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# Effectiveness of Several Biosorbents for Removing Endosulfan From The Environment in Water

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**Abstract:** The effectiveness of biosorbents made from diverse cultures for removing endosulfan from the aqueous environment was assessed in this study. For the biosorption of endosulfan from the aqueous environment, seven distinct biosorbents from the following organisms were synthesised and evaluated: Bacillus subtilis, Bacillus megaterium, Escherichia coli, Rhizopus arrihzus, Aspergillus nidulans, Cladosporium, and activated sludge biomass. Each biosorbent was used in biosorption tests with a 1 mg/l endosulfan solution at 250 rpm for three days, with biosorbent dosages ranging from 2.5 to 50 mg. The primary criterion for choosing the best biosorbent for further investigation was the biosorbent's saturation capacity. For all biosorbents, isotherms were created, and linear regression analysis was used to calculate saturation capacities using the BET, Freundlich, and Langmuir adsorption equilibrium models. The biosorbent made from the fungus Aspergillus nidulans was discovered to be the most suitable since, among all biosorbents, it had the highest saturation capacity of 43.6 mg/g of endosulfan.

**Keywords:** BET; biosorption; endosulfan; Freundlich; Langmuir.

## 1 Introduction

Pesticides are mostly chemical substances that are used to successfully and efficiently limit the growth of weeds, fungus, and insects that compete with people for crops by eating them. Only in agriculture, there are more than 500 distinct pesticide formulations in use (Arias-Estevez et al., 2008). They are intended to combat common diseases like malaria and encephalitis in addition to increasing crop yields and bringing down food prices. Nevertheless, crop spraying and irrigation runoff spread these dangerous chemicals to the habitats of unintended species. These animals' tissues can accumulate chemicals, and when people ingest the goods made from affected animals, the biomagnified pesticides cause health difficulties and, in some cases, death (Wandiga, 2001). Almost none of the pesticides applied to crops really reach the target pest; the remainder seeps into the environment and contaminates the soil, water, and air, as well as having negative effects on non-target animals (Krishna and Phillip, 2008). The fact that several organochlorine insecticides that were prohibited more than 20 years ago are still present in detectable amounts is proof that pesticides can linger in the environment for a very long time (Arias-Estevez et al., 2008).
Organochlorine insecticide endosulfan has been widely used in agriculture all around the world to control insect pests including whiteflies, aphids, leafhoppers, Colorado potato beetles and cabbage worms. It has also been used in wood preservation, home gardening and tse-tse fly control. It is hardly used for public health purposes. Cumulative global use of endosulfan for crops is estimated to be 338 kilo tons. The global endosulfan use has increased continuously since the time this pesticide was applied first time. India is the world's largest consumer of endosulfan with a total use of 113 kilo tons from 1958 to 2000 (GOC, 2009). Endosulfan I and II were recently reported in a passive air sampling study in India (Zhang et al., 2008) while residues of endosulfan I were found in

spruce needles from the central Himalayas (Wang et al., 2006). Agnihotri et al. (1994) reported endosulfan residues in the river Ganges water near Farrukhabad, India. Endosulfan is classified as a priority pollutant by many international environmental agencies (Dave and Dikshit, 2011b). The health and environmental concerns have led to an interest in detoxification of endosulfan in the environment. The guideline value for endosulfan and its metabolites in drinking water is set as 0.22 µg/l and 0.1–0.2 mg/l in agricultural products by USEPA (1999). As per Bureau of Indian Standards (BIS), the pesticide residue should be absent in drinking water and should not exceed 0.005 mg/l in surface waters (Dave and Dikshit, 2011a; Gora et al., 2006; Sudhakar and Dikshit, 2001). Single treatment method for removal of wide range of pesticides from water environment is not possible. Lots of efforts have been undertaken by researchers to develop technologies for the removal of various pesticides by photochemical or chemical treatment, volatilisation, biodegradation, reverse osmosis, adsorption mechanisms. Adsorption has been proven as well-established and best available technique for the removal of dissolved organic pesticides among these methods (Thacker et al., 1997; Gonzalez-Pradas et al., 1997; Kouras et al., 1998). Activated carbon has proved most effective in many research works because of its porous structure and the high surface area, which provide a good capacity for the adsorption of organic compounds (Perrich, 1981; Murayama et al., 2003; Brown et al., 2004). High operating and regeneration cost has led many researchers to search for cheaper and efficient adsorbents to remove organic contaminants from water and wastewater. Wood charcoal (Sudhakar and Dikshit, 1999), fly ash (Akgerman and Zardkoohi, 1996), baggase fly ash (Gupta et al., 2002), aquifer materials (Gullick and Weber, 2001) and porous polymeric absorbent (Kyriakopoulosa et al., 2005) have been tested for the adsorption of organic pollutants.

