Effect of Recycling Distillers' Solubles on Alcohol and Feed Production from Corn Fermentation

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A major cost and source of energy consumption during alcohol production from fermentation of corn are the evaporative concentration and drying of distillers's solubles. After the alcohol is distilled from the beer, the bulk of the insolubles is removed by screening and centrifugation, leaving 2–5% of solids in the solubles. Experiments were conducted in which the separated aqueous solubles were recycled and used for mashing and fermenting the grain without impairing fermentation. Series of 8-L fermentations of ground corn were conducted at approximately 20% glucose levels after conversion of gelatinized starch by commercial enzymes. After the first run in each series, most of the media water consisted of solubles from the prior run. Of the recovered solubles, 70% was recycled in one series of experiments and 100% in another. Alcohol production by the yeast remained fairly constant for 10 runs at 70% recycling and 7 runs at 100% recycling; yields averaged 9.4% and 9.2% w/w, respectively, or about 2.7 gal/bushel of corn. Glycerol content in the broth of each successive fermentation increased through five recycles and then declined. The solids content in the solubles rose during the initial five recycles but then leveled off. The effect of residual sugar on alcohol yields and byproducts was evaluated. This recycling process greatly reduced the energy requirement for drying stillage and yielded more concentrated distillers' solubles with greater feed potential.

Although ethanol production by fermentation of grain usually is regarded as an established art, the new economics of ethanol as a liquid fuel has required reexamination of alcohol production process and utilization of byproducts to attain greater efficiency. A need also exists to further increase the positive energy balance in alcohol production from grains. Some of the regulations and requirements that restrict beverage alcohol production do not apply to fuel production; modifications and improvements of existing process can result in significant savings. Major expenditures for energy and equipment are required for drying the residual stillage. Many of these expenditures can be reduced within the constraints of environmental regulations by recycling the solution remaining after particulate matter is removed from the stillage. We present data indicating that the remaining solution, when properly processed, can be used as the liquid in mashing and fermentation operations without impairing alcohol yields or feed quality.

For fermentation, corn is ground and dispersed in water, and its starch is gelatinized by heating. The starch is converted to soluble dextrins and then to glucose by added enzymes. After a 48–72-h yeast fermentation, the resulting ethanol is distilled. The residual stillage is screened or centrifuged to remove particulate solids, termed corn distillers' grains (CDG). The screened grains are pressed to remove excess liquid (Distillers' Feed Research Council, 1972). The turbid liquid that passes through the screen and the press effluent are combined to yield thin stillage, which may be further centrifuged to remove additional fine suspended solids including yeast. For every gallon of 95% (v/v) ethanol distilled, 7 gal of supernatant corn distillers' solubles (CDS) solution is produced. Multistage or recompression evaporators concentrate CDS to a syrup. In industry, the CDS concentrate is combined with the CDG and dried to 10% moisture; this product is sold as dried corn distillers' grains with solubles (CDGS), a high-protein feed product widely used in rations for dairy and beef cattle (Waller et al., 1980).

The composition and physical properties of CDG, CDS, and CDGS have been investigated by Wu et al. (1981). They determined that about 70% of the solid matter was removed from stillage by screening and centrifugation. The initial liquid CDS consisted of 2–4% dry matter that contained 5.1% N, 1.2% lipid, and 18.2% ash. Environmental regulations restrict the disposal of this material from industrial operations into sewage systems and bodies of water in the United States. Approximately \$0.12/gal of alcohol is expended for the purchase and operation of evaporators (Retzlaff, 1980). Concentration of solids from CDS by reverse osmosis (Wu et al., 1983) can reduce these costs but would require a larger initial investment. Costs for concentrating distillers' solubles in small plants (less than 500 000 gal of ethanol annually) often become prohibitive, and efforts to use the liquid stillage in animal feeds are restricted because large intakes of liquid may reduce feed uptake and growth rates.

