Original Article

Kinetic Models of Dichloroethylene Biodegradation by Two Strains of Aerobic Bacteria*

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Abstract

Objective In this study, we examined the biodegradation of Dichloroethylene (DCE) by two strains of aerobic bacteria.

Methods Using batch experiments, we measured the biodegradation rates of DCE and the residual concentrations of DCE for each bacterial strain. The varying trends in biodegradation rates with different initial concentrations of DCE were fitted to kinetic models.

Results The biodegradation kinetics of DCE by the strain DT-X, which uses toluene as co-metabolic substrate, fitted the Monod model (corresponding parameters: v_{max}=0.0075 h^{-1}, K_s＝2.12 mg/L). The biodegradation kinetics of DCE by the strain DT-M, which uses 1,1-Dichloroethylene as single substrate, fitted the Haldane model (parameters: v_{max} =0.0046 h^{-1}, K_s=4.25 mg/L, K_i=8.47 mg/L).

Conclusion The substrate removal rate constant of 1,1-Dichloroethylene of the co-metabolic strain DT-X was much higher than that of strain DT-M. The substrate removal rates obtained from both bacterial strains in this study were higher than those reported in similar studies.

Key words: Aerobic strain; DCE; Biodegradation; Kinetics

Biomed Environ Sci, 2011; 24(3): 261-267 doi: 10.3967/0895-3988.2011.03.009 ISSN: 0895-3988

www.besjournal.com(full text) CN: 11-2816/Q

INTRODUCTION

Chlorinated alkenes are a series of persistent organic compounds that are toxic or harmful to humans. They are widely used in dry cleaning, food production, etc., and are common environmental pollutants. These chemicals are considered as hazardous, and have mutagenic, carcinogenic, and teratogenic properties[1-8]. Chlorinated alkenes are volatile and fat-soluble, and so are easily absorbed through mucous membranes and skin. These compounds are particularly harmful to some organs such as the heart, blood vessels, the liver, and the kidney[9-16].

Dichloroethylene (DCE) is one of the products of partial dechlorination of perchloroethylene and trichloroethylene, but its toxicity is stronger than its matrix, and it is highly carcinogenic. For these reasons, it is listed as a top-priority pollutant for control by the Environmental Protection Agency of the United States[17-19]. In recent years, there have been some reports on biodegradation of DCEs[20-27], several of them were tested by isolated bacterial strains[28-30]. However, few reports have focused on the aerobic degradation of DCEs and uses DCE as the sole carbon source[31].

*This work was supported by the National Science Foundation of China (Grant No. 40102027; 50578151), the Beijing Natural Science Foundation (Grant No. 8052017; 8092025; 8102030), and the National S&T Major Project of China (2009ZX07207-008-4-02, 2009ZX07419-002-4, 2008ZX07007-008-4-2, 2011YXL016).

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Received: April 27, 2010; Accepted: January 26, 2011

The ultimate goal of studies on biodegradation of chlorinated hydrocarbons is to completely eliminate the toxicity of these hazardous compounds. In this paper, we studied the biodegradation kinetic models and rates of 1,1-Dichloroethylene by two bacterial strains.

MATERIALS AND METHODS

Experimental Strains

The experimental strains were screened from activated sludge acclimated by 1,1-Dichloroethylene. One strain, designated as DT-M, uses 1,1-Dichloroethylene as single substrate. The other, designated as DT-X, uses toluene as a co-metabolic substrate.

Activation of Strains

For both strains, the bacteria were cultured in liquid mineral medium in cultivation bottles. The medium contained liquid 1,1-Dichloroethylene at a final concentration of 300 μg/L. For DT-X, the liquid medium also contained the toluene as co-metabolic substrate, and its concentration was 42.4 mg/L. The cultures were grown in a water bath at constant temperature (30 °C) with shaking (150 rpm) and growth was monitored by measuring absorbance at 600 nm. The composition of the mineral medium is shown in Table 1.

Table 1. Composition of Liquid Mineral Medium (g/L)

Ingredient	Amount	Ingredient	Amount	Ingredient	Amount
$C_6H_{12}O_6$	1	$MgSO_4 \cdot 7H_2O$	0.059	Na_2CO_3	0.1
$CaCl_2$	0.0039	$FeSO_4$	0.0003	KH_2PO_4	0.338
$(NH_4)_2SO_4$	0.0593	$Na_2HPO_4 \cdot 12H_2O$	0.8907	-	-

Measurements of Bacterial Growth

To monitor bacterial growth, the absorbance of the culture suspension at 600 nm was measured using a spectrophotometer (Model 722; Shanghai, China). To ensure vigorous growth, additional substrate and/or co-metabolic substrate was added when the bacterial growth slowed.

