Natural groundwater of a gas field utilizable for a bioremediation of trichloroethylene-contamination

Mio Takeuchi · Kenji Nanba · Ken Furuya · Hisashi Nirei · Mitsuo Yoshida

Abstract Groundwater from a shallow aquifer in Mobara, a city in a natural gas field in Chiba Prefecture, Japan, was found to contain a significant amount of dissolved methane (<3.1 mM) along with nitrate, phosphate and methane-oxidizing bacteria (methanotrophs, $<9.9\times10^6$ MPN ml^{-1}) which can degrade trichloroethylene (TCE). This water exhibited high methanotroph growth activity and rapid degradation of TCE. This water was introduced into a TCE-contaminated aquifer. The concentration of TCE at the monitoring well 2 m down-gradient of the injection pit decreased from 128 μg L^{-1} before the injection to less than the lower detection limit of 12.5 μg L^{-1} after the injection, while it decreased only slightly (to 86 μg L^{-1}) when control water was injected. These results demonstrate the feasibility of utilizing a natural groundwater resource containing methane and methanotrophs without any additives for bioremediation of a TCE-contaminated site.

Keywords Bioremediation · Methane · Methanotrophs · Natural gas field · Trichloroethylene (TCE)

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Introduction

Trichloroethylene (TCE) is one of the major subsurface contaminants in Japan (Ministry of Environment, Japan 2001). Many methods to clean up TCE-contaminated sites efficiently have been developed to date. Among them, bioremediation has attracted much attention because it is expected to shorten the treatment period and reduce the cost. Of the known aerobic decomposers of TCE, such as toluene-, phenol- and ammonia-oxidizing bacteria (Nelson and others 1987; Vannelli and others 1990), methane-oxidizing bacteria (methanotrophs) are considered to be the most suitable for introduction into an aquifer because their substrate, methane, does not negatively impact groundwater quality. Methanotrophs are known to co-oxidize TCE with methane monoxygenase (Little and others 1988). Pilot studies on the bioremediation of TCE-contaminated sites using methanotrophs have already been conducted (Semprini and others 1990; Pfiffner and others 1997; Iwamoto and others 2000). In their studies, growth substrates for methanotrophs were injected into the aquifer, stimulating the growth of indigenous methanotrophs. However, only 10–30% of the TCE was degraded (Semprini and others 1990; Iwamoto and others 2000), indicating the need for more effective methods.

In a natural gas field area that covers about 3% of the total land area of Japan (Tennen-gas kogyokai 1980), groundwater contains high concentrations of methane. Especially in Mobara, which is located at the center of the southern Kanto gas field, biogenic methane (Igari and Sakata 1989) exists in a Tertiary formation extending to more than 1,000 m deep. In Mobara, methane is also emitted through the surface soil layer in some areas. Consequently, it is expected that a large number of methanotrophs, which are aerobic bacteria that utilize methane as a sole carbon and energy source, exist in the shallow groundwater of this area. If we could utilize this methane-rich groundwater for bioremediation of the TCE-contaminated site nearby, it might prove to be more cost-effective than standard methods.

In this paper, we conducted a fundamental analysis of the groundwater of Mobara and examined the feasibility of utilizing natural methane-rich groundwater for bioremediation.

Materials and methods

Groundwater sampling in a natural gas field area

Groundwater used for both in vitro and in situ experiments was obtained from wells in Mobara, Chiba, Japan (35.4°N, 140.3°E), which were screened at the first aquifer, 3–16 m below the ground level. Groundwater was sampled by a 5014-B water sampler (40 mm ID×500 mm; Rigo, Tokyo, Japan) or a pump.

