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EXPERIMENTAL ARTICLES

Metabolic Resistance of a Psychrotolerant VFA-Oxidizing Microbial Community from an Anaerobic Bioreactor to Changes in the Cultivation Temperature

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Abstract—A psychrotolerant microbial consortium from a low-temperature anaerobic EGSB bioreactor was grown separately on acetate, propionate, butyrate, and H_2/CO_2 at 30 and 10°C in glass flasks. In the course of the experiments, the cultivation temperature was changed at different time intervals. The initial rates of substrate utilization were higher at 30 than at 10°C. However, the microbial consortium was found to be well adapted to low temperatures; when grown at 10°C for 1.5-5 months, the rates of butyrate, propionate, and H_2/CO_2 utilization increased steadily. When grown at 30°C for 1.5–2.5 months, this consortium retained its ability to degrade VFA and H_2/CO_2 at 10°C. However, after long-term (150 days) cultivation at 10°C, its ability to utilize the substrates at 30°C decreased. In the consortium grown in the acetate-containing medium, a *Methanosaeta*-like methanogen was predominant; in media with propionate and butyrate, besides VFA-degrading bacteria, acetoclastic *Methanosaeta*-like and hydrogenotrophic *Methanospirillum*-like methanogenic archaea prevailed. A *Methanospirillum*-like strain predominated in the H_2/CO_2-containing medium. The *Methanospirillum* strain of this microbial community was presumably psychrotolerant. A method based on changes in the cultivation temperature is of practical interest and can be used to start up new bioreactors.

Keywords: anaerobic degradation of VFA at low temperatures, methanogenesis, psychrotolerant microbial consortium, metabolic resistance of a microbial community.

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Short-chain volatile fatty acids (VFAs)—acetate, propionate, and butyrate—are the main intermediate products of anaerobic degradation of organic matter. In freshwater ecosystems, some methanogenic archaea are able to utilize acetate and produce methane. Acetate, propionate, and butyrate are utilized by syntrophic consortia consisting of syntrophic bacteria and hydrogen- and acetate-utilizing methanogenic archaea [1–3], or, in the presence of electron acceptors in the media, by bacteria capable of reducing sulfate, iron, and other electron acceptors [1–4].

The main reactions of anaerobic transformation of acetate, propionate, butyrate, and hydrogen studied in this work are listed below [1, 3]:

$$\text{Acetate}^- + 4H_2O \longrightarrow 2HCO_3^- + H^+ + 4H_2 \quad (1)$$
$$\Delta G^{\circ\prime} = +104.6 \text{ kJ},$$

$$\text{Propionate}^- + 3H_2O \longrightarrow \text{Acetate}^- + HCO_3^- + H^+ + 3H_2 \quad (2)$$
$$\Delta G^{\circ\prime} = +76.0 \text{ kJ},$$

$$\text{Butyrate}^- + 2H_2O \longrightarrow 2\text{Acetate}^- + H^+ + 2H_2 \quad (3)$$
$$\Delta G^{\circ\prime} = +48.3 \text{ kJ},$$

$$4H_2 + HCO_3^- + H^+ \longrightarrow CH_4 + 4H_2O \quad (4)$$
$$\Delta G^{\circ\prime} = -135.6 \text{ kJ},$$

$$\text{Acetate}^- + H^+ \longrightarrow CH_4 + HCO_3^- \quad (5)$$
$$\Delta G^{\circ\prime} = -31 \text{ kJ},$$

$$\text{Propionate}^- + 1.75H_2O \longrightarrow 1.75CH_4 + 1.25HCO_3^- + 0.25H^+ \quad (6)$$
$$\Delta G^{\circ\prime} = -56.6 \text{ kJ},$$

$$\text{Butyrate}^- + 2.5H_2O \longrightarrow 2.5CH_4 + 1.5HCO_3^- + 0.5H^+ \quad (7)$$
$$\Delta G^{\circ\prime} = -81.7 \text{ kJ}.$$

Anaerobic waste water and animal waste treatment is widely employed in many countries and is carried out mainly at temperatures ranging from 30 to 50–55°C. Operating a bioreactor at an elevated tempera-

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ture increases energy consumption, which makes the processes of anaerobic treatment and microbiological methane production more expensive. To reduce energy consumption, it is possible to carry out these processes without heating, at ambient temperatures [5–14].

In our previous work, the degradation of VFA by granulated microbial biomass in a continuous anaerobic EGSB reactor at low temperatures was investigated. The biomass was adapted to low temperatures by cultivation at 3–12°C for 1.5 years in a bioreactor containing a mixture of acetate, propionate, and butyrate. The resultant rate of VFA utilization was comparable to that observed under mesophilic conditions. Subsequent determination of the temperature characteristics of the biomass in the course of batch cultivation on a VFA mixture demonstrated that 35–40°C remained the optimal temperature even after long-term adaptation to low temperature [10]. However, it remained unclear whether the entire microbial community was mesophilic or some acetate-, propionate-, or butyrate-utilizing microorganisms within this community became psychrotolerant. In natural environments or during operation of biogas bioreactors, some changes in the cultivation temperature may occur. Therefore, it was considered essential to study the level of metabolic resistance of a psychrotolerant microbial community to a temporary increase or decrease in temperatures in the course of batch cultivation.

