ELSEVIER

Bioresource Technology **59** (1997) 73–80

0960-8524/97 $17.00

PII:S0960-8524(96)00124-1

BATCH ANAEROBIC DIGESTION OF GLUCOSE AND ITS MATHEMATICAL MODELING. I. KINETIC INVESTIGATIONS

S. V. Kalyuzhnyi[a]* & M. A. Davlyatshina[b]

[a]*Department of Biotechnology of Enzymes, Chemistry Faculty, Autonomous University of Coahuila, 25000 Saltillo, Mexico*
[b]*Department of Chemical Enzymology, Chemistry Faculty, Moscow State University, Moscow 119899, Russia*

(Received 7 March 1996; revised version received 2 August 1996; accepted 7 August 1996)

Abstract

Kinetics of anaerobic digestion of different mixtures of glucose and intermediates of its acidogenic degradation (ethanol, butyrate, propionate and acetate) have been investigated in batch reactors. Hydrogen concentration and pH value constitute the primary modulating factors in the process. Slight inhibition of the methanogenic step by the excess of butyrate, propionate and ethanol was also observed. The obtained information, as well as the proposed stoichiometry, will be used in an accompanying paper as a basis for the development of a mathematical model of batch anaerobic digestion of glucose. © *1997 Elsevier Science Ltd. All rights reserved.*

Key words: Anaerobic digestion, kinetics, glucose, butyrate, propionate, acetate, ethanol, hydrogen, pH.

INTRODUCTION

Anaerobic digestion is a complex multistage process of organic compound degradation to methane and carbon dioxide by the action of numerous anaerobic microflora. Although this process has been studied for a long time, its kinetic regularities, as well as the interactions of different generic groups of bacterial species within an anaerobic ecosystem, are insufficiently clear yet. Understanding of these regularities and interactions is important for practical application of methanogenesis, especially for fuel production and waste utilization. In this regard, comprehensive mathematical modeling of this process is essential.

The growing interest in the kinetics of methanogenesis during previous years has resulted in developing a variety of mathematical models for the different anaerobic digestion processes (Andrews, 1969; Graef & Andrews, 1974; Hill & Barth, 1977; Hill, 1982; Mosey, 1983; Oi *et al.*, 1984; Bhatia *et al.*, 1985; Bryers, 1985; Bolle *et al.*, 1986; Dalla Torre & Stephanopoulos, 1986; Havlic *et al.*, 1986; Beba & Atalay, 1987; Mata-Alvares, 1987; Wang *et al.*, 1987; Yang & Okos, 1987; Denac *et al.*, 1988; Varfolomeyev *et al.*, 1989; Ozturk *et al.*, 1989; Young, 1989; Zavarzin *et al.*, 1990; Costello *et al.*, 1991a, b; Kalyuzhnyi *et al.*, 1991; McCarty & Mosey, 1991; Angelidaki *et al.*, 1993; Ryhiner *et al.*, 1993; Shimizu *et al.*, 1993).

Mathematical simulation of anaerobic digestion of glucose, one of the key natural organic compounds, has been carried out elsewhere (Mosey, 1983; Oi *et al.*, 1984; Denac *et al.*, 1988; Varfolomeyev *et al.*, 1989; Zavarzin *et al.*, 1990; Costello *et al.*, 1991a, b; Kalyuzhnyi *et al.*, 1991). Most of these models can be categorized into three groups, one where only simplistic Monod-type substrate consumption equations are used (Oi *et al.*, 1984; Denac *et al.*, 1988), one where the process is primarily controlled by the H_2 concentration in the reactor (Mosey, 1983; Zavarzin *et al.*, 1990; Kalyuzhnyi *et al.*, 1991), and one where the influence of pH and volatile fatty acids (VFA) on the process is taken into account (Varfolomeyev *et al.*, 1989; Costello *et al.*, 1991a, b). It is evident that the first group of models simplify the real processes and the second and the third groups give more comprehensive descriptions.

The aim of the present work was to study kinetically the methanogenesis of glucose and its intermediates in batch reactors (Part I) and to work out a mathematical model joining the approaches of the latter two groups of models mentioned above for a more detailed description of kinetic mechanisms of anaerobic digestion (Part II).

METHODS

Reactors

Kinetic investigations (batch cultivation) were performed in non-stirred, 525 ml serum flasks sealed

*Author to whom correspondence should be addressed. Present address: Dept of Chemical Enzymology, Chemistry Faculty, Moscow State University, Moscow 119899, Russia.

with a rubber septum retained with a screw-cap. As a mineral pool, the following medium was used (g/l): NH_4Cl, 2.0; $K_2HPO_4.3H_2O$, 3.6; KH_2PO_4, 2.8; $MgCl_2.6H_20$, 0.3; $Na_2S.9H_20$, 0.08; and trace elements, 2 ml/l (Pfennig & Lippert, 1966). The anaerobic sludge from a laboratory UASB reactor, operating on glucose–acetate synthetic medium, was used as a source of inoculum. The total volume of the liquid phase was 200 ml with initial concentration of volatile suspended solids (VSS) of about 0.3 g/l in all the flasks. The organic substrates for anaerobic digestion were added in concentrations of 1–5 g/l. At the start of each experiment the gas phase of the flasks was flushed with argon. The flasks were then placed in a thermostat at 35°C. All the experiments were carried out in four replicates.

