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Cadmium Biosorption by Free and Immobilized Live Biomass of *Rhizopus oligosporus*

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Abstract

Studies on the feasibility of using free and immobilized live cells of *Rhizopus oligosporus* as a biosorbent to remove cadmium from solution was carried out using shake flask experiments. The effect of various conditions such as pH, different initial cadmium concentrations, different biomass concentrations and initial cadmium concentration to biomass concentration ratio was investigated. The biosorption of Cd^{2+} was determined using several sorption isotherm models such as Langmuir and Scatchard plots. The Langmuir sorption model was found sufficient to describe the biosorption of cadmium by both immobilized and free cells, suggesting that the process was chemical, saturable and equilibrated mechanism similar to ion-exchange mechanism of metals adsorption. A curve of Scatchard transformation plot reflected the covalent nature of Cd^{2+} adsorption by live cells of *Rhizopus oligosporus*. Maximum uptake capacity for immobilized cells was about 2-fold higher (34.25 mg/g) than free cells. The immobilized cells projected a higher cadmium uptake capacity with increasing biomass concentration compared to free cells which reached optimum at 0.5 g/L. The initial cadmium concentration to biomass concentration ratio for immobilized cells was lower (33.3 mg/g) compared to free cells (200 mg/g) reflecting that effective removal of Cd^{2+} can be obtained with increasing immobilized biomass concentration. In bioreactor, the cadmium uptake capacity in comparison with shake flasks experiments for immobilized cells was not effected as observed for free cells. In fixed bed-column, packed-bed with immobilized cells permitted better process control with 2.5-fold higher (0.18 Lh^{-1}) influent feeding rate achieved compared to packed-bed with free cells. About 99 per cent of cadmium removal was achieved for influent containing 5 mg/L and 20 mg/L of cadmium indicating strong affinity of free and immobilized live cells of *Rhizopus oligosporus* towards Cd^{2+}.

Introduction

Pollution of the natural environment by heavy metals has become a serious problem in some industrialized countries and in a number of developing countries of Southeast Asia, including Malaysia. The first case of severe metal poisoning have been reported in Japan, the "Itai-Itai" disease caused by chronic cadmium poisoning (Flick *et al*., 1971). Cadmium is one of the most dangerous heavy metal both to human health and aquatic ecosystems. Cadmium can be released into the environment from various sources, such as in by-products from zinc refining, smoke from coal combustion, waste from electroplating process and mine wastes. In industrial wastewater, cadmium ion concentration can approach 200-250 ppm. This value is very high in relation to water-quality standards as the cadmium concentration of wastewater should be reduced to a value of 0.005-0.01 ppm (Crine, 1993).

Methods of removal of metals from industrial waste water includes chemical precipitation, solvent extraction, dialysis or electrodialysis, electrolytic extraction, reverse osmosis, evaporative methods, ion-exchange resins, carbon adsorption and dilution. The increasing problem of heavy metal contamination as well as increasing value of some metal has stimulated a search for new mechanisms for removal and recovery of these metals. In that attempts, biological methods using the metal-binding capacity of microorganisms such as bacteria (Shuttleworth and Unz, 1993), fungus (Volesky and Prasetyo, 1994), algae (Kuyucak and Volesky, 1988; Cho *et al*., 1994) and yeast (Volesky and May-Philips, 1995) have been reported to remove metals from aqueous solutions. This biological removal is commonly known now as biosorption.

Fungi are capable of sequestering and accumulating heavy metals. This feature may be a result of (i) active metal intake for metabolic purposes as well as passive metal uptake and (ii) binding due to the chemical makeup of the cell and its constituent parts, composing of mainly three major classes of fungi biopolymers that is the polysaccharides, nucleic acids and protein.

These biopolymers provide active sites at which the metal binding takes place (Volesky, 1990). The latter feature which is biosorption may actually be so strong that in some instances it may lead to instantaneous removal of metal from aqueous solution. This feature of fungi biomass when pragmatically turned around could serve as an useful purpose of extracting and concentrating metallic species from the aqueous environment. The ubiquitous nature of many types of fungal species, the biomass of which could be available in large quantities from established fermentation processes as waste product, makes fungi an interesting target for examination of their metal biosorbent potential. There is a possibilities to use fungal biomass as biosorbent for detoxification of industrial effluent streams by removing their toxic heavy metal components. As another aspect, by immobilizing the fungal biomass, the sequestered metals of value could be recovered and recycled or resold, thus offsetting the costs of the wastewater treatment process and actually of the entire industrial operation cost. After recovering the metal, the

biosorbent could be regenerated and used for subsequent removal of metals from industrial wastewater.