The goal of this work was to study the ability of a psychrotolerant microbial community to remain active at low temperatures (10°C) depending on the duration of preliminary cultivation (separately on acetate, propionate, butyrate, and H_2/CO_2) at 30°C. In addition, it was also interesting to know the effect of the duration of cultivation at 10°C on the metabolic activity of the psychrotolerant microbial community during subsequent growth on the same substrates at 30°C.

MATERIALS AND METHODS

Anaerobic granulated biomass from a pilot-scale anaerobic continuous expanded granular sludge bed (EGSB) bioreactor treating malting wastewater at temperatures from 12 to 20°C (The Netherlands) was used for inoculation of a laboratory EGSB bioreactor at the University of Wageningen. The bioreactor consisted of two sequentially connected fermentors. A bacterial consortium was grown on a mixture of volatile fatty acids (VFAs) (acetate, propionate, and butyrate) as substrates, at 3–12°C for 1.5 years [9, 10]. In the first stage, in which a mixture of all VFAs was added, only acetate and butyrate were utilized completely. Propionate was completely oxidized in the second stage.

In this work, we used the methanogenic granulated biomass adapted to low temperature from the second stage of the laboratory anaerobic two-stage bioreactor. The biomass was cultivated in Pfennig medium [15] containing the following (mg/l): NH_4Cl, 330; $MgCl_2 \cdot 6H_2O$, 500; $CaCl_2$, 168; KCl, 330; and KH_2PO_4, 330. The medium was supplemented with yeast extract, 200 mg/l, and $NaHCO_3$, 2000 mg/l, as well as with a trace element solution (according to Lippert [16]) and a vitamin solution (according to Wolin [17]). Sodium sulfide (0.5 g/l) was used as a reducing agent. The final pH was 6.8–7.0.

The granules of the biomass were crushed in a glass mortar under nitrogen flow and resuspended in a nutrient medium. The cell suspension (2 ml, 10% vol/vol) was transferred into 40- and 140-ml serum bottles(for experiments with VFA and H_2/CO_2, respectively); the bottles were then hermetically sealed. The volume of the medium was 18 ml. Acetate, propionate, and butyrate (10 mmol/l) were used separately as substrates. The gas phase in the VFA-containing flasks was a mixture of N_2 and CO_2. The bottles used for cultivation on the gaseous substrate (H_2/CO_2) were flushed with the gas mixture (80 : 20). In the course of the experiments, substrate utilization and methanogenesis, as well as of the production and utilization of intermediate products, were monitored. Portions (10 mmol/l) of organic substrates were added upon exhaustion. In the experiments with H_2/CO_2, the medium was flushed with the gas mixture. The growth temperature during the experiment varied from 30 to 10°C and vice versa. Figure 1 shows a schematic diagram of the experiment, analogous for each substrate. At the early stages of the experiment, six bottles (nos. 1–6) containing suspended microbial biomass were supplemented with the relevant substrates, and the bottles were incubated at 30°C in the thermostat. Three bottles (nos. 7–9) were incubated at 10°C. After 48-day incubation at 30°C, three bottles (nos. 1–3) were transferred to the 10°C incubator. After 36-day incubation (84 days from the beginning of the experiment) at 30°C, two bottles (nos. 5 and 6) were transferred to the thermostat set at 10°C. Then, 150 days after the beginning of the experiment, the remaining flasks (no. 7) incubated at 10°C were transferred to the thermostat set at 30°C. During the entire period of incubation, the bottles were supplemented with the relevant substrates upon their exhaustion. After each change in the cultivation temperature, each bottle was supplemented with the next portion of the substrate; then, a series of measurements were made to determine the dynamics of substrate utilization and methanogenesis, as well as of production and utilization of intermediate products.

The biomass activity was determined as the rate of substrate utilization (mmol l^{-1} day^{-1}).

The weight of dry biomass (dried to a constant weight at 105°C) was determined by the standard method.