Biosorption is an attractive and promising alternative which accumulates organic and inorganic matter and offers potential advantages such as low operating cost, minimisation of chemical or biological sludge (Ahluwalia and Goyal, 2007; Maurya et al., 2006). It has been used for the treatment of wastewaters rich in heavy metals for several decades (Apiratikul and Pavasant, 2006; Wang and Chen; 2006; Hammaini et al., 2007). Several researchers have focused on the removal of phenol and dyes by biosorption but very few have done work on removal of pesticides (Tsezos and Bell, 1989; Calace et al., 2002; Juhasz et al., 2002; Bhattacharyya and Sharma, 2004; Allen et al., 2005). Biosorbent derived from bacteria, fungi, algae, activated sludge, byproducts from fermentation industries or seaweeds were used for removal of organic or inorganic matter (Dave and Dikshit, 2011a). Microbial biomass, such as fungi, would be particularly cost effective as there are many food-processing plants in many countries that could provide wastewater as substrate at a very low cost for the cultivation of them (Dave and Dikshit, 2011b). Tsezos and Bell (1989) evaluated the biosorption of lindane, diazion and malathion on the dried activated sludge. Benoit et al. (1998) reported the equilibrium sorption isotherms and sorption kinetics of herbicides 2,4-dichlorophenoyacetic acid (2,2-D) and atrazine on live and freeze dried inactivated fungal mycelium. Hong et al. (2000) reported biosorption of 1, 2, 3, 4-tetrachlorodibenzo-*p*-dioxin (1, 2, 3, 4-TCDD) and some polychlorinateddibenzo-furans (PCDFs) on *Bacillus pumilus* dead biomass and concluded that dead biomass of microorganism could more effective than live cells in removal of these organic pollutants.

In the present work, efforts have been made to develop biosorbents based on the biomass obtained from the dead micro-fungi, bacteria and mixed cultures and to identify

the suitable one, which could be adopted for the removal of endosulfan from the water environment.

## 2 Materials and methods

### *Microorganism and its growth conditions*

Three bacterial cultures, *Bacillus subtilis* NCIM 2063, *Bacillus magaterium* NCIM 2087, *Escherichia coli* NCIM 2065 and three fungal cultures, *Rhizopus arrihzus* NCIM 997, *Aspergillus nidulans* NCIM 1211, *Cladosporium* NCIM1082 used in this study were procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India. Bacterial cultures were grown in nutrient medium containing 10 g/l beef extract, 5 g/l NaCl, and 10 g/l peptone. Fungal cultures were grown in potato dextrose medium (NCIM 44), which contained 200 g/l peeled potatoes, 20 g/l dextrose, and 0.1 g/l yeast extract. The incubation was carried out at ambient temperature for bacterial cultures and at 37°C for fungal cultures in orbital shaker incubator at250 rpm.

