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EXPERIMENTAL ARTICLES

Alkaliphilic Sulfidogenesis on Cellulose by Combined Cultures

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Abstract—Soda lakes are characterized by an intense sulfur cycle that begins with sulfidogenesis. Model laboratory experiments that involved combining of pure cultures showed that, during anaerobic decomposition of cellulose by *Clostridium alkalicellulosi*, the sulfate-reducing bacteria (SRB) of the species *Desulfonatronovibrio hydrogenovorans, Desulfonatronum lacustre*, and *Desulfonatronum cooperativum*, different in their nutritional requirements, may directly use the cellulose fermentation products for sulfidogenesis without mediatory microorgansims. In binary cocultures with SRB, the amount of the H_2S formed constituted from one-third to two-thirds of the cellulose [H] equivalents; acetate was among the products formed. When the syntrophic *Contubernalis alkalaceticum*, capable of acetate oxidation, was incorporated into the trophic chain along with hydrogenotrophic SRB, the amount of the H_2S formed exceeded by 33–42% the amount of the [H] equivalents in the utilized cellulose, water being the source of additional hydrogen. Thus, the trophic pathway from plant residues to sulfide, previously considered to be the longest in the alkaliphilic microbial community, may involve a minimal number of stages and do without intermediate participation of dissipotrophic fermenting organisms.

Key words: soda lakes, alkaliphiles, sulfidogenesis, anaerobic decomposition of cellulose, combined cultures.

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The excess of sulfate in mineralized soda lakes allows for intense operation of the sulfur cycle, which is most markedly pronounced in them. In this ecosystem, it is the final stage of organic matter decomposition, carried out by sulfate-reducing bacteria (SRB) of the genera *Desulfonatronovibrio* [1] and *Desulfonatronum* [2], and, in hypersaline soda lakes, by the novel representatives of the order *Desulfovibrionales* and the family *Desulfobacteriaceae* [3]. The sulfur cycle is normally completed in the photic zone with the development of various anaerobic phototrophic sulfur bacteria [4] and the aerobic haloalkaliphilic sulfur-oxidizing bacteria of the genera *Thioalkalivibrio, Thioalkalimicrobium*, and *Thioalkalispira* [5], which oxidize sulfide and regenerating sulfate.

The source of reducing power for sulfidogenesis is allochthonous and autochthonous vegetation. Decomposition of plant residues, which include cellulose as the main carbohydrate biopolymer is usually a multistage process carried out via a number of reactions catalyzed by specific microorganisms and completed by secondary anaerobes. For soda lakes, the sequence of the biochemical stages of cellulose decomposition by anaerobes was traced by us by the example of the microbial communities of Central Asia lakes having low mineralization and pH 10 [6]; the corresponding microorganisms have been identified and described [7–12]. Cellulose decomposition included the first, hydrogen, phase, determined by cellulose hydrolysis by *Clostridium alkalicellulosi* [11] and the development of the dissipotrophic saccharolytics *Anoxynatronum sibiricum* [7], *Alkalibacter saccharofermentans* [8], and *Alkaliflexus imshenetskii* [9], and the stage of direct or syntrophic decomposition of their fermentation products to sulfide with the involvement of the hydrogenotrophic SRB *Desulfonatronum cooperativum* [10] and the obligate syntroph *Contubernalis alkalaceticum*, which oxidized acetate in the presence of the hydrogenontroph [12].

Is the above chain of reactions always operative or are shorter pathways for sulfidogenesis also possible in the course of anaerobic decomposition of organic matter in the alkaliphilic community? This question can be answered by investigating combinations of pure cultures with known properties. Several such interactions in pairs composed of hydrogenotrophic sulfate reducers and sugar-utilizing spirochetes or other dissipotrophs were described by us earlier [8, 13]. When these microorganisms grew in combination on carbohydrates, complete removal of hydrogen and/or formate by SRB occurred with concomitant sulfide formation; this resulted in an acetogenic shift in the fermentation products of the saccharolytics and in acetate accumulation.

The aim of this work was to study the shortest trophic pathway from a solid hydrolysable material to hydrogen sulfide as exemplified by solid-phase fermentation of microcrystalline cellulose by combined cultures; this study became feasible with the isolation of

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Table 1. Strains of alkaliphilic anaerobic bacteria used in this work and their trophic capacities