Analyses

Glucose was determined spectrophotometrically using glucose oxidase–peroxidase method (Berezin *et al.*, 1977). VSS analysis was performed according to standard methods (APHA, 1975).

VFA and ethanol were measured using a Chrom-5 gas chromatograph (Czechoslovakia) equipped with a 1 m × 4 mm glass column packed with Chromosorb 101 (100–120 mesh). The column temperature was 180°C, while the injection port and FID detector temperature was 200°C. Argon was used as the carrier gas at a flow rate of 60 ml/min. Before injection, analyzed samples were acidified and centrifuged.

Methane, hydrogen and carbon dioxide were determined using a LHM-8MD gas-chromatograph (USSR) equipped with a thermal conductivity detector and a Porapack QS glass column (80-10 mesh, 2 m × 4 mm). Detector and column temperature was 50°C. Helium served as the carrier gas at a flow rate of 20 ml/min.

Solubility of hydrogen and methane in the medium was neglected. The overall content of CO_2 in the reactor was calculated on the basis of its solubility in the medium depending on temperature, pH and pressure. The pressure in the reactor, increasing constantly in the course of digestion, was measured with a manometer. For consistent presentation of experimental data, the concentration of gaseous products was expressed in relation to the medium in mmol/l (mM).

The tabular form of presentation of the experimental data was mainly used in this paper for supplying more comprehensive information about the process kinetics studied. The graph form of some part of these data will be presented under the validation of the mathematical model (Kalyuzhnyi, 1997).

RESULTS AND DISCUSSION

The kinetic study of batch anaerobic digestion of glucose (Table 1, initial pH 7.0) showed that, besides methane and carbon dioxide as the main final products, some intermediate byproducts (ethanol, acetate, propionate, butyrate and hydrogen), concentrations of which passed through maxima, were also detected in the reactor medium. This reflects the multistep nature of glucose anaerobic digestion which is believed to include three main steps: acidogenesis, acetogenesis and methanogenesis.

The carbon balance calculated on the basis of detected substances (last column of Table 1) had a deficiency through the experiment with a minimum at 23 h. The main reason of the observed deficiency was a consumption of organic matter for the microbial growth because the biomass yield for the glucose methanogenesis can reach 20% and above (Henze & Harremoes, 1983; Mosey, 1983). Thus, the fact that the carbon balance deficiency exceeded these values only in the vicinity of 15–23 h means that we succeeded in detecting practically all principal intermediates in the glucose methanogenesis.

Table 1. Kinetics of anaerobic digestion of glucose at the initial pH 7.0[a]

Time, h	Concentration, mM								pH	Carbon balance, %
	Glucose	Ethanol	Acetate	Propionate	Butyrate	H_2	CO_2	CH_4		
0	10.9	0	0.2	0	0	0	0.3	0	7.0	100
15	1.1±0.1	1.5±0.2	9.5±0.8	1.7±0.3	1.6±0.3	0.06±0.01	7.0±0.7	2.1±0.2	6.55±0.25	74
23	0	1.2±0.2	11.5±0.8	1.8±0.3	1.8±0.3	0.04±0.01	8.0±0.7	3.1±0.2	6.50±0.20	74
33	0	0.7±0.2	12.5±1.0	1.8±0.2	1.7±0.2	0.03±0.0	19.5±0.8	4.5±0.3	6.48±0.19	80
45	0	0.1±0.1	12.7±1.1	1.8±0.2	1.7±0.2	0.02±0.01	9.6±0.7	5.3±0.4	6.43±0.21	80
59	0	0	12.5±1.1	1.7±0.2	1.6±0.2	0.01±0.01	10.7±0.8	5.6±0.5	6.43±0.20	80
71	0	0	11.8±1.0	1.7±0.1	1.6±0.1	0	10.9±1.0	6.8±0.7	6.45±0.18	80
89	0	0	11.1±0.9	1.6±0.2	1.5±0.2	0	12.0±1.2	8.1±0.9	6.51±0.12	80
108	0	0	9.9±0.5	1.5±0.1	1.3±0.1	0	14.5±1.5	10.5±1.0	6.56±0.20	82
130	0	0	8.5±0.4	1.4±0.1	1.0±0.1	0	16.1±1.8	13.5±1.5	6.65±0.12	83
154	0	0	5.1±0.3	1.2±0.1	0.6±0.1	0	19.8±2.0	17.1±1.8	6.80±0.10	80
178	0	0	2.4±0.2	0.8±0.1	0.1±0.1	0	22.9±2.5	22.1±2.1	6.81±0.09	80
199	0	0	0.3±0.1	0.2±0.1	0	0	25.3±2.6	25.1±2.2	6.90±0.10	80

[a]Results expressed as means ± standard error.

The minimum at 15–23 h was probably due to the transient accumulation of lactate (Pipyn & Verstraete, 1981; Eng *et al.*, 1986) which could not be detected by the gas-chromatographic method used in the study.

