The Methane Fermentation of Carbohydrates[1,2]

BY G. E. SYMONS WITH A. M. BUSWELL

The effect of microbial action on carbohydrates has furnished a profitable field of study for biochemists and bacteriologists since the time of Pasteur. However, investigations of the methane fermentation of carbohydrates and similar substances have been few and scattered over the period since 1875. Reviews and summaries of the literature on carbohydrate fermentation[3-11] make no mention of the formation of methane except in a few instances where the classical work of Popoff,[12] Hoppe-Seyler[13,14] and Omelianski[15-18] are cited. For carbohydrates (other than cellulose) and related substances, there is little information on which gas ratios have been or may be calculated. Complete quantitative data have not been presented by previous investigators.[12-22] More recent studies on the methane fermentation include those on cellulosic materials,[23-26] various food materials,[27] saccharose[28] and fatty acids.[22,29] A comparison of the high yields of gas obtained in two of the latter investigations[25,29] (90-100% of the substrate fed) with the oft-quoted data of Omelianski[17] and Hoppe-Seyler,[13] who

(1) An abstract of a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry in the Graduate School of the University of Illinois, 1932.

(2) Presented before the Division of Biological Chemistry at the 84th meeting of the American Chemical Society, Denver, Colorado, August 22-26, 1932.

(3) Fulmer and Werkman, "Chemical Action of Microörganisms," Chas. C. Thomas, Springfield, Ill., 1930.

(4) Kendall, "Newer Knowledge of Bacteriology and Immunology," University of Chicago Press, Chicago, Ill., 1928, p. 227.

(5) Koser, ibid., p. 243.

(6) Hall, ibid., p. 198.

(7) Buchanan and Fulmer, "Physiology and Biochemistry of Bacteria," The Williams and Wilkins Co., Baltimore, Md., Vol. II, 1930.

(8) Buchanan and Fulmer, ibid., Vol. III.

(9) Thaysen and Bunker, "Microbiology of Cellulose, Hemicellulose. Pectins and Gums." Oxford University Press, 1927.

(10) Smyth and Obold, "Industrial Microbiology," Williams and Wilkins Co., Baltimore, Md., 1930.

(11) Stevenson, "Bacterial Metabolism," Longmans, Green and Co., New York, 1930.

(12) Popoff, Arch. ges. Physiol. (Pflügers), 10, 113 (1875).

(13) Hoppe-Seyler, Z. physiol. Chem., 10, 401 (1886).

(14) Hoppe-Seyler, ibid., 13, 66 (1889).

(15) Omelianski, Compt. rend., 125, 1131 (1887).

(16) Omelianski, Centr. Bakt. Parasitenk., 8, 353 (1902).

(17) Omelianski, ibid., 11, 178 (1904).

(18) Omelianski, ibid., 15, 673 (1904).

(19) Baginski, Z. physiol. Chem., 12, 434 (1888).

(20) Mazé, Compt. rend. soc. biol., 78, 398 (1915).

(21) Groenewege, Med. Geneeskindig. Lab. te Weltevreden, 3, 66 (1920).

(22) Buswell and Neave, Illinois State Water Survey Bulletin, No. 30, 1930.

(23) Heukelekian, Ind. Eng. Chem., 19, 928 (1927).

(24) Boruff and Buswell, ibid., 21, 1181 (1929).

(25) Buswell and Boruff, Sewage Works J., 4, 454 (1932).

(26) Boruff, Ph.D. Thesis, University of Illinois, 1931.

(27) Bach and Sierp, Centr. Bakt. Parasitenk., Abt. II, 62, 24 (1924).

(28) Coolhaas, ibid., 75, 161 (1928).

(29) Neave and Buswell, THIS JOURNAL, 52, 3208 (1930).

reported only 50% yield as gas, shows the effect of recent improvement in technique.

The mechanism of the methane fermentation seems to have occasioned less interest and speculation than the study of its other phases. Hoppe-Seyler[18] concluded from his fermentation of cellulose that one molecule of water entered the reaction to give three molecules each of carbon dioxide and methane. Omelianski[16] obtained different gas ratios and an abundance of fatty acids but offered no explanation for the disagreement with the former's data.

