Pilot test of biological removal of 1,4-dioxane from a chemical factory wastewater by gel carrier entrapping Afipia sp. strain D1

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**HIGHLIGHTS**

- Two pilot-scale biological 1,4-dioxane (1,4-D) treatment systems were operated.
- Gel cubes entrapping Afipia sp. strain D1 were used for real wastewater treatment.
- The maximum 1,4-dioxane removal rates of 0.72 kg m\textsuperscript{-3} day\textsuperscript{-1} was observed.
- Monod model describes 1,4-D degradation, showing half saturation constant is 28 mg L\textsuperscript{-1}.

**ABSTRACT**

A pilot-scale (120 L) bioreactor system using a gel carrier-entrapped pure bacterial strain, Afipia sp. strain D1, capable of degrading 1,4-dioxane as a sole carbon and energy source was constructed and applied to treat real industrial wastewater containing 1,4-dioxane from a chemical factory. Although the wastewater not only contained high concentrations of 1,4-dioxane but also considerable amounts of other organic compounds (73 mg TOC L\textsuperscript{-1}) on average, the bioreactor could efficiently remove 1,4-dioxane without significant inhibitory effects. The reactor startup could be completed within approximately 1 month by increasing the 1,4-dioxane loading rate (0.09–0.47 kg-dioxane m\textsuperscript{-3} d\textsuperscript{-1}) in a stepwise manner. Effective 1,4-dioxane removal was stably maintained for 3 months with an influent 1,4-dioxane of 570–730 mg L\textsuperscript{-1}, giving an average effluent concentration and removal rate of 3.4 mg L\textsuperscript{-1} and 0.46 kg-dioxane m\textsuperscript{-3} d\textsuperscript{-1}, respectively. A 1,4-dioxane loading fluctuation between 0.14 and 0.72 kg-dioxane m\textsuperscript{-3} d\textsuperscript{-1} did not significantly affect its removal, and more than 99% removal efficiency was constantly maintained. The Monod model could well describe the relationship between the effluent 1,4-dioxane concentration and 1,4-dioxane removal rates of the bioreactors, showing that the half-saturation constant (K\textsubscript{s}) was 28 mg L\textsuperscript{-1}.

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1. Introduction

It is known that 1,4-dioxane is widely used as a solvent or stabilizer in paints, lacquers, and pesticides. In addition, it is contained in detergents and cosmetics as an unwanted byproduct during manufacturing processes. It is discharged into the surface water via wastewater treatment plants (WWTPs) that receive industrial effluents. Thus, 1,4-dioxane has often been found in surface water and groundwater [12]. The U.S. Environment Protection Agency classified 1,4-dioxane as a group B2 probable human carcinogen. Moreover, the International Agency for Research on Cancer lists it as a group 2B carcinogen, possibly carcinogenic to humans. Thus, it is desirable to remove 1,4-dioxane from industrial effluents in WWTPs to reduce the risk of surface water and groundwater contamination. In fact, an effluent 1,4-dioxane standard for WWTPs is applied in Japan, which limits its discharge to 0.5 mg L\(^{-1}\). Because of its high solubility, it is difficult to remove 1,4-dioxane from wastewater by conventional physiochemical processes such as coagulation and activated carbon adsorption [3]. An advanced oxidation process (AOP), which is a combination of technologies including ozone oxidation, ultraviolet irradiation, and hydrogen peroxide, is the only effective method to decompose 1,4-dioxane [4,5] at present. However, AOP requires large amounts of chemicals and energy, entailing high operating costs. Although biological treatment is an effective method to remove organic chemicals from wastewater with low energy consumption, 1,4-dioxane is persistent against biodegradation in general and cannot be effectively removed by existing biological wastewater treatment processes such as the activated sludge process. It had been reported that the biodegradation efficiency of 1,4-dioxane was 0% in a 2-week test based on the Japanese standard test of the law concerning the examination and regulation of manufacturer, indicating its extreme persistency against biodegradation [6]. The biodegradation activity of several pure bacterial cultures such as Mycobacterium sp. and Pseudonocardia sp. [7–9] and fungi [10] has been reported. In particular, bacterial strains capable of utilizing 1,4-dioxane as a sole energy and carbon source, such as the Afipia sp. strain D1 that we have isolated, are very attractive biocatalysts that may be used in developing wastewater treatment technologies [10]. In a previous study [12], the 1,4-dioxane-degrading bacterium Afipia sp. strain D1 was immobilized in gel carriers to construct laboratory-scale bioreactors to be used as 1,4-dioxane treatment systems, and basic data describing the 1,4-dioxane removal performance of the bioreactors were investigated [12]. The experimental results indicated that the efficient removal of 1,4-dioxane in 1-L laboratory-scale bioreactors is possible. The highest removal rate of 0.67 kg-dioxane m\(^{-3}\) d\(^{-1}\) was achieved by treating synthetic wastewater composed of a simple mineral salt medium containing 1,4-dioxane as the sole organic component at an approximate 1,4-dioxane loading rate of 0.6 kg-dioxane m\(^{-3}\) d\(^{-1}\) [12]. However, this technology is at a preliminary stage. Before the industrial application of this novel biological 1,4-dioxane treatment technology, many further questions should be answered; for example, whether this technology can function with real industrial wastewater containing 1,4-dioxane in a complex matrix, whether the reactor can be scaled up without difficulty, and what is the stability of treatment performance over long-term operation and against fluctuations in loading. Thus, 1,4-dioxane removal performance should be further investigated with real industrial wastewater using a reactor on a larger scale. Here, we describe the 1,4-dioxane removal performance of a pilot-scale (120 L) bioreactor system using a gel-entrapped 1,4-dioxane-degrading bacterial strain D1 in the presence of industrial wastewater from a chemical factory. Two replicated pilot-scale bioreactors were started up and operated for 120 days to evaluate the long-term stability and response to influent fluctuation. The kinetics of 1,4-dioxane degradation in the bioreactors were also determined to optimize the design and operation of the treatment system.

