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Kinetic Regularities of Methane Production by a Methanogenic Association

Investigation of Methanol and Glucose Conversion Dynamics

S. VARFOLOMEYEV* AND S. KALYUZHNYY

Moscow State University, Moscow 119899, USSR

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ABSTRACT

The kinetic regularities of anaerobic conversion of glucose, ethanol, methanol, acetate, and carbon dioxide by a syntrophic methanogenic association were investigated.

The processes of formation of various metabolities from the above listed substrates under argon and hydrogen were studied in quantitative terms. The dependences of metabolite formation rates on the starting concentrations of the substrates and pH were investigated. Kinetic conversion schemes for the given substrates and the methanogenic association were evolved.

Index Entries: Kinetics; methanogenic association; glucose; methanol, acetate; carbon dioxide; hydrogen; methane.

INTRODUCTION

Anaerobic conversion of biomass to methane constitutes one of the few biotechnological processes capable of making a tangible contribution to our society's energy sufficiency within the next few decades. This process has been assimilated, and there are technologically acceptable conditions for its application on a wider scale (*1–10*). Despite the deep and ever growing interest in this field (*1–3, 11–16*), many problems related to the methane production mechanism have yet to be clarified.

*Author to whom all correspondence and reprint requests should be addressed.

To study the process and evolve well-premised concepts about the possibility of its intensification, we looked into the kinetic regularities of methane formation. As we know, under natural conditions, a complex consortium of microorganisms is responsible for biomass destruction and gasification that results in methane formation (*1*). Selection of a model system for analysis thus attains cardinal significance. The investigated system should satisfy the following two conditions: it should be sufficiently complex and reflect the most characteristic properties of the methane formation process and it should be simple enough for laboratory conditions.

As a model system, we used a methanogenic termophilic association that was earlier known as "Methanobacillus kuzneceovii." This association, which was isolated from a anaerobic digester, is a stable formation cultivated on methanol and acetate (*17–21*). It was shown in the course of preliminary experiments that a number of successive passages do not change the properties of the selected association, i.e., the dynamic regularities of methane formation are reproduced both qualitatively and quantitatively. Pantskhava assumed that "Methanobacillus kuzneceovii" consists of at least two microorganisms: one of them is a methylotroph unable to grow on the media with benzylpenicillinate sodium, and the other is a methanogen (*21*). This assumption seems to be true, as pure cultures of *Clostridium thermoautotrophicum* and *Methanobacterium thermoautotrophicum* were isolated recently from the similar associations (*22,23*).

The aim of the present work was to carry out an expeimental investigation into the regularities of chemical reactions proceeding under the effect of the biocatalytic systems of microorganisms contained in the given methanogenic association and to identify most significant reactions occurring in the course of methane formation. We also set the task of a theoretical modeling of the processes within biokinetic approximation (*24,25*).

METHODS

The methanogenic association inoculate was grown at 60°C in a 10-liter vessel fitted out with a hermetic system for measuring the gas formed. As a mineral pool, the following medium was used (g/L): NH_4Cl, 2.0; $K_2HPO_4 \cdot 3H_2O$, 3.6; KH_2PO_4, 2.8; $MgCl_2 \cdot 6H_2O$, 0.3; $CoCl_2 \cdot 6H_2O$, 0.08; $NiCl_2 \cdot 6H_2O$, 0.02; $FeSO_4 \cdot 7H_2O$, 0.04; $Na_2 \cdot 9H_2O$, 0.08; and methanol, 5 mL/L.

For inorganic salt solutions, sterilization was performed for 30 min at 1.5 atm at 110°C and cold sterilization for methanol.

The growing of the culture was arrested in 7–9 d, when the gas volume reached 17.5 L. The yield of the dry cells was nearly 10 g. The supernatant thus obtained was used as inoculate for kinetic research experiments.

For kinetic investigations, we used hermetic 60–100 mL flasks equipped with a vacuum rubber cork and hermetically screwed caps. The gas com-

partment of the flasks was filled with argon from which the residual oxygen was removed on a column with granulated copper. The liquid phase volume was 20–40 mL. The concentration of the inoculate (on dry weight) was 0.05–0.1 g/L. Reactors were thermostatted at 60°C. To prevent a rapid fall of pH, 0.17*M* phosphate buffers were used in the reactors in the process of metabolism in experiments with glucose.

H_2, CO_2 and CH_4 analysis was performed on a LXM-9MD chromatographer, model 3 with a catarometer; gas carrier, argon, the gas carrier rate—20 mL/min. Two meter long columns were filled with Porapak QS. At 50°C, hydrogen was evolved after 65 s; methane in 112 s; and carbon dioxide in 185 s. For each analysis, a 0.2 mL gas sample was selected.

The pressure in the reactor increased because of the gas evolution. To determine the amount of the gases formed, the calculation was made relative to the starting conditions when the pressure was 1 atm. Here, the formulas of Boyle's Law were applied. The inside pressure was measured with a manometer.

H_2 and CH_4 dissolution in water was neglected because of the poor solubility of these gases at 60°C, whereas a fairly significant solubility of CO_2 under these conditions was calculated as follows. The overall concentration of carbon dioxide in the system is made up of CO_2 concentration in the gaseous phase and CO_2 concentration in the liquid phase

$$[CO_2] = [CO_2]_{gas} + [CO_2]_{lp}$$

Under our experimental conditions, we measured the CO_2 concentration in the gas phase. But in the liquid phase, the CO_2 concentration is related to the CO_2 concentration in the gas phase according to the Henry Law

$$[CO_2]_{lp} = H \cdot [CO_2]_{gas}$$

where *H* is the Henry constant that depends on the temperature and pH (owing to biocarbonate and carbonate formation). There are works and reference literature data that give Henry coefficients at different temperatures and pH values (*27,28*). We used them to calculate the overall concentration of CO_2 in the system

$$[CO_2] = [CO_2]_{gas} (1 + H)$$

The products and substrates of the processes we investigated were ethanol, methanol, and acetate contained in the liquid phase in large quantities, and propanol, propionate, and butyrate contained in negligible quantities. The concentration of these substances was controlled on a Chrom-5 chromatographer with FID. Helium was the gas carrier, and the rate of the gas carrier was 60 mL/min. One-meter long columns were filled with Porapak QS. These products were clearly separated at 200°C. The retention time (in s): methanol, 26; ethanol, 51; propanol, 101; acetic acid, 130; propionic acid, 256; and butyric acid, 491.