In this study the effectiveness of cadmium removal by using *Rhizopus oligosporus* live biomass and its mechanism of removal was determined. The interest given to this fungus was due to the general characteristics of the fungus which was less susceptible to contamination during its cultivation, its ability to be easily immobilized (especially by means of passive immobilization) and it can easily be obtained as a starter culture from 'Tempe' (molded soy beans) production. Objectives of this study were to investigate the ability of *Rhizopus oligosporus* live biomass to remove cadmium from aqueous solution, compare the ability between immobilized live biomass and free live biomass of *Rhizopus oligosporus* in removing cadmium from aqueous solution and characterize the uptake of cadmium ion by using adsorption isotherm models such as Langmuir and Scatchard plots.

Materials and Methods

Microorganism: The fungus *Rhizopus oligosporus* used in this study was obtained from Malaysia Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia. The composition of complex medium used for cell cultivation was as follows (in g/L); glucose, 40; yeast extract, 10; KH_2PO_4, 1; $MgSO_4.7H_2O$, 0.5; $NaNO_3$, 1. Cells were cultivated in a l-L shake flask containing 500 mL medium, inoculated with 10 mL spore suspension and was incubated at 30°C, 250 rpm on an orbital shaker (CERTOMAT R, B. BRAUN). The initial medium pH was adjusted to 5.6. Cultures were harvested after 7 days of incubation.

Biomass Support Particles (BSP): About 5 mm cubic polyurethane foam BSP, having a pore size of 43 pores cm^{-1} were employed as carriers for immobilization of *Rhizopus oligosporus* cells. The BSPs were confirmed to have no sorption capacity towards Cd^{2+}.

Preparation of free and immobilized live cells: The biomass harvested was centrifuged (at 6000 g for 10 minutes) to remove unbound water, washed with deionized water and filtered to obtain free live cells. As for immobilized cells, autoclaved 400 BSPs were placed with the liquid medium (500 mL) in a l-L shake flask, inoculated with 10 mL spore suspension and agitated at 200 rpm for 7 days with temperature controlled at 30°C. A lower agitation speed was applied to allow maximum immobilization of cells within the BSPs. The immobilized cells were harvested, washed with deionized water and filtered in a vacuum filter unit to remove unbound water. Both the immobilized and free live cells were then stored in an air-tight container that was placed in a desicator and kept at 4°C to limit any biochemical activity within the cells. These biomass prepared was designated as resting or live cells.

Determination of amount of cells immobilized to BSP: To obtain a constant amount of cells immobilized to the support, immobilization was done in batches. A series of flask containing 400 BSPs were used for immobilization. 10 BSPs were withdrawn from each flask and average weight of the empty BSP were determined after placing 10 BSP in an over (90°C) overnight. After immobilization, 10 BSPs were withdrawn from each flask and dried in oven overnight to give (immobilized cells + BSP) dry weight. The amount of immobilized cells were thus determined by:

Dry weight of immobilized cells (g) per BSP

$$= \frac{\text{(Immobilized cells + BSP) - BSP dry weight (g)}}{10}$$

= Cells Immobilized (g)/BSP

From this immobilization procedure, 0.0046 g cells/BSP was immobilized.

Biosorpiton Experiments: Synthetic solutions of deionized water containing $CdCl_2.2H_2O$ metal salt (99% pure with molecular weight 228.34 g/mol) were used in all biosorption experiments. The biosorption experiments were carried out in batches using 250-mL shake flasks. Known amount of cells were added to 100 mL cadmium solution. Flasks were agitated in an orbital shaker (CERTOMAT R, B. BRAUN) at 200 rpm, and maintained at 30°C for 24 hours to allow each metal/biomass system to reach equilibrium. At equilibrium, the cells were separated from solution by centrifugation at 6000 g for 10 min. The concentration of cadmium in cells-free supernatant was determined using Atomic Absorption Spectrophotometer (PERKIN ELMER 3300). Several sets of biosorption experiments were carried out to investigate the effect of several biosorption conditions (initial cadmium concentrations (mg/L) and cell/biomass concentrations (g/L) on the capacity of cadmium adsorption by the biosorbents. Each experiments were carried out in triplicates and the results given are the average values.

