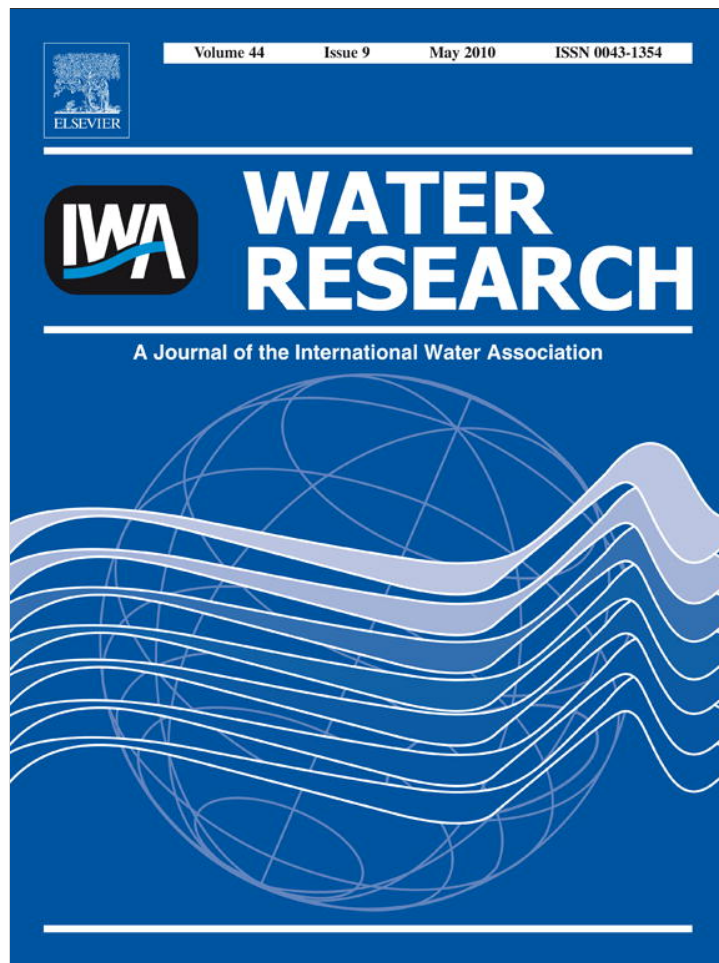


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1,4-Dioxane biodegradation at low temperatures in Arctic groundwater samples

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ABSTRACT

1,4-Dioxane biodegradation was investigated in microcosms prepared with groundwater and soil from an impacted site in Alaska. In addition to natural attenuation conditions (i.e., no amendments), the following treatments were tested: (a) biostimulation by addition of 1-butanol (a readily available auxiliary substrate) and inorganic nutrients; and (b) bioaugmentation with *Pseudonocardia dioxanivorans* CB1190, a well-characterized dioxane-degrading bacterium, or with *Pseudonocardia antarctica* DVS 5a1, a bacterium isolated from Antarctica. Biostimulation enhanced the degradation of 50 mg L⁻¹ dioxane by indigenous microorganisms (about 0.01 mg dioxane d⁻¹ mg protein⁻¹) at both 4 and 14 °C, with a simultaneous increase in biomass. A more pronounced enhancement was observed through bioaugmentation. Microcosms with 50 mg L⁻¹ initial dioxane (representing source-zone contamination) and augmented with CB1190 degraded dioxane fastest (0.16 ± 0.04 mg dioxane d⁻¹ mg protein⁻¹) at 14 °C, and the degradation rate decreased dramatically at 4 °C (0.021 ± 0.007 mg dioxane d⁻¹ mg protein⁻¹). In contrast, microcosms with DVS 5a1 degraded dioxane at similar rates at 4 °C and 14 °C (0.018 ± 0.004 and 0.015 ± 0.006 mg dioxane d⁻¹ mg protein⁻¹, respectively). DVS 5a1 outperformed CB1190 when the initial dioxane concentration was low (500 µg L⁻¹, which is representative of the leading edge of plumes). This indicates differences in competitive advantages of these two strains. Natural attenuation microcosms also showed significant degradation over 6 months when the initial dioxane concentration was 500 µg L⁻¹. This is the first study to report the potential for dioxane bioremediation and natural attenuation of contaminated groundwater in sensitive cold-weather ecosystems such as the Arctic.

