Efficiency of Rice Bran for Removal of Organochlorine Compounds and Benzene from Industrial Wastewater

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Rice bran was found to effectively adsorb several organic compounds, such as dichloromethane, chloroform, carbon tetrachloride, trichloroethylene, tetrachloroethylene, and benzene. Equilibrium adsorption isotherms conformed to the Freundlich type (log−log linear). The adsorption of dichloromethane and chloroform by rice bran was observed over the range of pH 1−11. Therefore, rice bran is applicable for treatment of wastewater over a wide pH range. Dichloromethane was successfully removed from water samples with an average removal efficiency of 70% after 60 min when rice bran was added to water samples containing from 0.006 to 100 mg/L dichloromethane. The removal of these organochlorine compounds and benzene by rice bran was attributed to the uptake by intracellular particles called spherosomes. Here, we report the results of a fundamental study of the efficiency of rice bran for removal of organochlorine compounds and benzene using a batch system on the laboratory scale, and describe elucidation of the mechanism of removal of these compounds by rice bran.

Keywords: *Dichloromethane; chloroform; benzene; rice bran; spherosome*

INTRODUCTION

In 1993, the Environment Agency of Japan brought into effect regulations concerning organochlorine compounds such as dichloromethane and tetrachloroethylene. To protect water sources, it is important to keep the concentrations of these compounds in groundwater as low as possible. To remove these compounds from chemical and industrial wastewater, adsorption on activated carbon (*1*−*4*), photochemical decomposition by ultraviolet irradiation (*5*−*8*), or aeration have usually been used. One problem with the use of activated carbon is its cost. Lykins et al. (*9*) reviewed the treatment data generated for the Ohio river from 1976 to 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 (*10*). Joyce (*11*) 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 (*12*) 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. This method is, however, flawed from the viewpoint of air pollution. On the basis of this information, we studied several adsorbents to find an effective alternative.

MATERIALS AND METHODS

Materials. Rice bran was purchased at a local market. Bentonite (colloidal hydrated aluminum silicate, predominant cation: Al^{3+}, Si^{4+}), charcoal bone (charcoal from bone), kaolin (hydrated aluminum silicate), diatomaceous earth (kieselgur), Japanese acid clay (hydrated aluminum silicate), and activated carbon (powder, coal base carbon) were purchased for the practical grade from Wako Pure Chemical Industries Ltd. (Amagasaki, Japan). Dichloromethane, chloroform, carbon tetrachloride, trichloroethylene, and benzene standard were purchased for water analysis from Wako. Chemical compounds recently noted for their toxicity were chosen.

Procedure for Removal. Samples of 100 mL of organochlorine compound solutions were placed into 100-mL glass-stoppered Erlenmyer flasks, to which 0.1−1 g of rice bran was then added. The samples were mixed with a stirrer. The reaction mixture was filtered through filter paper (quantitative ashless no. 5A Toyo Roshi, Ltd., Japan) to remove the rice bran. The initial 10 mL of filtrate was discarded because of the adsorption of chemical compounds by the filter paper. In control samples lacking rice bran, the subsequent filtrate after the discarded portion contained the same amount of chemical compounds as 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 analyze these compounds (*13*). To assess the evaporation loss of the chemical compounds, control experiments were performed according to the same procedure, except for the absence of rice bran (control). Maximum loss was about 5% (4.7 $\pm$ 0.22), although almost no loss was detected in most cases. The removal efficiency of rice bran was calculated by eliminating the contribution due to 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 $\times$ 0.53 mm) or Shimadzu model GC 6A gas chromatograph equipped with a flame ionization detector and glass column (3 m $\times$ 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. Other adsorbents such as bentonite, charcoal bone, kaolin, diatomaceous earth, Japanese acid clay, and activated carbon were tested by the same procedure as rice bran. Treatment with soybean oil was carried out by the same procedure for

