Biosorption of cadmium by fungi

Submitted as a major project in partial fulfillment of the requirements for the award of degree of

MASTER OF SCIENCE
IN
BIOTECHNOLOGY

By

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Candidate's Declaration

I, hereby declare that the work presented in the dissertation entitled **"Biosorption of cadmium by fungi"** in partial fulfillment of the requirement for the award of the degree of Masters in Biotechnology, Department of Biotechnology and Environmental Sciences, Thapar Institute of Engineering and Technology, Patiala, is an authentic record of my own work during the period of five months from January 2006 to May 2006, under the supervision of Dr. Dinesh Goyal, Associate Professor, Department of Biotechnology & Environmental Sciences, Thapar Institute of Engineering and Technology. The report has not been submitted for the award of any other degree or certificate in this or any other university.

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This is to certify that the thesis entitled “Biosorption of cadmium by fungi” submitted by Nandini Chatterjee in partial fulfillment of the requirements for the award of Degree of Masters of Science in Biotechnology to Thapar Institute of Engineering and Technology (Deemed University), Patiala, is a record of student’s own work carried out by her under my supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other University or Institute.

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ABSTRACT

The aim of this study was to investigate Cadmium removal potential of two different fungal biomass namely *Paecilomyces variotii* and *Cladosporium resinae*. Experiments were carried out under shake flask conditions i.e at different pH, temperature of 28°C and agitation rate of 120 rpm. The effect of biomass concentration, pH and metal concentration on the ability of dried biomass to remove metal from solution was investigated. Optimization of pH and biomass dosage for maximum removal was carried out. Non-living cells of *Paecilomyces variotii* were superior to *Cladosporium resinae. P.variotii* was found to be superior in metal uptake. Removal potential of cadmium by live cells of *P.variotii* was also superior to those of *C.resinae* at different metal concentrations and 96 hrs of incubation. Maximum adsorption was at 10-20 ppm after 96 hr. Column studies for metal removal from 50 ppm cadmium containing solution with non-living biomass of both the organisms were carried out till complete saturation. *P.variotii* showed saturation in 33 hrs whereas *C.resinae* showed saturation in 28 hours. For the same metal ion different adsorbents had different removal rates. Residual metal concentration decreased with time whereas metal uptake showed an increasing trend, which followed Langmuir and Freunlich isotherm.

SDS-PAGE analysis of cells grown with (test) and without (control) metal stress showed the presence of extra bands in the tracks, which were loaded with test samples. This confirmed the release of certain extracellular proteins by the cells when exposed to metal ions in the media, which may help in adsorption process. Out of live and dead cells for cadmium removal studies, use of dead cells is economical because it does not require any maintenance of sterile conditions and the dead biomass can be regenerated. Therefore there is plenty of scope for large-scale application of non-living biomass for metal ion removal.

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INTRODUCTION

A sudden boost in industrial activities has contributed quantitatively to the alarming increase in the discharge of metal pollutants into environmental sink, especially the aqueous environment. Dispersion of the metal ions in the water bodies leads to their biomagnification through the food chain and results in increased toxicity. This fact renders the removal of heavy metals from aqueous solutions indispensable. Metals discharged into water bodies are not biodegraded but undergoes chemical or microbial transformations, creating large impact on the environment and public health (Volesky, 1995). Metals and their free radicals are highly reactive attacking other cellular structures. The ability of metals to disrupt the function of essential biological molecules, such as protein, enzyme and DNA is the major cause of their toxicity. The alteration in protein can also lead to toxic consequences.

Cadmium use has been steadily increasing in growing applications such as in electroplating, pigments, paint, plastics, silver cadmium batteries (Volesky, 1992), smelting (Buchar, 1973), cadmium nickel batteries, stabilizer, phosphate fertilizer, mining and alloy industries (Low and Lee, 1991). One of the major sources of cadmium discharge into natural waters is from the electroplating industries, which accounts for about 50% of the annual cadmium consumption.

Cadmium, for example, has a half-life of 10-30 years in the human body. Therefore, their toxic effects are especially pronounced in animals of higher trophic levels, particularly in humans. It causes kidney damage, bone diseases and cancer. Chronic exposure to elevated levels of cadmium is known to cause renal dysfunction, bone degeneration, liver damage, and blood damage. The US Department of Health and Human Services has reported that there is sufficient evidence in humans for the carcinogenicity of cadmium and cadmium compounds (Agency for Toxic Substances and Diseases Registry (ATSDR), Toxicological profiles, 1999).

Removal of cadmium from effluents before they are discharged into the environment can be accomplished by processes such as, chemical precipitation, cementation, solvent extraction, reverse osmosis and ion exchange (Meena and Rajagopal, 2003). These processes are sometimes, neither selective nor effective and some of them are very expensive (Patterson, *et al.*, 1975).

Conventional methods for removing heavy metals from industrial effluents include chemical precipitation, chemical reduction, adsorption, and ion exchange (Chen and Yu, 2000; Chen and Lim, 2002; Chen *et al*., 2002; Chen, and Wang, 2004).

However, they are often less cost effective when applied to dilute effluents. It is desirable to develop novel approaches for treatment of heavy metals. Biosorption by inexpensive biomaterials promises to be an excellent alternative (Matheickal and Woodburn, 1999; Davis, *et al.,* 2000 Chen, *et al.,* 2002). As the biosorbants are inactive, no nutrition is needed. Furthermore, biosorption process eliminates the difficulties in maintaining a healthy microbial population against metal toxicity and other unsuitable environmental factors. Unlike precipitation, this process does not generate toxic chemical sludge. Many early studies have indicated that the non-living biomass may even be more effective in sequestering and accumulating metallic elements than in living organisms (Crist, *et al*., 1981**;** Fourest, *et al.,* 1994). Many types of dead organisms have been studied for their heavy metal uptake capacities and suitability, which bacteria, fungi, yeast, fresh water algae, and marine algae.

The present investigation envisages evaluation of two filamentous fungi namely *Paecilomyces variotii* and *Cladosporium resinae* isolated from contaminated sites for biosorption of cadmium from synthetic metal solutions.

REVIEW OF LITERATURE

Microorganisms are generally the first to be affected by the discharges of heavy metals into the environment. Microbial ecosystem can drastically alter the fate of the metal entering into aquatic or soil environments. Bacteria, cyanobacteria and fungi alter the form of occurrence of metal through methylation, chelation, complexation, catalysis or adsorption affecting their bioavailability and movement in the food chain. Many types of yeast, fungi, algae, bacteria and some aquatic plants have been reported to have the capacity to concentrate metals from dilute aqueous solutions and to accumulate them inside the cell structure (Kapoor and Viraghavan, 1995; Volesky and Holan, 1995; Modak and Natarajan, 1996). There are numerous industrial processes and related activities that result in the release of metals can be controlled before it enters in common waste streams, enormous saving in disposal costs of resulting sludge are possible. In fact, the detoxification of sludge can convert them from economies liability to a sellable resource.

Preliminary studies carried out on biosorption reveals it to be a complex interplay of the properties of the biomolecules of the cell wall and the chemical nature of the metal ion in question. Each of the microbial group is characterized by a distinct by a distinct cell wall structure and presence of different polymers/monomers like chitin, amino acid and carboxylic acids groups provide several functional groups as binding sites for heavy metal ions due to ion exchange phenomenon. The amino and carboxyl groups, nitrogen and oxygen of the peptide bond could be available for characteristic coordination bonding with metal ions (Nourbaksh *et al.,* 1992; Bai *et al*., 2001). Such bond formation could be accompanied by displacement of protons, dependent in part upon the extent of protonation as determined by pH.

Conventional treatment methods

- Precipitation
- Ion – exchange method
- Complexation
- Electrochemical cells
- Reverse osmosis
- Biological methods

Micro precipitation is the deposition of electrically neutral material (metal or metal salt) at the surface of the biomass, and does not necessarily involve a bond between the biomass and the deposited layer. It can however be facilitated by initial binding of metal ions to relative sites of the biomass, which serve as nucleation sites for further precipitation. Micro precipitation is based on interactions between the solute (dissolved solid) and the solvent, and occurs when the local solubility exceeds.

Ion exchange and adsorption can be the result of three different interactions, which act in combination: The main contribution for free metal ions (which are highly soluble in water) is usually the attraction of the sorbate (metal ions) to the sorbent (biomass). Additionally hydrophobic expulsion may also play a role.

Complexation plays an important role in both metal-ligand and sorbate-sorbent interactions. Complexes can be neutral, positively charged or negatively charged. The number of coordinating atoms in the ligands that are directly attached to the central atom is the coordination number. The bond between the central atom and the coordination groups can be arising from principal or auxiliary valence forces.

Electrodialysis is a process where the ionic components are separated through the use of a semi-permeable ion selective membrane. Application of an electrical potential between the two electrodes causes a migration of cations and anions towards respective electrodes. Because of the alternate spacing of cation and anion permeable membranes, cells of

concentrated and diluted salts are formed. The disadvantage is the formation of metal hydroxides, which clog the membrane.

Reverse osmosis is a process where heavy metals are separated by a semi-permeable membrane at a pressure greater than osmotic pressure caused by dissolved solids in wastewater. It is very expensive.

Disadvantages of such physicochemical processes:

- They are expensive
- Lack the required specificity required treating target metal against a background of competing ions.
- Unpredictable metal removal
- High reagent requirement & generation of toxic sludge, which are often difficult to dewater and require extreme caution in their disposal.
- Such approaches are not applicable to cost effective remediation of large-scale subsurface contamination *in situ.*

The cell wall polymers provide a multitude of chemical groups such as hydroxyl, carbonyl, carboxyl, sufhydryl, thioether, sulfonate, amine, imine, amide, imidazole, phosphonate and phosphodiester. These chemical groups of the biopolymers in turn harbour binding sites, which provide the ligand atoms to form complexes with metal ions. In general metal binding can be distinguished between ion exchange, sorption of electrically neutral material to specific sites and microprecipitation. These mechanisms are based on sorbate-solvent interactions, which in turn rely on some combination of covalent, electrostatic, and Vander Walls's forces.

