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Efficiency of Spherosome for Removal of Chloroform from Water

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Spherosomes were elucidated to be a key for removal of chloroform by rice bran (Adachi, A.; Okano, T. *Chemosphere* **2002**, *46*, 87–92). On the basis of the above findings, a large amount of spherosomes was separated from rice bran using an industrial scale machine in order to investigate direct removal of chloroform by spherosome from tap water. The efficiency of removal of chloroform from water by rice bran spherosome was evaluated. Equilibrium adsorption isotherms conformed to the Freundlich isotherm (log–log linear). Adsorption of this compound by spherosome was observed in the pH range of 1–12. At equilibrium, the adsorption efficiency of spherosome for chloroform was higher than that of rice bran or activated carbon in the high chloroform concentration range (concentration > 0.1 g/L). In the low chloroform concentration range (concentration < 0.08 g/L), activated carbon adsorbs more chloroform than spherosome, but the adsorbed amount by spherosome is of the same order as that by activated carbon, which is several orders higher than that by rice bran. Chloroform was removed from tap water with an average removal efficiency of 90% after 90 min when spherosome (2 g/L) was added to tap water containing 0.105 mg/L of chloroform.

KEYWORDS: Chloroform; activated carbon; rice bran; spherosome

INTRODUCTION

Chloroform is found in all chlorinated tap waters. This compound is formed as a result of the reaction between chlorine and organic precursors, typically naturally occurring substances such as humic acids. A National Cancer Institute (NCI) report showed that chloroform caused cancer in rats and mice under laboratory test conditions (*1*). Japanese law has defined the regulations on chloroform in tap water. Water is possibly now the major source of environmental exposure to chloroform (*2*). We have previously reported that rice bran was effective in the removal of organochlorine compounds such as chloroform (*3*), dichloromethane, and benzene. Furthermore, it was confirmed that the spherosomes isolated from rice bran were effective in removing these compounds (*4*). 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 (*4*). Spherosomes are intracellular particles about 10 μm in diameter and widely distributed among plants and fungi (*5*). Neither the function of spherosomes nor the analytical method is well understood.

No detailed adsorption studies of spherosomes for removal of chloroform from water have been reported. The results are reported herein.

MATERIALS AND METHODS

Apparatus. The assay of chloroform 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 × 0.53 mm). The column was maintained at 90 °C, with both the injection port and detector being maintained at 150 °C.

Materials. Rice bran was purchased at a local market. In this experiment, a large amount of spherosomes was separated from rice bran using an industrial scale machine, that is, a supermicron-mill (Hosokawa-micron Co. Ltd.; type M 52 NC) and a micron-separator (Hosokawa-micron Co. Ltd.; type MS-1). First, rice bran was supplied to the supermicron-mill to grind rice bran to 2–100 μm size particles using impact, shearing, and friction. Then the micron size particles were sent to a micron-separator to separate and extract spherosomes using centrifugal separating force or acceleration in a cyclone separator. The feed rate was 250 kg/h, and collecting particle size was 7–12 μm. The yield is about 17%. The composition and parameters of rice bran and spherosome are shown in **Tables 1** and **2**, respectively. Moisture content was determined by drying a sample for 6 h at 110 °C. The nitrogen content (percent) was analyzed by using the Kjeldahl method (*6*). The protein content (percent) was calculated from the nitrogen content by multiplying it by the nitrogen–protein conversion factor of 6.25.

Lipids were extracted according to the Bligh and Dyer method (*7*). 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 anthrone method (*8*). Dietary fiber was determined by AOAC method (*9*). The surface area was measured by the N_2 gas adsorption method. Particle size was measured by laser diffraction method. The pore diameter was determined by mercury intrusion porosimetry.

Activated carbon (powder, coal base carbon) was purchased for the practical grade from Wako Pure Chemical Industries Ltd. (Amagasaki, Japan). Chloroform standard was purchased for water analysis from Wako Pure Chemical Industries Ltd. (Amagasaki, Japan).

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Table 1. Composition of Rice Bran and Spherosomes

constituent	concentration (g/100 g) rice bran	spherosomes
water	13.5	9.8
protein	13.2	26.6
lipid	18.3	3.9
carbohydrate		
glucide	38.3	38.4
fiber	7.8	3.6
ash	8.9	17.4

Table 2. Properties of Rice Bran and Spherosomes

	rice bran	spherosomes
specific surface area (m^2/g)	0.14	1.59
particle size (μm)	243.7	8.01
pore volume (mL/g)	1.36	1.16
pore surface area (m^2/g)	14.3	6.81
average pore diameter (μm)	0.38	0.74