Strains	Substrate/product					
	MCC $n[C_6H_{12}O_6]$	Lactate $C_3H_6O_3$	Ethanol C_2H_6O	Acetate $C_2H_4O_2$	H_2	H_2S
	[H]-equiv: 12	[H]-equiv: 6	[H]-equiv: 6	[H]-equiv: 4	[H]-equiv: 2	[H]-equiv: 8
Cl. alkalicellulosi Z-7026^T	+	+*	+*	+*	+*	–*
Dnv. hydrogenovorans Z-7952	–	–	–	–	+	+*
Dn. lacustre Z-7951^T	–	–	+	–	+	+*
Dn. cooperativum Z-7999^T	–	+	–	–	+	+*
Syntrophic culture *Cn. alkalaceticum* + *Dn. cooperativum*	–	+	+	+	+	+*
Cn. alkalaceticum + *Dn. cooperativum* + *Dn. lacustre*	–	+	+	+	+	+*

the alkaliphilic anaerobic cellulolytic *Cl. alkalicellulosi* and the acetate-oxidizing syntroph *Cn. alkalaceticum* and due to the availability of a collection of SRB utilizing various sets of substrates. One of the tasks of the study was to assess the influence of different SRB and the syntroph on the completeness of cellulose decomposition in combined cultures.

MATERIALS AND METHODS

Strains. The following SRB strains were used: *Desulfonatronovibrio hydrogenovorans* Z-7952 [14] and *Desulfonatronum lacustre* Z-7951^T (= DSM 10312) [13], isolated from the Tuva lakes with a low mineral content (Central Asia, Russia); and strains *Clostridium alkalicellulosi* Z-7026^T (= VKM B 2349 = DSM 17461) [11], *Desulfonatronum cooperativum* Z-7999^T (= VKM B 2329 = DSM 16749) [10], and the obligately syntrophic *Contubernalis alkalaceticum* Z-7904 (= VKM B 2362 = DSM 18223) [12], isolated from Lake Verkhnee Beloe (Buryat Republic, Russia).

Cultivation conditions. The optimal media for the isolates from different soda lakes used in this work somewhat differed in composition but were all based on carbonate/bicarbonate buffer with pH 9–9.5 and had a low mineral content (20–30 g/l). Therefore, cocultivation was possible.

The cultivation medium used in this work had the following composition (g/l): NH_4Cl, 0.5; KH_2PO_4, 0.2; $MgCl_2 \cdot 6H_2O$, 0.1; KCl, 0.2; Na_2CO_3, 6; $NaHCO_3$, 10; NaCl, 1; Na_2SO_4, 3; yeast extract (Becton Dickinson), 0.2; trace element solution, 1ml/l [15]; sodium thioglycollate, 0.25; pH 9.0. Medium preparation, inoculation, and culture sampling were carried out according to the requirements of the anaerobic cultivation technique, in an atmosphere of N_2. The cultures were grown in 120-ml flasks with 70 ml of medium into which 70 mg of Sigmacell 101 microcrystalline cellulose (MCC) was introduced, which corresponded to 432 μmol of hexose. The amount of cellulose was chosen proceeding, on the one hand, from the necessity to ensure its full utilization and, on the other hand, by the necessity of avoiding change in medium pH.

Incubation. The flasks with the medium where MCC was the only donor and sulfate served as a possible electron acceptor for SRB were inoculated with 1% inoculum of *Cl. alkalicellulosi* (10^8 cells/ml), pregrown on medium of the same composition. Then, according to the combinations shown in Table 1, SRB (10^6 cells/ml) and/or a syntrophic association (Z-7904, 10^5 cells/ml + Z-7999, 10^7 cells/ml) were additionally introduced (1% inocula). Incubation was carried out in a thermostat at 35°C in the dark.

Analytical determinations. The formation and consumption of acids and hydrogen in the dynamics of culture growth were measured quantitatively using a Staier chromatograph (ZAO NPKF Akvilon, Russia) with an UV detector (220 nm). A REZEX ROA column (7.8 × 300 mm) (Phenomenex, USA) was used. The eluting agent was 0.2% H_3PO_4; the flow rate was 0.5 ml/min; the sample volume was 20 μl.

The analysis of alcohols was carried out on a Kristall-5000 gas chromatograph (ZAO Khromatek, Russia) equipped with a flame-ionization detector. A Superox-FA column (10 m × 0.53 mm × 1.2 mm) (Alltech, USA) was used; the temperature was programmed to change from 60 to 160°C; helium was the carrier gas; the flow rate was 50 cm/min without partition; the sample volume was 1 μl.

Dissolved sulfide was determined by methylene blue formation using a modified Pachmayr colorimetric method with N,N-dimethyl-*p*-phenylenediamine [16].