2. Material and methods

2.1. Afipia sp. strain D1 and its immobilization

An efficient 1,4-dioxane-degrading bacterium, Afipia sp. strain D1 [11], was used as a biocatalyst to treat 1,4-dioxane-containing wastewater. This strain was isolated from a soil sample which was contaminated with 1,4-dioxane through the conventional enrichment culturing method. Strain D1 is a useful bioremediation catalyst because it can grow on 1,4-dioxane as its sole carbon and energy source, although most of the other reported strains degrade 1,4-dioxane via co-metabolic turnover. Further, the degradation capability of strain D1 is very high compared with that of other degraders. A specific 1,4-dioxane degradation rate of 0.263 mg-1,4-dioxane (mg-protein)\(^{-1}\) h\(^{-1}\) has been reported [11]. Strain D1 was grown in a mineral salts medium containing (per L) K\(_2\)HPO\(_4\), 1000 mg; (NH\(_4\))\(_2\)SO\(_4\), 1000 mg; MgSO\(_4\)·7H\(_2\)O, 200 mg; CaCl\(_2\)·2H\(_2\)O, 50 mg; NaCl, 50 mg; and 1,4-dioxane, 1000 mg [11]. The sole carbon and energy source was 1,4-dioxane. The culture was grown in a 500 mL conical flask filled with 300 mL of medium sealed with a silicon cap and incubated at 28 °C with shaking at 120 rpm for immobilization. The concentration of the cells of Afipia sp. strain D1 in the culture was 4.5 × 10\(^7\) CFU/mL. The strain D1 culture was immobilized in a polyethylene glycol (PEG) gel carrier following the method described by Sumino et al. [13]. In brief, a PEG prepolymer of PEG dimethacrylate (Shin Nakamura Chemicals, Tokyo, Japan) and N,N,N,N-tetramethylenediamine (a promotor) were dissolved in water [13]. The resulting mixture and the culture of strain D1 were mixed in a beaker, followed by the addition of an initiator (potassium persulfate) to induce polymerization. The polymerized carrier gel was cut into 3 mm cubes. The gel carrier contained 10% (w/v) PEG, 0.5% (w/v) promoter, 0.25% (w/v) initiator, and 5% (v/v) culture. The compressive strength of the initial gel cubes was measured by EZ-Test 20N (Shimadzu Corp., Kyoto, Japan), and the gel cubes were broken with a compressive strength higher than 550 kPa/cm\(^2\).

2.2. Characteristics of wastewater from a chemical factory used in this study

Industrial wastewater containing 1,4-dioxane was collected from a chemical factory in Japan every week and used for treatment experiments in pilot-scale bioreactors constructed in our institute (Matsudo Research Center of Hitachi Ltd.). The characteristics of the wastewater are shown in Table 1. The chemical factory synthesizes many chemicals, including 1,4-dioxane. The wastewater used in this study had been pretreated by a conventional activated sludge process at the factory and was collected from a sedimentation tank.