Adsorption Experiment. A 100 mL aqueous chloroform solution (chloroform = 1.0 g/L) was placed into a 100 mL glass-stoppered Erlenmyer flask, to which 0.05–1.0 g of spherosome, rice bran, or activated carbon was then added. The solution was mixed by a tumbler. The reaction mixture was filtered through filter paper (quantitative ashless no. 5A; Toyo Roshi, Ltd.) to remove spherosome, rice bran, or activated carbon. The initial 10 mL of filtrate was discarded because of the adsorption of chloroform by the filter paper. The identical procedure was performed on a control sample solution, which had no spherosome, rice bran, or activated carbon. Concentrations of chloroform in water samples were determined according to the method described by our research group (*10, 11*). Fifty milliliters of this filtrate was placed in a separatory funnel, and 5 mL of *m*-xylene was added. The mixture was shaken for 1 min. The separated *m*-xylene layer was subjected to gas chromatography to assay the concentrations of chloroform. To quantify the evaporation loss of chloroform, control experiments were performed following the same procedure except for the absence of spherosome, rice bran, or activated carbon. Maximum loss was about 5 (4.7 ± 0.22)%, although almost no loss was detected in most cases. The removal efficiency of spherosome, rice bran, or activated carbon was calculated after elimination of the contribution due to the evaporation loss.

RESULTS AND DISCUSSION

Adsorption Isotherm. The amount of chloroform adsorbed in the equilibrium state was plotted against the concentration of chloroform in solution on a logarithmic scale. Chloroform adsorption isotherms were fit to the Freundlich equation: $q = kC^{1/n}$, where q is the amount of chloroform adsorbed at the equilibrium concentration C and k and $1/n$ are the empirical Freundlich constants, which can be calculated from the linear plot of log q versus log C. A linear relationship was obtained, indicating that the adsorption reaction was of a Freundlich type (**Figure 1**). **Table 3** shows $1/n$ and k values obtained from the Freundlich isotherm. These values indicated that the removal of the chloroform by spherosome was effective at high concentration. At equilibrium, the adsorption efficiency of spherosome for chloroform was higher than that of rice bran or activated carbon. The very high adsorption of chloroform to spherosome would be attributed to the specific affinity of hydrophobic molecules to spherosome as shown in our previous paper (*12*).

Effect of pH on Adsorption. Figure 2 shows the effect of pH on the adsorption of chloroform by spherosome or rice bran, using buffer solutions at reaction time of 90 min. Adsorption of spherosome was observed over the range of pH 1–12 and exhibited a fixed value. The removal efficiency of rice bran decreased as the pH was lowered. Therefore, spherosomes can be applied for treatment of water over a wide pH range.

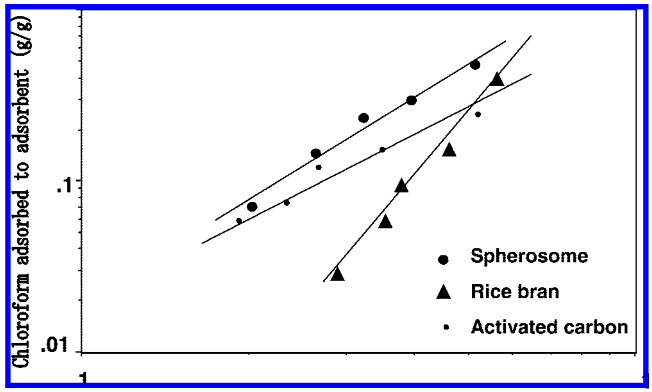

Figure 1. Freundlich's adsorption isotherms for chloroform. Data represent the mean ± SD of three separate determinations. Chloroform (1.0 g) was dissolved in buffer solution, and the solution was made up to 1000 mL with buffer solution. A 100.0 mL portion was used for the experiment. From 0.05 to 1.0 g of spherosome, rice bran, or activated carbon was added. Reaction time, 3 h; chloroform, 1.0 g/L; pH, 7.

Table 3. Freundlich Parameters for the Adsorption of Chloroform on Spherosomes, Rice Bran, and Activated Carbon

	$1/n$	k
spherosomes	1.98	1.92
rice bran	3.83	3.92
activated carbon	1.65	0.86

Effect of Amount of Spherosome or Rice Bran on Adsorption. Figure 3 shows the effect of the amount of spherosome or rice bran on the removal of chloroform. The residual chloroform decreases in response to the amount of spherosome or rice bran. The removal is initially fast, but after a reaction time of 60 min, the removal appears to plateau. The data show that the initial chloroform concentration of 1 g/L has been lowered approximately to 0.092 g/L (90.8% removal) at

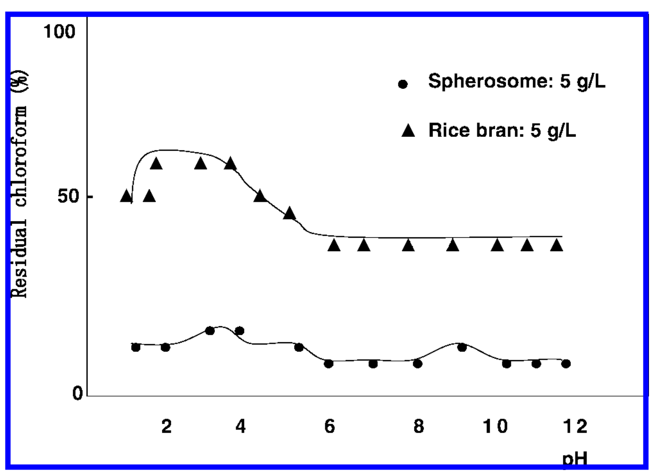

Figure 2. Effect of pH on the adsorption of chloroform by spherosome or rice bran. Data represent the mean ± SD of three separate determinations. Chloroform (1.0 g) was dissolved in buffer solution, and the solution was made up to 1000 mL with buffer solution. A 100.0 mL portion was used for the experiment. Solutions of HCl, citric acid−phosphate buffer, and carbonate buffer were used for preparation of pH 1–2, 3–7, and 8–11 solutions, respectively. Spherosome or rice, 5 g/L; reaction time, 90 min; chloroform, 1.0 g/L.