Later work indicates with more certainty the role of water in the reaction. Söhngen[30] shows water entering into the reaction for different fatty acid salts but its significance was apparently not evident to him. Mazé's[20] reaction for acetone and Coolhaas'[28] reaction for saccharose both show that water is necessary. Boruff and Buswell[24,30] presented data on gas recoveries that definitely showed the reaction for cellulose to involve the addition of water. Neave and Buswell[29] also were able to show definitely that water was the oxidizing agent in the anaerobic fermentation of the fatty acids and that the number of water molecules necessary for the reaction bore a simple relation to the number of carbon atoms in the acid.

The source of the methane itself has generally been considered to be the reaction[21,27,30,31,32]

$$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O \qquad (1)$$
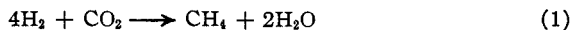
Acceptance of this idea followed the work of Söhngen,[30] who obtained practically quantitative yields according to the equation when he fed hydrogen and carbon dioxide to his cultures. Fischer, Lieske and Winzer[32] confirmed this work and showed that acetic acid was an intermediate in the reaction, and that methane was formed only in the presence of a colloidal surface (FeS). The methane fermentation belongs to the fifth class of anaerobic oxidative mechanisms as listed by Buswell and Neave.[22]

Consideration of past, and particularly of more recent, data,[22,26,33,34] together with those obtained in this investigation, indicates a very general reaction occurring in all methane fermentations. Briefly stated, the scheme proposed is an oxidation–reduction reaction involving the addition of water and decarboxylation, whereby acids, carbon dioxide and hydrogen are formed, the hydrogen combining with some of the carbon dioxide to form methane according to Eq. 1. In the course of this reaction, cellobiose, dextrose, succinic, butyric, propionic, lactic, pyruvic, acetic and formic acids may be formed as intermediates. Thus there are but two final end-products, carbon dioxide and methane, except for a small amount of hy-

(30) Söhngen, Proefschuft, Delft, 1906.
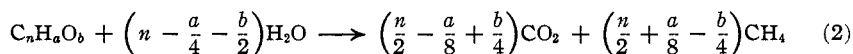(31) Imhoff, *Eng. News Rec.*, **91**, 512 (1923).
(32) Fischer, Lieske and Winzer, *Biochem. Z.*, **245**, 2 (1932).
(33) Pringsheim, *Z. physiol. Chem.*, **78**, 266 (1912).
(34) Neuberg and Cohn, *Biochem. Z.*, **139**, 527 (1923).

drogen which may be due to the incompletion of the reaction shown in Eq. 1 or to factors not yet understood.

For compounds of carbon, hydrogen and oxygen the general reaction has been shown to follow this simple equation

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \longrightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 \quad (2)$$

where C, H and O have their usual chemical significance and the subscripts refer to the number of the respective atoms. This equation has been derived in two ways. If water, carbon dioxide and methane are the only substances involved in the reaction besides the substrate, then their coefficients may be determined algebraically in terms of the number of atoms in the substrate molecule. The equation may also be developed in a stepwise manner, the first step being an oxidation–reduction by water to give carbon dioxide and hydrogen, and the second a combination of the hydrogen with part of the carbon dioxide to form methane and water; a summation gives Eq. 2.

Such an equation gives a simple method for writing the balanced reaction for that portion of substance which is converted completely into methane and carbon dioxide. This equation not only defines the gas ratios but also the weight of the gases produced. That small portion of the substrate not completely gasified, but which may collect as intermediate products, end-products in side reactions and as cell substance, affects the gas ratio in so far as these products differ in composition from the original substrate. They also affect the yield in proportion to the quantity accumulating during the fermentation.

Thermodynamic Considerations.—Acquisition of energy being the motivating force in bacterial degradation of organic matter some treatment of the energetics concerned in these reactions is of interest at this point. It has been shown that the use of heats of formation and heats of combustion do not give true information concerning the energy of a reaction and that the proper criterion is the free energy decrease.[8,35] It has further been shown that the use of free energies at standard state is not the true criterion because of the change in free energy with concentration and temperature.[36] Various other factors (not easily determinable) also enter the consideration and make the calculation of the true free energy decrease practically impossible. However, the free energy decrease in the reaction for all of those compounds for which the free energy of formation is known has been calculated at standard state. In all cases, the reaction is indicated to give off energy (*i. e.*, ΔF is negative).