Table 1

<table>
<thead>
<tr>
<th>Characteristic of wastewater from a chemical factory.</th>
<th>Average</th>
<th>Min.</th>
<th>Max. (mg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD(_{Mn})</td>
<td>115 (mg L(^{-1}))</td>
<td>77 (mg L(^{-1}))</td>
<td>205 (mg L(^{-1}))</td>
</tr>
<tr>
<td>BOD</td>
<td>21 (mg L(^{-1}))</td>
<td>8 (mg L(^{-1}))</td>
<td>38 (mg L(^{-1}))</td>
</tr>
<tr>
<td>TOC</td>
<td>210 (mg L(^{-1}))</td>
<td>68 (mg L(^{-1}))</td>
<td>443 (mg L(^{-1}))</td>
</tr>
<tr>
<td>TOC(_{CWWT})</td>
<td>73 (mg L(^{-1}))</td>
<td>39 (mg L(^{-1}))</td>
<td>109 (mg L(^{-1}))</td>
</tr>
<tr>
<td>TOC(_{DOX})</td>
<td>137 (mg L(^{-1}))</td>
<td>1 (mg L(^{-1}))</td>
<td>365 (mg L(^{-1}))</td>
</tr>
<tr>
<td>1,4-Dioxane</td>
<td>252 (mg L(^{-1}))</td>
<td>2 (mg L(^{-1}))</td>
<td>670 (mg L(^{-1}))</td>
</tr>
<tr>
<td>SS</td>
<td>40 (mg L(^{-1}))</td>
<td>17 (mg L(^{-1}))</td>
<td>110 (mg L(^{-1}))</td>
</tr>
<tr>
<td>T-N</td>
<td>139 (mg L(^{-1}))</td>
<td>55 (mg L(^{-1}))</td>
<td>340 (mg L(^{-1}))</td>
</tr>
<tr>
<td>T-P</td>
<td>2 (mg L(^{-1}))</td>
<td>1 (mg L(^{-1}))</td>
<td>5 (mg L(^{-1}))</td>
</tr>
<tr>
<td>Color</td>
<td>255 Unit</td>
<td>84 Unit</td>
<td>654 Unit</td>
</tr>
</tbody>
</table>

\(^{a}\) Calculated value as described in the text.
without sterilization. The data shown in Table 1 are for the wastewater after the pretreatment. The SS concentration of the wastewater was around 90 mg/L during the startup period (from days 0 to 50). According to long-term monitoring, the 1,4-dioxane concentration fluctuated widely between 2 and 670 mg L\(^{-1}\), although the average value was approximately 250 mg L\(^{-1}\). Because 1,4-dioxane is difficult to oxidize with potassium permanganate, the COD\(_{mn}\) value does not include the 1,4-dioxane-derived portion. Considering COD\(_{mn}\) and BOD, the wastewater was thought to contain appreciable amounts of persistent organic compounds other than 1,4-dioxane. The total organic carbon (TOC) consisted of organic carbon derived from real wastewater excluding 1,4-dioxane (TOC\(_{WWT}\)) and 1,4-dioxane (TOC\(_{DOX}\)). TOC\(_{WWT}\) was calculated from the difference between the influent TOC and TOC\(_{DOX}\) (Eq. (1)), whereas TOC\(_{DOX}\) was calculated from the 1,4-dioxane concentration using the molecular weight of 1,4-dioxane (C\(_4\)H\(_8\)O\(_2\)=88.11) and the atomic weight of carbon (C=12.01) (Eq. (2)).

TOC\(_{WWT}\) = TOC − TOC\(_{DOX}\) (1)

TOC\(_{DOX}\) = 1,4-dioxane concentration × (4C\(_4\)H\(_8\)O\(_2\))/12 (2)

The average TOC\(_{WWT}\) was 73 mg L\(^{-1}\). Phosphoric acid was added to the wastewater to adjust the total phosphorus concentration to 5 mg L\(^{-1}\).

2.3. Operational conditions of the pilot-scale bioreactors and 1,4-dioxane treatment experiments

Pilot-scale bioreactors were operated to treat the 1,4-dioxane-containing wastewater from a chemical factory in a continuous feeding mode. Two rectangular reactors (Reactors 1 and 2), containing strain D1 entrapped in gel carrier cubes as a biocatalyst, were constructed (Fig. 1).

