Bio adsorption of mercury using *Aspergillus niger*

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SUMMARY

The increasing awareness of dye toxicity and the biomagnifications through food chain are responsible for the demand for detoxification of industrial effluents prior to their discharge into natural streams. The potential of using fungal biosorbents has received considerable attention since this represents a significant by-product from several fermentations. In batch mode studies, the parameters such as contact time, adsorbent dosage and pH were analyzed to optimize the bioadsorption of mercury. Autoclaved *Aspergillus niger* was found to be efficient in mercury removal. Studies on adsorption isotherms revealed that adsorption of mercury by the fungal mycelium followed the Langmuir model and the process of adsorption is favourable. By using Langmuir isotherm, the adsorption capacity (Qo) of live and autoclaved adsorbents was determined with 0.80 and 0.75 mg/g, respectively. The adsorption rate constants (K_{ad}) were calculated from the slope of the linear plots and were observed to be in the range of 0.035 to 0.046 and 0.030 to 0.038 x10^{-2} L/min, respectively for live and autoclaved mycelium.

Key words :
Biosorption,
Adsorbents,
Langmuir,
Mercury,
Aspergillus niger

Pollution of water is no more a local, but it is a global phenomenon (Hedges and Purnaik, 1992). The loss of mercury in industrial effluents would affect the aquatic and terrestrial environment (Parks, 1988). Methyl mercury, a potent neurotoxin is a main form of mercury in fish. When men ingest the fish, it leads to the bioaccumulation of methyl mercury in human beings. This bioaccumulation of methyl mercury ultimately results in fatal neurological disorders like Minammata disease (Fergusson, 1990). Removal of heavy metals is usually achieved by physico-chemical processes like precipitation, coagulation, ion exchange, membrane process and adsorption. Formation of sludge and high cost of activated carbon are the problems for the same (Kapoor *et al*., 1999). Adsorption of heavy metals on fungi occurs as a result of ionic interactions and complex formation between metal ions and ligands, which include chitin, chitosan, phosphate, amine, hydroxyl groups and other pigments (Shumate and Strandberg, 1985; Kapoor and Viraraghavan, 1995). Exposure of microbial cell to metal ions results in the rapid binding of cations to negatively charged sites on the cell wall. Extracellular polysaccharide binds effectively and precipitates heavy metals. Since fungi are widely used in a variety of large-scale industrial fermentation processes, it can be easily procured as a by-product in a cost-effective way.

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MATERIALS AND METHODS

The test fungus, *Aspergillus niger* was isolated from metal contaminated soil from an electroplating industry by soil dilution plate technique. The fungus was sub-cultured and maintained on Czapek Dox agar medium at 27±2°C.

Adsorbent:

Live and autoclaved *Aspergillus niger.*

Preparation of adsorbate solution:

Various concentrations of metal solutions 5, 10, 15, 20 and 25mg/L of mercury (II) chloride were prepared. The wavelength maximum adsorption for Mercury (II) chloride was 565nm.

Batch mode adsorption studies:

Contact time:

1g of adsorbent was added to 50mL of various adsorbate concentrations and agitated on a rotary shaker (150 rpm) at room temperature (27±2°C). The flasks were withdrawn at predetermined time intervals at 15 minutes. The adsorbent and adsorbate were separated by centrifugation at 3000 rpm for 5 minutes. The remaining adsorbate concentration in the supernatant was determined spectrophotometrically at 565 nm.

Adsorbent dosage:

Adsorbate solutions were agitated with

various dosages of adsorbent (0.25g to 2.0g/50mL) at equilibrium time period. The adsorbent and adsorbate were separated and the amount of adsorbate adsorbed was determined.

pH:

The optimum pH of adsorbate solutions were determined at various pH levels (2.0 to 10.0) using either 1N HCl or 1N NaOH. All these experiments were carried out with both live and autoclaved *Aspergillus niger*. Adsorption isotherms such as Langmuir (Langmuir, 1918) and Lagergren rate constant (Lagergren and Svenka, 1898) were used.

RESULTS AND DISCUSSION

Aspergillus niger was isolated from metal contaminated soil. The effect of agitation time, adsorbent dosage and pH on adsorption of mercury has been assessed.