To obtain stoichiometric coefficients for the products of glucose acidogenic degradation, the bacterial activity in the acetogenic and methanogenic steps was suppressed by performing the experiments at pH 5.0 (Fig. 1). The detected products were hydrogen, carbon dioxide, ethanol, acetate, propionate and butyrate. No methane formation was observed. The dotted line in Fig. 1 corresponds to a supposed 20% consumption of glucose for the biomass growth. Though the carbon balance curve shows that some undetected products were also present within the time range of 15–40 h, we neglected their formation under elaboration of the chemical equation describing the acidogenic step. This was done in order to avoid an excessive complexity of the future mathematical model, taking into account a transient accumulation of these products and their limited influence on the glucose anaerobic digestion as a whole. This simplification, followed by quantitative analysis of the data of Fig. 1 under the conditions of maximal accumulation of the end products, leads to the following acidogenic step equation:

$$C_6H_{12}O_6 \rightarrow 1.14H_2 + 1.14CO_2 + 0.34C_2H_5OH + 1.31CH_3COOH + 0.19C_3H_7COOH + 0.2C_2H_5COOH \quad (1)$$

During anaerobic digestion of intermediates of glucose decomposition, ethanol, propionate and butyrate are first transformed into acetate and hydrogen followed by the methanogenic step (Tables 2 and 3). The corresponding figures are presented elsewhere (Kalyuzhnyi *et al.*, 1991; Davlyatshina, 1993). The following equations describe the chemical conversion in the acetogenic step:

$$C_2H_5OH + H_2O \rightarrow CH_3COOH + 2H_2 \quad (2)$$

$$C_3H_7COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 \quad (3)$$

$$C_2H_5COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 2H_2 \quad (4)$$

Thermodynamic calculations (Thauer *et al.*, 1977) show that reactions (2)–(4) may proceed at a partial pressure of hydrogen less than 0.15, 0.002 and 0.0008 atm, respectively, when concentrations of other substances are within the millimolar range. The shift of equilibrium to products in steps (2)–(4) is promoted by permanent consumption of hydrogen and acetate by methanogens according to known reactions:

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (5)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (6)$$

The second stage of our kinetic study consisted of the investigation of methanogenesis of glucose and its intermediates under variation of their initial concentrations. Some of these results are presented in Fig. 2 and Tables 2 and 3.

The experiments with variation of initial glucose concentration (Fig. 2) showed that there was no noticeable inhibition of methane production up to the initial glucose concentration of 11 mM. Further increase of the initial glucose concentration caused a decrease of the methane formation rate in the time range of 100 h (curves 5 and 7). This is related with the accumulation of VFA leading to a decrease of medium pH up to the values of 6.1–6.3 (curves 6 and 8) which are unfavorable for the methanogenesis. In the course of the following consumption of VFA, the medium pH values were gradually recovered and the methane formation rate was

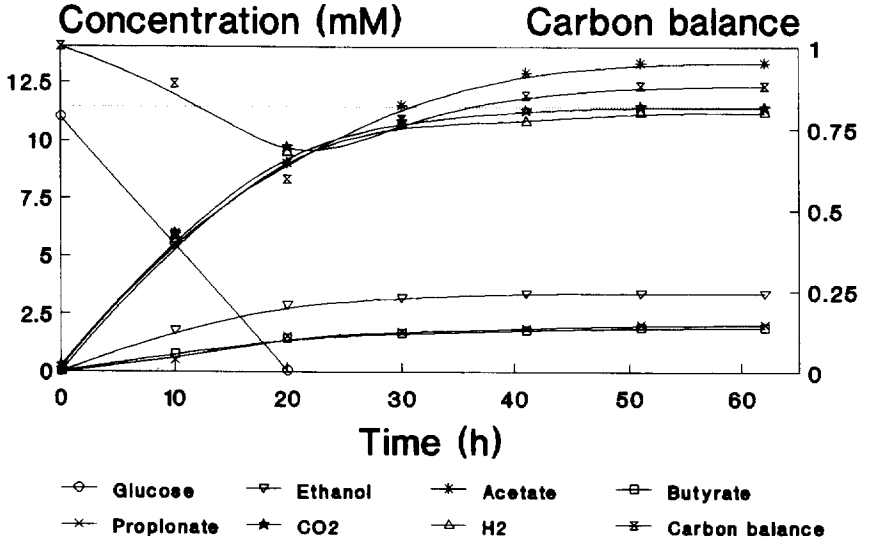

Fig. 1. Kinetics of anaerobic conversion of glucose at the initial pH 5.0.

increased. A strong delay of the methane formation (up to complete failure) was observed under the initial glucose concentration of more than 27 mM (data not shown) due to the fast acidification of the medium up to the pH values of 5.5–5.6.

The results of the variation of initial butyrate concentration are presented in Table 2. The initial rate of methane formation during the first 100 h was slightly inhibited by butyrate (compare, for example, methane concentrations at 87 h under various initial butyrate concentrations). With exhaustion of the butyrate level the inhibition was eliminated. At the same time, at the initial butyrate concentrations more than 47 mM, the inhibition effect became stronger and the conversion process proceeded over a much longer time. Analogous results were obtained under variation of initial propionate concentration (data not shown).