Biosorption experiments were also carried out using l-L stirred tank bioreactor with a working volume of 500 mL cadmium solution, equipped with a six-bladed Rushton turbine, to obtain data of cadmium uptake at different contacting times at controlled pH of 5.31 (controlled by pH controller, B.BRAUN DCU 2; pH probe, INGOLD) and uncontrolled pH. In all experiments, biomass concentration was 1 g/L and cadmium concentration was 100 mg/L. The bioreactor was agitated at 200 rpm and the temperature within the bioreactor was controlled at 30°C. Aliquots of 5 mL was withdrawn as samples for analysis at intervals of 2 minutes for the first 20 minutes, 5 minutes interval for the next 60 minutes and 20 minutes interval for the final 120 minutes.

A down flow packed-bed system was also used to perform biosorption experiments in a column operation. The

biomass/biosorbent was packed into a glass column (31 cm X 10.4 mm) with a fixed bed height of 22 cm. In order to maintain the temperature at 30°C, the column was fitted with a water jacket. 5 to 10 bed volumes of deionized water was added to rinse the column to wash out contaminants before cadmium solution was feed from top of the column. The feeding rate of the cadmium solution was controlled using a peristaltic pump (LKB BROMMA 2232 MICROPERPEX) fitted with a Teflon tubing (0.5 mm). Fraction/Effluents were collected periodically by fraction collector (PHARMACIA LKB RediFrac) at every interval of 1 hour for 100 hours and its cadmium concentrations were determined. The influent concentration was monitored at every 10 hours interval. Break through curves were developed by plotting effluent cadmium concentrations (mg/L) vs elution time (hr). Several sets of biosorption experiments were carried out to investigate the effect of several biosorption conditions (different cadmium influent concentrations (mg/L) and influent feeding rates (Lh^{-1}) on the capacity of cadmium adsorption by the biosorbents.

Data analysis: Cell metal-binding sites were characterized by the application of the Langmuir adsorption model (Langmuir, 1918) and Scatchard analysis (Scatchard, 1949) to the adsorption data.

Results

Biosorbents and initial Cd^{2+} concentrations: With the fixed cadmium concentration of 100 mg/L, the uptake of Cd^{2+} was decreased with increasing concentrations of free live cells and the highest cadmium uptake was observed at 0.5 g/L (Fig. 1). Conversely, a reverse trend was found for immobilized cells. From the plots (Fig. 2) of Cd^{2+} uptake against the initial cadmium concentration/cell concentration ratio, it was found that maximal cadmium uptake for immobilized cells was achieved at a lower ratio of 33.3 mg/g in contrast to free cells ratio of 200 mg/g.

Equilibrium biosorption isotherms of Cd^{2+} by the free and immobilized live cells of *Rhizopus oligosporus* are shown in Fig. 3. Biosorption isotherm represents the equilibrium distribution of Cd^{2+} between the aqueous and solid phases, when the cadmium concentration increases. The cadmium uptake by the cells of *Rhizopus oligosporus* increased with increasing initial Cd^{2+} concentrations and reached a maximum value at 100 mg/L (corresponding equilibrium cadmium concentration, C_{cq} = 78.8 mg/L). Result indicates that the free and immobilized live cells (1 g/L) were saturated with Cd^{2+} at 100 mg/L.

Adsorption mechanism: From linearised Langmuir plots (Fig. 4), the maximum uptake capacity of cadmium (q_{max}) for *Rhizopus oligosporus* cells and the dissociation constant of the equilibrium exchange (K_g), were calculated. Immobilized live cells have higher q_{max} (34.25 mg/g) and lower K_d value (10.72) compared to free live cells (q_{max} = 17.09 mg/g, K_d = 18.72).

