Studies on Defatted Seed Removal Efficiency for Organochlorine Compounds

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Defatted seeds were evaluated for effective adsorption of organochlorine compounds such as chloroform, dichloromethane, and trichloroethylene. The amounts of these compounds adsorbed were plotted against the equilibrium concentration of substances in solution on a logarithmic scale. A linear relationship was obtained, indicating that the adsorption reactions were of the Freundlich type. The removal of these organochlorine compounds by defatted seed was attributed to the uptake by intracellular particles called spherosomes.

Keywords: *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 nonintentionally by chlorination. Chloroform shows carcinogenicity in animal experiments. However, it is impossible to eliminate these chemical substances from the environment for as long as disinfection of tap water by chlorination 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 (Hager et al., 1965; Singley et al., 1979; Wood et al., 1980; Symons, 1978), photochemical decomposition by ultraviolet irradiation (Beltran, 1994; Latt, 1994; Glaze et al., 1987; Haag, 1992; Tanaka, 1997; Yamazaki, 1994), and aeration have usually been used. One problem with the use of activated carbon is cost; another is consistency. Lykins (1980) reviewed the treatment data generated from the Ohio river during 1976 and 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 such as TiO_2, PtO_2, and IrO_2. (Robin, 1982). Joyce (1979) reported that trichloroethylene was removed through an ammonia-stripping tower with an air-to-water ratio of approximately 3000 to 1, with an efficiency of removal ranging from 69 to 90%. McCarty's group (1979) reported 94% removal of tetrachloroethylene (average influent concentration of 2.8 μg/L) in ammonia-stripping towers fed with highly treated wastewater. The aeration ppprocess is based on transferring chemicals from water into the atmosphere through the water surface without treatment. Considering the potential impact with regard to air pollution, this method is also flawed. Based on this information, we studied several adsorbents to find an effective alternative to activated carbon.

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 (waste) from food oil extraction. A 100-mL sample solution including chemical compounds was placed in a 100-mL glass-stoppered Erlenmeyer 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 compounds as those in the original solution. This filtrate (50 mL) 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 (GC) 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 ± 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 was maintained at 130 °C. Active carbon (powder, Takeda Co.) was tested by the same procedure as defatted seed. The direct reaction with isolated spherosomes was done to compare defatted seed with spherosomes.

Sample Solution. Chemical compound (1.0 g) was dissolved in distilled water, and the solution was extended to 1000 mL with distilled water. In addition, it was diluted 10-fold, and 100.0 mL was used for the experiment.

Isolation of Spherosomes. Spherosomes were isolated according to a modification of the procedure of Moreau et al.

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

	Soybean			Sesame		
	concentration (mg/L)			concentration (mg/L)		
substance	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
	Rapeseed			Linseed		
	concentration (mg/L)			concentration (mg/L)		
substance	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

[a] Removal efficiency data represent the mean ± SD of four separate samples. Reaction time, 1.5 h; pH, 7.0. Soybean, sesame, rapeseed, linseed, 10 g/L.

