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Short communication

Adsorption behavior of lindane on *Rhizopus oryzae* biomass: Physico-chemical studies

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ABSTRACT

Rhizopus oryzae biomass (ROB), depending on the age of the culture has been found to adsorb lindane, an organochloro pesticide from its aqueous solution to the extent of 63–90%. The adsorption process does not depend on the pH of the solution or incubation temperature and is very rapid during the first 15 min and reaches equilibrium within 60 min following pseudo-second-order rate model. The adsorption capacity of 1 g ROB towards lindane is 107.5 μg as calculated from pseudo-second-order curve. The equilibrium isotherm data better fit to Freundlich ($R = 0.98$) than Langmuir model ($R = 0.54$). Removal of lipids from ROB with organic solvents reduces its adsorption capacity by 55–68% indicating the importance of hydrophobic interaction in the present adsorption process. SEM–EDX analysis reveals that lindane is adsorbed on the biomass. This study shows that ROB may be a good biosorbent for the removal of lindane from water.

1. Introduction

Lindane (1,2,3,4,5,6-hexachlorocyclohexane) (Fig. 1) is one of the most commonly used pesticides in India. It is recognized internationally as toxic, persistent and bio-accumulative in nature and is known to contaminate water through agricultural, domestic and industrial activities. Hydrolysis and photolysis are not considered to be important pathways of degradation for this pesticide and the half-lives in air, water and soil are reported to be: 2–3, 3–300 days and up to 2–3 years, respectively [1]. Due to its high solubility in lipid, lindane can accumulate in invertebrates, fish, birds and mammals easily through food chain [1]. Removal of this pesticide from environment therefore, is of immense importance. Recently researches have been focused on emerging sorption technology [2–6] to remove pesticides and other pollutants from water. Gupta et al. [7] reported removal of lindane from wastewater using bagasse fly ash. Reports are available in the literature on biosorption of lindane by some gram positive and gram negative bacteria, e.g., *Escherchia coli*, *Bacillus subtils* and adsorption of lindane onto humic acid a natural organic matter of soil [8,9].

Young and Banks [10] reported removal of lindane from its aqueous solution with *Rhizopus oryzae* biomass and proposed a possible mechanism of pesticide–biomass interaction. The study, however, was limited to determining the effect of pH, biomass concentration etc on removal of lindane from its aqueous solution evaluated on the basis of observed changes of Freundlich constant (K_F). The present investigation extends the previous limited information and reports detail studies on adsorptive behavior of lindane on *R. oryzae* biomass with special reference to the aspects not investigated earlier. Apart from the characterization of lindane uptake by the biomass, a mechanism of adsorption and desorption of the pesticide on and from *R. oryzae* cell surface, change in cell surface morphology associated with interaction with the pesticide and the prospect of *R. oryzae* biomass in lindane removal from various environmental samples have been described in this report.

2. Materials and methods

2.1. Chemicals and organism

Lindane (γ-BHC) used in this study was obtained from AccuStandard, Inc., USA (purity 98%). Solvents and other chemicals were of HPLC and analytical reagent grade, respectively and purchased from Mecrk, Germany. Water used in all experiments was ultrapure milliQ water. *R. oryzae* (MTCC 262) was obtained from the Institute of Microbial Technology, Chandigarh, India, and maintained on Potato dextrose agar (20% potato extract and 2% dextrose) slants. The organism was sub-cultured at regular interval of 30 days to maintain viability.

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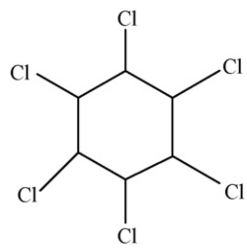

Fig. 1. Structure of lindane.