The scheme of the experiment and balance calculations. The trophic capacities of the strains used are shown in Table 1. The table does not list formate, which is utilized by all of the SRB strains used but which was revealed only in trace amounts when cellulose was fermented by *Cl. alkalicellulosi*, as distinct from the case with *Alkalibacter saccharofermentans* [8], where it was among the main metabolites. Cellulose was decomposed by *Cl. alkalicellulosi*, whose substrate could be native straw of the cereal *Nardus*, filaments of the green alga *Cladophora sivashensis*, and various other forms of cellulose, including MCC. The earlier determination of the balance of cellobiose fermentation by *Cl. alkalicellulosi* showed that the main products of its metabolism are lactate, ethanol, acetate, H_2, and trace amounts of formate [11], which, supposedly, could not provide for full-fledged sulfidogenesis in combination with hydrogenotrophic SRB. Cellobiose fermentation by *Cl. alkalicellulosi* approximately corresponds to the following equation [11]:

$$\underset{\text{cellobiose}}{6\,(C_{12}H_{22}O_{11})} = \underset{\text{lactate}}{12\,(C_3H_6O_3)} + \underset{\text{acetate}}{2\,(C_2H_4O_2)} + \underset{\text{ethanol}}{6\,(C_2H_6O)} + 6H_2 + xCO_2.$$

The fermentation balance is 97% closed by [H] but not by carbon (19 g-atoms of C deficiency) and oxygen (18 g-atoms of O deficiency), which would correspond to the formation of the formate, revealed, however, only in trace amounts. The element balance is as follows: 72 C, 132 H, and 66 O in the substrates and 53 C, 130 H, and 46 O in the products.

For each glucose molecule to be formed from cellobiose, a molecule of water is required, and, accordingly, 20% of [H] comes from water. In our experiments, 70 mg of cellulose $n[C_6H_{10}O_5]$ with a molecular mass of 162 corresponds to 0.432 mmol of hexose and contains 4.32 g-atoms of [H]. Hydrolysis involves additionally 0.86 g-atom of [H]. Hence, the [H] content of 70 mg of cellulose after hydrolysis corresponds to 5180 mg-atoms of [H].

Table 1 shows that various alkaliphilic SRB combined with a cellulose-decomposing anaerobe could use up all the cellulose degradation products in two- or three-stage reactions to form hydrogen sulfide if the syntrophic *Cn. alkalaceticum* was recruited, capable, in case of H_2 removal by hydrogenotrophic SRB, of carrying out the reaction

$$C_2H_4O_2 + 2H_2O = 2CO_2 + 8[H]. \quad (2)$$

The simplest calculation for alkaliphilic anaerobes is based on the hydrogen balance, because determination of CO_2 production in soda medium does not seem feasible. Hydrogenotrophic SRB carry out the following reaction:

$$4H_2 + SO_4^{2-} = 4H_2O + S^{2-}. \quad (3)$$

When calculating the reducing equivalents necessary for sulfate reduction, the 8 [H] value used, because what is formed is not only hydrogen sulfide but also water. Taking this into account, we can approximately calculate the balance of reducing equivalents during sulfidogenesis on fermented cellulose. The estimate of the [H] equivalents of cellulose $n[C_6H_{10}O_5]$ can be made in two ways, directly ([H] : 10) or considering hydrolysis ([H] : 12).

The actual pathway of hydrolysis in *Cl. alkalicellulosi* hydrolysis is not known; it might proceed via phosphorolysis, as in *Cl. thermocellum*. For calculations, we did not resort to the suggestions [17] about available electrons, since the system was simple enough, and we were only interested in the general result attainable with the method used. The [H] equivalents are shown in Tables 1–6.

When solid-phase fermentation is carried out, three phases—solid, liquid, and gaseous—must be taken into account. Therefore, apart from determining the fermentation products, it was necessary to know the loss of cellulose as well, which was only possible to determine at the end of the process, assuming the substrate was fully consumed. The biomass yield had to be neglected as an insignificant value and because of the possible hindrance from residues of unfermented cellulose.

Different growth rates of the organisms and their different initial numbers pose a formidable problem when experiments with combined cultures are performed; very different results may be obtained when a SRB is introduced into a culture of a cellulolytic and vice versa: in the latter case, the metabolism products are used up very quickly; in the former, they are initially accumulated. Judgments on the character of the process made based on product dynamics may be erroneous, since the concentration of quickly metabolized products is extremely low, while products utilized slowly accumulate, forming pools. Therefore, we took into account both the concentrations of dissolved products and the completeness of utilization of the solid phase as evaluated from hydrogen equivalents accumulated in H_2S.