Importance of the given group for biosorption of certain metal by certain biomass depends on various factors such as:

1) Quantity of sites in the biosorbent
2) Chemical state of the site
3) Accessibility of the site
4) Affinity between the site and the metal

Table 1. Different technologies for the removal of heavy metals from the industrial wastewater.

Technology	Concentration dependence	pH	Suspended solids	Effluent Concentration (mg/I)	Regeneration	Sludge generation
Biosorption	Yes	Yes	Yes	<1	Yes	No
Hydroxide Precipitation	No	No	Yes	2-5	No	Yes
Sulfide Precipitation	No	No	Yes	<1	No	Yes
Ion Exchange	Yes	Some	No	<1	Yes	Yes
Evaporation	Yes	Yes	Yes	1-5	----	No
Reverse Osmosis	No	Some	No	1-5	No	No
Adsorption	Yes	Some	Yes	1-5	Yes	No

Strong biosorbent behavior of certain types of microbial cells towards metallic ions is a function of the chemical make up of microbial cells of which it consists. This aspect is particularly important when it comes to the process application, whereby new biosorbents respective 'chemicals' are capable of sequestering a relatively large amount of the metal (Volesky 1987). Some types of biosorbents could have broad range binding of the majority of heavy metals with no specific prosity, while others can even be specific for certain types of metals (Volesky and Kuyvcak, 1988).

Bioremediation is to remove, sequester or solubilize the metals that are able to degrade compounds. This suggests that under the selective pressure of environmental pollution, a microbial capacity for the degradation of recalcitrant compounds exists that may be harnessed for pollutant removal by biotechnological process. Bioremediation using

microorganisms is less intrusive, less expensive and accumulates toxic for their removal and sequesters them for large-scale removal.

Biological methods

Microbial research and need for new methods of water cleanup has led to great deal of expansion in the field of biological methods of industrial effluent cleanup. Microbes require heavy metals as humans' require certain metals in their diet. The pathways by which microbes accumulate heavy metals are:

a) Binding to cell surface
b) Intracellular accumulation
c) Extra-cellular precipitation
d) Volatilization

Living biological systems are well suited for the treatment of water. The term 'biosorption' is used to describe the accumulation of metal ions from solutions by material of biological origin (microbe or plant). Biosorption is a process that uses inexpensive dead biomass to sequester toxic heavy metals and is particularly useful for the removal of contaminants from industrial effluents. It is a cost effective and highly specific process. This ensures reusability of biomass with short operation time especially if dead cells are used.

Bioremoval can be due to:

Biosorption	(by dead/live biomass)
Bioaccumulation	(by live biomass)
Enzymatic recovery	(biotransformations)

Biosorption by non-living cells

The use of dead cells offers the following advantages over living cells:

- Non-living cells are less sensitive to metal ion concentration (toxicity effects).
- Can be operated at ambient conditions of pH and temperature.
- Low operating cost.
- Volume of chemical or biological sludge can be minimized.
- Supply of nutrients not required.
- Dead biomass can also procured from industrial sources as a waste product from the fermentation process.
- Biosorbed metal can be easily desorbed and biomass can be reused.
- Much simpler process control and biomass can be stored for long periods of time.

Mechanism of biosorption

Biosorption by these microbes is attributed mainly to the ligands present in the biomolecules of their wall polymers.
Biosorption includes a combination of several mechanisms such as electrostatic attraction, complexation, ion-exchange, covalent binding,Van der Waal's forces, adsorption and microprecipitation.

The kinetics of metal uptake has been suggested to take place in two stages (Gadd and White, 1993; Sharma and Foster, 1993; Fude *et al.*, 1994 and Prakasham *et al.*, 1998).

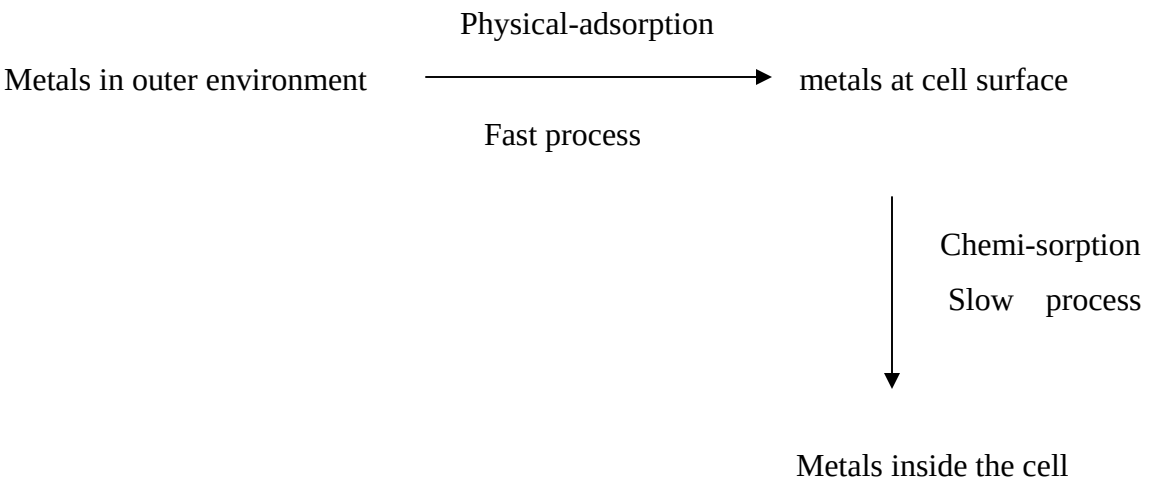

Fig. 1 Process of biosorption

Biosorbents are prepared from the naturally abundant and/or waste biomass of algae, fungi or bacteria. Varieties of uptake mechanism are involved including adsorption and ion exchange.

Commercial biosorsorbents need to fulfill a number of criteria such as:

- High biosorption capacity at equilibrium i.e. they should contain as little as possible of inert material in their binding sites.
- Favourable adsorption kinetics i.e. particles should be hydrophilic and porous in nature.
- Maintainance of smooth flow dynamics in a reactor-this prevents the use of either very small or strongly swelling particles in the column.
- Amenable to regeneration- this necessitates desorption by minimal possible volume of desorbing agent without damaging the biosorbent.
- Good mechanical strength.
- Temperature stability
- Resistance to chemicals.
- Availability of biosorbent

The need for economical, effective and safe methods for removing heavy metals from the wastewater has resulted in the search for unconventional materials that may be useful in reducing the levels or accumulation of heavy metals in the environment. The newly discovered metal sequestering properties of certain types of microbial biomass of fungi, bacteria and algae offers considerable promise (Volesky, 1987) and offer an alternative to the existing methods for metal detoxification and their recovery. The present investigation envisages use of dead or non-living biomass available in large quantities for removal of heavy metals from aqueous solution.

Biosorption by fungi as an alternative treatment option for wastewater containing heavy metal has been reviewed by Kapoor and Viraghavan (1995) and Modak and Natarajan (1996). Cd (II) biosorption to non-living biomass of *Rhizopus arrhizus* and *Schizomeris leiblenii* was studied in batch reactor. Maximum adsorption rates of Cd (II) ions to the biomass were at 30 C and at the optimum pH 5.0 for both microorganisms. The adsorption rates increased with increasing Cd concentration for both up to 100-150 mg/ml respectively. The adsorptions for *R.arrhizus* were higher than that of S.*leiblenii* (Ozer *et al*., 1997).

Dry cells of *Rhizopus arrhizus* has been used for the removal of iron(II), Pb(II), and Cd(II) ions from the industrial wastewater. Higher adsorption rates and adsorption capacities were obtained at initial metal concentration up to 100mg/ml in batch reactor. High concentration of heavy metals may be purified by using multistage batch reactor in series (Ozer *et al*., 1997) carried out using white-rot fungi *Polyporous versicolor* and *Phanerochaete chrysosporium* for Cu (II), Cr (III), Cd (II), Ni (II) and Pb (II) under same operating conditions. Results showed that both were effective in removing Pb (II) from aqueous solutions with maximum absorptive capacity of 57.5 and 110 mg Pb (II)/g dry biomasses (Yetis *et al*., 1998).

For the removal of Hg and Cd several brown seaweeds were tested for their ability to remove metal ions from aqueous solutions by biosorption and 90-95% removal level was found from industrial wastewater (Wilson and Edween, 1995).

Any fungi can tolerate high concentration of potentially toxic metals with other microbes; this may be correlated with decreased intracellular uptake or impermeability. A close relation between toxicity and intracellular uptake has been shown for Cu^{2+},Cd^{2+}, Co^{2+} and Zn^{2+} in yeast *Saccharomyces cerevisiae* (Gadd, 1986).

Waste mycelia from industrial fermentation plants (*A.niger*, *P.crysogenum* and *C.paspali*) were used to as a biosorbent for removal of Zn ions from aqueous environments, both batch wise as well as in column mode. Under optimized conditions *A.niger and C.paspali* were found to be superior to *P.chrysogenum* (Leuf *et al*., 1991).

Microbial biomass was used as an adsorbing agent for the removal and recovery of uranium present in industrial effluents and mine wastewater (Nakazima and Sukaguchi, 1986).

Dead cells of *S.cerevisiae* removed 40% more uranium or zinc than the corresponding live cultures. Biosorption of uranium by *S.cerevisiae* was a rapid process reaching 60% of the final uptake value within the 15min contact. The deposition differing from that of other heavy metals more associated with the cell wall, uranium was deposited as fine needle-like crystal both on inside and outside the *S.cerevisiae* cells (Volesky *et al*., 1995).

Biosorptive capacity of different biosorbent including dried mycelium of some species of fungi, baggase, rice husk and fermented baggase by selected fungal species or natural microflora was examined to remove cyanide from industrial effluent. The biomass of *Rhizopus sexualis* and the fermented baggase by *R.sexualis* or *A.terreus* showed higher sorption capacity than activated charcoal. The biomass of *R.sexualis* and *Mortierella ramanniana* exhibited higher cyanide sorptive capacity than ascomycetes e.g. *Aspergillus terreus* and *Penicillium capsulatum* (Azab *et al*., 1995).

