

# Evaluation of Kinetic Parameters of Endosulfan Biosorbed on Biosorbent Prepared from *Aspergillus nidulans*

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**Abstract** The biosorption of endosulfan from aqueous solution on biosorbent prepared from fungal culture *Aspergillus nidulans*(ANS) was studied in a batch system with respect to different initial concentrations, adsorbent size, agitation and interruption. The kinetic parameters of sorption process such as equilibrium times and different diffusion coefficients were evaluated. The pseudo-first-order, pseudo-second-order kinetic model and the intraparticle diffusion model were used to describe the kinetic data and the rate constants were estimated. The experimental data fitted very well the pseudo-second-order kinetic model and also followed the intraparticle diffusion model up to 12 hrs. The intra-particle diffusion along with the external diffusion was determinant of the overall rate of the biosorption of endosulfan on to ANS biosorbent.

**Keywords** *Aspergillus Nidulans*, Biosorbent, Biosorption, Endosulfan, Film Diffusion, Kinetics, Pore Diffusion

## 1. Introduction

Endosulfan, a hydrophobic chemical, is a broad-spectrum insecticide and acaricide first registered for use in the United States in 1954 to control agricultural insect and mite pests on a variety of field, fruit, and vegetable crops. Technical-grade endosulfan is composed of two stereochemical isomers,  $\alpha$ -endosulfan and  $\beta$ -endosulfan, in concentrations of approximately 70% and 30%, respectively. The chemical is marketed by many different companies and under a variety of names including: Agrosulfan; Aginarosulfan; Banagesulfan; Cyclodan; Endocel; Endoson; Endonit; Endomil; Endosol; Endostar; Endodaf; Endosulfer; E-sulfan; Endorifan; Hildan; Redsun; Seosulfan; and Thiodan. It is also comes under the list of most stable known pesticides. It has been detected in the atmosphere, soils, sediments, surface and rain waters, and food stuffs[1]. It has extreme toxicity towards fish, aquatic invertebrates and mammalian[2,3]. Its presence in the environment may be the cause of several health effects including genetic disorders[4] and neurological disorders[5]. These health and environmental concerns have led to an interest in detoxification of endo-

sulfan from the environment.

Many international organizations have enforced stringent limits of pesticides in water and food in order to prevent human risk and environmental pollution. As per USEPA (1999), the guideline value for endosulfan and metabolites in drinking water is 0.22  $\mu\text{g/L}$  and 0.1 to 0.2  $\text{mg/L}$  in agricultural products. According to Bureau of Indian Standards (BIS), no pesticide should be present in natural waters and should not exceed 0.005  $\text{mg/L}$  in surface waters[6]. World-wide lots of efforts have been put for removal or reduction of pesticides from water environment. Conventional methods for the removal of pesticides are found to be either uneconomical or insufficient[7-11]. Biosorption comprises binding of pollutants(solutes, colloids or suspensions) to the biomass by a process(via physico-chemical mechanisms such as adsorption or ion exchange) which does not involve metabolic energy or transport, although this process may also occur simultaneously where live biomass is used. Biosorption is generally used for the removal of heavy metals from aqueous phase[12-15]. Removal of organic and other pollutants from aqueous phase by biosorption has already been attempted and documented in literature[16-21].

In present study, biosorption technique was attempted for removal of endosulfan from water environment by biosorbent prepared from fungal culture, *Aspergillus nidulans*. The objective of the present work was to find out the process that is controlling the rate of sorption. The rate li-

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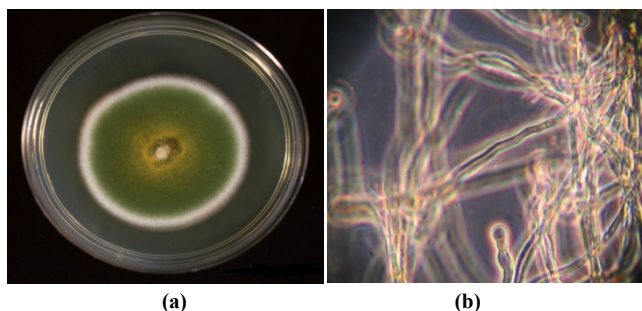
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miting step was evaluated to find the relative importance of film diffusion(external mass transfer) and pore diffusion(intra-particle diffusion) and also to decide which of the two dictates the overall rate of adsorbate transfer onto biosorbent sites. The rate limiting step was determined using a number of approaches viz. based on film and pore diffusion, based on external mass transfer, based on the effect of initial adsorbate concentration, based on the effect of adsorbent size, based on the effect of agitation and based on interruption on test.