Experimental Procedures

The cultivation bottles (125 mL volume) were filled with culture suspension and liquid mineral medium. The amount of culture suspension and medium were adjusted so that the final absorbance of the mixture at 600 nm was approximately 0.2. For the co-metabolic strain, the co-metabolic substrate toluene was also added. 1,1-Dichloroethylene was added to the cultivation bottles to achieve the initial concentrations shown in Figure 3 and Figure 8. The bottles were plugged with a Teflon membrane, and the mixtures were cultured in a water bath at a constant temperature of 30 °C[32], with shaking (150 rpm). To monitor the decline in the amount of 1,1-Dichloroethylene, the initial concentrations of 1,1-Dichloroethylene were higher than those found in the environment, and had a broader range.

The bacterial cultures were sampled at regular intervals with an air-tight injector. For sampling, the shaking was stopped and the bottles were kept in a stationary position for 2 min before samples were removed. Samples were filtered through ϕ0.45 μm Teflon filtration membranes into 4 mL headspace vials, and then these vials were shaken for 30 min for extraction. The upper liquid was transferred to 1.5 mL headspace vials, while the vials were immediately sealed, and then kept at 4 °C until measurement.

Determination of 1,1-Dichloroethylene

The concentration of 1,1-Dichloroethylene was determined using an HP6890 Gas Chromatograph (GC) with an HP-7684 auto-sampler. The temperature of injector and detector were maintained at 130 °C and 250 °C, respectively. The initial temperature in the GC oven was 35 °C, and maintained for 5min. The flow rate of the chromatograph column was 4.5 mL/min.

Models

Monod equation The Monod equation applies to conditions in which substrates have no self-restraint, toxicity, or harm. The equation is as follows[33]:

$$v = \frac{v_{\max} S}{K_s + S} \tag{1}$$

Transformed as follows:

$$\frac{1}{v}=\frac{1}{v_{\max}}+\frac{K_s}{v_{\max}}\cdot\frac{1}{S} \quad (2)$$

Where v is the specific substrate removal velocity, and v_{max} is the maximal specific substrate removal velocity, S is the substrate concentration, and K_s is the saturation constant.

Equation (2) is a linear equation. According to the slope and intercept, the kinetic parameters can be obtained as follows: slope=K_s/v, and intercept=$1/v_{max}$.

Haldane inhibitory kinetic equation High concentrations of substrate can inhibit cell growth, and therefore, estimation of substrate inhibition is an important aspect for biodegradation of toxic compounds. There are several models of cell growth kinetics that have been developed to predict the inhibitory effects of toxic substrates, most of which have been derived from enzymatic reaction kinetics. The Haldane equation[34], shown below, is a model of substrate inhibition of cell growth kinetics that is in common use.

$$\upsilon=\frac{\upsilon_{\max}}{1+\frac{Ks}{S}+\frac{S}{Ki}} \quad (3)$$

Where K_i is the inhibition factor.

RESULTS

Kinetic Model of 1,1-Dichloroethylene Degradation by Strain DT-X

Strain activation The growth of strain DT-X, which used toluene as co-metabolic substrate, is shown in Figure 1. This strain grew relatively fast, and the biomass required for experiments was obtained after 2 weeks of culturing.

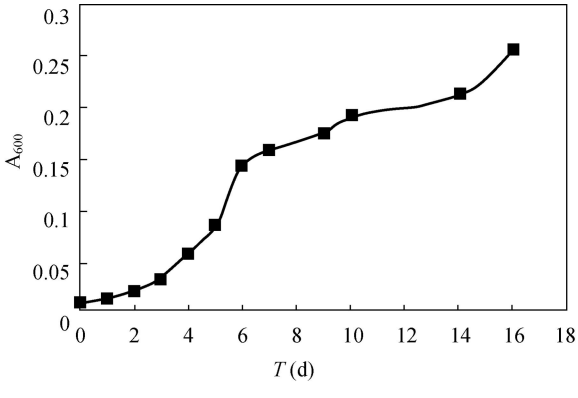

Figure 1. Growth curve of DT-X.

Scanning electron microscopy analysis of DT-X The morphology of DT-X is shown in Figure 2. DT-X is a *Bacillus* and its size similar to an insect egg. The length of the cell is 1.78-1.92 μm, and the diameter of the cell is approximately 0.35-0.5 μm.

Figure 2. Electron micrograph of strain DT-X.

