Seasonal changes in organic matter mineralization in a sublittoral sediment and temperature-driven decoupling of key processes

Keiichi Tabuchi[1], Hisaya Kojima[2*], and Manabu Fukui[1, 2]

1. Department of Biological Sciences, Graduate School of Science, Tokyo Metropolitan University, Tokyo, Japan

2. The Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan

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*Corresponding author.
E-mail: kojimah@pop.lowtem.hokudai.ac.jp
Fax: +81-11-706-5460

Abstract

Seasonal changes in the mineralization of organic compounds in sediments were investigated in temperate, sublittoral zone sediments (Tokyo Bay, Japan). The total mineralization rate and sulfate reduction rate showed large seasonal variations over the year, and although the fluctuations in both rates correlated with temperature, the latter was irregularly high in May. The concentration of organic carbon dissolved in interstitial water was specifically high in April. A culture-based experiment was also conducted under temperatures corresponding to the seasonal changes. In the culture incubated at a temperature corresponding to April (13°C), hydrolysis and fermentation proceeded, but terminal oxidation was hindered, thereby resulting in acetate accumulation. At a temperature corresponding to May (22°C), acetate oxidation coupled with sulfate reduction was observed. The temperature-related differences were also reflected in the bacterial community structure in the cultures analyzed by DGGE. In the culture incubated at the lower temperature, sulfate-reducing bacteria of incomplete oxidizers was detected, while sequence found in the culture incubated at the higher temperature was related to complete oxidizers. These results suggest that complete and incomplete oxidizing sulfate-reducing bacteria act as distinct functional groups, responding to temperature in different ways, particularly in environments characterized by large temperature fluctuations.

Introduction

In the global carbon cycle, organic carbon sedimented and buried in aquatic

environments plays a significant role as a major sink of carbon [7, 8]. Whether the sedimented organic carbon is recycled or remains buried permanently largely depends on microbial activity. With regard to marine systems, sedimentation mainly occurs in coastal areas including tidal flats and sublittoral zones which are one of the most productive marine ecosystems. In addition to active on-site primary production of organic matter, sublittoral zones receive the organic matter transported from terrestrial ecosystems. Thus, sublittoral sediments are exposed to a high input of organic compounds of both autochthonous and allochthonous origin.

Due to the limited availability of oxygen, degradation of the organic matter deposited in marine sediments mainly proceeds anaerobically [2, 5]. Under anaerobic conditions, high molecular weight organic matter is not mineralized at once, but is gradually degraded via hydrolysis, fermentation, and terminal oxidization of the low molecular weight organic compounds generated. These sequential processes are mediated by microorganisms specialized for each step. Therefore, the organic carbon mineralization rate in tidal flats might be affected by the activities of various microorganisms that respond to environmental changes in different ways. In a previous study, too high or too low temperatures were shown to induce decoupling of the key phases in anaerobic mineralization in marine sediments [30]. The imbalance was explained to result from the differences between the thermal responses of organisms responsible for terminal mineralization and other preceding processes [30]. This finding is very important for a comprehensive understanding of organic compound degradation in aquatic sediments, which is significant to the global carbon budget. The universality of this laboratory

phenomenon, however, has not been completely verified yet by sufficient field observations or other additional experiments.

In the present study, we investigated the seasonal changes in the mineralization of organic compounds in a temperate sublittoral zone. In marine sediments, the terminal step of mineralization is mainly undertaken by sulfate-reducing bacteria (SRB) [5, 11, 13, 17]. Hence, we monitored the total mineralization rate along with the sulfate reduction rate (SRR) throughout the year. In addition, a culture-based experiment was conducted in order to test a hypothesis to explain the observed seasonal fluctuations as consequences of the differences in the temperature characteristics of functional bacterial groups.

Methods

Study site and sampling

The study site (39°39.0'N, 139°55.5'E) was situated in Sanban-ze on the north coast of Tokyo Bay, Japan. The routine water depth at the study site was approximately 1 m, and sediments appeared above the water level only a few times a year. At the study site, accumulation of macroalgae was observed during summer. Monthly sampling was conducted from October 1999 to October 2000. Samples of the sediment core were manually obtained using acrylic tubes (inner diameter, 4.6 cm; length, 30 cm). The tubes containing the sediment core samples were sealed with rubber stoppers and transported to the laboratory in an ice-cooled box. All sediment core samples were

obtained during low tide on days of spring tide.

Measurements of physicochemical properties

On return to the laboratory (within a few hours after sampling), the sediment cores were sectioned (the top 2 cm of the sediment and 3-cm layers below it). The basic characteristics of the sediments, such as water content and loss-on-ignition, were determined. The sectioned sediment samples were weighed and then dried at 95°C for 24 h. The dried samples were weighed and then baked at 550°C for 2 h. The water content and loss-on-ignition were calculated from the decrease in weight after drying and baking, respectively. For chemical analyses, the interstitial water samples obtained by centrifugation of the sectioned sediment samples were used. The interstitial water samples obtained were filtered using a 0.22-μm filter and were stored at –20°C till chemical analyses. The concentrations of sulfate and chloride were determined using an ion chromatograph (DX-120; Dionex, Sunnyvale, CA) equipped with a column for anion analyses (AS4A; Dionex). To remove the interference caused by the excess chloride, the samples were diluted (1:400) prior to analysis. The dissolved organic carbon (DOC) concentrations were determined using a total organic carbon analyzer (TOC-5000; Shimadzu, Kyoto, Japan). Before analysis, the samples were acidified by the addition of HCl and were then purged with dinitrogen gas to remove the inorganic carbon.

Total mineralization rate

The total mineralization rate was determined from the incubation experiment as

described previously [11]. Undisturbed sediment cores were incubated under dark conditions at *in situ* temperature. Prior to incubation, the overlying water was replaced with filter-sterilized water to exclude the effect of planktonic organisms. After incubation for 20 h, the dissolved inorganic carbon concentrations in the overlying water and interstitial water were determined using an infrared gas analyzer. The total mineralization rate in each layer was calculated from the increase in inorganic carbon during incubation, and the change in the inorganic carbon content in the overlying water was assumed to be attributable to the mineralization in the surface layer of the sediment.

Sulfate reduction rate

The sulfate reduction rate for each layer of sediment was measured using a radiotracer as described previously [10, 27]. Briefly, a carrier-free solution of $Na_2{}^{35}SO_4$ was injected into each portion of the sediment. After incubation for 20 h at *in situ* temperature, the reduced inorganic sulfur compounds were converted into H_2S by chromium reduction and then volatilized by HCl. The generated H_2S was trapped using paper impregnated with zinc acetate solution in order to measure radioactivity. The sulfate reduction rate was calculated from the incubation time, amount of $^{35}S^{2-}$ generated, sulfate content in the sediment, and isotope fractionation factor.

