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Biodegradation of geosmin in drinking water by novel bacteria isolated from biologically active carbon

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Abstract

Three strains of Gram-negative bacteria capable of removing geosmin from drinking water were isolated from biologically active carbon and identified to be *Chryseobacterium* sp., *Sinorhizobium* sp. and *Stenotrophomonas* sp. based on physio-biochemistry analysis and 16S rRNA gene sequence analysis. Removal efficiencies of 2 mg/L geosmin in mineral salts medium were 84.0%, 80.2% and 74.4% for *Chryseobacterium* sp., *Sinorhizobium* sp. and *Stenotrophomonas* sp., respectively, while removal efficiencies of 560 ng/L geosmin in filter influent were 84.8%, 82.3% and 82.5%, respectively. The biodegradation of geosmin was determined to be a pseudo first-order reaction, with rate constants at 2 mg/L and 560 ng/L being 0.097 and 0.086 day^{-1}, 0.089 and 0.084 day^{-1}, 0.074 and 0.098 day^{-1} for the above mentioned degraders, respectively. The biomass of culture in the presence of geosmin was much higher than that in the absence of geosmin.

Key words: geosmin; biodegradation; pseudo first-order reaction; *Chryseobacterium* sp.; *Sinorhizobium* sp.; *Stenotrophomonas* sp.

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Introduction

A common and recurrent problem in drinking water is the formation of earthy-musty taste and odor (T&O) compounds (Durrer et al., 1999). T&O compounds episodes are the cause for most consumer complaints (Peter and von Gunten, 2007). Geosmin (trans-1,10-dimethyltrans-9-decalol), one of the most common T&Os, is a metabolite of two groups of aquatic microorganisms (cyanobacteria and actinomycetes), and has been considered to be the main cause of earthy-musty taints in drinking water (Ng et al., 2002; Robertson et al., 2005, 2006; Saadoun et al., 2001). This compound has an extremely low threshold odor concentration of 10 ng/L (Cook et al., 2001; Ho et al., 2007).

T&Os cannot be readily removed by conventional water treatment processes (Bruce et al., 2002; Ho et al., 2002, 2007; Rittmann et al., 1995; Izaguirre et al., 1982; Lalezary et al., 1986). Amongst the advanced treatment processes, activated carbon adsorption can remove most odorants (Cook et al., 2001), but may require relatively high doses and exhibit reduced efficiency in natural water due to the presence of natural organic matter (NOM) (Song and O'Shea, 2007). Ozone, a powerful oxidant, is efficient for degradation of geosmin (Nerenberg et al., 2000). Unfortunately, ozonation can produce hazardous oxidation byproducts, especially in waters containing bromide (Br^-), where carcinogenic bromate (BrO_3^-) is formed (Sagehashi et al., 2005). Therefore, a cost effective method for the treatment of geosmin episodes is required (McDowall et al., 2009). Several studies indicated that biological processes could be a viable treatment option for removing T&O compounds (Ho et al., 2007; Namkung and Rittmann, 1987; Saito et al., 1999). In general, biological processes are of low cost, require little maintenance and do not rely on the addition of chemicals which often results in unwanted by-products (McDowall et al., 2009).

A number of Gram-positive and Gram-negative bacteria can degrade geosmin, including Gram-positive strains of *B. cereus* (Narayan and Nunez III, 1974; Silvey et al., 1970), *B. subtilis* (Narayan and Nunez III, 1974; Yagi et al., 1988), *Arthrobacter atrocyaneus*, *Arthrobacter globiformis*, *Rhodococcus moris*, *Chlorophenolicus strain N-1053* (Saadoun and El-Migdadi, 1998), and Gram negative strains of *Alphaproteobacteria* and *Sphingopyxis alaskensis* (Hoefel et al., 2009). McDowall et al. (2009) reported a geosmin removal up to 75% through sand columns which had been inoculated with the geosmin-degrading bacteria.

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To date, only a few studies on the biodegradation rate of geosmin have been reported. Rittmann et al. (1995) determined that geosmin was utilized as secondary substrate in natural water due to the presence of NOM at milligram-per-liter level, and the biodegradation of geosmin in natural water was a second-order reaction. Ho et al. (2007) and Hoefel et al. (2009) found the biodegradation of geosmin to be a pseudo first-order reaction with a rate constant of 0.075 day^{-1} in the bioreactor experiments.

