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Short Communication

Glycerol accumulation while recycling thin stillage in corn fermentations to ethanol

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SUMMARY

By successive recycling of the thin stillage in mashing and fermenting fresh corn, the glycerol content in each fermentation increased by about 0.4% and accumulated to a high of 2.1% in the beer of the fifth recycle. Glycerol concentration declined after the fifth recycle. The original fermentation contained 0.8% glycerol.

INTRODUCTION

Glycerol is a trihydric alcohol (1,2,3-propanetriol) traditionally recovered as a by-product from animal and vegetable oils that have been saponified in the process of manufacturing soaps [1]. Currently, glycerol is more likely to be commercially produced by chemical synthesis from propylene and from sugars [1]. The U.S. market size is around 400 million lbs/yr and the world market is over 1 billion lbs/yr. Glycerol USP-grade sells for \$0.87–0.91/lb [1–3]. The consumption of glycerol in the U.S. is generally considered a good barometer of industrial activity because it enters into such a large number of industrial processes. The industrial uses of glycerol number in the thousands, with most going into the manufacture of synthetic resins, ester gums, tobacco processing, leather, textiles, cellulose films, meat casings, tooth pastes, cosmetics, pharmaceuticals, foods and explosives.

It is well known that glycerol is a principle by-product of the traditional alcohol fermentation process and that biologically produced glycerol has not been economically competitive [4–7]. However, considering the uncertainty of petroleum reserves, fluctuating oil costs, and the high surplus of corn in the U.S., industrial fermentation of corn to chem-

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*The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

icals is becoming more attractive. Kampen [2] proposed a scenario in which the economics of a financially troubled ethanol plant could be improved by modifying a 10 million gallon/yr ethanol plant to one producing 8.5 million gallons ethanol/yr and 3 million gallons glycerine/yr. Wall et al. [8] completed a comprehensive study on recycling Distillers' Solubles (DS) in traditional fermentation of corn to ethanol and found that glycerol content of successive fermentations first increased then declined. The research presented here focuses on the effect of recycling thin stillage (TS), rather than DS, on by-product glycerol accumulation.

MATERIALS AND METHODS

Preparation and fermentation of corn

Fig. 1 illustrates the fermentation procedures used in the TS recycling experiments. Standard No. 2 yellow dent corn of similar commercial batch number was fermented. First the corn was ground in a Fitzpatrick Homoloid Model J. T. mill through a 0.063-in round hole perforated screen. The resulting corn particle size distribution was $-12 + 20$ mesh, 30%; $-20 + 40$, 33%; -40, 33%. Composition of the ground corn was 11.26% moisture, 9.6% protein, 1.3% Ash, and 72% starch on a dry weight basis.

The fermentation medium consisted of 2356 g of the ground corn/8 l, yielding approximately 20% glucose. For medium preparation, the corn meal was dispersed initially in 5000 ml of tap water (following industrial practice) or TS in 20 l, stainless steel, temperature-controlled, jacketed fermentors equipped with stirrers. Tap water was added only in the first fermentation, after that, only TS was used to prepare the mash and to cool media prior to subsequent fermentations. The corn-meal-liquid-mixture was adjusted to pH 6.2, and then 6 ml of Taka-Therm (Miles Laboratory, Inc.) alpha-amylase was added. The Taka-Therm activity is 170 000 MWU/g (1 MWU is that amount of enzyme which will dextrinize 1 mg of soluble starch to a definite size dextrin in 30 min.). The corn meal-liquid-Taka-therm mixture was heated to 90° with stirring for 1 h to gelatinize the starch and degrade the soluble dextrins. Next, 1560 ml of tap water or TS was added to the now gelatinized cornstarch and it was allowed to cool to 60°C, and the pH adjusted to 4.0. At this time, 18 ml of Diazyme L-100 (Miles Laboratory, Inc.) glucoamylase was stirred into the corn to hydrolyse the dextrins to glucose, the mixture was incubated at room temperature for 2 h. The Diazyme activity is 100 units/ml (one Diazyme unit is that activity which will produce 1 g of glucose in 1 h).

For fermentation, the corn-glucose-mash was cooled to 30°C, adjusted to pH 4.5, and inoculated with 500 ml of culture medium containing a commercial *Saccharomyces cerevisiae* with the trade name Fermivin (G.B. Industries). The inoculum consisted of yeast extract 0.3%, peptone 0.5%, glucose 1.0%, and 9 g of Fermivin in 500 ml of tap water. The culture had been incubated at 30°C for 24 h and shaken at 200 rpm prior to inoculation.

