THE DISSIMILATION OF ORGANIC ACIDS BY AEROBACTER INDOLOGENES[1]

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In a previous paper by Reynolds and Werkman (1937) evidence was presented indicating that acetic acid plays an important intermediary rôle in the dissimilation of glucose by *Aerobacter indologenes*. It was suggested that the products of the anaerobic dissimilation of intermediately-formed acetic acid are acetylmethylcarbinol and 2,3-butylene glycol. In view of the indirect evidence previously reported and the possible importance of the behavior of acetic acid in the Voges-Proskauer reaction, it appeared desirable to provide direct proof of that suggestion.

METHODS

Fermentations were carried out in 1-liter flasks filled with medium and fitted with delivery tubes for removal of gases. Carbon dioxide was collected in "U" tubes filled with soda-lime, and weighed. Hydrogen was collected over water in graduate cylinders and quantitatively determined in a Hempel explosion pipette.

Other methods are described in a previous paper (1937).

EXPERIMENTAL

Investigation of the dissimilative action of *A. indologenes* on acetic, lactic and succinic acids was undertaken. Preliminary attempts to ferment these acids in the form of their sodium salts in a peptone medium, were unsuccessful. Clouding of the medium and microscopic examination indicated bacterial growth but

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chemical analysis demonstrated a negligible utilization of the acids. None of the media gave a positive acetylmethylcarbinol reaction. In succeeding experiments, dissimilation was obtained in media containing glucose in addition to the organic acids. In the presence of glucose conditions are more nearly those of a normal fermentation, particularly as regards possible hydrogen transfer. To the medium containing 2.0 per cent glucose, 0.1 per cent secondary ammonium phosphate, and 0.5 per cent sodium bicarbonate, was added 0.5 per cent of the acid as its sodium salt. A quantity of the same medium without addition

TABLE 1

*Fermentation of glucose plus an organic acid by Aerobacter indologenes**

ADDITION	INITIAL ACID	CO_2	H_2	FORMIC ACID	ETHYL ALCOHOL	ACETIC ACID	LACTIC ACID	ACETYL METHYL CARBINOL	2, 3-BUTYLENE GLYCOL	SUCCINIC ACID	CARBON RECOVERY	OXIDATION REDUCTION INDEX†
											per cent	
None	0	1.53	0.28	0.28	0.67	0.01	0.03	0.001	0.64	0	97	0.943
Acetic acid	0.86	1.74	0	0.09	0.55	0.50	0.03	0.022	0.88	0	98.6	0.953
Lactic acid	0.57	1.64	0.39	0.27	0.61	0.11	0.62	0.011	0.68	0	103.5	0.970
Succinic acid	0.43	1.49	0.31	0.38	0.65	0.04	0.02	0.008	0.72	0.28	95	0.875

* Quantities expressed as moles per mole of glucose fermented.

† Cf. Erb, Wood and Werkman: Jour. Bact., **31**, 595 (1936); a perfect index = 1.0.

of acid served as the control. Following inoculation, sterile, oxygen-free nitrogen was forced for fifteen minutes through the flasks, which were connected immediately to the gas train. Samples were removed for determination of the initial glucose, carbon dioxide and acid. Final analyses were made after seven days' incubation at 30°C. Typical results are shown in table 1. Fermentation of glucose was complete. Examination of the carbon balance and oxidation-reduction index indicates that the analyses were satisfactory.

The data show that added lactic acid was not attacked. The

increase in lactic acid over the original addition is comparable with the quantity produced by the control fermentation of glucose with no additions.

Comparison of the initial and final quantities of acetic acid in the glucose-acetate medium shows that 0.36 mole of the added acid has disappeared. Hence, a quantity of acetic acid equivalent to 40 per cent of the original addition plus that produced from the glucose was converted to some other product or products. The greatest variations from the control accompanying the utilization of acetic acid are in formic acid, acetylmethylcarbinol, 2,3-butylene glycol and hydrogen. Since the total quantities of 1-, and 3-carbon compounds are practically equal in the control and in the glucose-acetate medium, and the quantity of ethyl alcohol is less in the latter, the disappearance of acetic acid can be explained only on the basis of its conversion to 4-carbon compounds, i.e., 2,3-butylene glycol and acetylmethylcarbinol.

The increase in 2,3-butylene glycol in the acetate medium as compared with the control is 0.24 mole. The decrease in acetic acid calculated as 2,3-butylene glycol is 0.18 mole or equal to 75 per cent of the increase in yield of glycol.

