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EXPERIMENTAL ARTICLES

Butyric Acid Bacteria of the Genus *Clostridium* in the Bottom Sediments of Inland Basins of Different Types

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Abstract—The cell numbers and ecological characteristics of the distribution of certain species of butyric acid bacteria (BABs) of the genus *Clostridium* in the bottom sediments of inland basins of different types were studied using the optimal nutrient media. The seasonal dynamics of clostridial vegetative cells and spores in sediments with different ecological conditions were revealed. The cell numbers of the dominant BAB species were shown to depend on the redox potential of the sediments, the amount and composition of C_{org}, and the trophic state of the basin in general. *C. pasteurianum* was found to predominate in eutrophic lakes and reservoirs (5–11 × 10^6 cells/cm^3), *C. butyricum* and *C. felsineum* predominated in mesotrophic ones (2–11 × 10^6 cells/cm^3), and *C. acetobutylicum* was predominant in acidic chthonioeutrophic lakes and reservoirs (0.1–0.5 × 10^6 cells/cm^3). The lowest cell numbers of BABs were found in river sediments, whereas the highest numbers were recorded in the sediments of polysaprobic zones (0.1–1.0 × 10^3 and 0.5–2.0 × 10^7 cells/cm^3 respectively).

Key words: Butyric acid bacteria, ecological conditions, organic matter.

The carbohydrate-fermenting butyric acid bacteria (BABs) of the genus *Clostridium* perform an important step of anaerobic decomposition of organic matter in aquatic ecosystems, acting as precursors for sulfate reducers and methanogens [1]. Although the urgency of investigating BABs in water bodies has long been recognized [2], the published results, pertaining mostly to the cell numbers of *C. pasteurianum* in bottom sediments, are outdated [3]. Those works yielded underestimates [4] since nitrogen-free media, unsuited for determination of bacterial numbers, were used [5]. The main reason for this understatement is that only specific clostridial species grow well on "tough" mineral media [6]; only a small portion of BABs survive under these conditions [7], namely, those with a highly active nitrogenase complex [8]. The addition of reducing agents, growth factors, and vitamins increases survival of bacteria during the lag phase [8] and thus made it possible to substantially optimize the isolation and enumeration of clostridia from soils [5] and bottom sediments of water bodies [9]. Modern systematic ecological studies of this bacterial group are, however, still scarce [4, 10, 11].

The main objective of the present work was to generalize the data of many years' observations (mostly unpublished) of the abundance and ecological characteristics of the distribution of BAB species in different bottom sediments as dependent on their type, productivity, and anthropogenic influence.

MATERIALS AND METHODS

The investigation of the populations of butyric acid bacteria in bottom sediments (BSs) of different types of basins in European Russia and the Baltic region was performed during the summer seasons of 1975–1998. The basins investigated included over 30 lakes of varying productivity and physicochemical conditions; 11 major reservoirs on the Volga, Kama, and Don rivers, located in several climatic zones, from taiga to semidesert; and 12 small rivers of different levels of saprobity, some of them under strong anthropogenic influence.

The sediments were sampled with a dredger that preserved the sediment structure, after which samples of specific horizons for microbiological and chemical investigations were taken with sterile tubes. Immediately after sampling, Eh and dissolved O_2 were measured. Analysis of C_{org} forms and isolation of bacterial cultures on optimal media were performed in the laboratory of the Institute of the Biology of Inland Waters according the procedures described in [5, 12–14]. The equipment used included a KL-115 oxygen meter, a Radelkis ionometer, a CHN-1 gas chromatographic analyzer, and an Ergoval microscope.

