CLXVIII. THE UTILIZATION OF CO₂ BY THE PROPIONIC ACID BACTERIA¹

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THE utilization of CO₂ by heterotrophic bacteria was first reported by Wood & Werkman [1936, 1] in the case of the fermentation of glycerol by the propionic acid bacteria. Barker [1936] and van Niel [1937] have questioned the evidence for this physiologically important phenomenon. Van Niel states, "Wood and Werkman claim that carbon dioxide is reduced during the fermentation of glycerol by propionic acid bacteria. The published results cannot, however, be considered conclusive, although the data do seem to favor their claim." According to previous concepts of the physiology of heterotrophic, non-photo-synthetic bacteria the utilization of CO₂ by the propionic acid bacteria appears unlikely; thus confirmation by other workers is desirable. Neither Barker nor van Niel state the respect in which they consider our data inconclusive. It should be clearly recognized that although theories advanced concerning the mechanism of CO₂ assimilation are necessarily speculative and analyses of the fermentations will show experimental variation, proof of CO₂ utilization does not depend on these points. The proof is simply that a given quantity of CO₂ in the form of CaCO₃ was added to a medium which was fermented and analysed. Less CO₂ was accounted for than was originally present in the medium. Moreover, the recovery of the CO₂ from the CaCO₃ in an unfermented control medium was quantitative. The fact that the C recovery and oxidation-reduction balance were satisfactory when CO₂ utilization was assumed but were not when the principle of CO₂ utilization was not employed, was considered further evidence substantiating the occurrence of the phenomenon.

The purpose of the present investigation was to obtain additional information concerning the utilization of CO₂ in the dissimilation of glycerol by propionic acid bacteria. It is hoped that these studies will ultimately lead to an elucidation of the mechanism of this utilization.

METHODS

Two procedures have been used involving (1) serial analysis of the fermentation and (2) single analysis at the conclusion of the fermentation. The medium consisted of glycerol 2.5%, Difco yeast extract 0.4% and NaHCO₃ 1.5%. Each constituent was sterilized separately. The NaHCO₃ solution was made acid to phenolphthalein by saturation with CO₂ before adding it to the medium. Incubation was at 30°. The inocula were suspensions of cells in distilled water, obtained from glycerol yeast extract medium, used in a concentration of 3% by volume. During incubation the medium was agitated by shaking once each day.

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The apparatus used for the serial analysis is shown in Fig. 1. The fermentation was conducted under an atmosphere of CO₂ in a 2 l. Erlenmeyer flask (*A*) with 1900 ml. medium. The rubber stopper held an inlet (*B*) for CO₂-saturation of the medium, an outlet (*C*) leading to the absorption train (*D*), a syphon (*E*) for sampling, one connexion leading to a Hempel gas burette (*F*) and reservoir (*G*),

Fig. 1. Fermentation apparatus used in serial experiments.

a second connexion to a U-tube manometer (*H*) which was in turn connected to a closed flask (*I*) containing CO₂ and water to keep the gas saturated. The pressure in the fermentation flask was balanced against that of reference flask *I* by adjustment of the reservoir (*G*) before reading the volume in the burette and removing a sample. It is convenient to use a reference flask since the apparatus is then not affected by changes in temperature or pressure. There was a liberation of CO₂ during most of the fermentation, in spite of its utilization, owing to the reaction of the NaHCO₃ with acids formed by fermentation. This CO₂ was collected in 30% KOH in two Bowen bulbs in the drying train (*D*) and determined by weight. Hg was placed in the bottom of the first KOH bulb to cover the tip of the inlet tube to act as a trap between the alkali and CO₂ and prevent the alkali from being pulled back into the gas train. Only after the NaHCO₃ had completely reacted was the utilization of gaseous CO₂ apparent. At this time a partial vacuum was created in the fermentation flask and the gas utilized was measured in the burette.