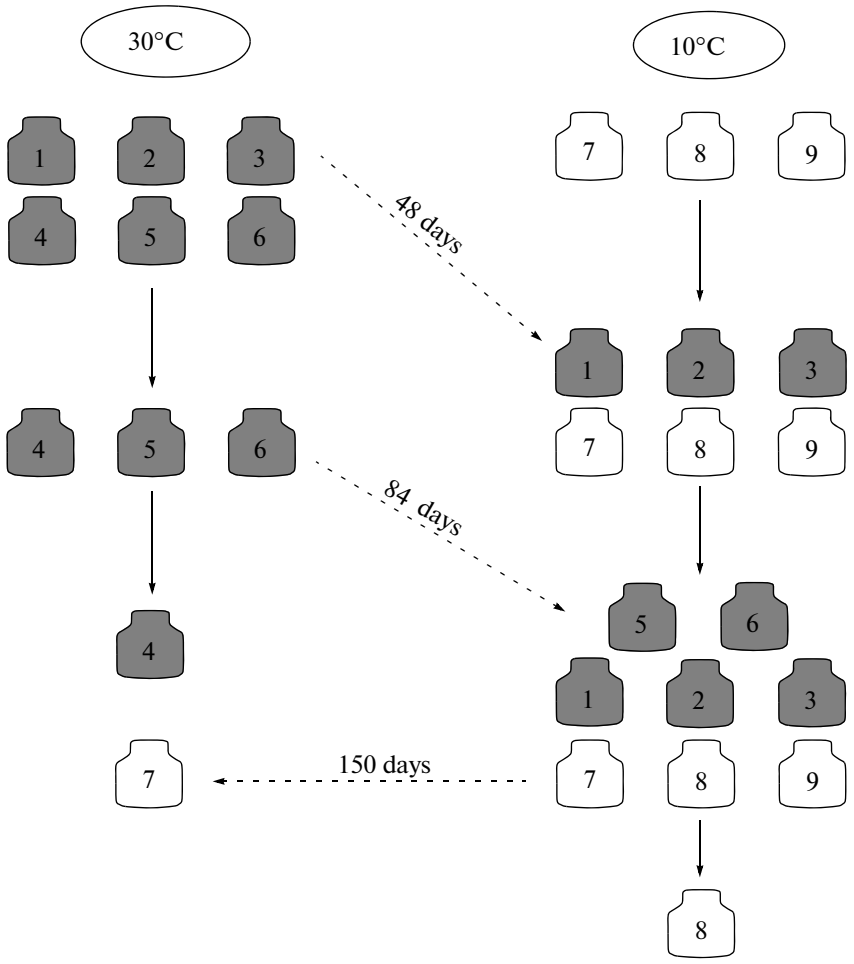

Fig. 1. Schematic diagram of the experiments.

Chromatographic analyses were performed on a Chrom-5 gas chromatograph equipped with a katharometer and a flame ionization detector, as described in [7].

RESULTS

Acetate utilization. At the early stages of the experiments, acetate was consumed by the microbial community at 30 and 10°C in 3 and 8 days, respectively (Figs. 2a and 2b). After 48 days, the activity of the biomass incubated at 30°C did not increase, but even decreased slightly as compared to the initial level (Table 1). After the first (48 days) and second (84 days) temperature shifts from 30 to 10°C, the rate of acetate utilization was 1.4 and 2.5 times lower, respectively, than at the beginning of the experiment (Table 1). The results obtained indicate that, after 48-day cultivation at 30°C, the biomass retained its ability to utilize acetate at 10°C. More prolonged cultivation (84 days) at 30°C resulted in a notable decrease in the consortium's ability to utilize acetate when the growth temperature shifted from 30 to 10°C (Table 1).

Earlier, we showed that a microorganism morphologically similar to *Methanosaeta* (*Methanothrix*) was the main acetate-utilizing methanogen of this community [10]. Microscopic examinations confirmed that this microorganism prevailed in the microbial community grown on acetate. After 48-day cultivation, *Methanosaeta* cells were opaque and intact, while after 84-day cultivation, many lysed cells were seen. Repeated addition of acetate into the same mineral base of the medium resulted in the exhaustion of some nutrient elements, accumulation of metabolites, partial lysis of the cells in the methanogenic monoculture, and loss of activity.

At the beginning of the experiment, the rate of acetate utilization at 10°C was more than 2.2 times lower than at 30°C (Fig. 2b, Table 1). After 55-day cultivation, the rate of acetate utilization was twice as low, and after 150 days, the rate of acetate utilization at 10°C was lower than the initial rate at 10°C (Table 1). This phenomenon may also be due to partial cell lysis. A return to the initial 30°C after 150-day cultivation at 10°C did not restore the rate of acetate utilization

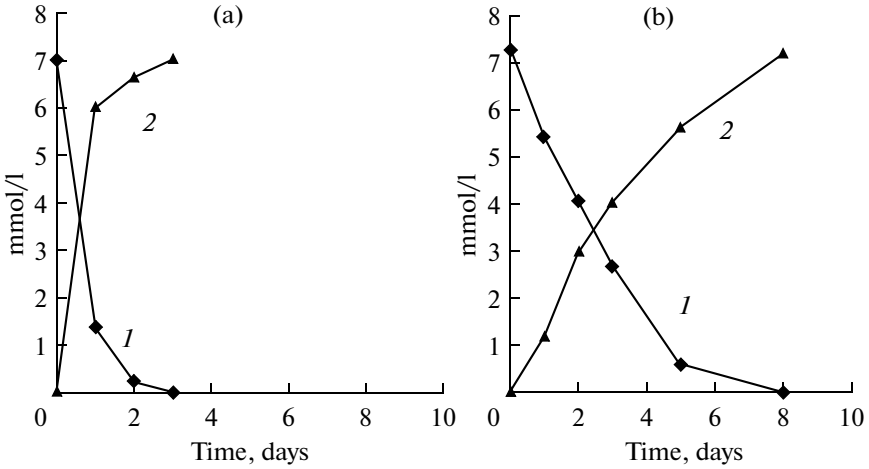

Fig. 2. Dynamics of acetate utilization and methane production: at the beginning of the experiment at 30 (a) and at 10°C (b): acetate (*1*) and methane (*2*).

observed at 30°C at the beginning of the experiment (Table 1).