RESULTS AND DISCUSSION

The investigation of lakes of different types using a modified [14] scheme of BAB study revealed that, in BSs of practically all basins, the cell numbers of

Table 1. Cell numbers of butyric acid bacteria in the bottom sediments of lakes of various trophic levels, 10^3 cells/cm^3

Lake	Location	Trophicity	Site	Eh, mV (0- to 2-cm layer)	C_{org}, mg/cm^3	C^*_{eh}, % C_{org}	*C. pasteurianum*	*C. butyricum*	*C. felsineum*	*C. acetobutylicum*
Tivera	Estonia	me	p	60	13	15	30	70	90	0.7
Linoyarv	The same	e	ol	55	6.4	–	10	50	70	1.2
″	″	″	p	–220	14–22	30–35	4000–11000	1700–4400	2000	3.1–10
Must'yarv	″	ce	ol	90	10	9.2	10	10	20	50
″	″	″	p	–40	9.3–11	15–18	30–100	10–70	10–90	100–250
Pikkayarv	″	″	p	–	17	18	100	50	–	500
Dridzas	Latvia	om	ol	160	1.2	–	10	50	–	–
″	The same	″	gl	40–110	5.6–7.8	20	40–90	140–250	–	1.2
″	″	″	p	105–120	6.1–9.8	9–12	50–200	50–500	–	–
Vishki	″	m	gl	90	14	14	250–700	700–1100	–	3.7
″	″	″	p	80–100	8.5–11	13–25	500–700	500–700	–	1.2
Dotkas	″	e	p	10–(–60)	16–19	26–30	700–7000	1100–3700	1700	5.6
Kivrenka	″	d	p	–	31	8	10	70	–	10
Drukshyai	″	e	ol	120	1.2–2.8	–	5–20	20–70	20	0.1
″	″	″	gl	60	6.6	12	100	500	100	7.0
″	″	″	p	10–(–80)	9.8–11	17–21	100–7800	200–2000	100–1000	2.7
Siverskoe	Vologda oblast	m	ol	120	13.1	20	40	130	170	2.5
″		″	p	60–(–20)	9.3–14	28–32	100–1000	1300–7000	100–2000	0.2–1.3
Kubenskoe	The same	me	gl	95	6.6–10	18–21	100–700	300–1100	100–700	0–0.1
″	″	″	p	40–80	12–14	25–30	200–1300	1000–7000	300–2000	0.1
Pokrovskoe	″	e	p	60	18	28	1000	70	130	0.1
Kishemskoe	″	d	p	90	15	9	10	50	70	7
Yugdem	Mari-El	o	p	–	9.7	20	1–10	10–90	–	0–0.1
Konon'er	The same	m	p	–	9.2	26	100	1000	–	0.7
Kichier	″	e	ol	–	15.5	17	100	100	–	1.3
″	″	″	p	–	14	27	11000	1000	–	7
Pleshcheevo	Yaroslavl oblast	me	gl	40–80	3.8–14	12–31	10–1000	90–2300	50–1700	0.01–0.2
″		″	p	20–(–40)	12–16	19–28	100–1200	150–1000	200–1100	0.2–2.3
Nero	The same	e	p	40–(–10)	24.4	32	5100	2700	2000	7
Lesnoe	″	d	p	90	28.3	8	1	20	–	20
Beloe	Moscow oblast	e	ol	105	4.4	22	100	90	–	0.1
″		″	p	60	16.1	30	1300	110	–	0.1

Note: Site designations: p, profundal; ol, open littoral; gl, overgrown littoral. Trophicity designations: o, oligotrophic; om, oligomesotrophic; m, mesotrophic; me, mesoeutrophic; e, eutrophic; ce, chthonioeutrophic; d, dystrophic. In all tables, dashes indicate absence of data.

* C_{eh}, easily hydrolyzable fractions of C_{org}.

