Chemical Action of Bacillus lactis aerogenes (Escherich) *on Glucose and Mannitol: Production of* 2 : 3-*Butyleneglycol and Acetylmethylcarbinol.*

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(Communicated by Dr. C. J. Martin, F.R.S. Received December 5, 1905,—Read February 1, 1906.)

(From the Chemical Laboratory, Lister Institute.)

I. *Action of B. lactis aerogenes on Glucose.*

In the course of an investigation on the chemical action on glucose of the lactose-fermenting bacteria of fæces* it was found that, whereas *B. coli communis* yields, with glucose, acetic acid and alcohol in approximately equal molecular proportions, *B. lactis aerogenes* produces a much smaller amount of acetic acid, relatively both to the alcohol and to the sugar fermented. It was, moreover, previously known that *B. lactis aerogenes* produces a greater volume of gas, containing a larger proportion of carbon dioxide than does *B. coli communis*.† These facts indicate that the fermentation of glucose by this organism is of a different type from that brought about by the *B. coli communis*.‡ A detailed examination of the products was therefore made. For this purpose the organism was grown anaerobically in a medium containing 1 per cent. of Witte peptone and 2 per cent. of pure glucose in the presence of chalk, the method of collecting the gases evolved and of examining the products being the same as that previously employed in the investigation of the action of *B. coli communis* on glucose.§

All the substances produced by *B. coli communis* from glucose were again found, viz., lactic acid, acetic acid, succinic acid, formic acid, ethyl alcohol, carbon dioxide, and hydrogen. A quantitative estimation, however, revealed the fact that only about two-thirds of the carbon of the glucose was thus accounted for. This is shown in the following tables, which embody the results of three separate estimations (Cols. 1, 2, 3), Table I giving the actual percentages by weight on the sugar fermented of the various substances produced, and Table II the number of carbon atoms per molecule of sugar decomposed represented by each product. The results of a typical

* Harden, 'Journ. of Hygiene,' 1905, vol. 5, p. 488.

† Theobald Smith, 'Centralbl. f. Bakteriol.,' 1895, vol. 18, pp. 1, 494, 589.

‡ Harden, 'Trans. Chem. Soc.,' 1901, p. 601.

§ Harden, *loc. cit.*

fermentation of glucose by *B. coli communis* are added (Col. 4) for the sake of comparison :—

Table I.

	1.	2.	3.	4.
Alcohol	17·1	16·4	18·2	12·85
Acetic acid	5·1	4·2	8·6	18·84
Lactic acid	5·5	4·7	9·1	31·90
Succinic acid	2·4	3·1	4·5	5·20
Formic acid	1·0	0·75	1·7	0·0
Carbon dioxide	38·0	—	35·2	18·09
Carbon dioxide, c.c. per gramme	198·3	—	178·5	91·8
Hydrogen, c.c. per gramme	82·4	—	92·4	110
Ratio H_2/CO_2	0·42	—	0·52	1·19
Percentage excess of l-lactic acid	50·0	66·0	86·0	Not determined

Table II.

	1.	2.	3.	4.
Alcohol	1·34	1·28	1·43	1·01
Acetic acid	0·31	0·25	0·52	1·13
Lactic acid	0·33	0·28	0·55	1·91
Succinic acid	0·15	0·19	0·27	0·32
Formic acid	0·04	0·02	0·07	0·00
Carbon dioxide	1·60	—	1·44	0·74
Total	3·77	—	4·28	5·11
Hydrogen, atoms per molecule	1·33	—	1·50	1·77

It will be observed that the ratio of hydrogen to carbon dioxide by volume is about 0·5 to 1, whilst these gases are produced by *B. coli communis* in approximately equal volumes. Theobald Smith,* using an ordinary fermentation tube, gives the characteristic ratio for *B. coli communis* as $H_2/CO_2 = 2:1$ and for *B. lactis aerogenes* $H_2/CO_2 = 1:1$. This difference is due to the solubility of the carbon dioxide in the liquid medium, and it must be remembered that while Smith's ratios give a perfectly satisfactory working test for the discrimination of the organisms, they do not represent the actual volumes or ratios of the gases produced.

Further examination of the fermentation products revealed the fact that

* *Loc. cit.*

no other acids had been formed, and search was therefore made for compounds of a different type. It was previously suggested that the deficiency of carbon observed in the fermentation produced by *B. coli communis*, amounting to only 0·25 to 0·9 of an atomic proportion of carbon, might possibly be due to the presence of reduction products of sugar, and compounds of this kind were therefore sought.