All samples were removed into a known volume of alkali to prevent loss of dissolved CO_2 . During removal of the sample, CO_2 was simultaneously introduced into the flask, thus preventing change in the equilibrium of free and dissolved gas. The liquor was thoroughly agitated before sampling to facilitate gaseous equilibrium. The original glycerol and CO_2 in the medium, i.e. dissolved CO_2 plus CO_2 bound as NaHCO_3 were determined on a sample removed immediately after the medium was inoculated and saturated with CO_2 .

The principles of the apparatus are described in more detail by Dixon [1934] and Wood *et al.* [1936]. No rubber tubing was used except at glass to glass joints where flexibility was necessary. Burette and manometer were filled with Hg.

In the second procedure when only a single analysis of the fermented medium was made, the apparatus shown in Fig. 2 was used. All connexions were sealed by Hg. The fermentation flask was a 300 ml. Erlenmeyer with a tube fitted by a

Fig. 2. Fermentation apparatus used in single analysis experiments.

ring seal into the neck. 250 ml. of medium were used in each flask. The incubation time was 30 days. Anaerobiosis was assured by passing O_2 - and CO_2 -free N_2 through the medium before the addition of the NaHCO_3 . After this addition the medium was again flushed with N_2 for about 5 min.; longer treatment caused the medium to become alkaline owing to the removal of CO_2 . The original CO_2 and glycerol in the NaHCO_3 and glycerol solutions were determined before addition to the medium. During the fermentation a slight vacuum was maintained in the apparatus (Fig. 2) thus preventing the development of pressure sufficient to break the Hg seals. After completion of fermentation, the CO_2 was flushed from the medium for 3 hr. with N_2 . Because of difficulty of including a drying train in the apparatus (Fig. 2), the CO_2 collected in the KOH was liberated and reabsorbed in a suitable train for determination by wt., adding excess H_2SO_4 (blue to Congo red), boiling the whole under a reflux condenser and aerating. A correction was made for the small amount of CO_2 contained originally in the 30% KOH.

Other quantitative determinations in both procedures were made as follows. The CO_2 (dissolved CO_2 and NaHCO_3) of the fermented medium was determined by wt. on an aliquot part of the sample. The volatile acids and propyl alcohol were determined on the same fraction after removal of the CO_2 . The alcohol was concentrated and separated from the volatile acids by distillation of one-half of the volume of the liquor and redistillation after neutralization. The alcohol was determined by oxidation with dichromate and analysis of the resulting acids (unpublished method). The volatile acids were obtained from the acidified

combined residues of the two alcohol distillations by steam distillation and were determined by the partition method of Osburn *et al.* [1933].

Succinic acid was extracted with ether from an aliquot part of the sample acidified with H₂SO₄ and determined gravimetrically as the Ag salt after removal of the volatile acids from the extract by steam distillation. The titratable acid in the distilled extract was equivalent to the succinic acid obtained in the Ag salt, also the Ag content of the salt was in agreement with the theoretical value. A separate aliquot part of the unheated liquor was extracted because apparently the glycerol and succinic acid react during the distillation. Previous results reported by Wood & Werkman [1936, 1] may show some error in the succinic acid since the determinations were made on extracts obtained from the residues of steam distillations which were taken up in Na₂SO₄. Recovery of succinic acid by this method is low when glycerol is present but complete when absent.

The unfermented glycerol was determined in an aliquot part of the medium by oxidation with periodic acid (unpublished method). This oxidation is specific for polyhydroxy and related compounds. The medium in the absence of glycerol gives a negligible determination and none of the products reacts. Pyruvic and lactic acids were not found at any time during the fermentation and there was no reduction of Fehling's solution by the fermentation liquor after hydrolysis with acid. Total C determinations were made by the method of Osburn & Werkman [1932] on the acidified medium from which residual CO₂ was removed. All analyses were made on the unfermented medium and the small blank values thus obtained were subtracted from the determinations made on the fermented medium.