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1. Introduction

1,4-Dioxane (dioxane) is a cyclic ether widely used as a stabilizer for chlorinated solvents, mainly 1,1,1-trichloroethane

(Mohr, 2001). Consequently, dioxane is an emerging groundwater contaminant commonly found at sites impacted by chlorinated solvent spills (Zenker et al., 2003). However, unlike chlorinated solvents, dioxane is highly hydrophilic and

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experiences extraordinary mobility in groundwater, leading to much larger regions of influence. Recently dioxane was included in the Final Third Drinking Water Contaminant Candidate List by U.S. EPA in September 2009 (U.S.EPA, 2009), due to its probable impact as human carcinogen (B2), as classified by the International Agency for Research on Cancer (IARC, 1999).

Conventional physical–chemical treatment methods are marginally effective to remove dioxane from impacted sites. Because of its low Henry's Law constant ($5 \times 10^{-6} \text{ atm m}^3 \text{ mol}^{-1}$ at 20 °C) and highly hydrophilic nature ($\log K_{ow} = -0.27$) (Schwarzenbach et al., 2003), dioxane is neither sufficiently volatile for air sparging nor efficiently absorbed onto activated carbon. Moreover, due to its small molecular weight of 88 g mol^{-1} , low-pressure reverse osmosis membrane may not be able to retain dioxane (Kishimoto et al., 2008). Advanced chemical oxidation (e.g., Fenton's process) and photocatalytic processes, utilizing hydrogen peroxide (Stefan and Bolton, 1998), zero-valent iron (Son et al., 2009), titanium dioxide (Yamazaki et al., 2007), ozone, electrolysis (Kishimoto et al., 2007), and sonication (Son et al., 2006) with or without UV irradiation can degrade dioxane in aqueous solution, but such techniques can be prohibitively expensive, and the contaminated groundwater needs to be pumped out from the subsurface for efficient treatment. Although plants such as hybrid poplars can assimilate and evapotranspire dioxane from aqueous solutions (Aitchison et al., 2000), the depth of contaminated groundwater typically exceeds root penetration and hinders the feasibility of phytoremediation. Furthermore, dioxane's heterocyclic ether structure makes it recalcitrant to biodegradation.

Recently, several bacterial pure cultures (Bernhardt and Diekmann, 1991; Parales et al., 1994; Mahendra and Alvarez-Cohen, 2005, 2006; Kim et al., 2009) and mixed cultures (Zenker et al., 2000, 2004; Kim et al., 2006; Shen et al., 2008; Han et al., 2009) and fungi (Nakamiya et al., 2005) were shown to degrade dioxane, and among aerobic bacteria, mono-oxygenases were implicated in metabolic (growth supporting) as well as co-metabolic (fortuitous transformation) processes (Mahendra and Alvarez-Cohen, 2006). The best-characterized dioxane-degrading strain is *Pseudonocardia dioxanivorans* CB1190, which was isolated from industrial sludge (Parales et al., 1994). CB1190 can aerobically mineralize dioxane and other cyclic ethers and use it as sole carbon and energy source (Parales et al., 1994; Mahendra and Alvarez-Cohen, 2005). Assays of acetylene irreversible inhibition (Prior and Dalton, 1985) and colorimetric naphthalene oxidation (Graham et al., 1992) confirmed that a monooxygenase initiated dioxane catabolism. A proposed mineralization pathway demonstrated that the major metabolite of dioxane in previous reports (Vainberg et al., 2006), 2-hydroxyethoxyacetic acid (HEAA), is quickly oxidized to CO_2 by CB1190 (Mahendra et al., 2007). In addition to CB1190, *Pseudonocardia* strains, such as *Pseudonocardia benzenivorans* B5 (Kampfer and Kroppenstedt, 2004; Mahendra and Alvarez-Cohen, 2006), *Pseudonocardia tetrahydrofuranoxydans* K1 (Kohlweyer et al., 2000; Kampfer et al., 2006), and *Pseudonocardia* ENV478 (Vainberg et al., 2006) have been reported to degrade dioxane.