Chemical and microbial analysis of groundwater

Groundwater was sampled from two depths in each of four wells (n=8) in Mobara area and analyzed as described below. Electric conductivity (EC), pH and redox potential (ORP) of collected water samples were measured on site using a model SC82 EC meter (Yokogawa, Tokyo, Japan), model PH82 pH meter (Yokogawa, Tokyo, Japan) and model PH82 ORP meter (Yokogawa, Tokyo, Japan), respectively. Dissolved oxygen (DO) was measured on site using OXYLIQUID 22 DO meter (IDORONAUT, Milan, Italy). Groundwater was collected in vials (120 mL) in triplicate for the measurements of dissolved methane in the laboratory. The groundwater was transferred to the vials through a tube and overflowed. The vials were sealed with unpunctured Teflon septa and aluminum caps. Samples were stored at 4 °C until analysis. After 6 mL of water were replaced with air, vials were incubated in a water bath at 50 °C for 40 min. The headspace was analysed on a GC-14B gas chromatograph (Shimadzu, Kyoto) equipped with a flame ionization detector and a 2-mm ID×2-m packed column (Chromosorb WAW DMCS, Silicone DC-550). The temperatures were as follows: injector, 150 °C; column, 90 °C; detector, 150 °C. Nitrogen was used as a carrier gas (50 mL min^{-1}). Samples for the anion analysis were filtered with a GS-25 (ADVANTEC, Tokyo, Japan) and stored at −20 °C until analysis by HPLC with a CDD-6A conductivity detector (Shimadzu, Kyoto, Japan). A Shodex I-524A column (Showa denko, Kanagawa, Japan) was used, with a column temperature of 40 °C and a pressure of 10 kg f cm^{-1}. The mobile phase contained 2.5 mM phthalic acid and 2.4 mM Tris and the pH was adjusted to 4.0. Phosphate was analyzed by a photometric method with ammonium molybdate (Kawamura 1996). Among methanotrophs, a certain subgroup has an enzyme, soluble methane monoxygenase (sMMO), which efficiently co-oxidizes TCE (Tsien and others 1989). Soluble methane monoxygenase is expressed only when the copper-to-biomass ratio is low (Stanley and others 1983; Koh and others 1993). Therefore, copper concentration in the groundwater was measured by photometric analysis with sodium diethyldithiocarbamate after chloroform extraction (Nishimura 1996). Samples for total bacterial counts were stained with DAPI, and filtered onto 0.2-μm pore-size black polycarbonate filters (Porter and Feig 1980). Counts were made using epifluorescence microscopy. At least 400 cells were counted per sample. Viable methanotrophs were enumerated by the most probable number (MPN) technique using nitrate mineral salts (NMS) liquid medium. Quadruple six-fold dilutions were prepared in 6×8 multi well plates and incubated in a 20% methane in air atmosphere at room temperature for 30 to 40 days. The groundwater of Abiko (hereafter referred to as Abiko water), Chiba, Japan, located at about 48 km north-west from Mobara where the in situ experiment was conducted, were also analyzed before the in situ experiment was conducted.

Trichloroethylene degradation ability of indigenous methanotrophs in groundwater

The potential of the indigenous methanotrophs in the groundwater of Mobara (hereafter referred to as Mobara water) to degrade TCE was measured. Abiko water was also tested. Twenty mL of groundwater were poured into 120-mL vials in duplicate. In order to prepare a favorable condition for a growth of methanotrophs, nitrate and phosphate were added to a final concentration of 10 and 4 mM, respectively, as generally used in NMS medium. Twenty mL of methane and 4.4 μg of TCE were added to the bottles. Vials were incubated at 17 °C with reciprocal shaking at 120 rpm. Methane and TCE concentrations in the head space were analysed on a GC-14B gas chromatograph (Shimadzu, Kyoto) as described above, and the growth of bacteria, as indicated by optical density (OD, A_{660}) was monitored for 16 and 29 days for Mobara water and Abiko water, respectively.

Growth ability of indigenous methanotrophs in Mobara water at low concentrations of nutrients

After the potential ability of the indigenous methanotrophs in Mobara water to degrade TCE was confirmed, growth of methanotrophs in Mobara water was checked with lower concentrations of nutrients considering a practical use. Twenty liters of groundwater were collected from Mobara, introduced into a polyethylene tank, and capped. Nitrate and phosphate were added to a final concentration of 12.5 and 0.5 μM, respectively, in case the groundwater contains no phosphate as sometimes observed in Mobara water (Table 1). Subsamples of the groundwater were taken repeatedly over time and analyzed in triplicate for dissolved methane concentration and number of methanotrophs by gas chromatograph and MPN technique, respectively.

In situ bioremediation test

An in situ bioremediation test was conducted at the TCE-contaminated site in Abiko (Fig 1), where the mechanism and extent of the contamination were investigated based on the geological structure. Geological (hydrostratigraphic) units consisted of surface soil, Kanto loam, clay, silty fine to medium sand (the first aquifer), clayey silt and silty fine sand (the second aquifer) (Fig 2). The contaminant penetrated into the first aquifer and spread along with the groundwater flow

Table 1
Characteristics of Mobara water, and Abiko water. The range of values observed at four wells (n=8) is shown for Mobara water

	Mobara water	Abiko water
Temperature (°C)	15.9–16.8	14.3
pH	6.48–7.97	6.14
Electric conductivity (mS cm^{-1})	0.460–1.469	0.223
ORP (mV)	−133–+97	174
DO (mg L^{-1})	0–0.8	4.3
CH_4 (±SD[a]) (mM)	0.2–3.1	9.8×10^{-5}
Cl^- (mM)	3.38–3.98	1.37
SO_4^{2-}	0.23–0.59	0.107
NO_3^- (μM)	8.1–33.9	95.2
PO_4^{3-} (μM)	ND[b]–16.7	ND
Cu (μM)	0.19[c]	–[d]
Methanotrophs (MPN mL^{-1}) 1.6×10^3–9.9×10^6	1.4×10^3	
Total bacterial number (cells mL^{-1}) 2.5×10^5–3.0×10^8	3.2×10^5	
The proportion of methanotrophs to total bacterial number (%)	0.8–100	0.4