Ni uptake capacity from aqueous solutions was also studied in filamentous fungi such as *Rhizopus sp., Penicillium sp.* and *Aspergillus sp*. The metal uptake was highest I by *Rhizopus sp*. (Gill *et al.*, 1996).

The yeast biomass of *S.cerevisiae*, which is a by-product from brewery industry, was used for the purification of water polluted by uranium ions, which had an efficiency to adsorb U 2.4mMol μg/dry biomass (Omar *et al.*, 1996).

Mucor meihi, a fermentation industrial waste was found to be effective biosorbent for the removal of hexavalent chromium from industrial tanning effluents. Sorption levels 1.15 and 0.7mmol/g were observed at pH 4 and 2 respectively. In comparative studies with the ion-exchange resins, *Mucor* biomass demonstrated Cr biosorption levels that correspond closely to those of commercial strongly acidic exchange resins in solutions. However, the Cr elution characteristic from the *Mucor* biomass was similar to those of both the weakly and strongly acid resins (Tobin and Roux, 1998).

Dead biomass of actinomycetes, which is the waste product from industrial fermentation, was mixed with wastewater as a free bacterial suspension and biosorption occurred. Cadmium cations bound to negatively charged sites on bacterial cell wall and could be desorbed (Butter *et al*., 1996).

Removal of lead ions from aqueous solutions by non-living biomass of *Penicillium chrysogenum* was studied and observed that lead was strongly affected by the pH in the range of 4-5. Uptake of lead was 116mg/g dry biomass, which was higher than that of activated carbon and some other microorganisms (Niu *et al*., 1993).

Table 2. Heavy metal removal efficiency of different microorganisms.

Organism Used	Metals removed	System	Reported efficiency and application
Pseudomonas flourescens	Pb, Zn	Immobilized on PVC & Packed in columns	Used in Hungarian Chemical Company
Pseudomons aeruginosa	Uranium, Plutonium	Immobilized in plasma treated Polypropylene	75-80% removal
Citrobacter sp.	Cd, Pb, Cu, U	Immobilized on PAG	80-90% removal
Bacillus subtilis, Saccharomyces	Pb, Zn, Cu, Ni, Cd, Hg, Ag, Au, Pd	Fixed Reactor	AMT-Bioclaim TM 98% removal
Streptomyces sp. Viridochromogenes sp.	Uranium	Trapped in Silica gel matrix	80-100% removal
Rhizopus arrhizus	Cr, Fe, Cu, U	----------	50-60% removal
Dead algae	Hg	Sorption column	95 uptake Alga SORBTM being used
Sargassum natans	Pb, Cd, Cr	---------	3 times more efficient than ion exchange resin
S. fluitans	Cu		80-90% removal
A.niger, P. chrysogenum	Ag, Zn		--------
A. oryzae	Cd	Immobilized on reticulated foam	95% removal

Instantaneous and equilibrium metal uptake performance of *Rhizopus arrhizus* was studied using aqueous solutions containing Cr (VI), Cu (II) and Cd (II) ions in ternary mixtures. Application of the multicomponent Langmuir model to describe the three metal systems revealed its non- ideal characteristics, whereby the values of the equilibrium constants and the maximum capacities for the metals differed for each system. For that reason, the ternary biosorption equilibria of Cr(VI), Cu(II) and Cd(II) ions with *Rhizopus arrhizus* were further investigated by using multicomponent Freundlich model. From the equations of the multicomponent Freunlich model, three dimensional (3-D) biosorption isotherm surfaces were simulated depicting the equilibrium behavior of multi metal system (Taylor *et al*., 2003).

Table 3. Metal removal by different conventional and non-conventional biosorbents

S. No.	Adsorbents	Metals	References
1	*Lemna minor*	Pb	Rahimani, *et al.*, 1999
2	*Amaranthus spinosus, Solanun nigrum*	Cu	Chen, *et al.*, 1996
3	*Sargassum natans*	Cr, Pb, Co, Cd	Volesky, 1995
4	Chicken feathers	Au, Pt	Suyama, *et al.*, 1996
5	Canola meal	Cr	Al-ashesh, *et al.*, 1996
6	Hyacinth roots	Cr	Low, *et al.*, 1997
7	Fly ash	Cr, Pb, Cd	Bhargava, *et al.*, 1989
8	Fly ash	Cr, Pb, Mn, Fe	Sharma, *et al.*, 1990
9	Agriculture residue	Cr, Cd	Orhan and Byakungar, 1993
10	Saw dust	Cr, Pb, Cd	Campanella, *et al.*, 1986
11	Coconut fibre	Cr	Tan, *et al.*, 1993

Chromium biosorption by non-living biomass of *Chlorella vulgaris, Cladophora crispate, Zooglea ramigera, Rhizopus arrhizus* and *Saccharomyces cerevisiae* was studied and observed that optimum initial pH (1.0-2.0) of the metal ion solution affected the metal uptake capacity of the biomass for all the microorganisms. Maximum adsorption rates of metal ions to microbial biomass were obtained at temperature in the range of 25-35 C. The adsorption rates increased with increasing metal concentration of *Chlorella vulgaris, C.crispate, Z.ramigera, Rhizopus arrhizus* and *S. cerevisiae* upto 200, 75, 125 and 100mg/ml respectively (Nourbaksh *et al.*, 1994).

The biosorption of Cu (II), Ni (II), Cd (II) and Cr (VI) from aqueous solutions on dried algae (*Chlorella vulgaris, Scenedesmus obliquus & Synechocystis sp.*) were tested under laboratory conditions as a function of pH, initial metal ion and biomass concentration. Experiment results showed that influence of the alga concentrations on the metal uptake of all the species. Both Freundlich and Langmuir adsorption models were found to be suitable for describing the short-term biosorption of Cu (II), Ni (II), Cd (II) and Cr (VI) by all algae species (Donmez *et al*., 1999).

Researchers have also reported the pretreatment of the biosorbent with acids, alkali, salt solutions, detergents or organic solvents to affect the biosorptive potential of the

biosorbent. (Kapoor and Viraghavan, 1996; Muzzarelli *et al*., 1980a, 1980b, 1981). The increased capacity results from deacetylation of chitin in the cell wall to form chitosan – glucan complexes with higher affinity for metal ions.

Dried non-living biomass of *Streptoverticillium cinnamoneum* was used for the recovery of Pb and Zn from the solution. The optimum pH of Zn and Pb was 3.5-4.5 and 5.0-6.0 respectively. The maximum loading capacity of *S.cinnamoneum* biomass was 57.7mg/g for Pb and 21.3 for the Zn with boiling water pre-treatment. The loaded metals could be desorbed effectively with dilute HCl, nitric acid and 0.1M EDTA. Treatment with 0.1M Na carbonate permitted reuse of desorbed biomass although the loading capacity in subsequent cycles decreased by 14-37% (Puranik *et al*., 1997).

Biosorption is the removal of metal or metalloid species, compounds and particulates, radio nuclides and organometalloid compounds from solutions by physicochemical interactions with biological agents. Biosorption technology is proposed in water pollution management now days (Leuch *et al*., 1995). The physiological state of the organism, age of the cells, availability of micronutrients during their growth and environmental conditions during the biosorption process (such as pH, temperature and presence of certain co-ions), are important parameters that affect the performance of a living biosorbent. Metal solution chemical features also influence the efficiency of metal concentration on the biosorbent (Volesky *et al*., 1990).

Waste biomass of from the pharmaceutical fermentation industry, i.e. non-living *Rhizopus nigricans* has been used for adsorption of lead over a range of metal ions concentration, adsorption time, pH and co-ions. The process of uptake obeys the Langmuir and Freundlich isotherms. Comparison of uptake between NaOH treated and untreated biomass shows that the adsorption takes place in the chitin structure of the cell wall (Zhang *et al*., 1998).

Non-living waste biomass from *Aspergillus niger* along with wheat bran was used as a biosorbent for the removal of Zn and Cu from aqueous solutions. The binding capacity of the biomass for Cu was observed to be higher than that of Zn. The metal uptake was

found to be a function of the initial metal concentration, the biomass loading and pH. The metal uptake of Cu by the biomass decreased in the presence of Co ion. The uptake of Cu by the biomass decreased in the presence of Zn and visa-versa. The decrease in the metal uptake depends on the concentration of metal ions in two compounds in aqueous solutions (Modak *et al*., 1996).

Non-living free and immobilized biomass of *Rhizopus arrhizus* was used to study the biosorption of Cr (VI). Chromium removal was slightly more in free biomass conditions over immobilized state. Stirred tank reactor studies indicated maximum chromium biosorption at 100 rpm and at 1:10 biomass-liquid ratio. Fluidized bed reactor is more efficient in chromium removal over stirred tank reactor. Immobilization of biomaterial has little effect on the Cr biosorption by *R.arrhizus* (Prakasham *et al*., 1999).

Fungal biomass offers the advantages of having high percentage of cell wall material with excellent cadmium binding properties particularly the genera *Rhizopus, Aspergillus*, and *Saccharomyces* (Panikar *et al*., 1993).

Removal of Cr (VI) from aqueous solutions was carried out in batch experiments using dead biomass of four fungal strains – *A.niger* NICM-501, *A.oryzae* NICM-637, *R..arrhizus* NICM-997, *R.nigricans* NICM-880.Out of these four *Rhizopus nigricans* and *R..arrhizus* possess high Cr(VI) uptake capacities (Bai and Abraham, 1998).

Recently some agricultural and forestry products/wastes have been recognized as new adsorbents. In general the cost of these biomaterials is negligible, compared to their cost of ion exchange and chemically prepared adsorbents. Wheat stem and babul bark, a raw material was used as agro waste carbons to remove the nickel metal from the effluent of the electroplating industry. Electroplating wastewater showed 2%-10% lower removal as compared to the synthetic solutions as the similar conditions. Almost 100% removal of Ni (II) was observed using wheat stem activated carbon at a pH value of 4.0 in adsorption of 4.0 hrs at 36±2 °C, carbon doses of 16.0hr/l when the initial nickel concentration was 25g/l (Verma and Shukla, 2000).

MATERIAL AND METHODS

3.1 Preparation of the biosorbent

The fungal biomass (isolated earlier from industrial effluents) were mass cultivated in potato dextrose broth and the mycelia was harvested by filtration, dried at 70°C overnight, pulverized using mortar and pestle and stored in polythene bags for further Cd uptake studies.

