韓國環境復元綠化技術學會誌 8(1) : 79～87(2005)
J. Korean Env. Res. & Reveg. Tech. 8(1) : 79～87(2005)

Direct Enrichment of EPTC-degrading Microorganisms in Soil and Its Use for the Acceleration of EPTC degradation in Soil

Kim, Sang-Jun[1)]

[1)] Department of Natural Sciences, Republic of Korea Naval Academy.

EPTC-분해 미생물의 토양 내에서의 직접 증식과 토양내의 EPTC 분해 촉진을 위한 그 이용

김 상 준[1)]

[1)] 해군사관학교 이학과

ABSTRACT

세계적으로 가장 널리 사용되는 제초제의 하나인 EPTC(*s*-ethyl-*N,N'*- dipropylthiocarbamate)에 대하여, 이를 분해하는 미생물의 토양 내에서의 직접 증식과 이를 함유한 토양(INOCULUM)의 토양내 EPTC 분해촉진을 위한 접종재(inoculant)로서의 효용성을 조사하였다.

한 차례의 EPTC(20mg EPTC/kg 토양)처리에 의해, 순수분리 없이 토양 내에서, EPTC-분해 미생물의 수가 10^2 수준에서 10^5 수준으로 약 10^3배 증식되었으며, EPTC 분해속도 또한, 토양으로부터 추출 가능한 EPTC가 초기 EPTC농도(20mg EPTC/kg 토양)의 20%까지 떨어지는데 걸리는 시간을 기준으로 할 때, EPTC처리 전의 20여 일에서 EPTC 처리 후에는 1일 이내로 빨라졌고, 이 토양의 EPTC 분해능력은 토양내의 EPTC 초기농도가 토양 kg당 2,000mg일 때까지도 크게 저해되지 않았다.

이 토양(INOCULUM)을 EPTC로 오염된 토양에 접종(0.05-5%, w/w)하였을 때, 오염된 토양 내에서의 EPTC 분해속도가 크게 향상되었다. 이 토양의 EPTC 분해능력은, 저온(10℃ 이하)의 습한 상태(수분함량 25%)에 보관하였을 때, 최소 6개월간 유지되었다. 본 연구는 EPTC-분해 미생물이 토양 내에서 쉽게 증식됨과, 이를 함유하는 토양(INOCULUM)이 토양내의 EPTC 분해 촉진을 위한 접종재로서 매우 효과적임을 확인하였고, 이 같은 방법은 다른 화합물과 그에 오염된 토양에도 적용될 수 있을 것으로 기대된다.

Key Words : *Soil contamination, pesticide, Bioaugmentation, Bioremediation.*

Ⅰ. INTRODUCTION

A variety of synthetic chemicals are used throughout the world, and environmental pollution by some of these compounds is a worldwide problem. Regulations have been imposed to ensure appropriate disposal of chemicals, to protect the environment, and to encourage the remediation of polluted environments.

Pesticides are indispensable tools for the control of weeds, insects, and fungi in agriculture, and aerobic microbial degradation is known as a major pathway of dissipation of pesticides. Unfortunately, some pesticides are quite persistent in the environment, and therefore, there has been considerable interest and need to develop cost-effective remediation practices that reduce or remove pesticides from soils before they reach other environments.

Conventionally, in the U.S., methods for detoxifying pesticides depend largely on incineration and landfills. However, incineration, an U.S. EPA-approved method, has experienced serious public opposition because of emissions of potentially toxic materials and high cost. Landfills are effective in the short term, but leaching of pesticides into surrounding soil and groundwater is of great concern. Recently, bioremediation, an application of either indigenous or exogenous microorganisms capable of degrading specific pollutants including pesticides, of contaminated sites is becoming more common(Chen and Mulchandani, 1998).