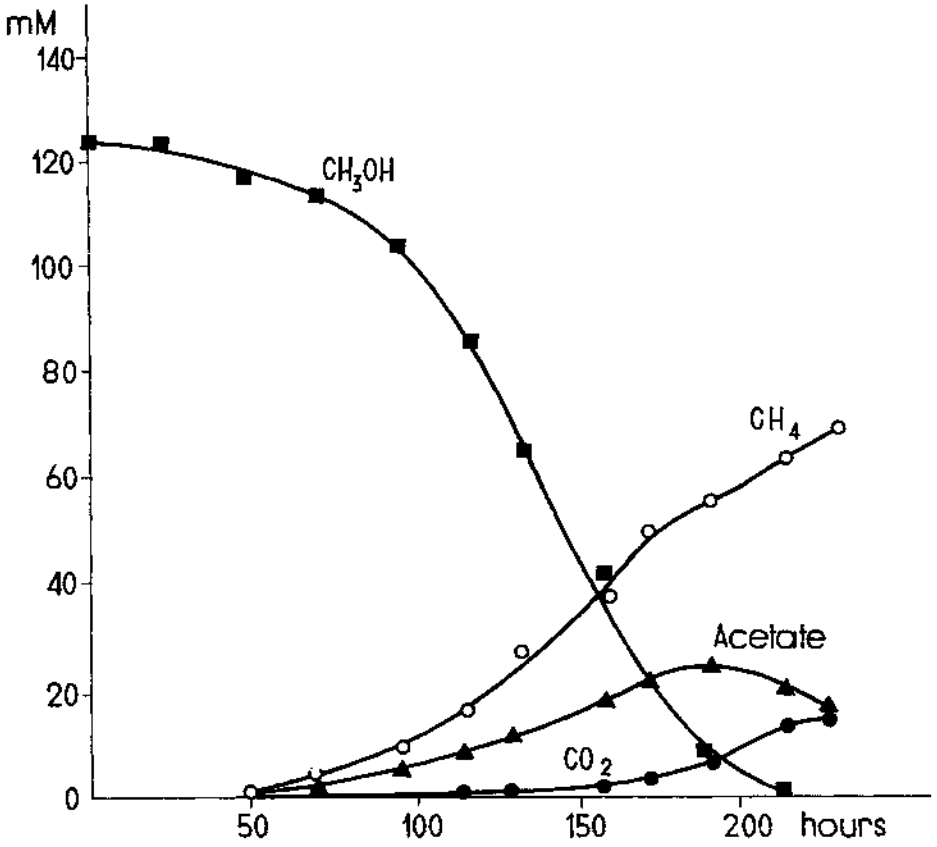

Fig. 1. Dynamics of anaerobic decomposition of methanol under the action fo a methanogenic association, 124 mM, CH_3, pH 7.0, 60°C.

Glucose concentrations were determined according to the standard enzymatic methods based on glucosoxidase and peroxidase, as previously described (*29*).

To obtain the concentration of all the substrate intermediates and products in single units, the concentration of the gaseous substances was likewise expressed in m*M*. For this purpose, the number of mols of gaseous substances was referred to 1 L of the cultural medium.

RESULTS AND DISCUSSION

Conversion of Methanol

A typical picture of anaerobic decomposition of methanol in the presence of a metanogenic association is given in Fig. 1. A study of the products of the reaction proceeding in the growing culture showed that apart from methane, the system generates acetate and carbon dioxide. Besides,

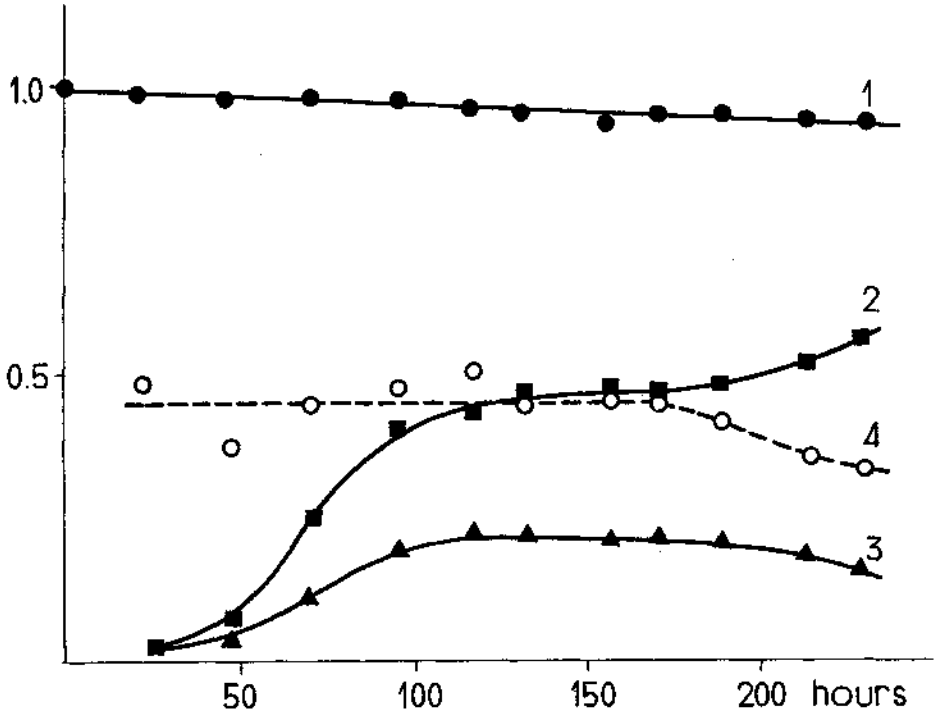

Fig. 2. Dependence of ($[CH_3OH] + [CH_4] + [CO_2] + 2[CH_3COO^-]$) / $[CH_3OH]_0$ (curve 1); $[CH_4]$ / ($[CH_3OH]_0 - [CH_3OH]$) (curve 2); $[CH_3COO^-]$ / ($[CH_3OH]_0 - [CH_3OH]$) (curve 3); and $[CH_3COO^-]$ / $[CH_4]$ (curve 4) on time.

small amounts of hydrogen are constantly evolved (not shown). Methane, acetic acid, and carbon dioxide production curves have lag periods owing to the development of a symbiotic system of microorganisms. Yet, already on d 6 or 7 of the process, vigorous methane production takes place. Then, pressure in the reactor rises to 3.5 atm, the medium grows turbid, and pH falls to 6.0 at the end of the process.

Let us try to single out basic chemical reactions of the investigated process. With this aim in view, let us transform the data shown in Fig. 1. Fig. 2 (curve 1) shows the time dependence of the ratio the sums of all the detected carbon compounds to the starting concentration of methanol (factor 2 is placed before the concentration of acetate, for it is a C_2- compound). We see that throughout the growth of the culture, this ratio is close to 1, though it naturally decreases toward the termination of the process (by 230 h the carbon balance deficit is equal to 7%). A part of the methanol in the process of its conversion to methane is expended for the growth of the association's biomass. Besides, curve 1 shows that within the accuracy of a microbiological experiment (10%), we may assert that other components, evolved into the cultural medium into the gas (which we failed to detect), are absent from the metabolism of the association. The other curves in Fig. 2 reflect the time sequence of the following rela-

tions: curve 2, the amount of the methane evolved to the amount of the methanol consumed; curve 3, the amount of the evolved acetate to the amount of the methanol consumed; and curve 4, the amount of the acetate evolved to the amount of the evolved methane. An analysis of these curves indicates that, barring the lag period effects, curve 2 tends to 0.5, curve 3 to 0.25, and curve 4 to 0.5 within 80–180 h. Thus, the overall chemical equation for the first step of methanol-to-methane conversion takes the form

$$4CH_3OH \rightleftharpoons CH_3COO^- + 2CH_4 + 2H_2O + H^+ \quad (1)$$

The formation of acetate from methanol may be carried out by methylotrophic bacteria similar to those previously described (*23,30–32*). According to the work of Pantskhava (*20,21*), methane formation from methanol by the methanogenic association proceeds via decomposition of methanol to CO_2 and H_2 with the subsequent reduction of CO_2 by hydrogen to methane. As noted above, in our experiments, we also observed hydrogen production, and the hydrogen concentration reached a maximum, whereas traces of hydrogen were detectable even 150–180 h after the process. This shows that hydrogen plays a significant role in the conversion of methanol to methane by the methanogenic association (33). Methane formation via methanol decomposition may proceed against a thermodynamic gradient on condition that the partial pressure of hydrogen in the system is low.