Batch mode adsorption studies:

Effect of contact time:

Time required by the live and autoclaved mycelium to reach adsorption equilibrium for mercury was 90 and 105 minutes, respectively (Table 1). Cell surfaces are mainly anionic due to the presence of ionized groups. Cells when subjected to autoclaving can suffer rupture that can allow free access of cell wall binding sites (Hughes and Poole, 1989; Polman and Breckenridge, 1996; Bell and Buckley, 2003). This could be the reason for enhanced mercury adsorption by autoclaved mycelium.

Table 1: Effect of contact time on removal of mercury

Sr. No.	Type of Adsorbent	Equilibrium time (minutes)	Adsorbate removal (%)
1.	Live	90	59-39
2.	Autoclaved	105	72-44

Equilibrium time and adsorbate uptake varied with adsorbates, which may be due to difference in affinity of various adsorbents for adsorbates. Equilibrium time is one of the important considerations for economical water and wastewater treatment (Shekinah *et al*., 2002). The removal of metal increased rapidly in the beginning and then more slowly until the equilibrium (Fig. 1 and 2). This was caused by strong attractive forces between the metal molecules and the sorbent, fast diffusion into the intraparticle matrix to attain rapid equilibrium (Asfour *et al*., 1985).

Effect of adsorbent dosage:

The study was carried out with different adsorbent dosages up to an equilibrium time (0.25 to 2.0g/50mL). Increase in adsorbent dosage increased the per cent removal of mercury (Fig. 3 and 4). The optimum adsorbent dosage for mercury was found to be 2.0g for both live and autoclaved mycelium. Availability of more surface area of the adsorbent could be the reason for the increase in per cent removal of adsorbate with the increase in adsorbent dosage (Kadirvelu *et al*., 2000).

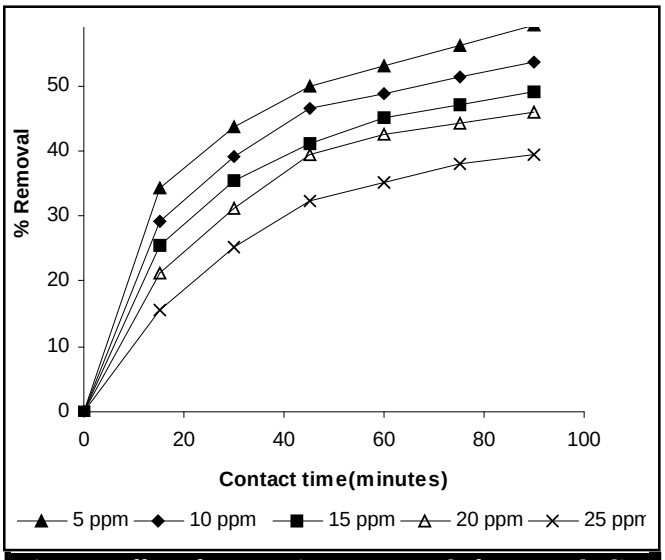

Fig. 1 : Effect of contact time on removal of mercury by live mycelium

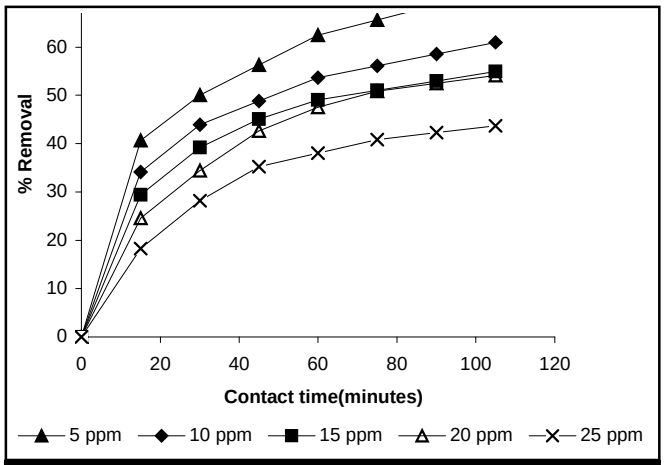

Fig. 2 : Effect of contact time on removal of mercury by autoclaved mycelium

Adsorption isotherms:

Langmuir isotherm:

These isotherms relate adsorption density q_e (uptake of adsorbate per unit weight of adsorbent) to equilibrium adsorbate concentration in the bulk fluid phase, C_e. The Langmuir isotherm treats surface sites analogous to

