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NOTE

Biodegradation of Trichloroethylene by Phenol-degrading *Pseudomonas putida*

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Abstract *Pseudomonas putida* KCTC 2401 degrades 1,1,2-trichloroethylene (TCE) using phenol as a cosubstrate. The initial TCE degradation rate decreased with the initial TCE concentration up to 20 mg/l of TCE at 30°C and pH 6.5. The initial degradation rate and total removal efficiency increased with inoculum size. The strain also degraded dichloroacetic acid, which was supposed to be a degradation by-product. Phenol monooxygenase apparently participates in the TCE degradation mechanism.

Key words: *Pseudomonas putida*, 1,1,2-trichloroethylene (TCE), phenol, cometabolism, biodegradation, aerobic condition

Chlorinated aliphatic compounds, such as 1,1,2-trichloroethylene (TCE), tetrachloroethylene (PCE), and chloroform are of major concern as potential health hazards in drinking water because of their toxicity and potential carcinogenicity [2, 6]. These compounds are persistent in the environment and are transported rapidly in ground water. Since 1996, TCE and PCE have been rated as a priority pollutant in Korea by the Environmental Department of Korea. TCE is a major industrial solvent used for degreasing and cleaning metal plates and electric parts. There have been many reports that cometabolism is responsible for the biodegradation of TCE. Under aerobic conditions TCE is easily cometabolized and, thus, requires the presence of cosubstrate, such as methane, ammonia, toluene, or phenol [3]. Thus, the relation and interaction between TCE and the cosubstrate are important variables in cometabolic biodegradation. Although much research on TCE biodegradation using the cometabolic scheme has been done [4-9, 11-13], much more data are required to apply microorganisms to practical biodegradation schemes. In addition, the search for a good strain is urgently necessary. We performed TCE degradation by *Pseudomonas putida* KCTC 2401 using aromatic compounds as cosubstrates. As a result of this test, a phenol was found to be a good cosubstrate. The purpose of this work was to investigate the TCE degradation ability of the phenol-degrading microorganism *P. putida* KCTC 2401, including study of the interaction between TCE and the cosubstrate compounds.

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Phenol as a Cosubstrate for TCE Degradation

P. putida KCTC 2401 was grown in a minimal medium containing 300 mg/l of phenol and 0.05% of yeast extract for 4 days, and then centrifuged. The pellet was transferred into fresh medium containing 300 mg/l of phenol and 3 mg/l of TCE. TCE degradation experiments were performed in 50 ml serum bottles sealed with butyl rubber stoppers and crimp caps containing a 10 ml solution of phenol, TCE, and inoculation medium. All inoculations were 10% of total culture volume. TCE degradation was monitored by measuring the TCE concentration in the aqueous phase of the bottle by the pentane extraction method and gas chromatography using ECD for TCE and flame ionization detector (FID) for phenol and other chlorinated aliphatics [8, 11]. The total TCE concentration was calculated from the liquid phase concentration. For control experiment, the total amount of TCE remained constant. After 35 h incubation, all TCE in the medium disappeared whereas 80 h were required for phenol to disappear (Fig. 1). Without phenol, TCE was not degraded at all (data not shown). From this result, it is considered that the monooxygenase which participated in the oxidation of phenol was responsible for TCE degradation. Besides phenol, benzene is a good carbon source, but is not a cometabolic substrate (data

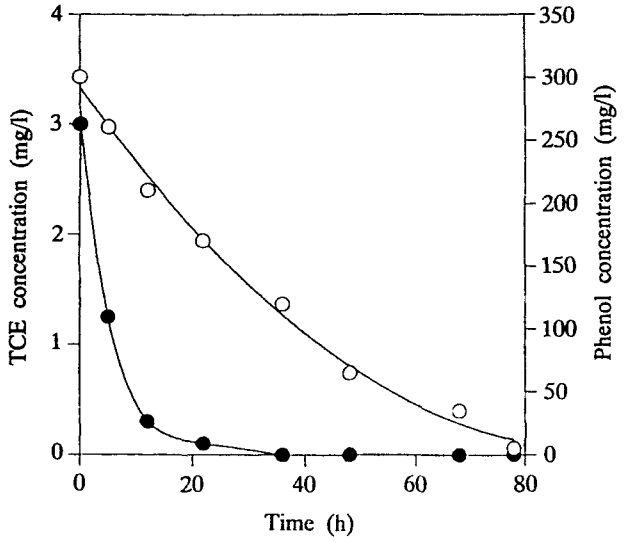

Fig. 1. Aerobic degradation of phenol (○) and TCE (●) by *Pseudomonas putida* KCTC 2401. Inoculum size was 10% (OD_{600} was 0.8).