The working volume of the reactors was 120 L, and 18 L of the gel carrier cubes were placed inside the reactors, with a packing ratio of 15%. Because the reactor was equipped with a screen on the effluent line, the gel carriers were separated from the effluent during the startup period, the two reactors were operated under nearly identical conditions at HRT of about 40 h. The 1,4-dioxane concentration and COD\(_{mn}\) in the wastewater from the chemical factory were 670 mg L\(^{-1}\) and 205 mg L\(^{-1}\), respectively. Fivefold-diluted wastewater was fed into the reactors in the first stage, during days 0–13. The 1,4-dioxane concentration in the feed wastewater was increased stepwise by changing the dilution ratio of the raw wastewater.

After startup, the HRT of Reactor 1 was set at about 40 h to evaluate the long-term stability of 1,4-dioxane removal performance. Because the 1,4-dioxane concentration in the chemical factory effluent gradually decreased, the influent 1,4-dioxane concentration used in this study was adjusted to approximately 600 mg L\(^{-1}\) by the addition of 1,4-dioxane. In Reactor 2, HRT was set at 29–41 h to increase the 1,4-dioxane loading rate, followed by repeated changes in influent 1,4-dioxane concentration between 180 and 730 mg L\(^{-1}\) to evaluate the effects of fluctuation in the loading rate.

The pH in the reactors was monitored and adjusted to 7.0 by the addition of 1 N NaOH. The water temperature of both reactors was controlled at 25°C. The DO concentration was kept higher than 3.0 mg L\(^{-1}\).

2.4. Analytical methods

Influent and effluent samples were filtered through a 0.45 μm syringe filter immediately after sampling. The 1,4-dioxane concentration of the samples was determined by solid phase extraction–gas chromatography–mass spectrometry [14]. The samples were passed through an AC2-activated carbon cartridge and a PS2 styrene divinyl benzene polymer cartridge (Waters Corp., Billerica, MA, USA) connected in a series, and 1,4-dioxane on the activated carbon cartridge was desorbed with 1 mL of acetone. A 1-μL aliquot of sample was injected into a QP-2010 gas chromatograph equipped with a mass spectrometer (Shimadzu). The mass spectrum was scanned at 58 and 88 m/z (1,4-dioxane) and 64 and 96 m/z (1,4-dioxane-d8). TOC was determined using a TOC-V TOC analyzer (Shimadzu). COD\(_{mn}\), BOD, and SS were measured using the Japanese Industrial Standard (JIS) Methods JIS K 0102 17, JIS K 0102 21, and JIS K 0102 14.1, respectively.

3. Results

3.1. Start up of the pilot-scale bioreactors

Fig. 2 shows the performances of both pilot-scale bioreactors during the startup period (days 0–50). Fig. 2(a) and (c) describe changes in influent and effluent 1,4-dioxane concentrations of Reactors 1 and 2, respectively, whereas Fig. 2(b) and (d) show the 1,4-dioxane loading and removal rates in each bioreactor. Because the bioreactors were operated under the same conditions, nearly identical trends in the change of treatment performance were observed for both bioreactors. Remarkable 1,4-dioxane removal activity was observed shortly after the start of operation, from day 10, and almost complete removal was achieved by day 13 to give effluent 1,4-dioxane concentrations of lower than 5 mg L\(^{-1}\). Thereafter, although the 1,4-dioxane loading rate was increased.
The concentration of 1,4-dioxane fluctuated widely between 2 and 670 mg L$^{-1}$, while TOCWWT was calculated from the organic carbon derived from real wastewater excluding chemical factory wastewater. The data shown in Table 1 are for the wastewater without sterilization. The pH in the reactors was monitored and adjusted to 7.0 by using phosphoric acid. The wastewater was thought to contain appreciable amounts of persistent organic compounds without sterilization.

### 2.4. Analytical methods

The 1,4-dioxane concentration in the feed stage, during days 0–13. The 1,4-dioxane concentration in the feed was around 90 mg/L during the startup period (from days 0 to day 50). The pH in the reactors was monitored and adjusted to 7.0 by using phosphoric acid.