EPTC(*s*-ethyl-*N,N'*-dipropylthiocarbamate) is one of the world's most widely used carbamothioate herbicides, and microbial degradation is thought to be the major route of carbamothioate dissipation in the environment(Casida et al., 1974; Chen and Casida, 1978; Fang, 1969). In some bacteria, the EPTC degradation is associated with an indigenous plasmid(Assaf, 1991; Anthony et al. 1987; Wang, 2002), which are generally mobile genetic elements. This fact opens up the possibility of rapid genetic transfer of EPTC-degrading genes among soil (micro)organisms. Enhanced degradation of carbamate herbicides in soils with a history of application of the same or similar compounds implies that the microorganisms involved in degradation of carbamothioates can be enriched even in natural media such as soil.

So far, microbial isolates, rather than natural media, were used for bioaugmentation. However, isolation of microorganisms with specific capacity from soils may require excessive time and effort, or not be successful at all, because not all microbial species in natural environments can be isolated using current culture-based methods(Torsvik et al., 1990). Thus, using soil, in which indigenous degraders have been enriched, has advantages as an inoculant in time and economics. In addition, soil may provide nutrients and supporting materials for the introduced microorganisms, and may provide them with shelter from predation by other organisms in the environment.

The specific objectives of this study were : (1) to investigate the possibility of direct enrichment of EPTC-degrading microorganisms in soil, (2) to evaluate the effect of inoculation with directly enriched microorganisms on EPTC degradation in soil, and (3) to find conditions for storage of directly enriched microorganisms.

Ⅱ. MATERIALS AND METHODS

1. Chemicals, soils, and soil analyses

Standard-grade EPTC(s-ethyl *N,N'*-dipropylcarbamothioate) and pesticide-grade toluene used for the extraction of residual EPTC were purchased from Chem Service, Inc. (West Chester, PA) and Sigma(St. Louis, MO), respectively.

A Brookston clay loam soil(designated as Soil-I) was sampled in 1988 from an experimental field, which had been previously treated with Eradicane (EPTC+antidote, 2,2-dichloro-*N,N'*-di-2- prophenyl acetamide) for four consecutive years at field application rate(approximately 3.5 kg active ingredient/ha),

near Canal Winchester, OH, U.S. The soil was then stored in an air-dried state at room temperature for 14 years without additional treatment(for more information on this soil, refer to Ankumah, 1988). Other surface soils(0 ~ 20cm depth) were obtained with different textures, pH values, organic matter content and land use. A Wooster silt loam(designated as Soil-Ⅱ) and a Fremont sandy loam(designated as Soil-Ⅲ) were sampled from agricultural areas near Wooster and Fremont, OH, respectively. These soils may have received occasional pesticide applications, but their pesticide application history is not known. A forest soil(designated as Soil-Ⅳ) was collected from Mohican State Park, OH, and had no known history of pesticide application.

Soil texture and soil pH were analyzed by the hydrometer method(Buoyoucos, 1962) and the glass electrode method(McKeague, 1978) in a 1/1(w/v) soil/water suspension, respectively. Total organic matter was analyzed using methods described by Combs and Nathan(1998), and total nitrogen was determined using a Carbon/Nitrogen Analyzer, Model MAX-CN from Elementar Americas, Inc.(Mt. Laurel, NJ). Characteristics of soil used are summarized in Table 1.

Soil-I was used for direct enrichment of EPTC-degrading micro-organisms because some EPTC-degrading microorganisms were isolated from this soil(Assaf, 1991) and because EPTC-degrading microorganisms had survived for several years when stored at 4℃(Behki, 1991).

Table 1. Soil characteristics.