It was found that when the neutral liquid, containing the products of fermentation along with peptone, was evaporated to dryness at 55° under diminished pressure and extracted with alcohol, a solution was obtained which yielded on fractionation a colourless liquid boiling at 181° to 183° (corr.) at 760 mm. pressure. The yield was very small, only amounting to about 1 gramme per litre of medium containing 20 grammes of glucose, but it was found possible to increase the yield by employing a medium containing 5 per cent. of glucose, and in this way 8 grammes of the new substance, containing 52·8 per cent. of carbon, were obtained per litre of medium containing 50 grammes of sugar. This only accounts for about two-thirds of the missing carbon, and a rough estimate of the amount lost during the process of distillation and extraction was, therefore, made by dissolving 8 grammes of the material in 500 c.c. of a medium containing 5 grammes of Witte peptone, 6 grammes of calcium lactate and 6·5 grammes of alcohol and then extracting it in the manner described above. Only 5·2 grammes were recovered, the loss per 500 c.c. being therefore about 2·8 grammes and the loss per litre about 5·6 grammes. This brings the total amount produced from 50 grammes of glucose to about 13·6 grammes, slightly in excess of that required. It is hoped that the actual yield may be increased by a careful fractionation of the fermentation products.

The new product is apparently a mixture, and it has not yet been found possible to separate and identify all the components, so that the following must be taken as only a preliminary account of the substance.

It boils at 181° to 183° (corr.), and solidifies in the cold to a transparent mass which melts indefinitely at about 28°. It is optically active, the value for $[\alpha]_D$ for different preparations varying from 0·46 to 0·71. The composition of the substance dried by quicklime is approximately that of a butyleneglycol, but the percentage of carbon is about 0·6 too low. It does not reduce Fehling's solution either in the cold or on heating. That this substance contains a large proportion of 2:3-butyleneglycol, $CH_3.CH(OH).CH(OH).CH_3$, is shown by the following facts :—

1. When the liquid is heated with phenylisocyanate dissolved in anhydrous ether, combination occurs and a mixture of urethanes is produced. The fraction of these which is least soluble in alcohol comprises about 90 per cent.

of the whole amount and has the composition of the diphenylurethane of butyleneglycol ($C_4H_{10}O_2.2C_6H_5NCO$):—

Analysis.

	Found.	Calculated.
C	65·79	65·85
H	6·21	6·09
N	8·57	8·53

It is sparingly soluble in cold alcohol, ether and benzene, crystallises in rosettes of needles and melts at 197° to 198° (uncorr.). When the urethane is boiled with baryta water or caustic soda solution it is decomposed and yields a glycol boiling at about the same temperature as the original material. This glycol has, however, not yet been isolated in the perfectly pure and dry state. A monourethane, $C_4H_{10}O_2C_6H_5NCO$, has also been prepared which is somewhat more soluble in cold alcohol than the diurethane, and crystallises in needles, melting at 100°:—

Analysis.

	Found.	Calculated.
N	6·89	6·65

2. Both the crude glycol and that recovered from the diurethane are converted by oxidation with bromine in the light* into diacetyl, $CH_3.CO.CO.CH_3$, which was recognised by its extremely characteristic appearance and smell, and by the formation of a phenylosazone melting at 242° to 242°·8 (uncorr.).† The formation of this substance shows conclusively that 2 : 3-butyleneglycol must be present in the fermentation product.

Detection of Acetylmethylcarbinol among the Fermentation Products.—It was further found that the distillate from the liquid in which the organism was grown reduced Fehling's solution in the cold and gave with phenylhydrazine the osazone of diacetyl, melting at 243°. These properties point to the presence in the distillate of acetylmethylcarbinol, $CH_3.CO.CH(OH).CH_3$, which has previously been detected in this way by Grimbert‡ and by Desmots§ in the products of the fermentation of glucose by several bacteria: *B. tartricus, B. mesentericus vulgatus, B. fuscus, B. flavus, B. niger, B. ruber, B. subtilis,* and *Tyrothrix tenuis.* It has also been found in vinegar.

This compound appears only to be formed in very small amount. Since it

* v. Pechmann, 'Ber.,' 1890, vol. 23, p. 2427.

† v. Pechmann, 'Ber.,' 1888, vol. 21, p. 2754.

‡ 'Compt. Rend.,' 1901, vol. 132, p. 706.

§ 'Compt. Rend.,' 1904, vol. 138, p. 581.

is likewise converted into diacetyl by oxidation with bromine in the light, it is important to notice that the glycol used for conversion into diacetyl, as described above, was quite free from any substance capable of reducing Fehling's solution, and yielded a relatively large amount of diacetyl.

II. *Action of B. lactis aerogenes on Mannitol.*

A quantitative examination of the products of fermentation of mannitol by *B. lactis aerogenes* showed that in this case also the action differed from that produced by *B. coli communis,* but that the deficit of carbon was only one-half of that found for glucose. This is shown in the following tables: Table III giving the percentages and Table IV the number of carbon atoms per molecule of mannitol represented by the products in two experiments (Cols. 1 and 2). As before, the products obtained by the action of *B. coli communis* are also given for the sake of comparison (Col. 3) :—

Table III.

