academicJournals

Vol. 12(40), pp. 5950-5955, 2 October, 2013
DOI: 10.5897/AJB2013.12937
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Water and energy saving bioprocess for bioethanol production from corn grain applying stillage liquid part recirculation

Małgorzata Lasik*, Małgorzata Gumienna, Katarzyna Szambelan and Zbigniew Czarnecki

Institute of Food Technology of Plant Origin, Department of Fermentation and Biosynthesis, Poznań University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland.

Accepted 5 September, 2013

The distillery stillage is a major and arduous byproduct generated during ethanol production in distilleries. The liquid part of this stillage was proved that can be recycled in the ethanol production from corn, without disturbing the fermentation process. The corn seeds were fermented employing the conventional non-pressure method for gelatinizing as well as including a novelty: Recirculation system of distillery stillage liquid part instead of process water. The efficiency of fermentation and main chemical parameters of stillage were estimated. The liquid part of stillage was recycled 28 times. At these conditions distillery yeast *Saccharomyces cerevisiae* efficiently produced ethanol yielding 79.80% of the theoretical, keeping the vitality and quantity on the same level. However, recirculation of the liquid part of stillage caused protein and potassium increase in the wet cake what makes this product more attractive for fodder supplementation. It was proven that the addition of stillage liquid fraction to the mashing process instead of process water and 28 recirculation cycles in ethanol production from corn constitutes the way which could significantly reduce the water and energy consumption, what essentially reduce whole general production costs without ethanol efficiency decreasing.

Key words: Corn, stillage liquid part, recirculation, ethanol yield.

INTRODUCTION

Bioethanol is the most promising biofuel and the starting material for various chemicals production. The addition of bioethanol in motor fuel enhances its octane number and decreases the negative ecological effects. Increase in the demand for ethanol as a fuel additive resulted in an increase in the amount of corn used for ethanol production. There are many technologies and materials applied for effective bioethanol production and stillage utilization (Mwithiga, 2013; Anwar et al., 2012; Marx et al., 2012; Shanavas et al. 2011; Mojovic et al., 2010; Sun et al., 2010; Gibreel et al., 2009; Krzywonos et al., 2009a; Nicolic et al., 2009; Cibis et al., 2006a). Corn is characterized by high crop (8.0 t ha^{-1}) and ethanol yield (417 L t^{-1}) from ha. Corn grain is very important cereal material containing starch. This cereal contains over 60% of starch and is easy to handle as a material for fermentation. Corn can be converted into ethanol by either wet or dry milling method (Belyea et al., 2004;

*Corresponding author: E-mail: lasik@up.poznan.pl; Tel. +48618487288; Fax: +486184387314.

Abbreviations: DDGS, Dry distiller's grains with solubles; **CDS,** condensed distillers' solubles; **WDGS,** wet distillers' grains with solubles; **DM,** dry matter.

Devantier et al., 2005; Kwiatkowski et al., 2006). The hydrolyzing process must be preceded by gelatinization of the starch. Pressure cooking is very effective for further fermentation of starchy materials but production costs are high due to the high energy consumption in the cooking process. The processes to reduce the high production costs are required and non-pressure cooking fermentation system has also been successfully used and reached a fermentation efficiency equal to that of the conventional pressure cooking system (Shigechi et al., 2004; Mojovic et al. 2009; Nicolic et al. 2009; Shunavas et al. 2011).

However, the bioethanol production also generates arduous byproducts. The great influence on distillery industry situation has a raw material cost, expense of energy, water and rational utilization of byproducts. In distillery industry major byproducts are carbon dioxide and stillage. Stillage contains residual oligosaccharides, organic acids and non volatile metabolic byproducts of the fermentation (Cibis et al., 2006b; Kim et al., 2008). 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 stillage liquid part can be concentrated to produce 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 (Kim et al., 2008; Mojović et al., 2009). Stillage has to be chemically analyzed, among others for mineral substances which can be used as fertilizer components (Cibis et al., 2006b). But high water content in stillage gives many problems like transporting such product to farms or storage (Krzywonos et al., 2009b).