Properties		Soils			
		Soil-Ⅰ	Soil-Ⅱ	Soil-Ⅲ	Soil-Ⅳ
Particle Size Composition (%)	Sand	31.0	12.1	53.7	36.1
	Silt	36.0	72.7	32.9	53.1
	Clay	33.0	15.2	13.4	10.8
pH		7.3	6.8	6.6	4.5
Organic Matter (%)		7.63	3.23	2.74	4.13
Total Nitrogen (%)		0.345	0.164	0.516	0.416

2. Application, extraction and determination of EPTC

EPTC was applied to prepare artificially EPTC-contaminated soils as follows, Due to low water solubility of EPTC(375mg/L at 25℃), a diluted (approximately 100g EPTC/L) commercial formulation of EPTC(i.e., Eptam) with serial dilution was used as stock solution. Corresponding diluted stock solutions were sprayed onto soil at a ratio of 1 : 10(v/w) while thoroughly mixing with a spatula.

EPTC was extracted from soil as follows. Ten g soil contained in a 125-mL high-density polyethylene (HDPE) bottle was added with eight mL of distilled water, and the contents in the bottle were vortexed for one minute to disperse soil particles. Ten mL of pesticide-grade toluene was added and the bottle was shaken on a horizontal shaker(120 strokes/min) for one hour. The mixture was transferred to a Teflon tube and centrifuged at 8,000×g for 10min, and a 2-mL portion of the toluene layer was transferred into a 5-mL glass vial to be stored at 4℃ until analyzed.

To extract EPTC from microbial culture, 0.8-mL aliquot of the culture was mixed with an equal volume of pesticide-grade toluene in an Eppendorf tube on a Vortex, and the mixture was centrifuged at 15,000 rpm(10,000×g) for 10min. A 0.5-mL portion of the toluene layer was transferred to a 5-mL glass vial, and stored at 4℃ until analyzed.

The concentration of EPTC in the extracts was determined with a Varian 3700 gas chromatograph equipped with a 3% OV-1 on 100/200 Supelcoport packed column(3m in length) and a nitrogen-specific thermionic detector. Helium was used as carrier gas at a flow rate of 28mL/min. Temperatures were set at 210℃ for injector, 160℃ for column, and 250℃ for detector. The retention time for EPTC at the above given condition was 1.60 min.

3. Rates of Degradation of EPTC in soils

The term “degradation of EPTC” in soil is defined

as EPTC disappearance based on extractable EPTC remaining in soil. Degradation of EPTC in soils was measured as follows. A batch of each soil treated with EPTC was adjusted to moisture content of 25%(w/w) with sterilized water, and the soil was divided into 10g(as dry weight) subsamples and put into 125-mL high-density polyethylene(HDPE) bottles. The bottles were incubated in a temperature-controlled(25±2℃) incubator. Triplicate samples were randomly selected at various times of incubation, and EPTC remaining in soil was extracted and analyzed.

The rates of degradation of EPTC in soils were compared by plotting the percentage of EPTC recovered from soil vs. incubation time. The averaged amount of EPTC extracted from triplicate samples at the very beginning of the incubation(i.e., at time zero of incubation) was regarded as 100%.

4. Direct enrichment of EPTC-degrading microorganisms

Soil-I(a Brookston clay loam) was pretreated by adjusting moisture content to 25%(w/w) with distilled water and placing it at room temperature(20 ~ 25℃) for 7 days. This soil was treated with EPTC at a rate of 20mg/kg soil, and was then incubated at 25±2℃. During incubation, degradation of EPTC in soil was monitored as previously described, and the number of total and EPTC-degrading bacteria were enumerated by the most probable number(MPN) method described later. At the end of the third treatment, the moisture content of the soil was adjusted to 25%(w/w) and the activated soil was stored in a refrigerator(4℃).

5. Most probable number(MPN)

The number of total or EPTC-degrading micro-organisms in soils was determined based on the most probable number(MPN) method modified from Alexander(1982). A 10g soil sample was suspended in 100-mL of sterilized water in a 250-mL Erlenmeyer flask and gently shaken for 20 min. Ten-fold dilutions were made by sequential transfer of 1-mL portion into 9mL of each medium : TGYe(0.5% tryptone, 0.1% glucose, and 0.25% yeast extract, w/v) for total bacteria and a BMN(Behki and Khan 1986) amended with EPTC at 50mg/L concentration for EPTC-degrading bacteria.