In all our experiments with acetate, we failed to detect syntrophic acetate oxidation. The hydrogen-utilizing *Methanospirillum*-like archaeon was not present in the culture. In addition to a small amount of coccoid cells, a microorganism morphologically similar to acetoclastic methanogenic archaeon of the genus *Methanosaeta* was predominant.

The type strain of *Methanosaeta concilii* has traditionally been considered as mesophilic, since its temperature optimum is 37°C. However, its description mentioned its capacity for weak growth at 3°C [18]. During cultivation, many *Methanosaeta* cells were accumulated in the biomass of the anaerobic bioreactor [10]; therefore, it is not surprising that the rate of acetate utilization was quite high during growth at 10°C (Fig. 2b). Long-term batch cultivation of the consortium on acetate at both temperatures without changing the mineral base of the nutrient medium resulted in partial cell lysis.

Acetate oxidation was accompanied by formation of trace amounts of propionate in the liquid (not shown on the figures). It is well known [19] that *Desulfobulbus propionicus* is capable of producing propionate from acetate in sulfate-free media. Hence, the presence of a sulfate reducer in the consortium, despite the lack of sulfate in the medium, is not unlikely.

Utilization of butyrate and propionate. Figures 3a and 3b show the dynamics of butyrate oxidation at 30 and 10°C, respectively. At both temperatures, small amounts of acetate, propionate, and *iso*-butyrate were gradually produced and then utilized. Hydrogen was not detected in the gas phase, which suggests that it was actively consumed. Accumulation of excessive acetate not detected either. An increase in the rate of butyrate utilization was observed during cultivation at 30°C for 48 days (Table 1). However, despite the fact that trace amounts of acetate were detected in the culture liquid at the early stages of the experiments after utilization of 7.5 mmol/l of butyrate at 30°C (Fig. 3a), the addition of 9 mmol/l of butyrate after 48-day incubation at 30°C resulted in accumulation of 4 mmol/l of acetate after 2 days. By day 6, acetate was completely utilized (figure not shown). The transfer of the cultures incubated at 30°C for 48 days with the thermostat set at 10°C resulted in a 2.5-fold decrease in the rate of butyrate oxidation and led to accumulation of acetate and iso-butyrate (4.5 and 0.75 mmol/l, respectively) (Fig. 3c, Table 1). Cultivation of the community at 10°C for 55 days resulted in a 2.5-fold increase in the rate of butyrate oxidation as compared

Table 1. Comparative analysis of the rates of substrate utilization depending on the time of incubation, as well as after changes of the growth conditions (temperature shifts)

Cultivation temperature	Rates of substrate utilization (mmol l^{-1} day^{-1})			
	Acetate	Butyrate	Propionate	H_2/CO_2
30°C (at the onset of the experiment)	2.8	3.5	1.25	19.0
30°C (48 days)	2.25	4.5	5.0	ND
30°C → 10°C (48 days)	2.0	1.42	1.67	4.47
30°C → 10°C (84 days)	1.1	ND	1.5	5.46
10°C (at the onset of the experiment)	1.25	0.53	0.3	5.03
10°C (55 days)	1.5	1.29	0.59	ND
10°C (150 days)	1.05	1.43	1.05	9.47
10°C → 30°C (150 days)	1.56	1.69	2.4	27.6

Note: ND – not determined.

to the initial rate measured at the same temperature (Fig. 3d); after 150-day incubation, the rate of butyrate oxidation increased still more (Table 1). On return to the initial 30°C after 150-day cultivation, the rate of butyrate oxidation was higher than the rate measured at 10°C; however, it was not restored to the initial value observed at 30°C (Table 1).

During syntrophic butyrate oxidation, more acetate and less hydrogen were produced than during propionate oxidation (reactions 2 and 3). During butyrate and propionate oxidation by syntrophic bacteria, the rates of production and, especially, of utilization of acetate were higher at 30 than at 10°C; the acetate produced after the temperature shift from 30 to 10°C was the residue left after the slow process of partial acetate utilization. The rate of acetate utilization depends on the activity of acetoclastic methanogens. In our experiments, the rate of acetate oxidation at 10°C by the butyrate-oxidizing population was lower than the rate of butyrate oxidation, indicating that the growth of *Methanosaeta* slowed down significantly at 10°C. It seems likely that butyrate-oxidizing syntrophic microorganisms and the hydrogen-utilizing methanogen are capable of rapidly reproducing, oxidizing butyrate, and utilizing the resultant hydrogen at low temperatures, whereas the activity of acetoclastic methanogens at 10°C is very low (Fig. 3c). The butyrate-oxidizing community was found to be more balanced at 10°C. After the flasks incubated at 10°C for 55 days were supplemented with a new portion of butyrate, the accumulation of acetate in the culture liquid became less pronounced than after the temperature shift from 10 to 30°C (Figs. 3c and 3d).