3.2 Chemicals and reagents

Stock preparation – the stock solution containing 1000mg/ml concentration of $Cd(NO_3)_2.4H_2O$ were prepared in distilled water and were used in the experiment. These chemicals were procured from Sd Fine Chem Ltd., Mumbai.

Potato dextrose broth/agar available commercially from Hi Media was used for isolation and growth of fungi. Whenever a solid media was required, 2% agar was added to the liquid media. PH adjusted to 5.0.

Polyacrylamide gel electrophoresis –

Running gel – Acrylamide, sterile water, Tris-HCl (pH 8.8), SDS, TEMED, ammonium persulphate (10%)
Stacking gel – Acrylamide, SDS, Tris-HCl, sterile water, APS, TEMED
Acrylamide stock solution – Acryamide (40%)
Stain– Coomassie brilliant blue
Staining solution – Acetic acid (5%), methanol (10%)
Destaining solution – Methanol, acetic acid, distilled water
Tracking dye – Bromophenol blue

3.3 Removal of cadmium by non-living biomass in batch mode

Non-living and dried biomass of *Paecilomyces variotii* and *Cladosporium resinae* were used for removal of Cd from aqueous solutions in batch mode or shake flask condition. These experiments carried out in Erlenmeyer flasks at room temperature and 120 rpm with a working volume of 100 ml. Samples were withdrawn after specific intervals of time for 24 hours and thereafter the samples were filtered with qualitative filter paper (equivalent to grade no 1). Then after appropriate dilution cadmium analysis was carried out using atomic absorption spectroscopy (*GBC 932 AA Australia*).

3.4 Optimization of pH and biomass

Fungus was grown in potato dextrose broth for 72 hours. The biomass raised was then filtered, dried and pulverized. This powderised biomass was used further for optimization in required quantity. The solution were adjust to the required pH values by using 1N HCl and 1N NaOH. Dried biomass and Cd was added in a fixed concentration respectively. Incubation was done at room temperature at 120 rpm.

Similarly varying amount of biomass from 0.25-2g was used to carry out optimization of initial biomass concentration. 5 ml of sample was drawn at different intervals of time up to 24 hrs, filtered using filter paper no.1 and analyzed residual Cd content in the filterate.

3.5 Metal uptake by living biomass – growth curves

Concentration dependent growth of both *Paecilomyces variotii* and *Cladosporium resinae* in presence of graded concentrations of cadmium ranging from 5-50ppm (5, 10, 20, 50 ppm) was studied. 100ml of Potato dextrose broth was inoculated with both the fungi and incubated at 28±2°C and 120 rpm for 96 hours. Samples were collected after specific intervals of time (8, 24, 32, 48, 56, 72, 80, 96 hr). These samples were then diluted accordingly and analyzed using atomic absorption spectroscopy.

3.6 Metal uptake by biomass

The metal laden biomass is dried and digested with aqua regia (HCl: HNO_3 in the ratio of 3:1) to dryness. Heavy metals are extracted by suspending the residue in 50% HCl and filtered through Whatman 42.

Specific metal uptake is calculated as follows:

$Q\ (mg/ml) = (C_i - C_f) \times V/1000 \times W$

C_i is the initial concentration of metal in the solution (ppm)
C_f is the final concentration of metal in the solution (ppm)
V is the volume of the solution (ml) and
W is the weight of the biosorbent (g) taken.

3.7 Continuous column sorption studies

Continuous sorption column experiment was conducted to study the removal of cadmium from synthetic metal solution. A column of 1.7cm diameter and 6cm in length was packed with 3g of fungal non-living biomass of both *P.variotii* and *C.resinae.* Synthetic metal solution of 50ppm of cadmium was run through it manually from the top and fraction of eluant were collected at specific intervals of time till the column is saturated and the residual cadmium concentration was analyzed using atomic absorption spectroscopy.

3.8 Cadmium analysis

The residual concentration of cadmium in the sorption medium was determined by atomic absorption spectroscopy using air-acetylene flame (*GBC 932AA, Austrailia*). The working standards were prepared from 1000ppm stock procured from Acros Organic Ltd., New Jersey, USA.

The residual concentration R (%) will be calculated (Zhang *et al.*, 1998) as:

$$R = 100 \times (C_i - C_f)/C_i$$

Where
C_i is the initial concentration of metal in the solution (ppm)
C_f is the final concentration of metal in the solution (ppm)

3.9 SDS-PAGE analysis

Continuous polyacrylamide gel electrophoresis was carried out to see if there are any new extracellular proteins produced when the cells are exposed to known amount of metal solutions. Both control and test were run on the gel. The cells were grown in potato

dextrose broth for 48 hours and then the filterate was loaded onto the gel to investigate the presence of extacellular proteins. Samples were prepared by mixing an equal amount of filtrate and bromophenol dye and kept in boiling water bath for 5 min and then loaded into the wells and run at 120volts. The gel was finally destained to view bands using acetone, methanol and water mixture.

RESULTS AND DISCUSSION

Feasibility of two different fungal biomasses (*Paecilomyces variotii* and *Cladosporium resinae)* was tested for removal of cadmium from synthetic solutions.

Removal of cadmium by non-living biomass *P.variotii* and *C.resinae*.

The efficiency of dead cells in the biosorbing metal ions can be greater than, equivalent to or less than that of the living cells. Biosorption of heavy metal ions by any material is affected by not only surface properties but also by physico-chemical parameters of metal ion solution. Therefore, the effect of initial metal ion concentration initial pH and varying amount of biomass on removal of cadmium was investigated. The biosorption potential of *Paecilomyces variotii* was found to be greater than that of *Cladosporium resinae* in all the cases discussed below.

The cells can be killed for biosorption by physical or chemical methods. These include vacuum drying and freeze-drying, boiling, autoclaving and mechanical disruption. Chemical include contact of the biomass with various organic and inorganic compounds (Siegel *et al*., 1990: Kapoor and Viraghavan, 1995). Chitin and chitosan present in the cell walls help to sequester metal ions (Muzzarelli, 1972; Tsezos, 1983). Tsezos and Volesky (1982a, 1982b) indicated that biosorption of radionuclides initiated from their association with the nitrogen of the chitin monomer N-acetyl-glucosamine. Muraleedharan *et al*., (1994a) further proposed that structural polysaccharides of the cell wall were probably the main sites of interaction and the complexing ligand is rich in oxygen. Most of the metal uptake was due to ion exchange.

Time course metal removal by different biomass concentration from synthetic metal solutions

Time course metal removal by different biomass concentration from synthetic metal solution in batch mode was studied. Table 4 (Fig. 3) and Table 5 (Fig. 4) gives the time course biosorption efficiency of *P. variotii* and *C.resinae* respectively at different biomass concentration. For the same metal different adsorbents had different removal rates. The adsorption of metal ions reached equilibrium in 30-60 minutes of contact time at the specific pH and room temperature by a specific amount of powderized biomass in 100 ml of synthetic metal solution with a continuous agitation of 120 rpm. This experiment shows that the removal rate occurs quickly in the first 30-60 minutes of initial contact time.

Residual metal concentration decreased with time where as metal uptake showed an increasing trend (Tables 4-11, Fig. 3-10), which followed Langmuir and Freundlich adsorption isotherms. The experiment shows that the metal uptake decreases when biomass concentration rises. This reaction is attributable to metal concentration shortage in the solution. Therefore it is not useful to increase the biomass beyond 1.5-2 grams per 100 ml to purify 20 ppm of cadmium solution. Reduction in biomass concentration in the suspension at a given metal concentration enhances the metal/biosorbant ratio and thus increases the metal uptake per gram of biosorbant as long as the later is not saturated. These results are of great interest in scale up process to optimize industrial effluent purification where the amount of biosorbent to be used in an optimum amount for maximal metal ion removal. *P.variotii* showed maximum sorption of 75.9% with 0.5g after 24 hr. *C.resinae* showed removal of 69.9% with 0.5g after 24 hr.

With an increase in the biosorbent concentration there results a corresponding increase in the total metal removal accompanied by a decrease in the specific uptake. This is due to the fact that total adsorption is dependent upon the number of available binding sites whereas specific uptake is calculated as the amount of metal adsorbed per weight of the biosorbent.

Time course effect of pH on biosorption efficiency

Removal of cadmium by dead biomass of *P.variotii* (Table 6, Fig 5) and *C.resinae* (Table 7, Fig 6) was studied in batch experiments at varying pH ranging from 3-8. *P.variotii* showed maximum removal at pH 4-5 whereas *C.resinae* showed an optimum removal at pH 6 respectively. There was no change in pH of final solution after completion of adsorption. The adsorption of metal ions depends upon solution pH, which influences the electrostatic binding of ions to corresponding functional groups. The result indicated that maximum adsorption of different metal species occur at different pH. However, from practical point of view pH of 5-6 was adequate.

The pH of the environment influences the biosorption process in several ways. The most important effect is the change imparted by it on active binding sites, which in biosorption are usually acidic in nature. Decrease in pH leads to their protonation thereby decreasing their negative charge and consequently the cation binding. On the other hand, an increase in pH increases the availability of the negatively charged free sites for electrostatic attraction of cations, thereby resulting in an increase in the cation binding capacity (Zakharova et al., 2003).

Effect of initial Cadmium concentration

Absorption capacities of *P.variotii* (Table 8, Fig. 7) and *C.resinae* (Table 9, Fig. 8) were determined at different initial Cd concentrations. Higher removal was observed at lower concentrations (10-20 ppm) of Cd. At lower metal/biosorbent ratio, complete saturation of biosorbent sites with all Cd ions in the solution occurs and facilitates maximum removal. As metal/biosorbent ratio increases, more and more cadmium is left unadsorbed in the solution due to saturation of biosorbent mainly due to non-availability of adsorption sites. The optimum adsorption by *P.variotii* was at pH 5 from 10ppm by 0.5% of biomass (Table 8, Fig 7). The optimum adsorption by *C.resinae* was at pH 6 from 50ppm by 0.5% of biomass (Table 9, Fig. 8).

