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ORIGINAL PAPER

# Evaluation of the biodegradation potential of 1,4-dioxane in river, soil and activated sludge samples

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**Abstract** To evaluate the biodegradation potential of 1,4-dioxane in natural environments, a total of 20 environmental samples including river water, activated sludge, soil from the drainage area of a chemical factory and garden soil were subjected to a 1,4-dioxane degradation test. The five soil samples from the drainage area of the chemical factory were capable of reducing 100 mg $l^{-1}$ of 1,4-dioxane to below the detection limit (0.8 mg $l^{-1}$) within 33 days. In one activated sludge sample, 100 mg $l^{-1}$ of 1,4-dioxane decreased by 69% within 14 days via cometabolic degradation in the presence of 100 mg $l^{-1}$ of tetrahydrofuran (THF). The ability of all samples to degrade 1,4-dioxane degradation with or without THF increased after repeated enrichment, except for one soil sample from the drainage area of the chemical factory that was no longer able to degrade 1,4-dioxane after the third cycle of enrichment. However, most of the samples (14/20) were not able to degrade 1,4-dioxane degradation. Thus, it can be concluded that the potential for 1,4-dioxane degradation is not ubiquitously distributed in natural environment.

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**Keywords** 1,4-Dioxane · Biodegradation potential · Environmental samples · Tetrahydrofuran (THF) · Enrichment culture

## Introduction

1,4-Dioxane is a cyclic ether that is widely used as a solvent in paints, lacquers, cosmetics, deodorants, fumigants and detergents, as well as a stabilizer for chlorinated solvents such as 1,1,1-trichloroethane. In addition, 1,4-dioxane is formed as an undesired by-product in many industrial processes, especially during the synthesis of polyester. This xenobiotic compound is highly soluble and mobile in water; however, there is little volatilization to the atmosphere or adsorption to solids when this compound is in aquatic systems. In addition, because 1,4-dioxane does not have functional groups that are susceptible to hydrolysis (Wolfe and Jeffers 2000) and does not absorb light in the environmental spectrum (>290 nm), it is believed that it is not hydrolyzed or photo-degraded in aquatic environments (Agency for Toxic Substances and Disease Registry 2007). Thus, the fate of 1,4-dioxane in the aquatic environment depends on biodegradation. 1,4-Dioxane is a serious pollutant in aquatic environments because of its acute and chronic toxicity, as well as its suspected carcinogenicity. Accordingly, 1,4-dioxane has been nominated for inclusion in the guidelines for drinking water produced by the WHO in 2003. Furthermore, the water quality standard for drinking water in the Water

Supply Law of Japan set a limit of 50 μg $l^{-1}$ for 1,4-dioxane in 2004. Despite this, 1,4-dioxane has been widely detected in wastewater from industries (up to 4,020 μg $l^{-1}$ in Japan), surface water (up to 260 μg $l^{-1}$ in the USA, 46 μg $l^{-1}$ in Japan and 10 μg $l^{-1}$ in the Netherlands), groundwater (up to 220 mg $l^{-1}$ in the USA) (Agency for Toxic Substances and Disease Registry 2007), and landfill leachate (up to 2,000 μg $l^{-1}$ in Japan; Namegaya et al. 2002). Because 1,4-dioxane is considered to be quite recalcitrant due to its heterocyclic ether structure, any 1,4-dioxane released into the environment could persist for a long time.

Despite the recalcitrant nature of 1,4-dioxane, there have been a considerable number of attempts to isolate and characterize 1,4-dioxane degrading bacteria (Bernhardt and Diekmann 1991; Parales et al. 1994; Kämpfer and Kroppenstedt 2004; Mahendra and Alvarez-Cohen 2005; Mahendra et al. 2007; Kim et al. 2009) or to treat wastewater containing 1,4-dioxane using biological processes (Burback and Perry 1993; Roy et al. 1994; Abe 1999; Zenker et al. 2004; Han et al. 2009). Although the results of these studies have demonstrated that 1,4-dioxane degrading bacteria exist, they have not provided any information regarding the 1,4-dioxane degradation potential in the natural environment, especially for environments that have not been contaminated with 1,4-dioxane. As a result, accumulation of information regarding the potential for 1,4-dioxane biodegradation in various environments is needed for accurate prediction of the fate of this compound in the environment.

