

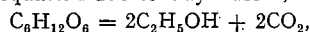
MANUFACTURE OF GLYCERIN FROM SUGAR BY FERMENTATION.

By J. O. DUCHENNE.

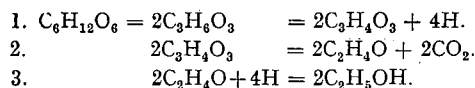
Glycerin has been known to be present amongst the products of ordinary fermentation since Pasteur's time. He established in 1857 his balance of substances formed to sugar consumed and found 3.6 grams of glycerin from 100 grams sugar fermented by his yeast. It was not until 1917 that chemists, studying the mechanism of fermentation, found a method of producing glycerin in large amounts.

Neuberg and Färber in 1917 found that in presence of alkalis the ethyl alcohol yield decreased, whilst glycerol, acetaldehyde, and acetic acid increased. Then Neuberg and Reinthur, and also Connstein and Lüdecke, found that in presence of sodium sulphite, the alcohol and carbon dioxide diminished in favour of acetaldehyde and glycerol. These two types of fermentation became known as Neuberg's third form and second form respectively, as distinct from the normal or first form.

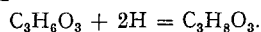
The classical equation due to Gay-Lussac,



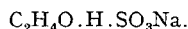
only shows the final products of fermentation as being alcohol and carbon dioxide. The intermediate reactions involved have been studied extensively by Harden¹ and his co-workers, and by Neuberg and others. It is now proved that pyruvic acid is formed, together with hydrogen, from some substance, $C_3H_5O_3$, derived from glucose. The pyruvic acid is then decomposed by the yeast enzyme carboxylase into acetaldehyde and carbon dioxide. The acetaldehyde is reduced by hydrogen from the first step of transformations, to form ethyl alcohol:—



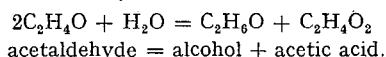
If the acetaldehyde, which is a highly reactive substance (boiling at 21°C.) is separated as formed—or combined to form a non-reactive compound—the hydrogen reacts with the unknown substance $C_3H_5O_3$, once supposed to have been methylglyoxal, to form glycerol:—



This is just what happens when sodium sulphite is added to the fermentation, the sulphite "immobilizes" the aldehyde as a bisulphite complex:—

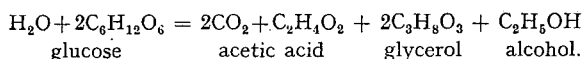


Addition of alkalis modify the course of fermentation by hydrolysis of the acetaldehyde, to alcohol and acetic acid:—



This reaction is known as the Cannizzaro Reaction.

Most known soluble alkaline salts will produce this modification of fermentation. The hydrolysis of the aldehyde allows the movement of the hydrogen atoms to proceed as in the second form, i.e., glycerol is formed. The equation for the third form is given by Harden¹ as:—



The alkali added, however, does not prevent the whole of the acetaldehyde formed from undergoing normal transformation into alcohol; the two forms of fermentation, therefore, co-exist. The result is made up of the simultaneous fermentations of the normal and the third forms. This also applies to the "sulphite" fermentation or second form, so that theoretical yields are never obtained, as is usual with bio-chemical methods.

The balance of products formed in the three types of fermentation can be set out from the equivalents in the empirical equations above, as follows: From 100 sugar fermented:—

	Normal or first form.	Second form with sulphite.	Third form with salts.
Ethyl alcohol	51.2	Nil	13.1
Glycerol	trace	51.2	51.2
Acetaldehyde	trace	24.4	Acetic acid 16.7
Carbon dioxide	48.8	24.4	24.4
	100.0	100.0	105.4

LARGE-SCALE PRODUCTION OF GLYCEROL AND COMMERCIAL PROCESSES USED.