MPN tubes(5 replicates) were prepared for each of the 10-fold serial dilutions, and tubes were incubated at 25±2℃ for 5 days. Positive tubes for total bacteria were identified by visual turbidity. Those for EPTC-degrading bacteria were determined by measuring EPTC remaining in the culture, and the tubes containing less than 20% of the initial EPTC recovered were considered positive.

6. Effect of Inoculation of contaminated soil with INOCULUM

Artificially EPTC-contaminated soil was inoculated with INOCULUM at different rates(0.05, 0.5, and 5%, w/w), and the rates of degradation of EPTC in soil were monitored and compared as described before.

7. Evaluation of storage conditions on the stability of INOCULUM

The stability of INOCULUM, in terms of EPTC-degrading activity, was investigated under different storage conditions. For the storage at room temperature (20 ~ 25℃) with and without moisture adjustment, INOCULUM was divided into two subsamples and each placed in a plastic container with lid. One of the subsamples had water content maintained at 25%(w/w) by adding sterilized water every other week. The other was allowed to naturally dry down over time, and soil water content in this soil dropped below 15% (w/w) after 1 month of storage. For the storage at low temperatures(10 or 4℃) or frozen state(-20℃), the INOCULUM was subdivided into 10g subsamples and placed in small(16-mL) vials and capped, vials were placed in a cold room (10℃), a refrigerator(4℃), and a freezer(-20℃).

Subsamples stored at different conditions were randomly taken out, and remaining activity at specific times of storage was compared to that of the beginning of the storage according to the following procedure. Ten g of each of these subsample was inoculated into 500g of artificially EPTC-contaminated (20mg/kg) Soil-II, and rates of degradation of EPTC were monitored. The subsamples stored at 4℃ and at frozen state(-20℃) were allowed to adjust to room temperature(20 ~ 25℃) for 1 and 2 days, respectively.

8. Statistical analysis

All statistical analyses were done using MINITAB (ver. 13.1) from MINITAB Inc., State College, PA. Analysis of Variance(ANOVA) was done using the General Linear Model menu in MINITAB, and the Least Significant Difference(LSD) was calculated at the 5% level.

III. RESULTS AND DISCUSSION

1. Direct enrichment of EPTC-degrading microorganisms in soil

Although high activity for EPTC degradation in Brookston clay loam(Soil-I), previously observed at the time of sampling(Ankumah 1988), was not recovered by water treatment only for 7days, both EPTC-degrading microorganisms and activity were readily enriched and recovered during subsequent EPTC treatments at a rate of 20mg/kg soil as shown in Figure 1.

Based on MPN, while total number of microorganisms remained relatively constant, the number of EPTC-degrading microbes increased significantly (>3 log units) and reached its maximum during the first cycle of treatment as shown in Figure 1. However, it might be possible to increase the number of EPTC degrading microorganisms in soil more by application of EPTC at a higher rate. In contrast, Moorman(1988) reported that populations of EPTC-degrading microorganisms in soils with

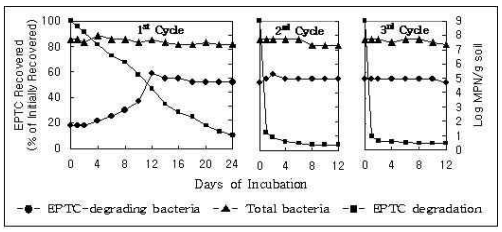

Figure 1. Direct enrichment of EPTC-degrading microorganisms in soil. The rate of EPTC at the beginning of each treatment cycle was 20mg/kg soil.

accelerated rates of EPTC degradation were not larger than those of other soils. The author suggested that increased rates of metabolism of EPTC, rather than increased populations of degraders, was apparently responsible for the increased rates of EPTC degradation.