If water is the only source of oxygen besides that in the compound undergoing fermentation, the greatest decrease in free energy occurs when carbon

(35) Wilson and Peterson, *Chem. Rev.*, **8**, 427 (1931).
(36) Fulmer and Liefson, *Iowa State J. Sci.*, **2**, 159 (1928).

dioxide and methane are the end products. In considering that the reaction is a stepwise breakdown, as suggested above, it appears that most of the intermediate steps (for which free energy data are available) take place with a decrease in free energy. The step involving pyruvic acid appears to require energy but this reaction may proceed with a decrease in free energy at the concentrations actually existing in the bacterial system.[35] It has also been suggested[37] that bacteria may do work on an intermediate reaction if they can obtain energy from the over-all reaction.

The amount of the energy available to the organism appears to be of the same order of magnitude as that previously reported.[35] In the present investigation but a small amount of the substrate was converted into bacterial cell protoplasm. The fermentation of the remainder was more than sufficient for the energy requirements of the bacteria as calculated by Wilson and Peterson.[35] Boruff and Buswell[24] calculated from heats of combustion that 6.7% of the total energy available from the complete oxidation of cellulose is lost to the bacteria by its fermentation to carbon dioxide and methane. A similar calculation using the free energy at standard state has been made for the substances in this investigation. The loss varied widely. For carbohydrates it averaged about 12%.

Experimental

Preliminary qualitative experiments of a type frequently used[22,24] were made for the purpose of ascertaining whether a particular substance could be fermented. Experiments in which a carbon balance was determined on the fermentation were carried out in inverted filter flasks connected to a gas collector and gasometer as described by Neave and Buswell.[29] The original inoculum for some of the experiments was a mixed culture from an anaerobic digestion tank treating domestic wastes and for the remainder the mixed culture was carried from one experiment to another.

Care in maintaining complete anaerobiosis was one of the factors contributing to the practically quantitative and nearly theoretical yields of gas. Control of the feedings was also important so that acids did not accumulate and side reactions were minimized. The small quantities of substrate fed daily were regulated so that the weight of the gas produced between feedings was approximately 85% of the theoretical yield. To supply the nitrogen requirement of the bacteria it was found necessary to add approximately 6 mg. of inorganic nitrogen as ammonium hydroxide per gram of substrate fed. The concentration of ammonia nitrogen in the liquor was maintained at 400–600 mg. per liter. As a resting place for the bacteria, 25 g. of finely chopped and freshly ignited asbestos was added to each liter of culture media in the flasks.[38] This served to eliminate the need for control experiments and the error introduced by large amounts of inoculating material[14,22] and thereby reduced the amount of organic matter in the inoculum to less than 5% of that fed. The organic nitrogen content of the inoculum indicated that this organic matter was practically all cell substance. Gas was collected over saturated salt brine and analyzed in a modified Orsat apparatus. Any gain of carbon dioxide in the culture medium due to solubility was added to the carbon dioxide determined in the gas. An added correction was made for a slight loss of carbon dioxide to the air due to manipulation; it amounted to but a few tenths of one per cent. Under the

(37) Aubel and Salabartan, Compt. rend., 180, 1784 (1925).

(38) Breden, J. Bact., in press.

conditions of the experiments small amounts of acetic and lactic acids were frequently present before and after fermentation. These were quantitatively determined and in the extreme case found to be only 0.3% of the amount of substrate digested. Ethyl alcohol was absent.

From a determination of the organic nitrogen and organic carbon in the material attached to the asbestos after an experiment was completed it was calculated that the relation of nitrogen to carbon in the cell protoplasm was 1:5. This agrees with the data of Buchanan and Fulmer.[39] Therefore, from the gain in organic nitrogen of the system during the fermentation, the amount of substrate carbon that had been synthesized into cell substance was calculated. When the amount of substrate fed was large and distributed over a long period of time, that converted into protoplasm was only 5–8%. When the total substrate fed was small in amount and over a short period to a young culture, the conversion into cell protoplasm varied from 10–20%. Knowing these data and the carbon content of the substrate fed it was possible to strike a complete carbon balance on the experiments. These experiments were carried out at a temperature of 33–35°.[15,21]

Such errors as were apparent in the data were due to the use of an average figure for the nitrogen–carbon ratio in the cell protoplasm. This error was small in itself and therefore not significant in the final balance, since well over 80–95% of the substrate was converted into gas which could be determined accurately. The molar ratios of the two gases were determined from the carbon content since these gases have different molar volumes. The methane was used as the base since it could be more nearly quantitatively determined.