Extracellular enzyme activity

The activity of β-glucosidase, the major extracellular enzyme hydrolyzing high molecular weight organic polymers, was determined using fluorogenic substrates. The

assay method was modified from that described in previous studies [2, 4, 14]. A slurry of the sediment was prepared by mixing 1.2 ml of the weighed sediment with 7.2 ml of filtered seawater. Stock solution of 4-methylumbelliferyl β-D-glucoside was added to the sediment slurry to obtain a final concentration of 50 μM. The stock solution was prepared by dissolving the substrate in ethylene glycol monomethyl ether. After incubation for 10 min to 20 min at *in situ* temperature, the reaction was stopped by addition of NaOH solution (final concentration, 90 mM). The sediment particles and suspended substances were removed by centrifugation and subsequent filtration. The fluophor in the supernatant was quantified by measuring the fluorescence (excitation, 365 nm; emission, 455 nm) using a fluorescence spectrophotometer (Hitachi 650-10; Hitachi, Tokyo, Japan). The calibration curve was obtained from a 4-methylumbelliferone (MFU) solution of a known concentration. The hydrolysis rate was calculated from the incubation time and the difference in the fluophor content between the incubated samples and controls (no incubation).

Enrichment culture experiment

An enrichment culture experiment was conducted to examine the effect of temperature on mineralization under sulfate-reducing conditions. Throughout the experiment, bicarbonate-buffered and sulfide-reduced medium was used as the basal medium [31]. The medium contained the following constituents (l^{-1}): 20.0 g NaCl; 4 g Na_2SO_4; 0.2 g KH_2PO_4; 0.25 g NH_4Cl; 3.0 g $MgCl_2{\cdot}6H_2O$; 0.5 g KCl; 0.15 g $CaCl_2{\cdot}2H_2O$; 1 ml trace element solution; 1 ml selenite-tungstate solution; 1 ml vitamin

mixture solution; 1 ml vitamin B_{12} solution; 30 ml of 1 M $NaHCO_3$ solution; and 1.5 ml of 1 M Na_2S solution [31]. To simulate winter conditions, the first enrichment culture was incubated at a low temperature. In a 250-ml flat bottle, 5 ml of the sediment obtained in May 2000 was inoculated in 220 ml of the medium with 0.5 g cellobiose added as the carbon source. The headspace was filled with an N_2/CO_2 mixture (80:20), and the sealed bottle was incubated at 7°C for 2 months. From the subsequent resultant culture, a second set of enrichment cultures were incubated at 4 different temperatures corresponding to seasonal fluctuations. The first enrichment culture was transferred to a new medium and incubated at 7°C, 13°C, 22°C, and 29°C. These temperatures were selected based on field observations (see below for detail). Each enrichment culture in the second set was prepared in 500-ml flat bottles containing 440 ml of the basal medium, 1 g cellobiose, and 10 ml of the first enrichment culture as an inoculum.

During incubation at the 4 temperatures, the changes in the concentrations of sulfide, DOC, and organic acids were monitored. The concentration of dissolved sulfide was determined by a colorimetric method [6], and DOC was quantified as described above. Organic acids (volatile fatty acids, VFAs) were analyzed using a high-performance liquid chromatography system. A column (SCR-101H; Shimadzu) was used for separation during organic acid analysis, and the column temperature was maintained at 50°C. Perchloric acid solution (pH 2.0) was used as an aqueous mobile phase at a flow rate of 1 $ml \cdot min^{-1}$. The absorbance at 210 nm was measured using a UV detector (Hitachi L-4000).

Denaturing gradient gel electrophoresis (DGGE) analysis of the enrichment cultures

The bacterial community structure in the cultures incubated at different temperatures was analyzed by DGGE. Cells were harvested from a 10-ml aliquot of each culture by centrifugation. Each culture was sampled 3 times in order to monitor the changes in the community structure throughout the incubation period. Total DNA was extracted using a method described previously [32]. The 16S rRNA gene fragments were amplified using the primers 341F-GC/907R under PCR conditions as described previously [21]. DGGE and subsequent sequencing of the major bands were performed as described previously [16]. Sequence-specific separation was performed using a 1.5-mm-thick 6% (w/v) polyacrylamide gel (acrylamide:bisacrylamide = 37.5:1). The denaturation gradient ranged from 20% to 60% (100% corresponding to 40% (v/v) formamide and 7 M urea), and the electrophoresis was conducted at 60°C for 240 min at a constant voltage of 200 V. The sequence of each DGGE band was subjected to the RDP Classifier [28] from the Ribosomal Database Project-II Release 10 (http://rdp.cme.msu.edu/index.jsp). Based on the phylogenetic affiliation inferred with the default settings, the noticeable bands were selected for further detailed analysis. The sequences of these bands and the related sequences retrieved from the database were aligned by using the program, ClustalX [26]. MEGA3 was used to calculate the genetic distances using Kimura's 2-parameter model and to construct the phylogenetic tree by the minimum evolution method [18]. The robustness of the tree obtained was examined by bootstrap testing of 1000 replicates.

Nucleotide sequence accession numbers

The nucleotide sequences obtained in this study were assigned the DDBJ/EMBL/GenBank accession numbers AB534762–AB534772.

Results

Characteristics of the sediments

During the study period, the recorded temperatures of bottom water ranged from 6.5°C to 32.8°C. At all sampling times, the temperature of the bottom water was virtually identical to that of the sediment surface. The chloride concentration in the bottom water ranged from 360 mM to 550 mM (Table 1).

At the study site, the upper portion of the sediment was mainly composed of silt and clay, but fine sand was predominant in those portions of the sediment that were deeper than 15 cm. The water content and loss-on-ignition did not show any obvious tendencies (Table 2). The sulfate concentration in the interstitial water varied depending on the sampling date; however, there was no significant vertical change in any of the core samples (Table 3). Even in the deep layers of the sediment, the sulfate concentrations were equivalent to those in the overlying water. As an exceptional case, in one of the core samples, i.e., the one obtained in October 2000, the sulfate concentration decreased with depth and was almost depleted over an 8- to 11-cm layer.

The changes in the DOC concentration are summarized in Table 4. In April 2000, outstanding accumulation of DOC was observed, and a high DOC concentration was also observed in May.

Microbial activity

The total mineralization rate showed large seasonal variations, but exhibited similar vertical profile independent of the sampling date (Fig. 1). On all sampling dates, the highest rate was observed in the 0- to 2-cm layer. The SRR also varied seasonally, but the vertical profiles showed no constant depth-related trend (Fig. 2). These depth-related changes could not be explained by any of the factors, including water content, loss-on-ignition, sulfate concentration, or DOC.