To further elucidate the kinetics of geosmin biodegradation process, it is necessary to isolate single bacterium with the ability to degrade geosmin. The objective of this study was to identify the geosmin degraders isolated from a granular activated carbon (GAC) filter in a commercial water plant and to examine their removal efficiency of geosmin at different concentrations with a focus on the kinetics of geosmin biodegradation.

1 Materials and methods

1.1 Materials

Solid geosmin (98%) used in this study was obtained from Wako Pure Chemicals, Ltd. (Osaka, Japan). Sterile geosmin solution (20 mg/L) was prepared by dissolving geosmin in purified water treated by a Milli-Q system (Millipore Pty Ltd., USA). The solution was then filtered through a sterile 0.45 μm-pore-size micro porous membrane (MPM) and stored in fridge at 4°C before use.

The glassware was washed three times with purified water, and then sterilized at 121°C for 30 min before use.

1.2 Enrichment procedures

Enrichment of geosmin degraders was conducted with a mineral salts medium (MSM, NH_4NO_3 0.1%, K_2HPO_4 0.1%, $MgSO_4{\cdot}7H_2O$ 0.05%, KCl 0.02% (*W*/*V*), pH 7) as reported by Tanaka et al. (1996). Portions of 100 mL MSM were placed in 250 mL Erlenmeyer flask sealed with absorbent gauze and then sterilized at 121°C for 30 min.

Activated carbon samples were obtained from a carbon filter of a water plant in Beijing, and stored in the bottle containing the influent of the filter at 4°C. About 5.0 g carbon was added into 10 mL sterile PBS buffer (0.1 mol/L). The buffer was shaken vigorously for 15 min.

The culture of PBS buffer for inoculation was poured into the Erlenmeyer flask filled with 100 mL MSM. The geosmin solution was spiked through the sterile 0.45 μm-pore-size MPM. The initial concentration of geosmin in MSM was controlled at 100 ng/L. The flasks were then covered with sealing film (12 × 12 cm, Solarbio, Beijing, China) and rotated in an incubator shaker at 30°C and 160 r/min for 5 days.

Five percent of the MSM culture was spiked into another 100 mL MSM to select geosmin degraders, and incubated repeatedly with the increase of geosmin concentration (200 ng/L, 500 ng/L, 1 μg/L, 2 μg/L, 5 μg/L, 10 μg/L and 20 μg/L).

In all tests, the vessels were tightly capped to prevent geosmin from volatilizing.

1.3 Isolation of geosmin degraders

For isolation of geosmin degraders, LB agar medium (peptone 1%, yeast extract powder 0.5%, NaCl 0.5%, agar 1.5% (*W*/*V*), pH 7) was supplemented and autoclaved (Tanaka et al., 1996). Followed by cooling to about 40°C the medium was poured into 90 mm vitreous plates. After application of 1 mL cultured MSM on LB agar medium in triplicate, these plates were covered with the lids, and sealed tightly. They were placed upside down and incubated at 30°C for 2 days. Various cultures were streaked onto the LB agar medium at least twice for purification. Three pure strains were isolated.

The pure cultures were dissolved in several vials of 1 mL sterile PBS buffer (0.02 mol/L, pH 7), and then centrifuged at 12,000 r/min for 3 min. The cells in each vial were washed twice with 1 mL sterile PBS buffer, and then stored at 4°C before geosmin degradation tests were conducted. Analysis of physio-biochemistry for geosmin degraders was carried out to investigate the characteristics of biological metabolism.

1.4 Identification of bacteria

The purified isolates from various cultures were identified by a commercial laboratory, TaKaRa Biological Company (China). The 16S ribosomal RNA (rRNA) gene was PCR-amplified from plasmid DNA isolated from the enriched culture. The purified culture of 10 μL was spiked into the sterile water, and then denatured at 99°C followed by centrifugation. The supernatant was taken as the template strand. The fragments of target gene were amplified using TaKaRa 16S rDNA Bacterial Identification PCR Kit (Code No. D310). PCR cycling parameters used were as follows: 1 μL of masterready reagent at 94°C for 5 min and circulated one time; 25 μL of PCR premix at 94°C for 1 min, 0.5 μL of forward primer (20 pmol/μL) at 55°C for 1 min, 0.5 μL of reverse primer 2 at 72°C for 1.5 min, and circulated for 30 times; 23 μL of 16S-free H_2O at 72°C for 5 min, and circulated one time.