C. pasteurianum, C. butyricum, and *C. felsineum* were very high, reaching 100–11 000 × 10^3 cells/cm^3 (Table 1) in profundal silts of productive lakes. The values were two to three orders of magnitude higher than the cell numbers determined earlier on nitrogen-free media [10]. Even in the aerated sediments of oligotrophic lakes and in littoral sediments, their cell numbers were 5–1100 × 10^3 cells/cm^3. On the contrary, the cell numbers of the acidophilic *C. acetobutylicum* were low in the majority of the BS samples. Only in the black coarse detritus silts of Estonian chthonioeutrophic lakes (water color index, 240–300^0, pH 4.6–5.4) did the cell numbers of *C. acetobutylicum* reach 100–500 × 10^3 cells/cm^3, making them the dominant clostridia. The highest levels of BABs were generally found in reduced profundal silts of eutrophic lakes, rich in easily hydrolyzed organic matter (C_{eh}); *C. pasteurianum*, which ferments simple sugars, dominated there [5, 12]. The lowest levels were recorded for the oxic sands of the open littoral (independent of the basin trophicity), where C_{org} reserves are low, and in the peaty sediments of dystrophic lakes, where organic matter is low in C_{eh} and consists of 85–90% lignin–humus fractions [10]. In specific BSs of the overgrown littoral with a high concentration of decomposing residues of aquatic plants, BAB cell numbers were unusually high for the aerated sites. In such BS, the polysaccharide-decomposing species, *C. butyricum* and *C. felsineum*, predominated (Table 1).

The ecological diversity of the lakes investigated made it possible to determine certain factors affecting the cell number and distribution of BABs in the sediments: redox conditions, total C_{org} pool, and its composition and availability. Significant fluctuations of the cell numbers and composition of this group in the BSs of the same basins from year to year (Table 1) were caused by the spotty distribution of the BSs types and their properties and also by seasonal changes, as will be considered below. The simultaneous enumeration of vegetative cells and spores revealed that the latter constituted in different lake BSs from 0.01 to 80% of the total BAB cell number [11] and probably depended on the same ecological factors. In the profundal silts of productive basins in summer, clostridia were mainly present as active cells; in the littoral sands and peat sediments of dystrophic lakes, the fraction of spores increased.

The water reservoirs studied belong to the same water system; are mesotrophic (except for the eutrophic Ivan'kovskoe and Tsimlyanskoe); and have similar redox conditions of the upper layers of the main part of BSs, i.e., from oxidized to slightly reduced [11]. The BSs, however, differed in C_{org} contents and composition even within the same basin, depending on the site location; this resulted in a number of peculiarities in the quantitative characteristics of the clostridial community. Although the BAB concentration in reservoir BSs was generally not less than in the lakes [11], their distribution along the bottom was different, characteristic rather for running water basins with a variable hydrological regime. The cell numbers of *C. pasteurianum, C. butyricum*, and *C. felsineum* at the river sites in summer varied from 0.1–10 × 10^3 in washed sands with low C_{org} to 100–1300 × 10^3 cells/cm^3 in the silted BSs of the eutrophic Ivan'kovskoe and Tsimlyanskoe reservoirs and in suburban areas. In the sediments of lakelike pools of most water bodies with elevated C_{org} and C_{eh} content, the cell numbers of the three dominant BAB species were 50–2500 × 10^3, and in detrital silts of eutrophic zones (C_{eh} > 20%), as high as 11 000 × 10^3 cells/cm^3. At all sites the cell numbers of *C. acetobutylicum* were very low. They reached 7–11 × 10^3 cells/cm^3 only in the peaty and coarse detrital BSs of certain sites of the upper Volga and Kama reservoirs overloaded with poorly hydrolyzable natural and anthropogenic compounds.

In spite of the BS diversity and significant fluctuations in clostridial cell numbers, the structure of BAB communities was sufficiently homogeneous along the Volga–Kama cascade. *C. butyricum* and *C. felsineum* dominated in the overwhelming majority of BSs formed mostly by terrigenous organic inflow. *C. pasteurianum*, which utilizes simple sugars, predominated only at certain sites in eutrophic basins with algal silts [10]. The spore content in reservoir water fluctuated within the range 0.1–90% and was generally higher than in most lakes.