[a]SD; standard deviation, n=3
[b]Not detected, lower detection limit; 0.5
[c]Cu concentration was measured at one well in Mobara
[d]Not determined

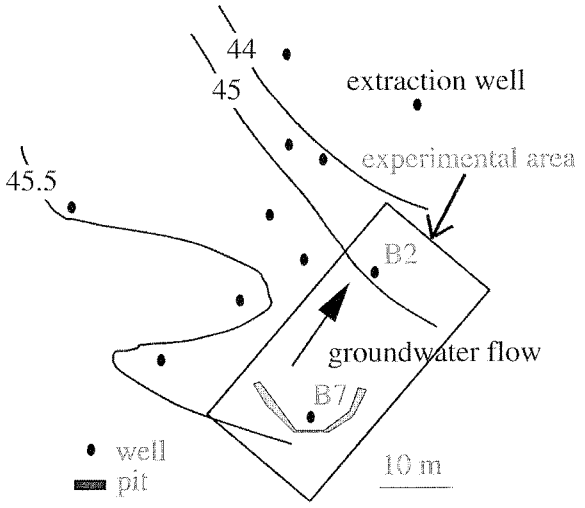

Fig. 1
Location of monitoring wells, extraction well and pit at the contaminated site in Abiko, Chiba, Japan. The experimental area is shown as a *square*. Groundwater level shown with a *contour* is indicated as an altitude (m) of water head

in the aquifer. Groundwater flowed in the direction from monitoring well B7 to well B2 (Fig 1). The groundwater flow was enhanced by extraction from the extraction well downgradient of the experimental area (Fig 1). During the experiment, groundwater velocity (v) was calculated according to

$$v = -K \cdot h/x/n_e,$$

where K is the hydraulic conductivity of the aquifer determined by pumping test (1.63×10^{-2} cm sec^{-1}), h/x is

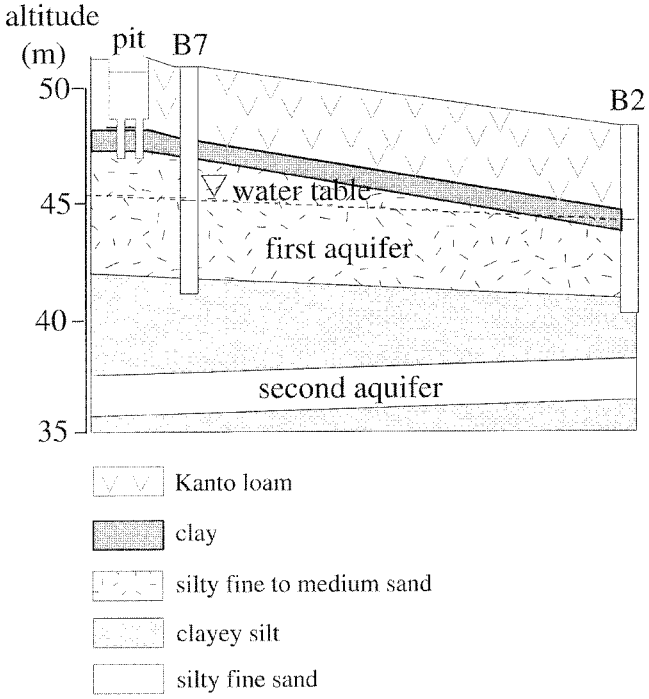

Fig. 2
Geological crosssection of the zone between B7 and B2 in the experimental area shown in Fig 1. The pit was installed in Kanto loam

the measured hydraulic gradient (average of the three measurements during the experiment, 0.044, was used) and n_e is an effective porosity (n_e=0.25 was used after (Kono 1989)). The average groundwater flow was calculated to be 260 cm day^{-1}. The experimental area included B7 and B2, which were installed at 2 and 20 m downstream of the pit, respectively (Fig 1).

Water was injected into the first aquifer through the pit installed in Kanto loam. The pit was 3.5 m deep, 1.5 m wide, and 10 m long. Water was allowed to penetrate into the first aquifer through small holes bored at the bottom of the pit through a clay layer. First, as a control, water meant for extinguishing fires obtained from a nearby factory and not contaminated with TCE (hereafter referred to as control water), was injected in order to evaluate a physical dilution of TCE. Thereafter, Mobara water was injected. Control water was injected daily from September 17, 1998 to October 9, 1998, at 4–6 tons day^{-1}. Mobara water was injected daily from October 15, 1998 to October 20, 1998, at 3–5 tons day^{-1}. The concentration of TCE was monitored at monitoring wells B7 and B2 by a head space-detector tube method (Suzuki and others 1995; detection limit: 12.5 μg L^{-1}; GASTEC, Kanagawa, Japan). Briefly, two hundred milliliters of groundwater were sampled in a 645-mL bottle and shaken for 1 min. After settling for 2 min at 25 °C, the headspace was analyzed with detection tubes. At well B7 and B2, concentrations of methane and anions as well as total bacterial numbers, and the number of methanotrophs were determined. Methanotrophs that have sMMO (sMMO-methanotrophs) were enumerated by MPN technique using copper-free NMS medium.