In Fig. 3, the cumulative total mineralization and sulfate reduction rates are shown in relation to temperature. For each sampling date, SRRs measured over 5 layers (from the surface to a depth of 14 cm) were added and then converted into the respective CO_2 production rates using the equation

$$SO_4^{2-} + 2CH_2O \rightarrow S^{2-} + 2CO_2 + 2H_2O$$

To evaluate the contribution of SRR, the total mineralization rates are also shown as a sum of values from the corresponding layers. As clearly shown in Fig. 3, the highest SRR measured in May 2000 was distinctly greater than the SRR expected from the temperature-related trend. In that month, the estimated contribution of sulfate reduction to the total mineralization reached 67%. All cumulative SRRs other than this showed a strong positive correlation with temperature ($r^2 = 0.962$; $p <$

0.00001; n = 9).

The seasonal variations in the potential activity of β-glucosidase are shown in Fig. 4 as MFU production rates. Higher β-glucosidase activity was observed in the upper layers of the sediment, particularly during spring and summer. The highest β-glucosidase activity, i.e., 1.61 μmol MUF·$cm^{-3}h^{-1}$, was observed in May 2000 in the 0- to 2-cm layer. In the deeper layers, the β-glucosidase activity was consistently low (0.04–0.41 μmol MUF·$cm^{-3}h^{-1}$) irrespective of the sampling date. These results indicated that active hydrolysis of organic polymers by extracellular enzymes was restricted to portions near the sediment surface, as demonstrated in previous studies in other localities [4, 20, 22].

Culture experiment

Changes in the concentration of sulfide, DOC, and VFAs in the cultures incubated at the 4 temperatures are shown in Fig. 5. In all cultures, only formate and acetate were detected as the major VFAs. In the culture incubated at 7°C, the decrease in DOC and sulfide production stopped after incubation for 10 d, despite the remaining sulfate. Formate accumulated in the early phase and then decreased rapidly, but acetate increased consistently during the monitoring. In case of the culture incubated at 13°C, the pattern of changes in DOC and sulfide concentrations was similar to that in the culture incubated at 7°C; however, the concentration of VFA appeared to peak earlier. In the culture incubated at 22°C, sulfide increased throughout the experiment and DOC decreased accordingly. The pattern of changes in the VFAs was similar to that in case of

the culture incubated at 13°C, but significant acetate consumption was observed in the latter stage. In the culture incubated at 29°C, active sulfide production was observed till depletion of sulfate in the medium. The decline in DOC was terminated along with stoppage of sulfide production. With regard to VFAs, formate was detected only on the first day, and acetate was consumed rapidly after accumulation in the first 5 days. For all cultures, the sulfide concentrations were monitored till day 47; however, no change was observed after day 30.

The bacterial community structure in the cultures was analyzed by DGGE. In all 4 cultures, shifts in the bacterial community structure were observed along the incubation period (Fig. 6). Temperature-related differences were also observed, despite the similarities between the bacterial community structure in the cultures incubated at lower temperatures (7°C and 13°C). The phylogenetic affiliations of the DGGE bands are summarized in Table. 5. Among the 11 sequenced bands, 4 were revealed to originate from SRBs belonging to the class Deltaproteobacteria (Fig. 7). One of these bands, i.e., TK3, was detected from the cultures incubated at lower temperatures (7°C and 13°C). In contrast, bands TK10 and TK12 were observed only in the cultures incubated at 22°C and 29°C. The other SRB band (TK13) was specific to the culture incubated at the highest temperature. Based on the phylogenetic analysis, the organism corresponding to the band TK12 was inferred to be a complete oxidizer, characterized by the ability to utilize acetate as an electron donor for growth. The other bands of SRB appeared to originate from incomplete oxidizers, which cannot grow on acetate.

Discussion

On August 30, 2000, an anomalously high total mineralization rate was observed (Figs. 1 and 3) in sublittoral zone sediments from Sanban-Ze, Tokyo Bay, Japan. At the study site, macroalgae of the family Cladophoraceae had flourished at the beginning of that month, but had been discolored and had begun to decay by the end of that month. Thus, a massive supply of organic compounds of algal origin might have induced the anomalous mineralization. By excluding this sampling data, significant correlations were detected between temperature and the total mineralization in the tests performed on individual layers. These significant correlations ($p < 0.05$; $n = 9$) were observed in all layers, except the 0- to 2-cm layer. The predominant effect of temperature on total mineralization has been demonstrated in previous studies [11, 19], and the importance of organic substrate availability has also been shown [1, 29]. The inconsistency in the temperature-activity relationships in the surface layer may be explained by the variations in the supply of organic matter from the sediment surface.

Seasonal changes in the SRR significantly correlated with temperature fluctuations, except in May 2000, when the highest activity was observed (Fig. 3). This irregular SRR appeared to be related to the accumulation of DOC in spring, although the highest DOC concentration was observed in April (Table 4). As a unified explanation for DOC accumulation and the subsequent drastic increase in SRR, the following hypotheses were formulated and tested in the culture-based experiment. (i) During winter, all microbial activity was suppressed, leading to accumulation of undissolved organic

material. (ii) In early spring, hydrolysis and/or fermentation were activated, but not terminal mineralization by SRB, thus resulting in the accumulation of DOC. (iii) With increasing temperature, the SRB community became active, and the accumulated DOC facilitated the irregularly high SRR. (iv) After depletion of the accumulated DOC, the SRR was controlled by temperature. The cultures were incubated at different temperatures to correspond to these presumed stages. Analysis of the cultures showed differences between the cellobiose mineralization processes in the cultures incubated at lower temperatures (7°C /13°C) and those incubated at higher temperatures (22°C /29°C). In the cultures incubated at lower temperatures, DOC consumption and sulfide production slowed, despite the remaining sulfate. When sulfide production stopped, approximately 20 mM acetate remained, corresponding to ca. 80% of the DOC left unutilized (each acetate molecule contains 2 carbon atoms). These results indicated that hydrolysis and fermentation had proceeded at the lower temperatures, whereas terminal oxidation of acetate was hindered. Sulfate reduction observed in the early phase would have been conducted by incomplete oxidizers by utilizing formate or another substrate. In contrast, in the culture incubated at 22°C, the sulfide content increased consistently, and considerable acetate consumption was observed. These results suggest that the threshold temperature for the activation of complete-oxidizing sulfate reducers within these sediments was between 13°C and 22°C, corresponding to the temperatures of April and May, respectively, at the study site. Therefore, the results of the culture experiment were consistent with our field observations. Although the hypotheses were partially supported, the difference between 7°C (corresponding to February) and 13°C

was not as significant as presumed. The high concentration of DOC observed in April might have resulted from DOC accumulation during the prolonged winter, rather than from the elevation in the activity in early spring. The seasonal variations in the supply of organic matter under actual field conditions must also be taken into account.