2.2. Preparation of *R. oryzae* biomass (ROB)

The organism was grown in potato dextrose broth (100 ml) up to 96 h at 35 °C under stationary condition. At the end of the desired incubation period, the biomass was harvested by filtration and washed thoroughly with water. Dead biomass was prepared by autoclaving the culture at 121 °C for 15 min before harvesting. The biomass was dried by soaking with blotting paper and kept in refrigerator at 4 °C until used. Moisture content of the biomass was determined by heating at 70 °C until constant weight.

2.3. Pesticide solution

Stock solution of lindane (100 mg/l) was prepared in hexane. Working solution was prepared by evaporating certain volume of stock solution under vacuum at 35 °C, dissolving the residual pesticide in minimum volume of methanol and finally diluting to 100 ml with water.

2.4. Analysis of lindane

Quantitative analysis of lindane was performed by Gas Chromatography (PerkinElmer Model: Autosystem XL) equipped with ECD detector and a Elite-5 (25 m × 0.2 mm ID) capillary column (PerkinElmer, USA), using split flow of 50 ml/min, at injector, detector and oven temperatures of 300, 350 and 210 °C, respectively. The flow rate of the carrier gas (N_2) was 0.5 ml/min. Concentration of lindane was obtained from the standard calibration curve.

2.5. Adsorption experiments

ROB (25 mg) was added to 15 ml aqueous solution (pH 6.0, normal) of lindane (200 μg/l) taken in 100 ml stoppered Erlenmeyer flask and incubated at 30 °C with shaking (120 rpm) for 5 h unless otherwise stated. Biomass was separated by filtration through GF/C (glass microfibre) filter disc under vacuum. Lindane was extracted from the filtrate as described by Pasha [11] and analyzed. In brief, 0.2 ml of saturated sodium chloride solution was added to 10 ml of the filtrate and lindane was extracted thrice with 10 ml of dichloromethane. The extracts were pooled and the solvent was removed by evaporation under vacuum at 35 °C after passing through anhydrous sodium sulfate column. Residue was dissolved in hexane and analyzed for lindane content. The lindane adsorbed per gram of biomass was calculated from the following mass balance equation:

$$Q = (C_i - C_f)\frac{V}{1000W}$$

where Q (μg/g), C_i (μg/1), C_f (μg/1), V (ml) and W (g) represent the lindane uptake, initial lindane concentration, lindane concentration at equilibrium, the volume of the pesticide solution and the weight of the biomass respectively.

2.6. Effect of pH and temperature on lindane adsorption

The effect of pH and temperature on the adsorption process was studied over a pH range 2–10 at 30 °C and temperature range 20–40 °C at pH 6.0.

2.7. Kinetics and equilibrium isotherm

Kinetics of adsorption of lindane by ROB was followed at regular interval of time up to 240 min. Equilibrium isotherm studies were conducted using lindane concentration 20–1000 μg/l and incubation period 120 min. Other conditions were same as described in the adsorption experiments.

2.8. Modification of the biomass

Lipids and hydrophobic components were extracted from ROB with nonpolar solvents, e.g., xylene and chloroform. Extraction was carried out by shaking the biomass (25 mg) with the solvent (50 ml) in a stoppered Erlenmeyer flask for 24 h. The solvent was removed by filtration through glass wool and the modified biomass was dried under current of air. Adsorption capacity of the modified biomass towards lindane was determined as described above.

2.9. Desorption of lindane from loaded biomass

Biomass obtained after equilibrium experiment with 200 μg/l of lindane was washed thoroughly with water, dried and extracted with hexane/acetone (50 ml) for 16 h with shaking (120 rpm). Lindane content of hexane or acetone extracts was determined as described above.

2.10. Scanning electron microscopy

The surface morphologies of ROB before and after adsorption of lindane were recorded with a FESEM–EDX (JEOL JSM-6700F). The samples were prepared as described by Sastry et al. [12].

2.11. Studies with simulated wastewater

Ground and surface water as well as effluents collected from different industries were simulated with lindane. Adsorption experiments with ROB were carried out as described above.