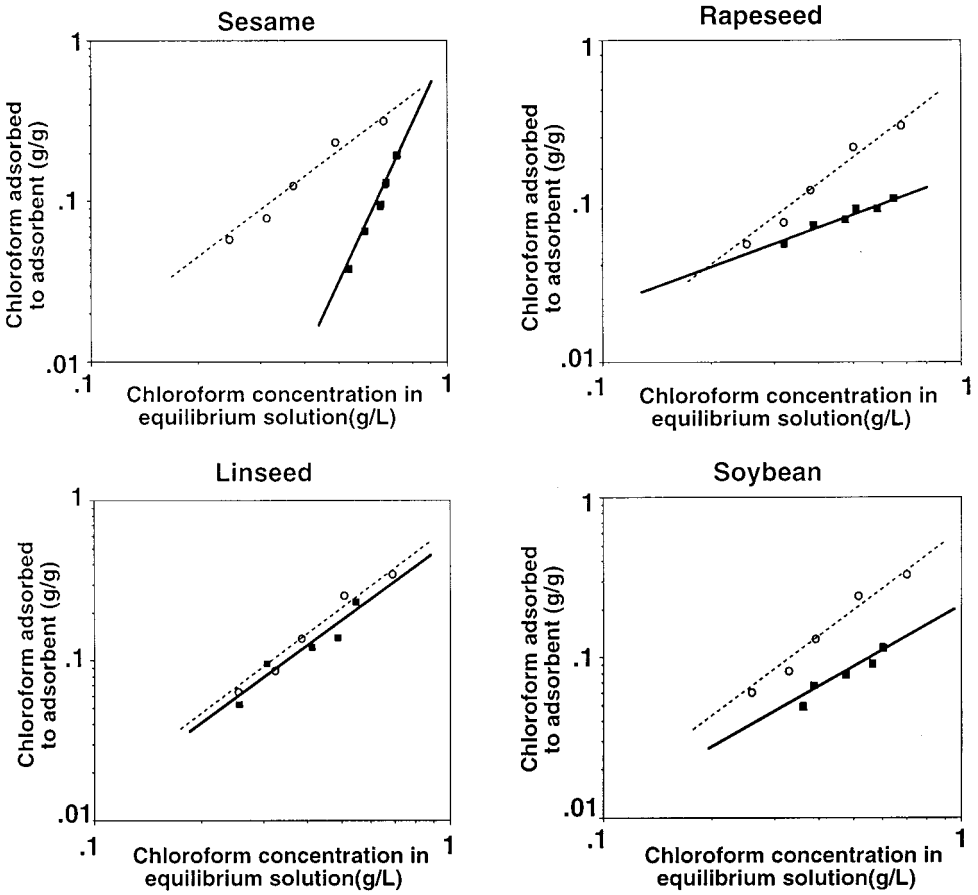

Figure 1. Freundlich's adsorption isotherm for chloroform. Data represent the mean ± SD of three separate samples. Reaction time, 6 h; $CHCl_3$, 1 g/L; pH, 7. ■, Activated carbon.

(1980). Defatted seed (5 g) was ground in 40 mL of grinding medium (20 mM sodium succinate, pH 5.6, containing 10 mM $CaCl_2$) with a mortar and pestle. The paste was filtered through four layers of cheesecloth, and the filtrate was centrifuged at 30 000*g* for 20 min. The spherosome pad was removed from the surface with a spatula. It was washed by resuspension in 40 mL of fresh medium, and recentrifuged at 30 000*g* for 20 min. This process was repeated four more times; the final pellet was the spherosome fraction.

Light Microscopy. Sudan III (0.1%) 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 an Olympus BX50WI laser scanning microscope.

Statistical Analysis. Values are shown as means ± SD. 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

Adsorption Rate. Table 1 shows the defatted seeds' removal efficiencies for chloroform, dichloromethane,