EXPERIMENTAL RESULTS

Results of serial analysis of the fermentation by *Propionibacterium pentosaceum* are presented in Tables I and II. The data in Table I are calculated on the basis of 1 l. medium for the period t_0 to the time of removal of the respective samples. The results in Table II are calculated on the basis of the fermentation occurring during the time between samplings and per 100 mM. glycerol fermented. Data obtained by single analyses of the fermentation are given in Table III.

The accuracy of the data can be judged from the C recovery and oxidation-reduction balance; the former (Table I) is low during the early period but increases as the fermentation proceeds. On the basis of the glycerol fermented and CO₂ utilized the C unaccounted for is substantially constant after the 9th day as shown in Table I. The maximum cell development is reached on the 7th or 8th day and remains quite constant thereafter. This simultaneous occurrence

Table I. *Serial analysis of the dissimilation of glycerol by Propionibacterium pentosaceum (49 W)*

Time days	Glycerol fermented per l. mM.	CO ₂ utilized per l. mM.	Products per l.				C un- account- ed for per l. mM.	C recovery %	Redox- index O/R
			Succinic acid mM.	Propionic acid mM.	Acetic acid mM.	Propyl alcohol mM.			
4	36.55	5.34	4.98	24.9	1.49	1.42	13.0	88.7	1.04
6	66.26	10.53	10.20	43.8	2.46	3.84	20.5	90.2	1.01
9	114.40	23.90	23.10	73.8	3.56	6.61	26.4	92.8	0.97
12.5	160.90	41.80	41.00	101.9	4.31	6.64	26.2	95.0	0.98
19.5	191.60	53.60	51.60	120.1	4.16	7.66	30.1	95.2	0.97
28.5	222.30	70.50	68.60	136.7	8.18	4.66	22.1	97.0	1.00
46.5	275.20	103.80	99.80	159.0	5.94	4.69	26.9	97.1	0.99

Table II. *Serial analysis of the dissimilation of glycerol by Propionibacterium pentosaceum (49 W)*

Interval days	Glycerol fermented per l. m.M.	CO ₂ utilized per 100 m.M. glycerol m.M.	Products per 100 m.M. glycerol				C recovery %	Redox-index O/R
			Succinic acid m.M.	Propionic acid m.M.	Acetic acid m.M.	Propyl alcohol m.M.		
0-4	36.55	14.6	13.6	68.3	4.08	3.87	88.7	1.04
4-6	29.71	17.5	17.6	63.6	3.26	8.15	92.0	0.96
6-9	48.10	27.8	26.7	62.2	2.29	5.76	96.2	0.94
9-12.5	46.50	38.3	38.6	60.5	1.61	0.06	100.2	1.01
12.5-19.5	30.60	38.5	34.7	59.4	-0.49	3.34	96.3	0.92
19.5-28.5	30.70	55.5	55.2	54.1	13.09	-9.77	106.4	1.12
28.5-46.5	52.90	62.9	59.0	42.1	-4.23	0.06	97.7	0.95

Table III. *Dissimilation of glycerol by the propionic acid bacteria.**

Culture*	Glycerol fermented per l. m.M.	CO ₂ utilized per 100 m.M. glycerol m.M.	Products per 100 m.M. glycerol				C recovery %	Redox-index O/R
			Succinic acid m.M.	Propionic acid m.M.	Acetic acid m.M.	Propyl alcohol m.M.		
11 W	214.3	12.6	16.6	80.00	3.97	1.42	101.9	1.06
34 W	212.5	10.4	15.0	76.40	3.30	3.32	98.4	1.08
35 W	212.3	13.5	16.9	77.10	2.74	2.77	99.7	1.04
49 W	211.5	17.2	20.2	76.60	1.38	1.95	100.7	1.03
52 W	206.7	21.1	25.1	74.19	2.08	0.19	102.0	1.07