3. Results and discussions

Biosorptive removal of lindane, a highly toxic organochloro pesticide from water was studied with both live and dead biomass of *R. oryzae* of varying culture age. Depending on the age of the culture, adsorption of lindane varied from 63% to 90% and decreasing trend was noted after the organism entered into the death phase (Fig. 2). This may be due to change in chemical composition of the cell surface with time [13]. However, no difference in adsorption was noted between live and dead biomass (data not shown). Due to maximum adsorption capacity, biomass obtained after 96 h of growth was selected for further studies.

3.1. Effect of pH and temperature

Adsorption of lindane by ROB was influenced by the change in pH only to a small extent (Fig. 3). Hydrogen ion concentration which modifies the ionic charge of the adsorbent influences its ionic interaction with the adsorbate. The present observation suggests that lindane having no ionisable functional group is not likely to bind ROB through ionic interaction. Our observations contrast with the findings of Young and Banks [10] and Ju et al. [8] who

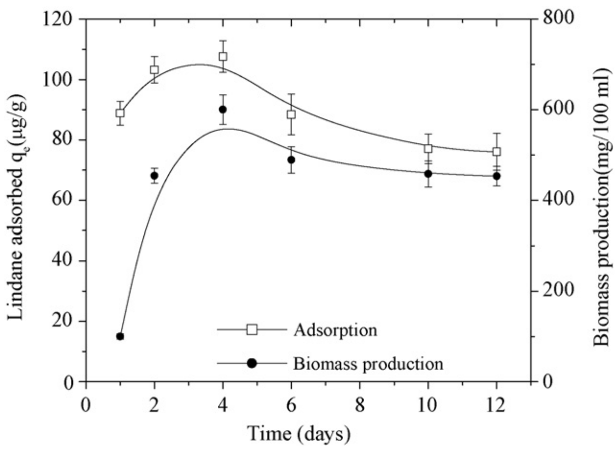

Fig. 2. Effect of culture age on biomass production and lindane adsorption by ROB. Data represent an average of four independent experiments ± SD shown by error bar.

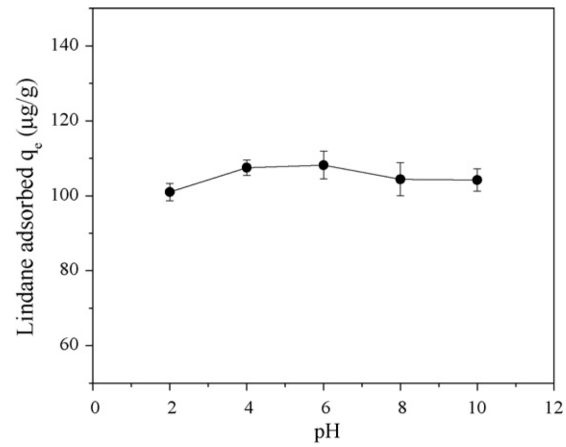

Fig. 3. Effect of pH on biosorption of lindane on ROB. Data represent an average of four independent experiments ± SD shown by error bar.

reported higher adsorption of lindane at lower pH while investigating adsorption of lindane by biomass of *R. oryzae* and certain gram positive and gram negative bacteria respectively. Interaction of lindane with humic acid however, was reported to be unaffected by the change in pH of the medium (Prosen et al. [9]). Unlike the observation of Young and Banks [10] who observed higher adsorption of lindane by *R. oryzae* biomass at lower temperature, in the present investigation lindane adsorption was not found to be influenced by change in temperature from 20 to 40 °C (data not shown). Ju et al. [8] also did not observe any general trend of the effect of temperature on biosorption of lindane by different gram positive and gram negative bacteria.

3.2. SEM and EDX analysis

Significant textural change towards the compactness and coherency in the surface morphology of the post-adsorbed ROB is observed (Fig. 4B) compared to that of pristine biomass (Fig. 4A). Further, morphological alterations are much more clearly depicted in the higher magnified SEM images (Fig. 4AH and BH). EDXA spectra of the biomass were recorded in area profile mode and show the signals of carbon, nitrogen and oxygen in the pristine biomass that are due to X-ray emissions for cell wall carbohydrates and proteins (Fig. 5A). Additional peak of chlorine after adsorption of pesticide on ROB indicates the incorporation of lindane on the cell surface (Fig. 5B).

