Studies on Removal Efficiency of Rice Bran for Pesticides

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Rice bran effectively removed pesticides from an aqueous solution. The removal by rice bran was examined using 22 different pesticides. The removal efficiencies varied from 22.2% to 98.8%. The variation in the removal efficiency of different pesticides was studied, and the pesticides with high lipophilicity were found to be easily removed by rice bran. The amount of captan adsorbed was plotted against the equilibrium concentration of substances in solution on a logarithmic scale, and a linear relationship was obtained, indicating that the adsorption reaction was a Freundlich-type. The mechanism of pesticides removal by rice bran was attributed to the uptake into intracellular particles called spherosomes.

Key words —— rice bran, pesticide, spherosome, captan

INTRODUCTION

There are increasing kinds of chemical substances in the environment that cause large social problems. Some of the pesticides are endocrine disrupting compounds.[1–3] However, it is impossible to eliminate these chemical substances from the environment, as long as the use of pesticides continues on its current path for maintaining life. Therefore, it is necessary to limit the chemical substances discharged into the environment as much as possible, and also to remove them as completely as possible. To remove pesticides from water in the environment, photochemical decomposition by ultraviolet irradiation,[4–6] ozonation[7] or alkaline hydrolysis[8,9] have usually been used. One problem with these methods is cost. 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] Furthermore, the problem following ozonation is the degradation product: Ohashi *et al.*[11] reported that in laboratory tests using standard chemicals, it formed substances with higher toxicity than before processing. Based on this information, we studied several adsorbents to find the most effective one. The removal efficiency of bentonite, charcoal bone, kaolin, diatomaceous earth, Japanese acid clay, soil, and rice bran for organochlorine compounds were examined. Rice bran was the most effective of the adsorbents.[12] In this study, we elucidate the removal mechanism of rice bran for pesticides using the batch system in laboratory tests.

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MATERIALS AND METHODS

Procedure for Removal —— Commercial rice bran was used. A 100 ml sample solution of pesticide was placed in a 100 ml glass stoppered Erlenmyer flask, to which 0.1–1 g of rice bran was added. The solution was mixed by a stirrer. The reaction mixture was filtered through filter paper to remove the rice bran. The initial 10 ml of filtrate was discarded because of the adsorption of pesticides 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 those in the original solution. Fifty ml of this filtrate was placed in a separate funnel and 5 ml *m*-xylene was added to the solution. The mixture was shaken for 1 min. The separated *m*-xylene layer was subjected to gas-liquid chromatography (GLC) or high-performance liquid chromatography (HPLC) to assay the concentrations of these compounds. GLC analysis 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). Both the column and injection port were maintained at 75°C, and the detector at 130°C. The analysis by HPLC was carried out in the method of Tonogai.[13] To assess the evaporation loss of the chemical compounds, the control experiments were performed following the same procedure as for the sample treatment, except for the absence of rice bran. The removal efficiency of rice bran was calculated by eliminating the percentage due to evaporation loss. Activated carbon (granular, Wako Pure Chemical Industries, Japan) was tested by the same procedure as rice bran. Treatment with soybean oil was carried out by the same procedure for removal of pesticides. The experimental conditions were as follows: defatted rice bran: 10 g/l, soybean oil: 0.1 g/l, reaction time: 1.5 hr.

Isolation of Spherosomes —— Spherosomes were isolated according to a modification of the procedure of Moreau *et al.*[14] Rice bran (5 g) was ground with a mortar and pestle in a 40 ml grinding medium of 20 mM sodium succinate, pH 5.6, containing 10 mM $CaCl_2$. The paste was filtered through eight 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 resuspending in 40 ml fresh medium, and the resuspension was recentrifuged at 30000 *g* for 20 min. This process was repeated four more times; the final pellet was the spherosome fraction. A yield of spherosome of about 0.34 (0.343 ± 0.016) g was extracted from 5 g rice bran. Isolation of spherosomes from defatted rice bran was performed following the same procedure as for rice bran except the resuspension process was not repeated.

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 in order to observe the shape of spherosomes and the fluorescence of anthracene with an Olympus BX50WI laser scanning microscope.