## 2. Materials and Methods

### 2.1. Microorganism and its Growth Conditions

Fungal culture, *Aspergillus nidulans* (NCIM 1211) was procured from National Collection of Industrial Micro-organisms (National Chemical Laboratory, Pune, India) and used to prepare biosorbent for removal of endosulfan. Slants and petri dishes were made using fungal potato dextrose agar medium (1 L distilled water, 200 g peeled potatoes, 20 g dextrose, 0.1 g yeast extract, 20 g agar) and potato dextrose broth (1 L distilled water, 200 g peeled potatoes, 20 g dextrose, 0.1 g yeast extract) was used as liquid medium. The liquified agar was pored into sterilized culture tubes and petri plates, and allowed to solidify. The culture were then streaked on slants and petri plates and incubated in a bacteriological incubator (Trishul Equipments, Mumbai, India) at 37°C for 4 days. After 96 h, colonies with dark green color started forming on the potato dextrose agar (Figure 1a). The microscopic view of the long aerial hyphae of *Aspergillus nidulans* using Zeiss image analysis microscope (model Axion Star Plus, Germany) is shown in Figure 1b. The subcultures were preserved in the cold room at 4°C.



**Figure 1.** *Aspergillus nidulans* (a) Colony with dark green colour on potato dextrose agar (b) Long aerial hyphae observed under the Zeiss image analysis microscope

### 2.2. Preparation of Biosorbent

*Aspergillus nidulans* was grown in an autoclaved NCIM 44 Potato dextrose medium with corresponding fungal spores (approximately 10% of the volume of medium). Test flasks were placed on rotary platform incubator shaker (Trishul equipments, Thane, Mumbai) maintained at 37°C and 250 rpm for 4 days. Spore inoculum was prepared under sterile conditions in 0.05% in Tween-80 using freshly grown and sporulating mat on potato dextrose agar plates. The

mycelia pellets were harvested through filtering. The biomass was then washed repeatedly with distilled water to remove the growth medium adhering on its surface. The mycelia pallets used in this study were autoclaved at 121°C for 20 minutes. The biomass was then dried in an oven at 103°C for 12 hrs and ground with a laboratory grinder and sieved to get particle size between 0.15–0.3 mm.

### 2.3. Chemicals

All chemicals and reagents used were of analytical grade (AR). Technical grade endosulfan of 96.14% purity was obtained from M/s Vijiyalaxmi Insecticides and Pesti cides Limited(Andhra Pradesh, India). N-Hexane and Acetone were purchased from Merk India Limited (Mumbai, India). Distilled water was used for making synthetic samples. Before every experiment, all glassware were cleaned with mixture of dilute chromic acid and soap solution followed by through washing with tap water and distilled water. Stock solution of endosulfan was prepared in acetone at a concentration of 100 to 1000 mg/mL. All solutions were stored in the dark at 4°C prior to use.

### 2.4. Extraction of Endosulfan from Water Samples

The flasks containing endosulfan was separated from the fungal biosorbent by filtering through filter paper no. 201. Prior to filtration, the filters were washed with 300 mL of distilled water to remove any leachable materials. About 40–45 mL of extracted solution was filtered through the filter paper to achieve biosorption equilibrium of filter paper with the solution. This portion of filtrate was discarded. The subsequent filtrate was then extracted by n-Hexane as prescribed in the following paragraph and collected for GC analysis in teflon sealed septum bottles.

Extraction of endosulfan from water was done by liquid-liquid partition method. Representative sample of 2 mL of aqueous solution spiked with endosulfan was extracted in a 20 mL test tube. Extraction was done three times with 2 mL, 2 mL, and 2 mL of n-Hexane, respectively. During the extraction process, the sample-hexane mixture was shaken for 60 seconds in multi tube vertex mixer(Trishul equipments, Thane, Mumbai). Then, allowed to settle for 1 minute. Hexane extract was collected in 20 mL test tube and made to 6 mL with n-Hexane(some hexane was lost due to evaporation during transfer of solvent). 1 mL from 6 mL extracted hexane was transferred to Teflon sealed septum bottles. 2 µL of extracted sample was used for injecting into GC.

### 2.5. Analytical Procedures

Gas Chromatograph (Agilent Technologies, model GC-6890N) with electron capture detector and Agilent HP-5 column(0.53 mm internal diameter, 1.5 µm film thickness and 30 m length) was used for endosulfan n-Hexane extracts. Temperature of column, injector and detector was maintained at 280°C, 250°C and 300°C respectively. Nitrogen (99.9% purity) was used as carrier gas at a flow rate of 30 mL/min.

## 2.6. Kinetic Experiments

All batch sorption experiments were carried out using synthetic samples in distilled water at room temperature with constant stirring using mechanical shaker (Trishul Equipments, Thane, Mumbai). A high agitation speed of about 240-250 rpm was maintained to keep the sorbents in suspension during the experimental studies. Screw-top flasks (Borosil, India) of 100 mL capacity were used in the kinetic experiments. Synthetic water sample (50 mL) of 1 mg/L concentrations of endosulfan was taken in separate sets of flasks and the adsorbents were added to these at a suitable dose. All experiments are carried out for 14 hrs and Samples were collected at 30 minutes interval up to first 3 hours; every one hour interval up to 12 hrs; every 2 hours interval up to 14 hrs. All flasks were withdrawn from the system after the required reaction time and biosorbent was separated by gravity filtration. Representative samples (5 mL) were taken from the flask, extracted and analyzed for pesticide as per the method outlined in earlier sections.