Significant differences were also observed in the bacterial community structure between the cultures incubated at 13°C and 22°C as revealed by DGGE analysis. Since only a limited number of bands could be identified, relationships between bacterial community compositions and degradation processes are still unclear. However, the results of the phylogenetic analysis of the sequenced bands did not conflict with the interpretation described above. The SRB detected in the culture experiment incubated at lower temperatures (band TK3) was related to sequences belonging to incomplete oxidizers, and sequence related to complete oxidizers was detected in cultures incubated at higher temperatures (band TK12).

The results of the culture experiment in the present study are consistent with the results of a previous experiment on sediment slurries that examined temperature responses [30]. In that study, the rate of hydrolysis/fermentation exceeded the terminal oxidation rate at low temperatures. In the present study, sulfate reduction occurred even at the lower temperatures, but was not coupled with acetate oxidation. In studies that recognize anaerobic degradation of organic matter as a multistep process, sulfate reduction has generally been regarded as the terminal mineralization step. As shown in the culture experiment, however, sulfate reduction can be accompanied with acetate production when it is mediated by incomplete oxidizers [31]. The results of the present

study also suggested that the contributions of complete- and incomplete-oxidizing sulfate reducers to the total SRR can vary depending on the temperature.

The results of the present study predict the predominance of incomplete oxidizers over complete oxidizers in low-temperature environments. However, this result may not always be consistent with the results of previous studies, particularly those conducted in permanently cold marine sediments. In such studies, complete oxidizers have been detected as the major members of the SRB community [23, 24], although the predominance of incomplete oxidizers has been reported from another site [15, 25]. Further, it has also been suggested that complete mineralization of organic matter can proceed without decoupling of the key steps [9]. The phenomenon observed in the present study might be specific to environments characterized by large temperature fluctuations and high organic carbon inputs

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Figure legends

Fig. 1. Total mineralization rate determined from incubation of undisturbed cores. Error bars indicate standard deviation. In 5 Feb and 30 Aug, measurements were conducted with single core.

Fig. 2. Sulfate reduction rate determined with the radiotracer methods. Error bars indicate standard deviation.

Fig. 3. Relationships between temperature and mineralization rate. Both total mineralization (open circle) and contribution of sulfate reduction (solid circle) are shown as cumulative rates calculated from measurement values of five layers (from surface to 14 cm depth).

Fig. 4. Vertical profiles of β-glucocidase activity.

Fig. 5. Changes in sulfide, DOC, and VFAs concentrations in enrich cultures of differing temperatures.

Fig. 6. DGGE band patterns of the enrichment cultures incubated at diffrent temperatures. Numbers on the lanes indicate incubation time (day).

Fig. 7. Minimum evolution tree showing the phylogenetic affiliations of DGGE bands identified as sulfate reducers. Numbers on nodes are percentage values of 1000 bootstrap resamplings (values greater than 50 are shown).

Table 1. Characteristics of overlying water

	1999			2000							
	29 Oct	24 Nov	22 Dec	5 Feb	6 Mar	7 Apr	6 May	3 Jun	1 Aug	30 Aug	14 Oct
Temperature (°C)	18.3	14.5	7.4	6.5	8.1	13.5	22.0	25.8	32.3	32.8	20.3
Dissolved oxygen (g O_2/l)	6.07	4.92	8.80	11.33	10.54	- [a]	-	-	-	-	-
Chloride (mM)	533	512	543	484	444	493	443	452	364	-	476

a) -, not determined

Table 2. Water content and loss-on-ignition of sediment

		1999			2000							
	Depth (cm)	29 Oct	24 Nov	22 Dec	5 Feb	6 Mar	7 Apr	6 May	3 Jun	1 Aug	30 Aug	14 Oct
Water content（g/cm^3)	0-2	0.53	0.69	0.67	0.65	0.73	0.74	0.68	0.65	0.66	0.59	0.62
	2-5	0.55	0.57	0.66	0.63	0.57	0.60	0.61	0.53	0.61	0.60	0.66
	5-8	0.56	0.56	0.53	0.62	0.53	0.53	0.53	0.52	0.60	0.52	0.71
	8-11	0.53	0.55	0.52	0.57	0.52	0.56	0.55	0.50	0.53	0.52	0.68
	11-14	0.41	0.57	0.50	0.55	0.51	0.51	0.54	0.53	0.50	0.47	0.63
	14-17	0.42	0.46	0.48	0.54	0.48	0.49	0.52	0.50	0.51	0.50	0.70
Loss-on-ignition (%)	0-2	4.86	4.84	4.99	5.12	5.59	4.79	4.46	4.42	4.88	4.73	4.10
	2-5	4.51	4.34	6.19	5.05	4.01	3.89	4.07	3.22	5.01	5.27	4.84
	5-8	4.59	3.58	3.64	4.93	3.10	3.26	3.06	3.41	5.24	3.96	6.86
	8-11	4.73	3.57	3.12	4.01	3.44	4.02	3.80	3.35	3.89	3.95	6.96
	11-14	3.51	3.66	3.08	3.81	3.29	3.13	3.47	4.43	2.97	2.95	5.71
	14-17	2.69	2.87	3.05	3.73	2.55	2.60	3.23	3.03	2.50	3.20	7.77

Table 3. Sulfate concentration in interstitial water (mM)

	1999			2000							
Sediment depth (cm)	29 Oct	24 Nov	22 Dec	5 Feb	6 Mar	7 Apr	6 May	3 Jun	1 Aug	30 Aug	14 Oct
0-2	21.2	24.5	24.7	25.4	24.3	24.6	25.8	23.0	17.1	-[a]	21.9
2-5	21.1	18.4	25.6	26.0	26.0	26.8	26.2	23.2	16.2	-	9.7
5-8	23.6	20.8	25.5	25.3	27.3	27.6	25.3	22.5	17.3	-	2.0
8-11	25.1	22.4	25.8	26.9	26.7	27.0	25.5	21.7	17.9	-	0.6
11-14	24.3	23.1	22.1	25.4	27.0	27.0	26.2	21.5	18.8	-	0.4
14-17	23.0	20.6	21.8	26.4	30.5	26.2	26.3	22.0	19.1	-	4.7

a) -, not determined

Table 4. Vertical and seasonal variations in DOC concentration (mg carbon per liter)