Results

Characteristics of Mobara water, control water and Abiko water

The results of the chemical and microbial analyses of Mobara water and Abiko water are given in Table 1. The maximum number of methanotrophs reached 9.9×10^6 MPN mL^{-1} in Mobara water while it was 1.4×10^3 MPN mL^{-1} in Abiko water. The fraction of total bacteria consisting of methanotrophs exceeded 10% in four samples and reached 100% in two samples (data not shown). The maximum dissolved methane concentration observed in Mobara water was 3.1 mM, which was more than the saturated concentration (1.6 mM, under 100% methane, atmospheric pressure, salinity=0, and 17 °C). The concentration of DO (0–0.8 mg L^{-1}) and ORP (−133–+97 mV) of the first aquifer of Mobara indicated a microaerobic condition. Phosphate, which is not often seen in groundwater, was found at <16.7 μM in Mobara water. The copper concentration was 0.19 μM in Mobara water. Compared to Abiko water, Mobara water contained less oxygen and nitrate, and more methane and phosphate.

Trichloroethylene-degrading ability of indigenous methanotrophs in groundwater

In Mobara water, 24% of methane was consumed after 3 days and 100% after 10 days (Fig 3b). At this point, methane was reinjected to the same concentration as the initial. After the reinjection, methane was again totally consumed in 6 days (Fig 3b). Microbial growth did occur, but the growth was less than the maximum observed in Abiko water (Figs. 3a, 4a). The TCE concentration decreased to 52% after 3 days of incubation as the methane was consumed and decreased to lower than the detection limit (<5 μg L^{-1}) after 16 days from the start of the incubation (Fig 3b). In Abiko water, significant growth of bacteria occurred after 29 days of incubation, at that time, 25% of the methane had been consumed (Fig 4a, b). However, TCE remained at 89% after 29 days of incubation (Fig 4b).

Growth ability of indigenous methanotrophs in Mobara water at low concentrations of nutrients

Methanotrophic growth occurred when Mobara water was incubated with lower concentrations of nitrate and phosphate added. The dissolved methane concentration decreased to less than 20% of the initial level after 3 days of incubation (Fig 5a). The population of methanotrophs increased from 8.5×10^2 MPN mL^{-1} to 1.5×10^4 MPN mL^{-1} in 3 days (Fig 5b).

In situ bioremediation test

Methanotrophs require methane, oxygen, nitrate and phosphate as major substrates. Because Mobara water contained more methane and phosphate than Abiko water, and Abiko water contained higher concentrations of oxygen and nitrate than Mobara water, an injection of Mobara water into a contaminated aquifer of Abiko through a pit in which oxygen would also be supplied through the air to

