Recovery of 2,3-Butanediol Produced by Fermentation

MURRAY SENKUS

Commercial Solvents Corporation, Terre Haute, Ind.

A 2,3-butanediol fraction produced by fermentation was recovered from the beer as the formal. The method consisted of distilling an acidified mixture of whole beer and formaldehyde into a decanter which permitted return of the lower aqueous layer to the distilling vessel. The top oil layer which was collected contained, as the formal, 98–100% of the butanediol present in the beer. The 2,3-butanediol formal which was present in the oil layer was isolated and resolved into two geometrical isomers. The formal was allowed to react with acid methanol and converted to 2,3-butanediol and methylal in 95% conversion. A method was developed for the recovery of formaldehyde and methanol from methylal.

THE recovery of many materials produced by fermentation is generally accomplished by either distillation or extraction of the fermentation liquors. Various modifications of these processes have been used for the recovery of 2,3-butanediol produced by fermentation. Blom, Reed, Efron, and Mustakas (*1*) developed a distillation process, and Othmer, Bergen, Schlechter, and Bruins (*2*) developed an extraction process. In these reports the authors emphasized the difficulty of recovery; they ascribed it to the high boiling point of the glycol and to the presence in the fermentation liquor of 2 parts nonvolatile solids to 1 part glycol.

As the difficulties of recovering the glycol produced by fermentation were recognized, the chemical properties of the glycol were examined in the hope that the material could be converted, in the liquor, to some readily isolable derivative. Polyhydric alcohols which have hydroxyl groups on carbon atoms alpha or beta to each other are distinguished by their pronounced tendency to form cyclic acetals. This property of 2,3-butanediol was recognized; it seemed possible that the diol could be converted to the formal in the 4–6% 2,3-butanediol beer (which can be produced by fermentation) and that distillation of this mixture would remove the formal from the beer.

A 3% aqueous solution of the glycol was prepared, and the experiment was tried. All of the glycol in solution was converted to the formal and the latter distilled as a constant boiling mixture (CBM) with water. Work was then started on the isolation of 2,3-butanediol formal from the beer and on the hydrolysis of the formal to the glycol. This paper describes the results of that work.

LABORATORY ISOLATION OF 2,3-BUTANEDIOL FORMAL FROM BEER

1. WITH AQUEOUS FORMALDEHYDE. Five pounds (2265 grams) of beer (2.7 weight % glycol) were distilled through a 1-foot Vigreux column at a 10:1 reflux ratio until the vapor temperature had reached 99.5° C. This distillation gave 276 grams of distillate, which contained all of the ethyl alcohol present in the beer.

One hundred eighty grams of 50% aqueous sulfuric acid and 225 grams of 36% aqueous formaldehyde were added to the residue; the mixture was distilled over the range 80–97° C. through the Vigreux column at total take-off. The distillate consisted of two layers. The top oil layer was separated from the water layer and stored; the latter was returned to the residue. Distillation was continued for 6 hours. At the end of this time the separation of oil in the distillate had ceased. The entire distillation gave 84 grams of oil; its composition was determined by rectification. The results of the oil analysis appear in Table I. The recovery of glycol as formal was 100% of theory.

2. FROM SYNTHETIC 25% GLYCOL BEER WITH TRIOXYMETHYLENE. An attempt was made to concentrate a 2.5% glycol beer to a 25% beer, but there was considerable decomposition of solids during the concentration. Therefore, a synthetic beer was made up.

Whole-grain residue from the ethyl alcohol beer still was filtered through a No. 30 U. S. Standard sieve. To 375 grams of this filtrate were added 125 grams of 2,3-butanediol, 18 grams of concentrated sulfuric acid, and 53 grams of trioxymethylene. The mixture was distilled into a decanter which permitted return of the lower aqueous layer to the reaction vessel. The top oil layer was collected and stored. The separation of oil ceased after 1.5 hours. Analysis of the oil layer showed that it contained 140.5 grams of 2,3-butanediol formal, 2 grams of water, and 1 gram of formaldehyde. The recovery of glycol from synthetic beer was 99% of theory.