Kinetic analysis of 1,1-Dichloroethylene degradation by strain DT-X Figure 3 shows the curve of 1,1-Dichloroethylene degradation by strain DT-X. The concentration of the co-metabolic substrate toluene was 42.4 mg/L. The maximum degradation rate was observed in the first 5 h after addition of 1,1-Dichloroethylene. The degradation rates slowed to an almost undetectable rates after addition of 1,1-Dichloroethylene for approximately 10 h, so the concentration of 1,1-Dichloroethylene barely changed from 10 to 24 h. The maximum rate of degradation was observed when the original concentration of 1,1-Dichloroethylene was 3.09 mg/L, and more than 60% of the 1,1-Dichloroethylene was degraded. At other initial concentrations of 1,1-Dichloroethylene, the amount of DCE degraded ranged from 40% to 60%.

In the first 30 min after addition of 1,1-Dichloroethylene, the growth of bacteria was too small to be significant. Thus, we can calculate the specific degradation velocity of 1,1-Dichloroethylene by strain DT-X (v) and then obtain the v-S curve. The kinetic curve of 1,1-Dichloroethylene degradation by strain DT-X is shown in Figure 4.

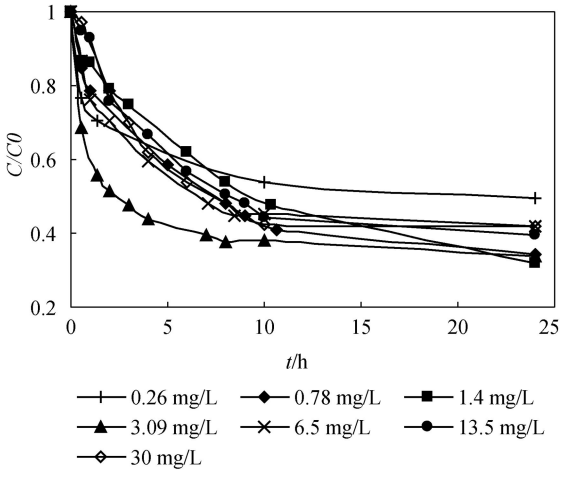

Figure 3. Biodegradation of 1,1-Dichloroethylene by DT-X.

As shown in Figure 4, the specific degradation velocity increased with increasing initial concentrations of 1,1-Dichloroethylene. When initial concentration of 1,1-Dichloroethylene reached a certain point, the specific degradation velocity reached its maximum (approximately 0.044 d^{-1}). After that, as the increasing of initial concentration of 1,1-Dichloroethylene, the specific degradation velocity remained the same; that is, it could not increase further. Thus, the degradation of 1,1-Dichloroethylene by DT-X was consistent with the Monod equation.

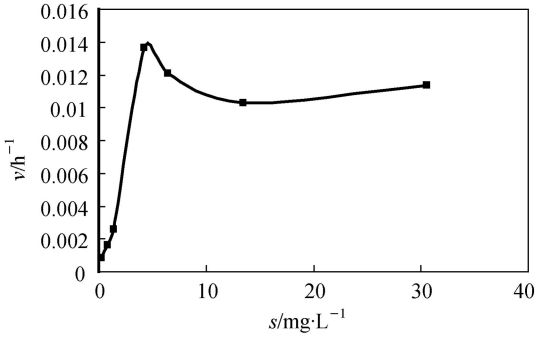

Figure 4. Kinetic curve of 1,1-Dichloroethylene biodegradation by Strain DT-X.

By plotting 1/*v* against 1/*S*, a linear curve can be obtained, as shown in Figure 5. The correlation coefficient of the linear was 0.9481.

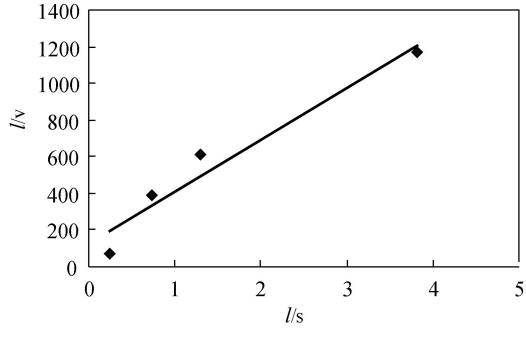

Figure 5. Relationship between 1/*v* and 1/*S*.

From this linear curve, we can obtain the slope K_s/v and the intercept $1/v_{max}$. Then, these values can be used to calculate the final parameters: v_{max}= 0.0075/h, K_s=2.12 mg/L. Thus, the kinetic equation of 1,1-Dichloroethylene degradation by strain DT-X is as follows: ·

$$v = \frac{0.0075 \cdot S}{2.12 + S}$$

Kinetic Model of 1,1-Dichloroethylene Degradation by Strain DT-M

Strain activation Figure 6 shows the growth of strain DT-M, which uses 1,1-Dichloroethylene as the single substrate. Strain DT-M grew relatively slowly, and it took 1 mon to obtain sufficient biomass for experiments.