	1.	2.	3.
Alcohol	32·5	32·5	28·1
Acetic acid	2·5	2·1	9·5
Lactic acid	8·6	8·6	18·6
Succinic acid	3·2	2·8	8·9
Formic acid	1·5	1·6	3·0
Carbon dioxide	35·5	35·5	28·44
Carbon dioxide, c.c. per gramme	180·3	180·3	143·0
Hydrogen, c.c. per gramme	138·3	143·6	167·0
Ratio H_2/CO_2	0·77	0·79	1·18
Percentage excess of 1-lactic acid	65·0	56·0	79·0

Table IV.

	1.	2.	3.
Alcohol	2·57	2·57	2·22
Acetic acid	0·15	0·12	0·58
Lactic acid	0·52	0·52	1·13
Succinic acid	0·20	0·17	0·55
Formic acid	0·06	0·065	0·12
Carbon dioxide	1·47	1·47	1·16
Total	4·97	4·91	5·76
H atoms per molecule glucose	2·26	2·34	2·7

Further examination has shown that in this case as in that of glucose, both acetylmethylcarbinol and a glycol are produced, but both in much less quantity. The amount of crude glycol actually isolated from the products of fermentation of 50 grammes of mannitol was only 0·75 gramme. Since, however, the loss in isolating may be roughly taken as about 5 grammes, this is approximately the yield which would be expected if 6 to 7 grammes were formed. The nature of these products and their quantitative estimation, as well as the study of their optical properties, is still under investigation, and search is also being made for these and similar substances among the fermentation products of other bacteria.

General Considerations.

The production of so large a proportion of 2:3-butyleneglycol in these experiments affords clear proof that this substance is derived from the glucose. The interesting question as to the mode of its production from the glucose or mannitol molecule will be best deferred until a more complete examination of the products, and especially of their optical relations, has been made. The close constitutional relation between the glycol and lactic acid, and the readiness with which its oxidation product—diacetyl—passes into an aromatic compound are also points of great interest. It may, however, be noted that the comparison of the fermentation products of *B. coli communis* and *B. lactis aerogenes* shows, firstly, that the alcohol produced by the latter organism is slightly greater in amount than that due to the former, and, secondly, that it is at the expense of that part of the molecule which in the *B. coli* fermentation yields acetic acid and lactic acid, that the *B. lactis aerogenes* forms the new products.

It may further be observed that both these bacteria produce twice as much alcohol from mannitol as from glucose, a fact which tends to confirm the suggestion previously made,* that the formation of alcohol in these reactions is related to the presence of the terminal $CH_2(OH).CH(OH)$ group, which occurs twice in the molecule of mannitol and only once in that of glucose.

A substance of the composition of butyleneglycol has previously been isolated from the products of fermentation of sugar by yeast,† and was also found in wine‡ and in brandy.§ This substance, boiled at 178° to 179°, yielded a diacetin boiling at 192° to 193°, and was considered to be identical

* Harden, 'Trans. Chem. Soc.,' 1901, p. 601.

† Claudon and Morin, 'Compt. Rend.,' 1887, vol. 104, p. 1109; Henninger and Sanson, 'Compt. Rend.,' 1888, vol. 106, p. 208.

‡ Henninger, 'Compt. Rend.,' 1882, vol. 95, p. 94.

§ Morin, 'Compt. Rend.,' 1887, vol. 105, p. 1019.

with the synthetical isobutyleneglycol of Nevolé,* which boils at 176° to 178°. The yield obtained from sugar was, however, very small, and only amounted to about 0·2 per cent. after allowing for the losses involved in the extraction of the compound.

In view of the properties of the crude glycol described above, it would seem advisable to re-examine Henninger's glycol, the constitution of which was not experimentally examined.

The Alcoholic Ferment of Yeast-Juice.

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(Communicated by Dr. C. J. Martin, F.R.S. Received December 8, 1905,—Read February 1, 1906.)

(From the Chemical Laboratory, Lister Institute.)

1. *Effect of the addition of Boiled and Filtered Yeast-juice on the Fermentation of Glucose Produced by Yeast-juice.*

In the course of some experiments on the action of various proteids on the fermentative activity of yeast-juice, it was observed that the alcoholic fermentation of glucose by yeast-juice is greatly increased by the addition of yeast-juice which has been boiled and filtered, either when fresh or after having undergone autolysis, although this boiled liquid is itself incapable of setting up fermentation. Thus, the total fermentation produced by yeast-juice acting on excess of glucose is, as a rule, doubled by the addition of an equal volume of the boiled juice, and a further increase is produced when a greater volume is added, the sugar concentration being kept constant.†

A similar observation was previously made by Buchner and Rapp‡ in a single experiment (No. 265).

The following table embodies a few of the results obtained, the yeast-juice being prepared and the amount of carbon dioxide evolved being estimated by

* 'Compt. Rend.,' 1876, vol. 83, p. 65.

† Harden and Young, Preliminary Note, 'Proc. Physiol. Soc.,' 1904, vol. 32, November 12.

‡ 'Ber.,' 1899, vol. 32, p. 2093.