Lawrie² describes how Connstein and Lüdecke adapted the sulphite process to large-scale production in Germany during the Great War, 1,000 tons of dynamite grade glycerin being made per month. Refined beet sugar in 10 per cent. solutions was fermented with 10 per cent. yeast in large vats of 80,000 gallons, with addition of sodium sulphite crystals up to 60 per cent. of the sugar present. After a few days fermentation, the filtered wash contained 2 to 3 per cent. glycerin, 1 to 2 per cent. alcohol, 1 per cent. acetaldehyde, salts, acetic acid and other impurities. After distillation of the wash, the residue was concentrated to a syrup containing 14 to 18 per cent. glycerol. Owing to difficulties in distilling this impure syrup, a purification of the wash was resorted to. This consisted of:—

- (1) Filtration and recovery of yeast.
- (2) Distillation of alcohol and acetaldehyde.
- (3) Precipitation of sulphite by calcium chloride and milk of lime, and filtration.
- (4) Acidification, followed by excess soda to remove lime and iron salts.
- (5) Re-acidification and concentration to syrup.

The losses were around 40 per cent. of the glycerol in the wash. The crude syrup contained 28 to 33 per cent. of glycerol, which was distilled in a Jobbins type still. It was necessary to re-distil the glycerol in order to obtain dynamite grade glycerol. This contained up to 4 per cent. trimethylene glycol, the sweet water fractions containing 25 per cent. The process of distillation was carried out in one central factory receiving dunder from 63 fermenting houses. The nett yield of glycerol was around 10 per cent.

THE AMERICAN SODIUM CARBONATE PROCESS.

In 1917 Eoff and his co-workers discovered that fermentation in presence of alkalis, such as sodium carbonate, bicarbonate, hydrate, etc., greatly increased glycerol formation. His results, later embodied in his patent, show that 20 to 25 per cent. glycerol was formed apart from alcohol, acetic acid and some acetone, on 100 sugar fermented. The amount of glycerol formed was proportional to the sodium carbonate added. His wash contained 17.5 to 20 grams per cent. sugars, to which he added up to 5 per cent. sodium carbonate in powdered form. The yeast he acclimated was *Saccharomyces ellipsoideus*, var. Steinburg No. 657, used first in 1 per cent. sodium carbonate culture medium. The soda was added in five doses in such a way as to stop "short of that amount which will inhibit further fermentation." In this way Eoff obtained a wash, after five days fermentation, containing 3.1 per cent. glycerol, 6.75 per cent. alcohol by volume, 3.6 grams alkali as sodium carbonate, and residual sugar 0.86 per cent. On a 425 gallon molasses wash, of 21.2° Balling containing 16.85 per cent. sugars, he recovered about 100 lbs. of crude glycerin from the purified dunder. This syrup, containing about 33 per cent. glycerol, distilled in a Jobbins-Van Ruymbecke type of still, eventually gave 50 lbs. dynamite glycerol, a nett yield of half a pound per gallon of molasses. Double distillation gave a glycerin nitrating normally. A large plant was put up in Illinois to work the process.³

The Cocking and Lilly process was developed in England in 1919. Its object was to obtain glycerol in almost theoretical yields by using a mixture of acid and normal sodium sulphite, in such proportions as to be neutral and relatively non-antiseptic.² The advantage was that the acetaldehyde was fixed to the bisulphite at an earlier stage previous to the formation of bisulphite by the normal sulphite reacting with carbon dioxide. The time of fermentation was shortened and yields up to 43 per cent. glycerol, 21 per cent. aldehyde and 8 per cent. alcohol were obtained on inverted raw sugars; molasses gave 35.5 per cent. yield on sugars fermented. As in the sulphite process, the yields are directly proportional to the amount of salts added.

Martin³ supplies the following table:—

Sulphite used.	Glycerol formed.	Alcohol.	Acetaldehyde.	Carbon dioxide
25	11.3	40.0	2.4	37.6
50	19.6	28.7	5.8	35.8
100	27.1	23.3	8.6	29.4

Other patented processes are quoted by Lawrie², whilst Owen, Levy and Owen⁴ describe some of the fermentation processes and the methods of recovery. These last three writers are of the opinion that:—"Glycerin produced by fermentation offers by far the greatest promise commercially," and that "there is every indication that fermentation glycerol would soon become a potent factor in glycerin production."