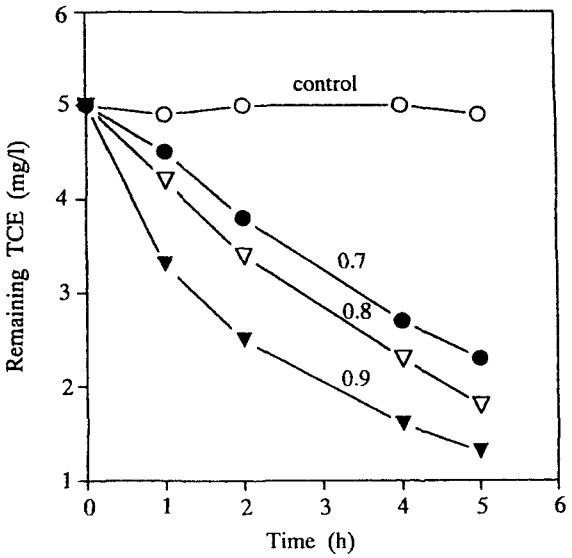

Fig. 3. Influence of inoculum concentration on TCE degradation. Inoculum size was 10% and phenol concentration was 100 mg/l.

not shown) presumably because the specificity of the oxygenase participating in the oxidation of benzene is not broad [9].

Effect of Temperature, pH, and Initial TCE and Phenol Concentration

The optimum temperature was 30°C, probably due to the mesophilic nature of *P. putida* [10]. The pH of 6.5 to 7.0 allowed better degradability than an alkali pH [10]. The initial rate of TCE degradation was apparently decreased with the initial TCE concentration, up to 20 mg/l (Fig. 2). Wackett *et al.* (1988) reported that the TCE degradation

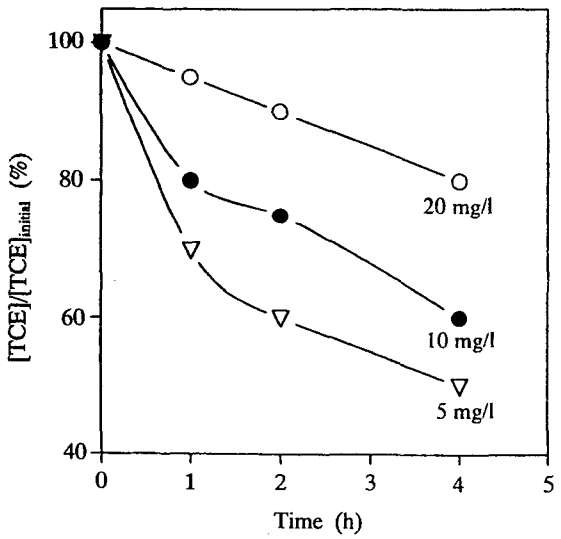

Fig. 2. Influence of TCE concentration on TCE biodegradation. Inoculum size was 10% (OD_{600} was 0.8) and phenol concentration was 100 mg/l.

rate increases with TCE concentration up to 10.16 mg/l-TCE and inhibition occurs at 40 mg/l with *P. putida* F1. Folsome *et al.* (1990) reported a constant rate of TCE degradation with a TCE concentration from 2.54 to 22.56 mg/l using *P. cepacia* G4. In the case of mixed microorganisms, the initial rate of TCE degradation decreased with the TCE concentration, as in this study [11]. Generally, the degradation potential depends on the degradation ability of the microorganisms used. A high concentration of TCE was thought to disturb the metabolic pathways and to induce other metabolic routes in the cell [1]. Figure 3 shows that 2 h were required to degrade 3 mg/l of TCE to half the original concentration when the optical density of the inoculum was 0.9. The best TCE degrader up to now was thought to be a mixed methanotroph, probably due to the simultaneous action of the mixed population [1]. The growth of *P. putida* KCTC 2401 was inhibited at phenol concentrations over 20 mg/l and at Monod-Haldane parameter values of 0.37 m 3.5 and 140 mg/l for V_{max}, K_s, and K_i, respectively [10]. Thus, the presence of phenol can prevent degradation of TCE whereas phenol is an inducer of monoxygenase for TCE degradation. In addition, TCE in the medium decreased the phenol disappearance rate significantly (data not shown). The two compounds were involved in a competitive degradation pathway. This phenomenon is common in TCE degradation using phenol as a cosubstrate [4, 11].

Degradation of Other Chlorinated Aliphatic Compounds

The degradation of several chlorinated aliphatic compounds other than TCE was investigated to estimate the

Table 1. Biodegradation of chlorinated aliphatics by *Pseudomonas putida* KCTC 2401.

Compounds	Remaining concentration (mg/l)[1]	Degradation (%)
2,2,2-Trichloroethanol	2.9	1
Chloroform	2.9	1
Tetrachloroacetic acid	2.8	1.5
Dichloroacetic acid	1.5	50

[1]The initial concentration was 3.0 mg/l, the inoculum was 10% (OD_{600} was 0.8) of the total volume and the reaction time was 5 h.

degradation ability of *P. putida* KCTC 2401. Reactions (9 ml) were carried out at 30°C and pH 6.5 for 5 h and then 1 ml of fresh cell solution (0.8 as OD_{600}) was added. Only dichloroacetic acid was degraded probably because the other compounds contained more than three chlorine atoms (Table 1). Compounds with four chlorine atoms, such as tetrachloroacetic acid and PCE, have not been degraded under aerobic conditions with strains of *Pseudomonas*. The rapid degradation rate of dichloroacetic acid suggests other compounds can be degraded by this strain. In summary, *P. putida* KCTC 2401 showed a substantial potential for TCE degradation in the presence of phenol as a cosubstrate. The ratio of TCE to phenol should be optimized to increase the degradation yield much more.

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