### 3. Results

#### 3.1. Startup of the pilot-scale bioreactors

The pH in the reactors was monitored and adjusted to 7.0 by using phosphoric acid. Phosphoric acid was added to evaluate the effects of fluctuation in the loading rate. Because the reactor was equipped with a screen on the effluent line, the gel carriers were separated from the effluent during the startup period. The two reactors were operated to contain strain D1 entrapped in gel carrier cubes as a biocatalyst, which appears to be an aggregate of strain D1, can be seen in the gel carrier, suggesting its significant growth. Similarly, an increase in the cell concentration of strain D1 in the gel carrier with increasing 1,4-dioxane removal performance has been reported in a lab-scale bioreactor [12]. A color change of the gel carriers to dark brown was also observed, probably because of the accumulation of colored components of the wastewater, but this showed no adverse effects on 1,4-dioxane treatment performance or operation. Although the real wastewater contained approximately 90 mg/L of SS derived from activated sludge used for pretreatment, indicating that a considerable amount of various bacteria had been fed into the bioreactors, the treatment performance was not adversely affected. Here, because the SS in the bioreactors was 50–75 mg/L, the activated sludge-derived bacteria seem to have grown only minimally or even decayed in the bioreactors, probably because of the lack of easily degradable organic compounds. Thus, these bacteria did not contribute to the removal of 1,4-dioxane, and only the contribution of strain D1 seems to be reflected in the treatment performance.

#### 3.2. Long-term stability of 1,4-dioxane treatment performance

After the startup period, Reactor 1 was set at HRT of about 40 h, and wastewater containing 570–730 mg L$^{-1}$ of 1,4-dioxane was fed continuously. The pH in the reactors was monitored and adjusted to 7.0 by using phosphoric acid. The pH in the reactors was monitored and adjusted to 7.0 by using phosphoric acid. Phosphoric acid was added to evaluate the effects of fluctuation in the loading rate. Because the reactor was equipped with a screen on the effluent line, the gel carriers were separated from the effluent during the startup period. The two reactors were operated to contain strain D1 entrapped in gel carrier cubes as a biocatalyst, which appears to be an aggregate of strain D1, can be seen in the gel carrier, suggesting its significant growth. Similarly, an increase in the cell concentration of strain D1 in the gel carrier with increasing 1,4-dioxane removal performance has been reported in a lab-scale bioreactor [12]. A color change of the gel carriers to dark brown was also observed, probably because of the accumulation of colored components of the wastewater, but this showed no adverse effects on 1,4-dioxane treatment performance or operation.

### Fig. 2

Fig. 2 shows the performances of both pilot-scale bioreactors stepwise by reducing the dilution ratio of the wastewater, effluent 1,4-dioxane concentration was always maintained at lower than 5 mg L$^{-1}$. The volumetric removal rate of the reactors reached 0.47 kg-m$^{-3}$d$^{-1}$ on day 38. These results indicate that the startup of the bioreactors could be completed within approximately 1 month. Moreover, the reproducibility of the startup was clearly demonstrated because the two reactors showed almost the same treatment performance. A micrograph of the gel carriers just after the startup period (day 51) is shown in Fig. 3. A small white cluster, which appears to be an aggregate of strain D1, can be seen in the gel carrier, suggesting its significant growth. Similar to the cell concentration of strain D1 in the gel carrier, with increasing 1,4-dioxane removal performance has been reported in a lab-scale bioreactor [12]. A color change of the gel carriers to dark brown was also observed, probably because of the accumulation of colored components of the wastewater, but this showed no adverse effects on 1,4-dioxane treatment performance or operation. Although the real wastewater contained approximately 90 mg/L of SS derived from activated sludge used for pretreatment, indicating that a considerable amount of various bacteria had been fed into the bioreactors, the treatment performance was not adversely affected. Here, because the SS in the bioreactors was 50–75 mg/L, the activated sludge-derived bacteria seem to have grown only minimally or even decayed in the bioreactors, probably because of the lack of easily degradable organic compounds. Thus, these bacteria did not contribute to the removal of 1,4-dioxane, and only the contribution of strain D1 seems to be reflected in the treatment performance.

### Fig. 3

Fig. 3. Micrograph of gel carrier in Reactor 1 after start up. Small white clusters of immobilized *Afipia* sp. D1 can be seen in the gel carriers.
The stable removal of 1,4-dioxane was observed for approximately 3 months at a relatively stable 1,4-dioxane loading rate. The removal of more than 99% of 1,4-dioxane was consistently maintained after startup, and the average influent and effluent 1,4-dioxane concentrations were 620 and 3.4 mg L\(^{-1}\), respectively (Fig. 4(a)). The average 1,4-dioxane removal rate from days 40 to 122 was 0.46 kg-dioxane m\(^{-3}\) d\(^{-1}\), and a maximum value of 0.53 kg-dioxane m\(^{-3}\) d\(^{-1}\) was observed on day 57 (Fig. 4(b)).