Data on the variation of initial ethanol concentration (Table 3) show that ethanol in the range studied also slightly inhibited the methanogenic step (compare, for example, methane concentrations at 87 h under various initial ethanol concentrations). At the initial ethanol concentration of 78 mM, the conversion process was retarded because of accumulation of hydrogen and acetic acid followed by a decrease of pH. The low pH values, inhibiting the methanogenesis, and high hydrogen concentrations, inhibiting the acetogenic ethanol conversion [eqn (2)], were mainly responsible for the process slowing down because the variation of initial acetate concentration (data not shown) at pH 7.0 did not lead to

Table 2. Kinetics of anaerobic digestion of butyrate under variation of its initial concentrations (initial pH 7.0)[a,b]

Time, h	Concentration, mM			
	Butyrate	Acetate	CO_2	CH_4
Initial butyrate concentration—10 mM				
0	10.0	0.2	0.3	0
16	9.8±0.1	0.8±0.1	0.3±0.1	0.2±0.1
39	9.2±0.4	1.8±0.2	0.6±0.1	1.3±0.2
64	8.2±0.5	2.5±0.2	1.5±0.2	3.4±0.3
87	6.9±0.7	2.8±0.3	2.0±0.2	5.4±0.5
136	3.8±0.5	3.0±0.3	5.5±0.4	11.7±1.2
159	1.8±0.3	2.3±0.3	7.9±0.5	14.9±1.3
184	0.4±0.2	1.5±0.2	10.0±0.6	19.3±1.6
207	0	0.4±0.1	11.9±1.0	21.7±1.7
232	0	0.1±0.1	12.1±1.1	22.2±1.1
Initial butyrate concentration—22 mM				
0	22.0	0.2	0.3	0
16	21.9±0.1	0.7±0.2	0.3±0.1	0.2±0.1
39	21.1±0.2	1.8±0.2	0.2±0.1	0.9±0.2
64	19.9±0.3	2.9±0.2	0.5±0.2	2.2±0.3
87	18.0±0.5	4.4±0.3	1.0±0.2	5.3±0.5
136	14.5±0.8	6.8±0.6	2.7±0.3	10.7±1.1
159	12.1±1.4	7.9±0.5	4.7±0.5	14.3±1.4
184	9.5±0.9	8.8±0.7	6.6±0.7	19.1±1.7
207	7.4±1.0	9.1±0.6	9.5±1.1	24.8±2.3
232	4.7±0.8	7.5±0.6	14.4±1.1	30.4±2.8
257	2.7±0.5	4.0±0.8	19.0±2.1	34.9±3.3
307	0	0.3±0.1	23.6±2.5	44.9±3.1
333	0	0.1±0.1	25.2±2.4	46.3±4.1
Initial butyrate concentration—47 mM				
0	47.0	0.2	0.2	0
16	46.8±0.3	1.3±0.1	0.2±0.1	0.1±0.1
39	46.6±0.5	2.4±0.2	0.1±0.1	0.5±0.2
64	46.3±0.6	3.5±0.3	0.1±0.1	1.4±0.3
87	44.9±1.1	4.9±0.4	0.1±0.1	2.9±0.4
136	41.9±2.8	9.0±1.3	0.2±0.1	7.8±1.0
159	39.7±3.4	11.1±1.4	0.3±0.1	11.1±1.3
184	36.3±3.9	13.7±1.4	0.6±0.2	13.9±1.6
207	33.1±3.0	15.5±1.5	1.0±0.3	17.1±1.2
232	30.7±2.8	17.5±1.8	1.5±0.3	21.9±2.1
257	27.0±2.5	19.9±1.8	2.0±0.3	25.0±2.1
307	21.7±2.0	23.7±2.0	5.6±0.7	35.9±4.0
333	16.8±1.7	25.1±2.2	7.3±1.1	41.4±4.1
377	11.7±1.2	25.1±2.0	14.6±1.4	47.0±3.1
404	8.2±1.0	22.8±1.5	32.6±3.5	57.2±4.1
464	1.0±0.4	13.1±1.9	38.6±2.5	78.1±6.5

[a]Results expressed as means±standard error.
[b]Concentration of hydrogen was negligible during all the experiments.

any inhibition of methane formation up to the acetate concentration of 83 mM.

In the third stage of our kinetic investigation, we studied methanogenesis of various mixtures of substrates to more exactly reveal their mutual influence. Some of these data are presented in Tables 4 and 5 and Fig. 3.

The conversion of the glucose–butyrate mixture was practically completed after 200 h (Table 4). The initial increase in butyrate level was due to its formation under acidogenic glucose decomposition. The two initial substrates were digested successively and the overall conversion process was composed of two stages, glucose methanogenesis (up to 50 h) followed by butyrate methanogenesis. The delay of butyrate conversion was related to transient accumulation of hydrogen up to its partial pressure, making reaction (3) thermodynamically unfavorable [for example, the hydrogen partial pressure in the system was less than 0.002 atm only after 23 h of the process (Table 4)].