Five micro liters of amplified sample was used to conduct an Agarose Gel Electrophoresis (AGE), followed by Gel Extraction Purification using TaKaRa Agarose Gel DNA Purification Kit Ver.2.0 (Code No. DV805A). Sequence analysis was performed with Seq Forward, Seq Reverse and Seq Internal as the primers.

1.5 Phylogenetic analysis of degraders

Sequence similarity searches were conducted using the National Center for Biotechnology Information BLAST network service (Blastn). Similar sequences, from previously cultured bacteria, were obtained from GenBank and aligned against the DNA sequences of GSM-1 to GSM-3. Neighbour-joining analysis was then performed using the software program MEGA (MEGA version 3.1; Arizona State University, Tempe, USA).

1.6 Geosmin degradation

In relative researches, 2 mg/L of 2-methylisoborneol, another T&Os, was employed for testing the degradation

ability of T&Os degraders (Izaguirre et al., 1988). However, there are knowledge gaps regarding the biodegradation of geosmin as the sole carbon source. In our study, the sterilized medium used for geosmin degraders was MSM with geosmin at about 2 mg/L. Portions of the 1000 mL medium were placed in 2000 mL Erlenmeyer flasks and sterilized at 121°C for 30 min. Active bacteria of the three geosmin degraders were inoculated into several flasks at a concentration of 2×10^2 cells/mL. They were covered with sealing films and rotated in an incubator shaker at 30°C and 40 r/min for 3 weeks. A control sample was also run under the same operating conditions in the absence of geosmin degraders. The samples were then analyzed by gas chromatography-mass spectrometry (GC-MS).

The geosmin removal experiments, with about 560 ng/L geosmin in the filter influent of the water plant, were prepared similarly to the aforementioned experiments. The objective of this experiment was to achieve geosmin degradation at levels similar to those in drinking water. The characteristics of the filter influent were as follows: pH 7.1, COD_{Mn} 2.0 mg/L, UV_{254} 0.033 /cm, NH_4^+-N 0.075 mg/L, NO_2^--N 0.0050 mg/L, and NO_3^--N 1.50 mg/L.

Samples of 40 mL were poured out from the Erlenmeyer flask at intervals of a few days. The samples were filtered through sterile 0.45μm-pore-size MPMs to eliminate the cells of geosmin degraders, and then transferred into crimp cap vials (total volume 40 mL). All these samples were stored in the dark at 4°C. The concentration of geosmin was determined within 7 days. The biomasses of geosmin degraders in the abovementioned experiments were also analyzed.

1.7 Analytical method

Geosmin was determined by headspace solid-phase microextraction (HS-SPME) coupled with GC-MS. Individual liquid sample (24 mL) was supplemented with 6 g NaCl and incubated in crimp cap vials, then incubated in a water bath at 65°C. After 30 min of incubation, SPME fiber was immersed into the headspace of the liquid sample vial, which was agitated so that the analyte was absorbed onto the SPME fiber. Geosmin concentrations were analyzed by injecting the fibers into the splitless operated injector of a GC (7890A, Agilent, USA) coupled with an MS (5740C, Agilent, USA) at 250°C for 2.5 min. An HP-5MS (30 m × 0.25 mm id, 0.25 μm film thickness) containing 5% phenyl methyl siloxane capillary column was employed. Helium was used as the carrier gas at a constant flow of 1 mL/min. The oven temperature was held at 60°C for 2.5 min from injection, increased to 280°C at 30°C/min, and held at 280°C for 5 min.

Biomasses of samples were detected by plate counting method (GB/T 5750.12-2006). Different dilutions were analyzed. The process was same as isolation of geosmin degraders. The plates with 30 to 300 colony forming units (CFU) were used for analysis.

2 Results and discussion

2.1 Isolation of geosmin degraders

Although geosmin is not encountered in nature as the sole carbon source or at levels sufficient to support bacterial growth, it can be metabolized by geosmin degraders. Three kinds of bacteria which were probably geosmin degraders (designated as GSM-1, GSM-2 and GSM-3) were observed from the agar after inoculation for 48 hr. Morphological characteristics and physio-biochemistry of homogenous colonies are summarized in Tables 1 and 2, respectively. All of these bacteria were Gram-negative.