The discovery of several dioxane-degrading strains has stimulated research on the feasibility of natural or enhanced

bioremediation for *in situ* remediation of dioxane-impacted sites. However, most dioxane biodegradation assays have been conducted under relatively warm temperatures ($>20 \text{ }^\circ\text{C}$) and the existence of microorganisms capable of participating in the remediation of dioxane-contaminated sites in cold regions has not been established. This is an important knowledge gap because of increasingly strict regulatory limits on dioxane concentration in groundwater in all environments, including cold-weather environments such as the Alaskan tundra, where a groundwater cleanup standard of $77 \text{ } \mu\text{g L}^{-1}$ was recently proposed (ADEC, 2008).

This study investigated the feasibility of dioxane biodegradation at low temperatures encountered in impacted Arctic groundwater. Different remediation strategies were considered, including biostimulation, bioaugmentation with different reference strains, and natural attenuation. In addition to varying incubation temperatures, various dioxane concentrations were considered to mimic source-zone bioremediation, where dioxane concentrations are relatively high (ppm levels), as well as natural attenuation in areas distant from the source, where lower (ppb) dioxane concentrations prevail. In doing so, novel insights were obtained into the kinetics, competitive advantages and limitations experienced by different organisms that could participate in the cleanup of dioxane contaminated sites.

2. Materials and methods

2.1. Chemicals

All reagents used in the medium preparation were of ACS grade or better. 1,4-Dioxane (99.9%, stabilized with 10 mg L^{-1} sodium diethyldithiocarbamate) was purchased from EM Science, Cherry Hill, NJ. Both 1-butanol (99.9%) and methylene chloride (99.9%) were obtained from Fisher Scientific, Fair Lawn, NJ. 1,4-Dioxane- d_8 (99%) was purchased from Sigma Aldrich, St. Louis, MO. 1,4-Dichlorobenzene- d_4 ($2000 \text{ } \mu\text{g mL}^{-1}$ in methanol) was purchased from Supelco Analytical, Bellefonte, PA. Methanol (99.9%, for GC, HPLC, Spectrophotometry, and Gradient Analysis) was purchased from EMD Chemical, Darmstadt, Germany.

2.2. Laboratory strains

Two reference strains, *P. dioxanivorans* CB1190 (ATCC #55486) and *Pseudonocardia antarctica* DVS 5a1 (DSMZ # 44749), were selected as bioaugmentation candidates. CB1190 is a well-characterized dioxane degrader (Parales et al., 1994; Kelley et al., 2001) while DVS 5a1 is an actinomycete isolated from a moraine sample from the Antarctic (Prabahar et al., 2004). Although DVS 5a1 has not been previously reported to degrade dioxane, it was chosen because it is taxonomically close to CB1190 (96% similarity in 16S rDNA) and belongs to a genus that includes various dioxane-degrading species (Mahendra and Alvarez-Cohen, 2006).

Both CB1190 and DVS 5a1 were grown in a R2A medium for 24 h to maximize the biomass at $24 \text{ }^\circ\text{C}$ while shaking at 150 rpm. Then cells were harvested by centrifugation at 8000 rpm for 15 min. The supernatant was decanted and the

pellets were washed three times with 25 mL of ammonium mineral salts (AMS) medium (Parales et al., 1994) to remove the dissolved organic carbon sources. The biomass of the resuspended cultures was quantified as total protein, and diluted to 20 mg protein L⁻¹ by AMS media.

One liter of AMS contained 100 mL of 10× salts solution, 1.0 mL of AMS trace elements, 1.0 mL of stock A, and 20 mL of 1.0 M phosphate buffer (added after sterilization). The AMS 10× salts solution contained 6.6 g of (NH₄)₂SO₄, 10.0 g of MgSO₄·7H₂O, and 0.15 g of CaCl₂·2H₂O. The AMS trace elements contained (L⁻¹) 0.5 g of FeSO₄·7H₂O, 0.4 g of ZnSO₄·7H₂O, 0.02 g of MnSO₄·H₂O, 0.015 g of H₃BO₃, 0.01 g of NiCl₂·6H₂O, 0.05 g of CoCl₂·6H₂O, 0.005 g of CuCl₂·2H₂O, and 0.25 g of EDTA. The AMS stock A contained (L⁻¹) 5.0 g of Fe-Na EDTA and 2.0 g of NaMoO₄·2H₂O. The 1 M phosphate buffer contained 113.0 g of K₂HPO₄ and 47.0 g of KH₂PO₄.