Defatted Rice Bran —— Defatted rice bran was prepared by removing oils with ether using Soxhlet extraction, and it was used after being completely defatted as confirmed by dyeing in Sudan III. The oil of the rice bran was analyzed and averaged 18.3 (18.3 ± 1.7) w/w%. It was confirmed by laser microscopy that the shape of spherosomes isolated from defatted rice bran was similar to that of intact rice bran.

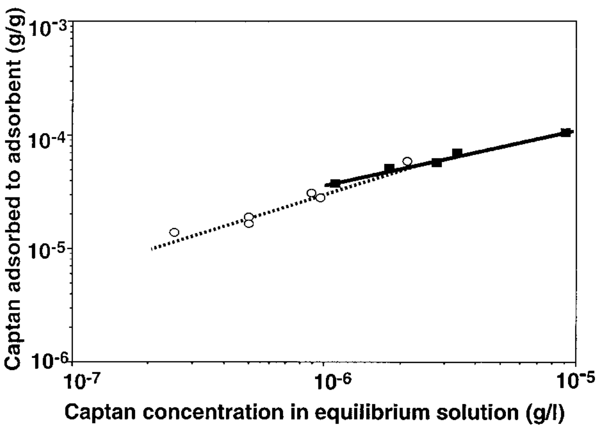

Fig. 1. Freundlich's Adsorption Isotherm of Captan
—■— Rice bran ····○···· Activated carbon (granular)

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

RESULTS AND DISCUSSION

The amount of captan adsorbed in the equilibrium state was plotted against the concentration of captan in solution on a logarithmic scale. Equilibrium was measured after at least three hours from contact. A linear relationship was obtained, indicating that the adsorption reaction was of a Freundlich type (Fig. 1). At equilibrium, the adsorption efficiency of rice bran was higher than that of activated carbon (granular).

Figure 2 shows the effect of pH on the adsorption of captan by rice bran using buffer solutions at a reaction time of 90 min. The adsorption was observed over the range of pH 1–9, and the removal efficiency of captan decreased as the pH was lowered. The decrease in low pH would be attributed to the renaturation of the proteins which constitute spherosomes.

The removal of pesticides by rice bran was examined using 22 different pesticides (Table 1) . The removal efficiencies varied from 22.2% to 98.8%. The cause of the differences in removal efficiency were examined. For the chemical properties of tested pesticides, the following were noted: molecular weight, vapor pressure, melting point and solubility in water. Of these properties, there were no correlations between removal efficiency and molecular weight, vapor pressure or melting point. However,

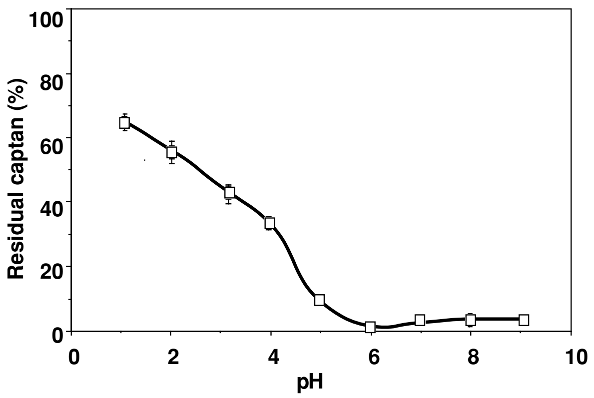

Fig. 2. Effect of pH on the Adsorption of Captan by Rice Bran
Data represent the mean ± S.D. of three separate determinations.
Rice bran: 10 g/l, Captan: 50 μg/l, Reaction time: 1.5 hr
Captan (10.0 mg) was dissolved in methanol 1 ml, the solution was extended to 100 ml with distilled water. In addition, it was adequately diluted with buffer solution, and 100.0 ml was used for the experiment. Each solution of HCl, citric acid-phosphate buffer and carbonate buffer was used for the preparation of pH1–2, pH 3–7 and pH 8–9 solutions, respectively. The rice bran (1.0 g) was added.

there was an inverse correlation seen for the solubility in water (Fig. 3). This finding indicates that the pesticides with high lipophilicity are more easily removed by rice bran. Next, we investigated the removal mechanism.

