	7.7	6.5-8.8
Residue starch, w/v	0.1	0.07-0.20
Total sugar, w/v	0.26	0.19-0.31
Reducing sugar, w/v	0.11	0.05-0.17
Total phosphates, mg/L	128.65	129.41-140.27
Total acids, g/100 mL	0.334	0.252-0.487
Total amino acids, g/100 mL	0.076	0.051-0.096

Methods

For mashing, 80 g of maize grits were mixed with 240 mL of water. The mixture was warmed at 65 °C with constant stirring in a mashing bath. After reaching the desired temperature, 0.064 mL (0.8 L/t of maize) of Termamyl 120L type S enzyme was added to the slurry. The slurry was incubated at 65 °C for 20 min to hydrolyze the starch. Then the temperature was raised to 95 °C. The slurry was held at this temperature for 30 min to complete the starch degradation. After that, the slurry was cooled to 55-60 °C and treated with 0.16 ml (2 L/t of maize) SAN Super 240 L enzyme. This temperature was kept for 30 min to prepare the starch for fermentation. The slurry was then cooled to 30 °C, filled with water to the initial mass and added to the yeast inoculums (1 g per each slurry sample). The fermentation was conducted at 30 °C for 60-70 h.

The following methods were used for the stillage analysis: the starch content after Ewers (% in dry matter) [11]; the dry matter content was determined by a standard method in the oven at 105 °C to constant mass; total and reducing sugars, as well as the phosphate content, were determined by standard methods [11]. The total acids in stillage were determined by titration with 0.1 M NaOH with phenolphthalein as the indicator. After the titration, 20 % of formaldehyde was added into the sample solution and this was titrated once more with 0.1 M NaOH to pH 8.2 for determination of total amino acids.

The slurry dry matter content after fermentation was determined. The slurry obtained after fermentation was filtered. The supernatant liquid was distilled. In the solid residue, remained after the filtration, the protein content was determined. The dry matter content was determined in the obtained stillage.

In order to investigate the efficiency of the thin stillage recirculation to the mashing phase, different amounts of stillage were added. In the present work, six cycles of mashing were performed in such a way that the stillage obtained after the first cycle was used in the second mashing and the stillage after the second cycle was used in the third mashing, *etc*.

RESULTS AND DISCUSSION

The particle size distribution of the maize grits is given in Table 2. The sample was ground so that 79.3 % of particles had diameter below 1 mm. This particle size distribution was found to be optimal for the dry-mill maize ethanol process.

Table 2. The particle size distribution of milled maize sample

$d\times10^{-3}$, mm	%
$d \geq 1.550$	9.70
$1.550 > d \geq 1.350$	5.30
$1.350 > d \geq 1.000$	5.70
$1.000 > d \geq 0.850$	11.00
$0.850 > d \geq 0.650$	16.90
$d < 0.650$	51.40
TOTAL	100.00

The summary of mashing conditions with the thin stillage recirculation is presented in Table 3.

The results obtained after fermentation are summarized in Table 4. In the first cycle, which did not include the stillage recirculation, the ethanol yield of 97.96 % was achieved as compared to the theoretical yield. This result implied that sustainable operational performances for degradation, saccharification and fermentation phases were reached. As the amount of recirculated stillage increased, higher bioethanol yields and starch utilization efficiencies were observed.

Table 3. Mashing conditions with thin stillage recirculation

Sample	Water quantity, g	Stillage quantity, g	% of stillage
1	240	0	0
2	216	24	10
3	192	48	20
4	168	72	30

Table 4. Bioethanol yields in fermentations with different quantity of recycled stillage

1st Cycle			
% of stillage	Bioethanol yield % DM	Bioethanol yield on starch, %	Yield, %[a]
0	43.41	70.08	97.96
10	44.48	71.80	100.36
20	44.12	71.22	99.56
30	44.83	72.37	101.16
2nd Cycle			
% of stillage	Bioethanol yield % DM	Bioethanol yield on starch, %	Yield, %[a]
0	42.17	68.08	95.16
10	44.83	72.37	101.16
20	42.88	69.22	96.76
30	42.71	68.94	96.36
3rd cycle			
% of stillage	Bioethanol yield % DM	Bioethanol yield on starch, %	Yield, %[a]
0	42.00	67.79	94.76
10	42.88	69.22	96.76
20	42.71	68.94	96.36
30	42.71	68.94	96.36
4th cycle			
% of stillage	Bioethanol yield % DM	Bioethanol yield on starch, %	Yield, %[a]
0	42.53	68.65	95.96
10	45.36	73.23	102.36
20	43.41	70.08	97.96
30	43.24	69.79	97.56
5th cycle			
% of stillage	Bioethanol yield % DM	Bioethanol yield on starch, %	Yield, %[a]
0	42.88	69.22	96.76
10	43.59	70.37	98.36
20	43.77	70.65	98.76
30	43.77	70.65	98.76
6th cycle			
% of stillage	Bioethanol yield % DM	Bioethanol yield on starch, %	Yield, %[a]
0	42.88	69.22	96.76
10	43.59	70.37	98.36
20	42.88	69.22	96.76
30	42.71	68.94	96.36