	1999		2000						
Sediment depth (cm)	29 Oct	24 Nov	22 Dec	5 Feb	6 Mar	7 Apr	6 May	3 Jun	1 Aug
Overlaying water	-[a]	3.0	3.6	5.8	-	3.1	5.8	3.2	3.0
0-2	-	-	18.4	24.0	-	38.7	31.2	31.0	22.9
2-5		-	35.2	27.0	-	130.5	58.1	16.8	18.6
5-8	14.6	-	23.7	27.5	44.9	107.9	61.9	35.9	14.5
8-11		-	27.9	53.0	45.2	172.2	64.5	31.3	14.6
11-14	29.0	-	23.6	26.4		51.0	48.1	20.7	13.2
14-17	19.5	-	23.2	37.2	26.5	22.6	36.9	22.4	19.4

a) -, not determined

Table 5. Phylogenetic affiliations of DGGE bands deduced by RDP Classifier

DGGE bans	phylum	class	order	family	genus	acc. number
TK1	*Bacteroidetes*	*Bacteroidetes*	*Bacteroidales*			AB534763
TK3	*Proteobacteria*	*Deltaproteobacteria*	*Desulfobacterales*	*Desulfobulbaceae*	*Desulfotalea*	AB534764
TK4	*Bacteroidetes*	*Bacteroidetes*	*Bacteroidales*			AB534772
TK5	*Proteobacteria*	*Gammaproteobacteria*	*Alteromonadales*	*Psychromonadaceae*	*Psychromonas*	AB534765
TK6	*Bacteroidetes*					AB534766
TK8	*Bacteroidetes*	*Flavobacteria*	*Flavobacteriales*	*Cryomorphaceae*	*Owenweeksia*	AB534767
TK9	*Bacteroidetes*	*Bacteroidetes*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	AB534768
TK10	*Proteobacteria*	*Deltaproteobacteria*	*Desulfobacterales*	*Desulfobulbaceae*	*Desulforhopalus*	AB534769
TK11	*Tenericutes*	*Mollicutes*	*Anaeroplasmatales*	*Anaeroplasmataceae*	*Asteroleplasma*	AB534770
TK12	*Proteobacteria*	*Deltaproteobacteria*	*Desulfobacterales*	*Desulfobacteraceae*	*Desulfobacter*	AB534762
TK13	*Proteobacteria*	*Deltaproteobacteria*	*Desulfovibrionales*			AB534771

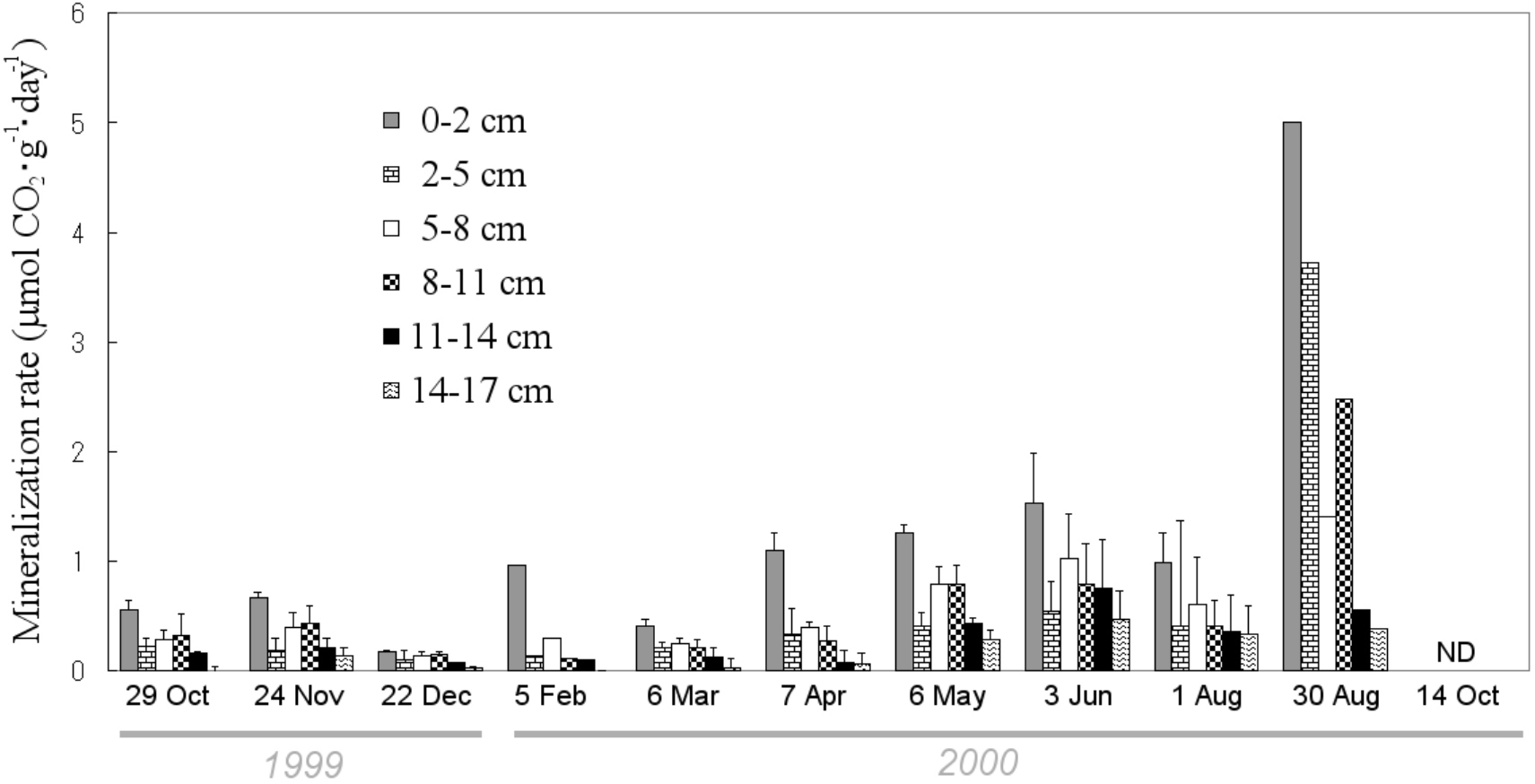
Mineralization rate (µmol $CO_2 \cdot g^{-1} \cdot day^{-1}$)
0
1
2
3
4
5
6
0-2 cm
2-5 cm
5-8 cm
8-11 cm
11-14 cm
14-17 cm
ND
29 Oct
24 Nov
22 Dec
5 Feb
6 Mar
7 Apr
6 May
3 Jun
1 Aug
30 Aug
14 Oct
1999
2000