PLANT ISOLATION OF 2,3-BUTANEDIOL FORMAL FROM BEER

1. WITH AQUEOUS FORMALDEHYDE. Three hundred sixty gallons of beer which contained 72 pounds of glycol by analysis were charged to a 500-gallon kettle connected to a thirty-plate column. The mixture was distilled at a 4:1 reflux ratio until the vapor temperature reached 99–100° C. This distillation yielded 24 gallons of distillate which contained 20% ethyl alcohol by volume.

The residue was cooled to 95° C. and the kettle was charged with 200 pounds of 50% aqueous sulfuric acid, 300 pounds of 36% aqueous formaldehyde, and 40 gallons of tails cut from a previous recovery. The mixture was distilled at total take-off into a decanter. The top oil layer which separated in the decanter was collected, and the lower aqueous layer was returned to the kettle. The separation of oil in the decanter ceased after 10 hours of distillation; the yield was 97 pounds. Recovery of glycol as glycol formal was 100% of theory (Table I).

Further distillation of the residue gave an aqueous distillate which contained some dissolved 2,3-butanediol formal. Hence, after oil had ceased separating in the decanter, 40 gallons of a tail cut was distilled at a 4:1 reflux ratio for 10 hours over the range 80–99° C. This tails cut was stored, and the oil was recovered in a subsequent run according to the method described.

2. WITH METHANOL-FREE AQUEOUS FORMALDEHYDE. The method of recovering 2,3-butanediol formal from beer described in the preceding section showed that the continued use of this method would lead to difficulties because of the presence of methanol in commercial aqueous formaldehyde. For this reason a series of 2,3-butanediol formal recoveries was carried out with formaldehyde from which all methanol had been removed by dis-

Table I. Composition of Oil from Beer

Material	% by Weight (Approx.) Laboratory	Plant
Methylal	3	6
Methanol	3	2
Water	8	3
2,3-Butanediol formal	83	84
Formaldehyde, biacetyl, and acetoin	3	5

tillation. A typical experiment of this series was carried out as follows:

> Two hundred pounds of 50% aqueous sulfuric acid[1] and 340 gallons of beer which contained 80 pounds of 2,3-butanediol and 5 gallons of ethanol were charged to a 500-gallon stainless steel kettle connected to a thirty-plate column. The mixture was distilled at a 6:1 reflux ratio for 6 hours over the range 80–99° C. This distillation gave 17 gallons of aqueous ethanol (30% ethanol by volume). The mixture was cooled to 95° C. The kettle was charged with 250 pounds of 40% (by weight) aqueous formaldehyde, from which all methanol had been removed by distillation, and 30 gallons of a tails cut from a previous experiment of this series. The mixture was then distilled, and the distillate collected in a decanter where the top oil layer and the aqueous layer separated. The aqueous layer was returned to the column just below the condenser[2], and the oil layer was collected and stored. The separation of oil ceased after 12 hours of distillation; the yield was 103 pounds. Finally, 32 gallons of a tails cut were distilled at a 6:1 reflux ratio for 6 hours over the range 80–99° C. This was stored for use in a subsequent isolation of formal from beer.

The analysis of the oil isolated in this experiment is given in Table II. The recovery of glycol as formal was 100% of theory.

Table II. Composition of Oil from Beer Obtained by Plant Procedure

Material	% by Wt.
Formaldehyde	3
Water	5
Biacetyl (probably some acetoin)	3
2,3-Butanediol formal	87

ANALYSIS OF OILS FROM GLYCOL BEERS

The compositions of oils listed in Table I were determined by rectification through a 4-foot laboratory column at a 10:1 reflux ratio.

The methylal (boiling point 42° C.) and methanol (boiling point 64° C.) distilled over and were collected. The vapor temperature then rose to 70° and a series of complex azeotropic mixtures containing biacetyl distilled over the range 70–78° C. This fraction was treated with 5% aqueous ammonia to remove the carbonyl compounds, formaldehyde, and biacetyl. This treatment converted the formaldehyde to hexamethylenetetramine, and the biacetyl to the nonvolatile quinoxaline derivative. The mixture was distilled to recover the 2,3-butanediol formal as a constant boiling mixture with water. The oil from this distillation was charged back to the residue from the original distillation. This distillation was resumed and a nonhomogeneous mixture distilled over the range 78–83° C. The lower aqueous layer was collected, and the top oil layer was returned to the column. The dissolved materials in the water layer were salted out with potassium carbonate and returned to the column. When all water had been removed in this manner, the vapor temperature rose to 97° C. The 2,3-butanediol formal then distilled over the range 97–102° C.