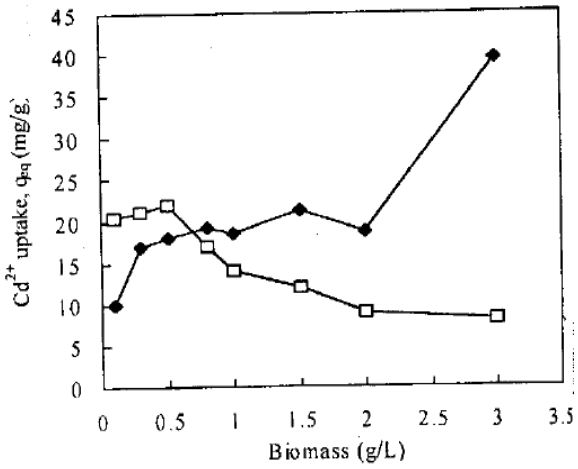

Fig. 1: Effect of increasing Rhizopus oligosporus biomass concentration on Cd^{2+} uptake capacity (□) Free cells; (◆) Immobilized cells.

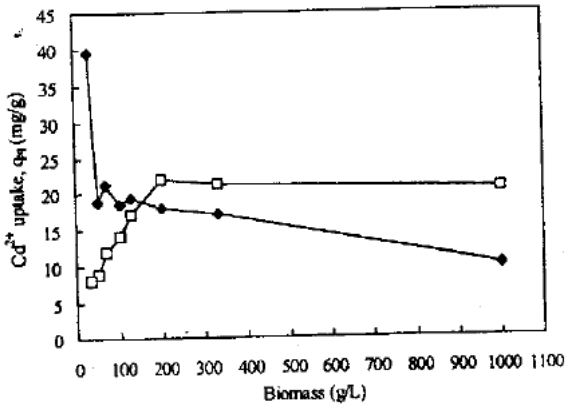

Fig. 2: Effect of (Initial Cd^{2+}/Biomass] ratio on Cd^{2+} uptake capacity (□) Free cells; (◆) Immobilized cells.

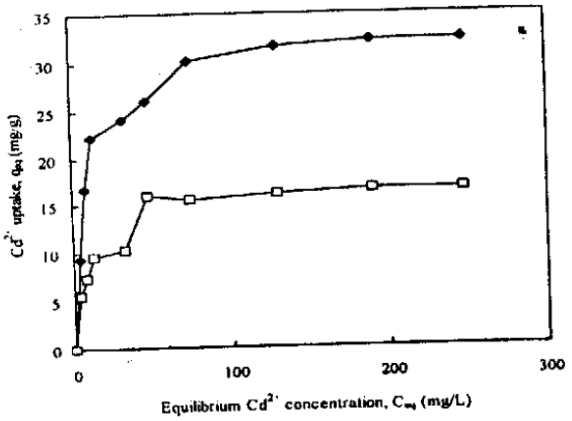

Fig. 3: Equilibrium Cd^{2+} biosorption isotherm of *Rhizopus oligosporus* biomass (□) Free cells; (◆) Immobilized cells

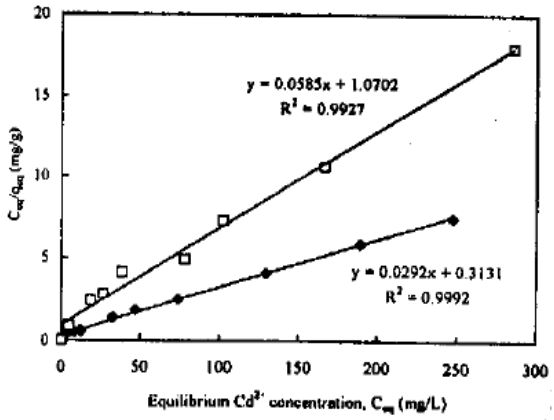

Fig. 4: Langmuir linearised biosorption isotherm of *Rhizopus oligosporus* biomass (□) Free cells (◆) Immobilized cells

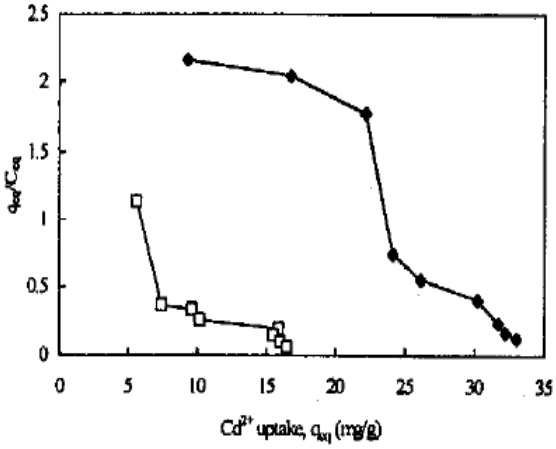

Fig. 5: Scatchard plot for Cd^{2+} biosorption by *Rhizopus oligosporus* biomass (□) Free cells; (◆) Immobilized cells.