As seen in Fig. 1, Eq. (1) does not describe all the variety of the processes in the system. A significant standout is the acetate formation curves passing through a maximum. Besides, the accumulation of carbon dioxide in considerable quantities takes place only after the exhaustion of the methanol pool and the descending branch of the acetate accumulation branch. All this indicates that, upon methanol conversion, the methanogenic association is shifted to acetate consumption, accompanied by the evolution of methane and carbon dioxide

$$CH_3COO^- + H^+ \rightleftharpoons CH_4 + CO_2 \quad (2)$$

Process (2) could be explained by the presence of a methanogene in the association, utilizing acetate as a carbon and energy source.

Thus, the conversion of methanol to methane by the methanogenic association is described well enough by this two-step scheme

$$4CH_3OH \overset{M_1}{\rightleftharpoons} CH_3COO^- + 2CH_4 + 2H_2 + H^+$$

$$CH_3COO^- + H^2 \overset{M_2}{\rightleftharpoons} CH_4 + CO_2$$

Two microorganisms must be responsible for the former reaction. We assume that they are *Clostridium thermoautotrophicum* and *Methanobacterium thermoautotrophicum*. Yet, for simplicity (of the kinetic scheme and subse-

quent mathematical modeling), let us believe that reaction (1) is affected by only one microorganism M_1, all the more so that the kinetic analysis data (Fig. 1) do not contradict this simplification. Reaction (2) proceeds during the growth of the acetate-utilizing microorganism M_2.

The methanol-to-methane conversion system was investigated at different starting concentrations of methanol and pH. The optimal conditions for the production process were found to be at pH 6.5–7.0 and methanol concentration of 100–200 m*M* at low buffer capacity of the media. It should be remarked that, at methanol concentrations above 200 m*M*, complications arise. Thus, at a starting concentration of CH_3OH equal to 320 m*M*, methane and acetate formation proceed vigorously at first, but then irreversible changes occur in the system, and the growth is arrested, with pH failing from 7.0 to 5.0 and lower, which must be the main paralyzing factor for methanol growth and conversion. The fall in pH is the consequence of acetate accumulation according to reaction (1). At starting methanol concentrations above 1*M*, neither methanol conversion nor growth in the system is observed, which must be owing to the inability of the methanogenic association to grow at such concentration of methanol.

The continuous cultivation of the selected association was investigated in the work (34). It was shown that the influent concentration of methanol can be markedly increased compared to optimal concentration for periodic cultivation. Maximal methane productivity was achieved at influent concentration of methanol 620 m*M*, pH 6.5, dilution rate 0.073/h.

It is also noteworthy that the process of anaerobic conversion of methanol in the presence of the methanogenic association has a fairly pH optimum (6.5–7.0), with methane formation being absent at the starting pH value 5.5 and 8.5. If the pH value is 6.0, the process of methanol-to-methane conversion starts vigorously, but very soon comes to a stop because of the pH fall. At pH 7.5, at initial steps, methanol is converted to acetate with little, if any, methane being evolved. Active methane production starts at pH 7.0. As we see, the investigated association, depending on the conditions of the medium, may vary somewhat the stoichiometric correlations between the products of the process for better growth conditions.

We attempted to convert methanol to methane under the hydrogen atmosphere in the presence of the methanogenic association. Under such conditions, methanol is virtually not converted to methane. This fact is yet another proof of the unusual pathway of methanol conversion to methane by the given association. Possibly, the hydrogen atmosphere significantly lowers the activity of microorganisms that decompose methanol to carbon dioxide and hydrogen.

Conversion of Glucose

Figure 3 shows a typical picture of glucose decomposition under argon in the presence of the methanogenic association. We see that apart from the principal product, methane, a number of intermediate products

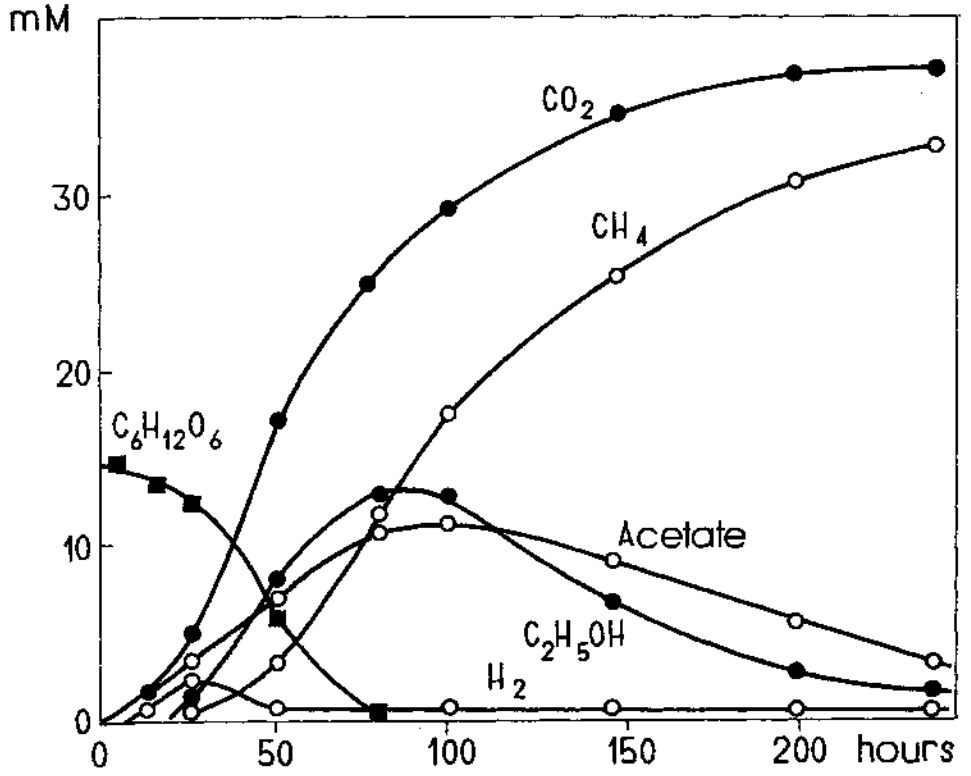

Fig. 3. Dynamics of anaerobic decomposition of glucose by the methanogenic association. Starting conditions: 16.5 m*M* glucose, pH 7.0, 60°C.

are formed in the reaction medium. Besides the metabolites shown in the figure, propanol, propionate and butyrate are also formed. Yet, the accumulation of these methabolites is insignificant. They contribute to the carbon material balance a share commensurate with the experimental error, and so we shall neglect them and consider hydrogen, CO_2, methane, ethanol, and acetate as the key metabolites of glucose decomposition. Production curves for these metabolites have small lag periods that are the results of both the development of the symbiotic system of microorganisms and the accumulation of metabolites, the methane precursors for the investigated association. Let us look into these two interconnected questions: (1) how is the carbon balance maintained throughout the process? and (2) are there any appreciable amounts of the other products of the reaction that we do not identify? Let us then transform the data shown in Fig. 3. Figure 4 depicts the time dependence of the ratio of the sum of all the detected products and the residual substrate to the initial concentration of glucose (the coefficients in the sum reflects the fact that glucose is a C_6-compound, propanol and propionate are C_3-compounds, butyrate is a C_4 compound, ethanol and acetate are C_2 compounds, and methane and carbon dioxide are C_1-compounds). It is seen that, throughout the culture growth, this ratio, as in the case of methanol conversion, is close