Initial metal concentration plays an important role in determining the biosorptive capacity of absorbent. Generally, it has been observed that an increases in the metal concentration results in an increase in the metal sorption capacity of the biosorbent, which culminates in a plateau at very high metal concentrations. The metal sorption capacity of an organism touches its peak at these high metal concentrations. At this point the sorption of the metal is limited by the availability of the number of binding sites in the biomass. Thus, at low metal concentrations the biosorptive capacity is not fully utilized (Bai and Abraham, 2001).

Biosorption by living cells

The metal ion uptake by living cells is a function of the cell age, composition of growth media, contact time, pH of metal solution, and temperature. The kinetics of biosorption of metals is usually biphasic in nature, consisting of an initial rapid phase, contributing up to 90% of biosorption, and lasting for 10 min. the second phase is slower and has been found to last for up to 4 hours. Increased biosorption has been observed during the lag period or early stages of growth and declined as cultures reached stationary phase

Growth of both *Paecilomyces variotii* and *Cladosporium resinae* in presence of graded concentrations of cadmium ranging from 5-50ppm was studied and the growth curves drawn. Maximum growth and removal by *Paecilomyces* cells were seen in 5ppm cadmium containing media after 96 hr (Table 10 and Fig. 9), whereas maximum removal in *Cladosporium* cells was seen from 10ppm cadmium containing media after 96 hrs (Table 11 and Fig. 10). Wet and dry biomass was taken after 96 hrs (Table 14 and 15). The maximum growth of both the fungi was seen in presence of 5ppm of cadmium in the media.

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Column studies

Continuous columns were run using dead biomass of both the organisms until the column was completely saturated. The column packed with *Paecilomyces variotii* biomass was saturated in 33 hr with an initial inflow metal solution of 50ppm and pH of 5 with a constant flow rate of 60-64 drops/min. (Table 12, Fig. 11).

The column packed with *Cladosporium resinae* biomass was saturated in 23 hr with an initial inflow metal solution of 50 ppm and pH of 6 with a constant flow rate of 60-64 drops/min. (Table 13, Fig. 12)

SDS - PAGE analysis

Fungi were grown in 20ppm of cadmium containing media for 48 hr, harvested and the filtrate was examined for extracellular proteins through SDS-PAGE. A new band was observed in the filtrate of each of the fungi (Fig. 13) on SDS-PAGE as a result of cadmium stress. However the nature and molecular weight of these proteins is yet to be determined.

CONCLUSION

1. Cadmium removal potential of two different fungal biomass namely *Paecilomyces variotii* and *Cladosporium resinae* were studied. Experiments were carried out under shake flask conditions i.e at different pH, temperature of 28°C and a agitation rate of 120 rpm. The effect of biomass concentration, pH and metal concentration on the ability of dried biomass to remove metal from solution was investigated. Optimization of pH and biomass dosage for maximum removal was carried out. Non-living cells of *Paecilomyces variotii* were superior to *Cladosporium resinae*. *P.variotii* was found to be superior in metal uptake.
2. Removal potential of cadmium by live cells of *P.variotii* was also superior to those of *C.resinae* at different metal concentrations and 96 hrs of incubation. Maximum adsorption was at 10-20 ppm after 96 hrs.
3. Column studies for metal removal from 50-ppm cadmium containing solution with non-living biomass of both the organisms were carried out till complete saturation. *P.variotii* showed saturation in 33 hrs whereas *C.resinae* showed saturation in 28 hours.
4. For the same metal ion different adsorbents had different removal rates. Residual metal concentration decreased with time whereas metal uptake showed an increasing trend, which followed Langmuir and Freunlich isotherm.
5. SDS-PAGE analysis of cells grown with (test) and without (control) metal stress showed the presence of extra bands in the tracks, which were loaded with test samples. This confirmed the release of certain extracellular proteins by the cells when exposed to metal ions in the media, which help in adsorption process.
6. Out of live and dead cells for cadmium removal studies, use of dead cells is economical because it does not require any maintenance of sterile conditions and the dead biomass can be regenerated. Therefore there is plenty of scope for large-scale application of non-living biomass for metal ion removal.

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Annexure I

Table 4: Effect of biomass concentration on cadmium removal by *P.variotii* (Temp: 28°C, rpm: 120)

S.No	TIME (hrs)	Biomass concentration (g)											
		0.25			0.50			1.00			2.00		
		C_i	C_f	%	C_i	C_f	%	C_i	C_f	%	C_i	C_f	%
1	0.25	13.8	8.90	35.5	13.8	7.58	45.1	13.8	7.96	42.3	13.8	6.70	51.4
2	0.5	13.8	8.32	39.7	13.8	7.32	46.9	13.8	6.70	51.4	13.8	7.14	48.2
3	0.75	13.8	7.36	46.7	13.8	7.90	42.7	13.8	5.92	57.1	13.8	5.30	61.6
4	1.0	13.8	6.66	51.7	13.8	6.12	55.6	13.8	5.20	62.3	13.8	6.00	56.5
5	1.5	13.8	6.76	51.0	13.8	6.72	51.3	13.8	6.28	54.5	13.8	5.94	56.9
6	2	13.8	6.12	55.6	13.8	6.12	55.6	13.8	5.32	61.4	13.8	5.14	62.7
7	3	13.8	6.00	56.5	13.8	5.68	58.8	13.8	4.90	64.5	13.8	5.16	62.6
8	4	13.8	7.32	46.9	13.8	5.68	58.8	13.8	5.38	61.0	13.8	6.16	55.3
9	5	13.8	7.24	47.5	13.8	5.54	59.8	13.8	4.98	63.9	13.8	5.60	59.4
10	6	13.8	6.84	50.6	13.8	4.92	64.3	13.8	4.98	63.9	13.8	5.16	62.6
11	24	13.8	6.78	50.9	13.8	3.32	75.9	13.8	3.42	73.2	13.8	4.38	68.2

Table 5: Effect of biomass concentration on cadmium removal by *C.resinae* (Temp: 28°C, rpm: 120)

S.No	TIME (hrs)	Biomass concentration (g)											
		0.25			0.5			1.0			2.0		
		C_i	C_f	%	C_i	C_f	%	C_i	C_f	%	C_i	C_f	%
1	0.25	25.6	22.7	11.5	25.6	21.2	17.2	25.6	21.9	14.4	25.6	25.2	1.70
2	0.5	25.6	14.7	42.6	25.6	16.9	33.8	25.6	21.7	15.3	25.6	24.1	6.10
3	0.75	25.6	14.3	44.2	25.6	16.6	35.0	25.6	21.4	16.5	25.6	24.4	4.75
4	1.0	25.6	13.9	45.7	25.6	14.9	41.7	25.6	20.5	20.1	25.6	24.2	5.68
5	1.5	25.6	13.5	47.1	25.6	14.8	42.2	25.6	18.7	27.0	25.6	23.1	9.73
6	2	25.6	13.3	47.8	25.6	14.7	42.6	25.6	17.1	33.2	25.6	23.0	10.2
7	3	25.6	13.2	48.4	25.6	14.4	43.6	25.6	16.9	34.1	25.6	22.4	12.5
8	4	25.6	12.8	49.9	25.6	14.2	44.5	25.6	16.2	36.6	25.6	22.2	13.4
9	5	25.6	12.4	51.4	25.6	14.1	45.0	25.6	15.8	38.2	25.6	22.1	13.9
10	6	25.6	11.5	55.0	25.6	13.3	48.2	25.6	15.4	39.4	25.6	17.9	30.2
11	24	25.6	9.19	64.2	25.6	7.72	69.9	25.6	13.4	47.5	25.6	15.1	40.9

Table 6: Effect of pH on cadmium removal by *P.variotii* (Temp: 28°C, rpm: 120)

S.No	TIME (hrs)	pH											
		4			5			6			7		
		C_i	C_f	%	C_i	C_f	%	C_i	C_f	%	C_i	C_f	%
1	0.25	12.8	11.1	13.6	13.1	9.12	30.5	14.0	9.16	34.5	9.42	9.84	-
2	0.5	12.8	10.1	21.7	13.1	10.2	22.1	14.0	8.08	42.2	9.42	10.0	-
3	0.75	12.8	9.90	23.2	13.1	8.80	32.8	14.0	9.56	31.7	9.42	10.0	-
4	1.0	12.8	9.26	28.6	13.1	8.60	34.3	14.0	7.62	45.5	9.42	8.66	8.00
5	1.5	12.8	7.68	40.4	13.1	8.74	33.5	14.0	11.0	21.4	9.42	8.20	12.7
6	2	12.8	9.34	27.5	13.1	9.44	28.2	14.0	9.60	31.4	9.42	8.74	7.40
7	3	12.8	8.48	34.2	13.1	8.30	36.6	14.0	7.82	44.2	9.42	8.52	9.50
8	4	12.8	9.82	23.8	13.1	8.46	35.8	14.0	8.74	37.8	9.42	7.44	21.2
9	5	12.8	8.44	34.5	13.1	7.06	46.5	14.0	8.24	41.1	9.42	7.20	23.4
10	6	12.8	8.18	36.5	13.1	7.90	39.6	14.0	8.20	41.4	9.42	9.26	-
11	24	12.8	3.76	70.8	13.1	4.80	63.3	14.0	6.10	56.4	9.42	5.52	41.4

Table 7: Effect of pH on cadmium removal by *C.resinae* (Temp: 28°C, rpm: 120)

S.No	TIME (hrs)	pH											
		4			5			6			7		
		C_i	C_f	%	C_i	C_f	%	C_i	C_f	%	C_i	C_f	%
1	0.25	19.4	18.8	5.10	19.6	17.6	10.2	20.6	19.9	3.30	21.4	18.7	12.6
2	0.5	19.4	18.0	7.60	19.6	17.3	11.7	20.6	17.6	14.0	21.4	18.2	14.9
3	0.75	19.4	17.9	8.20	19.6	16.9	13.6	20.6	17.7	14.1	21.4	18.1	15.4
4	1.0	19.4	17.5	10.2	19.6	16.8	14.2	20.6	17.6	14.5	21.4	18	15.8
5	1.5	19.4	17.2	16.4	19.6	16.4	16.0	20.6	17.1	16.9	21.4	17.5	18.1
6	2	19.4	16.3	16.9	19.6	16.1	17.6	20.6	15.9	22.8	21.4	16.9	18.2
7	3	19.4	16.0	17.9	19.6	16.0	18.3	20.6	14.4	29.8	21.4	15.9	25.5
8	6	19.4	15.8	18.9	19.6	15.3	21.9	20.6	14.0	31.7	21.4	15.7	26.3
9	24	19.4	15.3	21.0	19.6	14.8	24.2	20.6	13.9	32.5	21.4	12.0	43.9