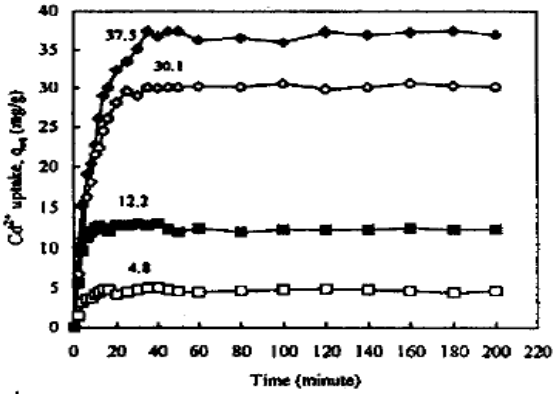

Fig. 6: Time courses of CcI^{2+} biosorption by *Rhizopus oligosporus* biomass (■) Free cells, uncontrolled pH (□) Free cells, controlled pH 5.31; (◆) Immobilized cells, uncontrolled pH (◇) Immobilized cells, controlled pH 5.31.

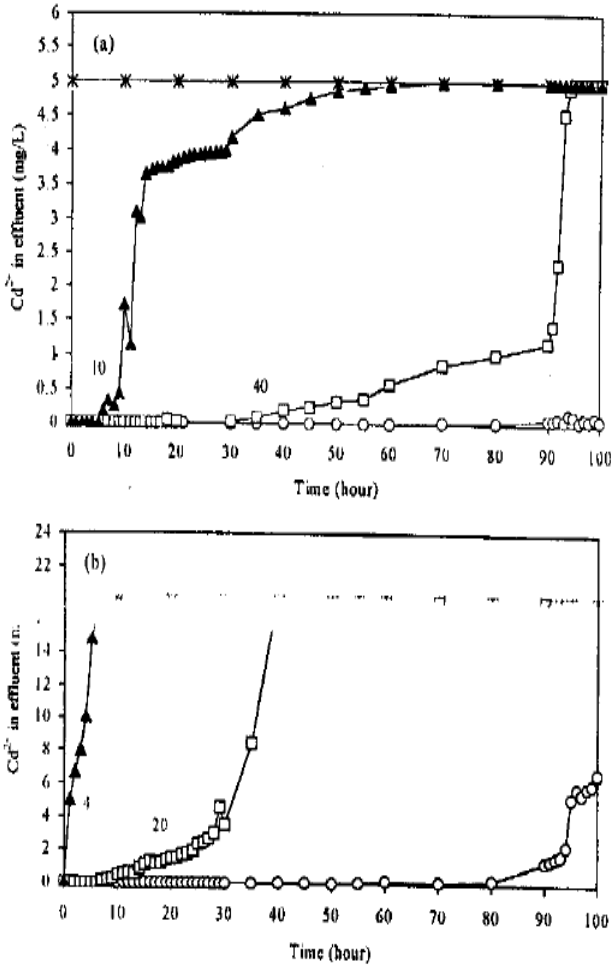

Fig. 7: Comparison between breakthrough curves for Cd^{2+} biosorption by *Rhizopus oligosporus* biomass for influent Cd^{2+} concentration (a) 5 mg/L and (b) 20 mg/L. (✳) Influent Cd^{2+} concentration; (O) Free cells, 0.007 Lh ^{1}; () immobilized cells, 0.007 Lh ^{1}; (▲) Immobilized cells, 0.018Lh ^{1}

The Scatchard plots of Cd^{2+} binding to the cell walls of *Rhizopus oligosporus* are presented in Fig. 5. The slopes of the Scatchard plots are biophasic or multiphasic and has been previously reported for metal binding by microbial biomass and are interpreted as indicating multiple nonequivalent binding sites (Scatchard, 1949; Tobin *et al*., 1994). If the plots curve concave to the origin, it reflect non-cooperative binding by multiple binding sites (Chamness and McGuire, 1975; Suen, 1997). The upper portion of the plot implies to strong primary binding (high affinity binding sites) at low Cd^{2+} concentrations due to covalent bonding and weaker secondary interaction (low affinity binding sites) is represented by the lower portion of the plot due to ionic bonding at elevated Cd^{2+} concentrations.

