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Effect of Spherosome on Degradation of Tetrachloroethylene in Soil

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The fate of spherosome on the degradation of tetrachloroethylene in soil was investigated for 38 days. The time needed to become the half value of the initial concentration is 8 days for the case with spherosome and 15 days for the case without spherosome. The time needed for complete degradation is 25 days for the case with spherosome and 38 days for the case without spherosome. The degradation of tetrachloroethylene appeared to be essentially due to the biological activity of the soil. Spherosome should enhance the rate of tetrachloroethylene destruction.

KEYWORDS: Tetrachloroethylene; soil; rice bran; spherosome

INTRODUCTION

Tetrachloroethylene pollution of the environment from manufacturing processes has resulted in wide contamination of soils and sediments. There are many reports of the contamination of water supplies by tetrachloroethylene, in both Europe and the United States (*1*–*4*).

A National Cancer Institute study reported an increase in liver tumors in B6C3F1 mice following long-term exposure to tetrachloroethylene (*5*). The World Health Organization (WHO) set a tentative guideline value of 0.01 mg/L for tetrachloroethylene in drinking water (*6*). In 2004, the Ministry of Health, Labour and Welfare in Japan set a quality standard of 0.01 mg/L for tetrachloroethylene in drinking water (*7*). Tetrachloroethylene is thought to be carcinogenic when ingested by animals. To protect water sources, it is important to keep the concentration of this compound in the soil as low as possible. To remove this compound from chemical and industrial wastewaters, adsorption on activated carbon (*8*, *9*), ultraviolet radiation/oxidation technology (*10*), or aeration have usually been used. One problem with these uses is their cost. The first of several roles is to provide a method for reducing the capital cost of the treatment plant. Joyce (*11*) reported that tetrachloroethylene in groundwater was removed through an ammonia-stripping tower with an air-to-water ratio of approximately 3000 to 1 and with an efficiency of removal ranging from 69 to 90%. The aeration process is based on transferring chemicals from water into the atmosphere through its surface without treatment. This method is, however, flawed from the viewpoint of air pollution. We have previously reported that rice bran and defatted seed were effective in adsorbing pesticides and organochlorine compounds such as chloroform, dichloromethane, and benzene (*12*).

Furthermore, it was confirmed that the spherosomes isolated from these adsorbents were effective in removing these organic compounds (*13*). Analytical and laser microscopic data have confirmed that the removal of organochlorine compounds and benzene is dependent on the uptake of these compounds into intracellular particles called spherosomes (*13*). Spherosomes are widely distributed among plants and fungi but have not been observed in animal cells. However, spherosomes occur prominently in seeds. Spherosomes are intracellular particles about 1 μm in diameter (*14*). The function of spherosomes is not well-understood. Our research has focused on the adsorption properties of spherosomes and their nutrient effects. The measurement of tetrachloroethylene levels in soil with or without spherosome is important for using spherosomes as adsorbent materials for the removal of tetrachloroethylene. The degradation of tetrachloroethylene in soil with or without spherosome was investigated. This paper provides the first report on the environmental fate of tetrachloroethylene in soil with or without spherosomes.

MATERIALS AND METHODS

Apparatus. The assay of tetrachloroethylene was performed on a Shimadzu model GC-14B gas chromatograph equipped with a flame ionization detector and a capillary column (ULBON HR-52, 30 m × 0.53mm). The column was maintained at 90 °C, and both the injection port and the detector were maintained at 150 °C.

Materials. Rice bran was purchased at a local market. The soil for these experiments was obtained from a farm in the Shiga Prefecture of Japan. Spherosomes were isolated from rice bran by a fractionation method developed by our research group. The composition of the spherosomes is shown in **Table 1**. The moisture content was determined by drying a sample for 6 h at 110 °C. The protein concentration was determined from the nitrogen content obtained by the method of Kjeldahl (*15*). Lipids were extracted by the Bligh and Dyer method (*16*). The mass of the total lipid was determined by drying an aliquot of chloroform extract in a vacuum oven overnight and weighing the resulting lipid residue. Carbohydrate (glucide) was determined by the Anthrone method (*17*). Dietary fiber was determined by an AOAC method (*18*). Tetrachloroethylene of analytical standard purity was

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Table 1. Composition of Spherosomes

constituent	concentration (g/100 g)
water	9.8
protein	26.6
lipid	3.9
carbohydrate	
glucide	38.4
fiber	3.6
ash	17.4

purchased from Wako Pure Chemical Industries Ltd. (Amagasaki, Japan).