The sorption kinetics was studied at different initial concentrations of endosulfan 1, 2 and 4 mg/L. The time required by endosulfan to reach the equilibrium condition was determined using the kinetic profiles. The effect of biosorbent size on sorption kinetics was studied by taking four different size ranges viz 0-0.075 mm, 0.15-0.30 mm, 0.425-0.60 mm and 0.60-0.85 mm. The effect of agitation speed on removal of endosulfan was also studied for two different agitation speeds of 175 rpm and 200 rpm.

## 2.7. Interruption Test

A series of 16 screw cap conical flasks of 100 mL were taken. The reaction mixtures containing 50 mL of distilled water spiked with 1 mg/L of endosulfan in flasks and 1 g/L of biosorbent were agitated in the mechanical shaker at an agitation speed of 250 rpm at room temperature. Interruption tests were conducted in eight screw cap conical flasks and eight flasks were maintain uninterrupted. After 15 min of shaking, one sample flask was taken out of the shaker, biosorbent was removed from the 1<sup>st</sup> sample flask and interrupted for a period of 30 min. After the completion of 30 min of interruption the separated biosorbent was reintroduced into the 1<sup>st</sup> sample flask, and the first sample flask was finally withdrawn after shaking for another 15 min.

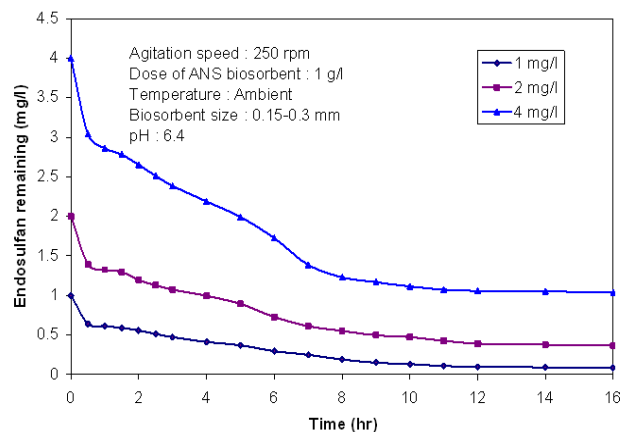
Therefore, the first sample was agitated for 30 min (15+15 min) with an interruption of 30 min. Similarly, the second sample flask was withdrawn for an interruption of 30 min after initial agitation of 30 min. The second sample flask was again agitated for another 30 min and withdrawn completely. Therefore, the second flask was agitated for 1 hr with an interruption of 30 min. Similarly, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup> and 8<sup>th</sup> sample flasks were withdrawn for an interruption of 30 min after an initial agitation of 90, 150, 210, 270, 330 and 390 min respectively and reintroduced for another period of 30 min agitation. All the flasks were interrupted for 30 min and had a total agitation of 30, 60, 120, 180, 240, 300, 360 and 420 min sequentially from 1<sup>st</sup> to 8<sup>th</sup> flask. The other eight

flasks on the shaker were withdrawn completely after 30, 60, 120, 180, 240, 300, 360 and 420 min of shaking without any interruption. The samples were collected from all the 16 flasks and initial concentrations of the sample were analyzed for endosulfan concentration.

## 3. Results and Discussion

### 3.1. Biosorption Kinetic Study and Modelling

The removal kinetics of endosulfan by the 1 g/L of ANS biosorbent (size 0.15-0.3 mm) for different endosulfan concentrations are shown in Figure 2. More than 60-70% of endosulfan was biosorbed within 5-6 hrs of reaction time in case of initial concentration of 1 mg/L. The removal rate was diminishing after 9 hrs of contact time and accounted only for 5.40% between 9 to 12 hrs. After 12 hrs of reaction time, the kinetics profiles gradually became horizontal indicating the final equilibrium. The removal of endosulfan beyond 12 hrs of reaction time was very slow; hence an equilibrium time of 12 hrs was standardized for the rest of the experiments. Figure 2 also shows that the initial percentage biosorption decreased 35.7%, 30.2% and 24.0% as the concentration was increased from 1 mg/L, 2 mg/L and 4 mg/L respectively.



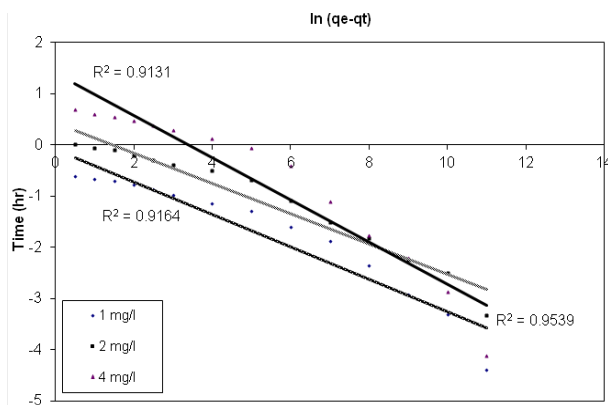
**Figure 2.** Change of concentration of endosulfan with time at different initial concentrations