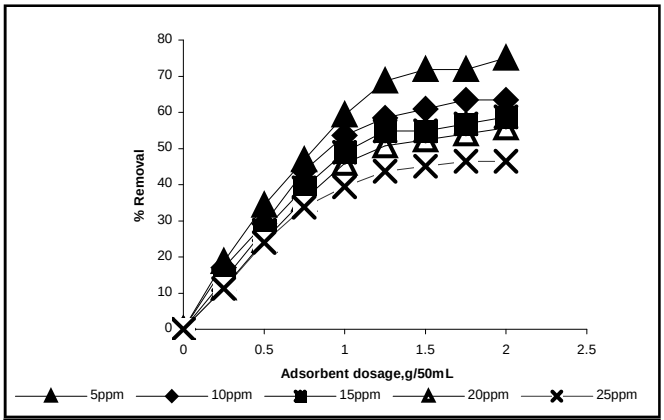

Fig. 3 : Effect of adsorbent dosage on removal of mercury by live mycelium

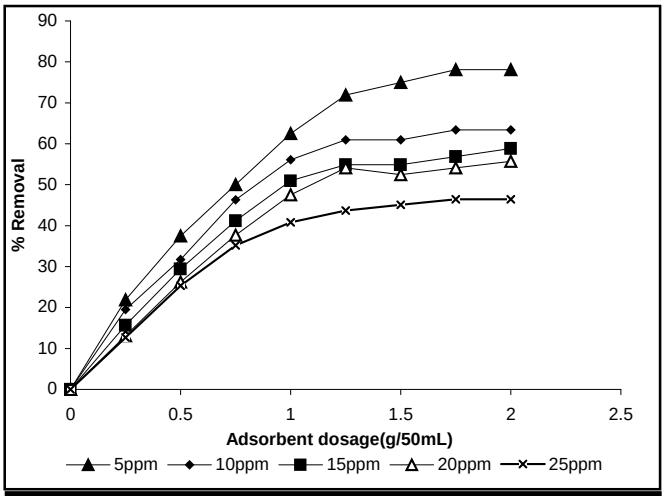

Fig. 4 : Effect of adsorbent dosage on removal of mercury by autoclaved mycelium

dissolved complex ligands. It is derived by combining sorption equilibrium constant with a mass balance on the total number of adsorption sites. The Langmuir isotherm is valid for monolayer adsorption onto a surface containing a finite number of identical sites (Namasivayam and Ranganathan, 1995). The model assumes uniform energies of adsorption onto the surface and no transmigration of adsorbate in the plane of surface (Langmuir, 1918).

The Langmuir isotherm is represented by the following equation:

$$C_e/q_e = 1/Q_o b + C/Q_o \quad \text{—— (1)}$$

where,

C_e is the equilibrium concentration (mg/L)

q_e is the amount adsorbed at equilibrium time (mg/g)

Q_o and b are Langmuir constants related to adsorption capacity and energy of adsorption, respectively.

In Langmuir plots for adsorption, the linear plots of C_e/q_e vs C_e (Fig. 6) confirm that the adsorption follows the Langmuir isotherm model. Langmuir constants, Q_o and b were determined from the slope and intercept of the respective plots (Table 2). The Q_o values for the adsorption of mercury in the present study were compared

Table 2 : Langmuir constants for mercury removal

Sr. No.	Biomass type	Langmuir constants	
		Q_0 (mg/g)	b
1.	Live	0.80	0.112
2.	Autoclaved	0.75	0.203

Table 3 : Comparison of adsorption capacity of *Aspergillus niger* biomass on mercury with other adsorbents

Adsorbent	Bio adsorption capacity (Qo)	References
Modified chitosan	329	Krishnan *et al*. (1988)
Cellulose	288	Navarro *et al*. (1996)
Rice straw	280	Friedman and Waiss (1972)
Peanut hull	220	Friedman and Waiss (1972)
Cellulose modified with ethylene diamine	188	Navarro *et al*. (1996)
Sugarcane bagasse	180	Friedman and Waiss (1972)
Polymerized saw dust	140.80	Raji and Anuirudhan, (1997)
Bicarbonate treated peanut hull carbon	109.89	Namasivayam and Periyasamy (1993)
Waste Fe (III)/Cr (III) hydroxide	37.30	Namasivayam and Senthilkumar (1997)
Commercial activated carbon	12.38	Namasivayam and Periyasamy (1993)
Dye stuff treated rice hull	12.13	Suemitsu *et al*. (1986)
Rice hull	8.67	Suemitsu *et al*. (1986)
Onion skin	7.10	Asai *et al.* (1986)
Waste rubber	4.0	Knock and Hemphill (1981)
Granular activated carbon	0.80	Huang and Blarikenship (1984).
Live *Aspergillus niger*	0.80	Present work
Autoclaved *Aspergillus niger*	0.75	Present work
Fly ash	0.73	Kapoor and Viraraghavan (1994),

with other reports (Table 3). Live mycelium of *Aspergillus niger* was highly efficient in adsorbing mercury when compared to autoclaved mycelium; the Q_o values of the live and autoclaved mycelium were 0.80 and 0.75 mg/g, respectively.