In the beverage spirits industry, it is standard practice to substitute about 20% of the recovered solubles for part of the water required for mashing and fermenting the grain. Recycling reduces concentration costs and appears to acclerate yeast growth and fermentation. There is some question as to the efficacy of more extensive recycling on subsequent fermentations. Hongo et al. (1967) suggested that the accumulation of protein metabolites in the stillage inhibited fermentation, and Panchal and Stewart (1980) found that a reduction in ethanol yield results from high osmotic pressure of the media. In contrast, Ronkainen et al. (1978) repeatedly used filtered distillers' solubles to replace 70–80% of the water used to prepare mash from wheat for fermentation. In their laboratory-scale and industrial trials, the yields of alcohol were not diminished by recycling. Since they successfully used wheat as a substrate at low levels, we conducted recycling trials with amounts of corn sufficient to produce alcohol yields similar to those obtained in the fuel alcohol industry. We herewith report the effect of recycling CDS on the yield of alcohol and other fermentation products and on the composition of the residual byproducts.

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MATERIALS AND METHODS

Grinding and Mashing of Corn. Standard no. 2 yellow dent corn was obtained from commercial sources. It was ground in a Fitzpatrick Homoloid Model J.T. mill through a 0.063-in. round-hole perforated screen to yield the following particle size distribution: −12 + 20 mesh, 30%; −20 + 40, 33.0%; −40, 33%. Composition of the corn after grinding was 11.26% moisture, 9.6% protein, 1.3% ash, and 72% starch on a dry weight basis. Small variations in composition occurred among batches of corn; therefore, corn of the same lot was used for each series of fermentations.

The medium was prepared in duplicate to approximate 20% glucose (2356 g of ground corn/8-L run). Corn meal was initially dispersed in 5000 mL of tap water or CDS in 20-L, stainless steel, temperature-controlled, jacketed fermentors equipped with stirrers. To the dispersed corn meal adjusted to pH 6.2 was added 6 mL of Miles Taka-Therm α-amylase. The temperature was elevated and maintained at 90 °C for 1 h with stirring until the starch gelatinized and degraded to soluble dextrins. Then 1560 mL of tap water or CDS was added, the temperature was cooled to 60 °C, and the pH was adjusted to 4.0. Next, 18 mL of Miles Diazyme L-100 glucoamylase was added to hydrolyze the dextrins to glucose during a 2-h incubation.

Fermentation. The mash was cooled to 30 °C and adjusted to pH 4.5, and 500 mL of yeast inoculum was added. The yeast inoculum consisted of 0.3% yeast extract, 0.5% peptone, 1.0% glucose, and 9 g of Fermivin dry yeast (G. B. Industries) in 500 mL of tap water. The culture was incubated for 24 h at 30 °C and shaken at 200 rpm prior to inoculation. Corrections were made on yields for dilution of the medium by inoculum. Samples of mash were taken at 0, 24, 48, and 66 h and assayed for glucose, ethanol, and glycerol. The fermentation was halted at 66 h.

Separation of Products. In initial experiments, alcohol was distilled from the fermentor by admitting steam from a jet for 15 min. Alcohol was not collected. Appreciable increase in volume of the residual stillage occurred due to steam condensation. In later experiments, the fermentor temperature was elevated to 100 °C for 30 min by circulating steam through the outer jacket and the alcohol was distilled. Since no condenser was used, some water was also lost. In both cases, the residual stillage volume and weight were measured.

The stillage was filtered through cheesecloth supported on a large perforated plate on a funnel inserted into a 10-L suction pot. Excess liquid was removed from the solids (CDG) remaining on the cheesecloth by means of a rubber dam drawn tight under vacuum. The filtrates or thin stillages were centrifuged with a Model L-1 Sharples. The wet CDG and centrifuge solids were collected at about 60% moisture. The CDG and centrifuge solids were weighed before and after drying in a forced-air oven at 90 °C. The volume of CDS from each run was measured. When CDS was not recycled within 1 day, it was stored at 4 °C.

Analyses. A measured portion of the CDS was dried to constant weight under vacuum at 100 °C, and the percent of solids was determined. CDG and centrifuge solids were further dried under vacuum at 100 °C to constant weight to determine dry matter content. Protein (6.25 × Kjeldahl nitrogen) and ash were determined on these dried materials (American Association of Cereal Chemists, 1967).

In initial experiments, 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 eluant at room temperture. In later experiments, analysis for glucose, glycerol, ethanol, and other metabolites were made by HPLC on a Bio-Rad HPX 87 H (300 × 7.8 mm) column with 0.01 N H_2SO_4 eluant at 45 °C. Determination of sugars, alcohols, polyols, and organic acids separated by HPLC was by refractive index difference.