### *Growth studies*

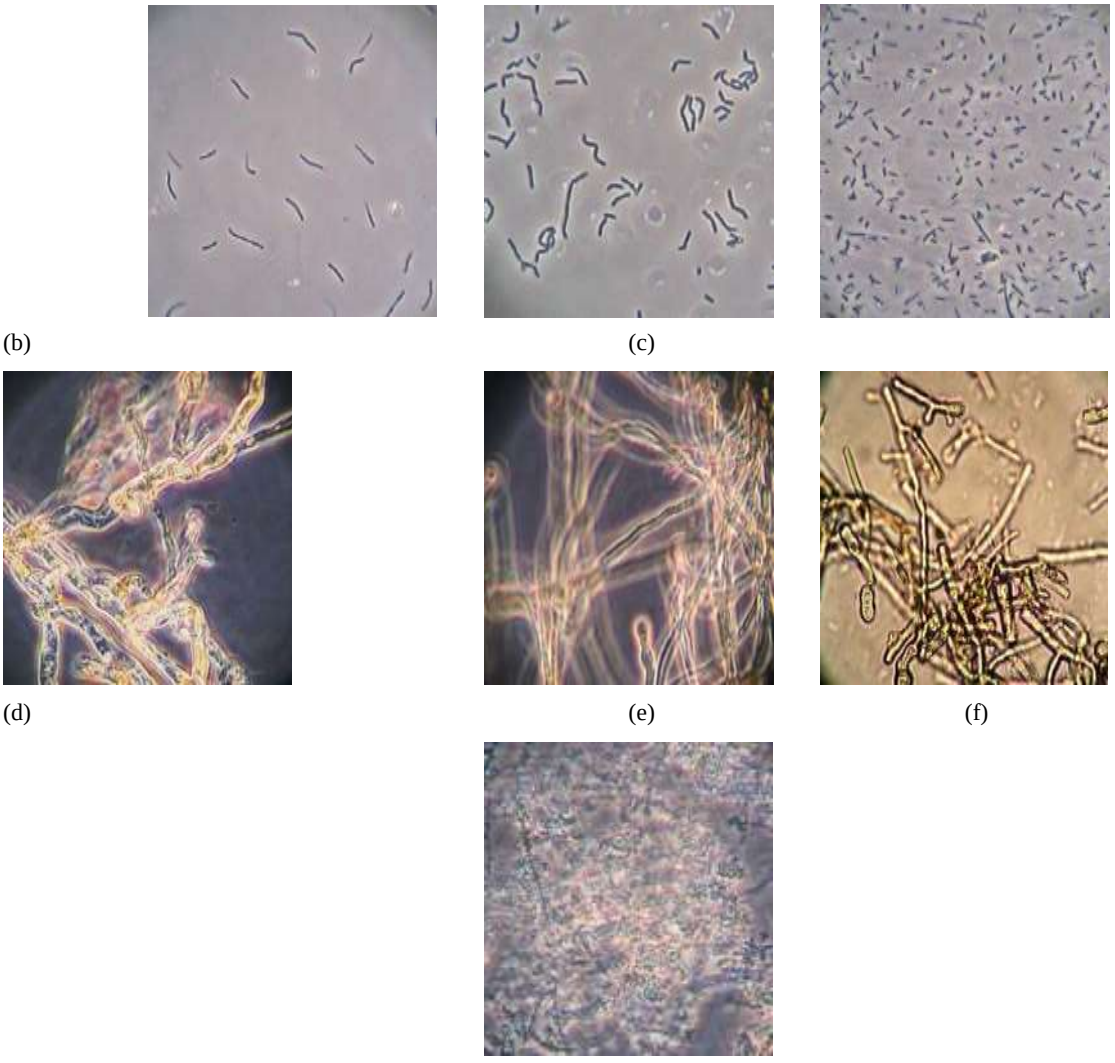
### *Bacterial growth*

Growth studies of all three bacterial cultures were done to identify the stationary phase of each bacterial culture. 1 ml of bacterial culture was inoculated in flask containing 100 ml nutrient broth. Immediately after inoculation, optical density of the sample was measured at 600 nm with Thermo Spectronic spectrophotometer (model Heλiose, USA). After optical density measurement, flask was kept in continuous rotary shaker at 250 rpm. After interval of two hr, optical density of the culture was measured again and then kept in shaker. This process was continued till the optical density of the culture reached to maximum and started decreasing. Optical density versus time graph was plotted to identify the optimum time required for stationary growth phase of bacterial culture. Same procedure has been done for all three bacterial cultures. Control experiments were also set up in the absence of bacterial inoculums to determine growth (if any) under biotic condition.

The growth curve of a bacterial culture can be divided into several distinct phases. The first, lag phase, occurs directly after dilution of the starter culture into fresh medium. During this phase, cell division is slow as the bacteria adapt to the fresh medium. The bacteria, then, start to divide more rapidly and the culture enters logarithmic (log) phase (4–5 hr after dilution), during which the number of cells increases exponentially. As the available nutrients in the medium are used up and the released metabolites inhibit bacterial growth, the culture becomes saturated, and enters stationary phase (~20 hr for *Bacillus subtillus*, 38 hr for *Bacillus megaterium*, and 14 hr for *Escherichia coli* after dilution), during which cell density remains constant. Eventually, the culture enters the phase of decline as cells start to lyse, the number of viable bacteria falls, and DNA becomes partly degraded. The microscopic views of *Bacillus subtilis, Bacillus megaterium*, and *Escherichia coli* using Zeiss image analysis microscope (model Axion

Star Plus, Germany) are shown in Figure 1(a), Figure 1(b) and Figure 1(c) respectively. All these images have been taken with 40X magnification.

**Figure 1** Microscopic view of (a) acillus subtilis (b) bacillus megaterium (c) escherichia coli (d) rhizopus arrhizus (e) aspergillus nidulans (f) cladosporium (g) activated sludge biomass (see online version for colours)



(b) (c)

(d) (e) (f)

(g)

## *Fungal growth*

Under ideal conditions, bacteria reproduce extremely rapidly, perhaps doubling in numbers every 20 or 30 min, but fungi grow more slowly. Growth studies were done for each fungal culture. Growth profile was analysed over a time period of 48–60 hr for *Rhizopus arrihzus* during which the complete growth is expected. Similarly, for