Adsorption rate constant :

The adsorption rate constant is determined from the following first order rate expression (Lagergren and Svenka, 1898).

$$\text{Log } (q_e - q) = \log q_e - K_{ad} / 2.303 \times t \quad \text{——(5)}$$

where,

q and q_e are amounts of adsorbete adsorbed (mg/g) at time, t (min) and at equilibrium, respectively.

K_{ad} is the adsorption rate constant (L/min).

The straight-line plots of log (q_e -q) Vs contact time (Fig. 7 and 8) showed the applicability of the above equation. The K_{ad} values were calculated from the slope of the linear plots and were observed to be in the range of 0.035 to 0.046, 0.030 to 0.038 $x10^{-2}$ L/min at 30^0C, respectively for adsorption of mercury using live and autoclaved mycelium (Table 4). The data revealed that the adsorbete concentration did not have any significant effect on the rate constant (K_{ad}).

Table 4 : Lagergren constant for mercury removal

Sr. No.	Initial mercury concentration (mg/L)	Adsorption rate constant K_{ad} (L/min)	
		Live	Autoclaved
1.	5	0.035	0.030
2.	10	0.038	0.031
3.	15	0.041	0.033
4.	20	0.046	0.039
5.	25	0.046	0.038

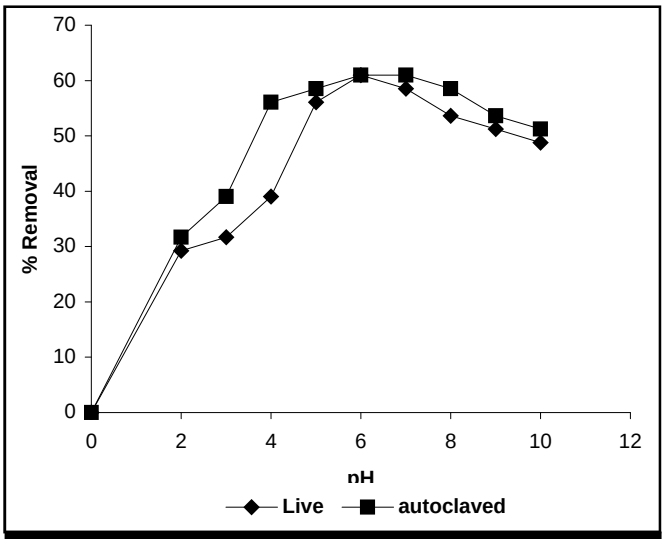

Fig. 5 : Effect of pH on removal of mercury by live and autoclaved mycelium

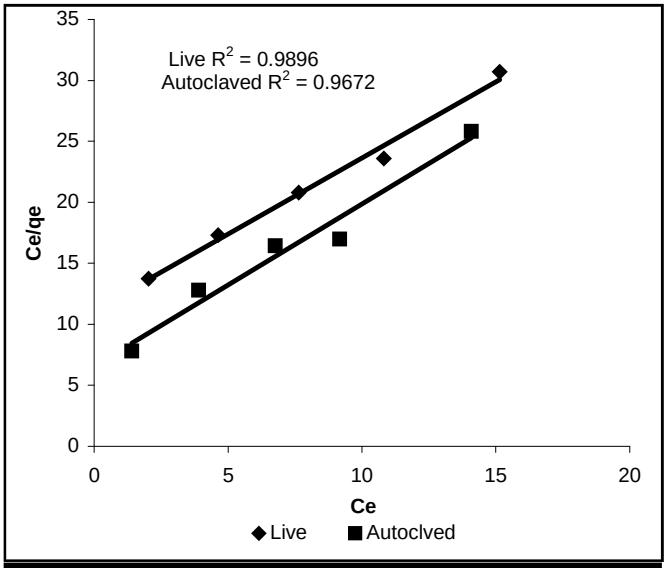

Fig. 6 : Langmuir plot for removal of mycelium

Effect of pH on adsorption of mercury:

The pH of the solution has a significant influence on the adsorption of the released charged groups onto the adsorbent surface (Ramakrishna and Viraraghavan, 1997). The effect of solution pH on removal of mercury was studied with and without adsorbent in order to determine whether the metal has been precipitated or not, by changing the pH of the aqueous solution. The data (Fig.5) revealed that in neutral pH, precipitation of mercury was increased whereas in both acidic and alkaline pH, the precipitation was reduced. As the pH of system increases, the number of positively charged surface sites decreases and the number of negatively charged surface sites increases (McKay *et al*., 1982). The biopolymeric adsorbents, have functional groups like - OH, - $NHCOCH_3$ and - NH_2 which aid in mercury adsorption through hydrogen bond or by ion exchange (Longhinotti *et al*., 1998).

Aspergillus niger is easily cultivable and also available as waste in large quantities from certain fermentation making the economical (Kuyucak, 1990; Edith *et al*., 1990; Fourest and Jean-Claude Roux, 1992). Moreover, as the biomass of *Aspergillus niger* is easily biodegradable, as metals can be desorbed from the biomass after adsorption (Tsezos and Volesky, 1981; Tobin *et al*., 1986). The adsorbent can also be used for land filling or composting after several cycles of adsorption (Muzzarelli *et al*., 1980; Brierley *et al*., 1986). Adsorption

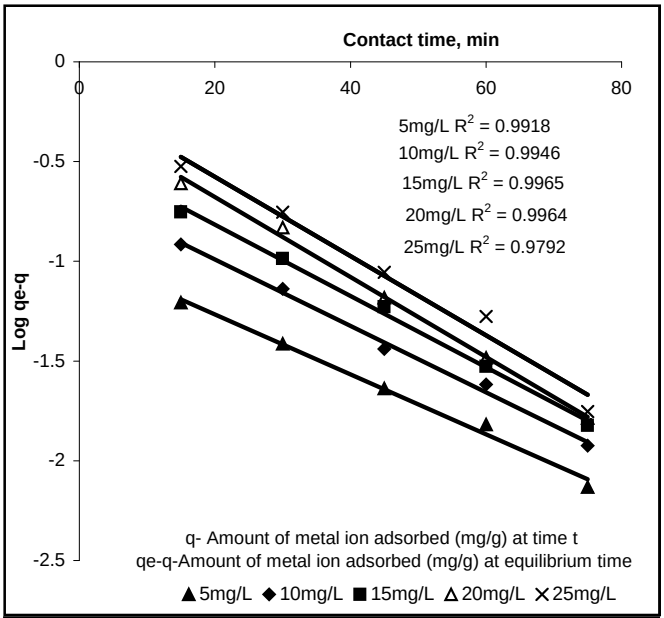

Fig. 7 : Lagergren plot for the removal of mycelium by live mycelium

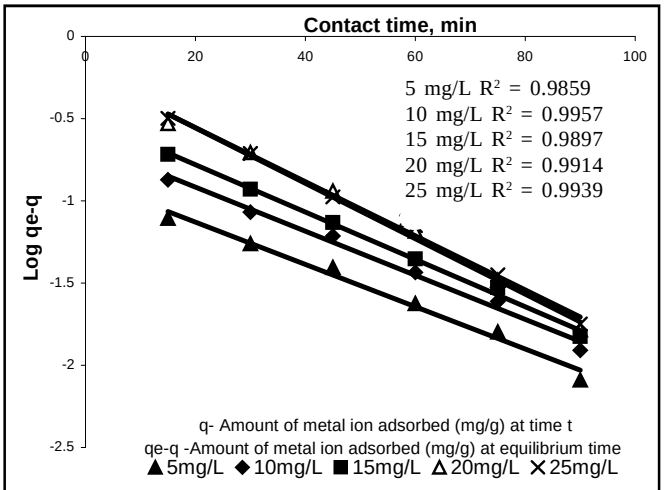

Fig. 8 : Lagergren plot for the removal of mycelium by autoclaved mycelium

isotherms revealed, adsorption of mercury by fungal mycelium followed the Langmuir model and process of adsorption is favorable. Thus, biological process for effluent treatment was more economical and eco-friendly.

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