Table I. Volume and Solids Content of Distillers' Solubles from Ethanol Fermentation following Distillation by Steam or External Heating

	distillation procedure			
	steam		external heating	
fermentation run[a]	volume, mL	solid content, %	volume, mL	solid content, %
1	9575	2.15	3112	4.04
2	8825	3.33	3138	5.89
3	7450	3.92	2800	7.72
4	8762	4.24	3655	6.40
5	8812	4.65	5325	5.76
6	9292	4.51	2975	9.15
7	9475	4.30	5105	5.83
8	8812	4.25	2812	9.51
9	8625	4.37	3237	7.62
10	8875	4.34	3800	6.91
av	8850	4.01	3596	6.88

[a] Average of duplicate fermentations.

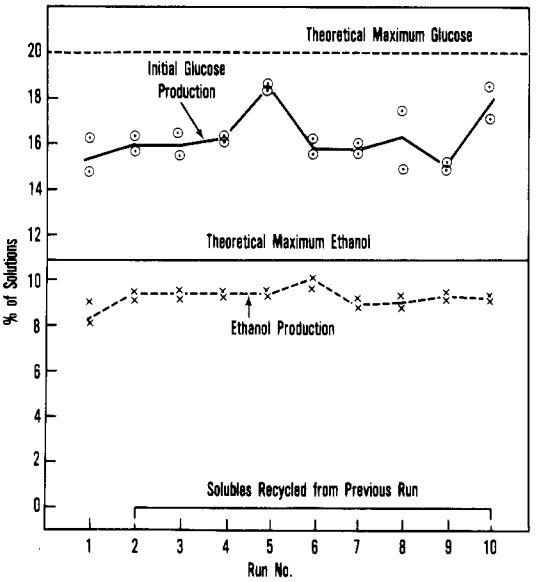

Figure 1. Effect of recycling distillers' solubles on glucose and ethanol yields from steam-distilled fermentation broth during mashing and fermenting of corn.

RESULTS

Recycling Solubles and Steam Distillation. Initial experiments in our laboratory involved steam distillation of the alcohol from the fermentation broth; this process was rapid and prevented caking of solids in the fermentor. However, as shown in Table I, steam distillation increased the volume of the CDS considerably over the initial volume of the liquid; the average volume of the CDS was 8850 mL. Consequently, only 70–80% of the CDS could be recycled to provide the 6560 mL of liquid used to make the 8 L of mash.

Figure 1 summarizes the results on ethanol and glucose production of recycling steam-heated solubles through nine

Table II. Linear Relations of Two Production Variables to Run Number

experiment	Y	runs	equation: $Y = a + bR^a$	P^b
recycle 70% (Figure 1)	glucose	1-10	15.97 + 0.0682R	ns
		1-7	15.88 + 0.1107R	ns
	ethanol	1-10	9.32 + 0.0064R	ns
		1-7	9.12 + 0.0696R	ns
recycle 100% (Figure 4)	glucose	1-10	17.44 - 0.2388R	<0.05
		1-7	17.26 - 0.2018R	<0.05
	ethanol	1-10	9.67 - 0.1439R	<0.01
		1-7	9.44 - 0.0714R	ns

[a] R = run number (1 to 10). Y = calculated glucose or ethanol concentration for run R. b = slope of the line. a = intercept. [b] ns = the linear trend is not significant at the 0.05 level. <0.05 = the linear trend is significant at the 0.05 level.

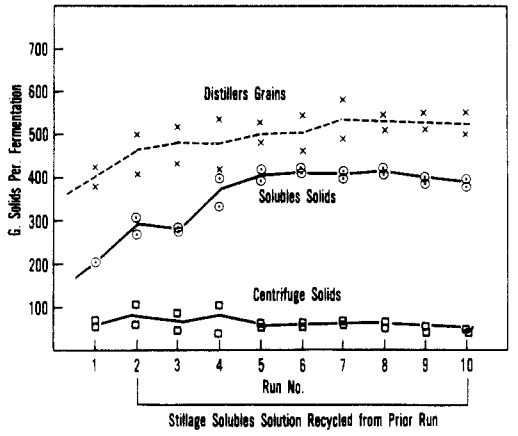

Figure 2. Effect of recycling distillers' solubles on stillage solids from steam-distilled fermentation broth.