The data show a significantly decreased production of alcohol in the presence of acetate.. Since it is probable that the alcohol arises through the reduction of intermediately-formed acetaldehyde, such a decreased yield should be expected in the presence of a competing hydrogen acceptor, i.e. acetic acid. As with the converted acetic acid, intermediately-formed aldehyde not reduced to alcohol must be accounted for among the 4-carbon compounds. The decrease in ethyl alcohol in the glucose-acetate medium as compared with the control is the equivalent of 0.055 mole of 2,3-butylene glycol and equal to 23 per cent of the increase in yield of glycol. Thus, 98 per cent of the increased yield of glycol in the presence of acetate can be accounted for by the converted acetic acid plus the condensation of intermediate aldehyde normally reduced to alcohol.

That acetic acid can be activated as a hydrogen acceptor by *A. indologenes* is shown by the absence of gaseous hydrogen in the presence of an excess of that acid. The utilization of hydro-

gen arising from the dissimilation of glucose by *A. indologenes* was substantiated by use of the Barcroft-Warburg respirometer. The technique is that described by Dixon (1935) with oxygen-free nitrogen substituted in the flasks for air. The results showed that in the presence of acetate no appreciable quantity of hydrogen may be liberated by *A. indologenes* whereas *Escherichia coli*, used for comparison, behaves otherwise. This is a fundamental difference between the two organisms.

Results obtained with the fermentation of added succinic acid are not as definite as those for acetic acid. About 40 per cent of the initial succinic acid was fermented and its conversion was

TABLE 2

Fermentation of a glucose-acetate medium by Aerobacter indologenes in the presence of hydrogen, and hydrogen and air

TREATMENT	INITIAL ACETIC ACID	FINAL ACETIC ACID	CARBON DIOXIDE	FORMIC ACID	ACETYLMETHYL CARBINOL	ETHYL ALCOHOL	2, 3-BUTYLENE GLYCOL	CARBON RECOVERY	OXIDATION REDUCTION INDEX
								per cent	
Hydrogen.....	1.63	1.36	1.72	0.28	0.010	0.53	0.86	100.1	1.02
Hydrogen and air..	1.63	1.47	1.73	0.14	0.01	0.44	0.82	97.4	1.08

Quantities in moles per mole of glucose fermented.

accompanied by appreciable increases in formic acid and 2,3-butylene glycol. The results indicate that the fermented succinic acid was converted largely to formic acid and 2,3-butylene glycol. Conversion to the glycol probably occurs subsequent to an initial decomposition to acetaldehyde or acetic acid.

Since less than half of the acetic acid initially present was hydrogenated, the reduction appears to have been limited by lack of available hydrogen. The data suggested the possibility of obtaining more complete reduction by providing an excess of hydrogen. To test that possibility the following experiment was arranged. One liter of medium containing 1.0 per cent of acetic acid as the sodium salt with 0.5 per cent Bacto-peptone was

inoculated with *A. indologenes*. Hydrogen was forced through the medium during six days' incubation at 30°C. Two other flasks containing the glucose-inorganic medium as previously described and 1.0 per cent of acetic acid as the sodium salt were inoculated with the same culture. Hydrogen was forced through one of these, and a mixture of equal parts of hydrogen and oxygen through the other during the six days' incubation at 30°C. The flasks were fitted with inlet tubes extending to the bottom. The end of the inlet tube was inserted tightly into a small basswood block. The gases, forced through the block, entered the medium as a fine spray.

The peptone-acetate medium showed good growth but the added acetic acid was entirely recovered unchanged after incubation. In the glucose-acetate medium (table 2), the fermentation of glucose was complete.

Comparison of the data in tables 1 and 2 shows that substantially equivalent quantities of acetic acid were reduced in the absence and in the presence of gaseous hydrogen. The results indicate that under the conditions molecular hydrogen was not activated to serve in the reduction of acetic acid. Apparently a more active form is required. In substantiation we have found by means of the Thunberg technique that molecular hydrogen is not activated by *A. indologenes* with methylene blue as acceptor. In the presence of a mixture of oxygen and hydrogen less acetic acid was reduced and reduction products (ethyl alcohol and 2,3-butylene glycol) were somewhat lower. Although hydrogen could not be determined on these fermentations the oxidation-reduction indexes, being close to 1.0, indicate that no appreciable quantities of hydrogen were liberated. The data in table 2 show that acetic acid is more readily activated as a hydrogen acceptor by *A. indologenes* than is molecular oxygen.

SUMMARY

Aerobacter indologenes dissimilates acetic or succinic but not lactic acid in the presence of glucose and under anaerobic conditions.

Although gaseous hydrogen is a product of the fermentation of

glucose by *A. indologenes*, no appreciable quantities are produced when glucose is fermented in the presence of sufficient acetate. *Escherichia coli* behaves differently.

The evidence indicates that in the case of *A. indologenes* acetic acid is reduced and condensed to 2,3-butylene glycol, probably through acetaldehyde and acetylmethylcarbinol as intermediary stages. Molecular hydrogen is not activated to reduce the acid.

REFERENCES

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