Table I includes a summary of all of the data on experiments in which a complete carbon balance was determined. In view of the excellent agreement of the gas ratios with the proposed theoretical equation (Eq. 2), the quantitative balances obtained, and the fact that such a high percentage of the substrate was converted into gas, another type of experiment was carried out in which only the gas yield and ratio were determined. Two different temperatures were used for these experiments (33–35° and 58–

TABLE I

SUMMARY OF DATA ON EXPERIMENTS IN WHICH A COMPLETE CARBON BALANCE WAS DETERMINED

A. Conditions of Experiment and Substrate

Substrate	Grams fed	Temp., °C.	Duration, days	Vol. of culture flask, liters	Asbestos, g.	Inoculum, g. Source	Ash free	Carbon in substrate fed, g.
Cellobiose	10.0	35.0	35	2.0	50	L[b]	2.60	4.25
Dextrose	1004.0	29.5	136	7.3	...	S[c]	136.80	402.0
Dextrose	682.0	33.1	185	7.3	135	L-A₃[e]	67.40	272.5
Ethylene glycol	38.7	35.0	75	2.0	50	L-A₄	1.96	14.98
d-Galactose	9.9	35.0	35	2.0	50	L	2.64	4.05[f]
Lactose	258.6[g]	34.7	74	7.3	175	L-B₂	24.80	108.90
Levulose	29.5	35.0	75	2.0	50	L-A₄	1.96	11.77
Maltose	10.0	35.0	33	2.0	50	L	2.64	4.33[f]
d-Mannitol	79.6	35.2	45	7.3	185	L-A₄	3.90	31.40
Raffinose	21.0[g]	35.0	100	2.0	50	L	2.80	9.00
Sucrose	70.8	35.0	105	2.0	50	L-A₄	6.4	29.80
Xylose	474.0	31.1	46	7.3	...	S	257.00	189.30
Xylose	242.5	30.7	70	7.3	185	L-A₃	14.00	97.09[h]

(39) Buchanan and Fulmer, "Physiology and Biochemistry of Bacteria," The Williams and Wilkins Co., Baltimore, Md., Vol. I, 1928.

TABLE I   (*Concluded*)

B.   Products

Substrate	CO₂. g.	CH₄, g.	G.	Total gas Per cent. of total fed	G.	Carbon accounted for as protoplasm[a] Per cent. of total fed	G.	Total carbon accounted for Per cent. of total fed	Moles CO₂/Moles CH₄ Actual	Theoretical
Cellobiose	1.60	1.6	3.2	75.3	1.01	23.8	4.21	99.1	1/1	1/1
Dextrose	174.43	175.05	349.48	87.0	48.50	12.1	398.75[d]	99.3	0.996/1	1/1
Dextrose	123.10	124.8	247.9	91.0	19.30	6.9	267.20	97.9	0.986/1	1/1
Ethylene glycol	4.83	8.06	12.89	86.1	1.75	11.7	14.54	97.8	2.994/5	3/5
d-Galactose	1.69	1.66	3.35	82.6	0.80	19.7	4.16	102.3	1.018/1	1/1
Lactose	50.50	49.7	100.2	92.1	7.40	6.8	107.60	98.9	1.028/1	1/1
Levulose	4.76	5.03	9.79	83.0	1.35	11.5	11.44	94.5	0.944/1	1/1
Maltose	1.79	1.78	3.57	82.4	0.79	18.2	4.36	100.6	1.005/1	1/1
d-Mannitol	13.00	15.20	28.20	89.5	4.10	13.0	32.30	102.5	11.08/13	11/13
Raffinose	3.91	4.00	7.91	87.9	1.00	11.1	8.91	99.0	0.978/1	1/1
Sucrose	12.20	13.10	25.3	85.0	3.9	13.1	29.2	98.1	0.953/1	1/1
Xylose	86.42	92.07	178.49	94.3	15.30	8.0	193.80	102.3	0.938/1	1/1
Xylose	46.11	45.98	92.09	94.7	4.95	5.1	97.04	99.8	1.003/1	1/1

[a] Gain in organic nitrogen × 5. [b] Liquor from anaerobic digestion of domestic wastes. [c] Sludge from anaerobic digestion of domestic wastes. [d] 0.75 gram carbon recovered as lactic acid. [e] Liquor from Expt. A₂. [f] Includes the carbon in 0.28 g. of acetic acid decomposed from inoculum. [g] Anhydrous. [h] Includes the carbon in 0.12 g. of acetic acid and 0.24 g. of lactic acid decomposed from inoculum.