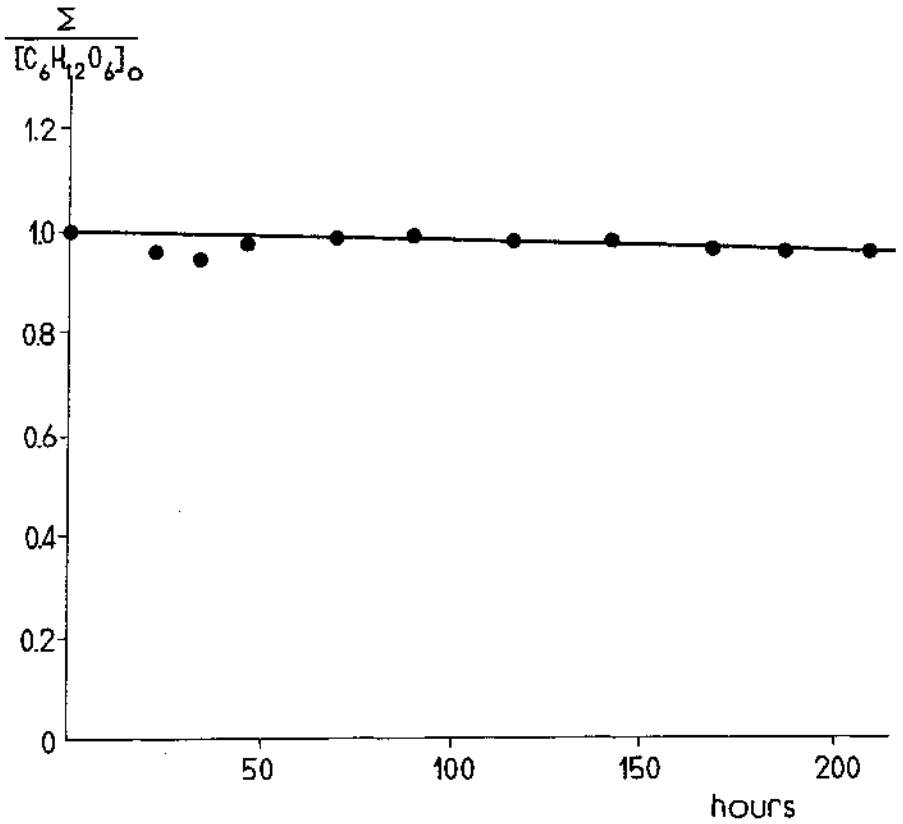

Fig. 4. Dependence of Σ / $[C_6H_{12}O_6]_0$ on time, where $\Sigma = [C_6H_{12}O_6] + 1/3[C_2H_5OH] + 1/3[CH_3COO^-] + 1/6[CO_2] + 1/6[CH_4] + 1/2[C_3H_7OH] + 1/2[C_2H_5COO^-] + 2/3[C_3H_7COO^-]$.

to 1, but it inevitably drops toward the end of the process (by 260 h the carbon balance deficit was equal to 6%). This deficit arises owing to carbon utilization for the cell biomass growth. On the whole, we may assert that within the accuracy of the microbiological experiment (10%) and at moderate concentrations of glucose, other, nondetectable carbon compounds, evolved into the cultural medium or the gas phase, are absent from the metabolism of the association.

To investigate the nonmethanogenic step of the process, we used the following property of the system: methane-generating cultures of the association do not grow at low pH values (under 5.5), whereas glucose conversion to ethanol, acetate, CO_2, and H_2 proceeds rather vigorously. Figure 5 depicts kinetic curves of the formation of the products at pH 5.3. In the first 80 h of the process, one can observe a pronounced exponential accumulation of the products of the premethanogenic decomposition of glucose—ethanol, acetate, CO_2, and H_2. These metabolites are the primary products of the anaerobic conversion of glucose by the given methanogenic association.

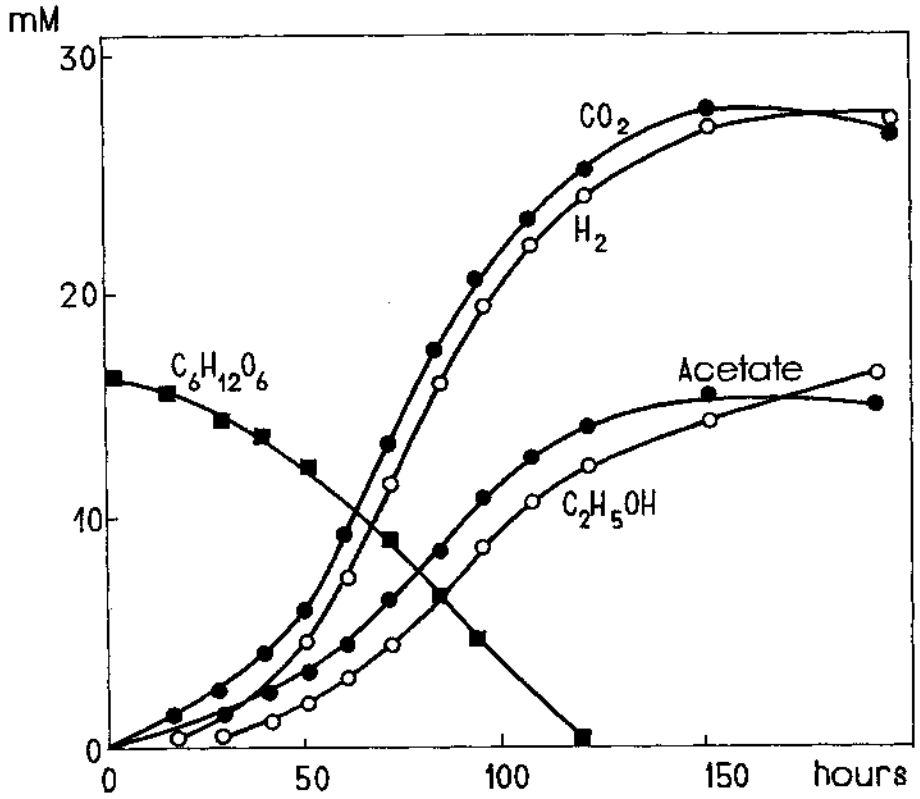

Fig. 5. Dynamics of anaerobic decomposition of glucose in the absence of the methanogenic phase. Starting conditions: 16.5 m*M* glucose, pH 5.3, 60°C.

An investigation into the stoichiometric correlation of ethanol, acetate, H_2, and CO_2 formation shows that the molar amounts of the substances thus formed are related as 1:1:2:2, i.e., ethanol and acetate are formed in equivalent and half of those for H_2 and CO_2. This follows both from the material balance data (Fig. 6) and from those on the accumulation kinetics in the exponential phase of growth (Fig. 7). The specific rates of growth obtained for various products have these values

$$q_{H_2} = 0.045 \pm 0.007 \text{ / h; } q_{CO_2} = 0.042 \pm 0.003 \text{ / h; } q_{C_2H_5OH} = 0.032 \pm 0.003 \text{ / h; } h^{-1}\text{; } hq_{CH_3COO} = 0.028 \pm 0.003 \text{ / } h^{-1} \quad (3)$$

Summing up these results, we may infer that the decomposition of glucose under the effect of the investigated methanogenic association obeys the equation

$$C_6H_{12}O_6 + H_2O \rightleftharpoons C_2H_5OH + 2CO_2 + 2H_2 + CH_3COO^- + H^+ \quad (4)$$

One microorganism is responsible for this process. Reaction (4) is a basic one for the given system. This chemical reaction, known as the