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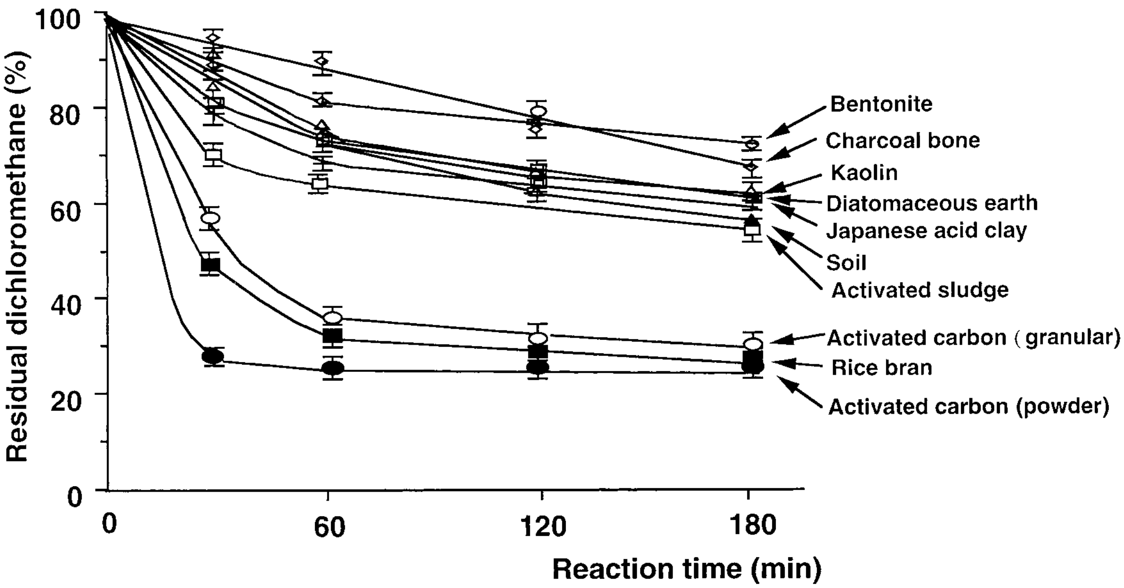

Figure 1. Efficiency of removal of dichloromethane by various kinds of absorbents. Data represents the means ± SD of three separate samples. Adsorbent, 0.1 g/L; CH_2Cl_2, 0.1 g/L; pH, 7. Dichloromethane (1.0 g) was dissolved in distilled water, and the solution was made up to 1000 mL with distilled water. In addition, it was diluted 10-fold, and 100.0 mL was used for the experiment.

removal of organochlorine compounds. The experimental conditions were soybean oil, 0.1 g/L; and reaction time, 1.5 h.

Isolation of Spherosomes. Spherosomes were isolated according to our improved method based on that of Moreau et al. (*14*). Samples of 5 g of rice bran or defatted rice bran were ground in 40 mL of grinding medium (consisting of 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 and washed by resuspending in 40 mL of fresh medium. This suspension was recentrifuged at 30 000*g* for 20 min. This process was repeated four more times, and the final pellet was used as the spherosome fraction. About 0.34 g (0.343 ± 0.016) of spherosomes was extracted from 5 g of rice bran.

Light Microscopy. Light microscopy was used to examine the uptake of oil into spherosomes. The spherosome fractions were placed on glass slides and Sudan III (0.1%) in glycerin−ethanol (1:1) was dropped onto the fractions to stain lipids. Samples were mounted in glycerin after blotting off of excessive reagent. Observations were carried out under a Lica DMLS optical microscope at a magnification of 400×.

Laser Microscopy. The isolated spherosome fractions were placed on glass slides and mounted in water to observe the shape of spherosomes and the fluorescence of anthracene with an Olympus BX50WI laser scanning microscope at a magnification of 400×.

Defatted Rice Bran. Defatted rice bran was prepared by removing oils by Soxhlet extraction with ether. Complete defatting was confirmed by dyeing with Sudan lll. The oil of the rice bran was analyzed with average 18.3% (18.3 ± 1.7). Laser microscopy confirmed that the shape of spherosomes isolated from defatted rice bran was similar to that of intact rice bran.