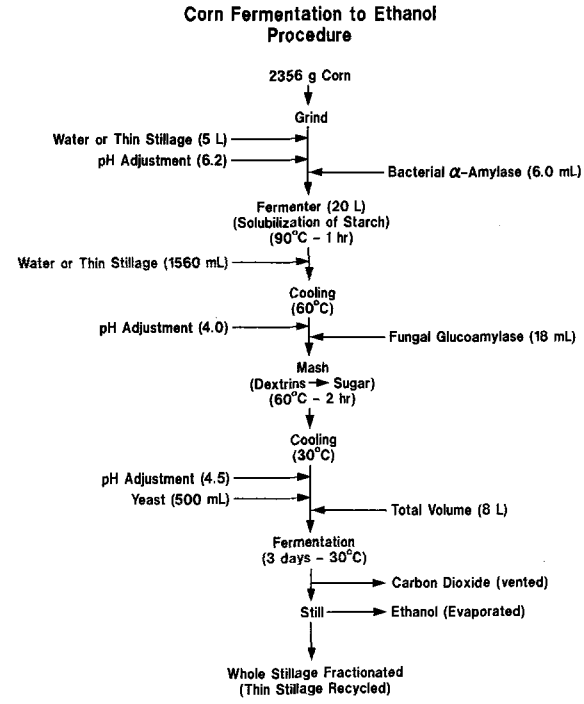

Fig. 1. Shown above is the procedure used for fermentation of corn to ethanol. The thin stillage (TS) was recycled in 7 separate fermentations subsequent to the first.

The mash fermentations were carried out in stainless steel fermentors mentioned above at 30°C for 3 days. Samples of the mash were taken at 0, 24, 48 and 66 h and assayed for glucose, ethanol, and glycerol. Maximum glycerol production and total glucose utilization were found at 48 h.

After the mash fermentation was completed, steam was circulated through the outer jacket of the fermentor for 30 min, thereby evaporating the alcohol from the mash. After evaporation, the whole stillage was removed from the fermentor and fractionated. A typical stillage fractionation can be seen in Fig. 2. The whole stillage was filtered through several layers of cheesecloth supported on a large perforated plate on a funnel inserted into a 10-l suction pot. Excess liquid was removed from the solids remaining on the cheesecloth by means of a rubber dam drawn tight under vacuum. Only the TS was recycled in these experiments. About 6560 ml TS liquid was recycled in each fermentation to make the final 8 l of mash. Where water loss occurred during the alcohol distillation process, resulting in slightly less than the required TS volume, tap water was added to retain the final 8 l mash.

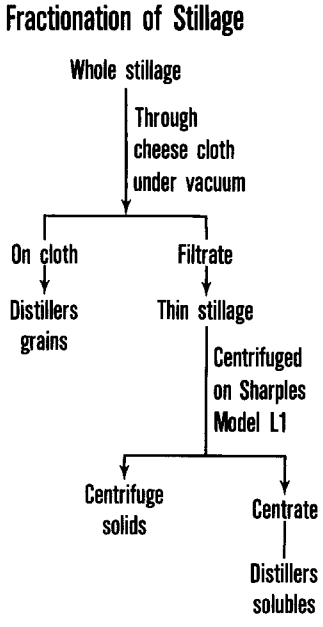

Fig. 2. Shown above is the procedure used to fractionate fermented corn mash into distillers' grains, thin stillage (TS), or distillers' solubles. Only the TS was recycled in the experiments presented.

Analyses

In each separate experiment, where appropriate, weights of mash solids and volume of stillage were measured. A measured portion of each TS was dried to constant weight under vacuum at 100°C, and the percent solids determined. About 1–2% solids remained in the TS. Ethanol was determined in the fermentation media by gas–liquid chromatography on a Poropak Q column (6 ft × 2 mm) at 180°C (detector temperature, 220°C; injection temperature, 200°C) with a flame ionization detector. Glucose was determined by high-performance liquid chromatography (HPLC) on a Bio-Rad HPX 87C column (300 × 7.8 mm) with water as eluant at room temperature. It was found that simultaneous analysis for glucose, glycerol, ethanol, acetic and lactic acid could be determined by HPLC on a Bio-Rad HPX 87H (300 × 7.8 mm) column utilizing 0.01 N. H_2SO_4 (1.0 ml per min) as eluant at 45°C. The analysis of the sugars, alcohols, polyols, and organic acids was carried out in 25 min with base line separations. The isocratic separations were carried out with a Waters Model 6000 pump, a 710B WISP automatic injection system and a Waters Model R401 Differential Refractometer as detector.