In the present study, a 1,4-dioxane degradation test was conducted using natural environmental samples and activated sludge samples to evaluate the biodegradation potential of 1,4-dioxane in various environments. Cometabolic degradation of 1,4-dioxane with tetrahydrofuran (THF), the structural analog of 1,4-dioxane and a possible primary substrate (Zenker et al. 2000; Vainberg et al. 2006), was also evaluated in some samples.

## Materials and methods

### Media

For the 1,4-dioxane and THF degradation and enrichment experiments, basal minimum medium (BMM; Parales et al. 1994) was prepared with known concentrations of 1,4-dioxane and THF unless otherwise stated (Table 1). The prepared BMM was autoclaved before inoculating environmental samples.

**Table 1** Basal minimum medium (Parales et al. 1994)

| Component | Concentration (mg/l) |
|---|---|
| $K_2HPO_4$ | 3,240 |
| $NaH_2PO_4 \cdot H_2O$ | 1,000 |
| $NH_4Cl$ | 2,000 |
| Disodium nitrilotriacetate | 123 |
| $MgSO_4 \cdot 7H_2O$ | 200 |
| $FeSO_4 \cdot 7H_2O$ | 12 |
| $MnSO_4 \cdot H_2O$ | 3 |
| $ZnSO_4 \cdot 7H_2O$ | 3 |
| $CoCl_2 \cdot 6H_2O$ | 1 |

### 1,4-Dioxane degradation experiment

#### *River water samples*

Four river water samples were collected from the Yamato River (two samples from midstream and downstream), Ina River (one sample from midstream) and Kanzaki River (one sample from downstream) in Osaka (Yamato River and Kanzaki River) and Hyogo (Ina River), Japan. One milliliter of each sample was inoculated into 10 ml of BMM supplemented with 0.1% (w/v) yeast extract as trace elements to support the 1,4-dioxane degradation and the growth of 1,4-dioxane degrading bacteria, and with 100 mg $l^{-1}$ of 1,4-dioxane in 20 ml glass vials.

#### *Soil samples from drainage areas of a chemical factory*

Six drainage surface soil samples (drainage soils A, B, K, L, M and N) were collected from six points of drainage in a chemical factory producing 1,4-dioxane in Japan. The drainage is set around the factory to remove rainwater fell in the factory area. Drainage soil sample A was collected from the border between the drainage water and soil. Drainage soil B was collected from 50 cm above the drainage water surface. Drainage soils K, L, M and N were collected from 20 cm above the drainage water surface and horizontally apart at a certain intervals. Three grams of each sample

were inoculated into 100 ml of BMM supplemented with 100 mg $l^{-1}$ of 1,4-dioxane in 300 ml glass flasks. In addition, autoclaved soil samples were inoculated into the same BMM with 100 mg $l^{-1}$ of 1,4-dioxane as controls.

*Activated sludge samples*

Three activated sludge samples (activated sludges A, B and C) were collected from the aeration tanks of two municipal wastewater treatment plants (WWTPs) in Japan that are used to treat both domestic and industrial wastewater. Activated sludges A and C were collected from the same WWTP, which utilized pseudo anaerobic–aerobic process, at different times. Activated sludge B was collected from a WWTP that adopted a conventional activated sludge process. The MLSS values of activated sludges A, B and C were 1,243, 3,360 and 1,100 mg $l^{-1}$, respectively. To evaluate the ability of the sludge to degrade 1,4-dioxane, 100 mg $l^{-1}$ of the compound was added directly to 100 ml of the activated sludge samples in 300 ml glass flasks. For activated sludge C, another test system supplemented with 100 mg $l^{-1}$ of 1,4-dioxane and THF was also prepared. Autoclaved activated sludge sample was also prepared in a same manner and served as a control.

*Garden soil samples*

Seven garden soil samples (garden soils A, B, C, D, E, F and G) were collected at random from the campus (c.a. 1 $km^2$) of Osaka University. Three grams of each sample were inoculated into 100 ml of BMM containing 100 mg $l^{-1}$ of 1,4-dioxane with or without 100 mg $l^{-1}$ of THF in 300 ml glass flasks.

All test systems were plugged with sponge plug, incubated at 28°C with rotary shaking at 120 rpm. The degradation of 1,4-dioxane and THF was monitored by gas chromatography (GC) periodically. The samples that showed obvious 1,4-dioxane degradation were successively inoculated into fresh experimental systems at 10% (v/v) after confirming the complete degradation of 1,4-dioxane and then enriched.