The formaldehyde and biacetyl were not collected and weighed in the above determinations. These materials were estimated by difference. The formaldehyde came off up to a temperature of 97° C. partly as a gas; part of it solidified in the condenser of the column head.

The oil composition given in Table II was determined by the method just described and also by another procedure in order to find the amounts of formaldehyde and biacetyl present in the oils obtained by the following procedure:

The oil (810 grams) was hydrogenated for several hours at 75–100° C. and 1500 pounds pressure in the presence of Raney nickel. The reduced mixture was then rectified through a 4-foot laboratory column as before. The formaldehyde was isolated as methanol[3] and the biacetyl as 2,3-butanediol.

The 2,3-butanediol formal was collected over the vapor temperature range 97–102° C. Some other properties of the 2,3-butanediol formal which was isolated from the oils are listed in the following table:

Refractive index n_D^{20}	1.4047
Density d_{20}^{20}	0.9599
Boiling range at 750 mm., ° C.	97–102
Soly. in 100 g. water at 20° C., grams	19
Soly. of water in 100 g. formal, grams	1
Soly. in kerosene	Miscible

The 2,3-butanediol formal whose properties are listed above was resolved into its geometrical isomers by rectification of the formal through a 4-foot laboratory column at a 20:1 reflux ratio. This rectification gave isomers I and II whose properties are listed in Table III. The weight ratio of isomer I to isomer II in the formal fraction was 1:5.

RECOVERY OF EXCESS FORMALDEHYDE FROM BEER

An excess of formaldehyde was used for the isolation of 2,3-butanediol as diol formal from beers. This excess formaldehyde was isolated from the spent beer according to the following procedure: Half a liter of methanol (386 grams) was added to the residue from 1000 grams of glycol beer resulting from the procedure described in section 1 of the laboratory method. The mixture was distilled through a 4-foot laboratory column at a 10:1 reflux ratio. This distillation yielded 54 grams of methylal and 336 grams of methanol.

One hundred grams of 36% aqueous formaldehyde were used for the recovery of glycol as formal from the beer. The oil which was isolated from the beer contained 12 grams of formaldehyde as 2,3-butanediol formal, 1 gram of formaldehyde as methylal, and about 1 gram of free formaldehyde. The methylal isolated in the recovery step contained 22 grams of formaldehyde. The recovery of formaldehyde from the beer residue was 100% of theory.

HYDROLYSIS OF 2,3-BUTANEDIOL FORMAL

In Presence of Hydrochloric Acid. A mixture of 1000 grams of oil (83% by weight of 2,3-butanediol formal, Table I), isolated from beer, 300 ml. of water, 25 ml. of concentrated hydro-

Table III. Properties of Isomers I and II of 2,3-Butanediol Formal Isolated from Glycol Beer

	Isomer I	Isomer II
B.p. (750 mm.), ° C.	97.0	101.5
B.p. of CBM (750 mm.) with water, ° C.	80.5	83.0
Density d_{20}^{20}	0.9417	0.9601
Refractive index n_D^{20}	1.3984	1.4055

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[1] In part 1 of the plant isolation procedure the sulfuric acid was added after the ethanol had distilled. It was found later that the addition of acid to beer before removal of ethanol improved heat transfer from the heating coils to the beer, throughout the entire procedure for recovery of the formal.

[2] In part 1 of the plant procedure the aqueous layer was returned to the kettle. It is believed that the present procedure is an improvement over this technique.

[3] The oils which were isolated according to the procedure described in section 2 of the plant procedure did not contain any methanol. This was proved by distillation of a sample of this oil which had not been hydrogenated.