In the butyrate-oxidizing consortium, microorganisms morphologically similar to *Methanomethylovorans* were detected; they were found in the granules as small cell aggregates.

In addition to acetate, propionate and *iso*-butyrate formed as a result of butyrate oxidation and were detected in the liquid (Fig. 3). Propionate formation by sulfate reducers was discussed above. *Desulforhabdus amnigenes* and some other sulfate reducers are capable of producing *iso*-butyrate from butyrate in the absence of sulfate [20]. Thus, sulfate reducers possibly participate in butyrate oxidation as well.

At the beginning of the experiment (30 and 10°C), propionate was completely utilized in 8 and 25 days, respectively. The intermediate accumulation of acetate at both temperatures was insignificant; it was then slowly utilized (Figs. 4a and 4b, Table 1). Cultivation at 30°C for 48 days resulted in a fourfold increase in the rate of propionate utilization (Table 1). The highest intermediate concentration of acetate was 2.3 mmol/l. After 48-day incubation and the temperature shift from 30 to 10°C, the rate of propionate utilization was higher than the initial rate at 30°C. However, the maximum intermediate concentration of acetate was 4 mmol/l (Fig. 4c). While the rate of propionate utilization remained virtually unchanged after the next temperature shift on the 84th day of incubation, the intermediate acetate accumulation reached 6 mmol/l (Table 1). A twofold increase in the propionate-utilizing activity (as compared to the initial rate of propionate oxidation at 30°C) was detected in one bottle after 150-day incubation and the temperature shift from 10 to 30°C (Table 1). After 55-day cultivation at 10°C, the rate of propionate utilization increased twofold as compared to the initial rate (Fig. 4d); during 150-day incubation, a threefold increase was detected (Table 1). Only trace amounts of acetate were detected (Fig. 4d). Hence, the propionate-oxidizing syntrophic consortium was found to be more psychrotolerant than the consortia growing on acetate and butyrate.

In the bottles with propionate, the biomass consisted of at least four of five main microorganisms, including, presumably, two syntrophs and two methanogens (morphologically similar to *Methanosaeta* and *Methanospirillum*). Moreover, microscopic examinations of propionate-oxidizing aggregates revealed the presence of microorganisms (in the form of small cell aggregates in the granules of biomass) with morphological properties similar to those of *Methanomethylovorans*. The number of aggregates was lower than during growth on butyrate. The role of *Methanomethylovorans* and the range of substrates that it utilizes during butyrate and propionate utilization is unknown. The described species of the genus *Methanomethylovorans*, *M. hollandica* and *M thermophila*, are obligate methylotrophs and cannot grow on acetate, propionate, butyrate, or hydrogen [21, 22]. Earlier, both described *Methanomethylovorans* species and uncultured species were detected in the microbial biomass of low-temperature bioreactors and in natural ecosystems using molecular biological techniques [23–25]. The range of substrates utilized by these microorganisms during growth in the studied microbial communities was not determined as well.

During syntrophic oxidation of VFAs, and of propionate to a greater extent, the role of hydrogen-utilizing methanogens was more important than during butyrate oxidation (reactions 2 and 3). We failed to detect hydrogen in the gas phase at all growth temperatures. That is, hydrogen was completely utilized by hydrogen-utilizing methanogens even at 10°C. It may be suggested that a significant increase in the propionate-oxidizing activity during long-term incubation of the community at 10°C up to values comparable to those detected at 30°C resulted from the accumulation of psychrotolerant hydrogen-utilizing methanogens in the consortium, since the mesophilic strains *Methanospirillum hungatei* JF 1^T [26] and GP 1 [27] cannot grow at temperatures below 20°C.

The microbial consortia grown on butyrate and propionate were found to be more resistant to inhibitory metabolites during batch cultivation without changing of the nutrient medium than acetate-oxidizing microorganisms (primarily *Methanosaeta* species).