The amounts of EPTC recovered at 1 day of incubation in the second and the third treatments dropped below 20% of initially recovered. This contrasts to a time of 20 days to achieve similar level of degradation during the first treatment. Thus a single treatment of EPTC at a rate of 20mg/kg soil was sufficient to achieve a high level of EPTC degradation activity. Similarly, Obrigawitch et al. (1983) observed that a single application of EPTC resulted in maximum increase in the rate of degradation of EPTC in the soils. According to Ankumah (1988), EPTC treatment at a rate of 4mg active ingredient/kg soil resulted in a dramatic self-enhancement of EPTC degradation after the second treatment, but further enhancement was not significant thereafter.

Although the use of indigenous microbial consortium has been proposed as a bioaugmentation method for enhanced biodegradation, direct enrichment of microorganisms capable of degrading specific compounds in natural media and its application for bioremediation have rarely been reported(Barbeau et al., 1997; Laine and Jorgensen, 1996).

The Soil-I(a Brookston clay loam) which had been treated with EPTC three times at a rate of 20mg/kg

soil in this study, and thus exhibited increased number of EPTC degraders and enhanced activity for EPTC degradation, is hereafter defined as INOCULUM.

2. Effect of initial EPTC concentration on the activity of INOCULUM

Chemicals become toxic to microorganisms at concentrations exceeding a threshold value, although they are not harmful at lower concentrations. Bioaugmentation may not be effective when the contamination exceeds the threshold because of contaminants' inhibitory effect on microbial growth or degradation activity.

There is no previous work on the effect of EPTC concentration on microbial growth or degradation of EPTC. In this work, degradation of EPTC in the INOCULUM was evaluated at three different initial EPTC concentrations(20, 200, and 2,000mg/kg soil). At all levels tested, degradation of EPTC in the INOCULUM proceeded very rapidly as shown in Figure 2. The amount of extractable EPTC remaining in soil after 8 days of incubation was about 5 to 15% of the EPTC initially recovered.

The effect of initial concentration of EPTC on degradation of EPTC, however, may be different in soil with EPTC at much higher initial concentrations (>>2,000mg/kg soil) or in soils with lower numbers of EPTC-degrading organisms.

3. Effect of inoculation of INOCULUM on EPTC degradation in soils

There have been numerous reports on the effect of introducing microorganisms, either cultured isolate(s), consortium of unknown members, enriched microbe(s), or commercial bioaugmentation product(bioadditive), to bioaugment degradation of pollutants under laboratory or field conditions(Barbeau et al., 1997; Boon et al., 2000; Mendoza-Espinosa and Stephenson, 1996; Pritchard, 1992; Rojas-Avelizapa et al., 2003; Schwartz and Scow, 2001). The results, however, have not always been promising, and several reasons for failure in bioaugmentation have been proposed (Fujita et al. 1994, Goldstein et al. 1985, McClure et al. 1989, McClure et al. 1991, Schmidt and Alexander 1985). The use of activated soil is proposed because it offers several advantages over that of cultured microorganisms. Soil serves as a carrier material that retains microorganisms introduced in required places, and it also provides inoculated microorganisms with shelter for survival from predation and/or nutrients, which may not exist in the environment introduced, essential for survival and/or activity.

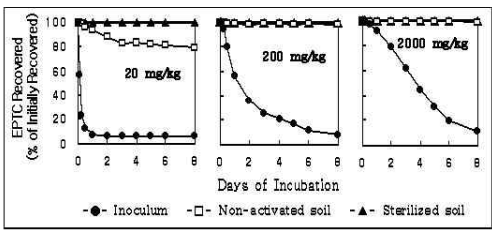

Figure 2. EPTC degradation in INOCULUM as a function of EPTC concentration.

The effectiveness of INOCULUM to bioaugment the degradation of EPTC in soils was studied by adding the INOCULUM at different rates(0.05, 0.5 and 5%, w/w) to artificially contaminated soils with 20mg EPTC/kg soil. Based on MPN method, a 5% inoculation corresponds to introduction of 10^3 EPTC-degrading microorganisms per g soil.