60°).   For these experiments seeded asbestos from previous experiments was used as the inoculum.   A greater recovery of gas is evident where such an inoculation was used.   The data are shown in Table II, A and B.

TABLE II

SUMMARY OF DATA ON EXPERIMENTS IN WHICH THE GAS YIELD ONLY WAS DETERMINED

Substrate	Grams fed	Recovery of carbon as gas, %	Moles CO₂/Moles CH₄ Actual	Theoretical
A. Experiments at Mesophilic Temperature (33–35°)				
Acetic acid	15.0	91.5	0.994/1	1/1
n-Amyl alcohol	25.2	88.4	0.985/3	1/3
Amyl alcohol (act.)	15.5	67.9	1/3	1/3
n-Butyl alcohol	27.6	95.4	0.996/3	1/3
Dextrin	10.0	87.0	0.948/1	1/1
Ethyl acetate	12.8	91.6	3.13/5	3/5
Ethyl alcohol	15.0	95.7	1.042/3	1/3
Ethyl alcohol	30.5	88.5	0.994/3	1/3
Formic acid	9.0	82.6	2.45/1	3/1
Glycerol	10.0	97.5	4.95/7	5/7
Inulin	10.75	100.0	0.993/1	1/1
Lactic acid	9.0	100.0	0.948/1	1/1
Methyl alcohol	14.0	93.6	0.989/3	1/3
Methyl alcohol	50.9	85.6	1.001/3	1/3
Oxalic acid	14.95	90.0	6.89/1	7/1
Propyl alcohol	25.0	75.3	0.997/3	1/3
Pyruvic acid	15.0	96.7	6.55/5	7/5
Starch	18.8	94.5	0.987/1	1/1
Succinic acid	5.0	90.0	8.64/7	9/7
Xylose	15.0	86.1	0.982/1	1/1

Table II (Concluded)

Substrate	Grams fed	Recovery of carbon as gas, %	Moles CO_2/Moles CH_4	
			Actual	Theorectical
B. Experiments at Thermophilic Temperature (58–60°)				
Acetaldehyde	21.0	95.4	3/5	3/5
Dextrose	24.5	98.1	0.996/1	1/1
Isoamyl alcohol	25.4	85.9	1/3	1/3
Lactose	23.7	88.2	0.966/1	1/1
Starch	25.2	80.1	0.965/1	1/1
Sucrose	25.0	81.2	0.995/1	1/1
Xylose	22.5	96.4	0.978/1	1/1
C. Preliminary Experiments at Mesophilic Temperatures[a] (30–33°)				
Acetone	7.0	89.5	0.975/2	1/2
Arabinose	7.0	105.0	0.77/1	1/1
Dulcitol	7.0	97.0	11/13	11/13
Rhamnose	7.0	58.7	9.7/13	11/13
Tartaric acid	8.0	89.9	9/5	11/5
Trimethylene glycol	7.0	46.8	0.77/2	1/2

[a] Gas ratios based on volume of gases formed.

Since the reaction consists so largely in gas formation, data on several compounds obtained in less carefully controlled preliminary experiments have been included here (Table IIC). From several experiments it appeared that the ethers (0.1% solution of ethyl, isopropyl and n-butyl) are not fermentable   Paraldehyde resisted fermentation due to its polymerization; the same concentration of depolymerized acetaldehyde fermented. This may also explain why formalin (0.01% soln.) also appeared to be unfermentable and in the two experiments tried, heptadecylamine hydrochloride (0.1% soln.) did not ferment.

One experiment on dextrose was carried out under an hydraulic pressure of 10,000 pounds per sq. in.   Practically no gas was formed but nearly 70% of the substrate fed was converted into propionic, lactic and acetic acids. Two experiments were carried out confirming Söhngen's[30] work on the bacterial reduction of carbon dioxide to methane by hydrogen.

Discussion of Results

The agreement of the actual gas ratio with the theoretical equation (Eq. 2) for such a wide variety of compounds and structures shows the applicability of the general equation to the fermentation. From the original data it has also been possible to show that the weight of gas produced in each experiment was practically that weight predicted by the equation (Eq. 2).
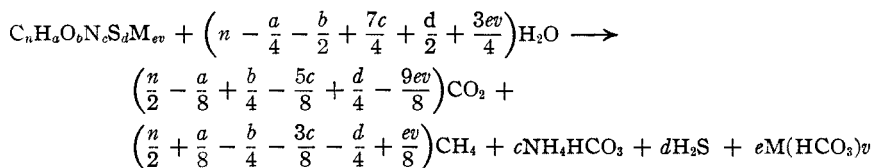
Compounds may be distributed into three classes in regard to the water involved in the reaction: (1) those adding water, (2) those losing water and (3) those showing neither gain nor loss. In all cases in which fermenta-

tion proceeded, only carbon dioxide and methane were produced regardless of the structure of the compound fermenting. However, structure did seem, in some instances, to have an effect on the rate of the reaction (*e. g.*, active amyl alcohol fermented much slower than other alcohols).