The distribution and composition of butyric acid bacteria in river sediments are practically not studied. Research performed on a number of tributaries of the Rybinskoe and Gor'kovskoe reservoirs with the use of optimal media revealed sharp fluctuations in the cell number of BABs in BSs of different sites, depending on their saprobity. The lowest total clostridial cell numbers were found in washed sand of the rivers with slightly polluted drainage basins; the highest, in the sediments under strong anthropogenic influence (Table 3). The abundance of individual species varied: for *C. pasteurianum*, 5–15 000; for *C. butyricum*, 9–17 000; and for *C. acetobutylicum*, 0–25 × 10^3 cells/cm^3, while the spore content was 0.1–80%. Representatives of *C. butyricum*, capable of fermenting a broad range of carbohydrates, dominated in all the BSs, independent of significant differences in physicochemical conditions [4, 12].

The data on the seasonal dynamics of clostridia in silts is scarce [2, 15]. The annual cycles for individual BAB species and their spores have been studied in the most complete way in various types of sediments of the dimictic mesoeutrophic Lake Pleshcheevo (1984–1985) and of the Rybinskoe Reservoir (1982–1983).

The black reduced silts of Lake Pleshcheevo are characterized by their stable redox conditions, which are disturbed only during the spring–summer circulation, and by constant availability of labile C_{org} [16]. The cell number of the dominant species *C. butyricum* and *C. pasteurianum* was therefore high throughout the annual cycle, with small seasonal fluctuations. The

Table 2. Cell numbers of butyric acid bacteria in bottom sediments of different sites in reservoirs of the Volga, Kama, and Don basins in summer, 10^3 cells/cm^3

Reservoir	Site	C_{org}, mg/cm^3	C_{eh}, % C_{org}	*C. pasteurianum*	*C. butyricum*	*C. felsineum*	*C. acetobutylicum*
Ivan'kovskoe	r	1.8–6.2	7–14	3–90	80–220	–	0–0.1
″	l	5.1–24	8–22	310–7000	100–1000	–	0.1–11
Rybinskoe	r	3.2–4.3	8–10	7–30	11–90	20–90	0–0.1
″	l	5.4–18	9–14	11–700	30–2000	100–2000	0.1–7
Sheksninskoe	r	8.4–14	–	5–70	90–1000	–	0.1–2
″	l	12–21	–	30–120	200–2000	–	0.1–7
Gor'kovskoe	r	2.8–7.1	8–12	10–90	1–120	11–100	0–0.1
″	l	9.5–25	10–22	60–700	100–2500	170–2000	0.1–0.7
Cheboksarskoe	r	2.6–6.9	13–19	1–10	20–1300	25–250	0.1–0.3
Kuibyshevskoe	r	2.2–5.7	7–9	1–10	60–110	30–100	0.1–0.3
″	l	9.2–18	8–19	1–110	110–7000	130–7000	0.06–0.3
Saratovskoe	r	1.2–4.3	7–9	0.1–7	1–9	0.7–9	0
″	l	7.2–17	6–12	30–130	210–900	130–700	0.02–0.3
Volgogradskoe	r	0.9–2.5	6–8	0.6–3	20–110	1–7	0
″	l	7.3–12	10–14	13–7000	130–11000	130–2500	0.03–0.3
Votkinskoe	r	7.2–9.9	6–8	1–20	10–110	–	–
″	l	12–24	8–12	10–210	50–250	13–200	0.3–7
Nizhnekamskoe	r	2.8–4.3	7–10	0.1–3	10–70	11–25	0.1–0.2
″	l	7.7–22	6–12	50–130	60–11000	25–1100	0.1–1.3
Tsimlyanskoe	r	3.1–7.7	15–28	70–1000	10–130	–	0
″	l	4.8–9.2	19–35	110–7000	100–1300	–	0.1–5

Note: r, riverlike; l, lakelike.

