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Removal Efficiency of Defatted Seed for Organochlorine Compounds

Atsuko Adachi,* Tomoko Komiyama, Tomoko Tanaka, Maki Nakatani, Ryuske Muguruma, and Toshio Okano

Kobe Pharmaceutical University, Higashinada-ku, Kobe 658–8558, Japan

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Defatted seeds effectively adsorbed organochlorine compounds such as chloroform, dichloromethane and trichloroethylene. The amounts of these compounds adsorbed was plotted against the equilibrium concentration of substances in solution on a logarithmic scale. A linear relationship was obtained, indicating that the adsorption reactions were Freundlich type. The adsorption of these compounds by defatted seed was observed over the range of pH 1-11. Chloroform was successfully removed from tap water with an average removal efficiency of 70% after 60 min when rapeseed was added to tap water that contained 0.0073 mg/l chloroform. The removal of these organochlorine compounds by defatted seed was attributed to the uptake by intracellular particles called spherosomes.

Key words —— soybean, sesame, rapeseed, linseed

INTRODUCTION

Various kinds of chemical pollutants have recently been detected in the environment, and this has created large social problems. By the treatment of tap water, chloroform is produced, which is one of the trihalomethanes that are created non-intentionally by chlorination, and shows carcinogenicity in animal experiments. However, it is impossible to eliminate these chemical substances from the environment for as long as disinfection of the tap water is necessary to maintain safe drinking water. Therefore, it is necessary to limit the amount of chemical substances that are released into the environment to as little as possible, and also to recover them as fully as possible. To remove these compounds from chemical and industrial wastewater, adsorption on activated carbon,[1–4] photochemical decomposition by ultraviolet irradiation[5–10] and aeration have usually been used. One problem with the use of activated carbon is cost. Lykins[11] reviewed the treatment data generated from the Ohio river between 1976–1977, and concluded that consistent removal of chloroform was not obtained with powdered activated carbon treatment. The photochemical reaction with ultraviolet irradiation barely occurs without expensive catalysts, and catalysts such as TiO_2, PtO_2 and IrO_2 have been used predominantly.[12] McCarty's group[13] reported 94% removal of tetrachloroethylene (average influent concentration of 2.8 μg/l) in ammonia stripping towers fed with highly treated wastewater. The aeration process is based on transferring chemicals from water into the atmosphere through its surface without treatment. From the view point of air pollution, this method is flawed. Based on this information, we studied several adsorbents to find an effective alternative.

MATERIALS AND METHODS

Procedure for Removal —— Defatted seeds provided by Nissin oil Mills Inc. were used. They included soybean, rapeseed, linseed and sesame that were the residue from food oil extraction. A 100 ml sample solution including chemical compounds was placed in a 100 ml glass stoppered Erlenmyer flask, to which 0.1–1 g of defatted seed was added. The solution was mixed by a stirrer. The reaction mixture was filtered through filter paper to remove the defatted seed. The initial 10 ml of filtrate was discarded because of the adsorption of chemical compounds by the filter paper. In control samples lacking defatted seed, the subsequent filtrate after the discard contained the same amount of chemical com-

*To whom correspondence should be addressed: Department of Hygienic Sciences, Kobe Pharmaceutical University, Motoyamakitamachi 4-chome, Higashinada-ku, Kobe 658–8558, Japan. Tel.: +81-78-441-7564; Fax: +81-78-441-7565; E-mail: a-adachi@kobepharma-u.ac.jp

Table 1. Removal Efficiencies of Defatted Soybean, Sesame, Rapeseed or Linseed for Chloroform, Dichloromethane and Trichloroethylene