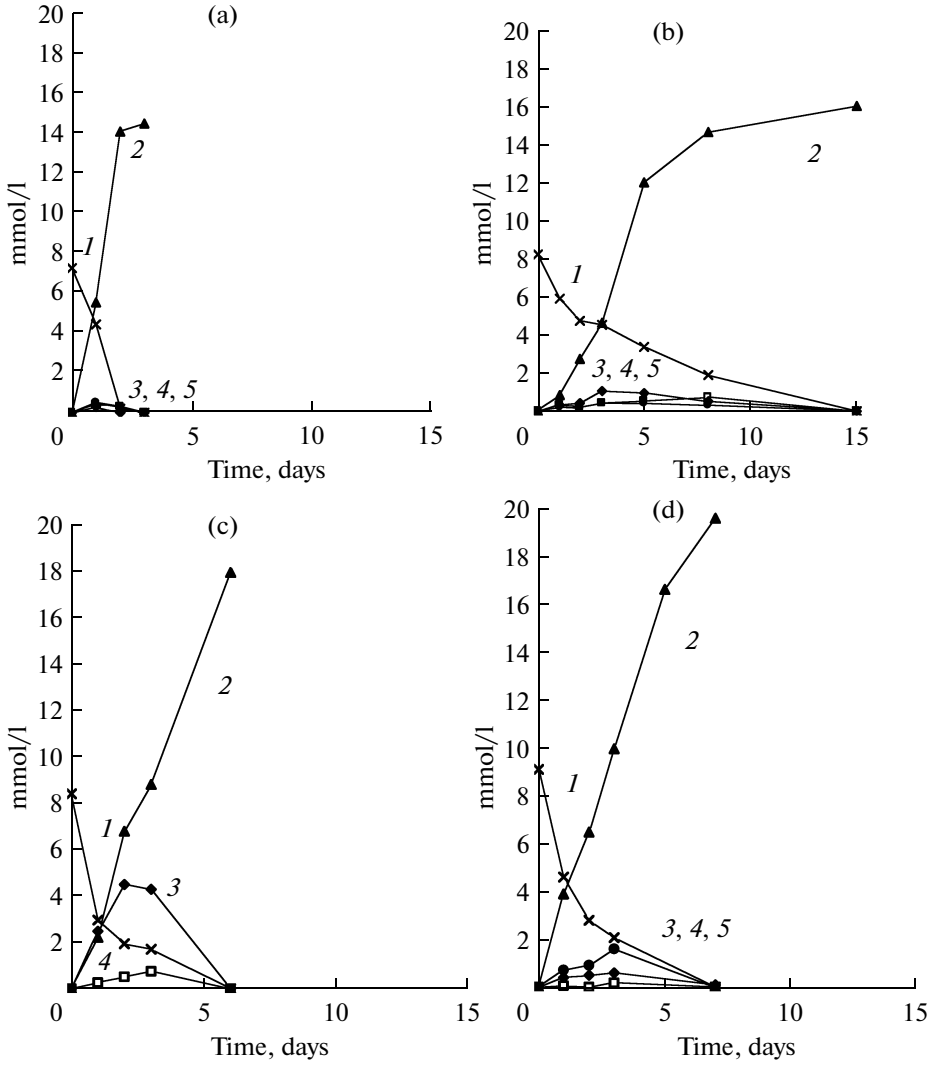

Fig. 3. Dynamics of butyrate oxidation: at 30°C at the beginning of the experiment (a); at 10°C at the beginning of the experiment (b); after 48-day cultivation at 30°C and the temperature shift to 10°C (c); and after 55-day cultivation at 10°C(d): butyrate (1), methane (2), acetate (3), iso-butyrate (*4*), and propionate (*5*).

Most probably, during the growth of microorganisms with different trophic requirements, the amount of accumulated inhibitory metabolites was lower. After the temperature shift from 30 to 10°C, the amount of accumulated acetate increased (Fig. 4c), indicating that the acetoclastic methanogen *Methanosaeta* sp. was more sensitive to the cultivation temperature and the duration of cultivation without changing of the nutrient medium. The rate of acetate utilization was lower than the rate of acetate production from propionate and butyrate. On the contrary, during long-term cultivation at 10°C, the acetate-utilizing activity of the microbial community was more balanced and acetate was utilized almost completely (Figs. 3b, 3c, 4b, and 4d).

H_2/CO_2 utilization. Figures 5a and 5b and Table 1 demonstrate the dynamics and initial rates of hydrogen utilization by the community at 30 and 10°C in our experiments with H_2/CO_2. A fourfold difference in the initial rates of substrate utilization was observed. The temperature shift from 30 to 10°C after 48-day cultivation resulted in a decrease in the hydrogen-utilizing activity (as compared to the initial activity measured at 30°C), which became similar to the initial rate of hydrogen utilization at 10°C (Fig. 5c, Table 1). The effect of the temperature shift from 30 to 10°C after 84-day cultivation was less dramatic (Table 1). Cultivation at 10°C for 150 days resulted in a twofold increase in the rate of hydrogen utilization, as compared to the initial rate at 10°C (Fig. 5c, Table 1). The hydrogen-utilizing microbial community accumulated at 10°C was found to be active at 30°C as well: the rate of hydrogen utilization after the temperature shift to 30°C following 150-day cultivation at 10°C was 1.5 times higher than that detected at 30°C at the beginning of the experiment (Table 1). The fact is