*Aspergillus nidulans* and *Cladosporium*, the studies were carried out for 120–132 hr and 172–192 hr, respectively during which the complete growth is expected. A set of flasks, each containing 100 ml of growth medium, was inoculated with 1 ml fungal spores suspension and placed on an incubator shaker at 250 rpm maintained at 37°C to initiate growth of the fungus. One flask from the set was sacrificed every 4 hr to harvest the fungal biomass pellets of *Rhizopus arrihzus*. Similarly, for *Aspergillus nidulans* and *Cladosporium*, the flask were sacrificed every 6 hr and 12 hr, respectively. The fungal pellets were dried at 105°C to determine the dry weight of the biomass. Dry weight versus time graphs were plotted to identify the optimum time required for active growth phase of fungus. Fungal biomass *Rhizopus arrihzus, Aspergillus nidulans* and *Cladosporium* entered into the log phase after 4 hr, 12 hr and 50 hr of spore inoculation. Stationary phase of *Rhizopus arrihzus, Aspergillus nidulans* and *Cladosporium* was 48 hr, 96 hr and 180 hr, during which fungal mass remained constant. Eventually the culture entered the phase of decline phase. The activated sludge biomass (ACE) was also used as a mixed culture for the research work. The microscopic views of *Rhizopus arrihzus, Aspergillus nidulans, Cladosporium* and mixed culture using Zeiss image analysis microscope at 40X magnification are shown in Figure 1(d), Figure 1(e), Figure 1(f) and Figure 1(g) respectively.

## *Preparation of biosorbents*

### *Bacterial biosorbents*

*Bacillus subtilis, Bacillus magaterium,* and *Escherichia coli* were grown in nutrient medium with a one full loop of cultured cells inoculation from sub cultured slants. Test flasks were placed on incubator shaker maintained at ambient temperature, and 250 rpm for 20 hr, 38 hr, and 14 hr for *Bacillus subtilis, Bacillus megaterium,* and *Escherichia coli*, respectively. Wet cells were harvested by centrifuging the fermentation broth at 5,000 rpm for 10 min. The cells were resuspended in distilled water and centrifuged at 5,000 rpm for another 20 min. Supernatant was discarded and the biomass was dried in an oven at 105°C for 12 hr and ground with a Remi laboratory grinder (Mumbai, India) and sieved to get particle size between 0.15–0.3 mm.

### *Fungal biosorbents*

*Rhizopus arrihzus, Aspergillus nidulans* and *Cladosporium* were grown in an autoclaved potato dextrose medium with corresponding fungal spores (roughly 10% of the volume of medium). Test flasks were placed on incubator shaker maintained at 37°C, and 250 rpm for 48 hr, 96 hr, and 180 hr for *Rhizopus arrihzus, Aspergillus nidulans* and *Cladosporium*, respectively. Spore inoculums were prepared under sterile conditions in 0.05% in tween-80 using freshly grown and sporulating mat on potato dextrose agar plates. *Rhizopus arrihzus, Aspergillus nidulans* and *Cladosporium* were recovered by filtering through filter paper no. 201, washed repeatedly with distilled water, and autoclaved at 121°C for 20 min. The biomass was, then, dried in an oven at 105°C for 12 hr, and ground with a laboratory grinder and sieved to particle size 0.15–0.3 mm.

### *Mixed culture biosorbent*

The sludge collected from Mahananda Dairy's wastewater treatment plant, Mumbai, India was allowed to settle and the supernatant was decanted. Repeated washings were given to the sludge for six times. The biomass was collected by centrifuging sludge at 5,000 rpm for 10 min. Supernatant was discarded and the biomass was dried in an oven at 105°C for 12 hr and ground with a laboratory grinder and sieved to get particle size between 0.15–0.3 mm.

### *Chemicals*

All chemicals and reagents used were of analytical grade. Technical grade endosulfan of 96.14% purity was obtained from M/s Vijiyalaxmi Insecticides and Pesticides Limited, Andhra Pradesh, India. n-hexane and acetone were purchased from Merck India Ltd., Mumbai, India. All glassware used was of Borosil. 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 at a concentration of 100 to 1000 mg/ml. All solutions were stored in the dark at 4ºC prior to use.

### *Biosorption experiments*

Seven different biosorbents from *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli*, *Rhizopus arrhizus*, *Aspergillus nidulans*, *Cladosporium* and ACE were evaluated for the biosorption of endosulfan from water environment. The saturation biosorptive capacity (or simply biosorptive capacity) of the biosorbent was chosen as the key parameter forthe selection of the most suitable biosorbent for further study.

Batch experiments were conducted for finding out the biosorption capacity of biosorbent. Biosorption experiments were conducted by contacting endosulfan solution of 1 mg/l with different quantities of biomass. Different biosorbent doses 2.5-50 mg were added to different 100 ml screw-top flask containing 50 ml endosulfan solution. In order to control loss of solute through experiments, equilibrium calculations were determined on the basis of organic recovered from duplicate blank samples handled in the same way as the regular contacts but without any biomass present in solution. The flasks were agitated on the shaker at 250 rpm for approximately three days at constant room temperature.