2.3. Microcosms preparation

Microcosms were prepared using North Slope tundra and pad samples (including soil and groundwater) collected in September 2008 at an industrial site in Prudhoe Bay, Alaska. The microcosms were prepared with 10 g soil and 50 mL groundwater, and were incubated in autoclaved 200 mL amber glass bottles capped with Teflon-lined mininert valves at 4 °C and 14 °C while shaking on a rotary table at 150 rpm. The groundwater collected from a monitoring well tapping a sandy-silt formation, using a peristaltic pump. The groundwater had relatively low dissolved oxygen content (DO = 1.7 mg L⁻¹, ORP = -85.4), was slightly acidic (pH = 6.8) and had low nitrogen content (total nitrogen <50 µg L⁻¹).

Various treatments were considered to investigate the feasibility for dioxane degradation in the Arctic aquifer through different in situ bioremediation strategies (i.e., unamended attenuation, biostimulation, and bioaugmentation) at 4 and 14 °C, which are within the range of groundwater temperatures in the tundra (Deming et al., 1992). Biodegradation was first evaluated at high dioxane concentrations (50 mg L⁻¹) representing source zone conditions, under various biostimulation and bioaugmentation conditions listed in Table 1. 1-Butanol (5 µL) was added monthly after the first 2 months to ensure the availability of a growth substrate in the microcosms. The dioxane biodegradation rate for each treatment (Table 2) was calculated at each temperature as the average of the removal rate (concentration versus time slope) for triplicate microcosms, corrected for the removal rate in autoclaved controls,

and normalized to the average total protein concentration over the six month incubation period.

Dioxane biodegradation was also evaluated at low concentrations (500 µg L⁻¹) typically found near the leading edge of the plume distant from the source. Treatments A, N, D, and E were repeated at 14 °C, which is representative of the groundwater at the Alaska site in summer. All microcosms were prepared in triplicate and differences in dioxane removal between treatments were assessed statistically using the student t-test at the 95% confidence level (Ang and Tang, 2006; Xiu et al., 2010).

2.4. Analytical methods

For dioxane quantification at higher concentrations (mg L⁻¹ range), 0.3 mL liquid samples were collected from each microcosm monthly with sterile 1 mL syringes with needles through the mininert valves. 0.2 µm 13 mm Nylon syringe filters were used to remove the cells and suspended soil in the samples. 2 µL aqueous samples were injected directly into an Agilent 5890 Chromatograph (GC) equipped with a Flame Ionization Detector (FID) and an Agilent 2 m × ¼ in × 2 mm glass column. The injector and detector temperatures were set at 200 °C and 250 °C, respectively. The oven temperature was initially held at 105 °C for 2 min, and then run with a ramp rate of 3.0 °C min⁻¹ to reach the final temperature of 150 °C. The retention time for dioxane and 1-butanol were 10.7 and 12.8 min, respectively. The detection limit was 1.0 mg L⁻¹ and the accuracy of dioxane concentration measurements was better than ±3%.

A novel method of sample preparation by frozen micro-extraction was developed for dioxane quantification at lower (µg L⁻¹) concentrations. Briefly, 200 µL aliquots of liquid culture from microcosms were collected, filtered, and subsequently spiked with 1 µL methanol mixture containing 40 mg L⁻¹ 1,4-dioxane-d₈ as internal standard and 20 mg L⁻¹ 1,4-dichlorobenzene-d₄ as surrogate. Equal volume of methylene chloride was added into the Agilent screw cap 1.5 mL glass vials. The capped vials were then gently shaken for 30 s and placed on glass plates inclined at an angle of 45° from the horizon. After frozen at -80 °C for 45 min, the liquid phase solvents (about 200 µL, mainly methylene chloride) were extracted out and stored at -20 °C for future analysis. Anhydrous sodium sulfate (0.05 g) was added in the final solvents to remove the influence of water for subsequent GC/MS analysis.

Table 1 – Initial ingredients for various microcosms spiked with 50 mg L⁻¹ dioxane.