We examined whether the adsorption mechanism of pesticides by rice bran was equivalent to that of activated carbon. Activated carbon as an adsorbent has been predominantly used in the treatment of volatile organic compounds in drinking water.[15,16] The surface area of activated carbon is very large, on the order of 300–900 m^2/g[17] effective in adsorbing chemical compounds. On the other hand, that of the rice bran is very small (Our measured value by Kr gas adsorption method is 0.14 m^2/g). The adsorption of captan to rice bran was independent of the rice bran particle size and reaction temperature. Methylene blue and iodine have been successfully used to check the adsorption efficiency of activated carbon, however rice bran was not effective in adsorbing either. These findings show 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 removal mechanism. Generally, plants store lip-

Table 1. Removal Efficiency of Rice Bran for Pesticides

Substance	Concentration (μg/l)		Removal efficiency (%)
	Before treatment	After treatment	
Captan	50.0	1.1–1.3	97.0 ± 0.2*
Chlomethoxyfen	50.0	4.2–6.7	87.2 ± 1.2*
CNP	50.0	2.1–2.9	93.5 ± 0.8*
Cypermethrin	2000	123–197	88.2 ± 4.2*
Dichlofluanid	50.0	0.55–1.1	98.8 ± 0.2*
Fenoxycarb	50.0	23–25	51.7 ± 2.4*
Fthalide	50.0	20–24	53.6 ± 3.4*
a-HCH	50.0	7.2–8.9	83.0 ± 2.4*
Hexachlorobenzene	50.0	6.6–7.8	86.4 ± 1.2*
Hexythiazox	50.0	11–14	70.6 ± 0.4*
Kelthane	50.0	2.0–6.9	88.9 ± 3.6*
Methoprene	50.0	9.4–13.2	77.7 ± 4.0*
Methyl-2- Benzimidazole carbamate	50.0	35–40	22.2 ± 5.0*
Oxadiazon	50.0	7.6–13.5	77.5 ± 2.0*
Phoxim	50.0	13–18	66.6 ± 4.4*
Propyzamide	50.0	27–28	39.5 ± 4.4*
Pyrazoxyfen	50.0	11–17	69.6 ± 3.2*
Tetradifon	50.0	2.7–3.3	92.2 ± 1.0*
Chlorobenzilate	50.0	9.2–13.2	77.9 ± 0.9*
Triuram	50.0	22–24	54.8 ± 3.3*
Clofentezine	50.0	1.9–2.6	96.2 ± 0.1*
Chlorothalonil	50.0	2.5–6.5	84.2 ± 0.1*

*Data represent the mean ± S.D. of four separate samples. Rice bran: 10 g/l, Reaction time: 1.5 hr, pH : 7.0. Pesticide (10.0 mg) was dissolved in methanol (1 ml), and the solution was extended to 100 ml with distilled water. In addition, it was adequately diluted with distilled water, and 100.0 ml was used for the experiment. The rice bran (1.0 g) was added.

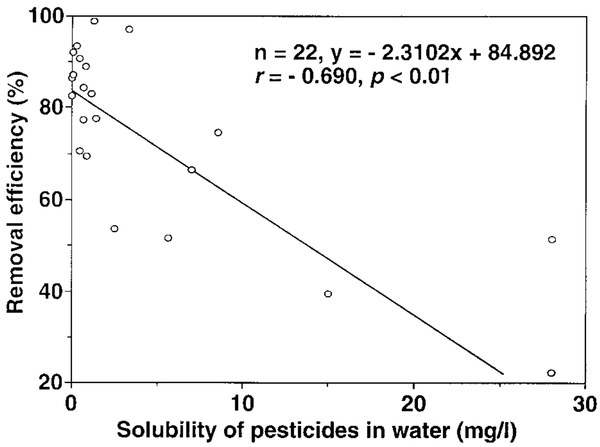

Fig. 3. Correlation between Removal Efficiency and Solubility of Pesticides in Water