Table 2. Cellulose fermentation by a pure culture of *Cl. alkalicellulosi*

Days of growth	$V_{liquid\ phase}$, ml	$V_{gaseous\ phase}$, ml	Measurement units	Metabolic products					Σ [H]	Balance, [H]%
				lactate	acetate	ethanol	H_2S	H_2*		
0	70	50	mM	0	0	0	1.62	2		
			µmol/V	0	0	0	113.4	4.5×10^{-3}		
			[H] equiv							
6	68	52	mM	1.2	0.5	3.5	0	24000		
			µmol/V	82	34	238	0	56		
			[H] equiv	492	136	1428	0	112	2168	42
9	66	54	mM	1.8	0.8	5.3	0	18700		
			µmol/V	119	53	350	0	45		
			[H] equiv	714	212	2100	0	90	3116	60
14	64	56	mM	2.2	0.9	5.6	0	18250		
			µmol/V	141	58	358	0	46		
			[H] equiv	846	232	2148	0	92	3318	64
20	62	58	mM	2.3	0.9	5.0	0	18000		
			µmol/V	143	56	310	0	47		
			[H] equiv	858	224	1860	0	94	3036	57
28	60	60	mM	2.4	0.8	5.7	0	18000		
			µmol/V	144	48	342	0	49		
			[H] equiv	864	192	2052	0	98	3206	62
40	58	62	mM	2.8	1.0	5.1	0	18000		
			µmol/V	162	58	296	0	50		
			[H] equiv	972	232	1776	0	100	3080	59

Notes: The initial amount of cellulose was 432 µmol per vessel, which corresponded to 5180 [H] equivalents taking into account hydrolysis. The amounts of H_2S and H_2 are given minus their concentration at the zero growth point. Σ denotes the sum of [H] equivalents in products. The balance shows in % the use of the cellulose [H] equivalents.

% H_2S shows % of [H] equivalents of H_2S in the sum of products.

* The H_2 concentrations are given in ppm.

RESULTS AND DISCUSSION

The results of the dynamics of cellulose fermentation by various culture combinations are shown in Tables 2–6 and in the figure.

When cellulose was fermented by a pure culture of *Cl. alkalicellulosi* (Table 2 and Fig. 1a), the end products contained about 60% of the substrate [H], which indicated incomplete fermentation. The process was virtually completed in nine days, with ethanol as the main reduced fermentation product. Hydrogen appeared at the first stage of hydrolysis, accumulated, and, after an insignificant decline on the ninth day, remained at a level of about 18000 ppm. No sulfide was formed, and even a small amount (1.6 mM) of it introduced into the medium with thioglycollate as a reducing agent gradually disappeared.

In a binary culture with *Dnv. hydrogenovorans*, which utilizes only H_2 (Table 3 and Fig. 1b), a more complete utilization of the cellulose [H] (approximately 90%) occurred, and the process was completed in 28 days. Hydrogen was utilized immediately; its content after 6–14 days exceeded the background 5 ppm only by a factor of 8–12, and it was not detectable later. In the process, a typical acetogenic shift with acetate accumulation occurred at the expense of a lesser amount of ethanol. The lactate content also increased in the course of fermentation. Sulfidogenesis was moderate, and the maximum amount of the H_2S formed corresponded to one-third of the cellulose [H]. Another one-third of the cellulose [H] was expended on the lactate, which was formed and not used; the remaining cellu-