Treatment	Initial ingredients
A. Autoclaved control	10 g soil + 50 mL groundwater (sterilized at 121 °C for 15 min)
N. Natural attenuation	10 g soil + 50 mL groundwater (no amendments)
B. Biostimulated with 1-butanol	10 g soil + 50 mL groundwater + 100 mg L ⁻¹ 1-butanol
C. Biostimulated with AMS & 1-butanol	10 g soil + 25 mL groundwater + 25 mL AMS + 100 mg L ⁻¹ 1-butanol
D. Bioaugmented, CB1190 + AMS & 1-butanol	10 g soil + 25 mL groundwater + 20 mL AMS + 100 mg L ⁻¹ 1-butanol + 5 mL <i>P. dioxanivorans</i> CB1190 cultures in AMS
E. Bioaugmented, DVS 5a1 + AMS & 1-butanol	10 g soil + 25 mL groundwater + 20 mL AMS + 100 mg L ⁻¹ 1-butanol + 5 mL <i>P. antarctica</i> DVS 5a1 cultures in AMS

Table 2 – Biodegradation rate (mg dioxane mg protein⁻¹ day⁻¹) in various microcosms with 50 mg L⁻¹ initial dioxane concentration.

Temperature	Biostimulated with AMS & 1-butanol	Bioaugmented with CB1190 + AMS & 1-butanol	Bioaugmented with DVS 5a1 + AMS & 1-butanol
14 °C	0.010 ± 0.002	0.155 ± 0.038	0.015 ± 0.006
4 °C	0.011 ± 0.003	0.021 ± 0.007	0.018 ± 0.004

2.5. Biomass quantification

Biomass was quantified as total protein concentration using a modified Bradford method (Bradford, 1976). Briefly, aqueous samples (0.5 mL) were collected every 3 months from each microcosm. Cells were digested by adding 0.1 mL 5 M NaOH and boiling at 98 °C for 10 min. Total soluble protein was measured in the supernatant obtained after centrifuging the digested cells for 15 min at 13,200 rpm. Several dilutions of bovine serum albumin were prepared to achieve final protein concentrations within the linear range of the assay. Then, 50 µL of each sample or standard were mixed with 1.5 mL of the dye reagent of the Coomassie Plus protein assay kit (Pierce

Chemical Company, Rockford, IL). Absorbance at 595 nm was measured immediately using an Ultrospec 2100 Pro UV/Visible spectrophotometer (Biochrom Ltd., Cambridge, England).

3. Results and discussion

Biodegradation of high (50 mg L⁻¹) dioxane concentrations was demonstrated at Arctic groundwater temperatures (e.g., 4 °C) by its significant disappearance in biologically active microcosms relative to autoclaved controls ($p < 0.05$) (Fig. 1(a)). The controls experienced losses of $15.0 \pm 8.7\%$ over six months due to abiotic processes such as evaporation,