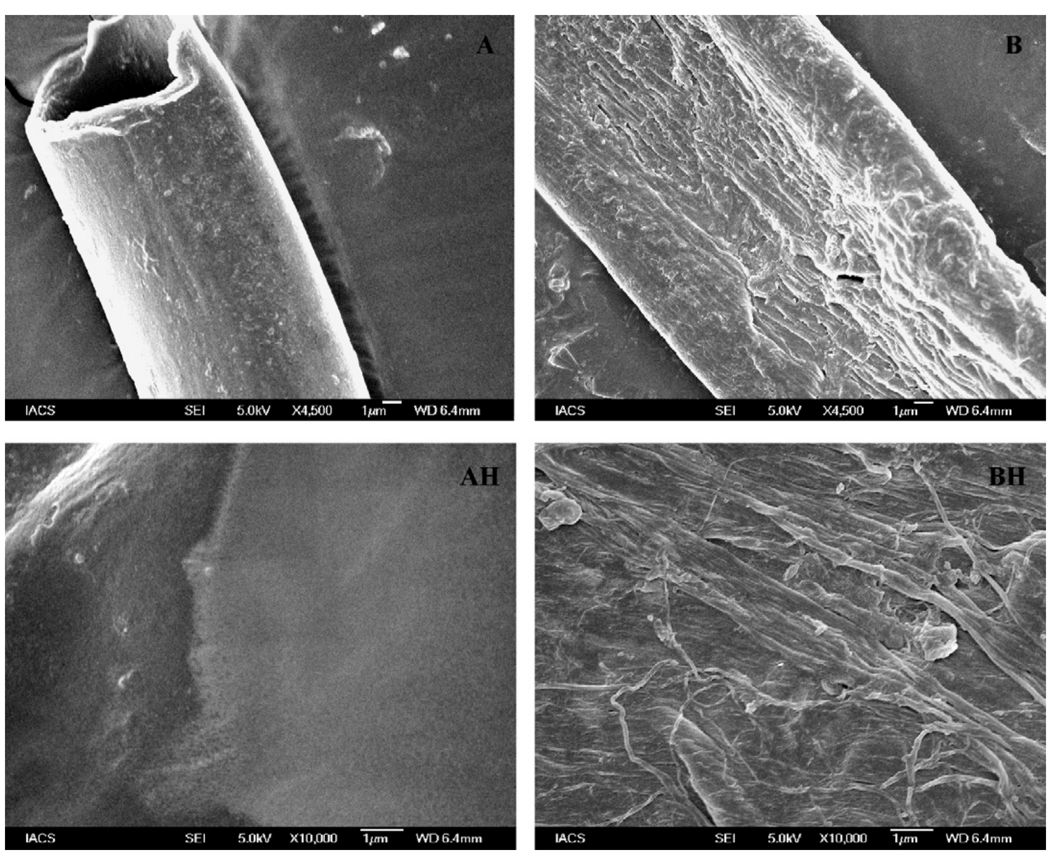

Fig. 4. SEM images showing surface morphologies of ROB before (A) and after (B) adsorption of lindane. Higher magnified images of ROB before (AH) and after (BH) treatment with lindane.