2.2 Identification and phylogenetic analysis of the bacteria

The 16S rRNA phylogeny results (Fig. 1) confirmed the placement of these isolates. According to the DNA sequences which were compared with the NCBI Gene Database, the isolated strain of GSM-1 shares 99.93% 16S rRNA gene sequence similarity with its nearest relative of *Chryseobacterium gambrini* (accession #AM232810.1), while GSM-2 shares 99.77% with *Sinorhizobium morelense* (accession #AM181737.1) and GSM-3 shares 99.98% with *Stenotrophomonas maltophilia* (accession #EU034540.1). Therefore, the species of geosmin degraders were identified as *Chryseobacterium* sp. (GSM-1), *Sinorhizobium* sp. (GSM-2), and *Stenotrophomonas* sp. (GSM-3), respectively. It may be concluded that these bacteria may have played a vital role in the biodegradation of geosmin within the Erlenmeyer flask. This is the first report of these bacteria capable of degrading geosmin.

Table 1 Morphology of geosmin degraders

Bacterial colony	Morphological characteristics
GSM-1	Orange, semitransparent, round with a glassy surface, flat, about 2 mm in diameter, smooth edge
GSM-2	White with a light pink in the center, semitransparent, minimal elevation, round with a glassy surface, about 3 mm in diameter, smooth edge
GSM-3	Cream with yellow in the center, semitransparent, minimal elevation, round with a glassy surface, about 3 mm in diameter, smooth edge

Table 2 Physio-biochemistry analysis of geosmin degraders

Subject	GSM-1	GSM-2	GSM-3
Gram stain	–	–	–
Citrate assimilation	–	–	–
Methyl Red	+	+	+
V-P	–	–	–
Indole	–	+	+
Fibrinolysis	+	+	+
Nitrate reduction	–	–	–
Hydrogen sulphide	+	+	+
Urea hydrolase	–	+	+
Amylorrhexis	–	+	+
Gelatin liquefaction	–	–	–
Salt tolerance	+	–	–
Coagulation of litmus milk	Alkaligenous and peptonized	Rennet concretionary	Acid-produced

"+": positive; "–" : negative.