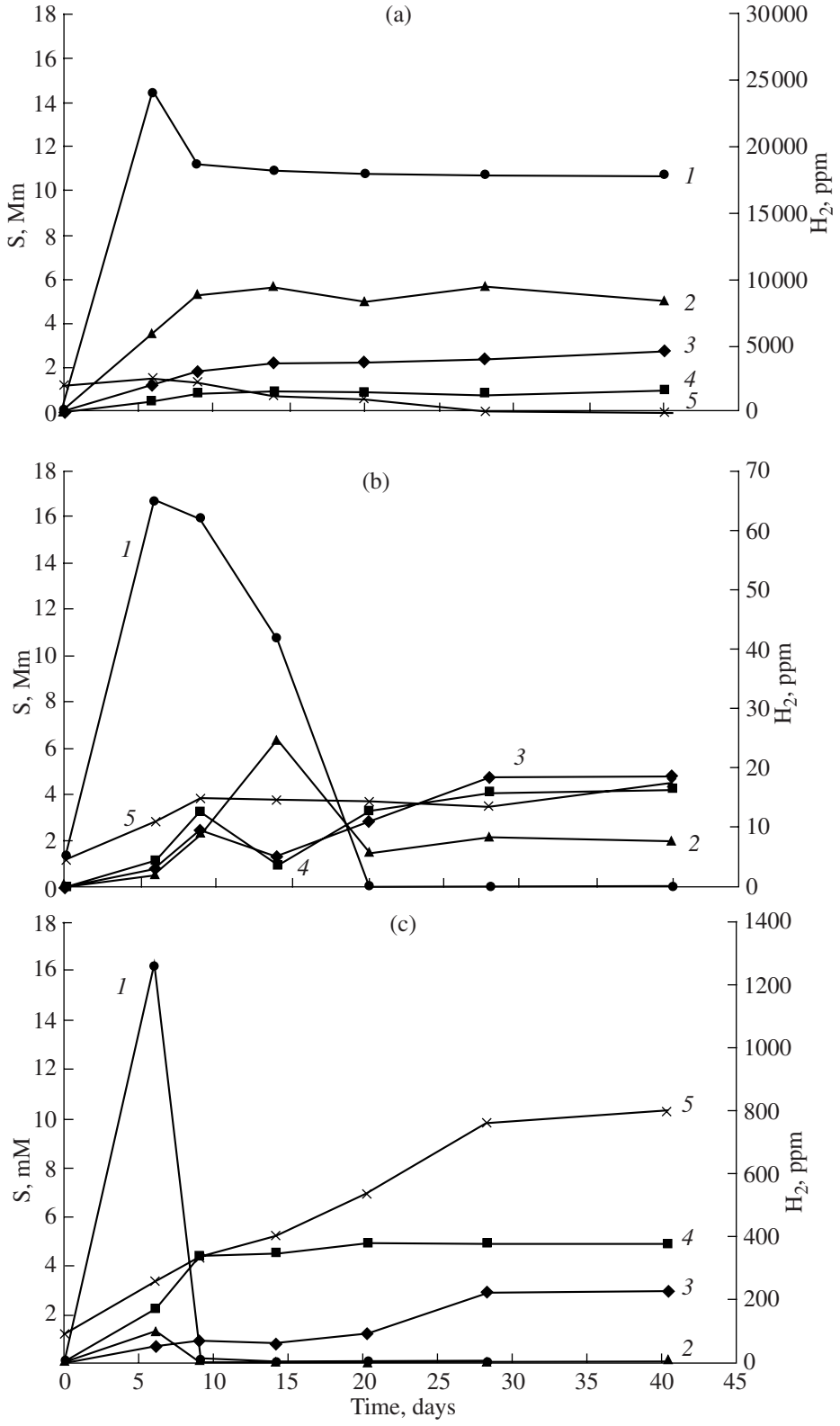

Fig. 1. Development of cultures of alkaliphilic anaerobes on cellulose. (a) *Cl. alkalicellulosi*; (b) *Cl. alkalicellulosi* + *Dnv. hydrogenovorans*; (c) *Cl. alkalicellulosi* + *Dn. lacustre*; (d) *Cl. alkalicellulosi* + *Contubernalis alkalaceticum* + *Desulfonatronum cooperativum*; (e) *Cl. alkalicellulosi* + *Contubernalis alkalaceticum* + *Desulfonatronum cooperativum* + *Dn. lacustre*. (*1*) H_2; (*2*) ethanol; (*3*) lactate; (*4*) acetate; (*5*) H_2S.

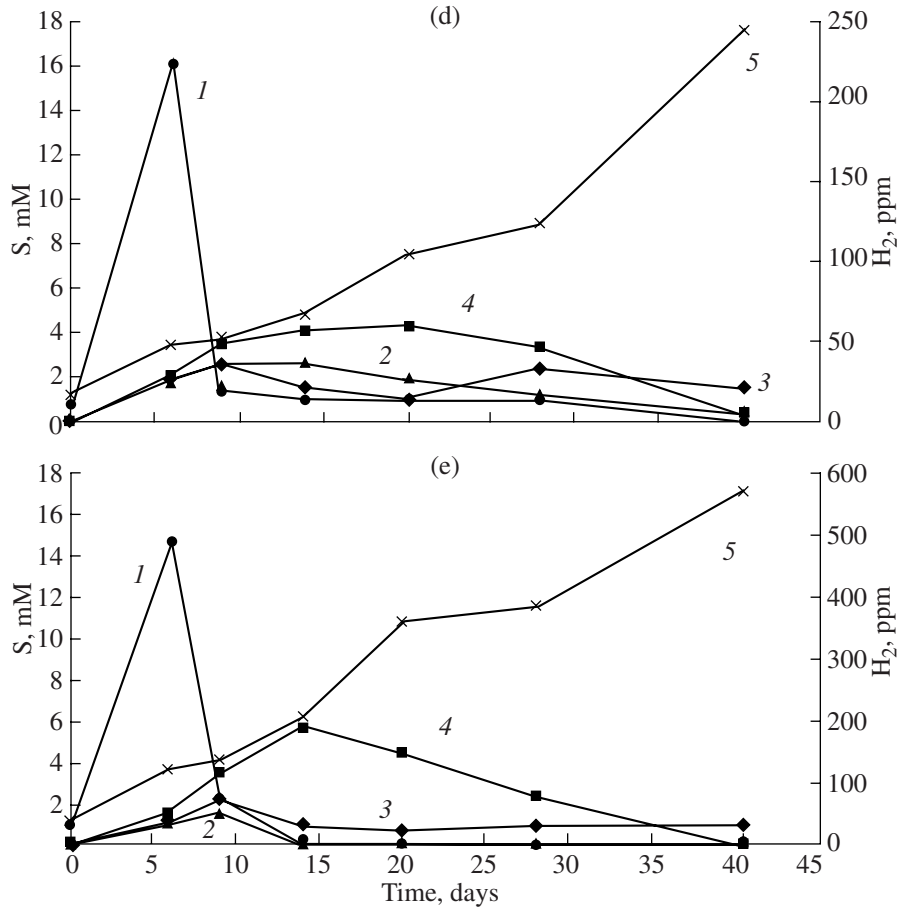

Fig. 1. (Condt.).

lose [H] equivalents were distributed between ethanol and acetate in approximately equal shares.