The biomass was separated from flasks containing biosorbents 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 adsorption equilibrium of filter paper with the solution. This portion of filtrate was discarded and the subsequent filtrate was, then, extracted by n-hexane as prescribed in Section 2.6 and collected for gas chromatographic (GC) analysis (as mentioned in Section 2.7) in Teflon sealed GC vials. All experiments were run in duplicate. Average values of observations were used provided that they were within 10% otherwise the experiments was repeated in duplicate till consistent observations were obtained.

### *Extraction of endosulfan from water*

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, Mumbai, India). Then, allowed to settle for 1 minute. Hexane extract was collected in 20 ml test tube. Total 6 ml n-hexane was collected as results of three times extraction. 1 ml from 6 ml extracted hexane was transferred to teflon sealed GC vials. 2 µl of extracted sample was used for injecting into GC.

### *Analysis of endosulfan*

Gas chromatograph (Agilent Technologies, model GC-6890N, USA) with electron capture detector and Agilent HP-5 column of 0.53 mm ID, 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 280ºC, 250ºC and 300ºC respectively. Nitrogen (99.9% purity) was used as carrier gas at a flow rate of 30 mL/min.

### *Saturation biosorption capacity*

There are several isotherm equations, which can be used to describe the equilibrium nature of adsorption. However, adsorption mechanisms are so complicated that no simple theory can adequately represent all experimental data. Most of the isotherm models used to describe adsorption in solutions is based on the semi-empirical equations. The saturation uptake capacity ($q_{max}$) was estimated for each biosorbent on the basis of isothermal studies. In this work, the Langmuir, Freundlich and BET equations that are most widely used were chosen to fit the experimental data. The assumptions of the Langmuir isotherm are that

1 adsorption energy is constant over all sites

2 adsorbed atoms or molecules are adsorbed at definite, localised sites

3 each site can accommodate only one molecule or atom

4 there is no interaction between adsorbates.

At adsorption equilibrium, a saturation point is reached at which no further adsorption can occur. Thus, the Langmuir isotherm reaches a plateau at the saturation point (Sohn and Kim, 2005). The Langmuir isotherm can be represented by following equation:

$$q_e = \frac{K_L q_{max} C_e}{1 + K_L C_e} \quad (1)$$

The following linearised form of above equation was used to calculate the maximum adsorption capacity of particular biosorbents.

$$\frac{1}{q_e} = \frac{1}{q_{max}} + \left(\frac{1}{q_{max} k_L}\right)\frac{1}{C_e} \quad (2)$$

where $q_e$ is adsorbate concentration sorbed per unit weight of adsorbent (mg/g); $C_e$ is adsorbate concentration in solution at equilibrium (mg/l), $q_{max}$ is the saturation abiosorptive capacity of the adsorbent (mg/g), and $K_L$ is the Langmuir adsorption constant (l/mg) and is related to the free energy of adsorption. The plots of $1/q_e$ versus $1/C_e$ can be used to calculate $q_{max}$.

The Freundlich isotherm is an empirical expression that encompasses the heterogeneity of the surface and an exponential distribution of the sites and their energies. This isotherm has been further extended by considering the influence of adsorption sites and the competition between different adsorbates for adsorption on the available sites. Isotherms of the Freundlich form have been observed for a wide range of heterogeneous surfaces including activated carbon, silica, clays, and polymers (Umpleby et al., 2001). The Freundlich equation is

$$q_e = K_f C_e^{\frac{1}{n}} \quad (3)$$

where $1/n$ is the heterogeneity factor of the adsorbent and $K_f$ is constant which represent sorption capacity. The surface heterogeneity is due to the existence of crystal edges, type of cations, surface charges, surface modification groups, and degree of crystallinity of the surface. The $1/n$ value indicates the relative distribution of energy sites and depends onthe nature and strength of the adsorption process. A linear form of the Freundlich equation is

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \quad (4)$$

BET model is applicable for multilayer adsorption. This model assumes that multiple layers of adsorbate molecules form at the surface of the adsorbent particles and Langmuir equation applies to each layer. However, a given layer need not form completely prior to the initialisation of subsequent layers (Milewska-Duda et al., 2000). BET equation takes the form

$$q_e = \frac{BCq_{max}}{(C_s - C_e)\left[1 + (B-1)\left(\frac{C_e}{C_s}\right)\right]} \quad (5)$$

where $C_s$ is saturation concentration of adsorbate in the solvent (mg/l) and $B$ is constant. The linearised form is written as follows:

$$\frac{C_e}{(C_s - C_e) q_e} = \frac{1}{bq_{max}} + \frac{(B-1)}{bq_{max}}\left(\frac{C_e}{C_s}\right) \quad (6)$$

