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INVESTIGATIONS OF THE POSSIBILITIES OF STILLAGE UTILIZATION FROM THE BIOETHANOL PRODUCTION ON CORN

ISPITIVANJE MOGUĆNOSTI KORIŠĆENJA DŽIBRE IZ PROIZVODNJE BIOETANOLA IZ KUKURUZA

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ABSTRACT

Bioethanol produced by fermentation of biomass, such as sugar, starch, or lignocellulosic materials, is one of the most important renewable energy resources. An average thin stillage amount produced in the bioethanol process is approximately 13 hL per hL of bioethanol. An adequate utilization of the stillage is of great importance in order to improve the economy of the bioethanol production. The aim of this paper was to study the possibilities of stillage utilization from the process of bioethanol production from corn. For this purpose the recirculation of thin stillage in the amount of 10-30% in mashing process was studied, while the solid phase remained after filtration was assessed for animal feed. The results have shown that the ethanol yield in the process can be significantly increased by the recirculation. The dry matter content in the slurry after the fermentation also increased with the increasing amount of recirculated stillage. Chemical composition of solid stillage remained after fermentation as well as of the solid stillage enriched with yeast (1 %of dry Saccharomyces cerevisiae yeast) was studied. The stillage enriched with yeast had a greater amount of proteins and generally can be used as a high quality feed.

Key words*: stillage, bioethanol, recirculation, animal feed.*

REZIME

Bioetanol proizveden postupkom fermentacije biomase na bazi šećera, skroba ili lignoceluloze predstavlja jedan od najznačajnijih obnovljivih izvora energije. Prilikom proizvodnje 1 hL etanola nastaje oko 13 hl bistre džibre. Da bi se povećala ekonomičnost ovog postupka od izuzetne važnosti je da se džibra koja nastaje kao otpadni proizvod adekvatno valorizuje. Cilj ovog rada je da se ispitaju mogućnosti iskorišćavanja džibre nastale prilikom proizvodnje bioetanola na kukuruzu. U radu je ispitivana recirkulacije bistre džibre u količini od 10-30% u fazu ukomljavanja kukuruza za proizvodnju etanola, dok je zaostala čvrsta faza nakon filtracije razmatrana za korišćenje u ishrani stoke. Utvrdjeno je da se recirkulacijom može značajno povećati koncentracija etanola u proizvodnom procesu. Sadržaj suve materije u džibri zaostaloj nakon fermentacije se takodje povećavao sa povećanjem količine recirkulisane bistre džibre. U radu je ispitan hemijski sastav čvrste džibre i džibre obogaćene kvascem (1 % suvog Saccharomyces cerevisiae kvasca) i analizirana nutritivna vrednost ovih uzoraka. Džibra obogaćena kvascem je imala značajno veći sadržaj proteina i generalno se može koristiti kao visoko kvalitetna hrana za životinje.

Ključne reči*: džibra, bioetanol, recirkulacije, stočna hrana.*

INTRODUCTION

Bioethanol produced by fermentation of biomass, such as sugar, starch, or lignocellulosic materials, is one of the most important renewable energy resources. Current annual world bioethanol production is around 50 million m^3 and the world and European production of bioethanol for fuel is constantly increasing, tending to double till 2012 *(Licht, FO, 2007; Radosavljević et al, 2009)*. 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 *(Mojović et al, 2007)*. 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 *(Mojović et al, 2009)*. An average stillage amount produced in the bioethanol process is approximately 13 hL per hL of bioethanol *(Kim et al, 1997)*.

There are many possibilities for valorization of stillage from bioethanol processing. Some of them are the stillage recirculation and reuse *(Mojović et al, 2007; Banković-Ilić et al, 2007)*, production of soil fertilizers *(Banković-Ilić et al, 2007)*, anaerobic fermentations for the production of lactic acid or butanol *(Vukašinović-Sekulić et al, 2009; Wilkie et al, 2000)* and the production of various types of animal feed *(Mojović et al, 2007; Rakin et al, 2009; Lan et al, 2008)*. In USA, around 85 % of the liquid stillage has been dried together with spent grains to produce dry distiller's grains with solubles (DDGS) which are being used as animal feed. In Europe, the most of the stillage for animal feed is used in wet form because the drying itself is a costly process which requires a lot of energy *(Banković-Ilić et al, 2007)*. In the majority of industrial facilities in Serbia, the bioethanol by-products have not been utilized posing therefore a hardly solvable environmental problem.