During the stable operation of Reactor 1, the TOC in the influent and effluent was monitored to determine whether the accumulation of the intermediates of 1,4-dioxane degradation occurred in the bioreactor (Fig. 5). The TOC in the influent wastewater was approximately 380–470 mg L\(^{-1}\), with an average value of 420 mg L\(^{-1}\). Considering the TOC derived from 1,4-dioxane (TOC\(_{\text{Diox}}\)) of 340 mg L\(^{-1}\) on average, the influent is expected to have contained approximately 80 mg-TOC L\(^{-1}\) of organic compounds other than 1,4-dioxane (TOC\(_{\text{WWT}}\)). Because the TOC in the effluent was between 44 and 86 mg L\(^{-1}\) (69 mg L\(^{-1}\) on average), the TOC removal efficiency was 84% on average. As shown in Fig. 5, the effluent TOC was almost the same as the TOC\(_{\text{WWT}}\). This result suggests that 1,4-dioxane was decomposed or mineralized in the bioreactor and that the accumulation of metabolites was nearly negligible, whereas the TOC\(_{\text{WWT}}\) in the wastewater seemed to be degraded to an extremely low extent and remained in the main effluent.

3.3. Effects of loading fluctuation on 1,4-dioxane treatment performance (Reactor 2)

The 1,4-dioxane loading into Reactor 2 was varied by changing the HRT and influent concentration after startup, and the effects on 1,4-dioxane removal performance were evaluated (Fig. 6). Although the 1,4-dioxane loading rate was increased by decreas-
ing the HRT from 38 to 28 h on day 58, no significant effect on the removal of 1,4-dioxane was observed. More than 99% removal and an average removal rate of 0.63 kg-dioxane m$^{-3}$d$^{-1}$ with a maximum value of 0.72 kg-dioxane m$^{-3}$d$^{-1}$ were stably maintained during days 59–76. The average effluent 1,4-dioxane concentration was 5.3 mg L$^{-1}$, even during high loading test. Then, the influent 1,4-dioxane concentration was suddenly decreased from 670 to 180 mg L$^{-1}$ on day 77 and increased from 200 to approximately 600 mg L$^{-1}$ on day 95 at HRT of 38 h. Additional drastic changes in the influent 1,4-dioxane concentration were repeated. Despite these abrupt loading fluctuations, removal of more than 99% of the 1,4-dioxane was maintained consistently, and the effluent concentration was maintained mostly below 6.0 mg L$^{-1}$, indicating the robust treatment performance of the reactor.

### 3.4. Kinetics of 1,4-dioxane removal

Fig. 7 shows the relationship between the 1,4-dioxane removal rate and the effluent 1,4-dioxane concentration obtained in the loading fluctuation tests of Reactor 2. An approximately proportional relationship was observed for these values, suggesting that 1,4-dioxane degradation in the reactor roughly obeyed the first-order kinetics. First-order and second-order substrate removal models are popular and basic models to determine kinetic constants, for example for nitrogen removal process [15,16]. Since first-order model well described the relationship between effluent 1,4-dioxane concentration and 1,4-dioxane removal rate rather than second-order model (data not shown), the curve fit to the first-order model was shown in the Fig. 7. The first-order kinetic constant ($k_1$) was calculated as $1.0 \times 10^2$ d$^{-1}$, and the coefficient of determination ($R^2$) was 0.945. Monod model is one of the best models to describe the kinetics of biological wastewater treatment processes [16,17]. A Monod model also well described the kinetics, and the saturation concentration ($K_S$) and maximum 1,4-dioxane removal rate ($R_{max}$) were calculated as 28 mg L$^{-1}$ and 3.5 kg-dioxane m$^{-3}$d$^{-1}$, respectively. $R^2$ for the Monod model was 0.958, indicating that the Monod model was suitable for describing the kinetics of 1,4-dioxane removal performance.

### 4. Discussion

The potential of some bacterial strains to remove 1,4-dioxane from real wastewater has previously been reported [18,19]. Han et al. [19] reported the biological 1,4-dioxane removal performance in wastewater from the polyester manufacturing process. A laboratory-scale bioreactor filled with tire chips and acclimated activated sludge was used in continuous feeding tests, resulting in a 1,4-dioxane removal efficiency of 71.4%. This report suggested that 1,4-dioxane could be biologically degraded by efficient 1,4-dioxane-degrading microbes in the bioreactor. Therefore, we have used a pure culture of *Afipia* sp. strain D1, which is capable of using 1,4-dioxane as its sole carbon source and has a very high degradation capability.