In South Africa, glycerin production from molasses on the industrial scale has started.⁵ The process is an alkaline fermentation, followed by distillation, purification, concentration and extraction by a solvent in which glycerin is soluble and impurities are precipitated. The extract is freed of the solvent, which is recovered for re-use.

The process which I developed at Umfolozi when studying ethyl alcohol production in 1938 consists of fermentation in presence of calcium sulphite in the powdered form; later, a mixture of soluble calcium bisulphite and sulphite was used, the fermentation being kept in the acid range.⁶ The mixture is added as a slurry with a sulphite to bisulphite ratio of from 15 to 1 to 5 to 1, and in gradually increasing amounts as fermentation proceeds. It is then added in diminishing quantities and the mixture allowed to ferment to the end. Slow stirring of the mixture is necessary.

An example of the calcium sulphite fermentation is as follows: 11 gallons of diluted syrup from old stock containing 139 grams per litre of total sugars and 21° brix were seeded with yeast (*S. ellipsoideus* acclimated to sulphites) from five consecutive fermentations. The temperature was kept at 35°C. Calcium sulphite slurry, made by sulphiting milk of lime, was added at intervals over a period of 90 hours, and the mixture agitated by hand stirring every 20 minutes. After five days (120 hours) the fermentation was taken as complete. The wash contained a trace of reducing sugars and was of 4.5 pH, with a total sulphite of 13.0 gr. SO₂ per litre. The wash was allowed to settle, the yeast was recovered and also the calcium sulphite, of which 16 lbs. 5 ozs. was weighed. It was then neutralized with milk of lime (1,200 ml. at 16° brix) containing 2 lbs. of lime. The slurry was again settled and removed. A one-litre sample gave on distillation 13.6 grams of alcohol and acetaldehyde. The dunder, on concentration and extraction with absolute alcohol, gave 154.4 grams of crude glycerin. On distillation in the superheated steam still, 21.2 grams of pure glycerin were collected. The crude syrup contained 13.7 per cent. glycerol. The yields on the basis of sugars present at the start were therefore: Alcohol 9.8 per cent., glycerol 15.3 per cent. The rest of the dunder on evaporation gave over 21 lbs. of syrup for extraction. Another test, using the same yeasts and the same calcium sulphite slurry, gave with a wash containing 16.9 per cent. total sugars, alcohol = 13.4 per cent., glycerol = 10.1 per cent. as pure glycerin (distilled).

After seven months continuous fermentations the following results were observed using calcium sulphite and bisulphite mixtures as aldehyde acceptor:—

F.XII, 26-6-40.—1420 ml. of wash: neutralized 1000 ml. and, after distillation, 18 grams calcium sulphite were filtered. Dunder concentrated and distilled: alcohol and acetaldehyde 51.76 grams = 20 per cent. yield; glycerol 13.13 grams = 10 per cent. yield.

F.XIII.—The wash analysed as total SO₂ = 8.34 grams per litre and free SO₂ = 1.36 grams per litre, leaving 6.48 as combined to acetaldehyde. As this latter is produced in a ratio of 1 to 2 glycerol, the calculated glycerol from aldehyde was 6.48 × 0.69 = 4.83 grams acetaldehyde = 9.66 glycerol. Actual distillation gave alcohol + acetaldehyde = 52.88 grams = 30.7 per cent. sugar. The dunder group gave glycerol distilled = 8.07 grams = 4.7 per cent.

It will be noted that the yields in glycerol decrease when less sulphite is added, whilst alcohol increases. Also, that the combined bisulphite is a measure of the glycerol formed. The reason why calcium sulphite is preferred is that its bisulphite combines with twice as much aldehyde as sodium bisulphite. Recovery is facilitated as solid sulphite is obtained after fermenting and distilling of the wash previously neutralized with lime, thus ensuring enough for re-use. The salt is easily sulphited to desired ratios of the acid salt in the slurry, with existing apparatus in the sugar factory. A large amount could be separated during juice clarification if necessary. On extracting dunder syrup (with alcohol produced during the previous step) a precipitate of gums, salts, etc., is obtained amounting to 55 per cent. of the weight of the syrup. Distilling off the alcohol leaves a crude glycerin containing 12 to 30 per cent. glycerol, depending on the yields obtained. The subsequent stage is the most important, as the method of obtaining pure glycerol must be chosen to give the highest yields and the least decomposition products, especially in such a biochemical process.