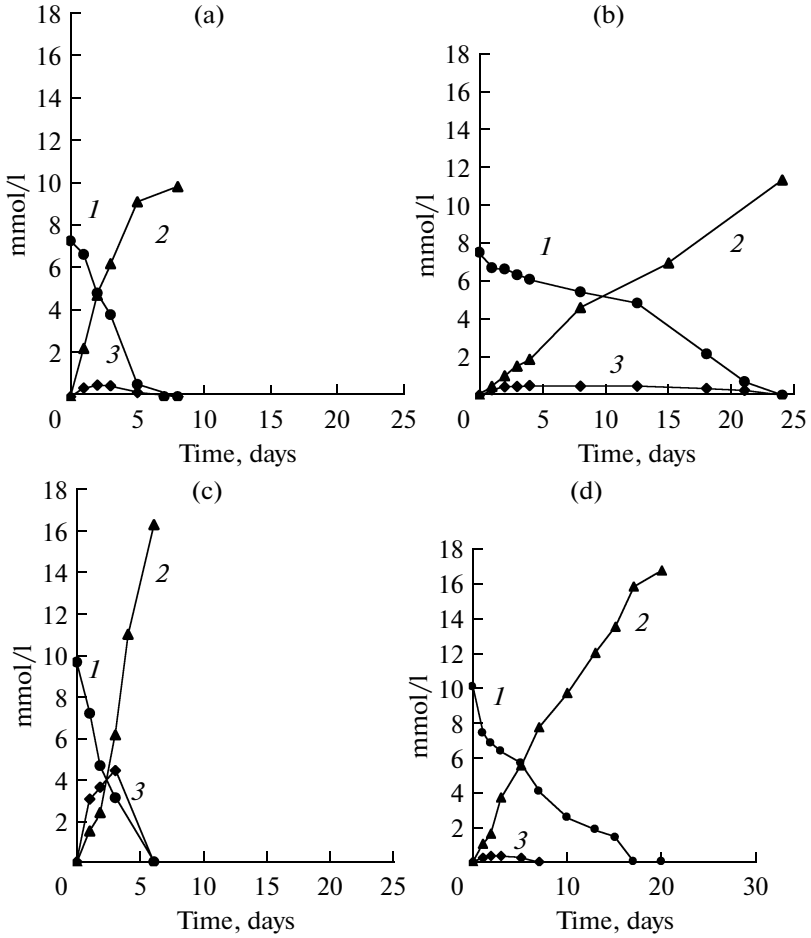

Fig. 4. Dynamics of propionate oxidation: at 30°C at the beginning of the experiment (a); at 10°C at the beginning of the experiment (b); after 48-day cultivation at 30°C and the temperature shift to 10°C (c); and after 55-day cultivation at 10°C(d): propionate (1), methane (2), acetate (3).

noteworthy that, at both cultivation temperatures, only trace amounts of acetate were detected. After cultivation on H_2/CO_2, both at 30 and 10°C, the biomass consisted primarily of *Methanospirillum* cells.

DISCUSSION

In the course of three-month cultivation, the average biomass yields on all organic substrates were relatively equal (Table 2). The effect of increasing biomass yield on the metabolic activity of the consortia was mostly positive, except for the microbial communities growing on acetate both at 30 and after transfer to 10°C.

The study of the dynamics of acetate, propionate, butyrate, and hydrogen utilization by the microflora of the psychrophilic anaerobic reactor at 30 and 10°C revealed that the rates of substrate utilization at the early stages of the experiment at 10°C were lower than the rates detected at 30°C. However, long-term cultivation at 10°C resulted in an increase in the metabolic activity; this suggests that members of the studied microbial community are capable of reproduction at 10°C. After relatively short (48 days) cultivation at 30°C, the consortium activity at 10°C did not decrease. Longer (84 days) cultivation at 30°C resulted in partial loss of the community psychroactivity (Table 1).

In our experiments, the acetoclastic *Methanosaeta*-like methanogen prevailed in the community grown in the acetate-containing medium. This strain was probably mesophilic; therefore, its activity decreased significantly at 10°C. In addition, *Methanosaeta* is known as a fastidious and difficult-to-cultivate methanogen. We also encountered the problem of *Methanosaeta* cell lysis during long-term cultivation without changing the nutrient medium.

In addition to bacteria, the consortia grown on the butyrate- and propionate-containing media included microbial cells with morphological properties similar to those of the archaea *Methanosaeta* and *Methanospirillum; Methanosaeta* prevailed in butyrate-containing media, whereas *Methanospirillum* cells were predominant in the media containing propionate.

The cells of the *Methanomethylovorans*-like methanogen accumulated in the aggregates formed in the butyrate- (more) and propionate-containing media (less). The growth substrates utilized by this microorganism in these consortia have yet to be determined.