Table 1: Cd^{2+} uptake capacity of *Rhizopus oligosporus* biomass in packed-bed column

Cd^{2+} uptake capacity (mg/q)			
Influent Cd^{2+} concentration			
5 mg/L		20 mg/L	
Feeding rate 0.007 Lh^{-1}	Feeding rate 0.018 Lh^{-1}	Feeding rate 0.007 Lh^{-1}	Feeding rate 0.018 Lh^{-1}
1.22	0.78	1.83	1.56

Time course of Cd^{2+} adsorption: The time courses of Cd^{2+} adsorption by free and immobilized live cells of *Rhizopus oligosporus* are shown in Fig. 6. The Cd^{2+} uptake increased rapidly with time and reached maximum after about 18 and 40 minutes for free and immobilized live cells respectively.

Cadmium biosorption in packed-bed column: Breakthrough curves of cadmium solution are displayed in Fig. 7. The breakthrough curves was analyzed for its break even point whereby at this paint complete biosorbent exhaustion occurs (Frank, 1985). Results obtained for immobilized live cells of *Rhizopus oligosporus* shows that when the feeding rate of influent was increased about 2.5-fold compared to 0.007 Lh^{-1} the break even point was achieved faster from approximately 40 to 10 hours. As for the effect of cadmium concentration, when the cadmium concentration fed was increased 4-fold (20 mg/L), a typically identical effect was also observed whereby time for break even point was reduced from 20 hours to over less than 4 hours. About 99 per cent removal of cadmium with a effluent concentration of 0.01-0.02 mg/L was obtained for both influent cadmium concentration treatment.

Based on the break even point, the cadmium uptake capacity calculated (Table 1) was also effected by increasing cadmium influent concentrations and feeding rates. The cadmium uptake capacity increased with increasing cadmium concentration but decreased when the feeding rate of influent was increased.

Discussion

Biosorbent and Initial Cd^{2+} concentrations: One of the parameter that greatly effected the adsorption capacity is the concentration of biosorbents whereby two contrasting' results were obtained. The reduction in Cd^{2+} uptake by free live cells was attributable to electrostatic interaction between free cells. Rome and Gadd (1987) and Fourest and Roux (1992) concluded that distance between cells decreased and cells aggregated easily to form clumps with increasing free cells concentration. This causes the three dimensional structure of the cell wall and the internal linkages between the reactive groups (COO^- and the NH^{3+}) to occur, thus reduces the Cd^{2+} diffusion through the structure and the accessibility of the binding sites for adsorption (Plette *et al.,* 1996). As for immobilized cells, the cells were positioned within the matrices of the BSP pores. This allowed very little or absolute no interaction with the rest of the immobilized cells in the cadmium solution and this did not permit cells from clumping. Therefore, the binding sites on the cell wall has maximum accessibility to Cd^{2+} and this attributed to increasing uptake of cadmium with increasing immobilized cells concentration, as long as the latter is not saturated.

The data presented in the plots of Cd^{2+} uptake against the initial cadmium concentration/cell concentration ratio (Fig. 2) can be useful to optimize the process of Cd^{2+} adsorption especially for further studies on treatment of industrial effluent. If the metal concentration in the effluent is known, then appropriate amount of biosorbent could be added according to the effective ratio.