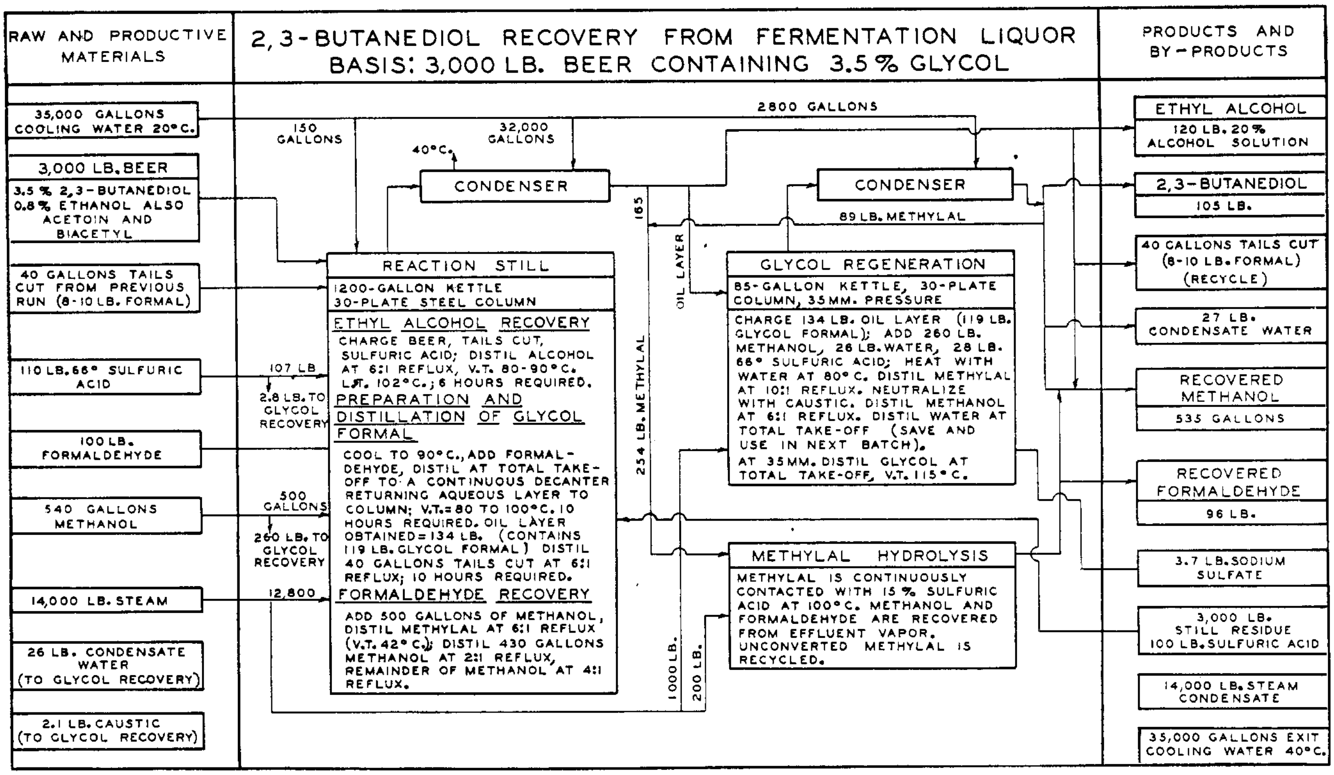
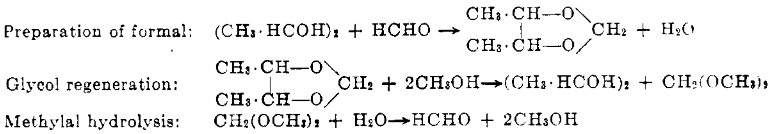


Figure 1. Flow Sheet for Glycol Recovery



Preparation of formal: $$(CH_3\cdot HCOH)_2 + HCHO \rightarrow \begin{matrix} CH_3\cdot CH—O \\ | \\ CH_3\cdot CH—O \end{matrix} \Big\rangle CH_2 + H_2O$$

Glycol regeneration: $$\begin{matrix} CH_3\cdot CH—O \\ | \\ CH_3\cdot CH—O \end{matrix} \Big\rangle CH_2 + 2CH_3OH \rightarrow (CH_3\cdot HCOH)_2 + CH_2(OCH_3)_2$$

Methylal hydrolysis: $$CH_2(OCH_3)_2 + H_2O \rightarrow HCHO + 2CH_3OH$$

chloric acid, and 1600 ml. of methanol, was distilled through a 4-foot laboratory column at a 10:1 reflux ratio. The results of this distillation are set forth in Table IV.