The study of conversion of the ethanol–butyrate mixture (Table 5) showed that butyrate was practically not converted until the ethanol level was exhausted. The main reason for the delayed butyrate conversion is again related to hydrogen, which plays an essential role in methanogenic systems. It is known that the hydrogen level in such systems is determined by the balance between the rates of hydrogen evolution and consumption (the latter is carried out by hydrogen-utilizing methanogens). Returning to the ethanol–butyrate case, one can say that increased hydrogen evolution according to the reaction (2) creates a hydrogen level sufficient to block the reaction (4). This is really so because the measurements showed that the partial pressure of hydrogen exceeded 0.002 atm until complete exhaustion of ethanol in the system (Table 5).

Table 3. Kinetics of anaerobic digestion of ethanol under variation of its initial concentrations (initial pH 7.0)[a]

Time, h	Concentration, mM				
	Ethanol	Acetate	H_2	CO_2	CH_4
Initial ethanol concentration—21.1 mM					
0	21.1	0.2	0	0.3	0
16	18.9±0.5	1.5±0.1	1.13±0.13	0.1±0.1	0.8±0.1
39	15.1±0.6	3.0±0.2	1.19±0.12	0.1±0.1	4.3±0.3
64	9.5±0.7	6.2±0.3	1.15±0.12	0.1±0.1	7.7±0.5
87	3.4±0.6	7.5±0.5	0.75±0.07	0.2±0.1	11.7±1.0
106	0	8.5±0.3	0.61±0.05	0.2±0.1	17.7±1.2
136	0	3.2±0.3	0	4.2±0.5	24.0±2.1
159	0	0.6±0.1	0	7.1±0.6	25.8±1.6
184	0	0.1±0.1	0	7.3±1.0	27.2±1.3
Initial ethanol concentration—38.5 mM					
0	38.5	0.2	0	0.3	0
16	37.4±0.9	1.5±0.1	1.25±0.13	0.1±0.1	0.3±0.1
39	35.9±0.8	3.6±0.1	1.52±0.12	0.1±0.1	4.4±0.4
64	32.9±1.3	5.9±0.3	2.11±0.12	0.1±0.1	5.2±0.5
87	27.5±1.5	7.2±0.7	1.65±0.12	0.1±0.1	10.2±0.8
106	24.5±0.8	8.3±0.8	1.51±0.11	0.1±0.1	14.2±1.1
136	18.1±1.4	9.5±0.8	1.41±0.11	0.1±0.1	21.3±1.7
159	9.9±0.9	10.6±1.4	1.28±0.16	0.1±0.1	25.3±1.9
184	4.6±0.8	12.1±1.5	0.62±0.05	0.1±0.1	32.4±2.8
207	0	13.7±1.6	0.51±0.05	0.2±0.1	39.2±3.8
232	0	5.9±0.4	0	6.9±0.8	42.8±3.4
257	0	0.5±0.1	0	12.8±1.1	47.2±4.2
Initial ethanol concentration—77.8 mM					
0	77.8	0.2	0	0.3	0
16	76.6±0.5	0.4±0.1	2.11±0.19	0.1±0.1	0.4±0.1
39	74.5±0.9	0.7±0.1	2.60±0.28	0.1±0.1	3.4±0.2
64	72.9±1.6	4.6±0.5	3.12±0.32	0.1±0.1	4.9±0.3
87	68.1±2.1	6.5±0.5	3.25±0.21	0.1±0.1	7.4±0.5
106	67.1±2.8	7.0±0.5	3.02±0.22	0.1±0.1	10.5±1.0
136	60.2±3.1	7.3±0.4	2.97±0.21	0.1±0.1	12.4±1.2
159	57.1±2.9	8.4±0.7	2.59±0.21	0.1±0.1	16.2±1.3
184	53.2±3.0	9.7±1.0	2.37±0.21	0.1±0.1	19.1±1.5
207	46.8±2.7	11.3±0.8	2.26±0.10	0.1±0.1	22.5±2.0
232	43.5±2.4	12.9±1.0	2.17±0.11	0.2±0.1	23.5±1.1
257	40.9±2.1	13.6±1.1	2.15±0.10	0.1±0.1	24.8±2.0
307	38.2±2.2	15.7±1.2	2.04±0.10	0.2±0.1	29.3±3.1
333	36.9±2.2	18.5±1.3	2.03±0.10	0.2±0.1	31.2±2.1
404	34.9±2.0	19.9±1.5	1.53±0.11	0.1±0.1	36.3±3.1
464	32.7±1.5	21.1±1.1	1.03±0.11	0.2±0.1	39.1±3.5

[a]Results expressed as means±standard error.