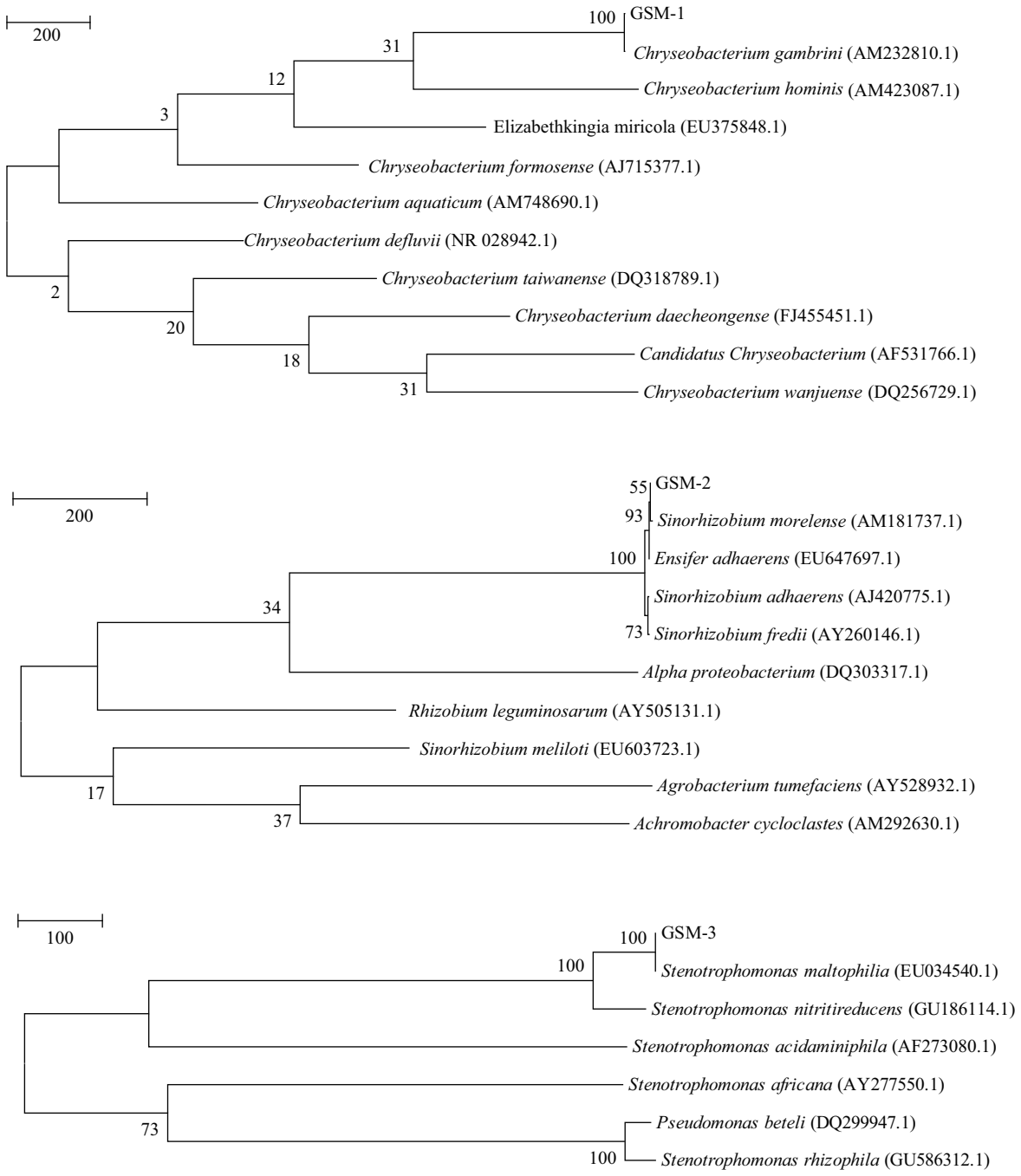

Fig. 1 Neighbor-joining trees showing the phylogenetic relationships of the predominant strains in the isolated culture with the closest-matching species.

2.3 Biodegradation of 2 mg/L geosmin

2.3.1 Removal efficiency

Geosmin in MSM, with an initial dose of 2 mg/L, was employed to investigate the biodegradation of geosmin as the sole carbon source. Figure 2 shows the change of geosmin concentration with time. The results indicated that the geosmin concentration was significantly reduced in the first 18 days, and tended to be stable during the next several days. Removal efficiencies of 2 mg/L geosmin by *Chryseobacterium* sp., *Sinorhizobium* sp. and *Stenotrophomonas* sp. were 84.0%, 80.2% and 74.4%, respectively. It can be seen from the control samples that about 33.7% of geosmin was volatilized. To the best of our knowledge, this is the first report demonstrating that geosmin at milligram-per-liter level can be removed by the pure strain of degraders as the sole carbon source.

Figure 3 shows the change of biomasses in the culture with and without geosmin. It indicates that biomass of the degraders in culture with geosmin was much higher than that without geosmin, suggesting that these bacteria were able to utilize geosmin as the sole carbon source.

It also can be seen from Figs. 2 and 3 that the removal efficiency of geosmin by the degraders was related to the biomass of single strain of bacterium. Measurable geosmin degradation appeared to begin immediately after

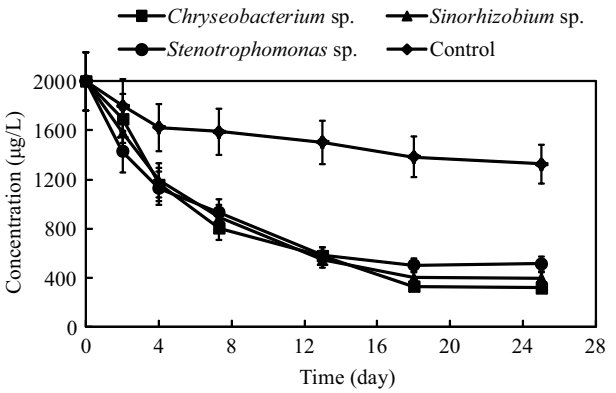

Fig. 2 Degradation of geosmin (2 mg/L). Error bars represent the standard deviation of triplicate observations.