In a combined culture with the *Dn. lacustre* strain (Table 4, Fig. 1c), whose preferential substrate is ethanol, the alcohol was utilized completely; like H_2, it was detected among the products only at the beginning of fermentation (6 days). The accumulation of acetate, both as the product of cellulose fermentation by *Cl. alkalicellulosi* and as the product of incomplete oxidation of ethanol by *Dn. lacustre*, was completed in nine days. Lactate continued to accumulate, indicating continuation of cellulose fermentation by *Cl. alkalicellulosi.* The cellulose [H] equivalents were utilized completely by the end of fermentation (28 days). H_2S formation corresponded to two-thirds of the cellulose [H], whereas the rest, approximately in equal shares, accounted for the lactate and acetate, which were not used.

The results obtained in combined cultures with involvement of the syntroph *Cn. alkalaceticum* are the most interesting (Tables 5, 6; Figs. 1d, 1e). In the triple combination *Cl. alkalicellulosi* + *Cn. alkalaceticum* + *Dn. cooperativum* (Table 5, Fig. 1d), H_2S production predominated; its [H] content in H_2S after 40 days exceeded that in cellulose by 42%. A source of excessive [H] may be the water protons in the process of acetate decomposition by the syntroph according to the formal reaction (2) (see Materials and Methods), which implies formation of 8 [H]. The approximate number of the [H] equivalents can be calculated from the decrease in acetate from its maximum value of 267 μmol after 20 days to the final 17 μmol after 40 days: $250 \times 8 = 2000$. Thus, an additional 2000 [H] equivalents result from acetate decomposition. The same can be said about the decrease in ethanol from the maximum to the final value after 40 days: $(172 - 17) \times 6 = 930$. As a result, the sum of [H] equivalents constitutes 5180 + 2000 + 930 = 8110. The latter value is close to the experimentally determined [H] sum after 40 days, which was 56% in excess of the cellulose [H] equivalents. A more detailed calculation of the dynamics is hardly worthwhile, since the current concentrations of the substances reflect their balance between formation and consumption.

A similar result was obtained with a combination of four cultures (Table 6, Fig. 1e), in which the additionally present *Dn. lacustre* used ethanol quickly and completely to form acetate, which was used more slowly but completely for sulfidogenesis with involvement of

Table 3. Cellulose fermentation in the *Cl. alkalicellulosi* + *Dnv. hydrogenovorans* combination

Days of growth	$V_{liquid\ phase}$, ml	$V_{gaseous\ phase}$, ml	Measurement units	Metabolic products					Σ, [H]	Balance, [H]%	% H_2S in Σ [H]
				lactate	acetate	ethanol	H_2S	H_2^*			
0	70	52	mM	0	0	0	1.2	5			
			μmol/V	0	0	0	84	0.01			
			[H] equiv								
6	68	54	mM	0.7	1.1	0.5	1.6	65			
			μmol/V	48	75	34	109	0.2			
			[H] equiv	288	300	204	872	0	1664	32	52
9	66	56	mM	2.4	3.2	2.3	2.6	62			
			μmol/V	158	211	152	172	0.2			
			[H] equiv	948	844	912	1376	0	4080	79	34
14	64	58	mM	1.3	0.9	6.4	2.6	42			
			μmol/V	83	58	410	166	0.1			
			[H] equiv	498	232	2460	1328	0	4518	87	29
20	62	60	mM	2.8	3.2	1.5	2.5	0			
			μmol/V	174	198	93	155	0			
			[H] equiv	1044	792	558	1240	0	3634	70	34
28	60	62	mM	4.7	4.1	2.2	2.5	0			
			μmol/V	282	246	132	150	0			
			[H] equiv	1692	984	792	1200	0	4668	90	26
40	58	64	mM	4.8	4.2	2.0	3.3	0			
			μmol/V	278	244	116	191	0			
			[H] equiv	1668	976	696	1528	0	4868	94	31

Notes: The initial amount of cellulose was 432 μmol per vessel, which corresponded to 5180 [H] equivalents taking into account hydrolysis. The amounts of H_2S and H_2 are given minus their concentration at the zero growth point. Σ denotes the sum of [H] equivalents in products. The balance shows in % the use of the cellulose [H] equivalents.

% H_2S shows % of [H] equivalents of H_2S in the sum of products.

* The H_2 concentrations are given in ppm.

the syntroph *Cn. alkalaceticum*, determining a 33% excess of H_2S over the cellulose [H] equivalents.