TABLE IV. CONVERSION OF 2,3-BUTANEDIOL FORMAL TO 2,3-BUTANEDIOL

Boiling Range, ° C.	Volume of Distillate, Ml.	Compn. of Distillate
42–48	1000	Mixt. of methylal and methanol
48–64	300	Mixt. of methylal and methanol
64	360	Methanol
64–105	410	Mixt. of methanol and water

When the vapor temperature in the above distillation reached 105° C., 20 grams of sodium carbonate were added to the residue to neutralize the hydrochloric acid. Distillation of the residue at atmospheric pressure gave 686 grams of 2,3-butanediol, boiling point 178–180° C. Conversion was 94% of theory.

IN PRESENCE OF SULFURIC ACID. A mixture of 25 pounds of oil isolated in the plant (procedure described in section 2), 0.75 pound of sulfuric acid dissolved in 3 pounds of water, and 12 pounds of methanol, was distilled through a 4-foot laboratory column at a 10:1 reflux ratio. After the methylal and excess methanol had been removed, the sulfuric acid was neutralized with caustic. The residue was distilled at atmospheric pressure until the pot temperature reached 140° C. This distillation removed most of the water in the mixture. Distillation of the residue in vacuo gave 17.5 pounds of 2,3-butanediol, boiling point 114–116° C. at 60 mm. Conversion was 94% of theory.

CONVERSION OF ISOMER II TO MESO-2,3-BUTANEDIOL IN PRESENCE OF HYDROCHLORIC ACID. A mixture of 204 grams of the higher boiling isomer of 2,3-butanediol formal (Table III), 10 ml. of concentrated hydrochloric acid, 180 grams of water, and 700 ml. of methanol was distilled through a 4-foot laboratory column at a 10:1 reflux ratio. After methylal and excess methanol had distilled, 20 grams of sodium carbonate and 300 ml. of benzene were added to the pot. The water was distilled as an azeotrope with benzene. After water had been removed, the residue was filtered and rectified, and the product was distilled in vacuo. It boiled at 83.5° C. at 10 mm. pressure. Conversion was 90% of theory. An undetermined amount of product was held up in the column.

The product isolated in this experiment melts at 32.5° C. This indicates that it is meso-2,3-butanediol, which is known to melt at 34° C. The *dl*-2,3-butanediol isomer melts at 4° C.

RECOVERY OF FORMALDEHYDE AND METHANOL FROM METHYLAL

The recovery of formaldehyde and methanol from methylal was confined to small laboratory experiments. At the time the present problem was discontinued, the procedure which gave the best results was as follows: After 250 grams of 10% aqueous sulfuric acid were heated to 90–95° C. in a 500-ml. round-bottom flask, methylal was introduced slowly at the bottom.

The vapors which escaped from the liquid were condensed and collected in a flask connected to a 4-foot laboratory column. The unconverted methylal was distilled through the column and recycled through the acid solution. Seventy-six grams of methylal were hydrolyzed for 2 hours according to this procedure. The acid solution was distilled into the column flask for 15 minutes in order to remove methanol and formaldehyde. The distillate in the column flask was fractionated, and unconverted methylal and methanol were collected. The residue from this fractionation weighed 141 grams and contained 17% by weight of formaldehyde. The conversion to formaldehyde and methanol was 80% and the yield 95% of theory.

COST OF RECOVERY

The chief items to be considered in the cost estimation of the present process are steam, cooling water, methanol, and formaldehyde. Methanol and formaldehyde are recoverable and, under ideal operating conditions, should not add much to the cost of recovery. Therefore, the economics of the present process, like those of distillation and extraction, depend on the consumption of cooling water and steam. Figure 1, a flow sheet for the recovery of glycol from 3000 pounds of 3.5% glycol beer, is shown here to allow a comparison of this process with other processes on a similar scale.

ACKNOWLEDGMENT

The 2,3-butanediol beer which was used throughout this work was provided by F. J. Rudert, now with W. S. Merrell Company, and by R. J. Hickey of the Bacteriological Research Division of Commercial Solvents Corporation.

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PREPARATION OF TECHNICAL DDT[1]

Harry S. Mosher, M. R. Cannon, E. A Conroy,
R. E. Van Strien, and D. P. Spalding
THE PENNSYLVANIA STATE COLLEGE, STATE COLLEGE, PA.