fermentation runs. Points are indicated for duplicate fermentations. After the first fermentation, only CDS was used to prepare the mash and to cool media prior to fermentation. As shown in Figure 1, the enzymatic conversion of starch to glucose was not effected by recycled CDS; mean initial glucose was 16.3% w/w (standard deviation ±1.55%), whereas theoretical is 20%. Glucose production continued concomitant with fermentation until all the starch was digested. Ethanol production averaged 9.4 ± 0.5% (w/w) which exceeded the yield of 8.7% (w/w) for the first two runs in which water was used. Ethanol production remained fairly constant through nine recyclings of solubles. Table II summarizes the analysis of deviation from the horizontal for the regression line for points in Figure 1. No significant trend for changes in initial glucose or ethanol yields with run was determined after either six or nine recycles. The theoretical ethanol yield is 10.7% (w/w). The attained yield is 88% of theory, indicating a low diversion of glucose to other metabolites and cell production.

During the partial recycling, solids in the CDS rose from 2.15% to 4.65% (Table I). The change in yields of dry solids in CDG, CDS, and centrifuge solids during successive recycling experiments is shown in Figure 2. Yields of CDS solids were calculated from data in Table I. Solubles increased from 200 g after the first fermentation to 400 g after five recycles but then leveled off. CDG increased from 400 to about 500 g due to inclusion of more solids in the adsorbed liquid as well as to precipitation of some solids as the concentration of the solubles increased. These results indicate that 70% recycling of centrifuged CDS does not impair the hydrolysis and fermentation of corn. Furthermore, there is a buildup in concentration of solids

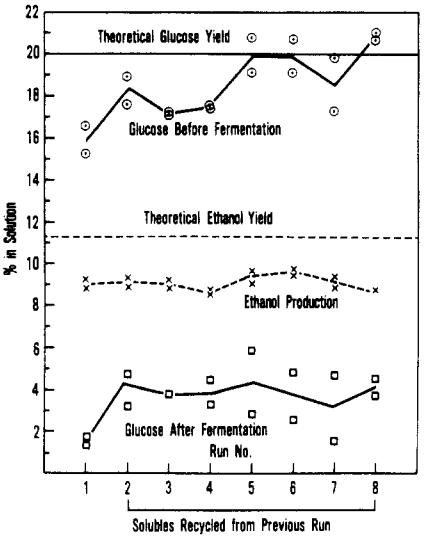

Figure 3. Results of fermentation lacking sufficient active yeast on glucose utilization and alcohol yields during fermentation of corn mash with recycled stillage.

in the solubles and distillers' grains that reduces recovery costs.

Effect of Yeast Culture on Alcohol Production and Byproduct Composition during Recycling. For effective recycling of the CDS, it is important that all glucose be utilized prior to termination of the fermentation in the allotted 66 h. Most fermentation operations are designed for a 3-day mashing, fermentation, and distillation cycle to utilize equipment efficiently. Use of 9 g of Fermivin dried yeast dispersed in and equilibrated with 100 mL of water as inoculum resulted in a lag in fermentation evidenced by lower ethanol production (8.3% w/w) in the media at 66 h and only 80% consumption of glucose. In contrast, when the dried yeast was added to 500 mL of culture medium and incubated for 24 h prior to inoculation of the mash, alcohol yield increased to 9.7% (w/w) and glucose was almost completely consumed in 48 h. After 24 h of incubation, the yeast is budding and is in an optimum stage for fermentation.

When the active yeast level is insufficient to ferment all the glucose, the level of glucose in the medium initially increases during recycling of the solubles (Figure 3). This buildup in glucose levels off with further recycling because yeasts produce more alcohol when exposed to higher concentrations of glucose. Thus, recycling can result in use of unfermented glucose. However, residual glucose causes excessive browning during drying of the CDG, reducing the already low lysine content of the protein (Wu et al., 1981). The hygroscopic sugars make drying more difficult and energy consuming. For best results, it is imperative that fermentation conditions, especially active yeast levels, be selected to ensure complete glucose utilization, as pointed out by Chen (1981).