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

RESULTS AND DISCUSSION

Figure 1 shows dichloromethane removal efficiencies as a function of time for several kinds of adsorbents. Bentonite, charcoal bone, kaolin, diatomaceous earth, Japanese acid clay, soil, and rice bran were examined as adsorbents. Rice bran was the most effective of these adsorbents. The adsorption by rice bran was similar to that by activated carbon (powder). The amount of dichloromethane adsorbed at equilibrium was plotted against the concentration of dichloromethane in solution on a logarithmic scale. Equilibrium was measured after at least 3 h of contact. At equilibrium, the adsorption efficiency of rice bran was higher than that of activated carbon. A linear relationship was obtained, indicating that the adsorption reaction was of a Freundlich type (Figure 2). Figure 3 shows the effects of pH on the adsorption of dichloromethane by rice bran using buffer solutions at a reaction time of 90 min. Adsorption was observed over the range of pH 1−11, and the removal efficiency increased with increasing pH. Therefore, it can be applied for the treatment of industrial wastewater over a wide pH range. The results shown in Tables 1 and 2 indicate that the adsorption of dichloromethane by rice bran was independent of the particle size of rice bran and the reaction temperature. Chemical compounds with high lipophilicity were found to be

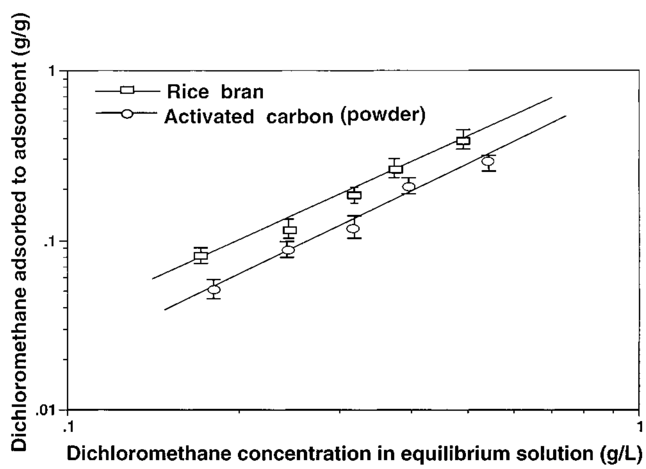

Figure 2. Freundlich's adsorption isotherm of dichloromethane. Data represent the means ± SD of three separate samples. Reaction time, 6 h; CH_2Cl_2, 1 g/L; pH, 7. Dichloromethane (1.0 g) was dissolved in distilled water, and the solution was made up to 1000 mL with distilled water. A 100.0 mL portion was used for the experiment. From 0.1 to 1.0 g of rice bran was added.

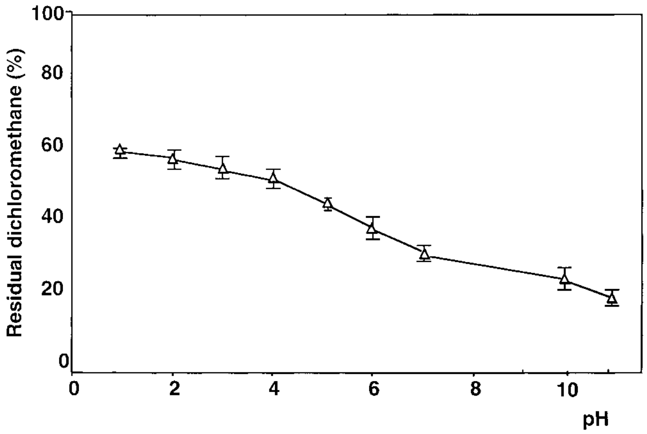

Figure 3. Effects of pH on the adsorption of dichloromethane by rice bran. Data represent the mean ± SD of three separate samples. Rice bran, 10 g/L; CH_2Cl_2, 0.1 g/L; pH, 7. Dichloromethane (1.0 g) was dissolved in buffer solution, and the solution was made up to 1000 mL with buffer solution. In addition, it was diluted 10-fold, and 100.0 mL was used for the experiment. A 1.0-g aliquot of rice bran was added. Solutions of HCl, citric acid−phosphate buffer, and carbonate buffer were used for preparation of pH 1−2, pH 3−7, and pH 8−11 solutions, respectively.