There is high surplus of stillage and that is why a lot of research is conducted aiming at creating technology for utilization or reusing this byproduct (Cibis et al., 2006b; Gibreel et al., 2009; Pejin et al. 2009; Mojovic et al., 2010; Gumienna et al., 2011). To lower the costs of water the distillery applies recirculation of liquid part of stillage. 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. Replacing water by the liquid part of stillage makes possibility to save great number of water, energy and decreases sewage quantity (Czupryński, 2004; Pejin et al., 2009; Gumienna et al., 2011). The remaining wet cake, mostly after drying, can be sold as an animal feed.

Composition of stillage and its fractions has been recently of great interest in the area of ethanol production (Mojović et al., 2009). Kim et al. (2008) calculated that corn DDGS and wet distillers (wet cake) grain were rich in glucan, xylan and arabinan, protein, crude fat and crude fibre. The liquid fraction of stillage can not be recycled continuously. During the recirculation the substances are accumulated what causes disturbances in the production process. In different systems, using various raw materials the ethanol fermentation process with stillage recirculation can give various effects and the limit of recirculation cycles is therefore unknown.

In the research, corn grain was tested as a raw material for ethanol production applying the technique of multiple recirculation of the liquid part of stillage. The aim of this study was to evaluate the effect of stillage liquid fraction recirculation in the mashing process for non-byproducts production and decreasing the amount of used water. The number of recirculation cycles which can be applied without disturbances in the ethanol fermentation process was determined.

MATERIALS AND METHODS

Raw material

Corn grain was obtained from Poznań University of Life Science Experimental Station in Swadzim, harvested in 2010. Raw material was milled before all analysis.

Microorganisms and enzymes

Distillery yeast, *Saccharomyces cerevisiae* ("Ethanol Red", Lesaffre, France) was used for fermentation experiments (0.5 g of the dry yeast per L^{-1} of mash). Spezyme Ethyl (EC 3.2.1.1), heat stable α-amylase from *Geobacillus stearothermophilus* was used for ground corn liquefaction. Fermenzyme L-400 (EC 3.2.1.3), *Aspergillus niger* glucoamylase was applied for starch saccharification. The enzymes were added in the amounts according to the producer (Genencor International) recommendation.

Fermentation

The ground corn grains were mixed with liquid part of stillage (75%) and water (25%) before hydrolysis process. The ethanol fermentation was performed in 500 ml glass flasks. Non-pressure cooking (100°C, 1 h) was used for gelatinizing the ground corn. Then liquefaction (80°C, 20 min) and saccharification (55-60°C, 100 min) were performed. Fermentation media after hydrolysis were inoculated with *S. cerevisiae* in the form of yeast milk. To assure a sufficient nitrogen and phosphate sources, the fermentation media were enriched with diammonium phosphate, in the amount of 0.4 g l^{-1}. Fermentation was run for 72 h at 30°C batch in stationary culture. After fermentation the distillation process was applied. The remaining stillage was centrifuged (4000 r min^{-1}, 15 min) to obtain the liquid fraction for next fermentation cycle.

Analytical methods

Dry matter was determined directly by drying at 130°C for ground seeds and two-step at 90°C and 110°C for stillage, liquid part of stillage and wet cake, to constant weight. The starch content was estimated with enzymatic method according to Holm et al. (1986). The content of reducing sugars was determined by DNS-method (Miller, 1959). Total protein content was analyzed by Kjeldahl method and potassium content with dr Lange cuvette test. Ash content was determined by combustion of the sample (Krełowska-Kułas, 1993). The ethanol concentration was assayed, in

Table 1. The efficiency of ethanol fermentation process from corn using stillage liquid part recirculation (75% instead of water).