Soil Sterilization. Soil to be heat-sterilized was placed in glass jars with loosely fitted lids and taken through three cycles of autoclaving for 15 min at 15 psi and 121 °C.

Recovery Test. To determine the method efficiency for tetrachloroethylene, tetrachloroethylene was added (50 mg/g) in the soil samples (10 g). The soil was extracted for 30 min by shaking with 30 mL of acetone. The total extracts were combined in a separatory funnel with 50 mL of 5% NaCl solution, and the extraction was repeated twice, using 10 mL of hexane each time. The hexane layer was analyzed by gas chromatography (GC) to determine the concentrations of tetrachloroethylene. Blank samples were used, and no interference was found in the determination of tetrachloroethylene. Recovery data represent four replications.

Determination of Tetrachloroethylen in Soil. Ten grams of the sample was weighed in a 200 mL Erlenmyer flask and was extracted with 30 mL of acetone. The soil was analyzed for tetrachloroethylene using the same procedures as described for the recovery test.

Soil Incubation. The nonsterile soil or sterile soil (10 g) were weighed into Pyrex glass flasks (100 mL). To each soil subsample was added an adequate amount of water, determined by weighing, to give a soil moisture content of 17%. The moisture content was maintained ranging from 15 to 17% throughout the experiment. In all of the soil samples, except the control, tetrachloroethylene was added (50 mg/g), following spherosomes (30 mg/g). The treated soil samples were then placed in the dark at 10 °C until sample analysis.

RESULTS AND DISCUSSION

Recovery Studies. The recovery of tetrachloroethylene can be checked according to the procedures for the recovery test. The recoveries of added tetrachloroethylene (50 mg/g) in the soil samples were 95.1–97.1%, with a maximum coefficient of variation (CV) of 5.5%. The limit of quantification was defined for GC as the sample concentration required to give a signal-to-noise ratio of 6:1. It was evaluated at 0.05 mg/g of soil.

Degradation of Tetrachloroethylene in Soil. The effect of spherosome on the degradation of tetrachloroethylene was examined using sterile and nonsterile soils. The residual concentration of tetrachloroethylene in soil is shown in **Table 2**. **Figure 1** shows the residual tetrachloroethylene (%) calculated by the following equation: residual tetrachloroethylene (%) = (X/M) × 100, where X is the tetrachloroethylene remaining in the soil after treatment and M is the initial tetrachloroethylene content. The time needed to become half value of the initial concentration is 8 days for the case with spherosome and 15 days for the case without spherosome. The time needed for complete degradation is 25 days for the case with spherosome and 38 days for the case without spherosome. The percentage of tetrachloroethylene in soil with spherosome was lower than that in soil without spherosome. Furthermore, no loss of tetrachloroethylene was observed in sterile soil samples. Evidence from the sterile soil experiment indicates that the mechanism of tetrachloroethylene degradation in soils is attributed to microbial degradation. We have previously reported that the efficiency of removal of organochlorine compounds by

Table 2. Concentration of Tetrachloroethylene in Soil with or without Spherosome

		residual tetrachloroethylene			
		with spherosome		without spherosome	
sample day	control	mg/g[b]	%	mg/g[b]	%
0	ND[a]	48.0 ± 0.2	100	49.0 ± 0.4	100
2	ND	42.5 ± 0.9	88.5	46.1 ± 1.4	95.4
4	ND	36.8 ± 2.1	76.7	41.8 ± 1.5	86.5
6	ND	29.0 ± 0.9	60.4	35.4 ± 1.3	73.3
8	ND	24.7 ± 0.6	51.5	31.7 ± 0.9	65.6
10	ND	22.3 ± 1.3	46.5	29.6 ± 0.9	61.3
13	ND	17.8 ± 1.3	37.1	26.7 ± 1.4	55.2
15	ND	14.0 ± 0.8	29.2	23.7 ± 0.7	49.1
18	ND	10.6 ± 0.8	22.1	20.1 ± 1.3	41.6
20	ND	8.0 ± 0.9	16.7	16.9 ± 0.9	35.0
22	ND	4.9 ± 0.4	10.2	14.6 ± 0.7	30.2
25	ND	ND	0	10.6 ± 1.7	21.9
28	ND	ND	0	6.3 ± 2.8	13.0
38	ND	ND	0	ND	0

[a] Not detected. [b] Each value represents the mean ± SD of three separate determinations.