* Culture numbers correspond to the following species: 11 W, *P. peterssonii*; 34 W, *P. arabinosum*; 35 W, *P. zeae*; 49 W, *P. pentosaceum* and 52 W, *P. shermanii*.

of cell development and low C recovery suggests that for the most part the C unaccounted for occurs as cell substance. The redox-index is satisfactory in almost every case in Table I. There is more variation in the balances shown in Table II than in those of Table I which is the result of the small values involved in the calculations of the data of Table II. The largest discrepancy is in the period between 19.5 and 28.5 days. When the data are considered as a whole and the C in cell material is taken into account the results are acceptable. The redox-indexes in Table III show some variations from 1.0; however, a small error in the analysis can cause such discrepancies.

A large utilization of CO₂, which started with initial fermentation and increased thereafter, is shown by Tables I and II. Since Barker and van Niel have previously questioned the validity of similar evidence of CO₂ utilization it seemed advisable to obtain further proof. The organic C in the medium should increase by an amount equivalent to the CO₂ utilized, since there is no loss of C as gaseous products. This relationship has been established by total C determinations on the medium before and after fermentation. The C determinations were made on the medium after bound CO₂ (NaHCO₃ and dissolved CO₂) had been removed. Aliquot parts of the original sample (unfermented) and the final sample (fermented for 46.5 days) equivalent to exactly 3 ml. undiluted medium were used in the determinations. The K₂S₂O₈ oxidation gave 0.1235 g. CO₂ per 3 ml. in the unfermented medium and 0.1355 g. CO₂ in the fermented medium, an increase of 0.0120 g. Experimentally 103.8 m.M. of CO₂ per l. were utilized (Table I, 46.5 days) or 0.0137 g. CO₂ per 3 ml. The fact that the CO₂ utilized, as determined experimentally, agrees closely with the increase in organic C, substantiates the utilization of CO₂ and is strong evidence for its conversion into organic compounds.

Further evidence of CO₂ utilization was shown by the formation of a partial vacuum in the fermentation flask in the interval between 12.5 and 19.5 days. During this time the NaHCO₃ in the medium was entirely decomposed and the utilization was no longer masked by evolution of CO₂; 123 ml. CO₂ at N.T.P. were utilized per l. medium.¹

Propyl alcohol was a product of the fermentation of glycerol in the present investigation. The authors have not found previous mention of the formation of this compound by propionic acid bacteria. Its isolation and identification will be described elsewhere. The alcohol obtained in the present investigation yielded after oxidation with dichromate a mixture of propionic and acetic acid in exactly the same proportion as from authentic propyl alcohol.

DISCUSSION

The experimental results show clearly that substantial quantities of CO₂ are utilized by the propionic acid bacteria in the dissimilation of glycerol. It is of primary interest to obtain an insight into the mechanism of CO₂ utilization and to determine whether the phenomenon occurs in other biological processes.

The results in Tables I, II and III bring out one point which may give an indication of the mechanism of CO₂ utilization. The *mM.* of CO₂ utilized and succinic acid formed show a close relationship in all the fermentations. This approximate equivalence of the succinic acid and CO₂ suggests that the former may originate by union of CO₂ with some 3-C compound formed from the glycerol. In this connexion the possibility must be considered of the formation of CO₂ from glycerol and its utilization together with that from the NaHCO₃. Since a 2-C compound (acetic acid) is formed from a 3-C substrate (glycerol), it is only reasonable to expect the formation of an equivalent concentration of a 1-C compound (CO₂). In this case the total CO₂ utilized will be equal to the sum of the CO₂ obtained from the NaHCO₃ (3rd column, Tables I-III) and that originating from glycerol with the acetic acid. Comparison of this sum (Table III) with the values obtained for succinic acid (16.6-16.6, 13.7-15.0, 16.3-16.9, 18.6-20.2, 23.2-25.1) discloses that the agreement is within limits of experimental error. The results shown in Table I² are not in complete agreement with this explanation. If to the CO₂ utilized from the NaHCO₃ a quantity is added equivalent to the observed acetic acid, then there is obtained a significant excess of total CO₂ over the succinic acid. It is probable that the results in Table III are more reliable than those in Table I inasmuch as the latter were obtained by a more complex procedure. However, the redox-indexes are more satisfactory in Table I. It is not deemed advisable to propose reactions to explain these small variations until additional evidence is available. Possibly part of the succinic acid was broken down to acetic acid without evolution of CO₂. There is, however, a general correlation of CO₂ utilization with succinic acid formation suggesting a synthesis involving CO₂ and succinic acid. Results reported in a previous paper [Wood & Werkman, 1936, 1] do not show an equimolar formation of CO₂ and succinic acid. However, as previously indicated, the method used in the determination of succinic acid was not accurate in all cases. For this reason present experiments are a better criterion of the CO₂-succinic acid relationship.