Table 1. Effects of Rice Bran Particle Size on the Adsorption of Dichloromethane

particle size (μm)	dichloromethane (mg/L) before treatment	dichloromethane (mg/L) after treatment	removal efficiency (%)
<150	100	25.8−28.0	73.1 ± 1.2[a]
150−300	100	22.3−27.2	75.8 ± 2.6[a]
300−500	100	21.2−22.9	78.2 ± 1.0[a]
500−850	100	19.5−27.1	75.9 ± 4.0[a]
>850	100	25.5−28.8	73.4 ± 5.4[a]
whole rice bran	100	18.8−23.4	78.9 ± 3.0[a]

[a] Data represent the means ± SD of four separate samples. Rice bran, 10 g/L; reaction time, 1.5 h; pH, 7.0. Rice bran with different particle sizes was prepared using Japanese industrial standard mesh. Dichloromethane (1.0 g) was dissolved in distilled water, and the solutions were made up 1000 mL with distilled water. The solution was diluted 10-fold, and 100.0 mL was used for the experiment. A 1.0-g portion of rice bran was added.

Table 2. Effects of Reaction Temperature on the Adsorption of Dichloromethane by Rice Bran

reaction temperature (°C)	dichloromethane (mg/L) before treatment	dichloromethane (mg/L) after treatment	removal efficiency (%)
4	100	22.8−25.0	76.8 ± 2.6[a]
10	100	21.2−23.9	77.2 ± 1.0[a]
20	100	20.5−27.1	77.9 ± 4.0[a]

[a] Data represent the means ± SD of four separate samples. Rice bran, 10 g/L; reaction time, 1.5 h; pH, 7.0 Dichloromethane (1.0 g) was dissolved in distilled water, and the solution was made up to 1000 mL with distilled water. The solution was diluted 10-fold, and 100.0 mL was used for the experiment. A 1.0-g portion of rice bran was added.

removed easily by rice bran. When rice bran was applied to chemical wastewater containing 0.1 g/L of dichloromethane (Figure 4), the removal efficiency was slightly better than that in pure water (Figure 1) because the pH of the wastewater was 10. Furthermore, chloroform was successfully removed from tap water containing 0.0064 mg/L of chloroform with an average removal efficiency of 70% after 60 min when rice bran was added (Figure 5). These observations indicated that rice bran

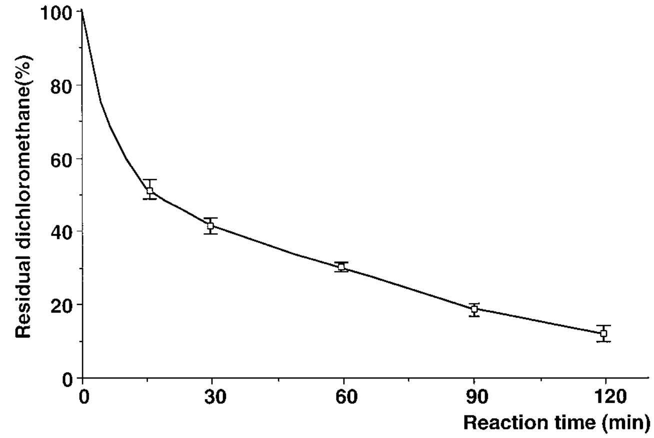

Figure 4. Efficiency of removal of dichloromethane in chemical wastewater by rice bran. Data represent the mean ± SD of three separate determinations. Rice bran, 10 g/L; CH_2Cl_2, 0.1 g/L; pH, 10. Dichloromethane (1.0 g) was dissolved in chemical wastewater, and the solution was made up 1000 mL with chemical wastewater. In addition, it was diluted 10-fold, and this 100.0 mL was used for the experiment. A 1.0-g aliquot of rice bran was added.