Number of recirculation cycle	Starch saccharification (%)	pH of the fermented mash	Yeast vitality (%)	Ethanol		
				(L kg^{-1} starch)[a]	(% of theoretical yield)	(L 100 kg^{-1} corn)
0	90.50	4.69	93.00	60.88	84.64[c]	38.26
1	96.41	4.28	99.00	54.96	76.44[a]	33.08
7	96.81	4.35	93.72	58.49	81.35[b]	35.74
14	80.85	4.35	99.00	60.38	83.98[c]	37.14
21	82.52	4.63	97.44	60.97	84.80[c]	38.26
28	77.24	4.59	91.00	58.29	81.07[b]	36.82

The coefficient of variation was below 5% in all cases. Means within column with different letters are significantly different ($p<0.05$). [a]The ethanol yield in L kg^{-1}starch was calculated to total starch.

accordance with polish national standards after distillation using areometric method. The composition and purity of the obtained raw distillates were checked on a Hewlett Packard HP gas chromatograph, using Supelcowax-10 (60 m x 0.53 mm x 1.0 μm) column and a FID detector.

The organic acids profile of fermented mashes was measured by high performance liquid chromatography (HPLC) method using Waters Alliance, HPX-87H BIO-RAD column with a RI detector, 30°C, flow speed 0.6 cm^3 min^{-1} as previously described (Lasik et al., 2008). This method with the same conditions was also used to confirm the results of ethanol concentration evaluated by traditional aerometric method. All experiments were carried out in triplicates. Significances and standard deviation were calculated using the analysis of variance ANOVA, Statistica 6.0 software (α=0.05).

RESULTS AND DISCUSSION

The chemical composition of corn grain was characterized. The protein content in corn was determined at 8.41%. According to the study of Aufhammer et al. (1996), substrates for ethanol production should contain no more than 11% of protein. Starch concentration in the corn grain used in the performed experiment was found at 631 mg g^{-1}, reducing sugars at 7.48 mg g^{-1} and ash at 1.3%.

In order to define the ethanol production efficiency in fermentation, using 75% stillage liquid part instead of process water for recirculation, there were 28 cycles conducted. Starch saccharification in sweet mashes reached average 84.96% what demonstrated good starch conversion to reducing sugars for further fermentation yield (Table 1). Ethanol yield from corn fermentations during all cycles ranged between 76.44 and 84.80% of theoretical yield (Table 1). Pejin et al. (2009) obtained higher ethanol yield, ranging 96.36-101.16% of theoretical yield, in the ethanol production from corn with liquid fraction of stillage recirculation. However, in their experiments the recirculation was repeated only six times with 10, 20 and 30% of liquid fraction instead of water.

In the present study, *S. cerevisiae* fermented corn mashes yielding average 82.05% of theoretical yield (differences statistically not significant at $p>0.05$, besides first recycling). It was observed that liquid part of stillage recirculated 28 times in 75% instead of process water did not cause ethanol fermentation yield decrease (Table 1). Increasing the number of recirculation cycles up to 28 times did not decrease ethanol production efficiency what could be explained by the fact that the liquid fraction enriched the medium with amino acids, vitamins and the products of yeast cells degradation. Gumienna et al. (2011) researched possibility of liquid part of stillage recirculation in bioethanol production from triticale. They also found high ethanol yield (mean 86.68% of theoretical yield) even the number of recirculation cycles was increased up to 40 times. The application of stillage liquid fraction recirculation (up to 28 times) did not statistically significantly ($p>0.05$) inhibit the yeast growth and activity. In the present experiments, yeast vitality always exceeded 91% (Table 1).

The analyses of volatile compounds in raw distillates were also performed. The percentage of ethanol in the distillates was always higher than 99% of all volatile compounds detected. The most common byproducts found were higher alcohols (3.011-5.383 g L^{-1} 100% spirit), esters (0.042-0.341 g L^{-1} 100% spirit), aldehydes (0.033-0.323 g L^{-1} 100% spirit) and methanol (0.031-0.066 g L^{-1} 100% spirit) (Table 2). Till the 28th recirculation cycle the quantity of higher alcohols and methanol decreased whereas the concentration of aldehydes and esters increased (differences statistically significant at $p<0.05$). The distillates obtained after fermentation with liquid part of stillage recirculation were characterized with volatile compounds. It was found that besides methanol, exceeded values acceptable for raw spirits in polish distillery industry were present. The requirements for raw spirits are very important when the spirit is intended for consumption. Taking into account application of the spirit for other needs, for example, bioethanol, higher content of volatile byproducts is not of great importance.