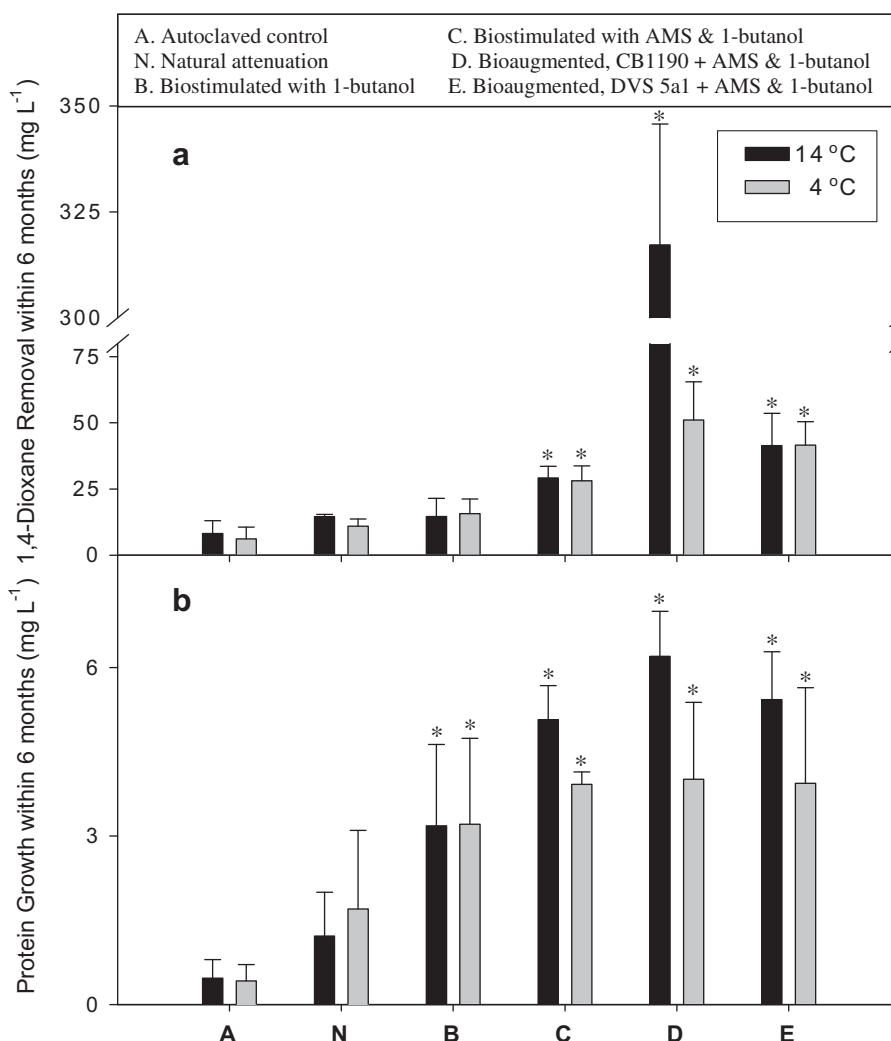


Fig. 1 – 1,4-Dioxane removal (a) within 6 months and microbial growth (b) in various microcosms at 4 and 14 °C.

* Indicates significant dioxane removal or protein growth ($p < 0.05$) relative to the autoclaved controls. The initial dioxane concentration was 50 mg L⁻¹. Treatments C, D, and E were re-spiked several times after dioxane was removed.

adsorption, and diffusion into fine grained pores or intralaminar storage in clay platelets (Mohr et al., 2010), but remained sterile and did not experience an increase in biomass (Fig. 1(b)). Microcosms amended with 1-butanol (100 mg L⁻¹) and inorganic nutrients (70 mg-N L⁻¹, 310 mg-P L⁻¹) removed significant amounts of dioxane ($p < 0.05$), which demonstrates the presence of indigenous dioxane degraders in these Arctic groundwater samples. However, neither significant dioxane removal nor protein growth was observed in the natural attenuation (unamended) microcosms. In microcosms amended with 1-butanol alone, protein growth occurred without significant dioxane depletion. Apparently, the low content of inorganic nutrients in the groundwater used to prepare the microcosms (e.g., Total N < 50 µg L⁻¹) was stoichiometrically limiting when dioxane and 1-butanol were present at such relatively high concentrations.

Several bacterial strains have been reported to utilize substrates such as methane, propane, butane, toluene, or tetrahydrofuran by inducing their corresponding mono-oxygenases (Wackett et al., 1989; Oldenhuis et al., 1991; Hamamura et al., 1997; McClay et al., 2000; Thiemer et al., 2003). These strains were recently reported to co-metabolically degrade dioxane (For review, see Mahendra and Alvarez-Cohen, 2006). In our study, 1-butanol had a stimulatory effect on indigenous microorganisms at 4 °C and 14 °C when inorganic nutrients were not limiting (Treatment C, Fig. 1), and the added 1-butanol was quickly consumed within the first 2 weeks at both temperatures (data not shown).

Bioaugmentation with CB1190 in the presence of nutrients and 1-butanol resulted in the fastest dioxane degradation rates (Fig. 1). The higher rates observed at a warmer temperature (i.e., 14 °C Table 2) are consistent with the fact that the optimum growth temperature for CB1190 is 30 °C (Parales et al., 1994; Mahendra and Alvarez-Cohen, 2005). At 4 °C, these microcosms exhibited a three-month lag period before appreciable degradation occurred (Fig. 2(a)), indicating that adaptation to cold temperatures was possible. The calculated biomass production for CB1190 (corrected for growth on 1-butanol from Treatment C, but not for cell decay) was 0.01 mg protein mg dioxane⁻¹ at 14 °C, which agrees with a previously reported yield coefficient at 20 °C (Kelley et al., 2001) and is slightly lower than values reported at 30 °C (0.02–0.09 mg protein mg dioxane⁻¹ (Parales et al., 1994; Mahendra and Alvarez-Cohen, 2006)).

