

Microbiology. — A. J. KLUYVER and H. J. L. DONKER : “*The formation of acetylmethylcarbinol and 2 : 3 butylene glycol in the fermentation of sugars by alcohol yeasts and true lactic acid bacteria.*” (Communicated by Prof. G. VAN ITERSON JR.)

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The formation of 2 : 3 butylene glycol and acetylmethylcarbinol in the fermentative decomposition of sugar under the influence of microbes was first demonstrated by HARDEN and WALPOLE in 1908 ¹⁾. These investigators found that in the fermentation of sugar under the influence of *B. lactis aerogenes*, the glycol, together with small quantities of acetylmethylcarbinol, was present among the fermentation products to the extent of 20 percent of the weight of sugar fermented.

The mode of production of these two compounds was explained in 1912 by HARDEN and NORRIS ²⁾ by the assumption of a condensation of immediately formed acetaldehyde.

In later years, a series of communications by LEMOIGNE ³⁾ has shown the occurrence of both the substances mentioned in the metabolism of very diverse microbes. Among these one finds both organisms, which possess exclusively an oxidative dissimilation and also those, which are able to bring about the splitting of sugars by fermentation. While we shall omit of consideration here the formation of acetylmethylcarbinol by the first mentioned organisms, we shall first consider to which of the groups given in the preceding communication ⁴⁾, the sugar fermenting microbes, which have been shown to produce these compounds, belong. It appears that all these microbes belong to groups IV and V. To this might be added immediately, that unpublished investigations, which we have carried out, have taught us that the products mentioned are also encountered very frequently among the normal fermentation products of microbes belonging to groups VI and VII. On the contrary these substances have not been found so far in the normal fermentative decomposition of sugar by microbes of groups I, II and III.

As will appear quite clearly from what is given below, this contrast is connected in a close manner with the fact, that free hydrogen is completely

1) HARDEN and G. S. WALPOLE, Proc. Royal Soc. Ser. B. Vol. 77, p. 399, (1906).

2) A. HARDEN and D. NORRIS, Proc. Royal Soc. Ser. B. Vol. 84, p. 492, (1912).

3) M. LEMOIGNE, Annales de l'Institut Pasteur T. 27, p. 856, (1913); Comp. rend. de la Soc. de Biol. T. 82, p. 984, (1919); Ibid. T. 83, p. 336, (1920); Comp. rend. de l'Acad. d. Sc. T. 177, p. 652, (1923); Compt. rend. de la Soc. de Biol. T. 88, p. 467, (1923).

4) These Proceedings 28, p. 297 (1925).

or almost completely absent among the fermentation products of the last mentioned groups of microbes.

A short time ago it was shown by NEUBERG and REINFÜRTH ¹⁾, however, that if acetaldehyde is added to a sugar solution undergoing fermentation by brewer's yeast or pressed yeast, the acetaldehyde added is converted almost quantitatively into acetylmethylcarbinol. The fact that the product so formed appears to be optically active leaves no doubt as regards the biochemical character of the condensation which occurs. In this condensation NEUBERG sees a special case of the carbon coupling action of yeast, which he has established with a number of other aldehydes and which should take place under the influence of what he describes as a carboligase enzyme.

No further proof is necessary that this carboligatic action on added acetaldehyde is precisely the same as the condensation, which has already been recognised by HARDEN as the cause of the occurrence of acetylmethylcarbinol (and indirectly of 2 : 3 butylene glycol) in the normal fermentation of sugar by *B. lactis aerogenes*.

While on the one side, from the researches of NEUBERG and REINFÜRTH it appears that alcohol yeast is able to bring about the carbinol condensation of acetaldehyde and on the other side it is established that acetaldehyde is an intermediate product in the normal fermentation of sugar by this organism, the question naturally presents itself, why carbinol, or its reduction product 2 : 3 butylene glycol, is not formed in the normal fermentation, as has also been stated by NEUBERG.

The only plausible explanation for this fact is, that the acetaldehyde formed intermediately undergoes other transformations so rapidly, that it is withdrawn from the simultaneously possible carbinol condensation.

The scheme, which we have developed for alcoholic fermentation and which may be gathered from Tables II and III of our preceding communication, clearly indicates that it is the protoplasm-hydrogen compound, which makes itself master of the acetaldehyde. Indeed, where protoplasm regeneration with the liberating of free hydrogen by the yeast cannot take place, the re-formation of protoplasm is left completely to the collaboration of a hydrogen acceptor, the acetaldehyde then functioning as such.