Table 3. Cell numbers of butyric acid bacteria (10^3 cells/cm^3) and their spores (%) in the BSs of some tributaries of the Rybinskoe and Gor'kovskoe reservoirs with different saprobity levels (SLs)

River and site	SL	C_{org}, mg/cm^3	C_{eh}, % C_{org}	*C. pasteurianum*		*C. butyricum*		*C. acetobutylicum*	
				cells	spores	cells	spores	cells	spores
Rybinskoe Reservoir									
Sit', near the settlement of Breitovo	++	12.3	18	70	17	250	10	0.1	20
The same, mouth	+	6.3	12	5	64	90	24	0	–
Suda, mouth	+	4.8	16	10	10	110	60	0.01	60
Yagorba, mouth near Cherepovets	++++	38.4	24	7000	1	12000	1	1.7	13
The same, 10 km upstream from the mouth	++	9.2	12	30	17	1000	10	0.1	10
Sheksna, mouth	+++	24.7	22	7000	10	7000	10	11	20
The same, upstream from Cherepovets	++	4.9	10	10	47	100	30	0.1	70
Ukhra, mouth	+	5.6	14	5	–	9	–	0.7	–
Gor'kovskoe Reservoir									
Cheremukha, near Rybinsk	++++	42.4	32	15000	0.1	17000	0.1	25	0.1
Kotorosl', near Yaroslavl	++	9.6	16	70	1	250	3	0.2	31
Kostroma, near Kostroma	+++	28.6	21	1000	–	7000	–	17	–
Poksha, mouth	+	3.5	14	5	80	11	17	0.1	80

Note: Saprobity levels: +, low; ++, moderate; +++, high; ++++, very high.