Ethanol Distillation from an Externally Heated Fermentor Vessel. Since repeated fermentations using 70–80% of the available solubles did not impair alcohol production, experiments were devised to allow a recycle of all the separated CDS. For this objective, it was necessary to maintain the volume of stillage during distillation. Alcohol was removed from the medium by elevating the temperature of the fermentor vessel to 100 °C by passing steam through the outer jacket. In Table I are listed the volumes and solids contents of the CDS obtained after filtering and centrifuging stillage prepared in this manner through 10 fermentations with 9 recycles. Under these conditions, loss of volume occurs and more of the distillers'

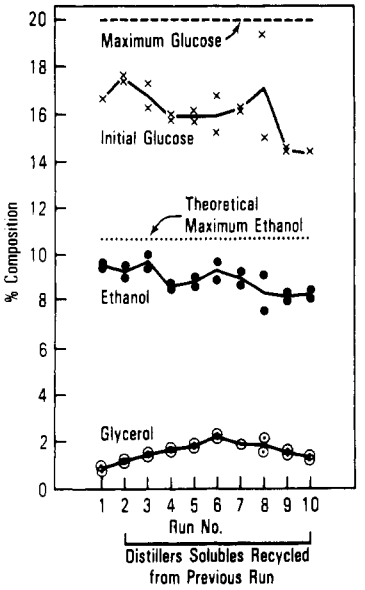

Figure 4. Effect of recycling distillers' solubles from externally heated pot distillation on glucose, ethanol, and glycerol yields after mashing and fermenting corn.

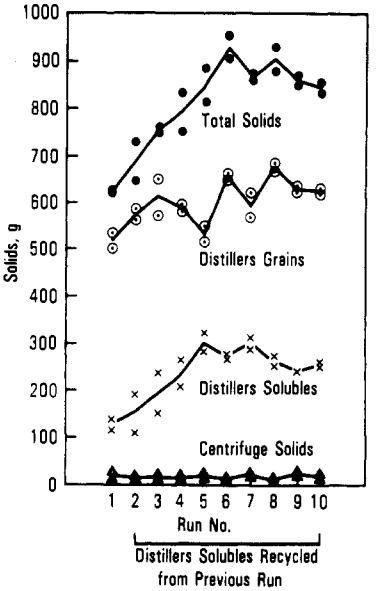

Figure 5. Effect of recycling distillers' solubles from externally heated pot distillation of fermentation broths on yields of stillage solids from yeast fermentation of corn.

solubles is retained in the grains. All of the recovered solubles are recycled, and additional tap water is added to retain the 8-L volume.

The yields of initial glucose and final ethanol in the fermentation media of 10 fermentations, in which the last 9 fermentations employed all of the distillers' solubles from the prior runs, are compared in Figure 4. Average initial glucose yield for 10 duplicate runs was 16.1 ± 1.2%. Glucose averaged 16.5 ± 0.6% (w/w) for the first seven duplicate fermentations but declined to 15.4 ± 1.7 (w/w) during the last three. Analysis of linearity (Table II) indicates that initial glucose production declined in succeeding runs as evidenced by the significant negative slopes of the equations for regression curves calculated for both 7 and 10 runs. Ethanol production averaged 8.8 ± 0.4% (w/w) for 10 duplicate runs. However, ethanol averaged 9.2 ± 0.4% (w/w) for the first seven duplicate fermentations but only 8.2 ± 0.6% (w/w) in the last three. As shown in Table II, only a small nonsignificant decline is calculated for ethanol production during the first seven duplicate runs. In contrast, when the last three duplicate runs are also considered, a significant trend to decline in ethanol yield is indicated. Fermentations proceeded rapidly, maximum yields of alcohol were obtained in 48 h, and no glucose was left after 66 h. The reasons for the decline in glucose and ethanol yields after six recycles are under study.

To determine if other fermentation products might account for reduced ethanol yields, HPLC analyses of the fermentation broth were conducted for acetic acid, lactic acid, and glycerol. Only production of glycerol was found to be significant. As shown in Figure 4, glycerol content of successive fermentation broths increased from an initial 0.7% to 2.2% in run 6 and then declined thereafter. Probably glycerol formation reduced the amount of ethanol produced more in initial recycles. After several recycles, it is possible that glycerol production was affected by other components in the media such as amino acids (Nordstrom, 1966).