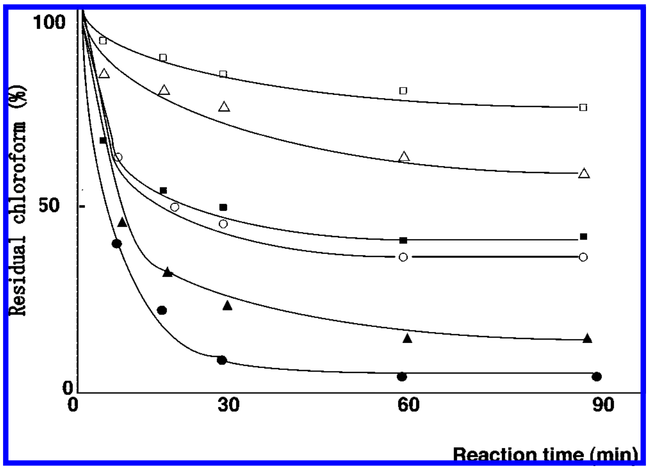

Figure 3. Effect of amount of spherosome or rice bran on the adsorption of chloroform. Data represent the mean ± SD of three separate determinations. Spherosome, (■) 1.0 g/L, (▲) 2.0 g/L, (●) 10 g/L; rice bran, (□) 1.0 g/L, (△) 2.0 g/L, (○) 10 g/L; chloroform, 1.0 g/L; pH, 7.

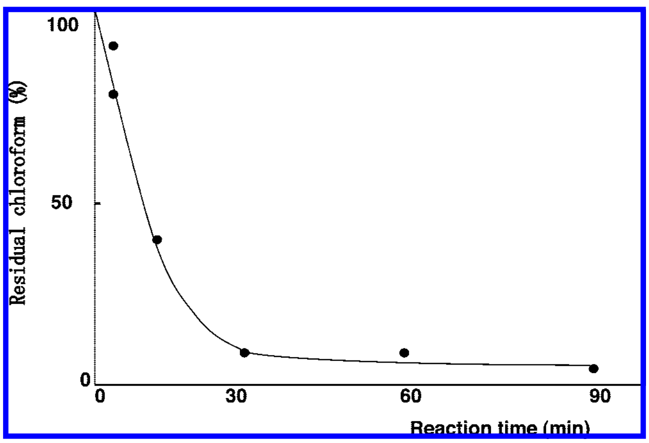

Figure 4. Efficiency of the removal of chloroform in tap water by spherosome. Data represent the mean ± SD of three separate determinations. Tap water containing 0.105 mg/L of chloroform was used. Spherosome, 2 g/L; pH, 7.

10 g/L of spherosome and 4.0 g/L (60% removal) at 10 g/L of rice bran after a reaction time of 60 min, respectively. In this experiment, 10 g/L of spherosome showed the highest efficiency. The adsorption efficiency of spherosome for chloroform was higher than that of rice bran. The specific surface area of activated carbon is 300–900 m^2/g (*13*), which is much larger than those of spherosome and rice bran (**Table 2**), indicating that the mechanism of adsorption by spherosome or rice bran is quite different from that of activated carbon. We have previously reported that the removal of organochlorine compounds and benzene by rice bran is dependent upon the uptake of these compounds into spherosomes (*4*). Spherosomes are organelles rich in lipid, and they differ in morphology and origin from large oil bodies (*14*). We regarded the special membranes to be related to the uptake of chemical compounds into spherosomes. The chemical nature of the spherosomes is uncertain.

Application toTap Water. Chloroform was removed from tap water with an average removal efficiency of 90% after 90 min when spherosome (2g/L) was applied to tap water that contained 0.105 mg/L chloroform (**Figure 4**). In conclusion, in the high chloroform concentration range (concentration > 0.1g/L), the adsorption efficiency of spherosome for chloroform was higher than that of rice bran or activated carbon. In the low chloroform concentration range (concentration < 0.08 g/L), activated carbon adsorbs more chloroform than spherosome, but the adsorbed amount by spherosome is of the same order as that by activated carbon, which is several orders higher than that by rice bran. The price of spherosome is $^{1}/_{50}$–$^{1}/_{60}$ of that of activated carbon. Moreover, spherosome is recovered from disused rice bran. Taking into account of reuse of spherosome from disused rice bran and the price of activated carbon in comparison with that of spherosome, the findings of this research are useful and contributes to the recycling of disused rice bran from an environmental viewpoint.

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