Inoculating contaminated soils with INOCULUM significantly(p<0.05) enhanced EPTC degradation at almost all incubation time with a little variation

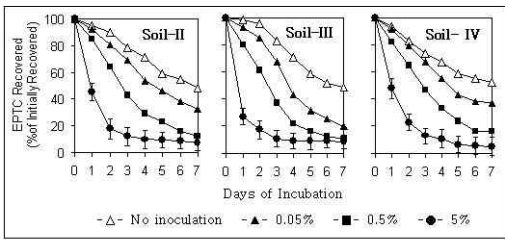

Figure 3. Effect of inoculation on degradation of EPTC in artificially EPTC-contaminated soils which were prepared from each soil treated with EPTC at a rate of 20mg/kg soil. Error bars represent Least Significant Difference(LSD) among treatment means at given incubation time.

among soils tested as shown Figure 3. As expected, the effect of inoculation on EPTC degradation in soils was proportional to the rate of inoculation. The EPTC-degrading activity was not significantly inhibited even in a soil with relatively low pH (Soil-Ⅳ, pH of 4.5).

The equivalent number of EPTC-degrading micro-organisms at 0.05% inoculation was 10cfu/g soil. It does not seem likely that only 10 microorganisms per gram of INOCULUM could enhance degradation greatly. There could be other EPTC-degrading microorganisms enriched but not enumerated by the MPN method which is based on culture technique, and this may ensure advantage of using natural media, such as soil, with enriched degraders.

4. Effect of storage condition on stability of INOCULUM

Since it is generally not convenient or practical to prepare the necessary quantities of inoculum immediately prior to use, inoculum is often prepared and then preserved so that it is immediately available when needed. Therefore, inoculum must be stable during storage.

The stability of INOCULUM, in terms of EPTC-degrading activity, was evaluated under various storage conditions, and the result is shown in Figure 4. When the INOCULUM was stored at room temperature(20~25℃), regardless of its moisture content, it lost its EPTC-degrading activity in a relatively short period(within 1 month). However, when stored at lower temperatures(below 10℃), degrading activity of the INOCULUM was preserved for at least 6 months. The stability of INOCULUM at these lower temperatures was not monitored more than 6 months.

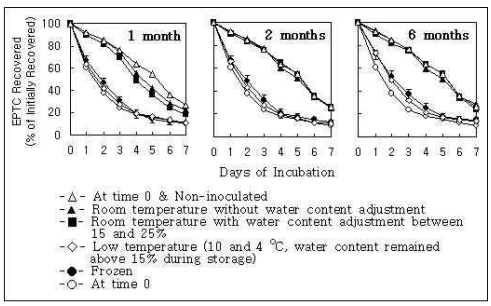

Figure 4. Effect of storage conditions on the stability in terms of EPTC degrading activity of INOCULUM Error bars represent Least Significant Difference(LSD) among treatment means at given incubation time.

Previously, Behki(1991) observed EPTC-degrading microorganisms survived for three years in a soil exposed to EPTC for four consecutive years, when the soil was stored at 4℃. It was evident in this study that EPTC-degraders could survive for a much longer period(14 years) even under harsh conditions(i.e., at room temperature and low moisture content) although degradation activity was lost. Furthermore, this study found that the INOCULUM, when stored at low temperature, was stable even in terms of EPTC-degrading activity. Therefore, the INOCULUM can be stored in a readily usable state if both the temperature is kept below 10℃ and moisture content is maintained above 15%(w/w). For longer term storage of soil, room temperature and air-dry conditions are probably more suitable as it is easier to preserve soil in this way and the number of degrader and activity readily respond to an environment which contains. Storing at room temperature and occasional treatment with EPTC can be another effective strategy for the storage of INOCULUM in a readily usable state.