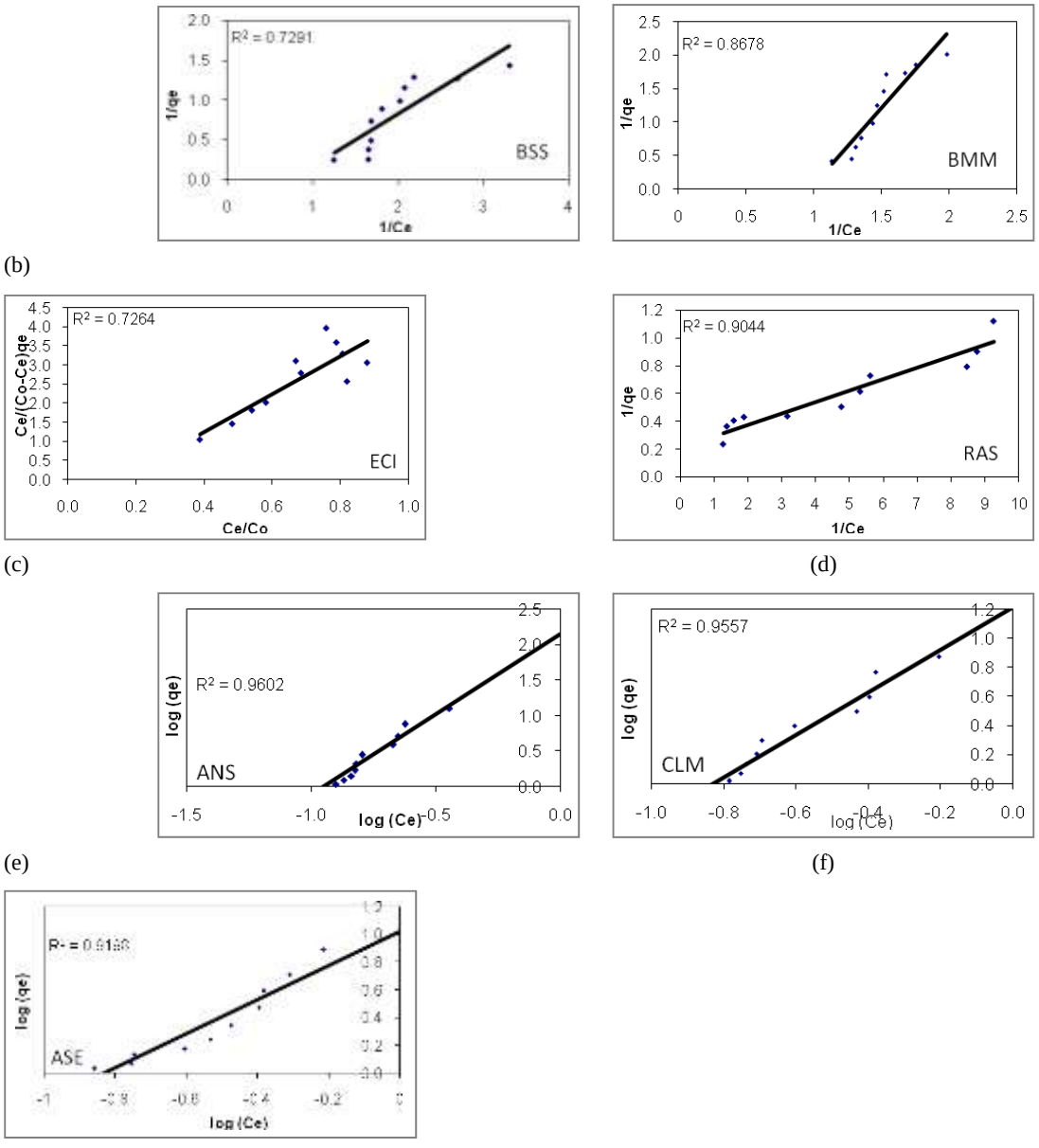
A linear plot between $C_e / (C_s - C_e)q_e$ and $(C_e / C_s)$ can be used to calculate $q_{max}$. In the present study, the isotherms were developed for all chosen biosorbents. To determine the saturation biosorptive capacity ($q_{max}$), linear plots were made with the corresponding parameters of various models. Linear regression analysis was carried out for all the candidate biosorbents with all three adsorption equilibrium models.