Analytical procedures

The 1,4-dioxane and THF concentrations were monitored using a GC-14B gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame-ionization detector (FID) and a 2.1 m (3.2 mm [i.d.]) glass column packed with Gaschropack 54 (GL Science, Tokyo, Japan). Aliquots (1 ml) of the samples were filtered through a 0.45 μm cellulose acetate filter (Advantec, Tokyo, Japan) and 2 μl of the filtered samples were then injected. Nitrogen gas was applied as the carrier gas at a flow rate of 33 ml $min^{-1}$. The injector, oven and detector temperature were all set at 200°C. The mechanical detection limit of 1,4-dioxane and THF was 0.8 mg $l^{-1}$.

## Results and discussion

Degradation of 1,4-dioxane by various environmental samples

None of the river water samples or garden soil samples could degrade 1,4-dioxane within 29 and 39 days, respectively. In addition, two of the three activated sludge samples (activated sludges A and B) could not degrade 1,4-dioxane within 29 days (data not shown). Moreover, cometabolic degradation of 1,4-dioxane with THF was not observed in the garden soil samples. Conversely, five of the six drainage soil samples (drainage soils B, K, L, M and N) could completely degrade 1,4-dioxane without the addition of exogenous carbon (Fig. 1). Specifically, drainage soil L degraded 1,4-dioxane to below the detection limit (<0.8 mg $l^{-1}$) within 14 days, while drainage soils B, K and M degraded the compound to below the detection limit

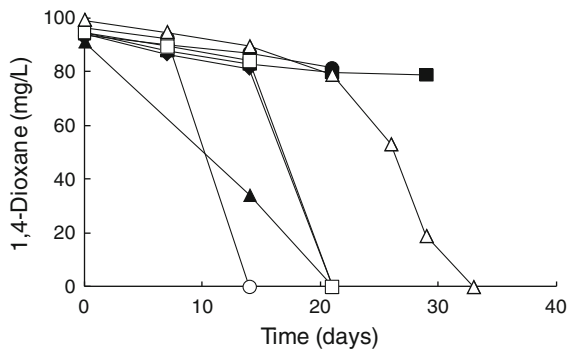


**Fig. 1** Degradation profiles of 1,4-dioxane using drainage soil samples. *Filled circle* control, *filled square* soil A, *filled triangle* soil B, *filled diamond* soil K, *opened circle* soil L, *opened square* soil M, *opened triangle* soil N

within 21 days, and drainage soil N required 33 days to completely degrade the added 1,4-dioxane. A certain lag period prior to 1,4-dioxane degradation was confirmed in most of the samples; 7, 14 and 21 days in drainage soils L, K and N, respectively. Additionally, less than 9% of the 1,4-dioxane from the sterilized control evaporated; therefore, the decrease in 1,4-dioxane in these samples could be attributed to biodegradation. Considering the previously reported half-life of 1,4-dioxane in surface water and soil (1–6 months; Kawasaki 1980), the ability of the drainage soil samples obtained in the present study to degrade 1,4-dioxane was high.

Activated sludge C degraded 69% of the added 1,4-dioxane (100 mg $l^{-1}$) after 14 days, but only when THF was also added (Fig. 2). The finding that this sample could not degrade 1,4-dioxane when it was added without THF strongly suggests that cometabolic degradation with THF occurred. Indeed, there have been several reports of bacterial 1,4-dioxane cometabolic degradation occurring when THF is used as the primary substrate (Kohlweyer et al. 2000; Zenker et al. 2000). This likely occurs because THF is a structural analog of 1,4-dioxane that has been shown to be biodegradable in aquatic environments (OECD 2000). Kohlweyer et al. (2000) also reported that they could enrich and isolate the THF degrading bacterium *Pseudonocardia* sp. K1 from sludge obtained from an uncontaminated community WWTP in Germany.

## Degradation of 1,4-dioxane by enrichment culture

The results of the 1,4-dioxane degradation test of various environmental samples revealed that five drainage soil samples (drainage soils B, K, L, M and N) and one activated sludge sample (activated sludge C) showed significant metabolic and cometabolic 1,4-dioxane degradation. Therefore, these samples were subjected to repeated enrichment by transferring 10% of the culture to fresh BMM supplemented with 100 mg $l^{-1}$ of 1,4-dioxane as the sole carbon source (drainage soils) or with 100 mg $l^{-1}$ of both 1,4-dioxane and THF (activated sludge C).