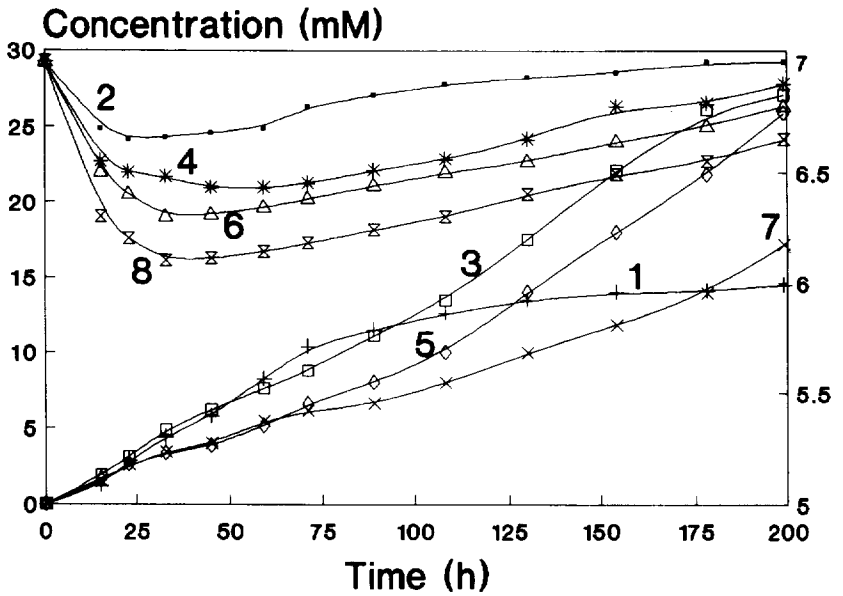

Fig. 2. Kinetics of methane formation and pH under variation of initial glucose concentrations: 5.6 mM (1—methane; 2—pH); 10.9 mM (3—methane; 4—pH); 12.9 mM (5—methane; 6—pH); 16.7 mM (7—methane; 8—pH).

Similar results were obtained in the study of methanogenesis of the ethanol–propionate mixture (Fig. 3), only the propionate conversion needed more strict conditions on hydrogen concentration. It is seen that for a decrease of the hydrogen level in the system during ethanol degradation, the metha-

Table 4. Kinetics of anaerobic digestion of glucose–butyrate mixture at the initial pH 7.0[a]

Time, h	Concentration, mM								P_{H_2}', atm
	Glucose	Butyrate	Ethanol	Acetate	Propionate	CO_2	CH_4	H_2	
0	5.6	10.0	0	0.2	0	0.3	0	0	0
15	0	11.0±0.3	0.1±0.1	4.4±0.6	0.6±0.1	1.7±0.1	0.8±0.1	0.14±0.03	0.00217
23	0	11.5±0.4	0	6.0±0.6	0.6±0.1	3.0±0.2	1.9±0.2	0.06±0.01	0.00093
46	0	10.8±0.4	0	6.8±0.5	0.6±0.1	4.6±0.4	4.8±0.3	0	
67	0	9.8±0.7	0	6.1±0.8	0.5±0.1	7.3±0.7	7.5±0.8	0	
111	0	6.9±0.5	0	6.0±0.5	0.5±0.1	9.3±0.9	12.6±1.4	0	
136	0	5.3±0.5	0	5.1±0.5	0.5±0.1	11.9±1.3	15.9±1.6	0	
165	0	3.2±0.3	0	4.6±0.3	0.4±0.1	15.9±1.5	22.2±1.9	0	
185	0	1.5±0.1	0	3.3±0.2	0.3±0.1	19.1±1.9	27.8±2.0	0	
208	0	0.3±0.1	0	2.3±0.2	0.1±0.1	21.7±1.8	32.5±1.5	0	

[a]Results expressed as means ± standard error.

Table 5. Kinetics of anaerobic digestion of ethanol–butyrate mixture at the initial pH 7.0[a]

Time, h	Concentration, mM						P_{H_2}, atm
	Ethanol	Butyrate	Acetate	CO_2	CH_4	H_2	
0	21.0	10.0	0.2	0.2	0	0	0
14	19.5±0.5	10.1±0.1	1.9±0.1	0.4±0.1	0.5±0.1	1.5±0.1	0.0235
45	16.0±0.8	10.2±0.3	4.8±0.3	0.3±0.1	3.5±0.3	1.7±0.2	0.0268
65	12.9±1.5	9.8±0.4	5.5±0.5	0.2±0.2	5.6±0.5	1.6±0.2	0.0246
110	6.7±1.2	9.6±0.4	7.2±0.7	0.2±0.2	10.3±1.1	1.4±0.1	0.0221
134	3.8±0.5	9.6±0.5	8.7±1.0	1.1±0.2	13.7±1.5	1.3±0.1	0.0204
163	0	9.8±0.5	10.1±1.5	1.3±0.3	17.9±2.2	0.7±0.1	0.0114
183	0	9.2±0.9	8.7±1.0	1.5±0.4	20.3±2.5	0.2±0.1	0.0026
206	0	8.8±0.8	7.6±0.8	2.2±0.3	23.6±2.9	0	0
229	0	7.6±0.5	5.8±0.6	5.4±0.7	29.6±3.0	0	0
282	0	5.8±0.5	2.9±0.4	10.7±1.0	35.1±3.1	0	0
309	0	4.2±0.3	1.6±0.2	12.4±1.1	39.2±3.2	0	0
339	0	2.1±0.2	1.2±0.1	14.5±1.2	41.9±3.6	0	0

[a]Results expressed as means ± standard error.