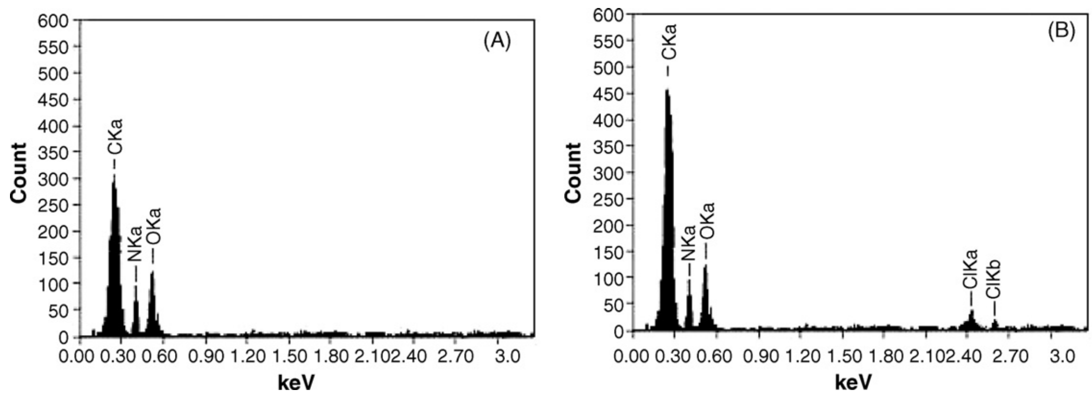

Fig. 5. EDX analysis data showing the elemental composition of the pristine ROB (A) and lindane loaded ROB (B).

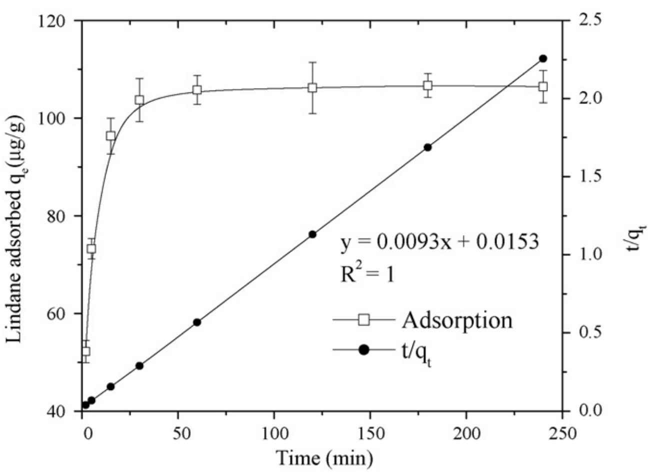

Fig. 6. Kinetics and pseudo-second-order plot of lindane adsorption on ROB. Adsorption (q_e) data represent an average of four independent experiments ± SD shown by error bar.

3.3. *Kinetics of lindane adsorption by R. oryzae biomass*

The rate of adsorption of lindane by ROB was very fast initially and more than 80% of adsorption took place within first 15 min and finally reaches equilibrium at about 60 min (Fig. 6). The initial high rate is due to the abundance of free adsorption sites. This has significant practical importance requiring smaller reactor volume ensuring high efficiency and economy. Initial analysis of the experimental kinetic data appears to fit a pseudo-second-order rate model. Hence this rate model was applied to understand the controlling mechanism of the adsorption process. The pseudo-second-order rate model can be written as [14]:

$$\frac{dq}{dt} = k_2(q_e - q_t)^2 \tag{1}$$

where q_e (µg/g) and q_t (µg/g) are the amounts of lindane adsorbed on ROB at equilibrium and at time t (min) respectively; k_2 (g/µg min) is the rate constant of pseudo-second-order model. Applying boundary conditions as $t = 0$ and $q_t = 0$ to $t = t$ and $q_t = q_t$ the integrated form of the Eq. (1) becomes:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \tag{2}$$

The plot of t/q_t vs t shows excellent linearity (Fig. 6) for all the experimental data indicating that the adsorption of lindane on ROB closely follows ($R^2 = 1$) pseudo-second-order kinetics. The adsorption capacity q_e and the rate constant k_2 determined from the slope and intercept of the plot respectively are shown in Table 1.