The amount of total solids in the CDS, as calculated by multiplying values for percent solids times volume from Table I, increased linearly during the first five pairs of fermentations in this recycling series, as shown in Figure 5. However, in the succeeding fermentations, the CDS

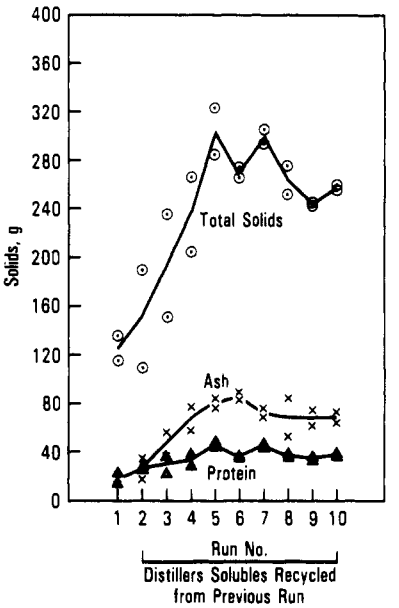

Figure 6. Changes in composition of distillers' solubles from yeast fermentation of corn during recycling of distillers' solubles.

solids did not increase and actually may have declined. The amount of CDG tended to increase in this series of fermentations from 500 to 650 g. Fluctuations in weight of recovered solids may be due to the variations in effectiveness of removal of solubles by vacuum filtration under a rubber dam; lower yields of CDG correspond to peaks in CDS recoveries. The recovery of centrifuge solids remained fairly constant and was much lower than that obtained from the steam-heated fermentation brew, averaging less than 20 g/fermentation. The combined yield of solids in the recycled stillage increased during the recycling process.

The compositional changes in the CDS during this series of recycling of the solubles are shown in Figure 6. Protein content remained fairly constant at about 15% of the CDS solids. Ash increased from 14.6% to about 31.3% of the solids. The largest component of the CDS appears to be nonfermentable soluble carbohydrates. The salts produced by the adjustment of pH for each fermentation are a major

Table III. Reagent Requirements for pH Adjustment during Recycling of Distillers' Solubles

mash no.	mash step	initial pH	adjusted pH	mequiv of NaOH	mequiv of HCl
1	α-amylase	5.9	6.2	12	
	glucoamylase	5.8	4.0		180
	ferment	4.0	4.5	56	
2	α-amylase	4.6	6.2	238	
	glucoamylase	5.7	4.0		318
	ferment	4.1	4.5	62	
3	α-amylase	4.7	6.2	250	
	glucoamylase	5.8	4.0		384
	ferment	4.1	4.5	91	
4	α-amylase	4.7	6.2	256	
	glucoamylase	5.8	4.0		444
	ferment	4.1	4.5	93	
5	α-amylase	4.7	6.2	300	
	glucoamylase	5.7	4.0		459
	ferment	4.1	4.5	119	
6	α-amylase	4.7	6.2	375	
	glucoamylase	5.7	4.0		528
	ferment	4.1	4.5	144	
7	α-amylase	4.8	6.2	300	
	glucoamylase	5.7	4.0		489
	ferment	4.1	4.5	106	
8	α-amylase	6.7	6.2	388	
	glucoamylase	5.7	4.0		519
	ferment	4.0	4.5	138	
9	α-amylase	4.8	6.2	275	
	glucoamylase	5.7	4.0		465
	ferment	4.0	4.5	125	
10	α-amylase	4.7	6.2	343	
	glucoamylase	5.9	4.0		480
	ferment	4.1	4.5	138	
	total used			3809	3777

cause of the increase in ash. Not only are these salts recycled but also increasing amounts of acid and base addition are required during each succeeding fermentation due to increased buffering capacity of the fermentation media, as summarized in Table III.

DISCUSSION

The amount of centrifuge solids recovered was much smaller in the series of experiments in which the fermentors were externally heated than when alcohol was distilled by introduction of steam. Therefore, it seemed logical to test the effect of recycling thin stillage obtained after filtering off CDG without centrifugation. Results thus far from these experiments have been inconclusive. Because low yields of alcohol were obtained in some experiments, we have not yet been able to rule out the need for the centrifugation in preparing the thin stillage for recycling. Additional experiments will be conducted to establish the cause of our variable results. On the basis of its feed value, recovery of the heated yeast contained in the centrifuge solids may justify the additional cost of centrifugation. However, elimination of increased capital investment and additional operating costs would be beneficial to small-scale alcohol production units if recycling can be carried out with thin stillage.