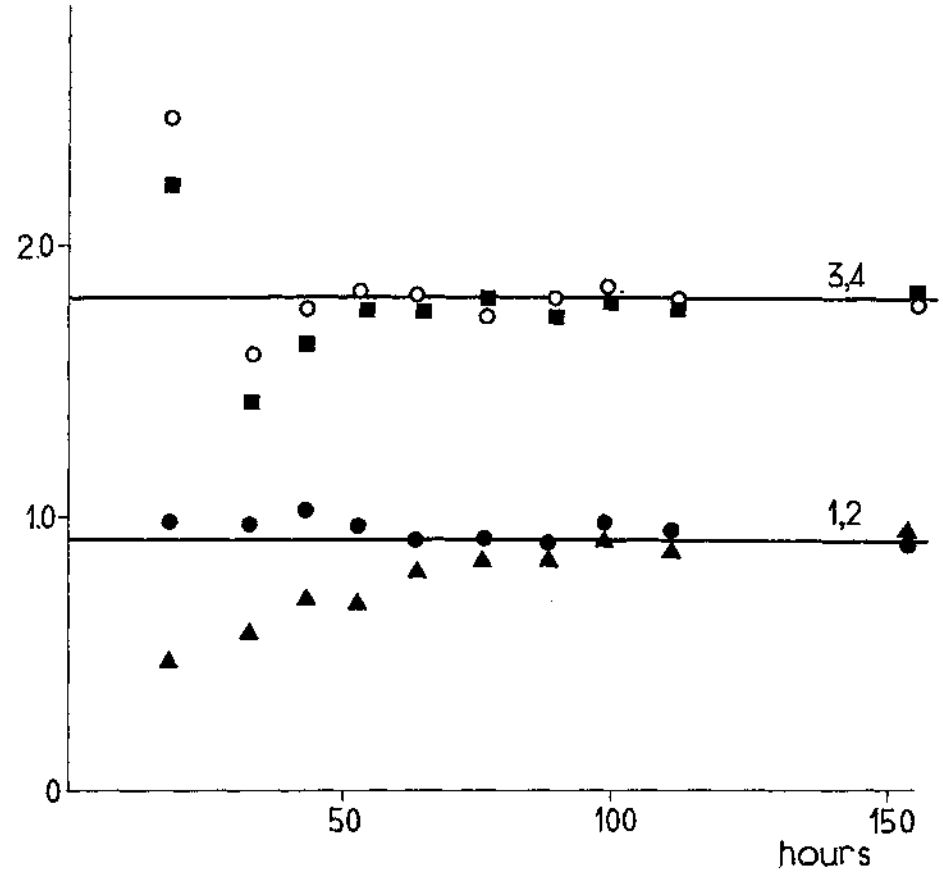

Fig. 6. Dependence of $[CH_3COO^-] / ([C_0H_{12}O_6])_0 - [C_6H_{12}O_6])$ (●); $[C_2H_5OH] / C_6H_{12}O_6]_0 - [C_6H_{12}O_6]$ (▲); $[CO_2] / ([C_6H_{12}O_6]_0 - [C_6H_{12}O_6])$ (○); $[H_2] / ([C_6H_{12}O_6]_0 - [C_6H_{12}O_6])$ (■) on time.

Embden-Meyerhoff pathway, is widespread in the metabolism of anaerobic microorganisms (*35*)

As seen from Fig. 3, the accumulation of ethanol and acetate in the process of methanogenesis passes through a maximum. This is a characteristic feature of the effect of a symbiotic association in which a metabolic product of one culture serves as a substrate for another (*25*). To check on the presence of microorganisms capable of acetate, ethanol, and CO_2 consumption in the given association of microorganisms, we carried out experiments in which the association was grown on these products used as substrates. Indeed, a microbial association is capable of converting ethanol, acetate, as well as carbon dioxide mixed with hydrogen, into methane.

Figure 8 shows kinetic curves of ethanol conversion to methane and CO_2 with acetate and molecular hydrogen (formed in small amounts) acting as intermediate products. This enables us to write down the basic chemical reaction:

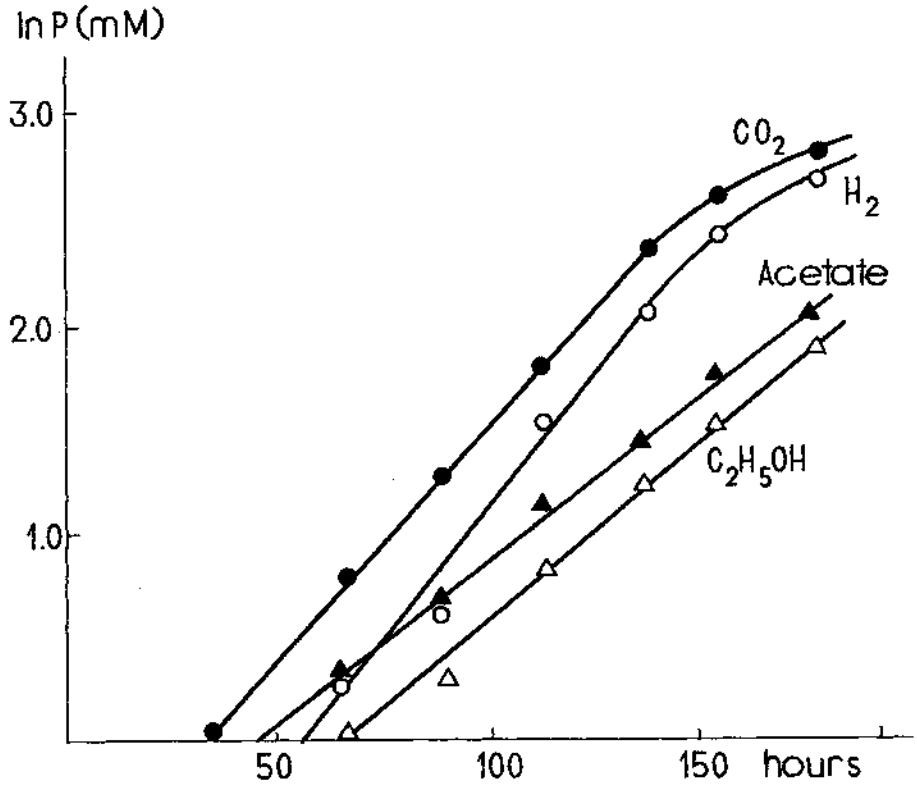

Fig. 7. Dynamics of accumulation of nonmethanogenic phase products in semilogarithmic coordinates. Conditions, as in Fig. 3.

$$C_2H_5OH + H_2O \rightleftharpoons CH_3COO^- + H^+ + 2H_2 \quad (5)$$

which is followed by the methane formation step. The thermodynamic conditions for the process are ensured by subsequent reactions of hydrogen binding and acetate removal from the system.

Figure 9 depicts kinetic data on acetate conversion under the action of the investigated methanogenic association. The process of methane formation is even less vigorous than in the case of ethanol with CO_2, as well ethanol and hydrogen in insignificant amounts, being formed. Ethanol is formed possibly as a result of the reverse reaction (5). Acetate formation accompanied by the production of carbon dioxide and hydrogen may be explained by the introduction of this reaction

$$CH_3COO^- + H^+ + 2H_2O \rightleftharpoons 2CO_2 + 4H_2 \quad (6)$$

It must be observed, however, that thermodynamically this is a very disadvantageous process: $\Delta G_0 = 94.9$ kJ/mol and reaction (4) may proceed only at very low partial pressures of hydrogen—below 10^{-3} atm (*36*). This conclusion about the nonacetoclastical transformation of acetate to methane in symbiotic associations was confirmed with the aid of isotope analysis in the work (*36*).