[a] Yield compared to the theoretical yield

Yields higher than 100 % could be explained by the fact that stillage enriched the slurry with surplus of products of carbohydrate (organic acids), amino acids, vitamins and yeast cells (phosphates) degradation. Yeast cells can utilize organic and amino acids as C-sources and N-sources. Analyzing the ethanol yields after the third cycle, it could be concluded that stillage recirculation did not adversely affect the bioethanol yields. The yields after the fourth and fifth cycles were higher than the average (>95 %). Recirculation of 20 and 30 % of stillage in the sixth cycle lowered the ethanol yields as compared to the previous cycles, but higher yields than the average were maintained. So far, it could be concluded that even the addition of 30 % of recirculated stillage to the mashing phase did not negatively affect the bioethanol production.

The dry matter content in the slurry after fermentation regularly increased with the increasing amount of recirculated stillage (Table 5). The highest dry matter content (9.40 %) was determined in the slurry after the fermentation obtained in the sixth cycle of the series with 30 % stillage recirculation. The dry matter remained after the slurry had been filtered could be used as cattle feed because of its high total protein content (Figure 1). However, from the present results it could not be unambiguously concluded that the stillage recirculation increased the protein content in the solid residue (Figure 2). The protein content in the stillage dry matter does not increase because the yeast cell number does not increase with the increased number of stillage cycles. This is in accordance with the results obtained by Castro and Gil [12]. Morin-Coualllier *et al.* [13] showed that during the stillage recycling, the contents of yeast cell inhibitors (formic, acetic, propionic, butanoic, pentanoic and hexanoic acids, furfural and 2-phenylethyl alcohol) increases. These inhibitors reduce the yeast specific growth rate and therefore the protein content in the stillage dry matter did not increase.

Table 5. Dry matter content in fermenting slurry

% of stillage	Dry matter content in fermenting slurry					
	Cycle					
	1st	2nd	3rd	4th	5th	6th
0	7.02	7.99	8.07	8.27	8.24	8.17
10	7.42	7.65	8.12	8.12	8.11	8.37
20	7.43	8.14	8.28	8.31	8.34	8.74
30	8.04	7.92	8.54	9.05	9.02	9.40

The most important co-products from the ethanol production process are the distillers dried grains (DDG) and distillers dried grains with solubles (DDGS).

The distillers dried grains with solubles (DDGS) are derived by separating the liquid portion (thin stillage) from the grain whole stillage by screening or centrifuging [14]. The stillage recycle is used to modify the conventional ethanol fermentation process thereby providing a more economical process. The method of the stillage recycle achieves less water consumption and a reduction in the stillage quantity [15].

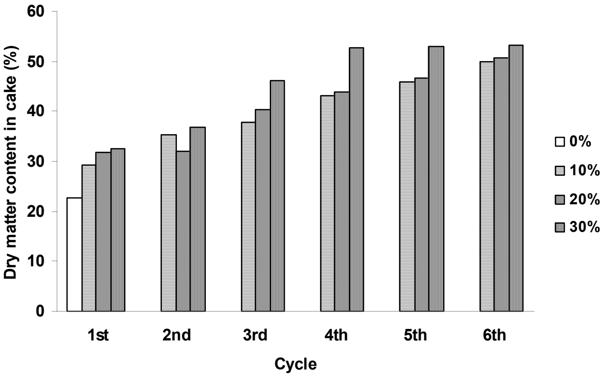

Figure 1. Dry matter content in cake (%).

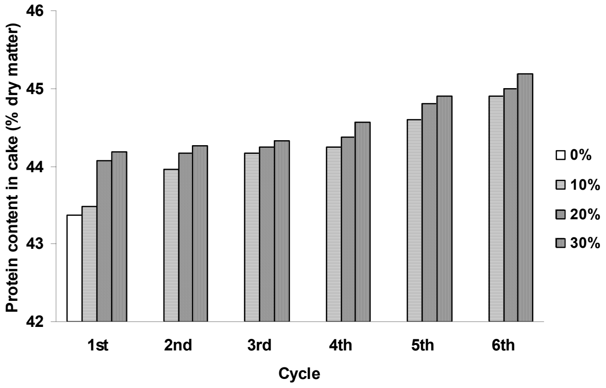

Figure 2. Protein content in cake (% dry matter).

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