Removal of 60-70% of endosulfan within 5-6 hr of reaction time was due to the availability of the number of free sites on to biosorbent surfaces and a high concentration gradient between the solution and the solid phase. The equilibrium time for organic pesticides in completely mixed batch reactor (CMBR) was found in the range of few minutes to several hours by many researchers [23-25]. Decreasing trend of biosorption with increased concentration of endosulfan (Figure 2) indicating the effect of loading of pesticides molecules on ANS biosorbent. However, the total amount of endosulfan biosorbed on the biosorbent increases with increase in endosulfan concentration in the initial solution. It is clear that the rate of biosorption of the pesticide depends on the initial concentration of pesticide. To analyze the biosorption rate of endosulfan on to the ANS biosorbent, the

pseudo first order and the pseudo second order kinetic model was evaluated based on the experimental data[26-29]. The linearized form the pseudo first order is as follows:

$$\ln(q_e - q_t) = \ln(q_e) - k_1 t \quad (1)$$

where,  $k_1$  is the pseudo first order kinetic constant,  $q_e$  is the amount of pesticide biosorbed on the ANS biosorbent at equilibrium(mg/g),  $q_t$  is the amount of pesticide biosorbed on the ANS biosorbent at any time t(mg/g). The slope of the linearized plot of  $\ln(q_e - q_t)$  versus t yields the first order rate constant  $k_1$  and the intercept of this plot gives the value of  $\ln(q_e)$ .



**Figure 3.** Linearized pseudo first order kinetic plots for the biosorption of different concentrations of endosulfan onto ANS biosorbent

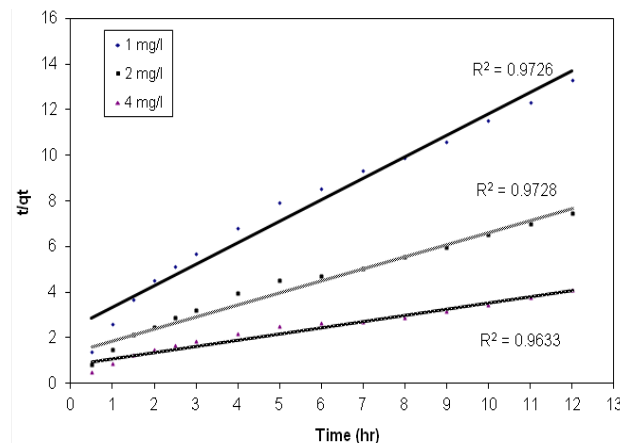
Figure 3 illustrates the linearized pseudo first order kinetic plots for the biosorption of 1 mg/L, 2 mg/L and 4 mg/L of endosulfan on ANS biosorbent. Table 1 summarizes the  $k_1$  values, the coefficient of regression( $R^2$ ) and the predicted and experimental  $q_e$  values for 1 mg/L, 2 mg/L and 4 mg/L of endosulfan biosorbed onto ANS biosorbent respectively.  $R^2$  values in all the cases are nearly 0.91, 0.95, 0.91 respectively and  $q_e$  values are also even reasonably close the value of experiments(except 4 mg/L). Next, an attempt is made to fit the biosorption data into pseudo second order kinetic model, to find out the more appropriate model by comparing the coefficient of regression( $R^2$ ) for both models.

The linearized form of the pseudo second order is as follows:

$$\frac{t}{q_t} = \frac{1}{h} + \frac{1}{q_e} t \quad (2)$$

where,  $h = k_2 q_e$  the initial sorption rate(mg/g hr),  $k_2$  is the pseudo second order rate constant(g/mg hr),  $q_e$  is the amount of pesticide biosorbed on the ANS biosorbent at equilibrium(mg/g), and  $q_t$  is the amount of pesticide biosorbed on the ANS biosorbent at any time t(mg/g). The values  $k_2$  and  $q_e$  are determined from the intercept and slope, respectively, from a plot of  $t/q_t$  and t.

Figure 4 illustrates the linearized pseudo second order kinetic plots for the biosorption of 1 mg/L, 2 mg/L and 4 mg/L of endosulfan on ANS biosorbent. Table 1 summarizes the  $k_2$  values, the coefficient of regression( $R^2$ ) and the predicted and experimental  $q_e$  values along with the initial sorption rate(h) for 1 mg/L, 2 mg/L and 4 mg/L of endosulfan biosorbed onto ANS biosorbent respectively. The correlation coefficient found was 0.97, 0.97, and 0.96 respectively which is on higher side than the correlation coefficient obtained from pseudo first order.



**Figure 4.** Linearized pseudo second order kinetic plots for the biosorption of different concentrations of endosulfan onto ANS biosorbent

The predicted values of  $q_e$  nearly matches the experimental values(Table 1) suggesting the goodness of the fit. It may be noted from Table 1 that the values of sorption rate(h) increased with increase in the initial concentration of endosulfan because the driving force for the mass transfer the permitted more pesticide molecules to reach the surface of on ANS biosorbent in shorter duration of time, suggesting that boundary layer resistance was not the rate controlling step[30].