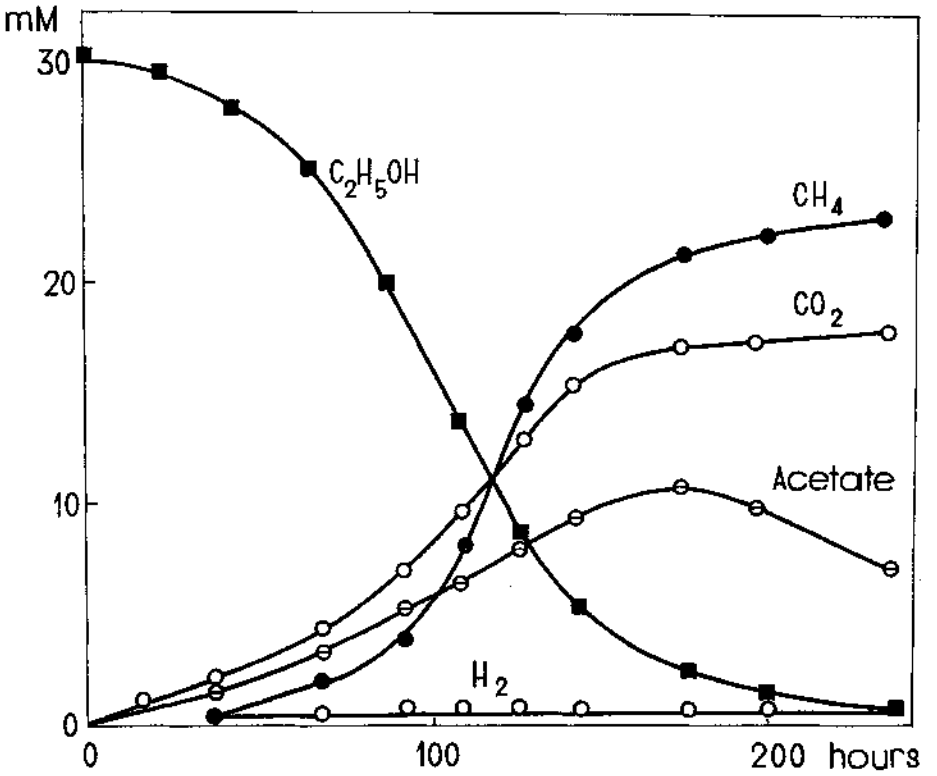

Fig. 8. Dynamics of anaerobic decomposition of ethanol. Starting conditions: pH 7.0, 30 m*M* ethanol, 60°C.

Acetate conversion to methane may proceed in accordance with the simple basic reaction

$$CH_3COO^- + H^+ \rightleftharpoons CH_4 + CO_2 \quad (7)$$

where CO_2 and CH_4 should be formed in stoichiometric quantities. Many methanogenic microorganisms can produce methane according to scheme (7) (*1,12,13,37,38*). As seen from Fig. 9a, the carbon dioxide formation rate exceeds the methane's. Apparently, in this case, the process according to step (6), with subsequent reduction of CO_2 to methane by hydrogen, is more rapid

$$CO_2 + 4H_2 \rightleftharpoons CH_4 + 2H_2O \quad (8)$$

Figure 9b shows curves for acetate conversion under hydrogen. In this case, methane production is significantly increased, and no accumulation of the detectable amounts of carbon dioxide is observed. Ethanol formation, as in the case of acetate conversion under the argon atmosphere, obeys Eq. (5).

These data allow us to infer that even though the hydrogen atmosphere inhibits the organism that decomposes acetate according to Eq. (7), the investigated methanogenic association also contains an organism that can reduce acetate according to the equation

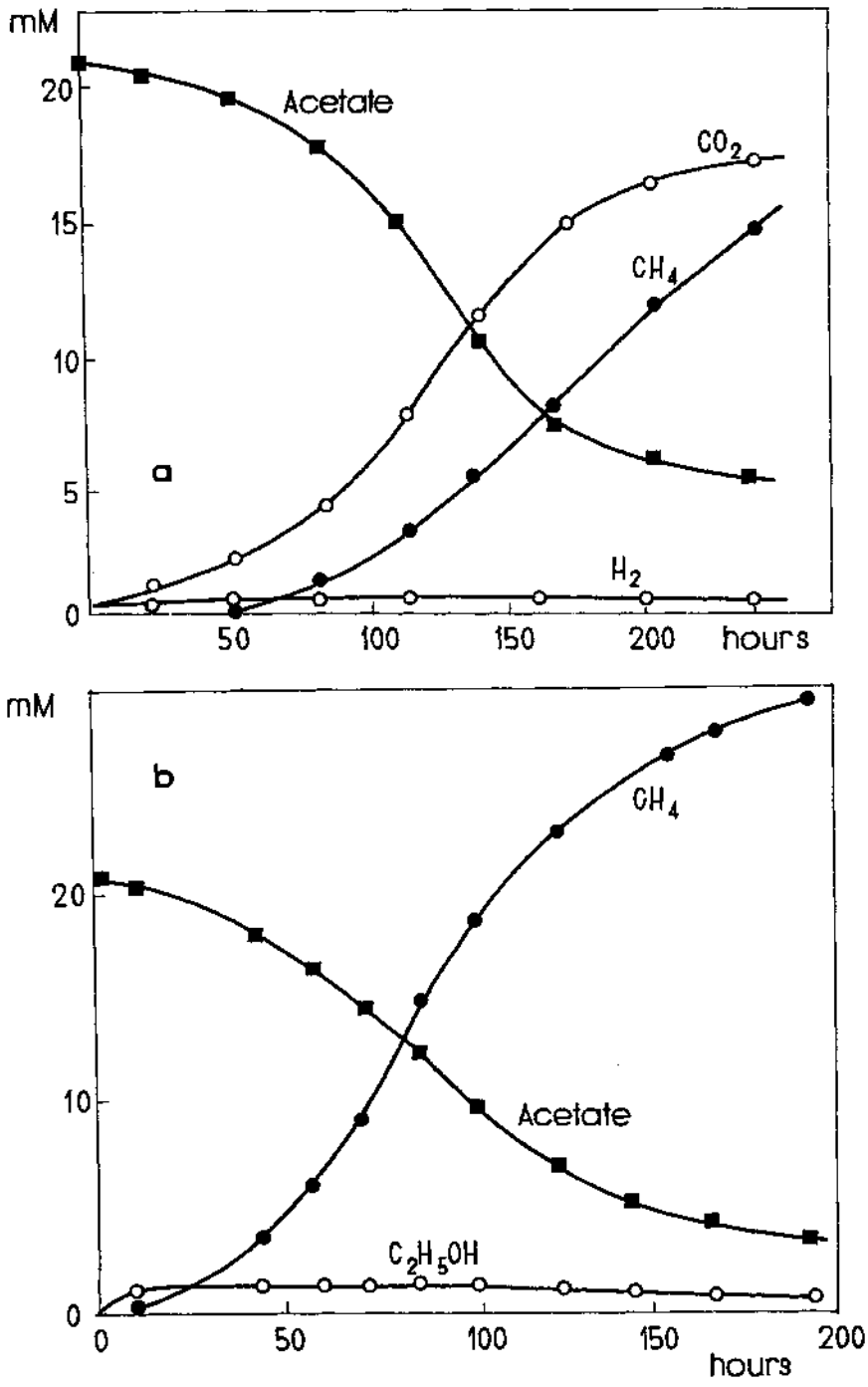

Fig. 9. Dynamics of anaerobic decomposition of acetate. Starting conditions: pH 7.0, 21 m*M* acetate, 60°C. (a), Under argon; and (b), under hydrogen.