Physical, chemical and nutritive characteristics of stillage are highly variable and dependent on the raw materials and various aspects of the ethanol production process *(Mustafa et al, 2000)*. It has around 7-10% of dry matter that originates 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, the 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 *(Yang, 1998)*. The complex composition of stillage causes high BOD_5 values which range from 15 to 340 g/L *(Kim et al, 1997)*. Such high concentrations of organic and inorganic substances prevent its disposal to water flows without previous treatments to lower the BOD_5.

The aim of this paper was to study two possibilities of the stillage utilization from the process of bioethanol production from corn. One is a thin stillage recirculation in the mashing process in order to decrease the amount of stillage and water used in the process and the other is its utilization for animal feed.

MATERIAL AND METHOD

Materials

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. The sample was ground so that 79.3 % of particles had a diameter below 1 mm.

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.

Methods

Starch hydrolysis and fermentation. 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 50-60 h.

Separation of stillage. 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.

Thin stillage recirculation. In order to investigate 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. The summary of mashing conditions with the thin stillage recirculation is presented in Table 1.

Animal feed samples. In order to investigate utilzation of the stillage for animal feed the solid residue, remained after the filtration were dried at 60°C and chemically analyzed.

Table 1. Mashing conditions with thin stillage recirculation

No.of sam.	Water quantity, g	Stillage quantity, g	% of stillage
1	240	0	0
2	216	24	10
3	192	48	20
4	168	72	30

Chemical analysis. The solid stillage samples were chemcially analized in order to determine the starch content; the dry matter content; total and reducing sugars, protein content, cellulose, minerals and microelements (P, Cu, Zn, Mn, Fe, Ca and Na) by standard methods (*Official Methods of Analysis, 1990)*.

At least three measurements were made for each sample analysis and the data given were averages.

RESULTS AND DISCUSSION

Thin stillage recirculation

The results of the yield of ethanol (expressed as percentage of the theoretical yield) obtained after fermentation are presented in Table 2. In the first cycle, which did not include the stillage recirculation, the ethanol yield of 97.96 % was achieved. 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 were observed.

Yields higher than 100 % could be explained by the fact that the stillage enriched the slurry with surplus of the 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 still 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.

Table 2. Ethanol yield obtained after fermentation of mash with various amount of recirculated stillage

% of stillage	Ethanol yield (% of the theoretical yield)					
	Cycle I	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
0*	97.96	95.16	94.76	95.96	97.76	96.76
10	100.36	100.16	100.36	100.36	98.36	98.36
20	99.56	96.76	96.36	97.96	98.76	96.76
30	100	96.36	96.36	97.56	98.76	96.36

*Control sample without addition of stillage

The dry matter content in the slurry after fermentation regularly increased with the increasing amount of recirculated stillage (Table 3). The highest dry matter content (9.36 %) was determined in the slurry after the fermentation obtained in the sixth cycle of the series with 30 % stillage recirculation

Table 3. Dry matter content in the fermenting slurry

% of stillage	Dry matter content in fermenting slurry					
	Cycle I	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
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

Chemical analysis of dried solid stillage and solid stillage enriched with yeast

The results of the chemical composition of the dried maize stillage and the maize stillage enriched with 1 % of dry yeast of *S. cerevisiae* are presented in Table 4.

Table 4. Chemical composition of the samples of solid stillage

Component	Maize stillage	Maize stillage enriched with yeast
Moisture, %	3.91	6.24
Proteins, %	39.96	42.90
Fat, %	13.14	14.28
Sugar, %	7.27	3.84
Cellulose, %	2.95	4.33
Phosphorus, %	1.12	1.49
Cu [mg/kg]	17.72	22.26
Zn [mg/kg]	49.57	47.90
Fe [mg/kg]	74.67	60.46
Se [mg/kg]	<2	<2
Mg [mg/kg]	4615.10	1971.18
Ca, %	0.20	0.31
Na, %	0.11	0.06

The protein content, which is the most important part of the cattle food, is rather high amounting 39.96 %. The protein content of the sample enriched with dried yeast is higher due to the contribution of the yeast biomass and its value is 42.90 %. The protein contents of the three samples of solid maize stillage (solid stillage, solid stillage after the fifth cycle with 30 % of recirculation and solid stillage enriched with 1% of dried yeast) are presented in Figure 1. The protein content value of the sample of the stillage after the fifth cycle with 30 % of recirculation (41.65 %) is in between of the content of nonrecirculated solid stillage and solid stillage enriched with yeast. It is important to note that the stillage recirculation itself does not increase the protein content in the solid residue in proportion with the degree of recirculation or the amount of recirculation. It is because the yeast cell number does not increase with the increased number of stillage cycles. Namely, *Castro and Gil (2007)* and *Morin-Couallier et al. (2008)* 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 proportionally.