HPLC analysis was conducted for determination of organic acid profile in fermented mashes. The analysis showed the increase of acids quantity along with the

Table 2. Ethanol and byproducts content of corn raw distillates from fermentations with stillage liquid part recirculation (75% instead of water).

Recirculation cycle number		0	1	7	14	21	28
Aldehydes	(g L^{-1} 100% spirit)	0.160^{c}	0.033^{a}	0.097^{b}	0.323^{e}	0.201^{d}	0.321^{e}
	(% Total compounds)	0.02	0.01	0.01	0.04	0.03	0.04
Esters	(g L^{-1} 100% spirit)	0.052^{d}	0.095^{b}	0.273^{e}	0.137^{c}	0.042^{a}	0.341^{f}
	(% Total compounds)	0.01	0.01	0.04	0.02	0.01	0.04
Higher alcohols	(g L^{-1} 100% spirit)	5.383^{f}	3.255^{b}	3.329^{c}	3.011^{a}	3.897^{d}	3.948^{e}
	(% Total compounds)	0.68	0.41	0.42	0.38	0.49	0.50
Methanol	(g L^{-1} 100% spirit)	0.066^{d}	0.031^{a}	0.048^{c}	0.042^{bc}	0.044^{c}	0.035^{ab}
	(% Total compounds)	0.01	0.01	0.01	0.01	0.01	0.01
Ethanol	(% Total compounds)	99.28^{a}	99.56^{c}	99.52^{c}	99.55^{c}	99.46^{bc}	99.38^{ab}

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

Table 3. Composition (mg mL^{-1}) of fermented corn mashes from fermentations with stillage liquid part recirculation (75% instead of water).

Recirculation cycle number	0	1	7	14	21	28
Lactic acid	0.087^{a}	0.064^{a}	0.138^{b}	0.292^{d}	0.293^{d}	0.217^{c}
Acetic acid	0.044^{b}	0.011^{a}	0.010^{a}	0.387^{e}	0.138^{c}	0.199^{d}
Propionic acid	0.406^{a}	0.477^{ab}	0.528^{b}	0.457^{b}	0.450^{ab}	0.445^{ab}

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

increased number of stillage liquid part recirculation cycles (Table 3). Lactic acid content ranged from 0.064 to 0.293 mg mL^{-1}, acetic acid from 0.010 to 0.387 mg mL^{-1} and propionic acid from 0.406 to 0.528 mg mL^{-1} (differences statistically significant at $p<0.05$). However, acid content detected in fermented mashes was not significantly important for further fermentations yield. Graves et al. (2006) reported that corn mashes contain at least 40 g L^{-1} of lactic acid caused final ethanol yield reduction.

It was observed that dry matter content increased significantly ($p<0.05$) with successive recirculation cycles both for stillage and its liquid part or wet cake (Table 4). Dry matter in stillage increased from 3.50% for the sample with no recirculation to 8.22% for 28th cycle; wet cake was characterized with an increase of dry matter from 14.54 to 21.69%, respectively. The present results showed that the recirculation of liquid fraction caused the protein content increase (differences statistically significant at $p<0.05$) in the stillage (from 14.00 to 45.74% dry matter (DM)) and wet cake (7.81 to 32.21% DM) (Table 4). Such protein condensation makes the wet cake a good product for fodder production. The liquid fraction of stillage was also characterized with higher quantity of protein after 28th recirculation cycle which increased from 8.99% DM to 38.73% DM (Table 4). 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 liquid fraction of stillage from non recycled sample was characterized with 1.27% DM potassium and after 28th cycle 2.32% DM (differences statistically significant at $p<0.05$) (Table 4).

Conclusion

The liquid part of stillage was proved that can be recycled in the process of bioethanol production from corn with no significant influence on ethanol production yield. A mean yield during all 28 tested recirculation cycles reached the level of 81.83% of the theoretical yield. Investigated solution of distillery byproducts utilization (liquid part of stillage) constitutes the way which could significantly reduce the bioethanol production costs due to both process water consumption and wastewater production.