Correlation between removal efficiency and solubility of pesticides in water was analyzed using 22 pesticides shown in Table 1.

ids in fat bodies or spherosomes. It was thought that the fat body might be related to the removal mechanism, thus removal was examined using defatted rice bran. Table 2 shows the removal efficiency of captan by both rice bran and defatted rice bran. Defatted rice bran effectively removed this compound by 98.2% after 90 min, comparable to the rice bran, which showed 97.0% removal. This finding shows that the fat body was not related to the removal of chemicals by rice bran. Next, spherosomes were examined; the uptake by spherosomes was studied by a sample reaction with soybean oil. The red color stained with Sudan III was confirmed only in the spherosomes after treatment with soybean oil by light micrography. In addition, the reaction was examined using anthracene as a fluorescent compound to clarify the uptake by spherosomes. Table 3 shows the removal efficiency of this compound by rice bran. Anthracene in solution at 2.5 mg/l was removed with 64.6% efficiency by rice bran, and 95.3% by defatted rice bran. The fluorescence of anthracene was detected only in the spherosomes after treatment by laser micrography. 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 efficiency of pesticides by spherosome isolated (0.343 ± 0.016 g) from 5 g rice bran. The removal by spherosomes was similar to that of rice bran. This finding shows directly that the pesticides are uptaken into spherosomes.

Rice bran is a by-product of making polished rice from brown rice. Therefore, rice bran is very inexpensive, costing 1/50–1/40 that of activated carbon, which would lower the cost of wastewater treatment significantly. Additionally, the use of rice bran is significant from the aspect of effective utilization of waste matter. Taken together, the findings of this study suggest that the use of rice bran as adsorbent is an efficient and cost-effective. Therefore, we are now investigating the removal efficiency using packed column for more practical use.

Table 2. Removal Efficiency of Rice Bran or Defatted Rice Bran for Captan

Substance	Rice bran			Defatted rice bran		
	Concentration (μg/l)		Removal efficiency (%)	Concentration (μg/l)		Removal efficiency (%)
	Before treatment	After treatment		Before treatment	After treatment	
Captan	50	1.1–1.3	97.0 ± 0.2*	50	1.0–1.3	98.2 ± 0.3*

*Data represent the mean ± S.D. of four separate samples. Rice bran: 10 g/l, Captan: 50 μg/l, Reaction time: 1.5 hr, pH : 7.0. Captan (10.0 mg) was dissolved in methanol (1 ml), and the solution was extended to 100 ml with distilled water. In addition, it was adequately diluted with distilled water, and 100.0 ml was used for the experiment. The rice bran (1.0 g) was added.

Table 3. Removal Efficiency of Rice Bran or Defatted Rice Bran for Anthracene

Substance	Rice bran			Defatted rice bran		
	Concentration (mg/l)		Removal efficiency (%)	Concentration (mg/l)		Removal efficiency (%)
	Before treatment	After treatment		Before treatment	After treatment	
Anthracene	2.50	0.75–0.93	64.6 ± 2.6*	2.50	0.05–0.19	95.3 ± 5.7*

*Data represent the mean ± S.D. of four separate samples. Rice bran: 10 g/l, Anthracene: 2.50 mg/l, Reaction time: 1.5 hr, pH : 7.0.

Table 4. Removal Efficiency of Spherosomes Isolated from Rice Bran for Pesticides

Substance	Concentration (μg/l)		Removal efficiency (%)
	Before treatment	After treatment	
Captan	50.0	0.94–1.6	97.0 ± 0.8*
CNP	50.0	9.9–1.5	73.9 ± 6.4*
Dichlofluanid	50.0	0.53–0.84	98.6 ± 1.0*
Tetradifon	50.0	8.7–10	81.0 ± 1.8*

*Data represent the mean ± S.D. of four separate samples. Reaction time: 1.5 hr, pH : 7.0. All spherosomes (about 0.34 g) obtained from rice bran (5 g) were used for this experiment. Pesticide (10.0 mg) was dissolved in methanol (1 ml), and the solution was extended to 100 ml with distilled water. In addition, it was adequately diluted with distilled water, and 100.0 ml was used for the experiment.

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