Thus, paired cultures of the cellulolytic and one of sulfate reducers did not provide for complete expenditure of cellulose for sulfidogenesis, although certain shifts in the product ratio were observed as compared to the pure cellulolytic culture. The sulfate reducers such as *Desulfonatronovibrio*, having a limited trophic potential and using only H_2, influenced the composition of the cellulose fermentation products only slightly. Complete utilization by *Dn. lacustre* of such a reduced product as ethanol in addition to H_2 led to more intense sulfidogenesis and to quick excessive acetate accumulation but influenced lactate formation insignificantly. In the *Cl. alkalicellulosi* + *Cn. alkalaceticum* + *Dn. cooperativum* combination, with the presence of *Dn. cooperativum*, capable of using lactate in addition to H_2, and *Cn. alkalaceticum*, capable of acetate and lactate oxidation in conditions of hydrogen withdrawal, the picture was somewhat different. The amount of lactate, although it was not utilized completely, was less than in the pure cellulolytic culture. Hydrogen sulfide and ethanol + acetate were the main products accumulated. Acetate and ethanol, despite the expectations, were used slowly, as if the syntroph did not work. The processes catalyzed by syntophs are a slow link in trophic systems. Ethanol oxidation preceded acetate oxidation, and only after 28 days, with the syntroph accumulation, did the picture change dramatically: the acetate and ethanol contents decreased sharply, virtually to the point of

Table 4. Cellulose fermentation in the *Cl. alkalicellulosi* + *Dn. lacustre* combination

Days of growth	$V_{liquid\ phase}$, ml	$V_{gaseous\ phase}$, ml	Measurement units	Metabolic products					Σ, [H]	Balance, [H]%	% H_2S in Σ [H]
				lactate	acetate	ethanol	H_2S	H_2^*			
0	70	50	mM	0	0	0	1.8	ç.Ó			
			µmol/V	0	0	0	126	ç.Ó			
			[H] equiv								
6	68	52	mM	0.7	2.2	1.3	1.6	1261			
			µmol/V	48	150	88	109	3			
			[H] equiv	288	600	528	872	6	2294	44	38
9	66	54	mM	0.9	4.4	0	2.5	14			
			µmol/V	59	290	0	165	0.03			
			[H] equiv	354	1160	0	1320	0	2834	55	47
14	64	56	mM	0.8	4.5	0	3.4	2.5			
			µmol/V	51	288	0	218	0			
			[H] equiv	306	1152	0	1744	0	3202	62	54
20	62	58	mM	1.2	4.9	0	5.1	2.6			
			µmol/V	74	304	0	316	0			
			[H] equiv	444	1216	0	2528	0	4188	81	60
28	60	60	mM	2.9	4.9	0	8	2.6			
			µmol/V	174	294	0	480	0			
			[H] equiv	1044	1176	0	3840	0	6060	117	63
40	58	62	mM	2.9	4.9	0	8.2	2.6			
			µmol/V	168	284	0	476	0			
			[H] equiv	1008	1136	0	3808	0	5952	115	64

Notes: The initial amount of cellulose was 432 µmol per vessel, which corresponded to 5180 [H] equivalents taking into account hydrolysis. The amounts of H_2S and H_2 are given minus their concentration at the zero growth point. Σ denotes the sum of [H] equivalents in products. The balance shows in % the use of the cellulose [H] equivalents.

% H_2S shows % of [H] equivalents of H_2S in the sum of products.

* The H_2 concentrations are given in ppm.

complete consumption after 40 days, and the H_2S content constituted 91% of the products formed. In the combination of four cultures (with the addition of *Dn. lacustre* actively using ethanol), a pronounced acetogenic shift was observed. As soon as after 14 days, ethanol could no longer be detected among the products, and decomposition of the main product, acetate, was completely channeled into sulfidogenesis due to the operation of the syntrophic pairs *Cn. alkalaceticum* + hydrogenotrophic SRB. In this case, the cellulose [H] equivalents was completely utilized in the short trophic chain involving the hydrolytic and sulfate reducer(s), with the recruitment of [H] equivalents from water by the syntroph. Thus, the involvement of syntrophic organisms results in the hydrogen sulfide production in the system significantly exceeding the [H] equivalents of cellulose itself.