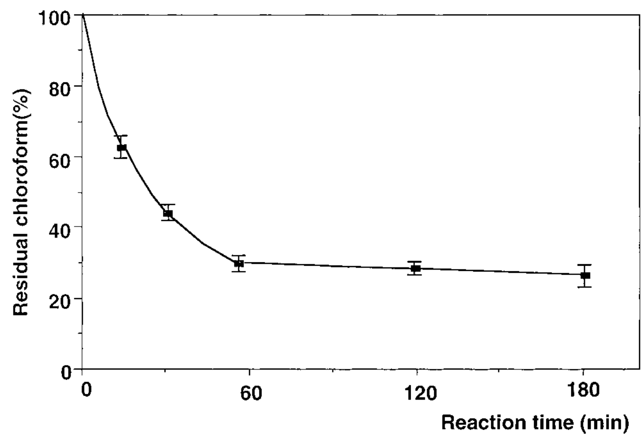

Figure 5. Efficiency of removal of chloroform from tap water by rice bran. Data represent the means ± SD of four separate samples. Tap water containing 0.0064 mg/L of chloroform was used as water samples. Rice bran, 2 g/L; pH, 6.8.

could be used for treatment of chemical wastewater and tap water. Sorption of other organic compounds such as chloroform, carbon tetrachloride, and trichloroethylene, and benzene also conformed to the Freundlich isotherm (Figure 6). Table 3 shows $1/n$ and k values obtained from the Freundlich isotherm. Almost equal values were observed for the $1/n$ and k values of rice bran and activated carbon except for chloroform and trichloroethylene. These values indicated that the removal of the chloroform and trichloroethylene by rice bran was effective at high concentrations.

Next, we investigated the mechanism of removal. We examined whether the adsorption of chemical compounds by rice bran was equivalent to that of activated carbon. Activated carbon has been predominantly used for removal of volatile organic compounds in drinking water as an adsorbent (*1, 2*). The surface area of activated carbon is very large, on the order of 300−900 m^2/g (*15*), making it effective for adsorbing chemical compounds. On the other hand, the surface area of rice bran is very small (our value measured by the Kr gas adsorption method was 0.14 m^2/g). Methylene blue and iodine have been used successfully to check the adsorption efficiency of activated carbon; however, rice bran