Bioaugmentation with DVS 5a1 also enhanced dioxane degradation relative to biostimulated microcosms (Fig. 1(a)). Unlike CB1190, DVS 5a1 experienced relatively long lags (i.e., 3 months at both 4 °C and 14 °C, Fig. 2(b)) before the onset of biodegradation, suggesting the need to adapt to such high initial dioxane concentrations and low temperatures.

Parallel biodegradation experiments were also conducted using lower initial dioxane concentrations (500 µg L⁻¹) that are characteristic of the leading edge of plumes, distant from the source. These experiments were conducted at 14 °C, which is commonly reached during summer months in the tundra with an annual average temperature of 6.3 °C. DVS 5a1 outperformed CB1190 at low dioxane concentrations (Fig. 3), suggesting that either DVS 5a1 is better adapted to low-carbon (oligotrophic) conditions and/or that CB1190 exhibits higher

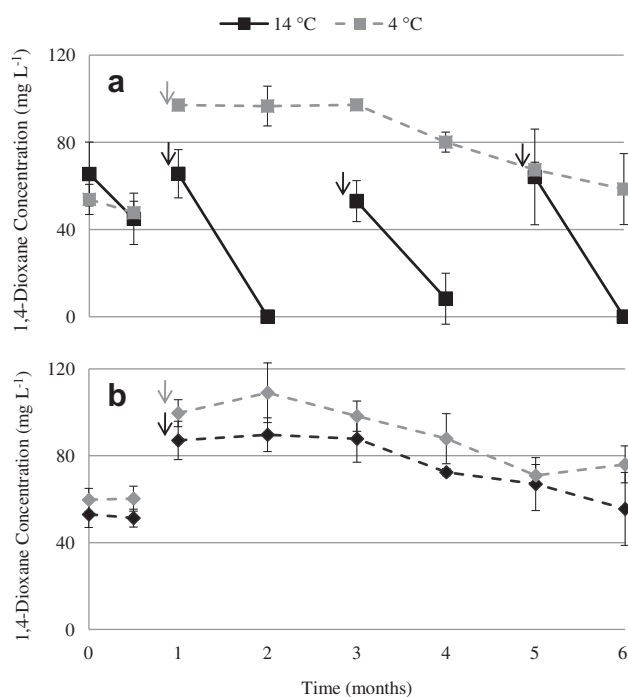


Fig. 2 – Degradation of high concentrations of 1,4-dioxane in (a) microcosms bioaugmented with CB1190 (Treatment D) and (b) microcosms bioaugmented with DVS 5a1 (Treatment E). Microcosms with CB1190 outperformed those with DVS 5a1 at high dioxane concentrations, especially at warmer temperatures. Arrows indicate times when microcosms were re-spiked.

tolerance to high dioxane concentrations, as evidenced by shorter lags (Fig. 2).

Significant dioxane biodegradation by indigenous microorganisms was also observed in aerobic microcosms mimicking natural attenuation, which removed dioxane from 500 µg L⁻¹ to 130 ± 4 µg L⁻¹ within six months of incubation (Fig. 3). Dioxane tends to migrate faster and form longer plumes than the associated chlorinated solvents, and the

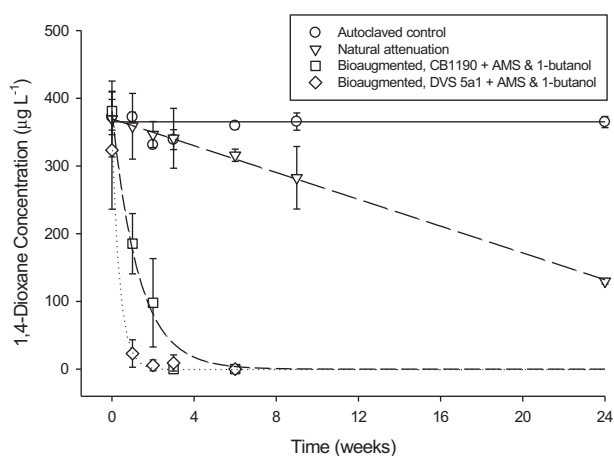


Fig. 3 – 1,4-Dioxane degradation with initial concentration of 500 µg L⁻¹ at 14 °C. Microcosms with DVS 5a1 outperformed those with CB1190 at low dioxane concentrations.