## 3 Results and discussion

In the present investigation, biosorptive capacities of all the biosorbents were calculated from the isotherm data. Detailed equilibrium uptake studies were conducted on biosorbents prepared from *Bacillus subtilis* (BSS), *Bacillus megaterium* (BMM), *Escherichia coli* (ECI), *Rhizopus arrihzus* (RAS), *Aspergillus nidulans* (ANS), *Cladosporium* (CLM) and Activated sludge biomass (ASE). BSS biosorbent, BMM biosorbent and ECI biosorbent were found to remove 69.8%, 49.6% and 61.1% of endosulfan from water respectively, while RAS biosorbent, ANS biosorbent and CLM biosorbent removed 89.2%, 90.3% and 86.4% respectively, over three days. ASE biosorbent showed removal of 88.2% endosulfan from water.

Next, the saturation biosorptive capacity of each adsorbent was estimated to compare the biosorption potential of various biosorbents. For which, the isotherms were developed for all biosorbents. Linear regression analysis was carried out with BET, Freundlich and Langmuir adsorption equilibrium models. The correlation of data with various models for different biosorbents is presented in Table 1. BSS biosorbent, BMM biosorbent, RAS biosorbent followed Langumir isotherm; ECI biosorbent followed BET isotherm; while ANS biosorbent, CLM biosorbent and ASE biosorbent followed Freundlich isotherm model. The isotherms for various models profiles of corresponding biosorbents are shown in Figure 2. To determine $q_{max}$, the model that showed better correlation with data, was selected. The correlation between data and the model was established using Microsoft Office Excel. The $q_{max}$ values for various biosorbents are shown in Table 2. The results in Table 2 indicate that the BSS, BMM and ECI bisorbents prepared from bacterial cultures *Bacillus subtilis, Bacillus megaterium* and *Escherichia coli* have comparatively less (2.1, 0.5, 0.2 mg/g, respectively) saturation biosorptive capacity. Ju et al. (1997) have reported low biosorptive capacity of 0.6 mg/g, 0.7 mg/g and 0.5 mg/g for biosorption of lindane onto bacterial biosorbents prepared from *Bacillus subtilis, Bacillus megaterium* and *Escherichia coli*, respectively. Thomas Johnson and Kennedy (1973) also reported low bisorptive capacity of 0.00356 and 0.00448 µg/g for biosorption of methoxychlor and DDT, respectively on *Bacillus subtilis* biomass. CLM biosorbent prepared from fungal culture *Cladosporium* has good biosorptive capacity of 16.36 mg/g for biosorption of endosulfan. Juhasz et al. (2002) have reported good biosorptive capacity of 17 mg/g for biosorbent prepared from *Cladosporium* for biosorption of p-p'DDT. Xinjiao (2006) reported biosorptive capacity of 28.31 mg/g for biosorbent prepared from *Cladosporium* for biosorption of $Cu^{2+}$ from aqueous solutions. Pethkara et al. (2001) reported 110 mg/g of golden loading capacity while using *Cladosporium* spp. as biosorbent. RAS biosorbent prepared from *Rhizopus arrhizus* has biosorptive capacity of 4.7 mg/g for the biosorption of endosulfan. Bell and Tsezos (1987) reported the biosorptive capacity of 2.7 mg/g, 0.5 mg/g and 13.2 mg/g for the biosorption of lindane, diazinon and malathion, respectively on biosorbent prepared from *Rhizopus arrhizus.* Lievremont et al. (1998) reported the bisorptive capacity of 4.6 mg/g for the bisorption of pentachloronitrobenzene on biosorbent prepared from *Rhizopus arrhizus.* Tsezos and Bell (1989) reported on the biosorption of pentachlorophenol with a biosorption capacity of 14.9 mg/g using *Rhizopus arrhizus* as a biosorbent. ASE biosorbent prepared from ACE has good bisorptive capacity of 10.6 mg/g for biosorption of endosulfan.

**Figure 2** Isotherms for different biosorbents (a) Langmuir for BSS (b) Langmuir for BMM (c) BET for ECI (d) Langmuir for RAS (e) Freundlich for ANS (f) Freundlich for CLM (g) Freundlich for ASE (see online version for colours)



Correlation coefficients for seven different biosorbents with different adsorptionequilibrium models
*Correlation coefficients of equilibrium model*

| *rbent* | *dlich* | *nuir* | *l chosen* |
|---|---|---|---|
| | | | nuir |
| | | | nuir |
| | | | |
| | | | nuir |
| | | | dlich |
| | | | dlich |
| | | | dlich |