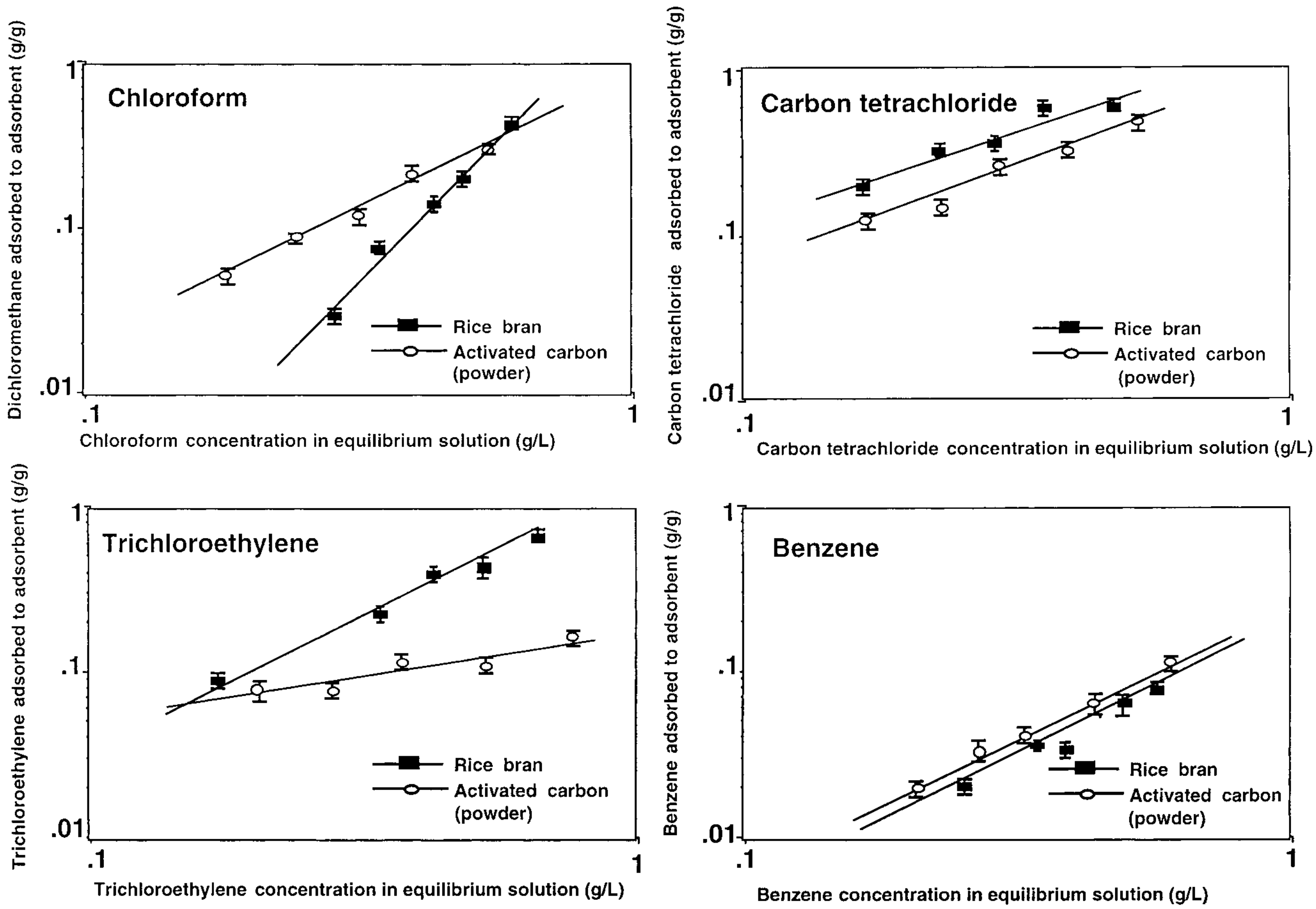

Figure 6. Freundlich's adsorption isotherms of dichlororomethane, chloroform, carbon tetrachloride, trichloroethylene, and benzene. Data represent the means ± SD of three separate determinations. Reaction time, 6 h; chemical compound, 1.0 g/L; pH, 7. Chemical compound (1.0 g) was dissolved in distilled water, and the solutions were made up to 1000 mL with distilled water. A 100.0-mL portion was used for the experiment. From 0.1 to 1.0 g of rice bran was added.

Table 3. Isotherm Constants of Chloroform, Dichloromethane, Carbon Tetrachloride, Trichloroethylene, and Benzene Adsorption on Rice Bran or Activated Carbon

	rice bran		activated carbon	
substance	$1/n$	K	$1/n$	K
chloroform	1.14	8.99	0.43	5.88
dichloromethane	0.35	3.86	0.38	4.31
carbon tetrachloride	0.44	6.50	0.40	6.74
trichloroethylene	1.30	11.8	0.29	8.10
benzene	0.43	5.20	0.45	6.50

was not effective in adsorbing either. Particle diameters in the rice bran samples were estimated to be in the range 10 − 700 μm (average particle diameter, 244 μm) by the light scattering method. Rice bran is not effective in the removal of phenol or ions such as CN^- and $PO_4{}^{3-}$, but rice bran is very effective in the removal of fat-soluble substances. These findings indicated that the mechanism of adsorption by rice bran is different from that of activated carbon. The special affinity for the removal of substances must be related to the mechanism of removal. Generally, plants store lipids in oil bodies or spherosomes. Spherosomes are organelles rich in lipid, and they differ in morphology and origin from large oil bodies (*16*). The oil body has been suggested to be related to the mechanism of removal, and therefore removal was examined using defatted rice bran. Table 4 shows the efficiency of removal of chloroform, dichloromethane, and benzene by rice bran or defatted rice bran. Defatted rice bran effectively removed these compounds within the range of 76−84% after 90 min, comparable to rice bran, which showed 72−84% removal. This finding indicated that the oil body was not related to the removal of chemicals by rice bran. Next,