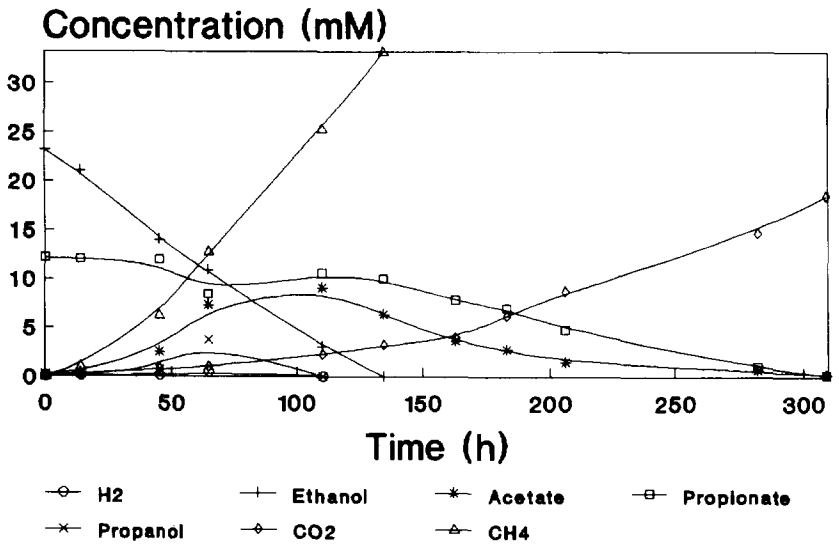

Fig. 3. Kinetics of anaerobic conversion of a mixture of ethanol and propionate at the initial pH 7.0.

nogenic bacterial consortium carried out the transient transformation of propionate into propanol:

$$C_2H_5COOH+2H_2 \rightarrow C_3H_7OH+H_2O \qquad (7)$$

In the course of decreasing hydrogen level in the system reaction (7) began to proceed in the reverse direction, as seen in Fig. 3. For a larger decrease of hydrogen partial pressure, the acetogenesis from propionate by equation (3) took place.

Some other kinetic experiments on the conversion of glucose and its intermediates (subsequent addition of substrates via definite time, variation of initial pH, sharp change of pH, etc.) have also been carried out (Davlyatshina, 1993) to more precisely define the impact of these factors on the methanogenesis process. Some of them will be presented in Part II of this investigation when establishing the credibility of the mathematical model (Kalyuzhnyi, 1997).

CONCLUSIONS

This study shows that chemical conversion under batch anaerobic digestion of glucose obeys, in general, the scheme (1)–(6). Among the factors influencing the process kinetics, H_2 concentration and pH value have the primary significance. For a decrease of the hydrogen level in a completely closed system during degradation of hydrogenic substrates, the methanogenic bacterial consortium can transiently form more reduced intermediates (for example, propanol). Slight inhibition of the methanogenic step by the excess of butyrate, propionate and ethanol was also noticed.

The mathematical model based on information obtained in this study will be described in Part II of this investigation (Kalyuzhnyi, 1997).

REFERENCES

APHA (1975). *Standard Methods for the Examination of Water and Wastewater*, 14th edn. American Public Health Association, Washington, DC.

Andrews, J. F. (1969). A dynamic model of the anaerobic digestion process. *J. Sanit. Engng Div. ASCE*, **95-SA1**, 95–110.

Angelidaki, I., Ellegaard, L. & Ahring, B. K. (1993). A mathematical model for dynamic simulation of anaerobic digestion of complex substrates: focusing on ammonia inhibition. *Biotechnol. Bioengng*, **42**, 159–166.

Beba, A. & Atalay, F. S. (1987). Mathematical models for methane production in batch fermenters. *Biomass*, **11**, 173–184.

Berezin, I. V., Rabinovich, M. L. & Sinitsyn, A. P. (1977). Investigation of possibilities of kinetic spectrophotometric method of determination of glucose. *Biokhimiya*, **9**, 1631–1641.

Bhatia, D., Vieth, W. R. & Venkatasubramanian, K. (1985). Steady-state and transient behavior in microbial methanification: II. Mathematical modeling and verification. *Biotechnol. Bioengng*, **27**, 1199–1207.

Bryers, J. D. (1985). Structured modeling of the anaerobic digestion of biomass particulates. *Biotechnol. Bioengng*, **27**, 638–649.

Bolle, W. L., van Breugel, J., van Eybergen, G. C., Kossen, N. W. F. & van Gils, W. (1986). An integral dynamic model for the UASB reactor. *Biotechnol. Bioengng*, **28**, 1621–1636.

Costello, D. J., Greenfield, P. F. & Lee, P. L. (1991a). Dynamic modelling of a single-stage high-rate anaerobic reactor—I. Model derivation. *Water Res.*, **25**, 847–858.

Costello, D. J., Greenfield, P. F. & Lee, P. L. (1991b). Dynamic modelling of a single-stage high-rate anaerobic reactor—II. Model verification. *Water Res.*, **25**, 859–871.

Dalla Torre, A. & Stephanopoulos, G. (1986). Mixed culture model of anaerobic digestion: application to the evaluation of startup procedures. *Biotechnol. Bioengng*, **28**, 1106–1118.