Ⅳ. CONCLUSION

EPTC-degrading microorganisms survived well for 14 years in a Brookston clay loam soil(Soil-I), that was sampled, air-dried, and placed at room temperature without any additional treatments for storage. After the soil was pretreated with addition of water and incubated at room temperature for 7 days, EPTC-degrading micro-organisms in this soil were readily enriched by repeated treatments of EPTC

at a rate of 20mg/kg soil. The number of EPTC-degraders increased by about 3 logs of magnitude after a single treatment with EPTC at a rate of 20mg/kg soil, and additional treatments of EPTC treatment at the same rate did not significantly increase the number of EPTC-degraders. This activated Brookston clay loam soil was defined as INOCULUM.

Degradation of EPTC in INOCULUM was very active at initial EPTC concentration up to 2,000mg/kg soil. Inoculation of EPTC-contaminated soil with the INOCULUM resulted in significant($p<0.05$) increase in rates of EPTC degradation in soil. Inoculation at a rate as low as 0.05% could result in considerable acceleration of EPTC degradation in soils, and rates of inoculation ranging from 0.05 to 5% were directly related to rates of EPTC degradation in soils. When soils were inoculated at a rate of 5%, the extractable EPTC remaining in soil after about 4 days in all three soils tested dropped to below 10% of EPTC initially added and recovered at time zero. The INOCULUM was found to be stable, in terms of EPTC-degrading activity, up to 6 months when it was stored in a moist state at temperature below 10℃.

This study suggests that use of this INOCULUM to bioaugment EPTC degradation in contaminated soils is effective, and that the same strategy may be applied for other contaminants and microorganisms.

LITERATURE CITED

Alexander, M. 1982. Most probable number method for microbial populations(In Page, A.L., R. H. Miller, and D.R. Keeney eds., "Methods of soil analysis, 2nd ed., Part 2"). Madison : American Society of Agronomy. pp. 815-820.

Ankumah, R. O. 1988. Enhanced degradation in soils of the herbicide EPTC and determination of its degradative pathway by an isolated soil microorganism. Ph.D. dissertation, The Ohio State University, Columbus.

Anthony, C. T., R. M. Behki, and S. U. Khan. 1987. Isolation and characterization of an s-ethyl-N, N'-dipropylthiocarbamate degradation. Appl. Environ. Microbiol. 53 : 1088-1093.

Assaf, N. A. 1991. Degradation of the herbicide EPTC by isolated soil bacteria. Ph.D. dissertation, The Ohio State University, Columbus.

Barbeau, C., L. Deschenes., D. Karamanev., Y. Comeau, and R. Samson. 1997. Bioremediation of pentachlorophenol-contaminated soil by bioaugmentation using activated soil. Appl. Microbiol. Biotechnol. 48 : 745-752.

Behki, R. M. 1991. Di-allate degradation by an EPTC-degrading *Rhodococcus*, and in EPTC-treated soil. Soil Biol. Biochem. 23 : 789-793.

Behki, R. M., and Khan. 1986. Degradation of atrazine by *Pseudomonas* : N-dealkylation and dehalogenation of atrazine and its metabolites. J. Agric. Food Chem. 34 : 746-749

Behki, R. M., E. Topp., W. Dick, and P. Germon. 1993. Metabolism of the herbicide atrazine by *Rhodococcus* strains. Appl. Environ. Microbiol. 59 : 1955-1959.

Boon, N., J. Goris., P. de Vos., W. Verstraete, and E. M. Top. 2000. Bioaugmentation of activated sludge by an indigenous 3-chloroaniline-degrading *Comamonas testosteroni* strain, I2gfp. Appl. Environ. Microbiol. 66 : 2906-2913.

Buoyoucos, G. J. 1962. Hydrometer method improved for making particle-size analysis of soils. Agron. J. 53 : 464-465.

Casida, J. E., R. A. Gray, and H. Tilles. 1974. Thiocarbamate sulfoxides : Potent, selective and biodegradable herbicides. Science. 184 : 573-574.