Substance	Soybean			Sesame		
	Concentration (mg/l)			Concentration (mg/l)		
	Before treatment	After treatment	Removal efficiency (%)	Before treatment	After treatment	Removal efficiency (%)
Chloroform	100	28.9–36.3	68.6±1.7*	100	46.4–49.2	52.4±0.6*
Dichloromethane	100	22.1–34.9	70.4±2.8*	100	58.4–66.3	38.6±1.8*
Trichloroethylene	50	8.9–13.3	78.6±1.2*	50	9.9–18.3	75.6±1.7*
Substance	Rapeseed			Linseed		
	Concentration (mg/l)			Concentration (mg/l)		
	Before treatment	After treatment	Removal efficiency (%)	Before treatment	After treatment	Removal efficiency (%)
Chloroform	100	22.5–31.9	72.4±2.0*	100	3.3–23.7	87.6±4.3*
Dichloromethane	100	28.6–34.6	69.5±1.4*	100	8.0–21.9	86.8±3.0*
Trichloroethylene	50	9.9–12.3	82.4±1.3*	50	4.5–8.5	95.6±1.7*

*Data represent the mean ± S.D. of four separate samples. Reaction time: 1.5 hr, pH: 7.0. Soybean, Sesame, Rapeseed, Linseed: 10 g/l. Chemical compounds (1.0 g) were dissolved in distilled water, and the solutions were extended to 1000 ml with distilled water. In addition, it was diluted 10 fold, and this 100.0 ml was used for the experiment.

pounds as those in the original solution. Fifty ml of this filtrate was placed in a separatory funnel and 5 ml of *m*-xylene was added to the solution. The mixture was shaken for 1 min. The separated *m*-xylene layer was subjected to gas chromatography (GLC) to assay the concentrations of these compounds. To quantify the evaporation loss of the chemical compounds, control experiments were performed following the same procedure except for the absence of defatted seed. Maximum loss was about 5% (4.7 ± 0.22), although almost no loss was detected in most cases. The removal efficiency of defatted seed was calculated by eliminating the contribution due to the evaporation loss. The assay of chemical compounds was performed on a Shimadzu Model GC-14B gas chromatograph equipped with an electron capture detector and a capillary column (ULBON HR-52, 30 m × 0.53 mm) or Shimadzu Model GC-6A gas chromatograph equipped with a flame ionization detector and glass column (3 m × 3 mm) packed with 20% silicon DC 550 on 60–80 mesh Chromosorb W. Both the column and injection port were maintained at 75°C, and the detector, at 130°C. Active carbon (powder) was tested by the same procedure as defatted seed. The direct reaction with isolated spherosomes was done to compare defatted seed with spherosomes.

Isolation of Spherosomes —— Spherosomes were isolated according to a modification of the procedure of Moreau *et al.*[14] Five g defatted seed was ground in 40 ml grinding medium with a mortar and pestle. The paste was filtered through four layers of cheesecloth, and the filtrate was centrifuged at 30000 *g* for 20 min. The spherosome pad was removed from the surface with a spatula. It was washed by resuspension in 40 ml fresh medium, and recentrifugation at 30000 *g* for 20 min. This process was repeated four more times; the final pellet was the spherosome fraction.

Light Microscopy —— 0.1% Sudan III in glycerin–ethanol (1 : 1) was used to stain lipids. The observation was carried out under a Lica DMLS optical microscope.

Laser Microscopy —— The spherosome fractions were placed on glass slides and mounted in water. They were observed with a Olympus BX50WI laser scanning microscope.

Statistical Analysis —— Values are shown as means ± S.D. Data were analyzed using one-way ANOVA and, when appropriate, by a Student-Newman-Keul test. Results were considered significant at $p < 0.05$.

RESULTS

Table 1 shows the removal efficiencies of the deffated seeds for chloroform, dichloromethane and trichloroethylene at reaction time 90 min. The average removal efficiency for chloroform by soybean, rapeseed, linseed and sesame were 68.6, 72.4, 87.6 and 52.4%, respectively. Similar adsorption rate for dichloromethane were seen. For trichloroethylene, a large removal efficiency was for all 4 defatted seed types.