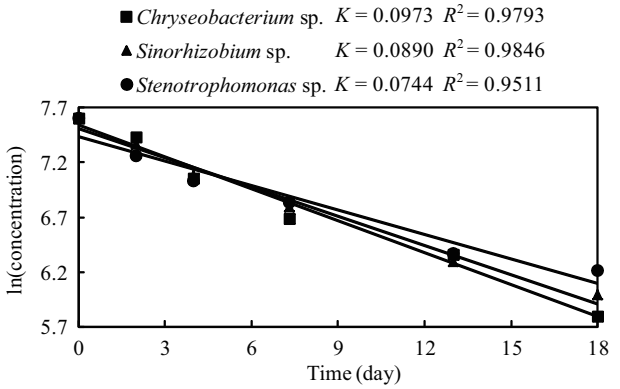

Fig. 4 Kinetics of geosmin biodegradation. The initial concentration of geosmin was about 2 mg/L.

the inoculation of geosmin degraders, with the maximum amount of geosmin degradation occurring when the bacteria were in exponential phase of growth. That agrees to the opinion of Hoefel et al. (2009). For *Chryseobacterium* sp. or *Sinorhizobium* sp., the geosmin concentration was significantly reduced as the biomass in the culture was increasing in the first 18 days; during the next 7 days, the biomass of degraders decreased rapidly and resulted in no detectable change in geosmin concentration. For *Stenotrophomonas* sp., the biomass began to decrease at day 13, while geosmin concentration still reduced with a slower rate in the next 5 days. The reason was that the removal efficiency was relative to the activity of enzyme, and the activity of enzyme in decline phase was low. Besides, the degradation rate also depends on the type of degrader. The removal efficiency of geosmin degraded by *Chryseobacterium* sp. was the highest, while its biomass was the lowest.

2.3.2 Kinetics of geosmin biodegradation

The geosmin degradation in the first 18 days is shown in Fig. 4. The numbers on *Y*-axis are the natural logarithm of the geosmin concentration (μg/L). The solids lines in Fig. 4 illustrate the simulation results with following equation:

$$\ln C = -Kt + \ln C_0 \quad (1)$$

where, t (day) is the biodegradation time; C (μg/L) and C_0 (μg/L) are the geosmin concentraitons at time t and initial time, respectively; K (day^{-1}) is the removal rate constants of geosmin.

From Fig. 4, it can be concluded that the biodegradation of geosmin by pure-culture degraders was a pseudo first-order reaction. This is in agreement with the finding of Ho et al. (2007) who reported a pseudo first-order mechanism for the biodegradation of geosmin by a mixed sand filter biofilm community suspended within a bioreactor. The rate constants were determined to be 0.097, 0.089 and 0.074 day^{-1} with R^2 of 0.9793, 0.9846 and 0.9511 for *Chryseobacterium* sp., *Sinorhizobium* sp. and *Stenotrophomonas* sp., respectively.

It is possible that biodegradation of geosmin occurs via an operon of genes in a similar way to that proposed for degradation mechanism of 2-methylisoborneol (Oikawa et al., 1995; Hoefel et al., 2009), which was also degraded as a pseudo first-order reaction (Yuan et al., accepted). To date, the best evidence for the pathway of geosmin degradation by bacteria has been provided by Saito et al. (1999), who identified four possible biodegradation products of geosmin (Hoefel et al., 2009). Two of these products were identified as 1,4a-dimethyl-2,3,4,4a,5,6,7,8-octahydronaphthalene and enone, both of which have been used in the chemical synthesis of (-)-geosmin (Hoefel et al., 2009).

2.4 Biodegradation of 560 ng/L geosmin

2.4.1 Removal efficiency

Figure 5 shows the biodegradation of geosmin in the presence of other nutrients when geosmin in filter influent with an initial concentration of 560 ng/L was employed.