In the 6th cycle of enrichment, drainage soils B, K and L degraded 100 mg $l^{-1}$ of 1,4-dioxane to levels below the detection limit within 10 days (Fig. 3). Although the time required for drainage soil M to degrade the 1,4-dioxane (21 days) did not decrease after repeated enrichment, the lag period prior to the start of degradation was obviously shortened (Fig. 3). Thus, the 1,4-dioxane degrading bacteria in these samples appeared to be successfully enriched and their 1,4-dioxane degrading ability was strengthened during enrichment. Additionally, white turbidity of the BMM due to bacterial growth was also confirmed in these samples. Conversely, although drainage soil N was capable of degrading 1,4-dioxane to below the detection limit within 15 days in the second enrichment, its ability to degrade 1,4-dioxane was lost after the third enrichment (Fig. 4).

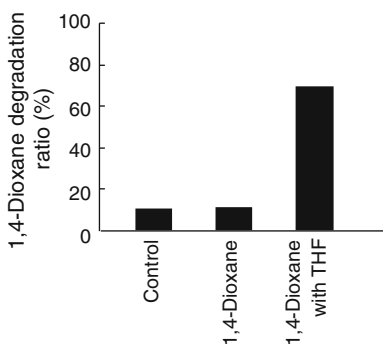


**Fig. 2** Degradation of 1,4-dioxane with/without THF using activated sludge sample C

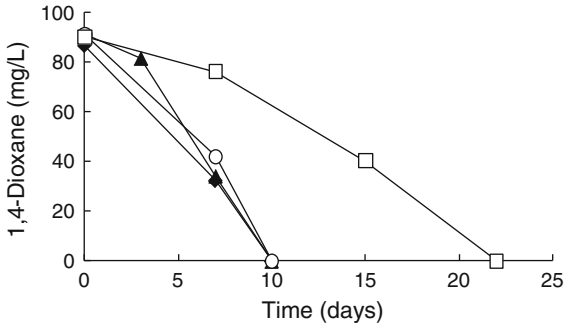


**Fig. 3** Degradation profiles of 1,4-dioxane during repeated enrichment using drainage soil samples (6th cycle). *Filled triangle* soil B, *filled diamond* soil K, *opened circle* soil L, *opened square* soil M

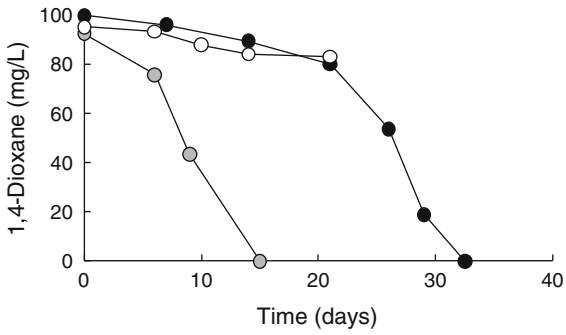


**Fig. 4** Degradation profiles of 1,4-dioxane during repeated enrichment using drainage soil N. *Filled circle* 1st cycle, *shaded circle* 2nd cycle, *opened circle* 3rd cycle

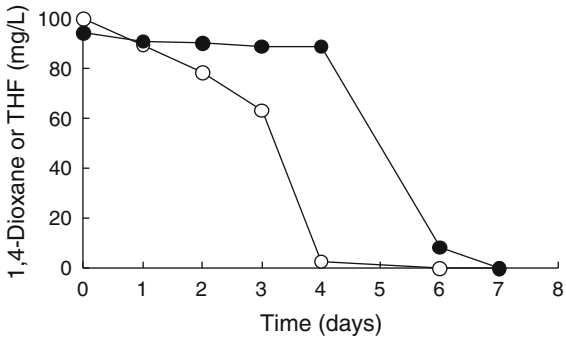


**Fig. 5** Degradation profiles of THF and 1,4-dioxane during repeated enrichment using activated sludge sample C (3rd cycle). *Opened circle* THF, *filled circle* 1,4-dioxane

Evaluation of activated sludge C revealed that both THF and 1,4-dioxane were degraded to below the detection limit within 7 days (Fig. 5) after the third cycle of enrichment. Specifically, THF, which was the primary substrate, was rapidly decreased and degraded to below the detection limit within 4 days, while 1,4-dioxane degradation began after the THF degradation was complete. This is a typical profile of cometabolic degradation with inhibition by the primary substrate. Zenker et al. (2000) reported the competitive inhibition of cometabolic 1,4-dioxane degradation by THF, which was used as a primary substrate, in a soil microcosm. Thus, 1,4-dioxane degradation in the enrichment culture of activated sludge C was considered to occur via the THF degrading enzyme (Fig. 5). It has been suggested that long-term enrichment on media containing THF will eventually lead to production of a mutant THF degrading bacterium with the ability to metabolize 1,4-dioxane (Parales et al. 1994). In the present study, in the range of 30-day enrichment of activated sludge C on 100 mg $l^{-1}$ of THF, we could not confirm the degradation of 1,4-dioxane when it was added to the enrichment culture.