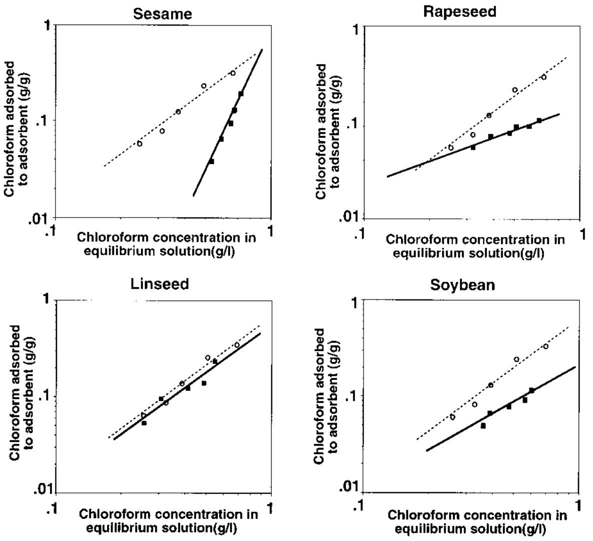

Fig. 1. Freundlich's Adsorption Isotherm for Chloroform

Data represent the mean ± S.D. of three separate samples. Reaction time: 6 hr, $CHCl_3$: 1 g/l, pH: 7. Chloroform 1.0 g was dissolved in distilled water, and the solution was extended to 1000 ml with distilled water. 100.0 mlwas used for the experiment. Defatted seed was added from 0.1 to 1.0 g. ■——■ Activated carbon

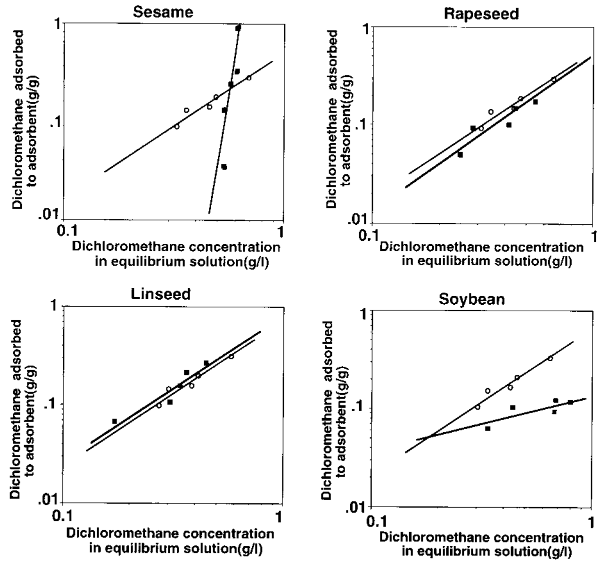

Fig. 2. Freundlich's Adsorption Isotherm for Dichloromethane

Data represent the mean ± S.D. of three separate samples. Reaction time: 6 hr, $CHCl_3$: 1 g/l, pH: 7. Dichloromethane 1.0 g was dissolved in distilled water, and the solution was extended to 1000 ml with distilled water. 100.0 ml was used for the experiment. Defatted seed was added from 0.1 to 1.0 g. ■——■ Activated carbon

The amount of chloroform adsorbed in the equilibrium state was plotted against the concentration of chloroform in solution on a logarithmic scale. Equilibrium state was measured after at least six hours from contact. A linear relationship was obtained, indicating that the adsorption reaction was of a Freundlich type (Fig. 1). Other organic compounds such as dichloromethane (Fig. 2) and trichloroethylene also followed Freundlich isotherms. The adsorption rate for defatted seed was similar to that of activated carbon (powder).

Figure 3 shows the effect of pH on the adsorption of chloroform by defatted seed using buffer solutions at reaction time 90 min. The adsorption was observed over the range of pH 1-11. The same findings were obtained for other organic compounds. Moreover, the adsorption of organochlorine compounds by defatted seed was independent of the particle size of defatted seed and reaction temperature.

Chloroform was successfully removed from tap water with an average removal efficiency of 70% after 60 min when rapeseed was added to tap water that contained 0.0073 mg/l chloroform (Fig. 4). Furthermore, when rapeseed was applied to chemical wastewater that contained 0.1 g/l dichloromethane (Fig. 5), dichloromethane was successfully removed.