3.4. *Equilibrium adsorption isotherm*

Adsorption isotherm depicts the adsorbent–adsorbate interaction and is important in optimization of the adsorbent which is essential for practical designing of an efficient adsorption system as well as its operation. In the present study, the equilibrium sorption of lindane by ROB (Fig. 7A) shows that the adsorption capacity of the biomass increases linearly with increase in equilibrium concentration up to 150 µg/l and ultimately approaches towards a saturated value. The experimental data have been analyzed by both the well known isotherm model, e.g., Langmuir and Freundlich [15,16]. The linearised form of Langmuir (Eq. (3)) and Freundlich isotherm (Eq. (4)) can be expressed as:

$$\frac{C_e}{q_e} = \frac{1}{K_L} + \frac{a_L}{K_L} \times C_e \tag{3}$$

$$\log q_e = \log K_F + \frac{1}{n} \times \log C_e \tag{4}$$

where q_e and C_e are the concentrations at equilibrium in the solid phase (µg/g) and aqueous phase (µg/l), respectively, and a_L (l/µg) and K_L (l/g) are the Langmuir isotherm constants (Eq. (3)) while K_F (l/g) is the Freundlich constant and $1/n$ is the heterogeneity factor (Eq. (4)) The theoretical monolayer saturation capacity Q_{max}, can be calculated from the straight line plot of C_e/q_e against C_e (Eq. (3)). Analysis of the equilibrium isotherm data according to Langmuir (Fig. 7B) and Freundlich (Fig. 7C) isotherm model indicates that the adsorption process ideally follows Freundlich model and the curve is linear over the entire concentration range with a correlation coefficient of 0.98. On the other hand isotherm data do not fit Langmuir model and shows poor linearity with correlation coefficient of 0.54. Thus Q_{max}, the theoretical saturation capacity of the biomass was

Table 1
Freundlich isotherm constants and the parameters of pseudo-second-order model.

Type of adsorbent	Freundlich isotherm			Pseudo-second-order kinetics		
	K_F (l/g)	n	r^2	q_e (µg/g)	$k_2 \times 10^3$ (g/µg min)	r^2
ROB	2.02	1.04	0.9557	107.5	5.65	1

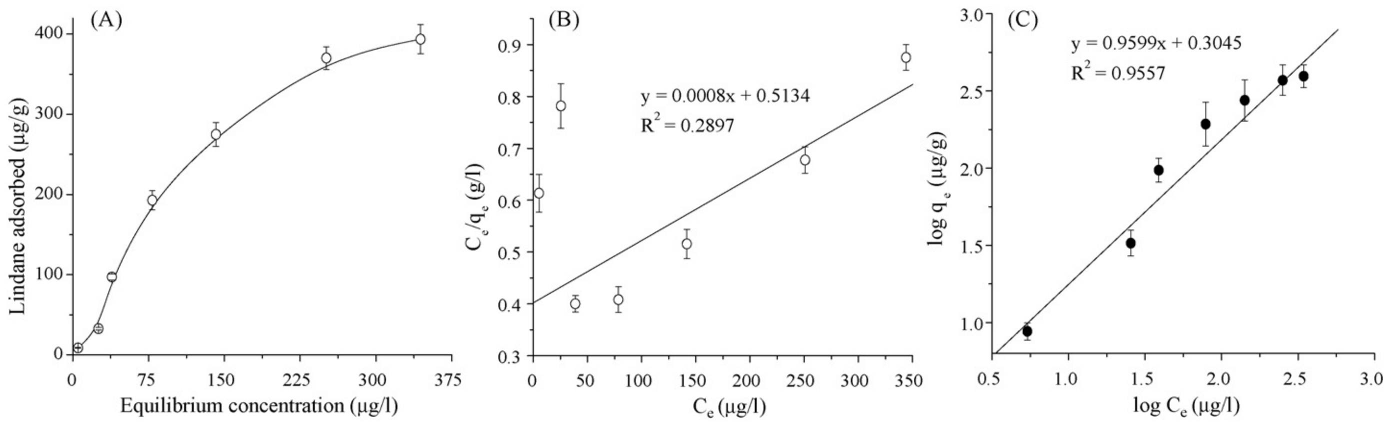

Fig. 7. Equilibrium adsorption isotherm of lindane (A); adsorption isotherm following Langmuir (B) and Freuendlich model (C). Data represent an average of four independent experiments ± SD shown by error bar.