Table 3 – Fitted models for dioxane degradation in treatments with 500 $\mu\text{g L}^{-1}$ initial dioxane concentration at 14 °C (Fig. 3).

Treatment	Model	Rate constants ^a	R ²
N. Natural attenuation	Zero order	$K_N = 1.4 \pm 0.02 \mu\text{g L}^{-1} \text{ day}^{-1}$	0.999
D. Bioaugmented with CB1190 + AMS & 1-butanol	First order	$K_D = 0.1 \pm 0.01 \text{ day}^{-1}$	0.982
E. Bioaugmented with DVS 5a1 + AMS & 1-butanol	First order	$K_E = 0.4 \pm 0.03 \text{ day}^{-1}$	0.999

a Subscripts assigned to rate constants denote treatment (Table 1).

leading edge of dioxane plumes is likely to encounter oligotrophic and aerobic conditions in Arctic aquifers. This result suggests that aerobic natural attenuation might be a feasible polishing approach to manage residual dioxane contamination in areas distant from the source, where dioxane might be present at trace levels over a relatively large area, and where more aggressive engineered remediation strategies that are more appropriate for source-zone remediation might be prohibitively expensive and/or marginally effective.

Biodegradation patterns in microcosms spiked with low dioxane concentrations provide insight into the relative affinity for dioxane exhibited by exogenous versus indigenous microorganisms. Indigenous microorganisms mediated a linear decrease in dioxane concentration versus time, with a zero-order removal rate of $1.4 \pm 0.02 \mu\text{g L}^{-1} \text{ day}^{-1}$ (Table 3). Zero-order kinetics indicates lack of significant microbial growth (as expected given the low concentration of dioxane available) and saturated enzymes kinetics, which occurs when the half saturation Monod constant (K_s) is relatively small compared to the substrate concentration (initially $500 \mu\text{g L}^{-1}$) (Alvarez and Illman, 2006). Such small values of K_s are indicative of high affinity for the substrate (i.e., dioxane), which is characteristic of oligotrophic bacteria (Atlas and Bartha, 1997). In contrast, the exogenous strains CB1190 and DVS 5a1 exhibited an exponential decrease in dioxane concentrations with time, indicating first-order kinetics. This pattern suggests a larger value of K_s (much greater than the substrate concentration), reflecting lower affinity for dioxane. This notion is corroborated by reported values of K_s for CB1190 ($160 \pm 44 \text{ mg L}^{-1}$ (Mahendra and Alvarez-Cohen, 2006)), which are much larger than the initial dioxane concentration of $500 \mu\text{g L}^{-1}$.

The identification of microbial populations adapted to degrade dioxane at high (source zone) or low (diluted down-gradient) concentrations is important for developing arguments for natural attenuation or formulating engineered bioremediation strategies suitable for each of these nutritionally-unique zones in aquifers. Fundamentally, source zones and aerobic fringes of plumes may respectively be suitable for different microorganisms (e.g., r- and K-strategists), implying that an engineered remedial strategy might be optimized by selectively augmenting with microbial strains targeted for high and low concentrations based on the strains' affinity and tolerance towards dioxane.

4. Conclusions

This is the first study to demonstrate the potential for dioxane bioremediation and natural attenuation of contaminated groundwater in cold-climate environments, such as the

Arctic, and to report the ability of *P. antarctica* DVS 5a1 to degrade dioxane. The higher tolerance to dioxane and higher degradation rates exhibited by CB1190 make it a better bioaugmentation candidate for near-source-zone bioremediation (e.g., to inoculate biobarriers or *in situ* reactive zones), especially at warmer temperatures. Overall, both indigenous and exogenous strains demonstrated the ability to degrade dioxane under a wide variety of conditions, and illustrated that different bacteria exhibit different competitive advantages and limitations in response to varying temperatures and substrate concentrations as they exploit dioxane biodegradation as a metabolic niche.

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