Fig. 1

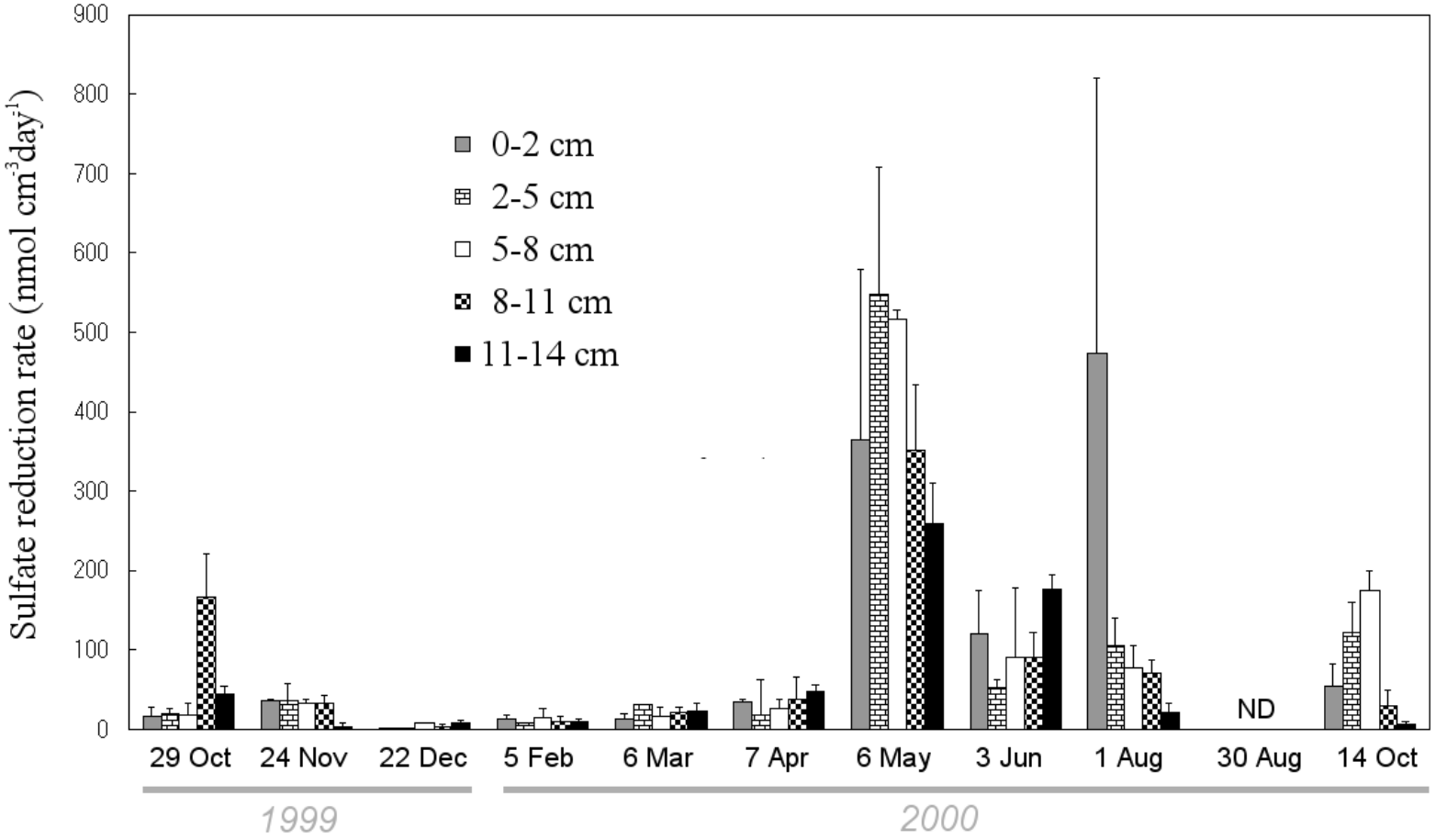
Sulfate reduction rate (nmol cm-3day-1)
900
800
700
600
500
400
300
200
100
0
0-2 cm
2-5 cm
5-8 cm
8-11 cm
11-14 cm
ND
29 Oct
24 Nov
22 Dec
5 Feb
6 Mar
7 Apr
6 May
3 Jun
1 Aug
30 Aug
14 Oct
1999
2000

Fig. 2

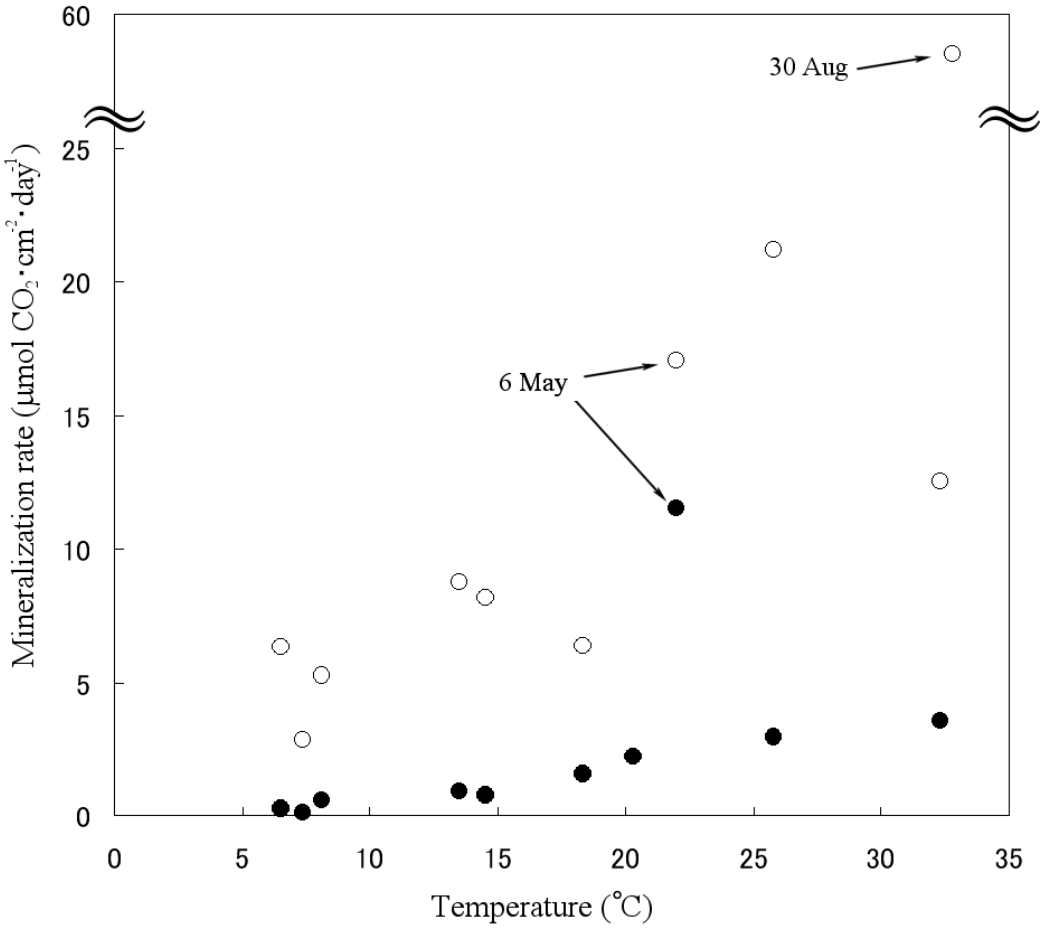
60
25
20
15
10
5
0
0
5
10
15
20
25
30
35
30 Aug
6 May
Mineralization rate (μmol $CO_2 \cdot cm^{-2} \cdot day^{-1}$)
Temperature (℃)