¹ The pH of the medium was 5.5 on the 19.5 day. Sufficient alkali was therefore added to buffer the medium throughout the remainder of the fermentation and it was again saturated with CO₂. A sample was then removed for determination of bound CO₂.

² Table II is not considered here because the method of calculation multiplies the experimental error.

During the preparation of this manuscript the publication of Elsdén [1938] appeared, showing that the rate of succinic acid formation by washed suspensions of *Bacterium coli commune* from sodium pyruvate, glucose and galactose is a function of the concentration of CO_2 in the medium. No direct evidence was obtained for fixation of CO_2 . It was suggested that possibly CO_2 is involved in the synthesis of succinic acid but the facts were too few to permit theorizing on the role of CO_2 in succinic acid formation. In the view of present results which show that succinic acid, a 4-C compound, is formed from the 3-C compound glycerol in amounts approximately equivalent to the CO_2 utilized, the definite possibility that succinic acid may be formed by synthesis from 3-C and 1-C compounds is evident. In the light of this suggestion an explanation is available for the results obtained by Virtanen [1925] and Virtanen & Karström [1931] who found succinic and acetic acids in equimolar quantities as the only products from glucose using preparations of propionic acid bacteria in the presence of toluene. These results could not be explained logically on the basis of a 3-C cleavage, i.e.

since CO_2 utilization was not considered at that time. The formation of succinic and acetic acids by a 4- and 2-C cleavage was therefore proposed to account for the formation of succinic and acetic acids unaccompanied by a 1-C compound. It is quite evident that Virtanen & Karström's results are precisely those to be expected in view of the present proposal, i.e. that one hexose yields two 3-C compounds; one is converted to acetic acid and CO_2 and the other unites with CO_2 or some derivative of CO_2 to give succinic acid.¹ Other investigators, Virtanen [1928], Scheffer [1928], Braak [1928] and Kluyver [1931], have applied the idea of 4- and 2-C cleavage to bacterial fermentations primarily because it offered an explanation for the shortage of 1-C compounds.

It is of interest that Butkewitsch & Gaewskaya [1935] and Wells *et al.* [1936] claim that the formation of citric acid from sugar by moulds cannot proceed by synthesis from 2-C compounds which originate from 3-C compounds. This view is maintained since they obtained more than the theoretical yield of citric acid, i.e. if 1-C compounds are not utilized. Gudlet & Makarowa [1935] proposed a 4- and 2-C cleavage of glucose with a subsequent synthesis as a possible mechanism in the formation of citric acid. Previously Bernhauer & Slanina [1934] showed that fungi can synthesize oxalic acid from formic acid and pointed out that there is no arbitrary limit to the quantity of citric acid which may be formed from glucose if synthesis occurs. The merits of the different schemes of citric acid formation are not within the scope of this investigation but it should be emphasized that it is not possible to limit the quantity of citric acid which can be formed from glucose since CO_2 utilization may also occur in mould metabolism. Woods [1936] has shown that *Escherichia coli* can reduce CO_2 to formic acid. This conversion does not involve a C to C linkage but does show that CO_2 can be activated by *Esch. coli*. Wieringa [1936] claims to have isolated an anaerobic spore-forming bacterium which almost quantitatively forms acetic acid from CO_2 and H_2 . The methods and data necessary to support the claim are not presented in his brief report but his discovery may be another example of the chemosynthetic activity of heterotrophic bacteria. If the propionic acid bacteria, and the anaerobic sporulating bacterium of Wieringa are oligocarbophilous there