A study has been made of the effect of time, temperature, acid strength, and excess chlorobenzene on the condensation of chloral and chlorobenzene in the presence of sulfuric acid to give DDT. When 98–99% sulfuric acid is used with 4 moles of chlorobenzene per mole of chloral and at a temperature of approximately 15° C. for 5 hours, 95–98% yields of DDT can be obtained.

DDT is most readily synthesized by some variation of the original Baeyer condensation (*1*) used by Zeidler when the synthesis of this compound was first reported (*20*). Since then various experimental conditions have been described for this reaction:

$$Cl_3C{-}CHO + 2C_6H_5Cl \longrightarrow Cl_3C{-}CH(C_6H_4Cl)_2 + H_2O \quad (1)$$

These appear in the Geigy patents (*15*) and, more recently, in articles by Darling (*6*), Bailes (*2*), Iris and Leyva (*13*), and Breckenridge (*3*). A survey of the literature prior to 1944 for other possible methods of preparing compounds such as DDT and for other catalysts such as aluminum chloride (*9*) did not reveal any promising alternative. Recently the use of chlorosulfonic acid as the condensing agent was described (*16*, *17*, *19*).

Thus the problem was to determine, first, the optimum conditions for the condensation of chloral and chlorobenzene with sulfuric acid to give DDT and, second, the best method of working up and purifying the reaction mixture. Our problem was not merely a matter of finding the conditions which would give a maximum yield of DDT, for these conditions might not be the most economical. Thus technical DDT can readily be obtained in 97–98% yields (based on chloral) by the use of a large excess of both chlorobenzene and condensing acid.

CONDITIONS FOR CONDENSATION

Preliminary experiments showed that DDT was readily formed according to Equation 1 at temperatures ranging from below 0° to above 60° C. and with sulfuric acid concentrations from 90 to over 100%. At the lower temperatures the reaction rate was slow, and at the higher temperatures some decomposition occurred. This could be counteracted to some extent by using higher acid concentration at the lower temperatures and more dilute acid at the higher temperatures; at either low or high temperatures, however, extensive sulfonation of the chlorobenzene took place. Thus one of the primary problems seemed to be a determination of the temperature and acid concentration at which production of DDT was at a maximum while sulfonation of the chlorobenzene was at a minimum, within the limits of reasonable reaction time and temperature.

In order that the different condensations could be accurately compared with one another, a standard method was devised for conducting the reaction and working up the product. The following section describes a typical run in which standard method *A* was employed for isolating the technical DDT. This standard method was not necessarily the best, but it gave reproducible results and required a minimum of operations which might result in loss of product or alteration in the properties of the product. The use of this standard procedure eliminated a number of variables and made it possible to study the effects of temperature and acid concentration on the DDT condensation. Since alterations of the additional variables, represented by mole ratios of chlorobenzene and chloral and by volume ratios of the condensing acid to both of these, would have expanded the work to an unmanageable extent, the mole ratio of chlorobenzene to chloral was standardized at 2.2 to 1.0 (10% molar excess of chlorobenzene), and a final volume of approximately 500 ml. of sulfuric acid was used per mole of chloral. After the optimum temperature and acid concentration were determined under these conditions, the effect of altering the mole ratio of chlorobenzene and chloral, the excess sulfuric acid, and the reaction time were studied.

TYPICAL DDT CONDENSATION

A mixture of 200 grams of 99.0% sulfuric acid, 74 grams (0.5 mole) of chloral purified through the crystalline hydrate, and 124 grams (1.1 moles) of technical chlorobenzene were stirred rapidly

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[1] The term DDT is an abbreviation for the generic name dichlorodiphenyltrichloroethane and refers to the product obtained from the condensation of chloral and chlorobenzene. This material is primarily 1-trichloro-2,2-bis-(*p*-chlorophenyl)-ethane, *p,p'*-DDT (approximately 75–80%), which melts at 108.5–109.0° C., uncorrected and is contaminated with 1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)-ethane, *o,p'*-DDT (approximately 15%), which melts at 73–74° C. uncorrected. The melting point of technical DDT ranges from 80–94° C.