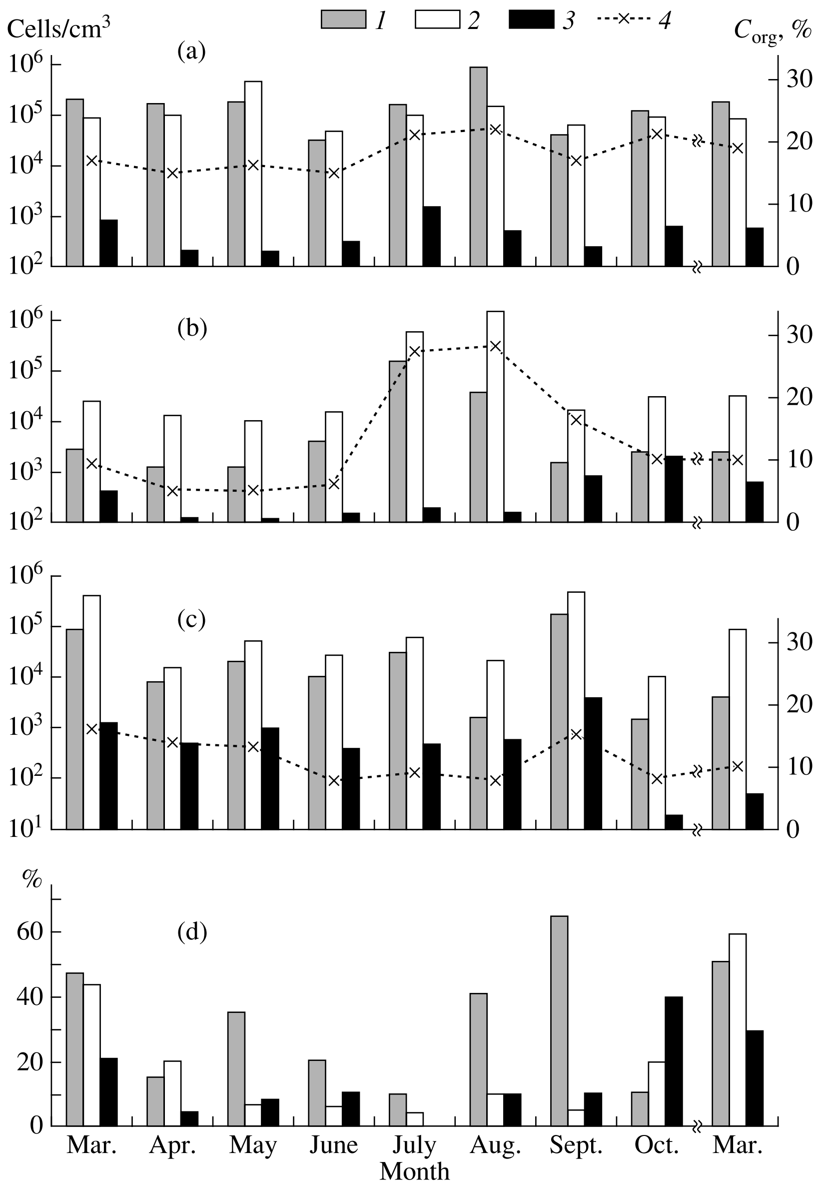

Fig. 1. Seasonal dynamics of individual BAB species in silts with different concentrations of labile organic matter (C_{eh}) ((a) profundal and (b) overgrown littoral of Pleshcheevo Lake and (c) central part of the Rybinskoe Reservoir) and of their spores, % of cells ((d) reservoir center). (*1*) *C. pasteurianum*; (*2*) *C. butyricum*; (*3*) *C. acetobutylicum*, cells/cm^3; (*4*) C_{eh}, % C_{org}.

former peaked in spring, when the profundal was warmed up (1×10^6 cells/cm^3); the latter, in late summer (1.2×10^6 cells/cm^3), growing in silts (Fig. 1a). The sharp seasonal fluctuations of C_{org} composition characteristic for the sediments of overgrown littorals [16] defined the annual dynamics of BAB development. *C. butyricum* was dominant throughout the cycle, peaking at 2×10^6 cells/cm^3 in the end of summer when C_{eh} content was highest; *C. pasteurianum* peaked in June (Fig. 1b). The cell numbers of *C. acetobutylicum* in the lake BSs have always been low. In the BSs of the meromictic Rybinskoe Reservoir, the highest BAB cell numbers were detected during the autumn–spring period (Fig. 1c), after sedimentation of dead algae finished and conditions favorable for anaerobic bacteriobenthos were established in the silts. The representatives of *C. butyricum* and *C. pasteurianum* dominated permanently in the main area of the basin (up to $100–1000 \times 10^3$ cells/cm^3) with a slight predominance of the first species, which ferments complex carbohydrates. A high abundance of *C. acetobutylicum* was found in the silts developed from flooded peat soil.

According to the spore counts, for most of the year they constituted not more than 1–10% of the number of vegetative cells in various BSs of Lake Pleshcheevo and of the Rybinskoe Reservoir; i.e., the clostridial commu-

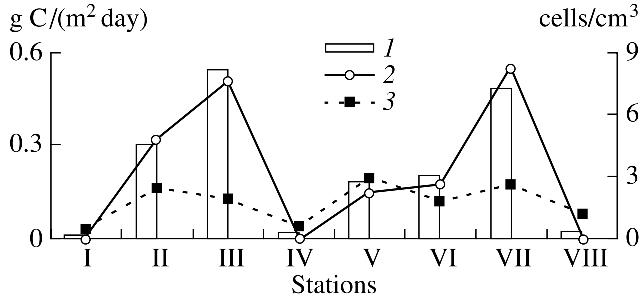

Fig. 2. (*1*) Anaerobic decomposition of organic matter, g C/(m^2 day); (*2*) total BAB cell numbers, $\times 10^5$ cells/cm^3; and (*3*) total bacterial numbers, $\times 10^9$ cells/cm^3 in different bottom sediments of (I–III) the Trubezh River, (IV–VII) Pleshcheevo Lake, and (VIII) the mouth of the Veksa River: (I) clayey sand; (II) silted sand; (III) coarse detritus silt; (IV) sand; (V) black sandy silt; (VI) black fine detritus silt; (VII) coarse detritus silt; (VIII) coarse sand.