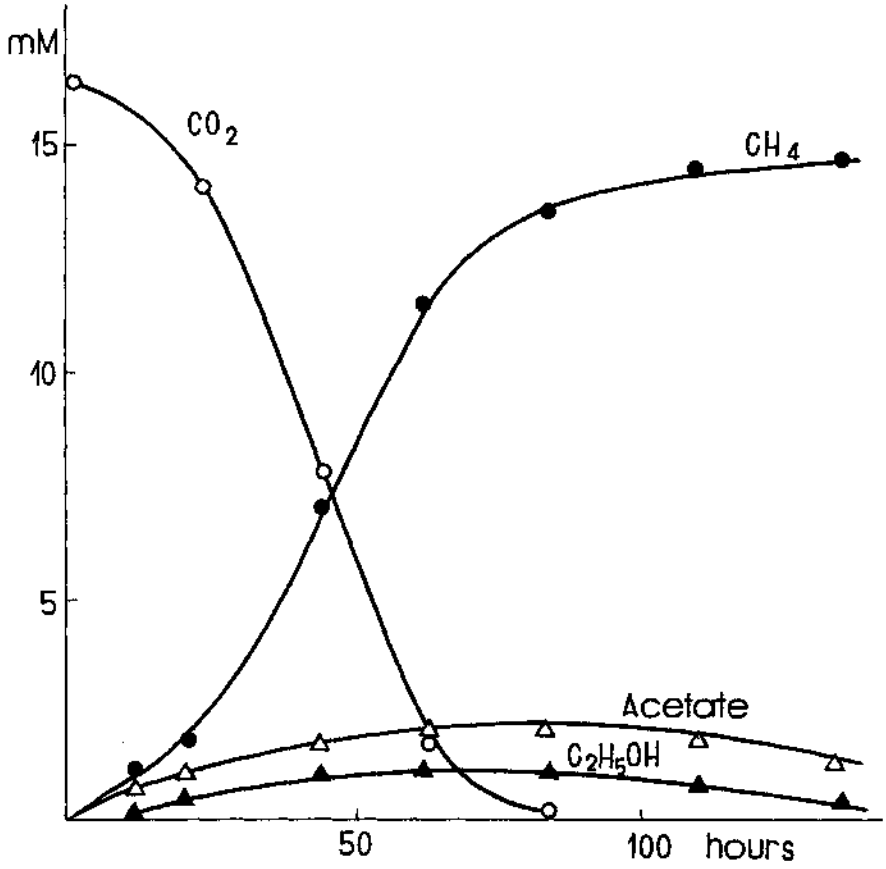

Fig. 10. Dynamics of methane synthesis from carbon dioxide under the hydrogen atmosphere, pH 7.0, 20 m*M* $NaHCO_3$, 60°C.

$$CH_3COO^- + H^+ + 4H_2 \rightleftharpoons 2CH_4 + 2H_2O \quad (9)$$

Process (8) in the system is confirmed by direct conversion of carbon dioxide under hydrogen (Fig. 10). Intermediate compounds, ethanol and acetate, are likewise formed. The synthesis of these compounds is probably owing to the presence in the given association of mixtures of a microorganism capable of synthesizing acetate from H_2 and CO_2.

The results of our quantitative analysis are summarized in the scheme

$$C_6H_{12}O_6 \overset{M_3}{\rightleftharpoons} C_2H_5OH + CH_3COO^- + 2H_2 + 2CO_2 + H^+$$

$$C_2H_5OH + H_2O \overset{M_3}{\rightleftharpoons} CH_3COO^- + 2H_2 \quad (10)$$

$$CH_3COO^- + H^+ \overset{M_2}{\rightleftharpoons} CH_4 + CO_2$$

$$CO_2 + 4H_2 \overset{M_4}{\rightleftharpoons} CH_4 + 2H_2O$$

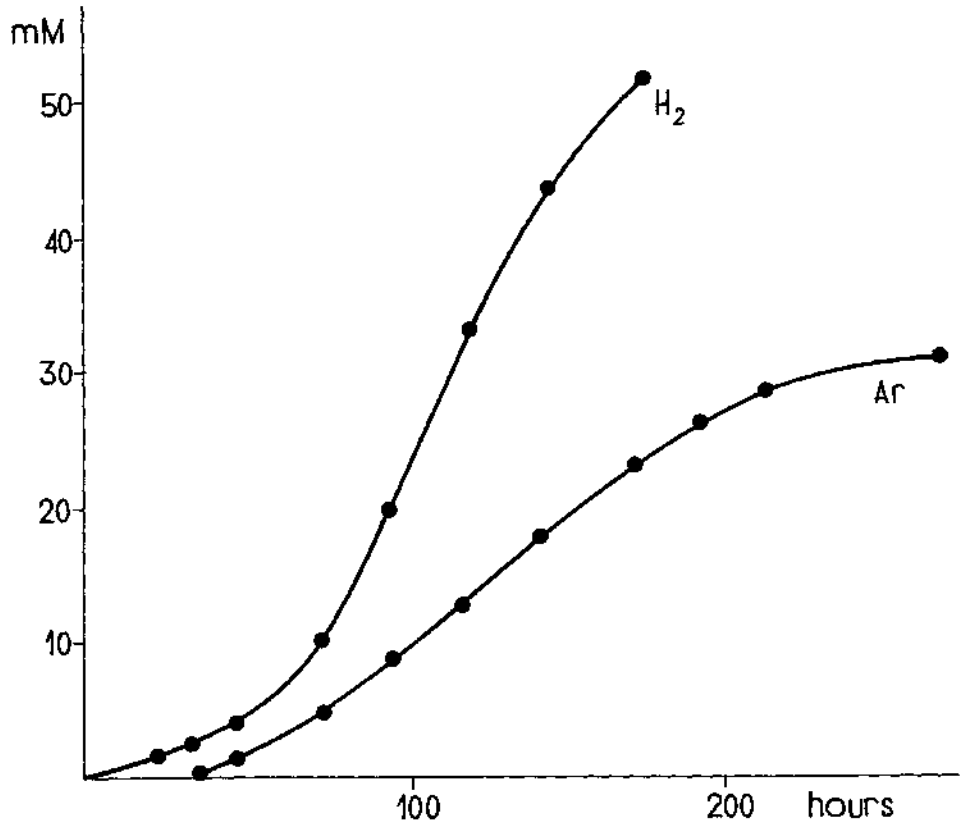

Fig. 11. Dynamics of methane production from glucose in the presence of the methanogenic association under the argon and hydrogen atmosphere, pH 7.0, 16 m*M* glucose, 60°C.