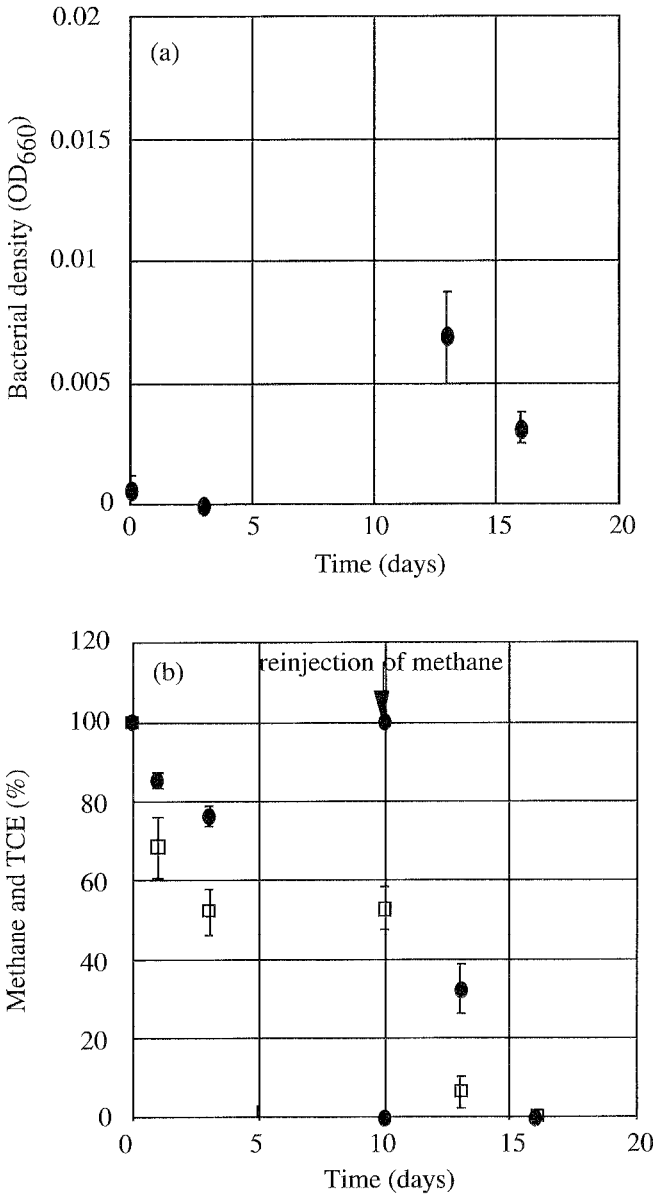

Fig. 3
a Growth of bacteria and b concentrations of methane (*circle*) and TCE (*square*) in Mobara water incubated under initial conditions of 20% methane, 220 μg L^{-1} of TCE, 10 mM of nitrate and 4 mM of phosphate. The mean value of the duplicates is shown. *Error bars* show the range of the duplicates

the Mobara water, would result in appropriate conditions for methanotrophs. Therefore, Mobara water was injected without any nutrient addition. Water injected into the pit penetrated into the first aquifer within a few hours through small holes. Injected water was expected to mix with the groundwater and be conveyed towards the monitoring wells. The distance of the monitoring wells from the pit was 2 and 20 m for B7 and B2, respectively. Assuming that groundwater flows at the calculated velocity of 260 cm day^{-1}, injected water was expected to reach well B7 after 0.8 day and well B2 after 8 days. Control water and Mobara water were injected for 23 days and 6 days, respectively. From these injection periods, the period of

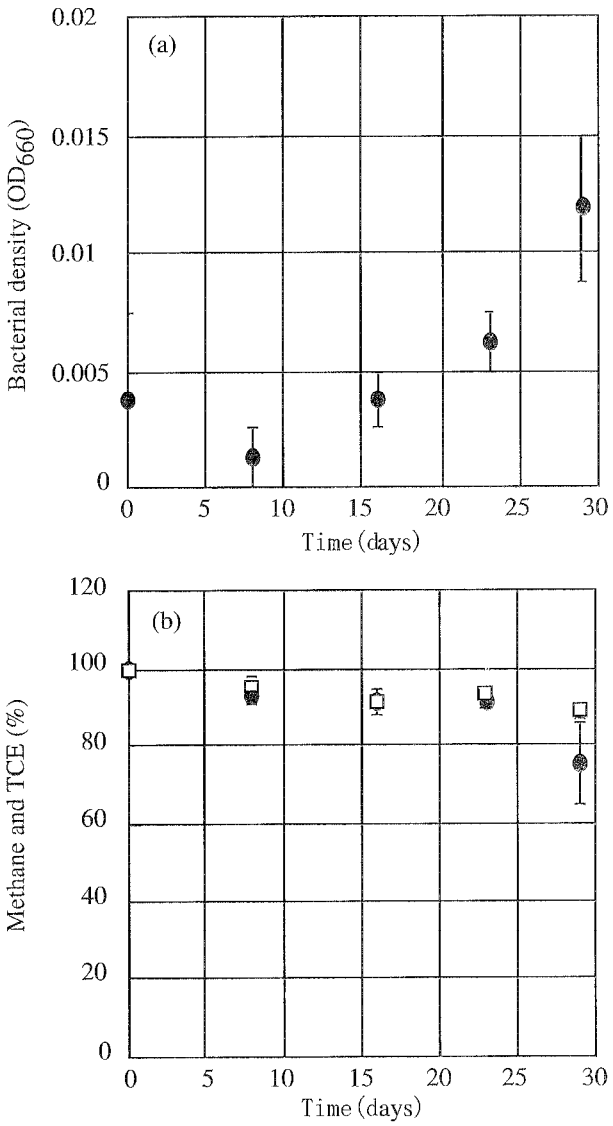

Fig. 4
a Growth of bacteria and b concentrations of methane and TCE in Abiko water incubated under initial conditions of 20% methane, 220 μg L^{-1} of TCE, 10 mM of nitrate and 4 mM of phosphate. The mean value of the duplicates is shown. *Error bars* show the range of the duplicates