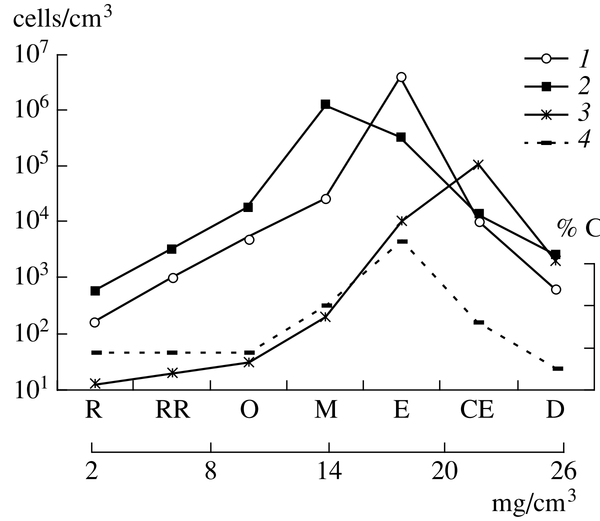

Fig. 3. Cell numbers of individual BAB species in bottom sediments of basins of different types and trophicity levels: (*1*) *C. pasteurianum*; (*2*) *C. butyricum* and *C. felsineum*; (*3*) *C. acetobutylicum*, cells/cm^3; (*4*) C_{eh}, % C_{org}. Along the abscissa: C_{org} content, mg/cm^3 wet BS; R, rivers (out of the urban influence zone); RR, riverlike parts of reservoirs; O, M, E, CE, D: oligotrophic, mesotrophic, eutrophic, chthonioeutrophic, and dystrophic basins, respectively.

nity was in an active state. The seasonal dynamics of sporulation in specific biotopes showed slight differences, depending on the physicochemical conditions and their stability. In the BSs of the main part of both basins, however, sporulation of most BABs peaked in early autumn and in winter, as is illustrated by the data on the central zone of the Rybinskoe Reservoir (Fig. 1d).

Analysis of the collected material revealed the presence of butyric acid bacteria in the BSs of a wide range of biotopes. Their cell number fluctuated from 0.1 to 17000×10^3 cells/cm^3, depending on the basin productivity and on the physicochemical conditions in the BSs; the ratio of spores in most of the samples did not exceed 1–20%. The geochemical activity of primary fermenters was previously believed to occur only in reduced silts of eutrophic lakes [1, 2]. The abundance of clostridia, often the dominant organisms in anaerobic bacteriocenoses [9, 17], and their activity throughout the main part of the annual cycle [11, 15, 16] lead, however, to the conclusion that BABs play an important part in the C_{org} turnover in the majority of internal basins. It was confirmed by experiments on a variety of BSs of Lake Pleshcheevo performed in summer 1996; they revealed a correlation between anaerobic C_{org} destruction and the total BAB cell numbers (Fig. 2), while no correlation with the total bacteriobenthos cell number was present.

As a result of investigation of a number of basins with a broad spectrum of hydrological, hydrochemical, and productivity characteristics, the ecological peculiarities of the distribution of individual BAB species were revealed. The concentration, composition, and availability of C_{org} compounds, reflecting the trophic state of the basins, were the main factors determining the predominant bacterial species in the sediments. *C. pasteurianum*, fermenting simple sugars, dominated in the silts of eutrophic lakes and in the polysaprobic BSs with high concentrations of easily hydrolyzed compounds. In mesotrophic lakes and reservoirs with BSs formed under the influence of allochthonous flow, *C. butyricum* and *C. felsineum*, which ferment various polysaccharides, predominated. In the BSs of acidic chthonioeutrophic lakes, *C. acetobutylicum* reached its maximum, being capable of utilizing not only carbohydrates and amino acids [5, 12] but also lignin–humus compounds [23] (Fig. 3). This vast array of data, new for water microbiology, provides unequivocal evidence of the geochemical role of butyric acid bacteria, an important component of bacterial communities in the bottom sediments of different types of internal basins.

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