The model experiments presented in this work allow a conclusion as to the possibility of operation of a short trophic chain from cellulose to sulfidogenesis in the alkaliphilic anaerobic community; this chain involves a cellulolytic and sulfate reducers and no intermediate fermenters. Sulfidogenesis is carried out by a group of alkaliphilic sulfate reducers differing in their nutritional requirements. However, acetate remains an unused product, formed by both the cellulolytic and

Table 5. Cellulose fermentation in the *Cl. alkalicellulosi* + *Cn. alkalaceticum* + *Dn. cooperativum* combination

Days of growth	$V_{liquid\ phase}$, ml	$V_{gaseous\ phase}$, ml	Measurement units	Metabolic products					Σ, [H]	Balance, [H]%	% H_2S in Σ [H]
				lactate	acetate	ethanol	H_2S	H_2^*			
0	70	51	mM	0	0	0	1.7	9.5			
			µmol/V	0	0	0	119	0.02			
			[H] equiv								
6	68	53	mM	1.8	2.1	1.8	1.7	225			
			µmol/V	122	143	122	116	0.5			
			[H] equiv	732	572	732	928	1	2964	57	31
9	66	55	mM	2.5	3.6	2.6	2.1	18			
			µmol/V	165	238	172	139	0.04			
			[H] equiv	990	952	1032	1112	0	4086	79	27
14	64	57	mM	1.5	4.1	2.6	3.2	13			
			µmol/V	96	262	166	205	0.03			
			[H] equiv	576	1048	996	1640	0	4260	82	38
20	62	59	mM	1.0	4.3	1.9	5.8	13			
			µmol/V	62	267	118	360	0.03			
			[H] equiv	372	1068	708	2880	0	5028	97	57
28	60	61	mM	2.4	3.3	1.2	7.2	13			
			µmol/V	144	198	72	432	0.03			
			[H] equiv	864	792	432	3456	0	5544	107	62
40	58	63	mM	1.5	0.3	0.3	15.9	13			
			µmol/V	87	17	17	922	0			
			[H] equiv	522	68	102	7376	0	8068	156	91

Notes: The initial amount of cellulose was 432 µmol per vessel, which corresponded to 5180 [H] equivalents taking into account hydrolysis. The amounts of H_2S and H_2 are given minus their concentration at the zero growth point. Σ denotes the sum of [H] equivalents in products. The balance shows in % the use of the cellulose [H] equivalents.

% H_2S shows % of [H] equivalents of H_2S in the sum of products.

* The H_2 concentrations are given in ppm.

sulfate reducers that are incapable of complete acetate oxidation. The inclusion of a syntrophic acetate-utilizing organism leads to excessive, in relation to the cellulose used, hydrogen sulfide formation and complete substrate utilization. Due to the involvement of the slowly growing syntrophs, the process rate depends, in large measure, on the number of the microorganisms already present in the system. Demonstration of the existence of the short pathway from cellulose to sulfidogenesis poses the question as to the expediency of the large number of the bypass metabolic pathways that involve dissipotrophic fermenters.

Table 6. Cellulose fermentation in the *Cl. alkalicellulosi* + *Cn. alkalaceticum* + *Dn. cooperativum* + *Dn. lacustre* combination

Days of growth	$V_{liquid\ phase}$, ml	$V_{gaseous\ phase}$, ml	Measurement units	Metabolic products					Σ, [H]	Balance, [H]%	% H_2S in Σ [H]
				lactate	acetate	ethanol	H_2S	H_2^*			
0	70	49	mM	0	0	0	2.2	14			
			µmol/V	0	0	0	154	0.03			
			[H] equiv								
6	68	51	mM	1.1	1.5	1.0	1.5	493			
			µmol/V	75	102	68	102	1			
			[H] equiv	450	408	408	816	2	2082	40	39
9	66	53	mM	2.1	3.4	1.6	1.9	71.5			
			µmol/V	139	224	106	125	0.2			
			[H] equiv	834	896	636	1000	0	3366	65	30
14	64	55	mM	0.9	5.7	0	4	2.5			
			µmol/V	58	365	0	256	0			
			[H] equiv	348	1460	0	2048	0	3856	74	53
20	62	57	mM	0.7	4.4	0	8.6	0			
			µmol/V	43	273	0	533	0			
			[H] equiv	258	1092	0	4264	0	5614	108	76
28	60	59	mM	0.9	2.3	0	9.4	0			
			µmol/V	54	138	0	564	0			
			[H] equiv	324	552	0	4512	0	5388	104	84
40	58	61	mM	0.9	0	0	14.8	0			
			µmol/V	52	0	0	858	0			
			[H] equiv	312			6864		7176	139	96

Notes: The initial amount of cellulose was 432 µmol per vessel, which corresponded to 5180 [H] equivalents taking into account hydrolysis. The amounts of H_2S and H_2 are given minus their concentration at the zero growth point. Σ denotes the sum of [H] equivalents in products. The balance shows in % the use of the cellulose [H] equivalents.

% H_2S shows % of [H] equivalents of H_2S in the sum of products.

* The H_2 concentrations are given in ppm.

Sulfidogenesis on cellulose appeared to be a very efficient process for the community of alkaliphilic anaerobes. It is not surprising that the sulfur cycle with the involvement of sulfate-reducing, phototrophic, and sulfur-oxidizing bacteria [18, 19] operates so intensely in soda lakes.

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