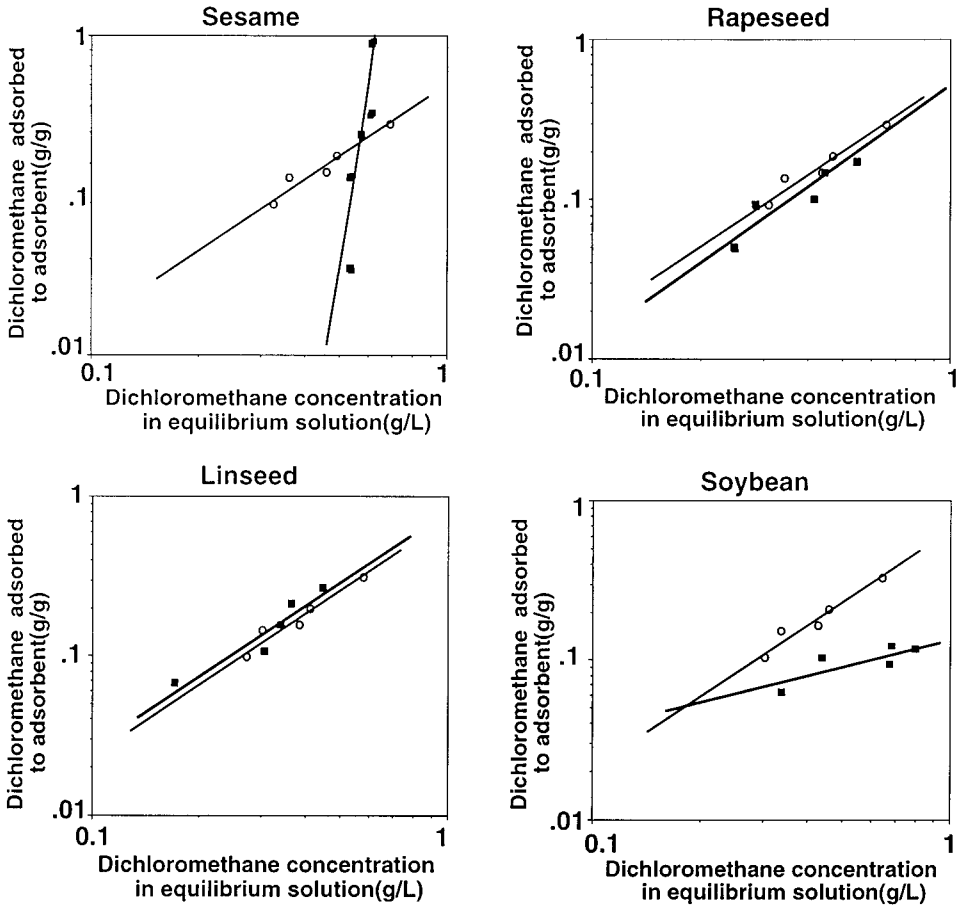

Figure 2. Freundlich's adsorption isotherm for dichloromethane. Data represent the mean ± SD of three separate samples. Reaction time, 6 h; $CHCl_3$, 1 g/L; pH, 7. ■, Activated carbon.

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

Adsorption Isotherm. 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 6 h after contact. A linear relationship was obtained, indicating that the adsorption reaction was of a Freundlich type (Figure 1). Other organic compounds such as dichloromethane (Figure 2) and trichloroethylene also followed Freundlich isotherms. The adsorption rate for defatted seed was similar to that of activated carbon (powder).

Effect of pH on Adsorption. 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 the defatted seed and the reaction temperature (Table 2).

Application to Sample. 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 (Figure 4). Furthermore, when rapeseed was applied to chemical wastewater that contained 0.1 g/L dichloromethane (Figure 5), dichloromethane was successfully removed.

Adsorption Mechanism. Next, we investigated the removal mechanism. In general, plants store lipids in fat bodies or spherosomes. The fat bodies are not found in the defatted seed because 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 treatment 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 3 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% efficiency 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. Uptake was further examined by the direct reaction of isolated spherosomes and chemical compounds to confirm this mechanism. Table 4 shows the removal efficiencies of spherosomes isolated from 5 g of 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.