Recycling of the solubles of six subsequent fermentations resulted in no major impairment of saccharification or ethanol yields. Admittedly, these experiments were laboratory or small pilot-plant scale where efficient screening, rapid handling, and refrigerated storage of the stillage were possible. Under such conditions, no major contamination by microorganisms was transferred to subsequent cultures via the distillers' solubles. Heating by steam or by an external source at 100 °C after distillation should destroy most non-spore-forming organisms. Also, the high-temperature incubation with Taka-Therm α-amylase enzyme should eliminate contamination. Some gum-producing organisms can impair filtration rates. Should evidence of contamination occur, such as gumminess or reduced pH of stillage, recycling should be halted.

A possible cause of alcohol yield reduction during recycling is the inhibitory effect of metabolic products that accumulate in the CDS. Hongo et al. (1967) have shown that yeasts produce derivatives of tryptophan and phenylalanine that inhibit alcohol production during manufacture of sake. Possibly most of the tryptophol and phenylethanol is removed by distillation as part of fusel oils or is adsorbed to zein protein in CDG. Hongo et al. (1967) claim these alcohols are adsorbed to hydrophobic rice protein so as to limit their inhibitory action in sake fermentations.

Another factor considered to be a deterrent to recycling is the effect of increasing salt concentration in the mash and its effect on the osmotic pressure of the medium. Increased osmotic pressure has been shown to reduce yeast growth and alcohol production (Panchal and Stewart, 1980). Although in the present experiments the ash content of the media increased considerably during the first seven runs, when CDS from externally heated stillage was recycled (Figure 6), this increase did not appear to impair fermentation during those runs (Figure 4).

The formation of metabolic products that might reduce yields of ethanol during fermentation does not appear to increase during recycling of the solubles. Only insignificant quantities of acetic acid and lactic acid are formed. Glycerol content of broths actually decreased in the later fermentations of the recycling series. There are three plausible explanations for this phenomena: (1) inhibition of glycerol production at higher levels of glycerol in the recycled media; (2) utilization of glycerol by yeast at higher glycerol concentrations; (3) decreased formation of glycerol due to availability of amino acids for cell synthesis from recycled solubles (Nordstrom, 1966). Holzer et al. (1963) have shown that dihydroxyacetone phosphate is a hydrogen acceptor from reduced nicotinamide adenine dinucleotide in early stages of fermentation and, consequently, the glycerol content increases rapidly during the initial anaerobic fermentation.

In our experiment where total solubles were recycled for six runs, yields of ethanol which averaged 9.2% (w/w) were possibly not as high as when only 70–80% of the solubles was recycled for nine runs, 9.4% (w/w). However, even the lower value could produce 2.7 gal of alcohol/bushel of corn, which is currently regarded as an economically feasible yield. Theoretical yield is 3.1 gal/bushel of corn. If recycling is carried out for six to nine fermentations following the initial alcohol production, then investment in concentrators can be reduced and energy and labor are expended for only a single concentration. For on-farm or farmers' cooperative small-scale operations, recycling could increase solids in CDS 2–3-fold; thus, the last batch could be incorporated into animal feeds without exceeding liquid consumption limits.

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Registry No. Glycerol, 56-81-5; ethanol, 64-17-5.

LITERATURE CITED

American Association of Cereal Chemists "Methods of Analysis"; American Association of Cereal Chemists: St. Paul, MN, 1967.
Chen, S. L. *Biotechnol. Bioeng.* **1981**, *23*, 1827.

Distillers' Feed Research Council "Distillers' Feed Research"; Distillers' Feed Research Council: Cincinnati, OH, 1972.
Holzer, H.; Bernhardt, W.; Schneider, S. *Biochem. Z.* **1963**, *336*, 495.
Hongo, M.; Hayashida, A.; Inoue, S.; Koizumi, R.; Kawaharada, H. *Nippon Nogei Kagaku Kaishi* **1967**, *41* (12), 629.
Nordstrom, K. *Acta Chem. Scand.* **1966**, *20*, 1016.
Panchal, C. J.; Stewart, G. G. *J. Inst. Brew.* **1980**, *86*, 207.
Retzlaff, R. E. J. In "Ethanol Production and Utilization for Fuel"; Cooperative Extension Service, University of Nebraska: Lincoln, NE, 1980; p 71.
Ronkainen, P.; Leppanen, O.; Harju, K. *J. Inst. Brew.* **1978**, *84*, 115.
Waller, J.; Klopfenstein, T.; Poos, M. *J. Anim. Sci.* **1980**, *51*, 1154.
Wu, Y. V.; Sexson, K. R.; Wall, J. S. *Cereal Chem.* **1981**, *58*, 343.
Wu, Y. V.; Sexson, K. R.; Wall, J. S. *Cereal Chem.* **1983**, in press.