The values of pseudo second order rate constant  $k_2$  were found to decrease with increasing initial endosulfan concentration. It can be concluded that the biosorption of endosulfan on to ANS biosorbent followed pseudo second order kinetic and that the biosorption of endosulfan onto ANS biosorbent was controlled by not ideal physical sorption as, involving valency forces through the sharing or exchange of electrons between the biosorbent and adsorbate as covalent forces and ion exchange[31, 32].

In recent years, the pseudo-second-order rate expression has been widely applied to the adsorption of pollutants from aqueous solutions. In addition, the initial adsorption rate can also be obtained from the model.

**Table 1.** Kinetic Parameters for the Biosorption of Endosulfan on ANS Biosorbent at Various Initial Concentrations

Conc. (mg/L)	$q_{e(\text{exp})}$ (mg/g)	Pseudo first order				Pseudo second order				
		$k_1$ (hr <sup>-1</sup> )	$R^2$	$q_{e(\text{cal})}$ (mg/g)	$k_2$ (hr <sup>-1</sup> )	$R^2$	H mg/(g min)	$q_{e(\text{cal})}$ (mg/g)	U (mg/g hr)	$t_{1/2}$ (hr)
1	0.9036	0.3174	0.9164	0.9233	0.3749	0.9726	0.4222	1.0612	0.4229	2.5114
2	1.6084	0.2959	0.9539	1.5452	0.2057	0.9721	0.7389	1.8950	0.7386	2.5650
4	2.9413	0.4118	0.9131	4.0148	0.0870	0.9633	1.1838	3.6886	1.1837	3.1160



Based on the second-order model, the initial adsorption rate( $u$ ) and half-adsorption time( $t_{1/2}$ ) are calculated in Table 1 according to the following equations[33].

$$u = k_2 q_e^2 \quad (3)$$

$$t_{1/2} = \frac{1}{k_2 q_e} \quad (4)$$

Half-adsorption time is the time required for the biosorption to take up half as much endosulfan as its equilibrium value. This time is an indicator for the biosorption rate.

### 3.2. Calculation of Film and Pore Diffusion Coefficient

The film diffusion coefficient ( $D_f$ ) and pore diffusion coefficient( $D_p$ ) are two important parameters of the kinetic study of the adsorption process. The diffusion coefficients were calculated by method based on adsorption rate proposed by Helfferich[34], which assumes the spherical geometry of adsorbent particles and uses the following equations to estimated the film and pore diffusion coefficients.

By assuming a spherical geometry for the adsorbent particles, Helfferich[34] derived the following half time equations:

$$t_{1/2} = 0.03 \frac{r^2}{D_p} \quad (5)$$

$$t_{1/2} = 0.23r \left( \frac{\delta}{D_f} \right) \left( \frac{C}{C_e} \right) \quad (6)$$

where,  $r$  is the radius of adsorbent particle (cm),  $D_f$  is film diffusion coefficient( $\text{cm}^2/\text{sec}$ ),  $D_p$  is pore diffusion coefficient( $\text{cm}^2/\text{sec}$ ),  $C_e$  is the concentration of adsorbate at equilibrium( $\text{mg/L}$ ),  $C$  is the concentration of adsorbate in solution( $\text{mg/L}$ ),  $\delta$  is the film thickness( $\text{cm}$ ),  $t_{1/2}$  is the time necessary to obtain half the initial concentration( $\text{sec}$ ).

Assuming a film thickness of 0.001 cm[34] and  $t_{1/2}$  values given in Table 1, the values of  $D_f$  and  $D_p$  have been determined for different initial concentrations of endosulfan and are tabulated in Table 2. These values of film and pore diffusion were used to determine the rate-limiting step[35].

In order to find out the rate limiting steps, many researchers[36, 37] used different models and estimated diffusion coefficients in the range of  $10^{-11}$  to  $10^{-12} \text{ cm}^2/\text{sec}$ . After the study, on heavy metal, Michelson *et al.*[35] suggested if the diffusion coefficient lies between  $10^{-6}$  -  $10^{-8} \text{ cm}^2/\text{sec}$ , the film diffusion will be rate limiting while if pore diffusion coefficient is in the range of  $10^{-11}$  -  $10^{-13} \text{ cm}^2/\text{sec}$ , the pore diffusion coefficients will be rate limiting. Generally, the diffusion of ion is faster than that of molecules[38]. It was found that the estimated film and pore diffusion were in the range of  $8.67 \times 10^{-7}$  to  $2.96 \times 10^{-9} \text{ cm}^2/\text{sec}$  and  $3.35 \times 10^{-10}$  to  $4.16 \times 10^{-10} \text{ cm}^2/\text{sec}$  for endosulfan. Thus, it may be concluded from the calculated diffusion coefficients from this study that as the diffusion values were more or less equal and were also in range within the two limits, either of the two i.e. that film diffusion or pore diffusion or both might be rate controlling.