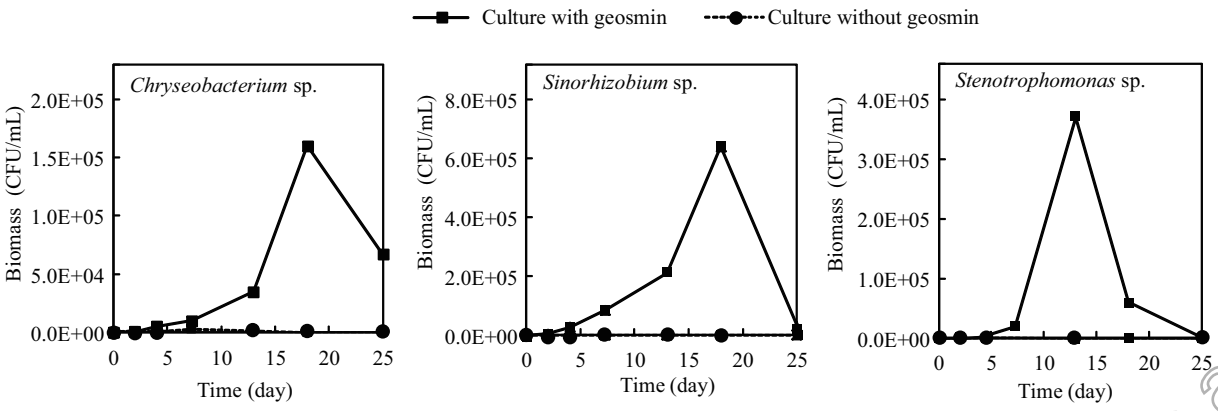

Fig. 3 Biomass of geosmin degraders. The initial concentration of geosmin was about 2 mg/L.

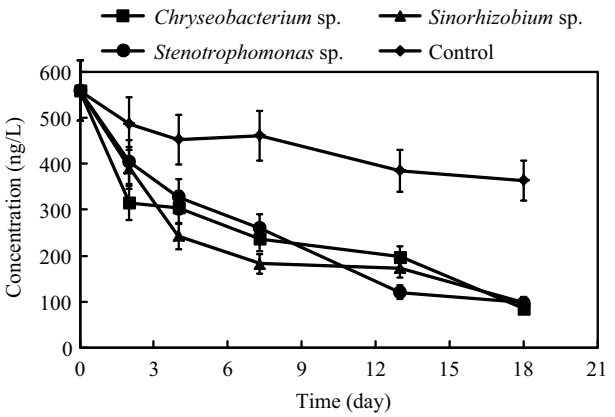

Fig. 5 Degradation of geosmin (560 ng/L). Error bars represent the standard deviation of triplicate observations.

The change in biomass with and without geosmin is shown in Fig. 6.

Removal efficiencies of geosmin by *Chryseobacterium* sp., *Sinorhizobium* sp. and *Stenotrophomonas* sp. were 84.8%, 82.3% and 82.5%, respectively, while about 35% of geosmin was volatilized. The data reported here clearly suggest that naturally occurring geosmin was degraded biologically. It is proposed that biodegradation of geosmin and perhaps other naturally occurring substances in drinking water can be applied as a method of choice for removal of odors in such water (Saadoun and El-Migdadi, 1998).

Figures 5 and 6 indicate that the removal efficiency of geosmin decreased when the biomass of single strain of bacteria was extremely low. The prolific increase in cell numbers was monitored in the earlier stage, and geosmin concentration reduced rapidly. Due to the presence of NOM, however, the degradation of geosmin did not always vary with the biomass. Consistent with the biodegradation of 2 mg/L geosmin, the removal efficiency of geosmin by *Chryseobacterium* sp. was also the highest. Because geosmin was degraded in days to weeks instead of hours, it was considered somewhat resistant to bacterial attack (Izaguirre et al., 1988).

As we know, the substrate concentration of 560 ng/L geosmin was lower than that generally considered capable of supporting microbial growth (Schmidt et al., 1985). The results shown in Fig. 2 and Fig. 5 indicate that the removal efficiency of geosmin at 560 ng/L (84.8%, 82.3% and 82.5% for *Chryseobacterium* sp., *Sinorhizobium* sp. and *Stenotrophomonas* sp., respectively) was slightly higher than that of geosmin at 2 mg/L (84.0%, 80.2% and 74.4%, respectively). This was because the degradation of geosmin at trace level would proceed in the presence of other organic compounds; mineralization of substrate at trace level in water could be sometimes enhanced by other nutrients (Rubin and Alexander, 1983). In addition, biomass in culture with geosmin was higher than that without geosmin (Fig. 6). In other words, geosmin in nanogram-per-liter level did not inhibit the bacterial growth.