Fig. 3

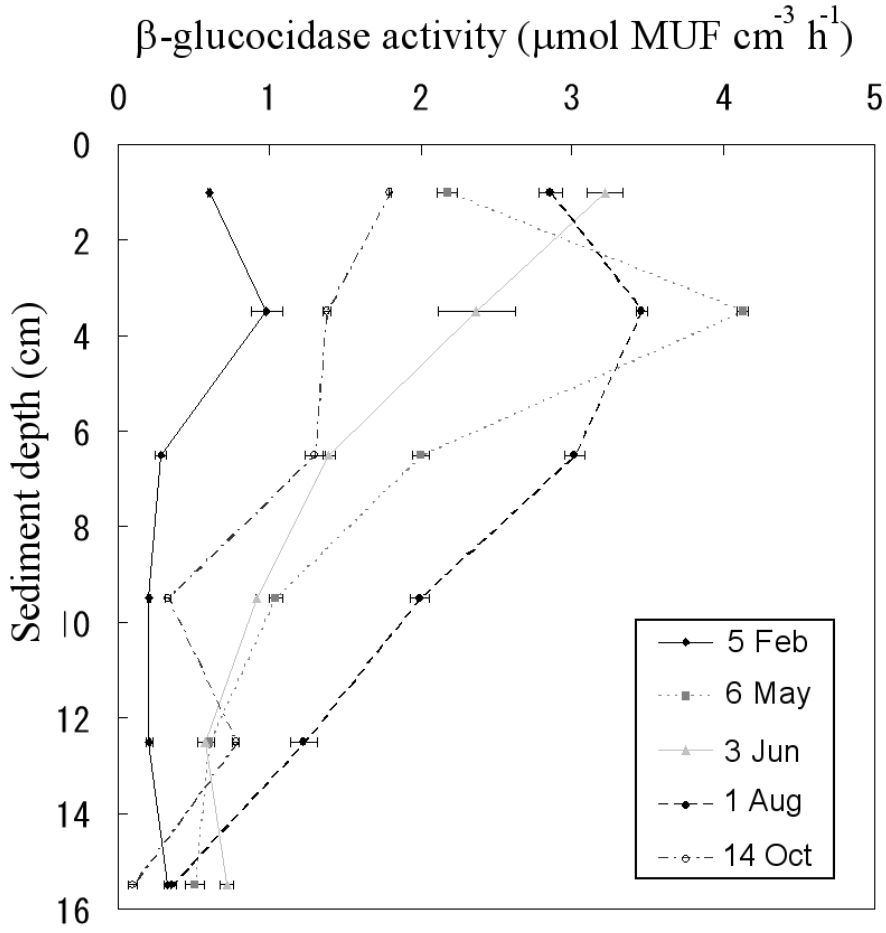
β-glucocidase activity (μmol MUF cm-3 h-1)
0
1
2
3
4
5
Sediment depth (cm)
0
2
4
6
8
10
12
14
16
5 Feb
6 May
3 Jun
1 Aug
14 Oct

Fig. 4

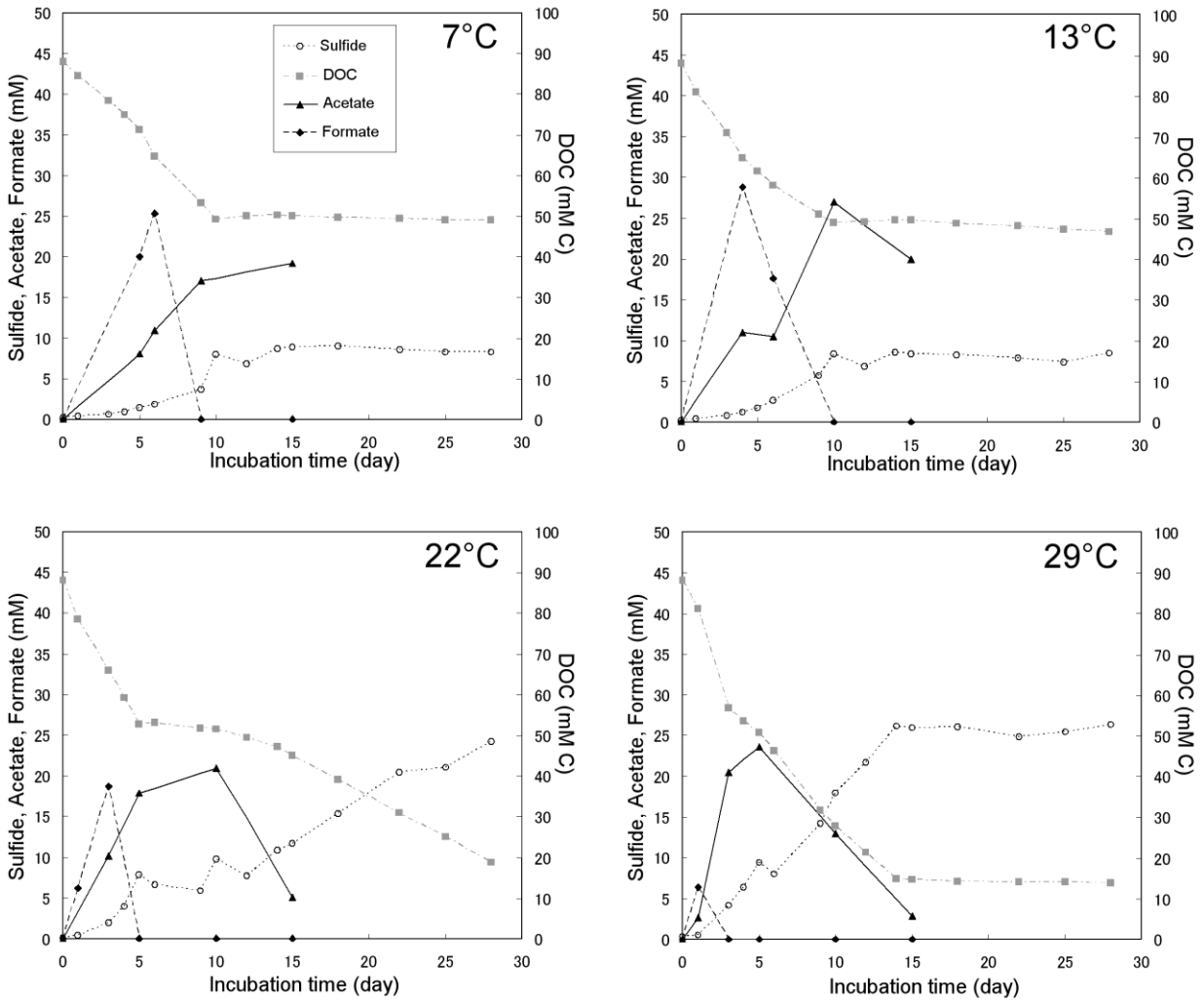
7°C
13°C
22°C
29°C
Sulfide
DOC
Acetate
Formate
Sulfide, Acetate, Formate (mM)
DOC (mM C)
Incubation time (day)