Chen, W. and A. Mulchandani. 1998. The use of biocatalysts for pesticide detoxification. Trends Biotechnol. 16 : 71-76.

Chen, Y. S., and J. E. Casida. 1978. Thiocarbamate herbicide metabolism : Microsomal oxygenase metabolism of EPTC involving mono- and

dioxygenation at the sulfur and the hydroxylation each alkyl carbon. J. Agric. Food. Chem. 26 : 263-266.

Combs, S. M., and M. V. Nathan. 1998. Chapter 12, Soil organic matter(In "Recommended chemical soil test procedures for the North Central Region. NCR Publication No. 221"). Columbia : Missouri Agricultural Experiment Station. pp. 53-58.

Fang, S. C. 1969. Thiocarbamates(In Kearney, P. C. and D. D. Kaufman eds., "Degradation of herbicides"). New York : Marcel Dekker. pp. 147-164.

Fujita, M., M. Ike, and K. Uesugi. 1994. Operation parameters affecting the survival of genetically engineered microorganisms in activated sludge processes. Wat. Res. 28 : 1667-1672.

Goldstein, M. G., L. M. Mallory, and M. Alexander. 1985. Reasons for possible failure of inoculation to enhance biodegradation. Appl. Environ. Microbiol. 50 : 977-983.

Laine, M. M., and K. S. Jorgensen. 1996. Straw compost and bioremediated soil as inocula for the bioremediation of chlorophenol-contaminated soil. Appl. Environ. Microbiol. 62 : 1507-1513.

McClure, N. C., J. C. Fry, and A. J. Weightman. 1991. Survival and catabolic activity of natural and genetically engineered bacteria in a laboratory-scale activated sludge unit. Appl. Environ. Microbiol. 57 : 366-373.

McClure, N. C., A. J. Weightman, and J. C. Fry. 1989. Survival of *Pseudomonas putida* UWC1 containing cloned catabolic genes in a model activated-sludge unit. Appl. Environ. Microbiol. 55 : 2627-2634.

McKeague, J. A.(ed.). 1978. Manual on soil sampling and methods of analysis. Can. Soc. Soil Sci.

Mendoza-Espinosa, L., and T. Stephenson. 1996. Grease biodegradation : Is bioaugmentation more effective than natural populations for start-up? Wat. Sci. Tech. 34 : 303-308.

Moorman, T. B. 1988. Populations of EPTC-degrading microorganisms in soils with accelerated rates of EPTC degradation. Weed Sci. 36 : 96-101.

Obrigawitch, T., A. R. Martin, and F. W. Roeth. 1983. Degradation of thiocarbamate herbicides in soil exhibiting rapid EPTC breakdowns. Weed Sci. 31 : 187-192.

Pritchard, P. H. 1992. Use of inoculation in bioremediation. Curr. Opin. Biotechnol. 3 : 232-243.

Rojas-Avelizapa, N. G., J. Martinez-Cruz, J. A. Zermeno-Eguia Lis, and R. Rodriguez-Vazquez. 2003. Levels of polychlorinated biphenyls in Mexican soils and their biodegradation using bioaugmentation. Bull. Environ. Contam. Toxicol. 70 : 63-70.

Schmidt, S. K., and M. Alexander. 1985. Effects of dissolved organic carbon and second substrates on the biodegradation of organic compounds at low concentrations. Appl. Environ. Microbiol. 49 : 822-827.

Schwartz, E., and K. M. Scow. 2001. Repeated inoculation as a strategy for the remediation of low concentrations of phenanthrene in soil. Biodegrad. 12 : 201-207.

Torsvik, V., J. Goksoyr, and F. L. Daae. 1990. High diversity in DNA of soil bacteria. Appl. Environ. Microbiol. 56 : 782-787.

Wang, Y. 2002. characterizing the structure and diversity of the EPTC-degrading microbial community in soils. Ph.D. dissertation, The Ohio State University, Columbus.

接受 2004年 12月 15日