In the consortium grown in the medium with H_2/CO_2, a microorganism with morphological properties similar to those of hydrogen-utilizing methanogenic archaea of the genus *Methanospirillum* was predominant. The rates of the metabolic activity of the psychrotolerant syntrophic community at 10°C and higher temperature, depend on the activity of the hydrogen-utilizing methanogen to a great extent. At low temperatures, methanogens are able to utilize hydrogen to a lower residual concentration [1, 28]. An increase in the numbers and activity of hydrogen-utilizing methanogens in the biomass of anaerobic bioreactors at 10–15°C has been reported earlier [10, 14, 24, 25]. According to the results of our experiments (Table 1), the temperature limit for growth of the hydrogen-utilizing strain *Methanospirillum* sp. in the investigated biomass was lower than that of the type strain [26, 27]. It was, therefore, psychrotolerant.

Table 2. Comparative analysis of the biomass yield on various substrates during three-month cultivation

	Biomass concentration (g/ml)	Substrate added (mM)	Biomass yield (g/ml mM substrate)
Beginning of the experiment	16.98×10^{-4}	ND	ND
Acetate	66.25×10^{-4}	180	27.4×10^{-6}
Propionate	62.5×10^{-4}	130	35.01×10^{-6}
Butyrate	65×10^{-4}	170	28.25×10^{-6}
H_2/CO_2	25×10^{-4}	ND	ND

Note: The presented results are averages of replicate data.

During short-term (48 days) batch cultivation of the psychrotolerant community at 30°C, the meta-

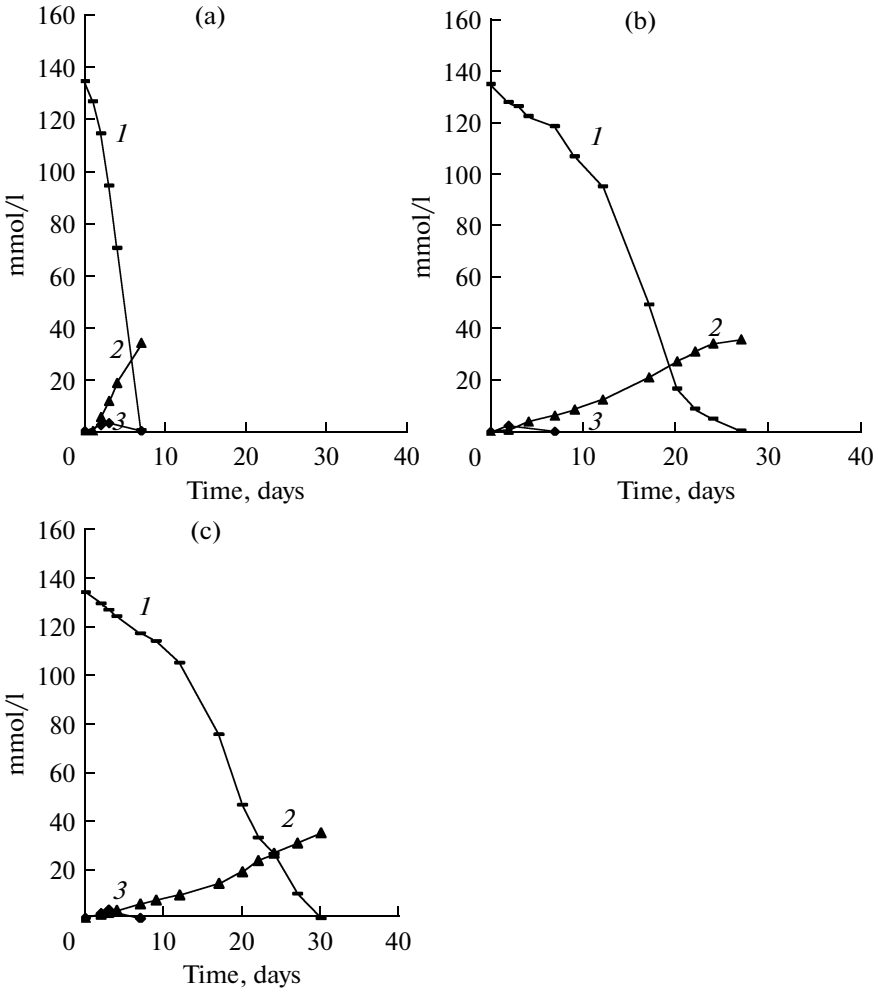

Fig. 5. Hydrogen utilization: at 30°C at the beginning of the experiment (a); at 10°C at the beginning of the experiment (b); after 48-day cultivation at 30°C and the temperature shift to 10°C (c): hydrogen (*1*), methane (*2*), and acetate (*3*).

bolic activity of the cultures grown on acetate and propionate was higher after the temperature shift from 30 to 10°C than that of the cultures initially grown at 10°C for 5 months (Table 1). However, the ability of the microbial community to utilize the substrates at 30°C after long-term (150 days) cultivation at 10°C decreased in comparison to the activity of the biomass cultivated at 30°C for 48 days. This indicates that both mesophilic and psychrotolerant microorganisms were present in the community. The psychrotolerant microorganisms of this microbial community are able to grow both at 30 and 10°C, whereas mesophilic bacteria do not grow at 10°C, which results in a decreased ability of the community to function at 30°C after long-term cultivation at low temperatures.

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