In our previous study, a biological wastewater treatment system using a pure culture of D1 entrapped in a gel carrier was proposed as a novel, cost-effective, and energy-saving alternative to AOP, which is the only existing technology for the treatment of wastewater containing 1,4-dioxane [12]. The high potential of our system for efficient 1,4-dioxane removal was shown using a 1-L lab-scale bioreactor and synthetic wastewater with a simple composition. For the future industrial application of this system, we investigated the applicability of this system to real 1,4-dioxane-containing wastewater from a chemical factory using 120-L pilot-scale reactors in the present study.

Although the wastewater treated not only contained a high concentration of 1,4-dioxane but also considerable amounts of other organic compounds, 73 mg L$^{-1}$ of the TOCWWT on average, the removal of more than 99% of 1,4-dioxane was consistently and reproducibly achieved after the startup period. It appeared that the removed 1,4-dioxane was degraded without an appreciable accumulation of metabolites. These results confirmed that the 1,4-dioxane degradation by strain D1 was effectively performed without any pretreatment, even in the presence of other organic compounds. Sei et al. [11] reported that 1,4-dioxane degradation by strain D1 was not diminished even in the presence of easily biodegradable ethylene glycol at up to 3000 mg L$^{-1}$, in accordance with the results of this study. These facts suggest that 1,4-dioxane degradation of strain D1 is catalyzed by enzyme(s) having high substrate specificity so that negligible competitive inhibition in the presence of other organic compounds occurred. This feature constitutes a great advantage of this novel biological treatment system for 1,4-dioxane over AOPs because 1,4-dioxane degradation by AOPs, a nonspecific reaction, is expected to be severely inhibited by the co-presence of other organic compounds in the wastewater, leading to more expensive treatment costs. However, it should be noted that significant microbial contamination can occur in the bioreactors when easily biodegradable organics are abundant in the wastewater along with 1,4-dioxane, possibly leading to the inhibition of 1,4-dioxane degradation by strain D1 through competition for nutrients and oxygen. Also, their attachment onto the gel carriers as a thick biofilm can significantly reduce the reactor performance.

The long-term stability and robustness during loading fluctuation were investigated in two pilot-scale bioreactors, namely Reactors 1 and 2, respectively. Because the wastewater treatment system should be long-lasting, the influent can fluctuate qualitatively and/or quantitatively according to the operating conditions of the chemical factory. Stable 1,4-dioxane removal performance was observed for approximately 3 months after the startup of Reactor 1. The removal of more than 99% of 1,4-dioxane was consistently obtained, and the effluent 1,4-dioxane level was kept lower than 4.5 mg L$^{-1}$ against an average influent of 620 mg L$^{-1}$. In addition, although the influent 1,4-dioxane concentration was drastically varied over 180–730 mg L$^{-1}$ (0.14–0.72 kg-dioxane m$^{-3}$d$^{-1}$) in Reactor 2, an effective treatment performance with the removal of more than 99% of 1,4-dioxane and an effluent concentration of less than 6 mg L$^{-1}$ was maintained during the loading fluctuation test. The 1,4-dioxane removal rate obtained in this study was relatively higher than previously-reported chemical biodegradation processes, for example, phenol degradation rate of 0.2 kg m$^{-3}$d$^{-1}$.
The startup period is one of the important factors in applying a biological process to a large-scale plant. The instability of biological treatment performance is another problem sometimes observed during the startup operation of wastewater plants. Although only 5% of the culture of strain D1 was immobilized in the gel carrier at the beginning in this study, the startup of the bioreactors could be completed within approximately 1 month by feeding real wastewater with a stepwise increase in loading rate and without an external carbon source. The 1-month startup period of the pilot-scale bioreactors is nearly equal to that obtained for the laboratory-scale reactor used in our previous study and is considered to be acceptable in practical industrial applications [12]. Reactors 1 and 2, started up with the same operational conditions and showed similar behavior and reproducible results, indicating that steady startup can be performed by a prescribed scheme. The short-term and reproducible startup of the bioreactors may reflect the efficient growth of strain D1 in the gel carrier using 1,4-dioxane in real wastewater, even during the startup period. This period can be regarded as the adaptation period of the bacteria to a new environment and tends to cause adverse effects on bacterial growth. The successful startup of the pilot-scale bioreactors indicates that the startup may not be a hurdle for applying this biological 1,4-dioxane treatment system, even at full scale.