The protein content of the samples investigated here are good enough considering the values officially prescribed by the regulations for animal feed mixes. According to the regulations, maximal protein content of 50 % is recommended only for full feed mixes for young trout, while the common values of proteins in animal feed mixes are in the range from 15- 40 % *(The regulations of the quality and other requirements for animal feed)*.

The cellulose content of the complete animal feed mixes is limited to 10% due to its low digestibility. In the stillage samples tested its content was within the officially allowed limits.

The fat content of the stillage samples was in the range from 13.14-14.3 % (Table 4). The fat content is very important for fatlings` feed and according to the official food regulations it shouldn't be below 5% *(The regulations of the quality and other requirements for animal feed)*.

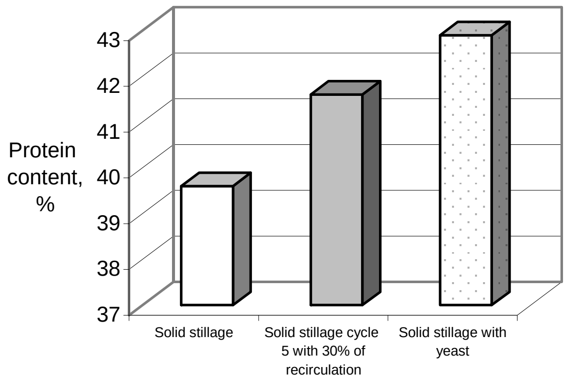

Fig. 1. Protein content of the solid maize stillage, solid maize stillage after 5 recirculation cycles with 30% of recirculation and the maize stillage enriched with S. cerevisiae yeast (1% of dried yeast)

The phosphor content of the samples is in the range from 1.12 to 1.49% with much greater value detected in the sample of the stillage enriched with yeast (Table 4). The value of phosphor content of 1.49% is higher from the majority of upper phosphor limits prescribed by the national feed regulations (average recommended values are from 0.6-0.8%), with an exception for complete animal feed mixes for the fatlings, where the upper phosphor value limit is 1.5%

For a good animal feed it is important that it contains particular minerals in a proper amount *(Bekrić, 1999)*. The content of minerals, such as Mg, Zn, Cu, Fe, Se, contributes to a higher quality of animal feed, especially in the case of mixes based on corn stillage. The enrichment of the stillage with yeast contributed to higher copper content (22.26 mg/kg), which is far above of the officially recommended value (5 mg/kg). In the sample of corn stillage enriched with yeast, a low sodium content of 0.055% may be noticed. This is acceptable only for a few animal feed mixes officially prescribed by food regulations. The content of calcium is higher in dried maize stillage sample (0.195%), but still remained of poor quality compared to the calcium content prescribed for animal feed mixes by animal feed regulations *(The regulations of the quality and other requirements for animal feed)*.

CONCLUSSION

The recirculation of thin stillage in the mashing process may be used as an efficient way to decrease the amount of stillage and water used in the production of ethanol from maize.

Our study showed that as the amount of recirculated stillage increased (from 10 to 30 %) the higher ethanol yields was observed. By the recirculation the ethanol yield could be increased from 97.9 % to more than 100 %, which could be explained by the fact that stillage enriched the slurry with amino acids, vitamins and the products of yeast cells degradation. The dry matter content in the slurry after the fermentation also increased with the increasing amount of recirculated stillage. The highest dry matter content (9.40 %) was determined in the slurry after the fermentation obtained in the sixth cycle with 30 % stillage recirculation.

The dry matter remained after filtration of the slurry could be used as animal feed because of its high total protein content.

Chemical analysis of the samples of dried solid maize stillage and dried solid maize stillage enriched with 1% of dried *S. cerevisiae* yeast has shown that the maize stillage samples contained a high percentage of proteins (close to 40% and more). Maximum protein content (42.90%) contains the stillage enriched with 1% of *S. cerevisiae* yeast. The addition of the yeast contributed to an increase in protein content, fat content and the content of particular minerals such as P, Ca and Cu.

The chemical composition of the solid stillage indicated that it can be a complete feed mix for several animal categories. The enrichment of the solid maize stillage with *S. cerevisiae* yeast can contribute to develop a new animal feed of a high quality. In addition, its valorization can significantly improve the economy of the bioethanol production on corn.

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