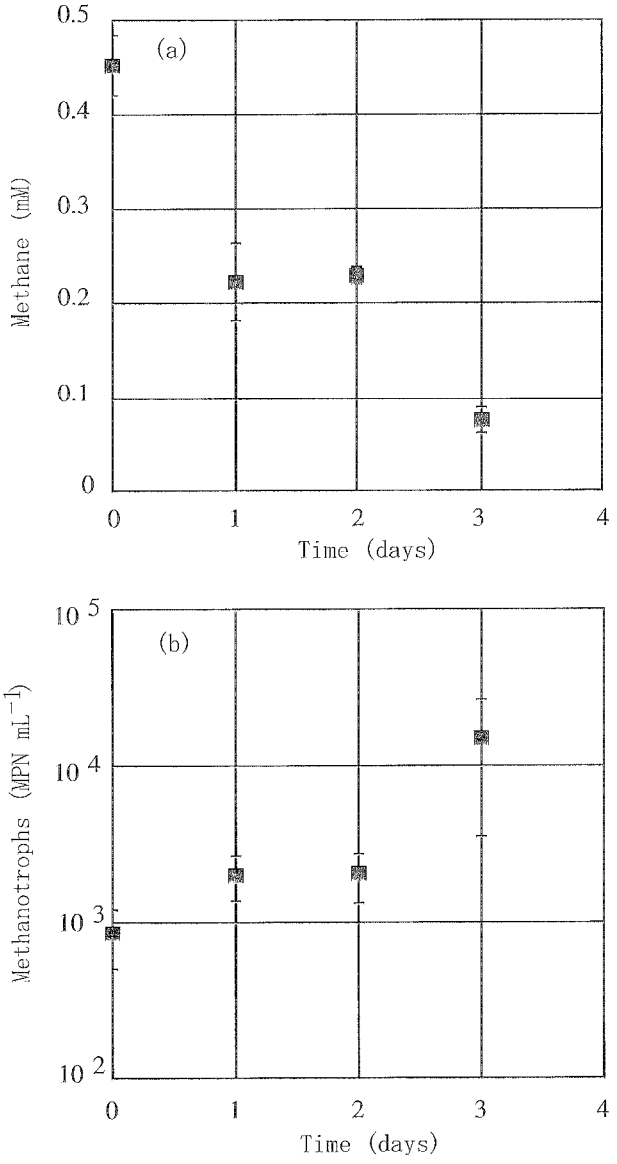

Fig. 5
a Dissolved methane concentration and b the number of methanotrophs in Mobara water incubated with 12.5 μM of nitrate and 0.5 μM of phosphate in a polyethylene tank. *Error bars* show standard deviation of triplicates

time when the injected water mixed with groundwater would run through each of the monitoring wells was calculated. These predictions are indicated on Fig 6, which shows the TCE concentrations at the monitoring wells during the study period. At B2, the average concentration of TCE was 56 μg L^{-1} during control water flow and 39 μg L^{-1} during Mobara water flow. At B7, the concentration of TCE was 128 μg L^{-1} before the injection, and the average concentration was 86 μg L^{-1} in the period of control water flow (Fig 6), while it decreased to less than the detection limit (<12.5 μg L^{-1}) in the period of Mobara water flow. After the period of Mobara water flow, the TCE concentration at B7 remained below the detection limit for about one week and gradually increased (Fig 6).
Chemical characteristics and microbial numbers in groundwater at well B7 and B2 in each period, injected control water and Mobara water after introduced into the pit were presented (Table 2). Injected Mobara water was characterized by higher concentrations of dissolved methane, phosphate, chloride and sulfate than control water and Abiko water (Tables 1, 2). Chloride and sulfate concentrations increased in the period of Mobara water flow than in the period of control water flow both at B7 and B2. The concentration of dissolved methane was 1.1×10^3 nM in the period of control water flow and decreased to 98 nM in the period of Mobara water flow at B7. No decrease was observed in the period of Mobara water flow at B2. The number of methanotrophs at B7 was 1.7×10^4 MPN mL^{-1} in the control water period, and 5.7×10^3 MPN mL^{-1} in the period of Mobara water flow. The number of sMMO-methanotrophs was 1.1×10^4 MPN mL^{-1} in the control