**Table 2.** Values of Film Diffusion and Pore Diffusion Coefficients for Different Initial Concentration based on Biosorption Rate

Endosulfan Concentration (mg/L)	Film diffusion coefficient, $D_f(\text{cm}^2/\text{sec})$	Pore diffusion coefficient, $D_p(\text{cm}^2/\text{sec})$
1	$2.96 \times 10^{-9}$	$4.16 \times 10^{-10}$
2	$1.42 \times 10^{-9}$	$4.08 \times 10^{-10}$
4	$8.67 \times 10^{-7}$	$3.35 \times 10^{-10}$

### 3.3. External Diffusion Analysis

External diffusion, internal diffusion (or intraparticle diffusion), and actual adsorption are the three stages for the adsorption process on a porous adsorbent[39]. Rapid diffusion and adsorption generally occur in the macro-pores and the remaining slow approach to equilibrium occurs in the micro-pores. The concentration decay curves for batch adsorption systems incorporate three mass transport rate- controlling steps viz. external film mass transfer, diffusion in the adsorbent macro-pores and diffusion in the adsorbent micro-pores. The shape of the concentration decay curves with time varies according to the value of each of the above-mentioned three terms and their relative magnitudes[40].

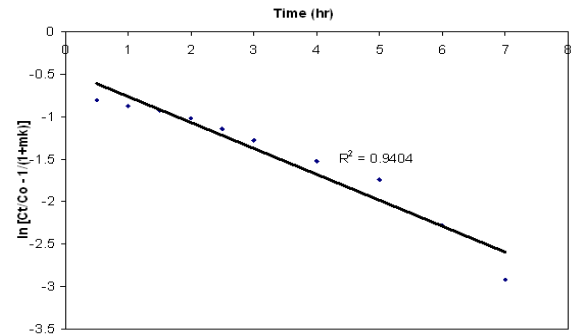
The effect of external mass transfer on adsorption kinetics can be investigated using the McKay equation valid for negligible intra-particle diffusion.

$$\ln \left( \frac{C_t}{C_0} - \frac{1}{(1+mk)} \right) = \ln \left( \frac{mk}{(1+mk)} \right) - \left( \frac{1+mk}{mk} \right) \beta_1 S_s t \quad (7)$$

where,  $m$  is the mass of biosorbent( $\text{g/L}$ ),  $C_0$  is initial concentration of adsorbate ( $\text{mg/L}$ ),  $C_t$  is concentration of adsorbate at any time  $t$  ( $\text{mg/L}$ ),  $k$  is the constant in the Langmuir equation defined as  $K_L x_m (1/\text{g})$  where  $K_L$  is the Langmuir constant( $1/\text{mg}$ ) and  $x_m$  is the monolayer saturation value( $\text{mg/g}$ ),  $S_s$  is surface area of biosorbent and  $\beta_1(\text{cm/s})$  is the mass transfer coefficients.  $S$  is given by:

$$S_s = \frac{6W}{d_p \rho_p (1 - \varepsilon_p)} \quad (8)$$

where  $W$  is the weight,  $d_p$  is the diameter,  $\rho_p$  is the density,  $\varepsilon_p$  is the porosity (subscript p refers to particles).



**Figure 5.** Application of McKay equation for analyzing effect of external mass transfer coefficient for endosulfan on ANS biosorbent

The values of  $\beta_1$  were found out to be  $7.384 \times 10^{-5} \text{ cm/sec}$  for endosulfan from the plot between  $\ln [C_t/C_0 - 1/(1+mk)]$  versus time shown in Figure 5. It was apparent from the linearity of the plots that external mass transfer played an important role in the biosorption process in the case of endosulfan. The deviation at the early stages indicated the

involvement additional rate controlling steps in biosorption process[41].

### 3.4. Internal Diffusion Analysis

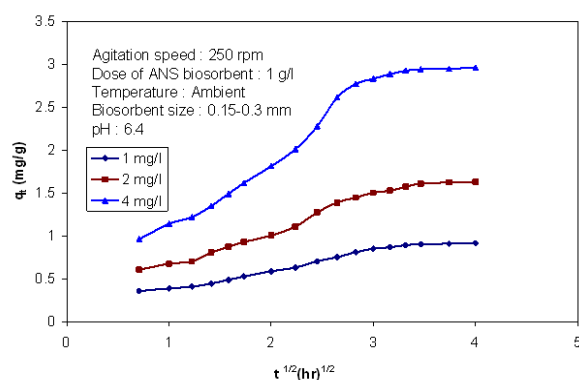
Compared to the external or internal diffusion step, the adsorption step is usually very fast for the adsorption of organic compounds on porous adsorbents[42] and it is known that in the absence of internal diffusion, the adsorption equilibrium is reached within several minutes[43]. Thus, the long adsorption equilibrium time in present experiments suggests that the internal diffusion may be the rate-limiting step. To see this more clearly, an internal diffusion model based on Fick's second law is used to treat the experimental data:

$$q_t = k_{id} t^{1/2} + C \quad (9)$$

where,  $q_t$  is the amount of pesticide biosorbed on the fungal biomass at any time  $t$ (mg/g) and  $k_{id}$  is the intraparticle diffusion rate constant(mg/g hr<sup>1/2</sup>). A plot of  $q_t$  vs  $t^{1/2}$  should give a straight line if the adsorption is limited by the internal diffusion process[44].