¹ There is one inconsistency in Virtanen & Karström's results. They obtained an oxidized product, succinic acid, with no corresponding reduced product, acetic acid being neither oxidized nor reduced.

is no reason *a priori* to assume that moulds and other tissues are not similar. There is no direct proof of the occurrence of a 4- and 2-C cleavage in biological reactions and many of the past concepts of fungous and bacterial metabolism may have to be re-evaluated on the basis of CO₂ utilization. Krebs & Johnson [1937] have recently shown that citric acid is synthesized by avian tissue from oxaloacetic acid and some unknown compound. It is possible that this synthesis involves utilization of CO₂.

Previously Wood & Werkman [1936, 1, 2] and Wood *et al.* [1937] have shown that certain glucose fermentations by propionic acid bacteria yield relatively large quantities of succinic acid and CO₂ and a small quantity of acetic acid. These results are readily adaptable to a scheme of fermentation involving the formation of succinic acid from acetic acid. On the contrary, if the succinic acid is assumed to be formed by union of CO₂ (or some other 1-C compound) and a 3-C compound, it is difficult to explain the origin of the large quantities of CO₂ in comparison with the small amount of acetic acid. Two mechanisms have previously been proposed for the formation of CO₂; one by the breakdown of pyruvic acid to acetic acid and CO₂; the other by decarboxylation of succinic acid to propionic acid and CO₂. If these reactions were the only sources of CO₂, and succinic acid was formed by union of CO₂ and a 3-C compound, the CO₂ would of necessity be less than the acetic acid provided that the latter was not broken down. If succinic acid formation occurs in glucose dissimilation solely by CO₂ synthesis it is probable that there is a mechanism of CO₂ formation which has not been considered as yet. Stone *et al.* [1936] and Wood *et al.* [1937] have shown that acetic acid is activated and dissimilated by propionic acid bacteria. In fact the results in Table I indicate there may have been some breakdown of acetic acid between 28.5 and 46.5 days. Aerobically, resting cells of propionic acid bacteria can attack acetic acid slowly (unpublished results). At present it seems probable that there may be two mechanisms of succinic acid formation in glucose dissimilation: one by CO₂ synthesis and another by synthesis involving acetic acid [Wood & Werkman, 1936, 2].

Although the present observations showing the equivalence of CO₂ and succinic acid indicate that the latter is formed predominantly by CO₂ synthesis in glycerol fermentation, other interpretations can be given. The formation of the oxidized product, succinic acid, must be accompanied by a simultaneous reduction. Since the utilization of CO₂ involves reduction, the relationship of CO₂ and succinic acid may arise in the establishment of the oxido-reduction balance.

The close connexion of CO₂ to succinic acid formation has also been shown by fermentation of glycerol under N₂ with phosphate as a buffer. Numerous experiments have been conducted but in no case have substantial quantities of succinic acid been found as a product under these conditions, i.e. in the absence of CO₂. Propionic, acetic, pyruvic and lactic acids and propyl alcohol are formed with no gas. These experiments indicate that CO₂ is necessary for the formation of succinic acid and are in complete agreement with the investigations of Elsdon [1938].