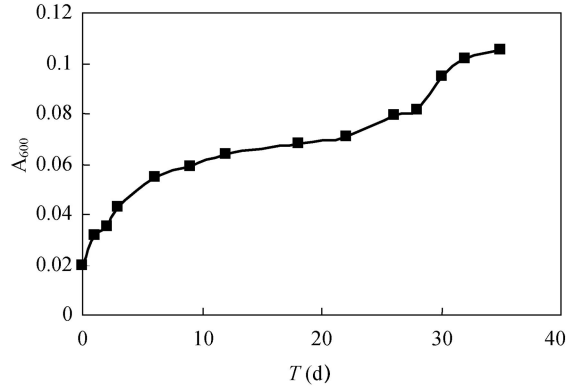

Figure 6. Growth curve of DT-M.

Scanning electron microscopy analysis of DT-M The micrograph in Figure 7 shows the morphology of strain DT-M. The cells have a column shape with a smooth surface. The cell length is approximately 2.85-3.07 μm and the diameter is approximately 0.93-1.07 μm.

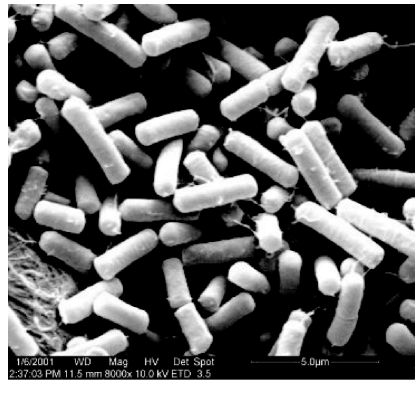

Figure 7. Electron micrograph of strain DT-M.

Kinetic analysis of 1,1-Dichloroethylene degradation by strain DT-M Figure 8 shows the curve of 1,1-Dichloroethylene degradation by strain DT-M. The degradation rate slowed at 6-7 h after addition of 1,1-Dichloroethylene, and thereafter the concentration of 1,1-Dichloroethylene barely changed. The maximum degradation was observed with an initial concentration of 5 mg/L 1,1-Dichloroethylene, and more than 90% was degraded. Increasing initial concentrations of 1,1-Dichloroethylene were associated with decreasing degradation ratios. At an initial 1,1-Dichloroethylene concentration of 32 mg/L, the degradation ratio decreased to less than 50%. This indicates that increasing initial 1,1-Dichloroethylene

concentrations was harmful to DT-M, and thus, the metabolism of strain DT-M decreased to some extent.

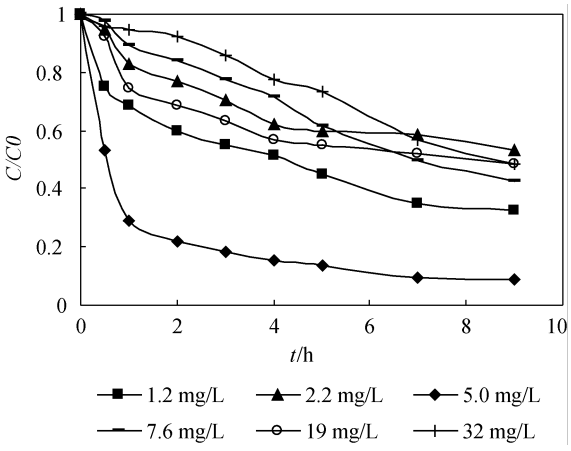

Figure 8. Degradation of 1,1-Dichloroethylene by strain DT-M.

The specific degradation velocity (v) of 1,1-Dichloroethylene by strain DT-M was calculated and used to obtain v-S. Thus, the kinetic curve of 1,1-Dichloroethylene degradation by strain DT-M is shown in Figure 9.

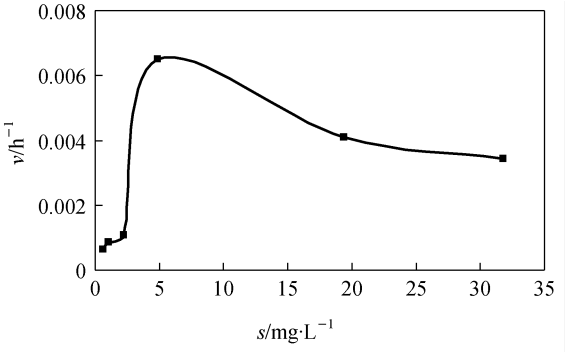

Figure 9. Kinetic curve of 1,1-Dichloroethylene biodegradation by strain DT-M.