Table 4. Efficiency of Removal of Chloroform, Dichloromethane, and Benzene by Rice Bran or Defatted Rice Bran

	rice bran			defatted rice bran		
	concentration (mg/L)			concentration (mg/L)		
substance	before treatment	after treatment	removal efficiency (%)	before treatment	after treatment	removal efficiency (%)
chloroform	100	11.6 − 16.1	84.3 ± 2.1[a]	100	10.4−19.3	76.1 ± 3.5[a]
dichloromethane	100	19.2 − 26.9	77.4 ± 2.9[a]	100	6.8−22.1	84.0 ± 3.9[a]
benzene	100	25.1 − 31.1	72.4 ± 3.3[a]	100	10.8−20.3	79.5 ± 3.5[a]

[a] Data represent the means ± SD of four separate samples. Rice bran, 10 g/L; reaction time, 1.5 h; pH, 7.0. Chemical compounds (1.0 g) were dissolved in distilled water, and the solutions were made up to 1000 mL with distilled water. The solution was diluted 10-fold, and 100.0 mL was used for the experiment. A 1.0-g portion of rice bran was added.

Table 5. Efficiency of Removal of Anthrancene by Rice Bran or Defatted Rice Bran

	rice bran			defatted rice bran		
	concentration (mg/L)			concentration (mg/L)		
substance	before treatment	after treatment	removal efficiency %)	before treatment	after treatment	removal efficiency (%)
anthracene	2.50	0.75−0.93	64.6 ± 2.6[a]	2.50	0.05−0.19	95.3 ± 5.7[a]

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

Table 6. Efficiency of Removal of Organochlorine Compounds and Benzene by Spherosomes

	concentration (mg/L)		
substance	before treatment	after treatment	removal efficiency (%)
dichloromethane	100	30−34	67.6 ± 2.1[a]
chloroform	100	25−39	67.0 ± 7.0[a]
trichloroethylene	50.0	19−26	53.2 ± 7.5[a]
benzene	100	47−54	51.0 ± 3.5[a]

[a] Data represent the means ± SD of three separate samples. Reaction time, 1.5 h; pH, 7.0. Spherosomes obtained from rice bran (5 g) were used for this experiment.

spherosomes were examined; the uptake by spherosomes was studied by a sample reaction with soybean oil. The red color obtained by staining with Sudan III was confirmed only in the spherosomes after treatment with soybean oil by light microscopy. We have already found that pesticides with high lipophilicity are more easily removed by rice bran (*17*). Therefore, the reaction was examined using anthracene as a fluorescent compound with high lipophilicity to clarify the uptake by spherosomes. Table 5 shows the efficiency of removal of this compound by rice bran or defatted rice bran. Anthracene in solution at 2.5 mg/L was removed with 65% efficiency by rice bran, and 95% by defatted rice bran. The fluorescence of anthracence was detected only in the spherosomes after treatment by laser micrography. Uptake was further examined by the direct reaction of isolated spherosomes and chemical compounds to confirm this mechanism. Table 6 shows the efficiency of removal of organochlorine compounds and benzene by spherosomes isolated (0.343 ± 0.016 g) from 5 g of rice bran. The removal by spherosomes was similar to that of rice bran. These observations indicated directly that organochlorine compounds and benzene are taken up into spherosomes. Sphrosomes are intracellular oil-containing particles about 1 μm in diameter, with an osmiophilic matrix, and are bound by unusual single-line membranes (*18*). We regarded these special membranes to be related to the uptake of chemical compounds into spherosomes. The chemical nature of the spherosomes is uncertain. Based on these results, we concluded that removal of organochlorine compounds and benzene by rice bran is dependent on the uptake of these compounds into spherosomes. In this paper, we report our fundamental study on the removal of organochlorine compounds and benzene by rice bran using a batch system on the laboratory scale and our elucidation of the mechanism of removal of these compounds by rice bran. We are now investigating the removal efficiency using packed columns for practical use.