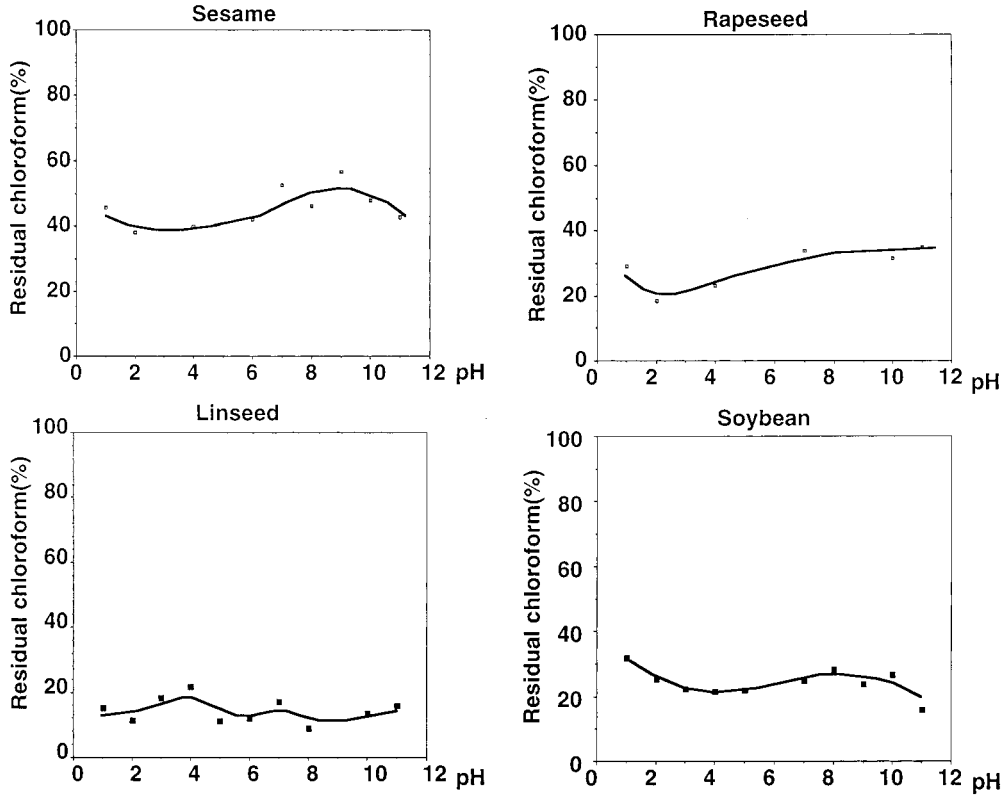

Figure 3. Effect of pH on the adsorption of chloroform by defatted seed. Data represent the mean ± SD of three separate determinations. Rice bran, 10 g/L; CH_2Cl_2, 0.1 g/L; pH, 7. Dichloromethane (1.0 g) was dissolved in buffer solution, and the solution was extended to 1000 mL with buffer solution. 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 pH 1−2, pH 3−7, and pH 8−11 solutions, respectively.

Table 2. Effect of Reaction Temperature on the Adsorption of Dichloromethane by Rapeseed

reaction temperature (°C)	dichloromethane (mg/L)		removal efficiency (%)
	before treatment	after treatment	
4	100	21.8−25.2	75.9 ± 1.6[a]
10	100	21.2−23.8	77.6 ± 1.3[a]
20	100	21.5−26.1	76.9 ± 3.0[a]

[a] Data represent the mean ± SD of four separate determinations. Rice bran, 10 g/L; reaction time, 1.5 h; pH, 7.0.

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 (Singley, 1979). 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 methylene blue or iodine. These findings show that the mechanism of adsorption by defatted seed is different from that by 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 4). Based

Table 3. Removal Efficiency of Defatted Soybean, Sesame, or Linseed for Anthracene[a]

	Soybean			Sesame		
	concentration (mg/L)			concentration (mg/L)		
substance	before treatment	after treatment	removal efficiency (%)	before treatment	after treatment	removal efficiency (%)
anthracene	2.5	0.91−1.10	55.6 ± 2.0	2.50	0.46−0.84	71.3 ± 3.7
	Rapeseed			Linseed		
	concentration (mg/L)			concentration (mg/L)		
substance	before treatment	after treatment	removal efficiency (%)	before treatment	after treatment	removal efficiency (%)
anthracene	2.5	0.41−0.55	77.8 ± 1.8	2.50	0.82−0.98	59.0 ± 1.2

[a] Removal efficiency data represent the mean ± SD of four separate samples. Soybean, sesame, rapeseed, linseed, 10 g/L. Reaction time, 1.5 h; anthracene, 2.5 mg/L.