Davlyatshina, M. A. (1993). Kinetic regularities and mathematical modeling of methanogenesis. Ph.D. Thesis, Moscow State University, Moscow, Russia.

Denac, M., Miguel, A. & Dunn, I. J. (1988). Modeling dynamic experiments on the anaerobic degradation of molasses wastewater. *Biotechnol. Bioengng*, **31**, 1–10.

Eng, S. C., Fernandes, X. A. & Paskins, A. R. (1986). Biochemical effects of administering shock loads of

sucrose to a laboratory-scale anaerobic (UASB) effluent treatment plant. *Water Res.*, **20**, 789–794.

Graef, S. P. & Andrews, J. F. (1974). Mathematical modelling and control of anaerobic digestion. *AICHE Symp.*, **136**, 101–131.

Havlic, I., Votruba, J. & Sobotka, M. (1986). Mathematical modeling of the anaerobic digestion process: application of dynamic mass-energy balance. *Folia Microbiol.*, **31**, 56–67.

Henze, M. & Harremoes, P. (1983). Anaerobic treatment of wastewater in fixed film reactor—a literature review. *Water Sci. Technol.*, **15**, 1–101.

Hill, D. T. (1982). A comprehensive dynamic model for animal waste methanogenesis. *Trans. ASAE*, **25**, 1374–1380.

Hill, D. T. & Barth, C. L. (1977). A dynamic model for simulation of animal waste digestion. *J. Water Pollut. Control Fed.*, **10**, 2129–2143.

Kalyuzhnyi, S. V., Gachok, V. P., Varfolomeyev, S. D. & Sklyar, V. I. (1995). Kinetic investigation and mathematical modeling of methanogenesis of glucose. *Appl. Biochem. Biotechnol.*, **28–29**, 183–195.

Kalyuzhnyi, S. V. (1997). Batch anaerobic digestion of glucose and its mathematical modeling. II. Description, verification and application of model. *Biores. Technol.* (in press).

Mata-Alvares, J. (1987). A dynamic simulation of a two-phase anaerobic digestion system for solid wastes. *Biotechnol. Bioengng*, **30**, 844–851.

McCarty, P. L. & Mosey, F. E. (1991). Modelling of anaerobic digestion process (a discussion of concepts). *Water Sci. Technol.*, **24**, 17–33.

Mosey, F. E. (1983). Mathematical modeling of anaerobic digestion process: regulatory mechanisms for the formation of short-chain volatile acids from glucose. *Water Sci. Technol.*, **15**, 209–217.

Oi, S., Tamura, S., Nukina, Y., Tanaka, T. & Taniguchi, M. (1984). A typical methane fermentation of glucose. *Agric. Biol. Chem.*, **48**, 1329–1331.

Ozturk, S. S., Palsson, B. O. & Thiele, J. H. (1989). Control of interspecies electron transfer flow during anaerobic digestion: dynamic diffusion reaction models for hydrogen gas transfer in microbial flocs. *Biotechnol. Bioengng*, **33**, 745–757.

Pfennig, N. & Lippert, K. D. (1966). Über das Vitamin B_{12}–Bedürfnis phototropher Schwefelbakterien. *Arch. Microbiol.*, **55**, 245–256.

Pipyn, P. & Verstraete, W. (1981). Lactate and ethanol as intermediates in two-phase anaerobic digestion. *Biotechnol. Bioengng*, **28**, 1145–1154.

Ryhiner, G. B., Heinzle, E. & Dunn, I. J. (1993). Modeling and simulation of anaerobic wastewater treatment and its application to control design: case whey. *Biotechnol. Prog.*, **9**, 332–343.

Shimizu, T., Kudo, K. & Nasu, Y. (1993). Anaerobic waste-activated sludge digestion—a bioconversion mechanism and kinetic model. *Biotechnol. Bioengng*, **41**, 1082–1091.

Thauer, R. K., Jungerman, K. & Decker, K. (1977). Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. Rev.*, **41**, 100–180.

Varfolomeyev, S. D., Kalyuzhnyi, S. V. & Spivak, S. I. (1989). Kinetic regularities of methane production by a methanogenic association, II. Mathematical modeling. *Appl. Biochem. Biotechnol.*, **22**, 351–360.

Wang, Y.-T., Suidan, M. T. & Rittman, B. E. (1987). Modeling biofilm kinetics for a low-loaded expanded-bed anaerobic reactor. *Biotechnol. Bioengng*, **30**, 15–21.

Yang, S. T. & Okos, M. R. (1987). Kinetic study and mathematical modeling of methanogenesis of acetate using pure cultures of methanogens. *Biotechnol. Bioengng*, **30**, 661–667.

Young, A. (1989). Mathematical modeling of landfill degradation. *J. Chem. Technol. Biotechnol.*, **46**, 189–208.

Zavarzin, G. A., Kalashnikov, V. V., Kevbrin, V. V. & Petrov, S. T. (1990). Modeling of methanogenic community, Izvestiya AN SSSR. *Ser. Biol.*, **N1**, 116–124.