As shown in Figure 9, at low initial concentration of 1,1-Dichloroethylene (0-6 mg/L), the specific degradation velocity increased with increasing concentrations. When the initial 1,1-Dichloroethylene concentration reached a certain point, the specific degradation velocity reached its maximum. However, if the original 1,1-Dichloroethylene concentration increased further, the specific degradation velocity decreased. Thus, the biodegradation of 1,1-Dichloroethylene by DT-M was consistent with the Haldane equation.

For the Haldane equation, the calculation of important parameters such as v_{max} and K_s can be obtained from the Monod equation. The inhibitory factor K_i can be obtained as shown below. When substrate inhibition exists, the maximum degradation rate (v_{max}) occurs at the optimal concentration $[S]_{opt}$. Thus, the Haldane model is a differential equation, with its maximum at the point ($[S]_{opt}$, v_{max}), that is:

$$\left.\frac{dv_{max}}{d[S]}\right|_{[S]_{opt}} = 0$$

$$\text{or} \quad [S]_{opt} = \sqrt{K_s \cdot K_i}$$

These equations were used to calculate the parameter K_i. The final parameters were as follows: v_{max} =0.0046/h, K_s =4.25 mg/L, and K_i=8.47 mg/L. Thus, the inhibitory kinetic equation of 1,1-Dichloroethylene degradation by DT-M is as follows:

$$v = \frac{0.0046}{1 + \frac{4.25}{S} + \frac{S}{8.47}}$$

DISCUSSION

In recent years, there have been considerable achievements in research on the biodegradation of DCEs. Kassenga and coworkers[22] studied removal of chlorinated volatile organic compounds from upflow wetland mesocosms, and obtained first-order removal rate constants for *cis*-1,2-DCE of 0.84±0.36d^{-1} in a mixture of sand, peat, and Bion Soil, and 0.37±0.13 d^{-1} in a mixture of sand and peat. Olaniran and coworkers[20] reported the aerobic biodegradation of DCEs by an indigenous mixed culture inoculum in soil and water collected from contaminated sites in South Africa. In addition, they studied the effects of biostimulation and bioaugmentation on the biodegradation of pollutants in soil and water microcosms under aerobic conditions. With biostimulation and bioaugmentation, the degradation rate constants ranged significantly between 0.0152 and 0.0911 d^{-1} for *cis*-DCE, and between 0.0307 and 0.0676 d^{-1} for *trans*-DCE, respectively. They further studied DCE degradation under aerobic conditions by seven bacteria indigenous to contaminated sites in Africa[29], and obtained degradation rate constants ranging between 0.167 and 0.198 d^{-1} for *cis*-DCE and *trans*-DCE.

In the present study, the biodegradation kinetics of strain DT-X were consistent with the Monod equation. When the initial concentrations of

1,1-Dichloroethylene were low, the Monod equation is represented as follows: $v=(v_{max}/K_s)$. That is, it follows first-order kinetics. It is important to note that here, the parameter v is the specific substrate removal rate, which is obtained by dividing the normal substrate removal rate by the numerical value of biomass (X). Thus, the substrate removal rate constant (K) is equal to $v_{max}\cdot X/K_s$. Here, v_{max} was 0.0075 h^{-1}, K_s was 2.12 mg/L, and the biomass (suspended solids) was 142.5 mg/L. Using these values, the calculated substrate removal rate constant of 1,1-Dichloroethylene biodegradation by strain DT-X (K_{DT-X}) was 0.504 h^{-1}, or 12.096 d^{-1}. Similarly, for strain DT-M, when v_{max} was 0.0046 h^{-1}, K_s was 4.25 mg/L, and biomass (suspended solids) was 8 mg/L, the calculated substrate removal rate constant of 1,1-Dichloroethylene biodegradation by strain DT-M (K_{DT-M}) was 0.157 h^{-1}, or 3.768 d^{-1}.

From the data above, the substrate removal rate constant of 1,1-Dichloroethylene was much higher for the co-metabolic strain DT-X than for the strain DT-M, which uses 1,1-Dichloroethylene as the single substrate. The substrate removal rate constants of both bacteria were higher than those reported in similar studies. This demonstrates that these strains are effective and efficient for 1,1-Dichloroethylene biodegradation. Alternatively, these differences may be due to the different isomers of DCEs used in various studies.

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