The lifetime of the gel cubes is also an important factor for applying this gel entrapment technology to full-scale wastewater treatments. We estimated the lifetime of the gel cubes to be more than 10 years, which is nearly equal to those of other reactor equipment such as pumps and motors. Actually, this immobilization technology has been practically applied to the immobilization of nitrifying sludge, and more than 40 full-scale wastewater treatment plants have been installed in Japan. The first full-scale plant using nitrifying gel cubes was established at the Munakata sewage treatment plant (Fukuoka, Japan) in 1994, and a stable nitrification performance had been observed without requiring the supplementation of additional gel cubes for 15 years, indicating that the lifetime of the gel cubes is long enough for practical application. In fact, no broken gel carriers were observed during this experiment.

Our kinetic study of 1,4-dioxane degradation in the bioreactors indicated that it is well-described by the Monod model, which is one of the most popular models applicable to bioreactors and wastewater treatment systems [15,22]. The $K_v$ and the $V_{max}$ were estimated at 28 mg L$^{-1}$ and 3.5 kg-dioxane m$^{-3}$ d$^{-1}$, respectively. Sei et al. [11] previously reported from flask studies that the $K_v$ of 1,4-dioxane degradation by strain D1 was 25.8 mg L$^{-1}$, which is almost the same as the $K_v$ obtained in this study. The $K_v$ estimated in this study is relatively high compared with that estimated for the biodegradation of other chemicals [23,24], indicating the relatively low affinity of the catalytic enzyme(s) of strain D1 for 1,4-dioxane.

The pathway of the 1,4-dioxane biodegradation by $Afipia$ sp. strain D1 remains unclear. Vainberg et al. [25] reported that the biodegradation of 1,4-dioxane by $Pseudomonas$ sp. strain ENV478 results in the accumulation of 2-hydroxyxethylic acid. Sales et al. [26] recently reported the genome sequence of $P$. dioxanivorans CB1190, and they further showed that glyoxylate metabolism is a key feature of metabolic 1,4-dioxane degradation by this strain based on gene expression analysis using DNA microarray and quantitative reverse transcription-PCR [27]. They found that a monoxygenase gene cluster located on plasmid pSEDO2 is responsible for initial 1,4-dioxane oxidation, and that genes encoding putative aldehyde dehydrogenases, an aldehyde reductase, and an alcohol oxidoreductase, all of which were located on pSEDO2, engaged in 1,4-dioxane metabolism together with the glyoxylate carboxylase gene located on the chromosome. Because, the accumulation level of intermediates by $Afipia$ sp. strain D1 was very low in the synthetic wastewater treatment tests [12], suggesting that strain D1 can achieve complete mineralization of 1,4-dioxane without the significant accumulation of intermediates. In the present study, an almost complete removal of 1,4-dioxane-derived TOC (TOC$_{DOX}$) was clearly observed even in real industrial wastewater. This may be an eminent characteristic of strain D1 as an agent for 1,4-dioxane degradation.

On the other hand, strain D1 seems ineffective for treating water with lower 1,4-dioxane concentrations. In fact, although the removal of more than 99% of 1,4-dioxane was obtained in this study against high influent 1,4-dioxane concentrations, 1–6 mg L$^{-1}$ of 1,4-dioxane remained in the effluent, and complete removal below the detection limit could not be achieved. Therefore, post-treatment is still required to meet the effluent standard, which is 0.5 mg L$^{-1}$ in Japan. AOP or activated carbon processes are effective methods of 1,4-dioxane post-treatment processing to meet this effluent standard. Even in this case, because 99% of 1,4-dioxane was treated in the present study, the operation costs for a post-treatment system could be minimal.

5. Conclusions

In this study, the novel biological wastewater treatment technology using a pure culture of 1,4-dioxane-degrading bacteria entrapped in a gel carrier was applied to real industrial wastewater in 120-L pilot-scale bioreactors to evaluate its practicability. The startup of the reactors was completed within an acceptable short period, i.e., 1 month, and the long-term (3 months) stability and robustness as well as the loading fluctuation of the culture were confirmed. These experimental results strongly suggest that our biological 1,4-dioxane treatment system can be easily applied to real wastewater at full-scale, as an alternative cost-effective and energy-saving system to AOP, which is the only existing technology for the treatment of wastewater containing 1,4-dioxane. Further, gel-entrapped specific catabolic microbes may be applicable not only for 1,4-dioxane removal but also for the treatment of other recalcitrant or hard-to-biodegrade hazardous chemicals.
1,4-dioxane as a sole carbon and energy source, Biodegradation (2013) 665–674.