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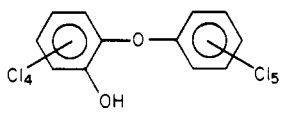
Photochemistry of Polychlorinated Phenoxyphenols: Photochemistry of 3,4,5,6-Tetrachloro-2-(pentachlorophenoxy)phenol

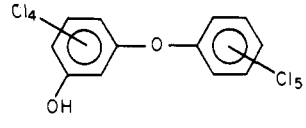
Peter K. Freeman* and Ramanujan Srinivasa

The 3,4,5,6-tetrachloro-2-(pentachlorophenoxy)phenol, a major contaminant in technical pentachlorophenol, was synthesized and its photochemistry studied at 300 nm in cyclohexane and acetone solvents. Its photochemistry was also studied in cyclohexane with *m*-methoxyacetophenone as a sensitizer. The results show that in the direct irradiation cleavage of the ether bonds and reductive dechlorination represent the major reaction pathways. On the other hand, photocyclization occurs in both acetone and cyclohexane in the presence of *m*-methoxyacetophenone as a sensitizer. The extent of photocyclization is much higher in acetone. The presence of an electron-transfer agent, triethylamine, enhances cyclization in the sensitized process. The mechanistic implications are discussed.

The impetus for an investigation of the excited state chemistry of chlorinated phenoxyphenols stems from the fact that the three isomeric perchlorophenoxyphenols (1, 2, and 3) represent major impurities in pentachlorophenol (Rappe and Nilsson, 1972; Jensen and Renberg, 1972; Nilsson and Renberg, 1974; Deinzer et al., 1978, 1979, 1981). Since these species all absorb in the sunlight range and their chemical construction is such as to suggest ready cyclization, the potential for photochemical conversion to highly toxic chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans is clear. This report describes our exploration of this potential for ortho isomer 1.



1



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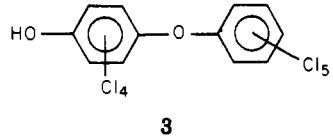

3

EXPERIMENTAL SECTION

Materials. Spectrograde cyclohexane (Mallinckrodt) was redistilled and no detectable impurity was found by GLC. Spectrograde acetone (Baker) and *m*-methoxyacetophenone (Aldrich, 99%) were used as sensitizers without further purification.

Product Analyses. The photoproducts were identified by comparing their GLC retention times with those of known compounds and by mass spectrometry. Gas–liquid chromatographic analysis of the photolysates was carried out on a Varian 3700 gas chromatograph equipped with a flame ionization detector. A 5-ft copper column of 0.56% SE-30 on Anakrom-AS (110–120 mesh) was used. Unless otherwise mentioned, the temperature of the column was programmed from 55 to 220 °C at 10 °C/min. Helium carrier gas flow rate was approximately 60 mL/min. The response factors for known compounds were determined by using dodecane as the internal standard. Whenever authentic standards were unavailable, response factors were estimated on the basis of similarities in structure and molecular weight, either to pentachlorophenol or to predioxin 1. For example, the response factor for octachloro-2-phenoxyphenol **22** was considered the same as that for predioxin 1.

Mass spectral analyses of the photolysis mixtures were done at 70 eV electron energy either on Varian CH-7 mass spectrometer equipped with a System Industries 150 data analyzer and a Varian 1200 series gas chromatograph or on a Finnigan 4023 mass spectrometer equipped with a Finnigan 9610 gas chromatograph. The column and the GLC conditions were the same for both GLC and GLC–MS analyses.

In the case of the lower chlorinated dioxins and predioxins, an effort was made to identify them with the limited data available. Wherever authentic samples were available the GLC retention times were compared and the mass spectra matched to confirm the structures. However, in certain cases, owing to the unavailability of authentic samples, mass spectral analysis was used to determine the structures partially (i.e., from which of the two phenyl rings the chlorine atom was lost).

Thus, the octachlorodibenzodioxin **27** was identified by comparing both its GLC retention time and the mass spectrum with those of an authentic sample. Comparison of the mass spectra and the GLC retention times of the

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