It was observed that when the amount of carbohydrate added to the culture was greater than the daily fermenting capacity, there was acid formation and concomitant evolution of a greater amount of carbon dioxide and a lesser amount of methane than predicted by the equation. If the addition of substrate was interrupted the gas ratio then shifted in the other direction and the acids disappeared. This confirms Hoppe-Seyler's observation[13] and indicates that fatty acids are among the first intermediates and that as suggested by Neave and Buswell[29] these decompose by an oxidation on the α-carbon atom.

The data obtained in this investigation confirm the findings of Johnson, Peterson and Fred[40] that the more reduced the compound the more reduced are the end-products and *vice versa*.

The applicability of the general equation has led to the formulation of one still more general that should be applicable to a wider variety of compounds undergoing this fermentation. It is as follows

$$
C_nH_aO_bN_cS_dM_{ev} + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{7c}{4} + \frac{d}{2} + \frac{3ev}{4} \right)H_2O \longrightarrow
$$
$$
\left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} - \frac{5c}{8} + \frac{d}{4} - \frac{9ev}{8} \right)CO_2 +
$$
$$
\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8} - \frac{d}{4} + \frac{ev}{8} \right)CH_4 + cNH_4HCO_3 + dH_2S + eM(HCO_3)v
$$

where the letters C, H, O, N and S have the usual chemical significance, M stands for any metal ion; v the valence of M, and the other subscripts the number of the respective atoms. This equation has been shown to apply to all the quantitative data available except the salts of formic and oxalic acids where there is not sufficient carbon dioxide formed to convert the positive ion completely to the bicarbonate stage.

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(40) Johnson, Peterson and Fred, *J. Biol. Chem.*, **91**, 569 (1931).

Summary and Conclusions

1. A study has been made of the methane fermentation of 45 substances, including carbohydrates, alcohols, acids, aldehydes, ketones, etc. For this work approximately 1900 separate feedings, 700 gas analyses and 800 analyses of culture medium were made.

2. It has been shown that the fermentation is an anaerobic oxidation-reduction reaction involving water and catalyzed by bacteria. Carbon dioxide and hydrogen appear to be primary products, the hydrogen combining with a portion of the carbon dioxide to give methane and water. Organic acids are indicated to be intermediates in the reaction.

3. This reaction follows a simple equation in which the number of molecules of water necessary for the reaction and the amounts of carbon dioxide and methane produced bear a mathematical relation to the formula of the compound undergoing fermentation.

4. Thermodynamic calculations show that if water is the only source of oxygen beside that in the compound undergoing fermentation, carbon dioxide and methane are the end-products that might be expected since the greatest decrease in free energy occurs with their formation.

5. The reaction proceeds in the same manner at both mesophilic (33–35°) and thermophilic (58–60°) temperatures.

The Condensation of Oxalic Esters with Benzyl Cyanide

BY GLENN S. SKINNER

Recently the methyl ester of phenylcyanopyruvic acid was prepared by the action of dimethyl oxalate on benzyl cyanide using sodium in absolute ether as the condensing agent.   This compound had previously been made by Bougault[1] using a different procedure but no analysis was reported. Also in view of the fact that it melts 15° lower than the well-known ethyl ester it was examined with the result that the analysis, molecular weight and chemical behavior definitely show it to be the compound indicated. Preparation of the higher homologs including the n-octyl ester now reveals the fact that the esters with alkyl groups containing an even number of carbon atoms invariably melt higher than the preceding or succeeding member.

The condensation of methyl oxalate with sodium in absolute ether proceeds so slowly that approximately three weeks are required for the reaction to go to completion.   The reaction of the higher homologs, however, proceeds so rapidly that the reaction mixture must be efficiently cooled.   It was observed that the crude odd alkyl phenylcyanopyruvic

(1) Bougault, J. Pharm. Chim., 10, 309 (1914).