ACKNOWLEDGEMENTS

This work was financed by the Ministry of Science and Higher Education in Poland, project No 0619/R/1/P01/07/02.

Table 4. Total protein and potassium content in corn stillage after fermentation (30°C, 72 h) with liquid fraction recirculation (75% instead of water).

Recirculation cycle number		Dry matter	Total protein		Potassium	
		(%)	(%)	(% DM)	(%)	(% DM)
0	Stillage	3.50[a]	0.49	14.00[a]	0.03	0.75[a]
	Liquid part	1.50[a]	0.14	8.99[ab]	0.02	1.27[a]
	Wet cake	14.54[a]	1.14	7.81[b]	0.05	0.33[a]
1	Stillage	5.41[bc]	0.79	14.60[a]	0.07	1.33[c]
	Liquid part	2.72[b]	0.21	7.72[a]	0.07	2.65[e]
	Wet cake	20.13[c]	1.13	5.61[a]	0.07	0.35[a]
7	Stillage	6.84[d]	0.91	16.52[ab]	0.09	1.30[c]
	Liquid part	3.74[cd]	0.41	9.86[ab]	0.09	2.33[d]
	Wet cake	19.18[b]	1.33	5.70[a]	0.09	0.47[b]
14	Stillage	5.60[bc]	1.07	19.04[b]	0.09	1.61[d]
	Liquid part	4.16[d]	0.41	14.47[b]	0.08	1.92[b]
	Wet cake	23.27[e]	1.86	7.97[b]	0.11	0.47[b]
21	Stillage	7.76[e]	3.69	47.49[c]	0.09	1.22[bc]
	Liquid part	3.60[cd]	1.51	41.95[d]	0.08	2.21[c]
	Wet cake	19.92[c]	5.86	29.39[c]	0.10	0.50[b]
28	Stillage	8.22[e]	3.76	45.74[c]	0.09	1.09[b]
	Liquid part	3.46[c]	1.34	38.73[c]	0.08	2.32[d]
	Wet cake	21.69[d]	6.99	32.21[d]	0.10	0.46[b]

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

REFERENCES

Anwar Z, Gulfraz M, Javaid Asad M, Imran M, Akram Z, Mehmood S, Rehman A, Anwar P, Sadiq A (2012). Bioethanol productions from rice polish by optimization of dilute acid pretreatment and enzymatic hydrolysis. Afr. J. Biotechnol. 11(4):992-998.

Aufhammer W, Pieper H, Kasser J, Schafer V, Senn T, Kubler E (1996). The suitability of grains from cereal crops with different N supply for bioethanol production. J. Agronom. Crop Sci. 177:185-196.

Belyea RL, Rausch KD, Tumbleson ME (2004). Composition of corn and distillers dried grains with solubles from dry grind ethanol processing. Bioresouce Technol. 94:293-298.

Cibis E, Krzywonos M, Miśkiewicz T (2006a). Aerobic biodegradation of potato slops under moderate thermophilic conditions: effect of pollution load. Bioresource Technol. 97(4):679-85.

Cibis E, Krzywonos M, Miśkiewicz T (2006b). Ethanol in the world – usage directions and byproducts. Przem. Chem. 85:1263-1267.

Czupryński B (2004). Actual problems in agricultural distillery industry. Fermentation, Fruit and Vegetable Industry. 9:33 (in Polish).

Devantier R, Pedersen S, Olsson L, (2005). Characterisation of very high gravity ethanol fermentation of corn mash. Appl. Microbiol. Biotechnol. 68:622-629.

Gibreel A, Sandercock JR, Lan J, Goonewardene LA, Zijlstra RT, Curtis JM, Bressler DC (2009). Fermentation of barley by using *Saccharomyces cerevisiae*: examination of barley as a feedstock for bioethanol production and value-added products. Appl. Environ. Microbiol. 75(5):1363-1372.

Graves T, Narendranath NV, Dawson K, Power R (2006). Effect of pH and lactic or acetic acid on ethanol productivity by *Saccharomyces cerevisiae* in corn mash. J. Ind. Microbiol. Biotechnol. 33:469-474.