The relative change in the course of the fermentation with time as shown in Table II is of interest. On the basis of the glycerol fermented, the CO₂ utilized and succinic acid formed during each interval progressively increased whereas the propionic acid decreased. Comparing the first with the final analysis, the m*M.* CO₂ utilized increased from 14.6 to 62.9 and the succinic acid from 13.6 to 59.2 m*M.* while the propionic acid decreased from 68.3 to 42.1, per 100 m*M.* glycerol. The cause of this change is not clear but it illustrates how large the utilization of CO₂ can be and also the relative changes in the other products.

The CO_2 amounted to 17.3 % of the total C converted, on the basis of the glycerol fermented and CO_2 utilized between 28.5 and 46.5 days.

The complete mechanism of the fermentation of glycerol by propionic acid bacteria is not clear. The role of phosphate esters in this fermentation has not been investigated extensively. Werkman *et al.* [1937] have shown that the fermentations of glycerophosphoric and phosphoglyceric acids are inhibited by 0.02 *M* NaF. On the contrary, the fermentation of glycerol with proliferating cells proceeds in this concentration of fluoride (unpublished results). Apparently glycerophosphoric acid and phosphoglyceric acid are not necessary intermediary compounds. Wiggert & Werkman [1938] have recently shown that propionic acid bacteria grown in the presence of fluoride do not have an enzyme system for the breakdown of phosphoglyceric acid, whereas in the absence of fluoride this enzyme system develops. Perhaps this also occurs in the fermentation of glycerol, in which case, in the absence of fluoride, phosphoglyceric acid and glycerophosphoric acid may be intermediates. Wood & Werkman [1938] have shown that glycerophosphoric acid is fermented to the same final products as glycerol and, on this basis, they could not exclude the ester as an intermediate of glycerol fermentation. Whether or not CO_2 uptake is connected with phosphorylation is not known at present. Pyruvic acid probably occurs as an intermediate since it may be isolated from glycerol fermentations by sulphite fixation [Wood *et al.* 1937]. This compound may be the point of entrance for CO_2 . Apparently propyl alcohol can be dissimilated by propionic acid bacteria. There was a decrease in the actual amount of propyl alcohol at the conclusion of the fermentation (Table I). Propyl alcohol is probably formed by reduction of propaldehyde which has been isolated from glycerol fermentation by fixation with sulphite and dimedon [Wood & Werkman, 1934].

Recently Fromageot & Bost [1938] have reported that the succinic acid which they obtained in propionic fermentation probably originated from compounds of the yeast extract. The CO_2 was not determined in their experiments but was calculated from arbitrary equations. Wood & Werkman [1936, 2] have shown that similar equations are not completely adaptable to the propionic acid fermentation of glucose. The excessive C recoveries (based on calculated CO_2 values) obtained by Fromageot & Bost are not a strictly reliable indication that products are being formed from the yeast extract, inasmuch as no consideration was given to the possibility of CO_2 utilization. It is probable that small quantities of different products are formed from the yeast extract; however, there is no evidence that these products are confined to succinic acid. In our experiments in which Difco yeast extract (0.4 %) has been used as the source of N there has been no evidence that the quantities of products formed from the yeast extract are significant when compared with a fermentation of 2.5 % substrate.

SUMMARY

Previous experiments showing utilization of CO_2 by propionic acid bacteria fermenting glycerol have been confirmed and extended. The C of the utilized CO_2 has been found in the products, which are succinic, propionic and acetic acids and propyl alcohol.

Total C determinations before and after fermentation show that there is an increase in the total organic C of the medium equivalent to the decrease in inorganic C (CO_2).

The uptake of gaseous CO_2 was sufficient to create a partial vacuum in the apparatus.

The succinic acid formed and CO₂ utilized are approximately equimolar. It is suggested that the formation of succinic acid is by synthesis from a 3-C compound through addition of CO₂.

In the absence of CO₂ little or no succinic acid is formed.

The relative proportions of the products varied with time and the CO₂ utilized. The succinic acid formed increased while the propionic acid decreased.

Utilization of CO₂ by heterotrophic bacteria is considered in connexion with other reactions proposed for the formation of succinic and citric acids.

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