Table 8: Effect of initial metal concentration on its removal by *P.variotii* (Temp: 28°C, rpm: 120)

S.No	TIME (hrs)	Metal concentration (ppm)											
		5			10			20			50		
		C_i	C_f	%	C_i	C_f	%	C_i	C_f	%	C_i	C_f	%
1	0.25	3.43	2.02	41.1	6.9	3.05	55.7	13.8	5.47	60.3	37.1	14.3	61.5
2	0.5	3.43	2.15	37.3	6.9	3.11	54.9	13.8	4.42	68.1	37.1	14.1	62.0
3	0.75	3.43	2.02	41.1	6.9	2.64	61.7	13.8	4.97	64.4	37.1	11.5	69.0
4	1.0	3.43	1.56	54.5	6.9	2.80	59.4	13.8	5.22	62.3	37.1	11.4	69.3
5	1.5	3.43	1.55	54.8	6.9	2.67	61.3	13.8	4.31	68.8	37.1	11.1	69.9
6	2	3.43	1.49	56.5	6.9	2.54	63.1	13.8	4.60	66.6	37.1	9.79	73.6
7	3	3.43	1.56	54.5	6.9	2.52	63.4	13.8	3.60	73.9	37.1	11.5	69.0
8	4	3.43	1.46	57.4	6.9	2.45	64.4	13.8	3.62	73.9	37.1	8.74	76.5
9	5	3.43	1.25	63.5	6.9	1.68	75.6	13.8	2.66	81.1	37.1	10.3	72.3
10	6	3.43	1.37	60.0	6.9	2.25	67.3	13.8	3.13	77.3	37.1	10.6	71.5
11	24	3.43	1.03	69.9	6.9	1.72	75.0	13.8	3.09	78.2	37.1	6.25	83.1

Table 9: Effect of initial metal concentration on its removal by *C.resinae* (Temp: 28°C, rpm: 120)

S. No	TIME	Metal concentration (ppm)											
		5			10			20			50		
		C_i	C_f	%	C_i	C_f	%	C_i	C_f	%	C_i	C_f	%
1	0.25	6.90	6.35	7.20	10.2	9.83	3.60	15.9	15.4	3.30	38.8	37.6	3.00
2	0.5	6.90	6.40	7.97	10.2	9.79	4.90	15.9	15.3	4.00	38.8	36.1	7.20
3	0.75	6.90	6.30	8.62	10.2	9.37	8.13	15.9	15.3	4.00	38.8	35.9	7.40
4	1.0	6.90	6.25	9.42	10.2	9.05	11.2	15.9	15.2	4.60	38.8	35.7	7.90
5	1.5	6.90	6.15	10.8	10.2	8.69	14.8	15.9	14.9	6.50	38.8	35.6	9.00
6	2	6.90	5.95	13.7	10.2	8.68	14.9	15.9	14.6	8.40	38.8	33.5	13.6
7	3	6.90	5.73	17.3	10.2	8.51	16.5	15.9	14.3	10.2	38.8	30.5	21.3
8	4	6.90	5.20	24.6	10.2	8.30	18.6	15.9	13.6	14.6	38.8	25.2	35.0
9	5	6.90	5.20	24.6	10.2	7.60	25.4	15.9	13.3	16.5	38.8	24.9	35.8
10	6	6.90	5.00	27.5	10.2	7.20	29.4	15.9	12.9	19.0	38.8	24.7	36.3
11	24	6.90	4.30	37.6	10.2	6.80	33.3	15.9	12.3	22.8	38.8	24.0	38.1

Table 10: Removal of cadmium by live cells of *P.variotii* (Temp: 28°C, rpm: 120)

S.No	TIME (hrs)	Metal concentration (ppm)											
		5 ppm			10ppm			20ppm			50ppm		
		C_i	C_f	%	C_i	C_f	%	C_i	C_f	%	C_i	C_f	%
1	12	6.50	5.90	9.20	14.9	10.6	30.1	14.9	17.8	10.5	43.4	42.2	2.70
2	24	6.50	5.20	18.7	14.9	9.04	39.6	14.9	17.2	13.2	43.4	42.1	2.80
3	36	6.50	5.10	21.5	14.9	9.00	39.8	14.9	17.0	14.5	43.4	41.2	5.00
4	48	6.50	4.94	24.0	14.9	8.81	41.1	14.9	16.6	16.4	43.4	40.8	5.90
5	60	6.50	4.62	28.9	14.9	8.63	42.3	14.9	16.6	16.5	43.4	40.7	6.10
6	72	6.50	4.57	29.6	14.9	8.54	42.9	14.9	15.7	20.8	43.4	40.2	7.30
7	96	6.50	4.50	30.7	14.9	8.20	45.2	14.9	10.8	45.3	43.4	39.5	8.90

Table 11: Removal of cadmium by live cells of *C.resinae* (Temp: 28°C, rpm: 120)

S.No	TIME (hrs)	Metal concentration (ppm)											
		5			10			20			50		
		C_i	C_f	%	C_i	C_f	%	C_i	C_f	%	C_i	C_f	%
1	8	8.24	8.15	1.09	16.2	11.8	27.0	19.6	17.8	9.18	49.7	40.5	18.5
2	24	8.24	8.06	2.10	16.2	10.3	36.5	19.6	17.6	10.2	49.7	40.3	18.9
3	32	8.24	7.8	5.30	16.2	9.69	40.4	19.6	17.6	10.2	49.7	39.0	21.5
4	48	8.24	7.50	8.90	16.2	9.45	41.5	19.6	17.2	12.2	49.7	38.6	22.3
5	56	8.24	7.20	12.6	16.2	9.29	42.8	19.6	17.1	12.7	49.7	38.5	22.6
6	72	8.24	7.20	12.6	16.2	9.29	42.8	19.6	17.0	13.2	49.7	38.5	22.6
7	80	8.24	6.80	17.4	16.2	8.82	45.7	19.6	17.0	13.2	49.7	38	23.6
8	96	8.24	6.70	18.6	16.2	8.67	46.6	19.6	15.2	22.4	49.7	36.5	26.6

Table. 12: Cadmium removal by *P. variotii* in continuous sorption column

Time (hrs)	Initial conc(C_i)	Final conc(C_f)
0.08	1.583	1.583
0.25	1.572	1.572
0.5	1.567	1.567
0.75	1.559	1.559
1.0	1.466	1.466
1.5	1.419	1.419
2.0	1.168	1.168
2.5	1.10	1.10
3	0.793	0.793
4	0.554	0.554
5	0.529	0.529
6	0.451	0.451
7	0.401	0.401
8	0.371	0.371
9	0.360	0.360
10	0.315	0.315
11	0.312	0.312
12	0.265	0.265
13	0.262	0.262
14	0.222	0.222
15	0.218	0.218
16	0.205	0.205
17	0.171	0.171
18	0.162	0.162
19	0.150	0.150
20	0.141	0.141
21	0.064	0.064
22	0.027	0.027
23	0.673	16.82
24	1.070	26.75
25	1.168	29.20
26	1.419	35.47
27	0.849	42.45
28	0.917	45.85
29	0.898	44.90
30	0.951	47.55
31	1.065	53.25
32	1.568	78.40
33	1.601	80.05

Table 13: Cadmium removal by *C. resinae* in continuous sorption column

Time (hrs)	Initial conc(Ci)	Final conc(C_f)
0.08	0.682	20.46
0.25	0.663	19.89
0.5	0.653	19.59
0.75	0.598	17.94
1.0	0.567	17.01
1.5	0.563	16.89
2	0.534	16.02
3	0.526	15.78
4	0.515	15.45
5	0.426	12.78
6	0.426	12.78
7	0.422	12.66
8	0.322	9.66
9	0.279	8.37
10	0.265	7.95
11	0.734	22.02
12	0.743	22.29
13	0.776	23.28
14	0.795	23.85
15	0.811	24.33
16	0.815	24.45
17	0.820	24.60
18	0.823	24.69
19	0.845	25.35
20	0.872	26.16
21	0.913	27.39
22	0.914	27.42
23	1.054	31.62
24	1.110	33.30
25	0.923	46.15
26	0.946	47.30
27	0.980	49.00
28	1.09	54.50

Table 14: Growth of *P.variotii* after 96 hr in presence of Cd

Cd (ppm)	Wet weight (g)	Dry weight (g)
0	9.02	0.464
5	0.661	0.212
10	0.354	0.140
20	0.264	0.150
50	0.197	0.128

Table 15: Growth of *C.resinae* after 96 hr in presence of Cd

Cd (ppm)	Wet weight (g)	Dry weight (g)
0	18.27	0.633
5	8.861	0.358
10	4.243	0.159
20	2.315	0.103
50	1.884	0.078