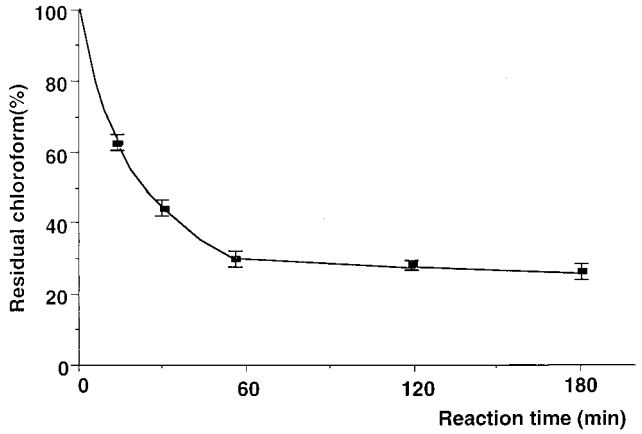

Figure 4. Removal efficiency of rapeseed for chloroform from tap water. Data represent the mean ± SD of four separate samples. Tap water that contained 0.0073 mg/L chloroform was used as water sample. Rapeseed, 2 g/L; pH, 6.8.

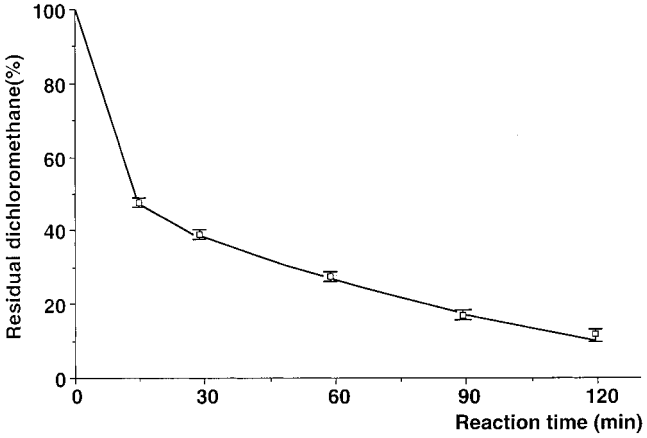

Figure 5. Removal efficiency of rapeseed for dichloromethane added to chemical wastewater. Data represent the mean ± SD of three separate samples. Rice bran, 10 g/L; CH_2Cl_2, 0.1 g/L; pH, 10. Dichloromethane at 1.0 g was dissolved in chemical wastewater, and the solution was extended to 1000 mL with chemical wastewater. In addition, it was diluted 10-fold, and 100.0 mL was used for the experiment. Rapeseed (1.0 g) was added.

Table 4. Removal Efficiency of Spherosome Isolated from Defatted Seed for Organochlorine Compounds

Rapeseed			
	concentration (mg/L)		
substance	before treatment	after treatment	removal efficiency (%)
dichloromethane	100	29−34	66.6 ± 2.1[a]
chloroform	100	24−39	66.0 ± 5.0[a]
trichloroethylene	50	19−26	72.2 ± 6.5[a]
Linseed			
	concentration (mg/L)		
substance	before treatment	after treatment	removal efficiency (%)
dichloromethane	100	28−34	63.6 ± 3.1[a]
chloroform	100	25−38	68.0 ± 6.0[a]
trichloroethylene	50	18−25	78.2 ± 6.5[a]

[a] Data represent the mean ± SD of four separate samples. Reaction time, 1.5 h; pH, 7.0. All spherosome obtained from defatted seed (5 g) were used for this experiment.

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 (Figure 2). The function of spherosomes is not known.

The application examples of rapeseed to tap water (Figure 4) and chemical wastewater (Figure 5) demonstrate practical uses. Defatted seed is the residue from the process of extracting edible oil, and is therefore a waste 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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