#### 3.4.1. Based on the Effect of Initial Adsorbate Concentration

The effect of initial concentration also gives a general idea about rate limiting steps. Figure 6 show the amount of endosulfan biosorbed per g of ANS biosorbent versus  $t^{1/2}$  for initial concentration of 1 mg/L, 2 mg/L, and 4 mg/L for endosulfan. Initially in all the cases studied, a linear relationship between  $q$  versus  $t^{1/2}$  with a zero intercept was found, suggesting that the internal diffusion step dominated the biosorption process before the equilibrium was reached.

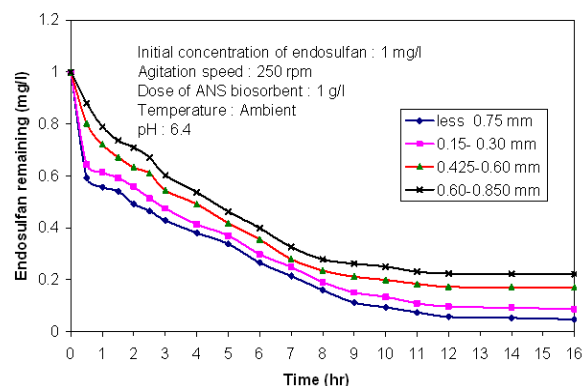


**Figure 6.** Intraparticle diffusion plots for endosulfan on ANS biosorbent at various initial concentrations

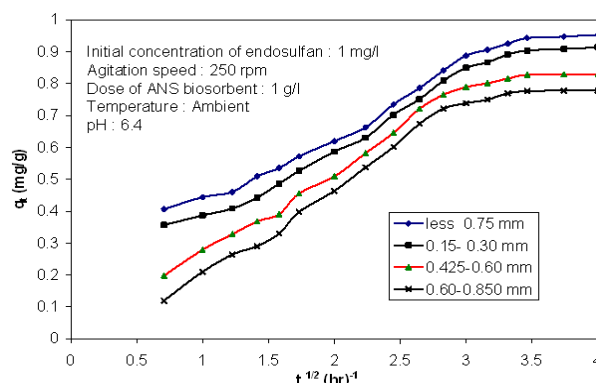
#### 3.4.2. Based on the Effect of Biosorbent Size

It is possible to get an idea about rate limiting step from the change of pesticide concentration with time and biosorbent size. Figure 7 and 8 show the effect of biosorbent size on removal of endosulfan. As the size increased, percentage removal and removal rate decreased. The removal rates( $k$ ) obtained from the linear portion of the curves were(tabulated in Table 3) plotted against reciprocal of the adsorbent diameter as shown in Figure 9. According to Helfferich[34], for film diffusion to be rate limiting, the removal should

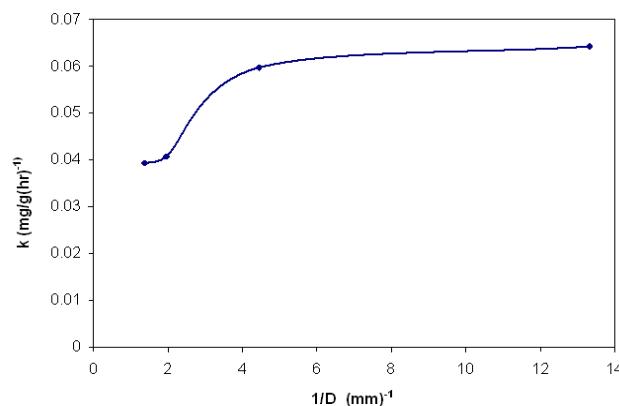
linearly vary with reciprocal of the diameter of adsorbent. It is evident from graphs(Figure 8) that the film diffusion alone does not appear to play a significant role, on biosorption process in the case of biosorption of endosulfan on ANS biosorbent. The trend of curve indicates that the inclusion of both the external mass transfer diffusion and intra-particle diffusion may give more precise expression.



**Figure 7.** Effect of biosorbent size on change of concentration of endosulfan with time



**Figure 8.** Intraparticle diffusion plots for endosulfan on ANS biosorbent at various biosorbent sizes



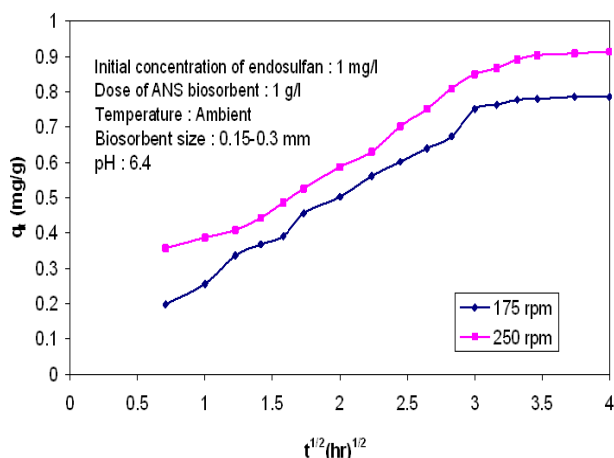
**Figure 9.** Variation of rate constant with reciprocal of diameter of biosorbent