Rice bran is a byproduct of making polished rice from brown rice. Therefore, rice bran is very inexpensive, with a cost of 1/50 − 1/40 of that of active carbon, and thus its use would significantly lower the cost of wastewater treatment. In addition, the use of rice bran would represent effective utilization of waste matter. Taken together, the findings of this study suggested that the use of rice bran as an adsorbent is an efficient and cost-effective method for removal of organochlorine compounds and benzene from wastewater.

LITERATURE CITED

(1) Dobbs, R. A.; Cohen, J. M. *Carbon Adsorption Isotherms for Toxic Organics*; Wastewater Treatment Division, U.S. Environmental Protection Agency: Cincinnati, OH, 1976.

(2) Singley, J. E.; Beaudet, B. A.; Ervin, A. L. Use of powdered activated carbon for removal of specific compounds. Presented at 99th Annual National AWWA Conference, San Francisco, CA, June, 1979.

(3) Wood, P. R.; Demarco, J. M. *Effectiveness of various adsorbents in removing organic compounds from water*; Ann Arbor Science: Ann Arbor, MI, 1980; pp 85−114.

(4) Symons, J. M. *Interim Treatment Guide for Controlling Organic Contaminants in Drinking Water using Granular Activated Carbon*; Drinking Water Research Division, U.S. Environmental Protection Agency: Cincinnati, OH, January, 1978.

(5) Beltran, F. J.; Carcia Araya, J. F.; Acedo, B. Advance oxidation of atrazine in water−II. Ozonation combined with ultraviolet. *Water Res.* **1994**, *28*, 2165−2174.

(6) Latt, J. D.; Tace, E.; Dore, M. Degradation of Chloroethanes in dilute aqueous solution by H_2O_2/UV. *Water Res.* **1994**, *28*, 2507−2519.

(7) Tanaka, K. Degradation of volatile organochlorine compound, mechanism and application. *J. Jpn. Soc. Water Environ.* **1997**, *20*, 2−5.

(8) Yamazaki, S. Destruction of groundwater contaminations photocatalyst. *Chem. Chem. Ind.* **1994**, *47*, 152−155.

(9) Lykins, B. W. An Overview of the Use of Powdered Activated Carbon for Removal of Trace Organics in Drinking Water. Drinking Water Research Division, U.S. Environmental Protection Agency: Cincinnati, OH, 1980.

(10) Robin, H. B.; Jochen, L.; Michael, G. In vitro analogues of photosystem, combined flash photolytic and conductometric study of light-induced oxygen evolution from water mediated by colloidal RuO2/TiO2. *J. Am. Chem. Soc.* **1982**, *104*, 422−425.

(11) Joyce, M. Delaware solves a water problem. *J. Environ. Health*. **1979**, *42*, 72−74.

(12) McCarty, P. L.; Sutherland, K. H.; Graydon, J.; Reinhard, M. Volatile organic contaminants removal by air stripping. Presented at 99th Annual National AWWA Conference, San Francisco, CA, June, 1979.

(13) Adachi, A.; Kobayashi, T. Comparison of trihalomethane in tap water with and without activated carbon adsorption during the preparation process of tap water. *Bull. Environ. Contam. Toxicol.* **1995**, *54*, 440−443.

(14) Moreau, R. A.; Kitty, F. L.; Huang, H. C. Spherosomes of castor bean endosperm. *Plant Physiol.* **1980**, *65*, 1176−1180.

(15) Tuji, Y. Removal of hazardous substances by adsorption on activated carbon. *PPM* **1996**, *27*, 95−102.

(16) Jelsema, C. L.; Morre, D. J.; Ruddat, M.; Turner, C. Isolation and Characterization of the lipid reserve bodies, spherosomes, from aleurone layers of wheat. *Bot. Gaz.* **1977**, *138*, 138−149.

(17) Adachi, A.; Takagi, S.; Okano, T, Studies on removal efficiency of rice bran for pesticides. *J. Health Sci.* **2001**, *47*, in press.
(18) Yatsu, L. Y.; Jacks, T. J. Spherosome membranes. *Plant Physiol.* **1972**, *49*, 937−943.

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