Fig. 5

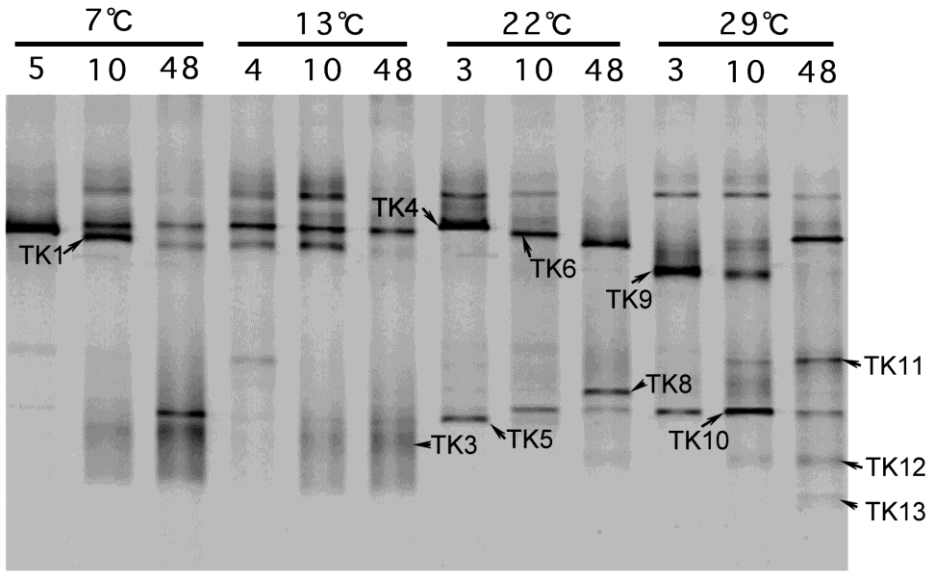
7℃
13℃
22℃
29℃
5 10 48
4 10 48
3 10 48
3 10 48
TK1
TK4
TK6
TK9
TK11
TK8
TK3
TK5
TK10
TK12
TK13

Fig. 6

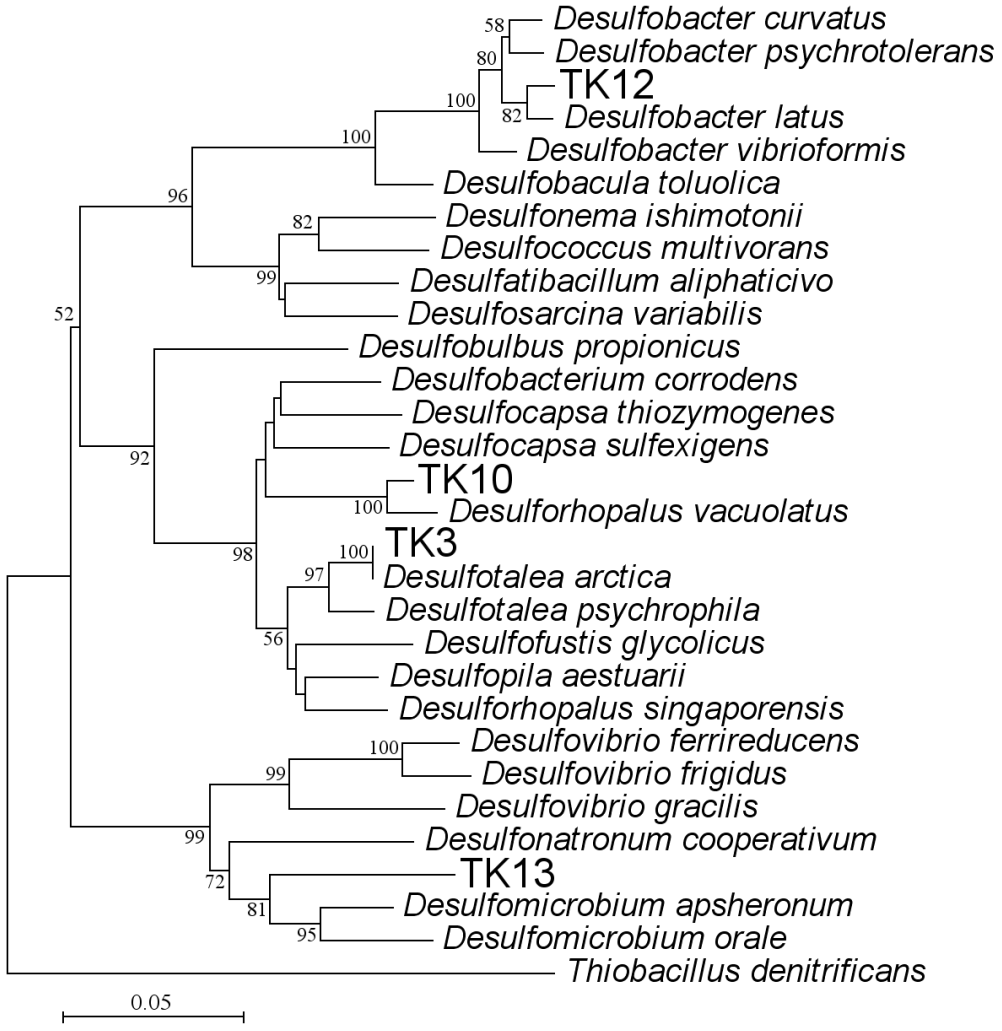
Desulfobacter curvatus
Desulfobacter psychrotolerans
TK12
Desulfobacter latus
Desulfobacter vibrioformis
Desulfobacula toluolica
Desulfonema ishimotonii
Desulfococcus multivorans
Desulfatibacillum aliphaticivo
Desulfosarcina variabilis
Desulfobulbus propionicus
Desulfobacterium corrodens
Desulfocapsa thiozymogenes
Desulfocapsa sulfexigens
TK10
Desulforhopalus vacuolatus
TK3
Desulfotalea arctica
Desulfotalea psychrophila
Desulfofustis glycolicus
Desulfopila aestuarii
Desulforhopalus singaporensis
Desulfovibrio ferrireducens
Desulfovibrio frigidus
Desulfovibrio gracilis
Desulfonatronum cooperativum
TK13
Desulfomicrobium apsheronum
Desulfomicrobium orale
Thiobacillus denitrificans
58
80
100
82
100
96
82
99
52
92
100
98
100
97
56
100
99
99
72
81
95
0.05

Fig. 7