Next, we investigated the removal mechanism. In general plants store lipids in fat bodies or spherosomes. The fat bodies are not found in the

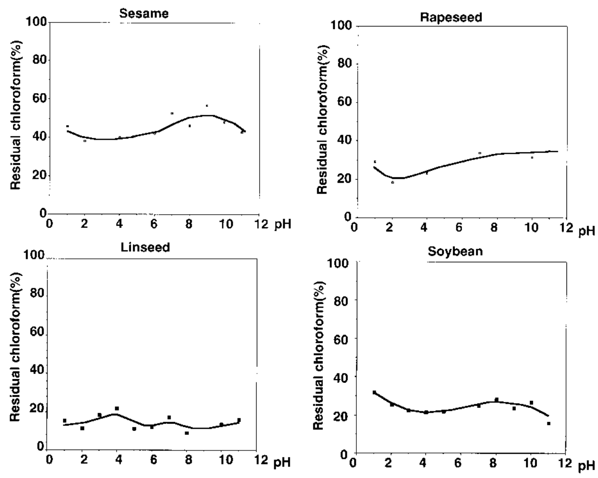

Fig. 3. Effect of pH on the Adsorption of Chloroform by Defatted Seed

Data represent the mean ± S.D. of three separate determinations. Rice bran: 10 g/l, CH_2Cl_2: 0.1 g/l, pH: 7. Dichloromethane 1.0 g was dissolved in buffer solution, and the solution was extended to 1000 ml with buffer solution. In addition, it was diluted 10 fold, and this 100.0 ml was used for the experiment. Defatted seed (1.0 g) was added. Each solution of HCl, citric acid-phosphate buffer and carbonate buffer was used for the preparation of pH1-2, pH3-7 and pH8-11 solutions, respectively.

defatted seed since it is a defatted sample. Thus, spherosomes were considered. The uptake by spherosomes was examined by the reaction of sample with soybean oil. The red color stained with sudan III was confirmed only in spherosomes after treat-

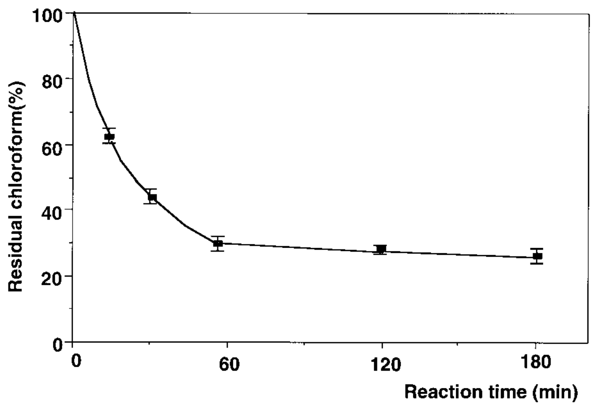

Fig. 4. Removal Efficiency of Rapeseed for Chloroform from Tap Water

Data represent the mean ± S.D. of four separate samples. Tap water that contained 0.0073 mg/l chloroform was used as water sample. Rapeseed: 2 g/l, pH: 6.8.

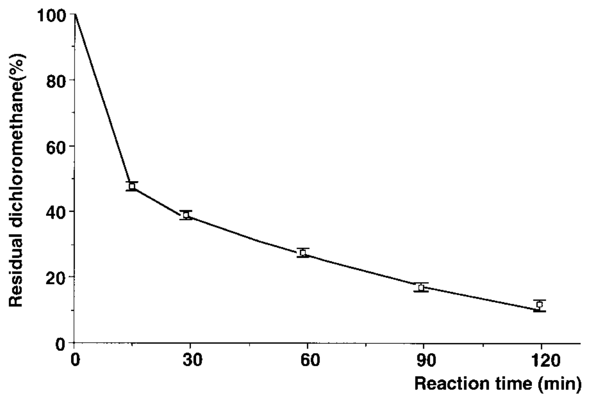

Fig. 5. Removal Efficiency of Rapeseed for Dichloromethane Added to Chemical Wastewater

Data represent the mean ± S.D. of three separate samples. Rice bran: 10 g/l, CH_2Cl_2: 0.1 g/l, pH: 10. Dichloromethane at 1.0 g was dissolved in chemical wastewater, and the solution was extended to 1000 ml with chemical wastewater. In addition, it was diluted at 10 fold, and 100.0 ml was used for the experiment. Rapeseed (1.0 g) was added.