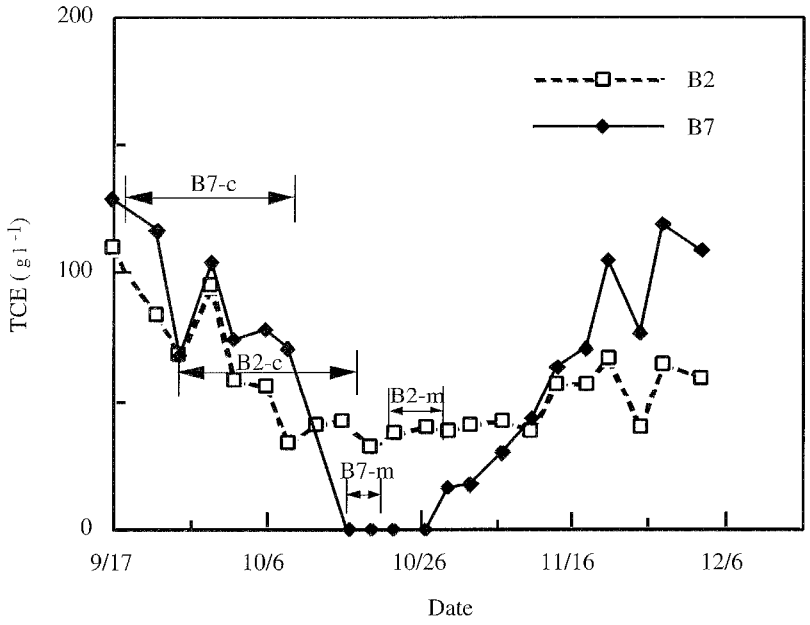

Fig. 6
Trichloroethylene concentration at monitoring wells B7 and B2. *Arrows B7-c* and *B2-c* show the period of time when control water was predicted to run through each well according to the calculated groundwater velocity. *Arrows B7-m* and *B2-m* show the period of time when Mobara water was expected to run through each well

water period, and 1.4×10^3 MPN mL^{-1} in the period of Mobara water flow at B7.

Discussion

The maximum population of methanotrophs observed in the groundwater of Mobara was higher than those reported for other groundwater systems (Heyer and others 1984; Bowman and others 1993). Methanotrophs comprised more than 10% of the total population of bacteria at four of eight points, and at two points, the number of methanotrophs equaled the total bacterial number. This indicates that the groundwater of Mobara is a suitable habitat for methanotrophs.

Indigenous methanotrophs in Mobara water was shown to have TCE degradation ability as methane and TCE in Mobara water decreased concomitantly to 76 and 52% of the initial level after 3 days of incubation (Fig 3b). Although microbial growth was not detected by OD measurement after 3 days (Fig 3a), MPN analysis showed that growth of methanotrophs can occur in one day and that the population of methanotrophs can increase twenty fold in 3 days in Mobara water even under lower concentrations of nutrients (Fig 5b). This indicates that the decrease in methane and TCE observed upon incubation of Mobara water was due to the activity of methanotrophs. The initial population of methanotrophs (8.5×10^2 MPN mL^{-1}) at the growth test was lower than those observed when the groundwater quality was investigated preliminarily (Table 1), which was considered to show a seasonal variation in methanotrophic population in groundwater as already reported for other groundwater (Takeuchi and others 2000). However, they started to grow soon after the incubation compared to Abiko water (Figs. 4a and 5b). This

Table 2
Chemical characteristics and the numbers of microorganisms at well B7 and B2 during the flow of control water and Mobara water, control water and Mobara water after introduced into the pit

	Control-B7	Mobara-B7	Control-B2	Mobara-B2	Mobara water in the pit	Control water
CH_4 (±SD[a]) (nM)	$1.1(\pm0.1)\times10^3$	98(±7)	420(±48)	710(±21)	$4.4(\pm1.9)\times10^3$	$7.4(\pm1.6)\times10^{-6}$
NO_3^- (μM)	40.0	150.3	203.3	186.4	39.9	40.3
PO_4^{3-} (μM)	ND[b]	ND	ND	ND	43.7	ND
Cl^- (mM)	1.40	1.46	1.20	1.28	1.92	1.68
SO_4^{2-} (mM)	0.11	0.18	0.22	0.24	0.25	0.18
Methanotrophs (MPN mL^{-1})	1.7×10^4	5.7×10^3	1.1×10^4	1.1×10^4	-	2.3×10^3
sMMO-methanotrophs (MPN mL^{-1})	1.1×10^4	1.4×10^3	1.1×10^4	9.6×10^3	-	-
Total bacterial number (cells mL^{-1})	2.3×10^5	1.1×10^5	2.3×10^5	5.1×10^5	-	1.5×10^5

[a]SD; standard deviation, $n=3$
[b]Not detected, lower detection limit; 0.5
- Not determined

showed that methanotrophs in Mobara water maintain their activity even if the population becomes smaller. In general, the activity of bacteria in the subsurface environment is not high, probably because of the low supply of substrates (Ghiorse and Wilson 1988). In the experiment with Abiko water (Fig 4a) and also with groundwater from other areas in Chiba Prefecture, Chikura (Takeuchi and others 2000), a long lag time was observed for methanotrophic growth compared to that in Mobara water experiment. Methanotrophs are known to lose their activity when starved of their substrates, but can recover after a lag time (Roslev and King 1994). Methanotrophs in Abiko water and groundwater of Chikura are considered to be starved and have lost their activity. But methanotrophs in Mobara water maintain their activity for some reason. Mobara water contained methane, nitrate and phosphate but was deficient in oxygen (Table 1). Therefore, oxygen was considered to be a limiting factor for methanotrophic growth in Mobara water. Oxygen is supplied to the groundwater of the first aquifer through the air or by precipitation (Takeuchi and others 2000). Such an oxygen supply could occasionally produce appropriate condition for methanotrophic growth in the first aquifer of Mobara and therefore methanotrophs can maintain their activity there. The results of the in vitro experiments showed the possibility of utilizing Mobara water that contain high concentration of methane and methanotrophs with high activity, in bioremediation of a TCE-contaminated site.