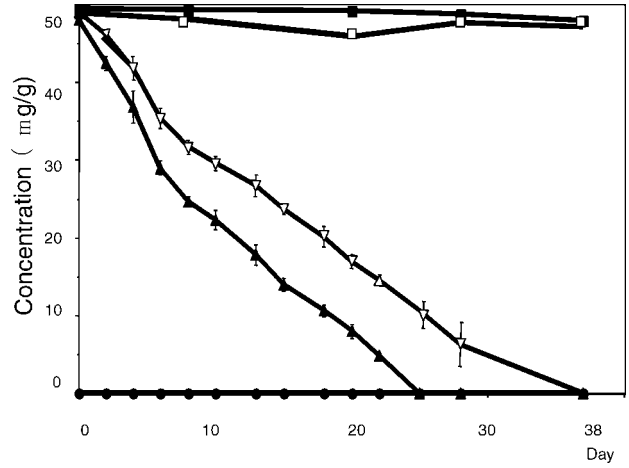

Figure 1. Concentration of tetrachloroethylene in soil with or without spherosome. Tetrachloroethylene, 50 mg/g; spherosome, 30 mg/g. With spherosome (nonsterile soil) (▲), without spherosome (nonsterile soil) (▽), with spherosome (sterile soil) (■), without spherosome (sterile soil) (□), and control (nonsterile soil) (●).

spherosomes isolated from rice bran was similar to that of rice bran (*13*). **Figure 1** shows that the degradation rates increased with the uptake of tetrachloroethylene into spherosomes. Our hypothesis is that spherosoms enhance microbial numbers and activity. Environmental factors can greatly influence the degradation rate of chemical compounds in soil, the most important being moisture, pH, and organic carbon content. Garcia-Valcarcel and Tadeo (*19*) reported that degradation rates increased with soil moisture content for hexazinone and simazine, which is in agreement with the results of Walker and Blacklow (*20*) for atrazine and simazine and those of Bowmer (*21*) for atrazine. Therefore, we keep constant the soil moisture. In sterile soil with and without spherosome, tetrachloroethylene remained almost unchanged during the incubation period. Evidence from the sterile soil experiment indicates that the mechanism of tetrachloroethylene degradation in soils is attributed to the microbial degradation. Pfaender and Alexander (*22*) reported that the numbers of microorganisms potentially able to cometabolize DDT were high in raw sewage as a result of the addition of glucose and diphenylmethane. The majority of approximately 300 isolates from water and soil could

metabolize DDT (*23*). Several soil microorganisms isolated from soil contaminated with pesticides have been reported to be active in the degradation of dieldrin (*24*).

Jacks et al. (*25*) reported that spherosomes isolated from peanuts are composed of 98.1% total lipids and 1.27% protein. Spherosomes isolated from wheat aleurone have also been reported to have a neutral lipid content of 86.9%, 8.5% of the phospholipid and 1.8% protein (*26*). Adans and Novellie (*27*) reported that spherosomes from linseed have an average composition of 27% protein, 12% phosphorus, and 8.6% metals. Their protein value is similar to our spherosomes (**Table 1**). Spherosomes were considerably high in protein and carbohydrate (**Table 1**). On the basis of the present data, it seems more likely that spherosomes might have a role as a nutrient for the growth of microorganisms. Therefore, spherosomes should be active in the growth of microorganisms. Furthermore, we consider that the effect of spherosomes, which adsorb tetrachloroethylene, on the microorganisms is similar to that of spherosomes without adsorption.

Our study showed that spherosomes should enhance the destruction rate of tetrachloroethylene. Therefore, this suggests that spherosomes may be applied to soil to reduce tetrachloroethylene. The spherosome that we examined is a residue from rice bran. Rice bran is a waste product in the process of making polished rice from brown rice and is very inexpensive. From this perspective, the use of spherosomes has merit in bioremediation.

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