Gumienna M, Lasik M, Szambelan K, Czarnecki Z (2011). Reduction of water consumption in bioethanol production from triticale by recycling the stillage liquid phase. Acta Sci. Pol. Technol. Aliment. 10(4):467-474.

Holm J, Björck A, Drews A, Asp NG (1986). A rapid method for the analysis of starch. Starch/Stärke. 38(7):224-226.

Kim Y, Mosier NS, Hendrickson R, Ezeji T, Blaschek H, Dien B, Cotta M, Dale B, Ladish MR (2008). Composition of corn dry-grind ethanol by-products:DDGS, wet cake, and thin stillage. Bioresource Technol. 99:5165-5176.

Krełowska-Kułas M (1993). The control of food products quality. PWE Press, Warsaw, Poland (in Polish).

Krzywonos M, Cibis E, Lasik M, Nowak J, Miśkiewicz T (2009a). Thermo- and mesophilic aerobic batch biodegradation of high-strength distillery wastewater (potato stillage) – Utilisation of main carbon sources. Bioresource Technol. 100:2507-2514.

Krzywonos M, Cibis E, Miśkiewicz T, Ryznar-Luty A (2009b). Utilization and biodegradation of starch stillage (distillery wastewater). Electron J. Biotechnol. 12(1). (www.ejbiotechnology.info/content/vol12/issue1/full5/)

Kwiatkowski JR, McAloon AJ, Taylor F, Johnston DB (2006). Modeling the process and costs of fuel ethanol production by the corn dry-grind process. Ind. Crops Prod. 23:288-296.

Lasik M, Gumienna M, Nowak J, Czarnecka M (2008). Application of ammonia treated triticale for ethanol production by simultaneous saccharification and fermentation of high gravity mashes. Research and Teaching Equipment. 4:97-103 (in Polish).

Marx S, Brandling J, van der Gryp P (2012). Ethanol production from

tropical sugar beet juice African J. Biotechnol. 11(54):11709-11720.
Miller G (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31(3):426-428.
Mojović L, Pejin D, Grujić O, Markov S, Pejin J, Rakin M, Vukasinovic M, Nikolic S, Savic D (2009). Progress in the production of bioethanol on starch-based feedstocks. Chem. Ind. Chem. Eng. Q. 15(4):211-226.
Mojović L, Pejin D, Rakin M, Vukasinovic M, Pejin J, Grujić O, Nikolic S, Radosavljevic M (2010). Investigations of the possibilities of stillage utilization from the bioethanol production on corn. J. Process. Energy Agricult. 14(1):54-57.
Mwithiga G (2013). The potential for second generation bio-ethanol production from agro-industrial waste in South Africa. African J. Biotechnol. 12(9):871-879.
Nicolic S, Mojovic L, Rakin M, Pejin D (2009). Bioethanol production from corn meal by simultaneous enzymatic saccharification and fermentation with immobilized cells of *Saccharomyces cerevisiae* var ellipsoideus. Fuel. 88:1602-1607.
Pejin D, Mojović L, Grujić O, Pejin J, Rakin M (2009). The bioethanol production with the thin stillage recirculation. Chem. Ind. Chem. Eng. Q. 15(1):49-52.
Shanavas S, Padmaja G, Moorthy SN, Sajeew MS, Sheriff JT (2011). Process optimization for bioethanol production from cassava starch using novel eco-friendly enzymes. Biomass Bioener. 35:901-909.
Shigechi H, Fujita Y, Koh J, Ueda M, Fukuda H, Kondo A (2004). Energy-saving direct ethanol production from low-temperature-cooked corn starch using a cell-surface engineered yeast strain co-displaying glucoamylase and α-amylase. Biochem. Eng. J. 18:149-153.
Sun F, Mao Z, Tang L, Zhang H, Zhang C, Zhai F, Zhang J (2010). Exploration of water-recycled cassava bioethanol production integrated with anaerobic digestion treatment. Afr. J. Biotechnol. 9 (37):6182-6190.