The first step of the process takes in glucose decomposition according to the Embden-Meyerhoff pathway accompanied by the growth of the nonmethenogenic microorganism, M_3, in the association. The second reaction is performed by the M_3 enzymatic system according to the Embden-Meyerhoff scheme, but is probably not accompanied by biomass growth. The two latter steps (10) are methane-generating ones and are carried out by two independent methanogenic microorganisms, M_2 and M_4. M_2 is an acetate-utilizing organism with slow methane generation, M_4, a hydrogen-utilizing organism with fast methane generation. This conclusion matches the date of a purely microbiological experiment (22, 23,33). Under the conditions of glucose conversion under argon, the growth of M_4 and methane formation are limited by the shortage of hydrogen. The latter provision was illustrated by glucose fermentation by the methanogenic association in the hydrogen atmosphere. Experimental data are shown in Fig. 11. It is seen that the lag period of methane production decreases considerably, whereas the very process of methane forma-

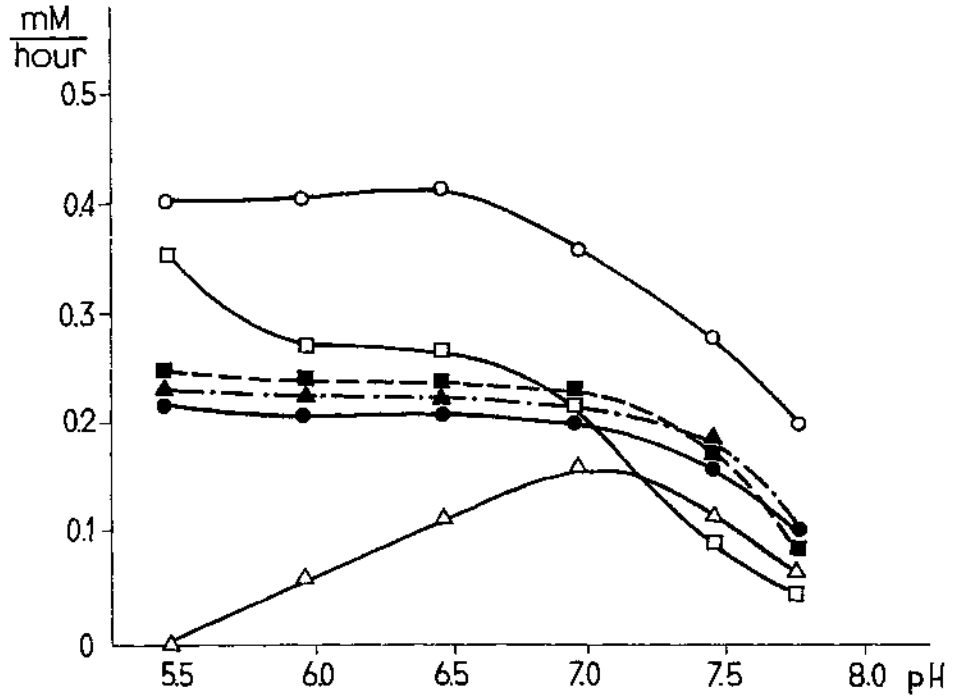

Fig. 12. pH dependence of steady-state rates of ethanol (▲), acetate (●) CO_2 (○), H_2 (□), CH_4 (△) formation, and glucose consumption (■); starting concentration of glucose, 16.5 m*M*.

tion is several times as efficient. In glucose conversion, reaction (6) may be excluded from the kinetic scheme for thermodinamic considerations.

The dependences of the rates of product formation and glucose consumption on the starting pH values in the medium were investigated. We used the steady-state rate of the process in the linear section of product formation as a measurable arbitrary unit. We found that the range of pH values optimal for methane formation are within 6.5–7.0 (Fig. 12). No methane was evolved at pH 5.5 and lower; however, significant amounts of carbon dioxide and hydrogen were accumulated, their pH optimum lies within 5.5–6.0. The position of the curve for the steady-state rate of hydrogen evolution (*12*) is much lower than of a similar curve for CO_2. This is owing to the difficulty of determining the steady-state rate of hydrogen evolution because of H_2 rapid consumption for methane generation. Moving into the alkaline region (from pH 7.0), the steady-state rates—both of nonmethanogenic and methanogic processes—exhibit a fairly rapid decrease.

We also investigated the dependence of steady-state rates of product formation and glucose utilization on the starting concentration of glucose at 0.17*M* phosphate buffer capacity. Experimental data are shown in Fig. 13. The methane formation optimum is observed within the glucose con-

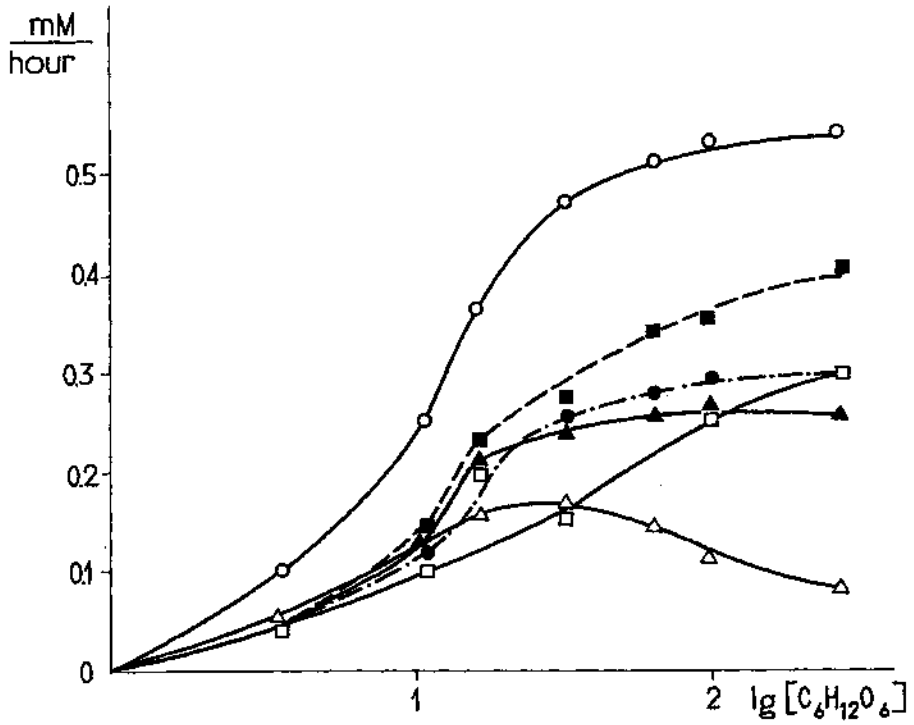

Fig. 13. Dependence of the steady-state rates of ethanol (▲), acetate (●) CO_2 (○), H_2 (□), CH_4 (△) formation, and glucose consumption (■); on log. of the starting concentration of glucose (m*M*), pH 7.0, 60°C.

centration range of 20–30 m*M*. If the starting concentration of glucose is above 40 m*M*, the processes of glucose destruction are intensified and enhanced amounts of organic acids are produced. As a result, pH is shifted into the acidic region when methane formation processes are decelerated or arrested altogether. Besides, at starting concentrations above 100 m*M*, the carbon balance deficit increases, probably because of the formation of lactic and other organic acids not detectable by the gasochromatographic method.

CONCLUSION

Thus, in this work, we have investigated into the processes of methanol and glucose anaerobic conversion in the presence of a specially selected methane-generating system. We believe that the results of these experiments may be summed up in the form of simple kinetic schemes (3) and (10) predicated on chemical reactions (*1,2,4,5,7,8*) performed by the four microorganisms—M_1, M_2, M_3, and M_4—present in the investigated association. Other microorganisms in this association make a far smaller con-

tribution to anaerobic conversion of methanol and glucose. The kinetic schemes (3) and (10) suggested by us do not contradict the presently available data in the literature (*17–23,30–34,36–38*).

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