Table 2
Removal of lindane from simulated wastewaters by adsorption on ROB.

Sample[a]	Concentration of lindane (μg/l)	Concentration of lindane (μg/l) after treatment with ROB	Adsorption (%)
Ground water	50.21 ± 1.3	6.14 ± 0.59	87.76 ± 1.17
River water	49.63 ± 2.4	5.03 ± 0.51	89.88 ± 1.03
Agricultural run-off	51.50 ± 2.2	6.65 ± 0.62	87.08 ± 1.21
Effluent from paper industry	48.95 ± 2.5	6.10 ± 0.75	87.54 ± 1.54
Effluent from soft drink industry	50.84 ± 1.4	7.03 ± 0.77	86.18 ± 1.52

Results represent average of four independent experiments ± SD.
[a] Samples were simulated with lindane (50 μg/l).

not determined from Langmuir curve. K_F and n values determined from Freundlich isotherm curve are shown in Table 1.

3.5. Mechanism of adsorption

Young and Banks [10] reported reduced adsorption capacity of *R. oryzae* biomass when the solution pH was increased, they explained this to be due to scarcity of positively charged hydrogen ion at higher pH which according to their proposition acts as bridging ligands between the negatively charged chlorine group of lindane and the negatively charged binding sites on the fungal cell surface in the adsorption process. However, as reported by these authors the rise in pH from 2 to 10 resulted only a 14% drop of lindane adsorption. Therefore the mechanism proposed could explain a small fraction of the total amount of lindane adsorbed on the *R. oryzae* cell surface.

In the present investigation adsorption of lindane by ROB is not significantly influenced by the change in hydrogen ion concentration and more importantly the extent of lindane adsorption was found to be almost identical at pH 2 and pH 10 (Fig. 3).

Being predominantly independent of pH, binding of lindane with ROB is likely to take place mainly through interaction other than ionic type and physical forces possibly play major role in this type of interaction. To confirm our presumption we extracted lipids from ROB with nonpolar solvents like chloroform and xylene and used this modified biomass for adsorption experiments. Adsorption of lindane per gram of the biomass has been decreased to 34.4 and 48.4 μg due to treatment with chloroform and xylene, respectively compared to 107.6 μg obtained with the unmodified biomass. This registers a decrease in adsorption by 55–68% and indicates the major role of lipid vis-à-vis hydrophobic interaction in the present adsorption process. Further, both the organic solvents, e.g., acetone and hexane desorbed lindane from the loaded ROB to the extent of 85–100% confirms our presumption. Our observations also corroborated the earlier report of Prosen et al. [9] regarding interaction of lindane with humic acid. These authors also did not observe any effect of pH on the interaction between lindane and humic acid. It appears therefore that more than one mechanism may be involved in the interaction between lindane and ROB, however, hydrophobic interaction of the pesticide with biomass lipids seems to play a predominant role.

3.6. Adsorption of lindane from simulated effluent

The adsorptive removal of lindane by ROB was investigated with simulated wastewater in order to study the influence of different matrix on the present adsorption process. Results (Table 2) of our preliminary studies indicate that ROB adsorbs lindane from wastewaters with almost identical efficiency to that from its aqueous solution. This indicates that *R. oryzae* biomass may be an efficient adsorbent for the removal of lindane from wastewater.

4. Conclusion

R. oryzae biomass is a good adsorbent for the removal of organochloro pesticide, lindane from water. The process is very fast reaching equilibrium within 60 min and follows Freundlich isotherm model. Hydrophobic interaction is mainly responsible for this adsorption process. ROB can be used for the adsorptive removal of lindane from wastewater containing different matrices.

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