In order to examine the feasibility of bioremediation of TCE-contaminated sites utilizing natural groundwater of Mobara, Mobara water was injected into a contaminated aquifer. After the injection of Mobara water, the TCE concentration at well B7 sank lower than the detection limit, which is attributed to TCE degradation rather than a physical process such as dilution because a similar decrease was not observed when larger amount of control water was likewise injected (Fig 6). The observed decrease from 128 $\mu g\ L^{-1}$ of TCE to less than the detection limit was comparable to the result obtained in vitro for Mobara water: a decrease of TCE of 110 $\mu g\ L^{-1}\ 3\ days^{-1}$ was observed. Abiko water showed little TCE degradation when indigenous methanotrophs were stimulated (Fig 4b). Therefore, the decrease of TCE is considered to be caused by methanotrophs in Mobara water and not by a stimulation of methanotrophs already present in Abiko water. Chloride and sulfate concentrations at B7 and B2 increased in the period of Mobara water flow compared to those in the period of control water flow, but lower than those of injected Mobara water (Table 2). These results suggest that the injected Mobara water was mixed with the contaminated groundwater in the aquifer (Table 2). This mixture of the two waters is considered to be achieved by the injection through small holes bored in clay layer (Fig 2). The concentration of dissolved methane at well B7 was 1.1×10^{3} nM during the period of control water flow and decreased to 98 nM during the period of Mobara water flow (Table 2) although Mobara water contained more than one thousand times more methane than control water (Table 1). This suggests that methane was consumed before reaching the monitoring well. Although a twenty-fold increase in methanotrophic population was observed in the in vitro experiment, the number of microbes was less during the Mobara water injection period than the control water period at B7 (Table 2). In a previous bioaugmentation study (Steffan and others 1999), *Burkholderia cepacia* ENV435, which was injected at a concentration of 1×10^{11} cfu mL^{-1}, decreased to a concentration of 2.2×10^{7}–1.9×10^{8} cfu mL^{-1} at a monitoring well 2 m downgradient of the injection well presumably due to attachment to sediment particles. We don't have data on the adhesion properties of indigenous methanotrophs in Mobara water, but they are probably not any less adhesive to sediment particles than the adhesion-deficient culture, ENV435. Hydraulic conductivity was larger in our study site (1.63×10^{-2} cm sec^{-1})than that in Steffan and others (1999) (1.3–3.1×10^{-3} cm sec^{-1}). However, considering that about 0.1% of the injected bacteria reached 2 m downgradient in the previous study and assuming that only twenty-fold increase of methanotrophs has occurred in situ as observed in the in vitro experiment, it is not surprising that we did not observe an increase in the number of methanotrophs at monitoring well B7. It appears that microbial growth near the pit may have caused a plugging, and the numbers of microorganisms at B7 became lower during the Mobara water period than the control water period (Table 2). TCE concentrations retained below the detection limit for about one week after the period of Mobara water flow and increased again to the initial level after about 30 days. This also suggests that biofilter of methanotrophs was formed near the pit. In the previous bioaugmentation study utilizing methanotrophs, it took 40 days until the concentration of the contaminant was back to the initial level when 5.4×10^{9} cells mL^{-1} of methanotrophs were injected (Duba and others 1996). This longevity of the effect was similar to the result of this study although injected population of methanotrophs and hydrogeologic characteristics of the site differ in two studies. The copper concentration was 0.19 and 0.21 μM in Mobara water and control water, respectively (Tables 1 and 2), which were less than the reported value (<0.25 μM, Tsien and others 1989) which suppresses the production of sMMO. As far as the reported threshold is applicable to the indigenous methanotrophs in groundwater, methanotrophs grew in situ are considered to be producing sMMO. The decrease in TCE concentrations was not obvious at well B2 during Mobara water flow although an increase in chloride and sulfate concentrations in the period of Mobara water flow suggested that the mixed water reached B2. Since B2 is located at 20 m downgradient of the pit, TCE might be supplied from aquifer materials in the pass of B7 and B2.

Taken together, these results suggest the following scenario for the in situ experiment. Injected Mobara water was supplied with oxygen in the pit or in the aquifer, and this caused a growth of methanotrophs near the pit. Methane was consumed and TCE was degraded by sMMO, but most of the grown methanotrophs were attached to the sediment and did not reach the monitoring well. Therefore, the groundwater at B7 contained less methane, TCE and methanotrophs after Mobara water was injected.

In conclusion, methane-rich groundwater from a natural gas field area was shown to be valuable not only chemically but also microbially for use in bioremediation of a TCE-contaminated site. As natural gas fields are found in many parts of Japan, remediation of TCE-contaminated sites utilizing natural groundwater resources might be a cost-effective technique although more work will be needed before a practical application.

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