Values of saturation biosorptive capacities ($q_{max}$) and correlation coefficients forvarious biosorbents

| *Sr. no.* | *Biosorbents* | *Model followed* | *Correlation coefficient (r)* | *Saturation capacity, $q_{max}$ (mg/g)* | *biosoptive Relative rank* |
|---|---|---|---|---|---|
| 1 | BSS | Langmuir | 0.729 | 2.1 | 5 |
| 2 | BSM | Langmuir | 0.867 | 0.5 | 6 |
| 3 | ECI | BET | 0.726 | 0.2 | 7 |
| 4 | RAS | Langmuir | 0.904 | 4.7 | 4 |
| 5 | ANS | Freundlich | 0.960 | 43.6 | 1 |
| 6 | CLM | Freundlich | 0.955 | 16.4 | 2 |
| 7 | ASE | Freundlich | 0.919 | 10.6 | 3 |

Biosorptive capacity of 16.9 mg/g was observed by Bell and Tsezos (1987) for biosorption of malathion on biosorbent prepared from activated sludge from a municipal biological wastewater treatment plant. Jianlong et al. (2000) characterised the adsorption behaviour of pentachlorophenol from aqueous solution on ACE collected from a local biological wastewater treatment plant and reported 2.56 mg/g of biosorptive capacity. ANS biosorbent prepared from *Aspergillus nidulans* has shown the maximum biosorptive capacity of 43.6 mg/g for biosorption of endosulfan as compared to other biosorbents (Table 2). Major components of *Aspergillus nidulans* cell walls are reported to be chitin and β-1, 3-glucan, which are believed to be the important structural polysaccharides that confer rigidity and strength to the cell wall. Other polysaccharides and proteins are also present in the cell wall as minor components (Bull, 1970; Zonneveld, 1971). According to Zhou and Banks (1993), the fungal cell wall components, particularly chitin and chitosan, are the most important components responsible for the biosorption. The percentage of chitin and chitosan in fungi varies with fungal species, strain, age and other conditions. Li et al. (2006) reported the presence of velvet gene, veA, which coordinates asexual-sexual development in *Aspergillus nidulans*, also regulates morphological differentiation for cell wall integrity, cell surface hydrophobicity, hyphal polarity and

conidiation pattern of *Aspergillus nidulans.* The presence of polysaccharides and proteins along with velvet gene, veA, responsible for the cell wall composition, might be the one of the reason for the higher uptake of endosulfan. Surface of ANS biosorbent is interwoven with different groups like carboxyl, hydroxyl, amines, and amides etc. All groups are hydrolysed differently in aqueous phase. pH of the aqueous biosorbent suspension is the measure of the cumulative effect of the hydrolysis of different surface groups. The pH of the ANS biosorbent suspension was 6.35 ± 0.05. It implies that in aqueous phase, biosorbent surface carries a net negative charge, which indicates the possibility of coulombic attraction between the biosorbent (negatively charged surface in the aqueous phase) and the endosulfan might be the another reason for higher biosorption.

In this study, all bacterial biosorbents showed low biosorption capacity due to hydrophobic interaction and van der Waals forces responsible for the biosorption (Ju et al., 1997) while biosorbents prepared from fungal culture showed comparatively higher biosorption capacity due to chitin and chitosan components of the cell wall (Zhou and Banks, 1993). The good biosorptive capacity of biosorbent prepared from ASE could be due to the higher population of the filamentous bacteria as they may have higher surface area. Since biosorption capacity of ANS biosorbent was maximum in comparison to all other biosorbents, it has been selected for the further research.

Conventionally, activated carbons have been used as adsorbents in the treatment of water and wastewater. So, the endosulfan uptake capacity of the activated carbon could be taken as the benchmark for comparing the biosorption capacity of the ANS biosorbent. Sudhakar and Dikshit (2004) reported sorption capacity of activated carbon (particle size 0.15–0.3 mm) for endosulfan under the identical experimental condition as 2.15 mg/g which shows that the sorption potential of ANS biosorbent is about 20 times higher than that of activated carbon for endosulfan removal. As a substitute for activated carbon, Sudhakar and Dikshit (2004) had also developed a low cost acid treated adsorbent prepared from wood charcoal for the removal of endosulfan. They reported the sorption capacity of above mentioned sorbent as 1.77 mg/g for endosulfan removal, which is about 25 times lesser than that of ANS biosorbent. Muraleedharan (1994) has also found similar removal of metals by *Ganoderma lucidum*, which was 13 times higher than commercial activated carbon, named Filtrasorb 400.

## 4 Conclusions

Seven different biosorbents prepared from bacterial cultures, fungal cultures and mixed culture were tried for the uptake of endosulfan. Biosorbent prepared from the fungal culture *Aspergillus nidulans*, which was named as ANS biosorbent, proved to be excellent in removal of endosulfan from water environment on the basis of its saturation biosorptive capacity among all biosorbents. It showed 20 times more uptake capacity than that of activated carbon under the identical experimental conditions. Thus, biosorbents could be a better and economical substitute for activated carbon. It also indicates that the biosorption mechanisms operative in the present system could be different from that of the activated carbon.

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