The numerical results shown are means of 3–5 separate experiments. Each experiment contained a minimum of 6 TS recycles.

RESULTS AND DISCUSSION

Glycerol

Recycling of TS through six subsequent fermentations resulted in no major changes in ethanol yields (7.9–8.3%) while glycerol content increased. Fig. 3 summarizes the results. Routinely, 0.8% (w/w) glycerol was recovered from the TS of the initial corn mash fermentation. Average glycerol accumulation was about 1.4%, 1.7%, 2.0%, 2.1%, 1.8%, and 1.7% in run 2 through run 7, respectively. In run 2 through run 5 where TS was recycled, glycerol content increased an average of about 0.4% in each successive fermentation. Total cumulative glycerol declined in run 6 and run 7. Three

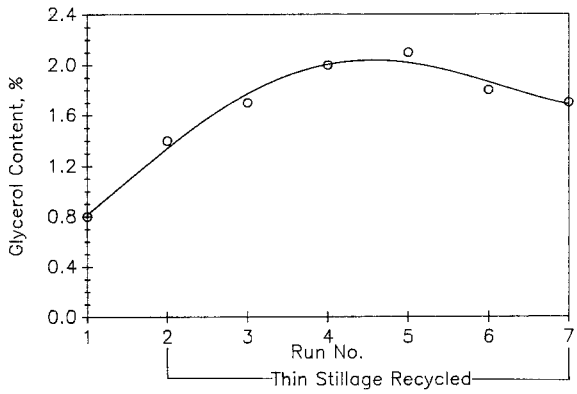

Fig. 3. Glycerol production in 7 separate 48 h corn mash fermentations, 6 of which contained recycled thin stillage (TS). The results represent the means of 3–5 experiments, each experiment contained 7 separate fermentations.

probable explanations for the low glycerol production and its subsequent decline during TS recycle are: (1) inhibition of glycerol production at higher levels of glycerol in the recycled media; (2) utilization of glycerol by yeast at higher glycerol concentrations and (3) decreased formation of glycerol due to availability of amino acids for cell synthesis from recycled solubles [4].

Ethanol

The beverage spirits industry routinely substitute about 20% of the recovered solubles for part of the water required for mashing and fermenting grain while the ethanol fuel industry uses 30–75% backset. The recycling reduces concentration costs and appears to accelerate yeast growth and fermentation. However, extensive recycling is considered by some to inhibit fermentation and reduce ethanol yield, while others found no effect upon ethanol yield [8].

In comparing ethanol produced from recycled DS [8] with data presented here on recycled TS, we found approximately a 1% (w/w) reduction in average ethanol yield. This reduction would be partially offset by circumventing the cost of centrifugation and drying of distillers solubles. However, experiments to optimize (culture conditions, inoculum level, etc.) ethanol production while recycling TS and accumulating glycerol were not conducted. Such optimization would probably increase ethanol yield without affecting glycerol accumulation [8].

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REFERENCES

1 Faith, Keyes, and Clark's Industrial Chemicals 4th edition. 1976. pp. 430–441, A Wiley-Interscience Publication, John Wiley and Sons, Glycerine (Glycerol).
2 Kampen, W.H. 1986. The extraction of corn proteins and the production of ethanol/glycerol or neutral solvents. In: Proceedings of the International Conference on Fuel Alcohols and Chemicals from Biomass (Kampen, W.H., ed.), pp. 211–215, Denison Newspapers, Inc., Miami, Florida.
3 Kirk-Othmer Encyclopedia of Chemical Technology, 2nd edition. 1976. pp. 619–631, Food Additives to Heterocyclic compounds, volume 10, Glycerol.
4 Nordstrom, K. 1966. Yeast growth and glycerol formation. Acta Chem. Scand. 20: 1016–1025.
5 Oura, E. 1977. Reaction products of yeast fermentations. Process Biochemistry 12: 19–22.
6 Tajima, K. and H. Yoshizumi. 1973. Mechanism of glycerol and 2,3-Butanediol formation by yeast in highly salted media during alcohol fermentation. J. Ferment. Technol. 51: 560–565.
7 Vijaikishore, P. and N.G. Karanth. 1986 Glycerol production by fermentation—a review. Process Biochemistry 21: 54–57.
8 Wall, J.S., R.J. Bothast, A.A. Logada, K.R. Sexson and Y.V. Wu. 1983. Effect of recycling distillers' solubles on alcohol and feed production from corn fermentation. J. Agric. Food Chem. 31: 770–775.