

XCII. CO₂ UTILIZATION DURING THE DISSIMILATION OF GLYCEROL BY THE PROPIONIC ACID BACTERIA¹

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WOOD & WERKMAN [1936] reported that propionic acid bacteria reduced CO₂ (obtained from CaCO₃) during the dissimilation of glycerol. They found that the amount of CO₂ present following fermentation was much less than the amount that had been added as CaCO₃. They also found that the total organic C content of the fermented culture was greater than the total organic C content of the unfermented medium [Wood & Werkman, 1938]. These were the first reports concerning such an unusual metabolic reaction of the propionic acid bacteria. Barker [1936] and van Niel [1937] seemed reluctant to accept, without confirmation, the original report regarding the propionic acid bacteria. However, the later evidence presented by Wood & Werkman [1938] should remove all doubts.

During the course of other work upon the propionic acid bacteria an indication of CO₂ utilization was noted and the more carefully controlled experiments recorded here were performed. In view of the unusual nature of this reaction, the controversial interest accorded the first reports and the desirability, as suggested by Wood & Werkman, of confirmation by other laboratories, it seemed worth while to report these results.

EXPERIMENTAL

Cultures, medium and methods

The cultures were *Propionibacterium pentosaceum*, P₁₁ (van Niel's 4 and Wood & Werkman's 49 W); *P. technicum*, P₁₂ (van Niel's 22); and *P. shermanii*, P₁₉ (Wood & Werkman's 52 W).

The medium contained 20 g. glycerol, 20 g. CaCO₃, 500 ml. yeast water,² and distilled water to make 1 l. Equivalent amounts of sterile CaCO₃ were added to the flasks just before inoculation. A 3% inoculum of washed 48 hr. cells from a glucose-yeast water medium was used. Air in the system was displaced with O₂-free N₂ immediately after inoculation. These fermentations were incubated at 30° for 28 days.

The residual glycerol, following extraction from the culture medium, was determined by the method of Woolley (unpublished) which is a modification of the method of Wagenaar [1911]. CO₂ was determined on both the culture and a sterile control. The CO₂ produced during fermentation and that liberated by

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² The clear extract from 200 g. pressed yeast autoclaved in 1 l. water.

acidification with H₃PO₄ and removed by aeration at the end of the fermentation was absorbed in KOH solution. The residual KOH was titrated after the addition of an excess of BaCl₂. The CO₂ utilized during the fermentation was then equivalent to the CO₂ from the CaCO₃, as determined from the control flask, minus the CO₂ from the fermentation flask. Volatile acids, propionic and acetic, were determined by a modified Duclaux distillation after steam distillation from an acidified (pH 2) aliquot of the culture. The non-volatile acids, lactic and succinic, were removed from the residue of the volatile acid distillation by continuous extraction with ethyl ether. Lactic acid was determined by the method of Friedemann & Graeser [1933] and succinic acid by the following modification of the method of Moyle [1924]. An acidified aliquot of the residue of the volatile acid distillation, containing 10–75 mg. succinic acid, was extracted with ethyl ether for 24 hr. The extract was taken up in 30–40 ml. water containing 1 ml. 95% H₂SO₄, heated and 0.1N KMnO₄ added until a permanent brown precipitate appeared in the boiling solution. This oxidation destroyed the lactic acid. The solution was evaporated to a convenient volume, cooled and extracted with ethyl ether for 24 hr. The extract was taken up in 15–20 ml. water and 5 ml. 10% AgNO₃ added. This solution was adjusted to pH 6.0–6.5 with 0.5N NH₄OH and bromocresol purple (other indicators in this range are unsatisfactory), filtered on a Gooch crucible, and washed with four or five 3 ml. portions of 50% alcohol. The precipitate was dissolved with two 10 ml. portions of hot 1:4 HNO₃, cooled, and titrated with 0.07N KCNS and saturated ferric alum indicator. Two mol. of KCNS are equivalent to one mol. of succinic acid. Recovery of known samples averaged 98–99%.

RESULTS

The data are recorded in Table I. Uniformly the principal course of the reaction has been the production of propionic and succinic acids accompanied by small amounts of acetic and lactic acids. In each fermentation there has been

Table I. *Glycerol dissimilation by the propionic acid bacteria*

Culture No.	P ₁₁	P ₁₁	P ₁₂	P ₁₂	P ₁₉
Glycerol fermented mM. per l.	205.3	212.8	183.9	186.5	92.5
CO ₂ utilized per 100 mM. fermented glycerol	21.40	25.6	9.22	14.36	25.41
Products per 100 mM. fermented glycerol					
Propionic acid	75.50	70.00	87.14	90.07	66.26
Acetic acid	2.92	8.75	0.92	0.99	5.84
Succinic acid	23.60	23.78	9.90	13.21	25.48
Lactic acid	—	—	0.27	0.99	0.70
C recovery (%)					
Basis, glycerol plus CO ₂	101.8	99.2	98.2	104.2	96.7
Basis, glycerol	108.9	107.5	101.2	109.3	105.0
Oxidation-reduction index					
Basis, glycerol plus CO ₂	1.04	1.02	1.04	0.95	1.05
Basis, glycerol	1.64	1.77	1.26	1.26	1.89

a definite utilization of CO₂, a fact which may be demonstrated in several ways. The strongest evidence appears in the CO₂ determinations. In every case the total CO₂ remaining in the flask and absorber following fermentation is less than the amount of CO₂ originally added as CaCO₃. In the fermentation showing the smallest CO₂ utilization the observed difference in CO₂ concentrations is some 25 times the experimental error; in other fermentations the observed CO₂ uptake is well over 100 times the experimental error.

Supplementary evidence is offered by the C recoveries and the oxidation-reduction indices. When the C recoveries are based upon glycerol alone they are uniformly high, for the C content of the products is greater than the C content of the glycerol fermented. However, when the C recoveries are based upon glycerol plus CO₂ the values approach 100%. The oxidation-reduction indices indicate a large excess of oxidized products when calculated upon the basis of glycerol alone. However, if the utilized CO₂, an oxidized compound, is entered in these calculations, the oxidation-reduction indices approach the ideal value of 1.00.

In each of these fermentations, as in most of those reported by Wood & Werkman, the succinic acid is, on a molar basis, approximately equal to the CO₂ utilized.

SUMMARY

The report of Wood & Werkman that the propionic acid bacteria utilize CO₂ during the dissimilation of glycerol has been confirmed.

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