## Biodegradation potential of 1,4-dioxane in the natural environment

Degradation of 1,4-dioxane was confirmed in six of 20 samples collected from various environments, although one sample lost its ability to degrade 1,4-dioxane after repeated enrichment. Four river water and seven garden soil samples from areas that had not been exposed to 1,4-dioxane did not degrade the compound, even by cometabolic degradation with THF (Table 2). All five samples that were capable of degrading 1,4-dioxane to below the detection limit without the addition of THF and enrichment were collected from the drainage area of a chemical factory (drainage soils B, K, L, M and N). Thus, the potential for 1,4-dioxane degradation seems not to be ubiquitously distributed in the natural environment. All previously reported 1,4-dioxane degrading bacterial consortia (Roy et al. 1994) and isolates (Bernhardt and Diekmann 1991; Parales et al. 1994; Kämpfer and Kroppenstedt 2004; Mahendra and Alvarez-Cohen 2006; Kim et al. 2009) have been obtained from sites contaminated with 1,4-dioxane such as sludge from WWTPs used to treat 1,4-dioxane or contaminated soil and sediment. Indeed, there have been no reports of the biodegradation of 1,4-dioxane or isolation of 1,4-dioxane degrading bacteria from uncontaminated

**Table 2** Degradation potential of 1,4-dioxane in various environments

| | River water | Drainage soil | Activated sludge | Garden soil | Total |
|---|---|---|---|---|---|
| Complete degradation | 0/4 | 5/6[a] (0)[b] | 0/3 | 0/7 | 5/20[a] (0)[b] |
| Incomplete degradation | 0/4 | 0/6 | 1/3 (1)[b] | 0/7 | 1/20 (1)[b] |
| No degradation | 4/4 | 1/6 | 2/3 | 7/7 | 14/20 |

[a] One sample lost 1,4-dioxane degradation activity after the third enrichment

[b] The number in parenthesis represents the number of the samples that showed co-metabolic degradation with THF

sites. However, 1,4-dioxane degrading bacteria do not always exist in contaminated environments. Indeed, Taylor et al. (1997) evaluated the metabolic 1,4-dioxane degrading ability of 180 aerobic and 100 anaerobic bacteria isolated from a 1,4-dioxane contaminated well and found that none of the bacteria could degrade 1,4-dioxane.

In the case of cometabolic degradation of 1,4-dioxane with THF (activated sludge C), repeated enrichment strengthened the THF and 1,4-dioxane degradation ability of the sample. Similarly, Zenker et al. (2000) evaluated cometabolic 1,4-dioxane degradation with THF using enrichment culture and found a typical acclimation profile in which the THF and 1,4-dioxane degradation abilities were strengthened by repeating cycles of enrichment. Although THF degrading bacteria eventually exist even in uncontaminated environments, we could not confirm cometabolic 1,4-dioxane degradation with THF in the garden soil samples. Nevertheless, the OECD test revealed that THF is a readily biodegradable compound in aquatic environments, THF is generally classified as relatively non-biodegradable over time (Yao et al. 2009). Thus THF degrading bacteria do not seem to be widely distributed in the natural environment, especially at uncontaminated sites. One possible reason for the uneven distribution of THF degrading bacteria is that the OECD involves application of a high concentration of seed microorganisms to degrade the target chemicals.

In conclusion, the potential for 1,4-dioxane degradation appears not to be ubiquitously distributed in natural environment. It is interesting to note that, although they were sampled from soils subjected to drainage from the same chemical factory, the 1,4-dioxane degradation profiles of drainage soil samples obtained in the present study were different, even after enrichment. These findings indicate that these soil samples contain different types of 1,4-dioxane degrading bacteria. Further studies to isolate and characterize 1,4-dioxane degrading bacteria should be conducted to understand the fate of 1,4-dioxane in detail.

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