**Table 3.** Values of Endosulfan Removal Rates( $k$ ) for Different Sizes.

Diameter(mm)	Removal rate $k$ (mg/g hr <sup>1/2</sup> )
0.075	0.064
0.15-30	0.062
0.425-0.60	0.040
0.60-0.850	0.039

### 3.4.3. Based on the Effect of Agitation

The rate of agitation is one of the prominent factors for biosorption process as it helps keep adsorbent in suspension and gives proper mixing to solutes. Especially in the case of pesticides, which are hydrophobic in nature, agitation speed has a significant effect on the removal efficiency. Figure 10 depicts the influence of agitation speed from 175 to 250 on biosorption of endosulfan. The biosorption rate calculated from the agitation speeds of 175 rpm and 250 rpm were found to be 0.197 mg/g.hr<sup>1/2</sup> and 0.22 mg/g.hr<sup>1/2</sup> respectively. Both the curves had features like an initial curve portion, followed by a linear portion and later a plateau. Thus intra-particle diffusion along with film diffusion may be the controlling rate-limiting steps in the biosorption process.



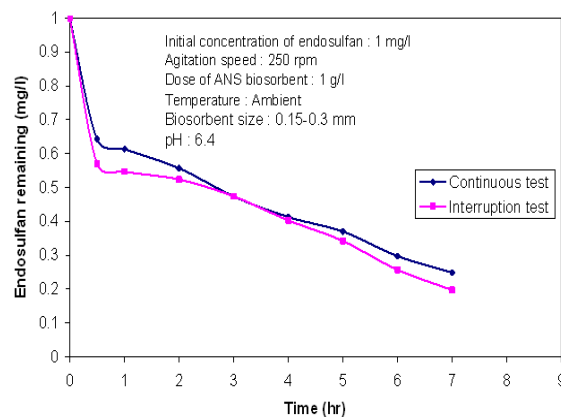
**Figure 10.** Intraparticle diffusion plots for endosulfan on ANS biosorbent at various agitation speeds

### 3.4.4. Based on Interruption Test

Several factors of the adsorbents, adsorbate and solution phase are of importance in determining the rate-limiting step. The factors include the particle size of the adsorbent, concentration of adsorbate, degree of mixing, affinity of adsorbate for adsorbent and diffusion coefficients of the adsorbate in bulk solution and within the porous adsorbent. In an interruption test, the adsorbent is periodically removed from the adsorbate solution for a brief period and reimmersed. When concentration gradients are present, the pause gives time for the gradients to level off within the pores of the adsorbent. When intraparticle transport is the rate limiting step, the rate of removal immediately after reimmersion is greater than the rate prior to interruption. However, the interruption period has no influence on the rate of biosorption after re-immersion when the external transport is rate limiting [34,22].

Figure 11 shows the effect of interruption test. A slight increase in the removal found after the interruption test indicated that intra-particle diffusion was rate limiting in biosorption process. From above all experimental results and discussion, it can be concluded that there are four steps involved in the entire biosorption process (1) migration of adsorbate molecules from bulk solution to the surface of the

biosorbent; (2) diffusion through the boundary layer to the surface of the biosorbent; (3) biosorption at a site and (4) intraparticle diffusion into the interior of the biosorbent [45]. As temperature increased, the rate constant for intraparticle diffusion also increased, indicated that in the process of biosorption intraparticle diffusion played a significant role. From these observations, it can be concluded that diffusion needs some energy to overcome the mass transfer resistance. The results of other studies [45,46] also support this observation. The interruption test confirmed that intra-particle diffusion (pore diffusion) was important in determining the overall rate of the transfer of the adsorbate onto ANS biosorbent.



**Figure 11.** Kinetic profiles of endosulfan biosorption onto ANS biosorbent interruption and un-interruption tests

## 4. Conclusions

In present study, sorption of endosulfan on ANS biosorbent was observed. The sorption capacity increased with an increase in initial endosulfan concentrations i.e 0.9636 mg/g, 1.6084 mg/g, 2.9413 mg/g for 1 mg/L, 2 mg/L and 4 mg/L respectively. The biosorption of endosulfan onto ANS biosorbent followed the pseudo-second-order kinetic model with high correlation coefficient and also fitted well the intraparticle diffusion model upto 12 hrs. It is evident from McKay equation that biosorption process on ANS biosorbent was mixture of different rate controlling steps. Plots of  $q_t$  vs  $t^{1/2}$  indicated the effect of intra-particle diffusion on the process and also indicated that the pore diffusion alone may not be the rate controlling step. Interruption test confirmed that the pore diffusion played the most important role for the transfer of the adsorbate to the biosorption sites.

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