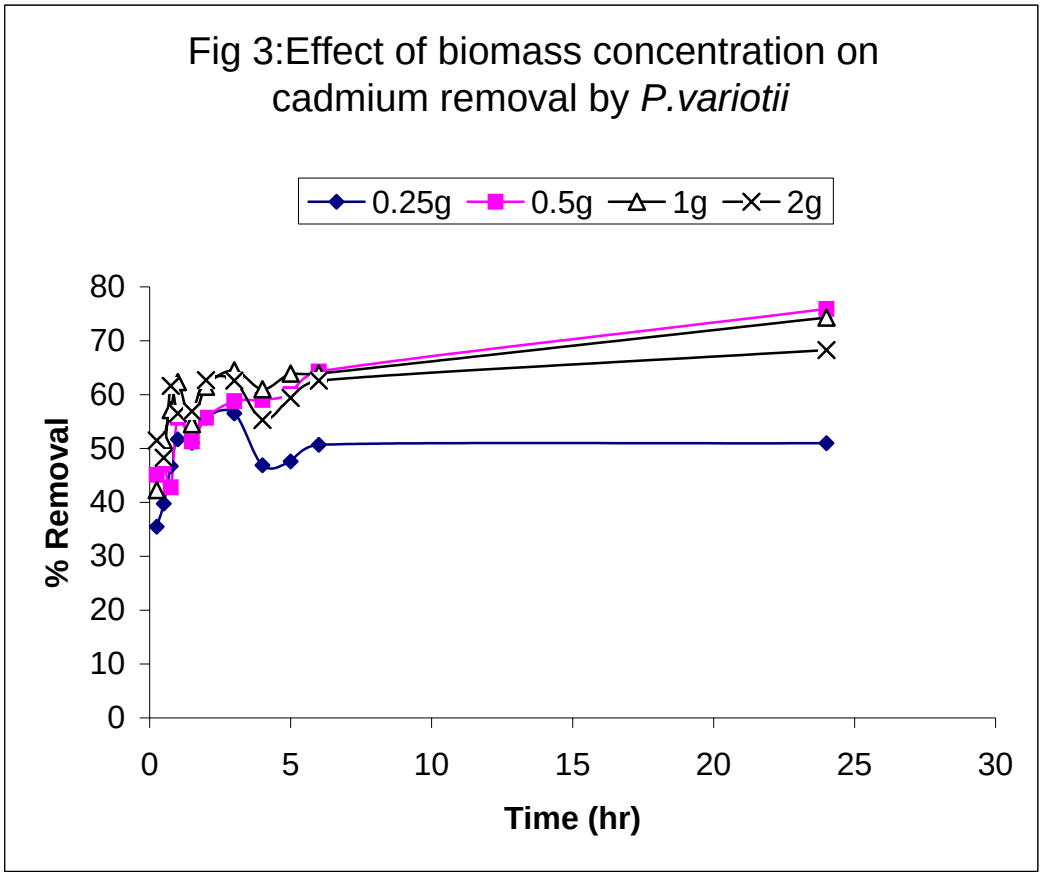


Fig 3:Effect of biomass concentration on cadmium removal by *P.variotii*
0.25g
0.5g
1g
2g
% Removal
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
Time (hr)