Laboratory distillations in a pilot plant during 1940-1941 have shown that the still has to be carefully designed for the type of crude used. It is preferable to purify the crude to the utmost before distillation, to avoid polyglycerols and trimethylene glycol, as well as fatty acids and esters formation at the high temperatures used. The analysis of a sample of once-distilled glycerin by the above process is included for comparison in the table below, showing the usual composition of commercial samples:—

	Dynamite grade.	Saponification crude.	Soap works crude.	CaSO ₄ Fermentation crude.
Glycerol per cent.	98.5	88.0	80.0	80.0
Ash per cent.	0.15	0.5	10.0	0.11
Carbonaceous residue per cent.	trace	1.0	3.0	0.34
Moisture per cent.	1.5	10.0	10.0	16.20
Specific gravity $\frac{15.5^\circ\text{C.}}{15.5^\circ\text{C.}}$	1.262	1.241	1.300	1.231
Free fatty acids	1 ml. $\frac{N}{10}$	—	—	>1 ml. N/10
	NaOH per 50 ml.			NaOH per 50 ml.
Combined fatty acids	0.22% NaOH	—	—	>0.22% NaOH
AgNO ₃ reduction test	Nil	—	—	Positive.
Colour	Light straw.	Yellow to dark brown.	Dark brown.	Light straw to brown.

A sugar factory producing 8,000 tons of molasses a year could manufacture 6 tons of glycerol and 1,000 gallons of alcohol per day, valued at £650. If only alcohol were produced 3,200 gallons per day, valued at £160. The problem of dunder disposal would not arise, as the non-glycerol organic matter is a solid (containing all the "ash" from the molasses) which would be disposed of with the filter cake.

References.

- ¹ Harden, Arthur (1932): *Alcoholic Fermentation*. Fourth edition.
- ² Lawrie, J. W. (1928): *Glycerol and the Glycols*.
- ³ Martin, G. (1926): *Modern Soap and Detergent Industries*.
- ⁴ Owen, William L., Levy, H. A., and Owen, W. Ludwell (1940): "The Production of Glycerin by Fermentation from Sugar and Molasses," *I.S.J.*, 248.
- ⁵ Viljoen, J. A. (1940-41): "The Organic Chemical Industry." *Proc. Assoc. Sci. and Tech. Soc. of S.A.*, 123.
- ⁶ — (—): Union patent 341.40.

Mr. DYMOND asked whether perfectly dry and pulverized dunder as produced by recent plants in Britain using film roller driers, would not facilitate the distillation of the glycerin?

Mr. DUCHENNE replied that he was at present investigating the problem. It seemed to him very promising, as the crude syrup had to be brought nearly to absolute dryness before distillation of the glycerin.

Dr. HEDLEY, replying to a question by Mr. Hendry, said that the samples of both sulphitation and carbonatation South African exhausted molasses sent to America were found to be perfectly suitable for acetone and butyl alcohol manufacture. The proportion of these two products could be varied.

He thought Mr. Duchenne had tackled a very difficult problem. During the last war the Germans made glycerin from pure beet sugar and only obtained a yield of 10 per cent. and about 45 per cent. of the glycerin could not be recovered. It must be even more difficult to produce glycerin commercially from an impure product such as molasses.

Dr. Hedley did not agree with Mr. Dymond that it would be advisable to dry the dunder before distillation. It simply meant additional expenses of drying and then wetting it again during distillation.

Mr. DUCHENNE, in reply to a question by Mr. Rault, said that to get the glycerin colourless the acids were first neutralised, the colour then absorbed and the glycerin redistilled.

He agreed with Dr. Hedley that it was an arduous task to get a good yield of pure glycerin. It was, however, possible to get a yield of 16 to 18 per cent. sugars and of 97 per cent. purity.