ment with soybean oil on a light micrograph. In addition, it was examined using anthracene as a fluorescent compound to clarify the uptake by spherosomes. Table 2 shows the removal efficiency of this compound by 4 kinds of defatted seeds. Anthracene in solution at 2.5 mg/l was removed with 55.6% efficiency by soybean, and 77.8% by rapeseed. Fluorescence of anthracene can be detected only in spherosomes after treatment with anthracene on a laser micrograph. This clearly shows the uptake of anthracene by spherosomes. It was further examined by the direct reaction of isolated spherosomes and chemical compounds to confirm this mechanism. Table 3 shows the removal efficiencies of spherosomes isolated from 5 g defatted seed for organochlorine compounds. The removal by spherosomes was similar to that of defatted seed. This finding shows directly that the organochlorine compounds are taken into spherosomes.

DISCUSSION

Figure 1 shows that the adsorption rate for defatted seed was similar to that of activated carbon (powder) except for sesame. Thus, we examined whether the adsorption mechanism of organochlorine compounds by defatted seed was equivalent to activated carbon. Activated carbon has been predominantly used in the treatment of volatile organic compounds in drinking water as an adsorbent.[2] The adsorption of dichloromethane to rapeseed was independent of the particle size of rapeseed and reaction temperature. Methylene blue and iodine have been successfully used to check the adsorption efficiency of activated carbon. Defatted seed was not effective in adsorbing either. These findings show

Table 2. Removal Efficiency of Defatted Soybean, Sesame, Rapeseed or Linseed for Anthracene

Substance	Soybean			Sesame		
	Concentration (mg/l)			Concentration (mg/l)		
	Before treatment	After treatment	Removal efficiency (%)	Before treatment	After treatment	Removal efficiency (%)
Anthracene	2.50	0.91–1.10	55.6±2.0*	2.50	0.46–0.84	71.3±3.7*
Substance	Rapeseed			Linseed		
	Concentration (mg/l)			Concentration (mg/l)		
	Before treatment	After treatment	Removal efficiency (%)	Before treatment	After treatment	Removal efficiency (%)
Anthracene	2.50	0.41–0.55	77.8±1.8*	2.50	0.82–0.98	59.0±1.2*

*Data represent the mean ± S.D. of four separate samples. Soybean, Sesame, Rapeseed, Lineseed: 10 g/l, Reaction time: 1.5 hr, Anthracene: 2.50 mg/l.

Table 3. Removal Efficiency of Spherosome Isolated from Defatted Seed for Organochlorine Compounds

[Rapeseed]

Substance	Concentration (mg/l)		Removal efficiency (%)
	Before treatment	After treatment	
Dichloromethane	100	29–34	66.6±2.1*
Chloroform	100	24–39	66.6±5.0*
Trichloroethylene	50.0	19–26	72.2±6.5*

[Lineseed]

Substance	Concentration (μg/l)		Removal efficiency (%)
	Before treatment	After treatment	
Dichloromethane	100	28–34	63.6±3.1*
Chloroform	100	25–38	68.0±6.0*
Trichloroethylene	50.0	18–25	78.2±6.5*

*Data represent the mean ± S.D. of four separate samples. Reaction time: 1.5 hr, pH: 7.0. All spherosome obtained from defatted seed (5 g) were used for this experiment.

that the mechanism of adsorption by defatted seed is different from that of activated carbon. The special affinity for the removal substances must be related to the removal mechanism. Our investigation showed that soybean oil and anthracene were accumulated within spherosomes after incubation. Furthermore, it was confirmed that the spherosomes isolated from defatted seed were effective in removing organochlorine compounds (Table 3). Based on these findings, we concluded that the removal of organochlorine compounds by defatted seed depends on the uptake of these compounds into spherosomes. Next, the adsorption to defatted seed was observed over the range of pH 1-11 (Fig. 2). The function of spherosomes is not known.

The application examples of rapeseed to tap water (Fig. 4) and chemical wastewater (Fig. 5) demonstrate practical uses. Defatted seed is the residue from the process of extracting edible oil, and is therefore a waster product. This process also offers a significant use for defatted seed in terms of recycling. From this perspective, the use of defatted seed as adsorbents is effective.

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