Fig 4:Effect of biomass concentration on cadmium removal by *C.resinae*

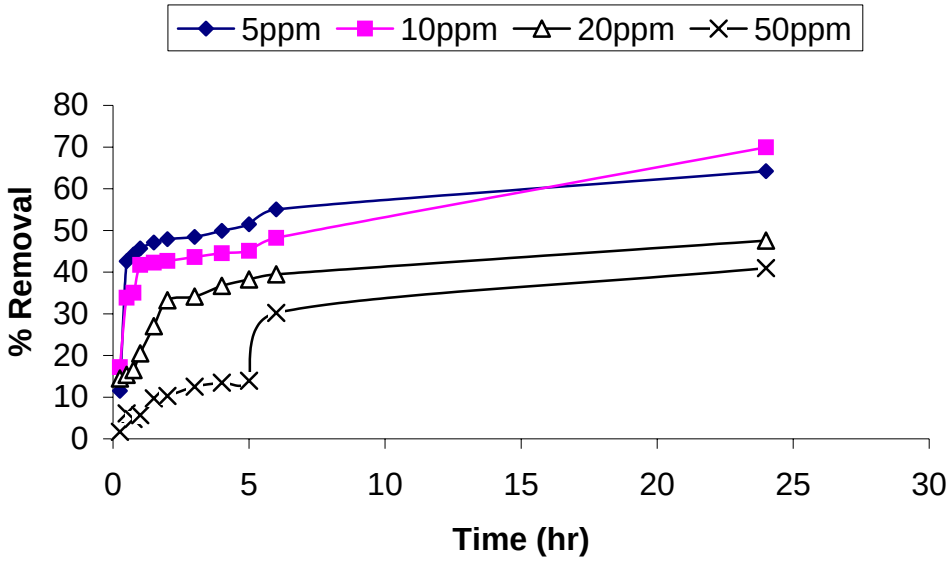


Fig 5: Effect of pH on cadmium removal by *P.variotii*

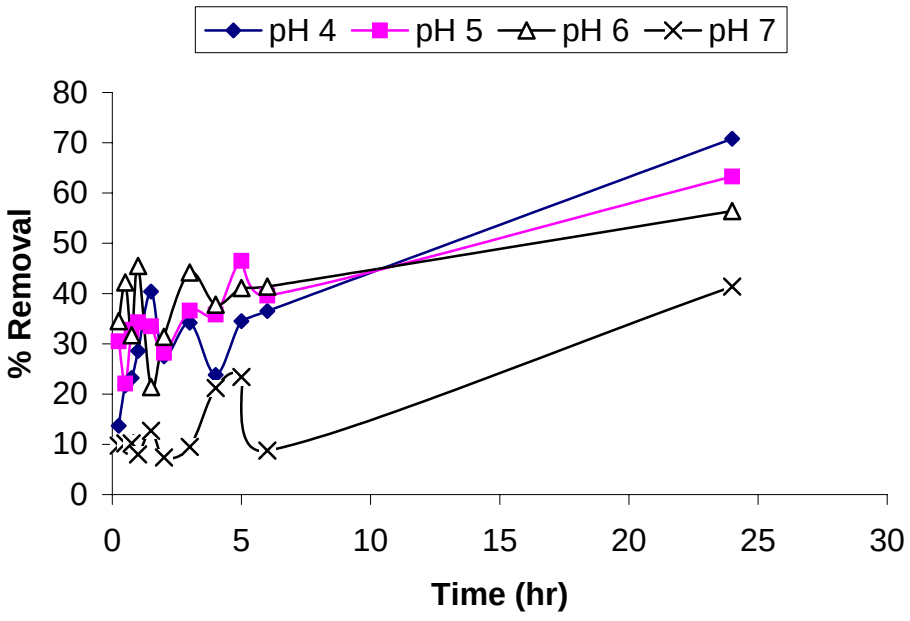


Fig 6:Effect of pH on cadmium removal by *C.resinae*

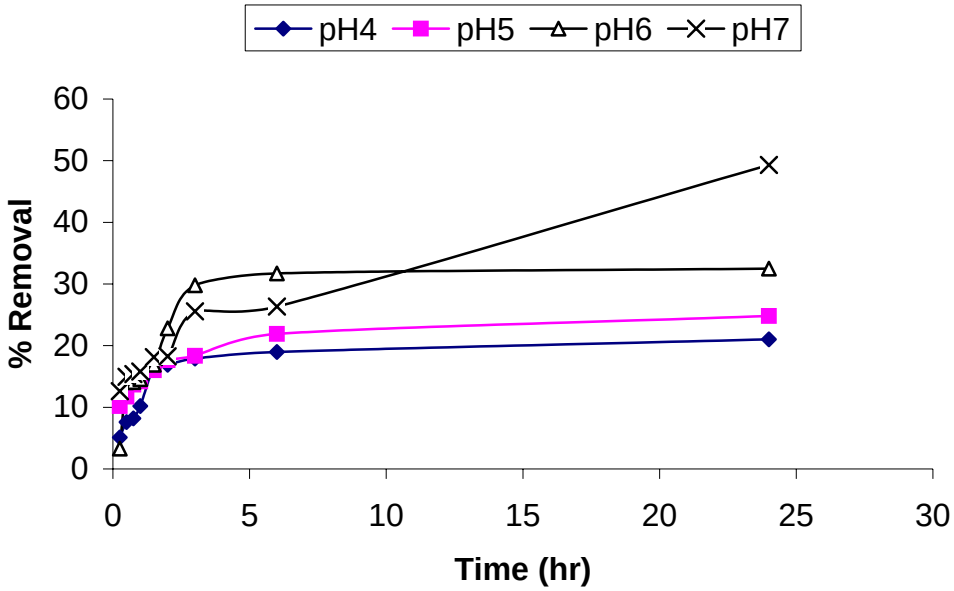


Fig 7:Effect of initial cadmium concentration on its removal by *P.variotii*

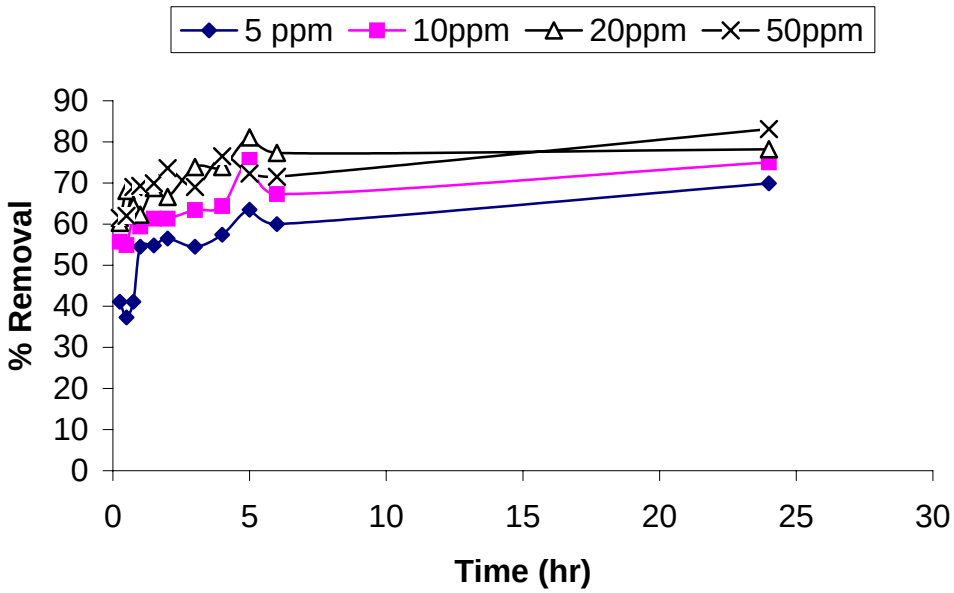


Fig 8:Effect of initial cadmium concentration on its removal by *C.resinae*

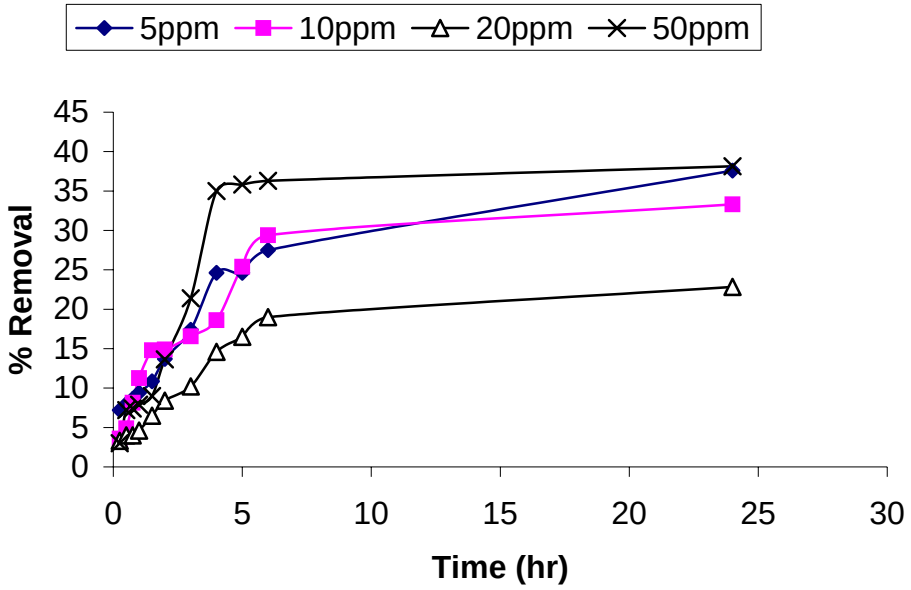


Fig 9:Removal of cadmium by live cells of *P.variotii*

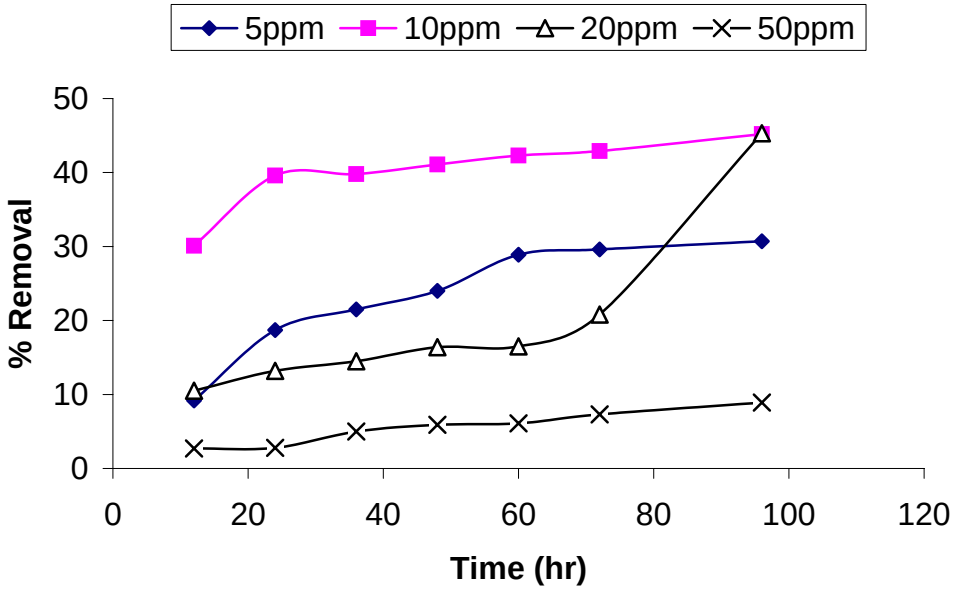


Fig 10:Removal of cadmium by live cells of *C.resinae*

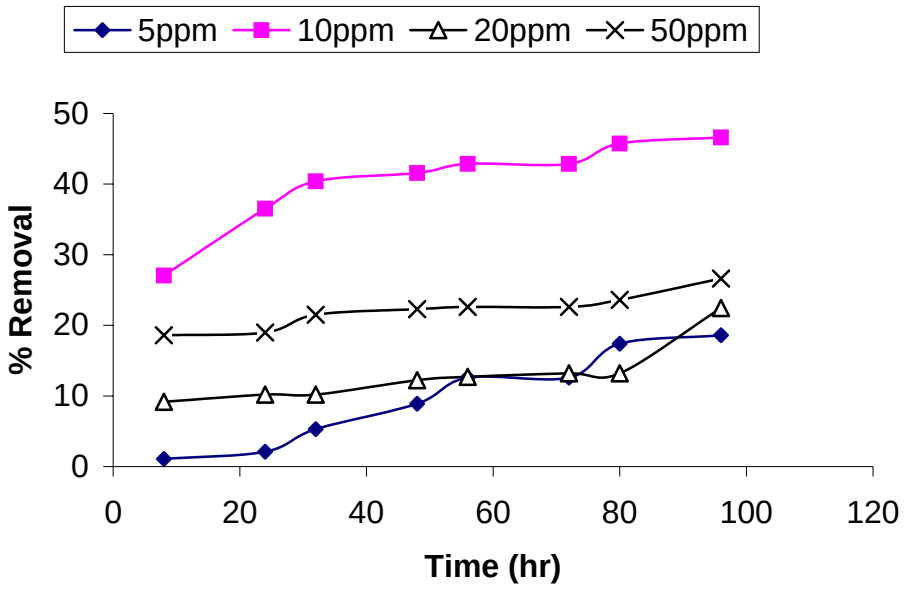
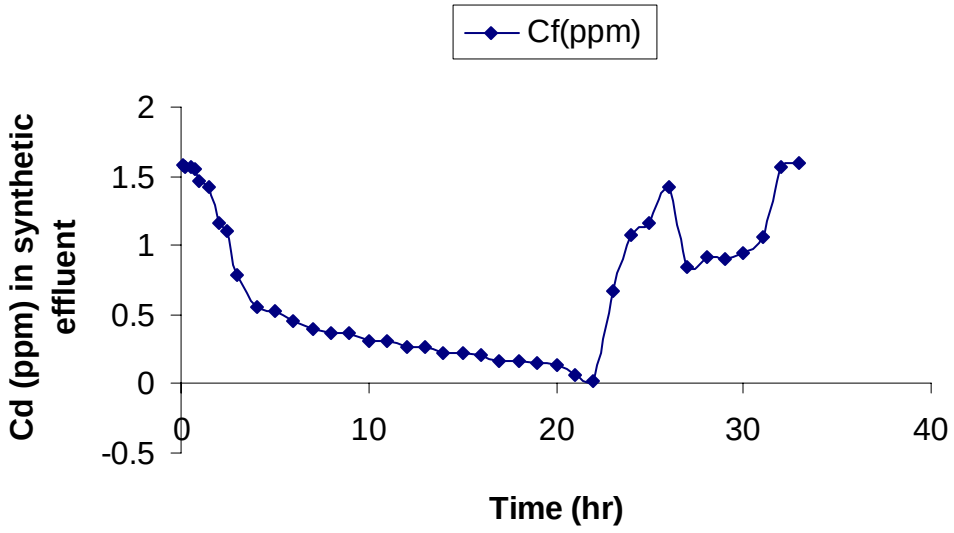


Fig 11:Cadmium removal in continuous sorption column by *P.variotii*



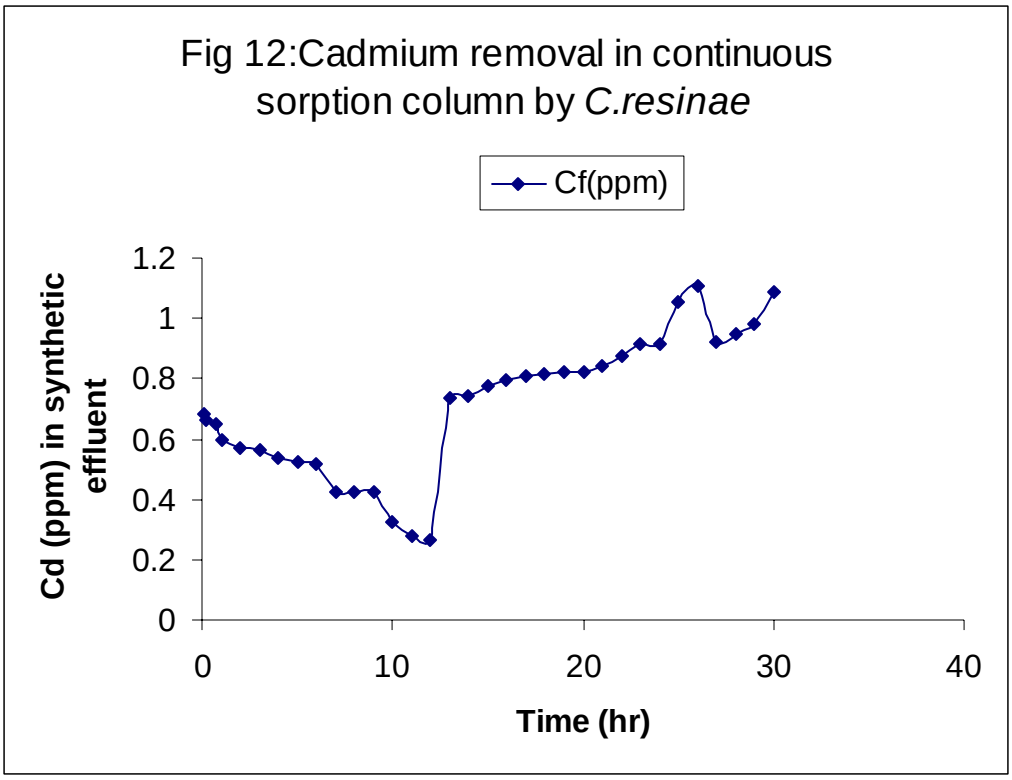


Fig 12:Cadmium removal in continuous sorption column by C.resinae
Cf(ppm)
Cd (ppm) in synthetic effluent
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
Time (hr)