2.4.2 Kinetics of geosmin biodegradation

The linear relationship between the natural logarithm values of the fraction of remaining geosmin versus time (Fig. 7) was consistent with that of a pseudo first-order mechanism of degradation. The rate constants were 0.086, 0.084 and 0.098 day^{-1} with R^2 0.9085, 0.8830 and 0.9758 for *Chryseobacterium* sp., *Sinorhizobium* sp. and *Stenotrophomonas* sp., respectively. This mechanism of degradation has also been reported by Schmidt et al. (1985) who described the mechanism for the aerobic biodegradation of geosmin, when present as secondary substrates, being of pseudo first-order. Besides, Hoefel et al. (2009) found the degradation of 100 to 1000 ng/L geosmin in surface water followed the same mechanism, with the concentration of geosmin compounds considerably lower than that of the primary substrate NOM. The rate constants in our study were quite similar with those reported for the removal of geosmin through sand filter columns, where a

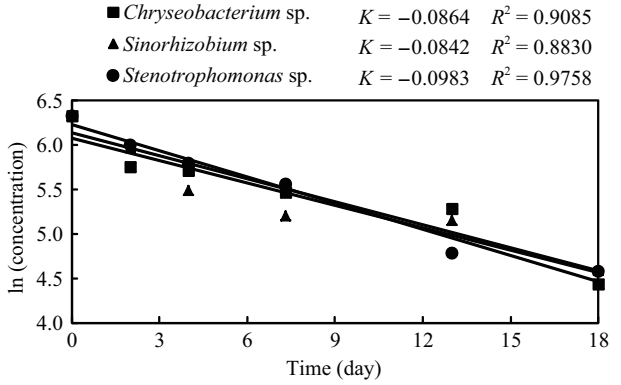

Fig. 7 Kinetics of geosmin biodegradation. The initial concentration of geosmin was 560 ng/L.

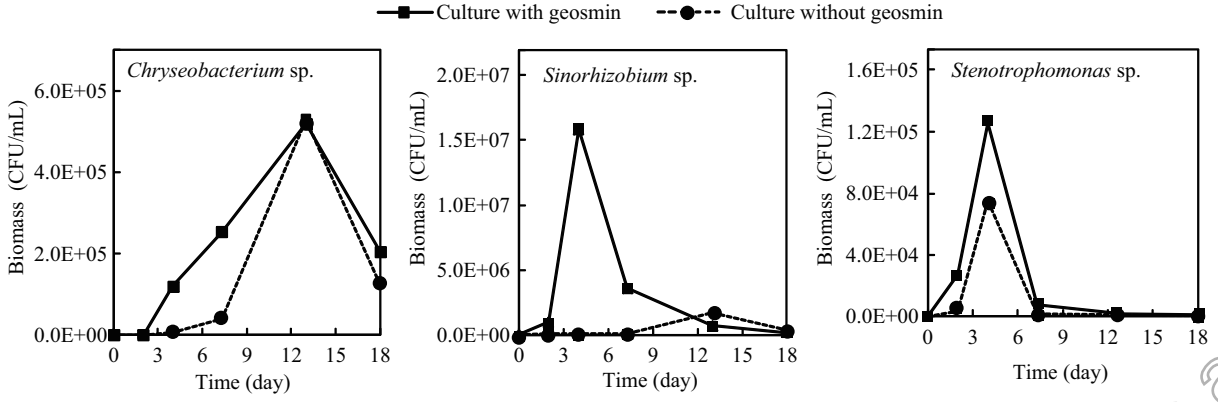

Fig. 6 Biomass of geosmin degraders. The initial concentration of geosmin was about 560 ng/L.

rate constant of 0.075 day^{-1} was reported previously for the biofiltration of geosmin (Ho et al., 2007).

3 Conclusions

This study showed that geosmin can be readily removed from water through biodegradation processes. Three bacteria were identified as *Chryseobacterium* sp., *Sinorhizobium* sp. and *Stenotrophomonas* sp. and they are most likely to be geosmin degraders. The biodegradation of geosmin was determined to be a pseudo first-order reaction. The removal efficiency of geosmin in filter influent at 560 ng/L was slightly higher than that of geosmin in MSM at 2 mg/L because the degradation of geosmin in nanogram-per-liter level was enhanced by other nutrients. The biomass of culture with geosmin was much higher than that without geosmin.

Acknowledgments

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