If this view is correct, then interference with the metabolism, the direct consequence of which is the introduction at least partially of another protoplasm regeneration reaction, must result in an excess of acetaldehyde in first instance. Now where acetaldehyde added externally is converted by yeast into acetylmethylcarbinol, one might expect that the method of interference, described above, will lead in like manner to the production of the carbinol or to its reduction product 2 : 3 butylene glycol.

In this line of thought we have investigated the result of the addition of hydrogen acceptors on the course of the normal fermentation of sugar.

¹⁾ Bioch. Zeitschr. Bd. 143, p. 553, (1923).

In complete agreement with expectation it appears, that if a ten percent glucose solution, to which 0.1 percent of methylene blue or a few percent of sulphur has been added, is allowed to ferment at a temperature of 30° C. by means of 10 percent pressed yeast (Koningsgist der Ned. Gist- en Spiritus-fabriek, Delft) with the exclusion of air, the formation of 2 : 3 butylene glycol can be demonstrated with certainty in the completely fermented liquid within three hours. It is wellnigh superfluous to remark, that the added methylene blue was completely reduced to the leuco-compound, while in the other experiments the added sulphur was converted into hydrogen sulphide to a noticeable extent.

In these and all subsequent experiments the presence of acetylmethylcarbinol or 2 : 3 butylene glycol respectively, was demonstrated by the excellent method of LEMOIGNE¹⁾, in which first the carbinol present is converted into diacetyl by oxidation with ferric chloride and afterwards any glycol is oxidised by bromine²⁾ in neutral solution to diacetyl also, the latter being indentified by steam distillation into an ammoniacal solution of nickel chloride and hydroxylamine, as the highly characteristic nickel dimethylglyoxime.

This confirmation of our expectations led us to the suggestion, that probably also with the heterofermentative, true lactic acid bacteria the absence of carbinol and glycol among the normal products of glucose fermentation — concerning which we especially reassured ourselves — must be ascribed neither to a lack of power to form carbinol. We found it to be possible to force the production of carbinol in sugar fermentation, in the same way as given above for alcoholic fermentation, for a couple of representatives of this groups, namely, *Lactobacillus fermentum* (BEIJERINCK) and *Betabacterium breve* (ORLA-JENSEN). As it is not possible to start with a large quantity of bacteria material, one must choose the added hydrogen acceptor for these bacteria in such a way, that there is no retarding influence on the growth. As appears from our scheme, laevulose is pre-eminently suitable for bacteria of the group mentioned, to serve both as dissimilation substratum and hydrogen acceptor. In a sterile culture liquid, consisting of yeast extract with 10 percent of laevulose, inoculated with one of the bacteria strains after incubating for several days (at 30°—35° C. for *L. fermentum* and at 25° C. for *B. breve*.) 2 : 3 butylene glycol could be demonstrated.

The positive results of these experiments opened up the possibility of investigating in this way whether, be it in a very small degree, laevulose also acts as a hydrogen acceptor in alcoholic fermentation.

As a matter of fact we found that 2 : 3 butylene glycol (together with

¹⁾ M. LEMOIGNE, Annales de l'Institut Pasteur, T. 27, p. 856, (1913); Compt. rend. T. 170, p. 131, (1920).

²⁾ To us it appeared that in this oxidation the presence of ferric chloride as a catalyst is necessary.

some unchanged acetaldehyde) can be shown in the fermentation of a 10 percent solution of laevulose with 10 percent pressed yeast after three hours. Under the same conditions no glycol or carbinol and at most a trace of acetaldehyde appears to be formed in the glucose fermentation.

By fermenting 10 percent laevulose in LEBEDEW's maceration extract prepared from brewer's yeast, the hydrogen acceptor function of the laevulose according to the strong acetylmethylcarbinol reaction, becomes even more prominent, which is in every respect conceivable, on account of the direct contact of the laevulose in high concentration with the fermentative agent. By means of a special control experiment we convinced ourselves that by using glucose instead of laevulose no production of carbinol or glycol took place.

Thus we see how the general scheme for the fermentative decomposition of sugar, which we have put forward, has led to the fact, that in future the formation of acetylmethylcarbinol or 2 : 3 butylene glycol from laevulose under quite normal conditions of fermentation of this sugar by means of alcohol yeast must be taken into account.

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