Adsorption mechanism: In all cases, the typical linearity of the linearised Langmuir adsorption model (Fig. 4) and a hyperbolic equilibrium biosorption isotherm (Fig. 3) suggests that adsorption of cadmium by *Rhizopus oligosporuslive* cells was a chemical, equilibrated and saturable mechanism which reflected the predominantly ion-exchange mechanism of metals adsorption. In this type of adsorption mechanism, it is assumed that each Cd^{2+} binding site accommodates a single Cd^{2+} which indicates monolayer, single site-type adsorption. Immobilized cells has a higher/larger; binding capacity (q_{max} = 34.35 mg/g) and affinity/strength (K_d = 10.72) towards Cd^{2+}. The higher affinity of immobilized cells towards Cd^{2+} lies in the strength of binding between binding sites with Cd^{2+}. For immobilized cells, the cells are confined in a matrix provided by the biomass support particles (BSP) pores. This limits the cells from being susceptible to shearing caused by agitation of the' surrounding solution, When the surface area of cells with binding sites comes in contact with Cd^{2+} from solution, Cd^{2+} adsorption occurs and as the cells surface area comes in contact with the agitated solution against, the shear force developed in solution does not come in direct contact with Cd^{2+} bound sites because it is shielded by the matrices of the BSPs. As the Cd^{2+} is strongly bound, a higher cadmium uptake capacity is obtained as more Cd^{2+} adsorbed to the "shear-force protected" immobilized cell binding sites on the surface area of the cell wall as no consequences of Cd^{2+} desorption occurring.

Time course of Cd^{2+} adsorption: The rapid Cd^{2+} uptake by both free and immobilized live cells proves that removal of cadmium by *Rhizopus oligosporus* live cells is due mainly to adsorption or biosorption which typically occurs rapidly (Gadd, 1986; Hafez *et al*., 1996). When compared to shake flasks, the maximum cadmium uptake capacity for immobilized live cells was not effected in bioreactor operation as compared to free live cells which projected decrease in its cadmium uptake capacity. This result prove that immobilized cells could withstand the shear force of the bioreactor impeller and could further be used in bigger contactors. As for the effect of pH on cadmium uptake capacity, by controlling the pH at 5.31 incidently contributes to addition of H^+ to the solution which may compete with Cd^{2+} for adsorption to the binding sites. A elevated concentration, H^+ displaces Cd^{2+} causing significant reduction in cadmium uptake capacity.

Cadmium biosorption in packed-bed column: Result obtained for immobilized live cells of *Rhizopus oligosporus* shows that when the feeding rate was increased, the brea even point was achieved faster. This was due to the flowrate in the column which increased resulting in the adsorption rate of Cd^{2+} to the biosorbent increase

Therefore, the biosorbent is exhausted faster but a larger volume of influent was treated. As for the effect of cadmium concentration, higher concentration causes rapid saturation of the biosorbent binding sites with Cd^{2+} which is higher in concentration in its surrounding. Strikingly, an absolute high removal efficiency (99%) of cadmium from influent was achieved regardless of the effect of higher flowrate and cadmium concentration indicating that a higher flowrate and cadmium concentration did not effect the affinity of the immobilized *R. oligosporus* cells whereby this biosorbent exhibits strong affinity towards Cd^{2+}.

The cadmium uptake capacity increased with increasing cadmium influent concentration but decreased when the influent feeding rate was increased. The uptake capacity was higher at higher cadmium concentration due to higher degree of dispersion of Cd^{2+} in the packed-bed causing more binding sites per surface area from the packed-bed coming into contact with Cd^{2+}. As for the contrasting effect of influent feeding rate, a higher feeding rate means that the contact time between Cd^{2+} and the binding sites in the packed-bed was reduced and this contact time was not sufficient for Cd^{2+} to be adsorbed to the binding sites. Furthermore, higher feeding rate contributes to a higher flowrate of influent in the column causing a 'flushing' effect to take place within the binding sites and the Cd^{2+} that comes in contact. But the result also shows that at a higher influent feeding rate with a higher cadmium concentration, the uptake capacity was higher. This indicates that treatment of a larger volume of influent containing higher concentration of cadmium is possible using immobilized cells packed column.

As for free live cells, there as no column breakthrough even after 100 hours for both experiments, so the uptake capacity of cadmium could not be calculated for free cells. Nevertheless this result indicates that free cell of *Rhizopus oligosporus* too has a strong affinity for cadmium but is not exhausted very fast. This may be due to a higher amount of free cells (8 g) packed in the column compared to immobilized cells (1.15 g). But, an apparent disadvantage was exhibited by free cells whereby the column operation could not be operated at higher feeding rate (at 0.018 Lha^{-1}) of influent due to the compaction of the cells that caused plug-flow. Based on this results obtain, it can be concluded that immobilized cells of *Rhizopus oligosporus* permitted better process control of biosorption in fixed packed-bed column.

As an overall conclusion, it can be concluded that the live biomass of Rhizopus oligosporus was found to have excellent Cd^{2+} uptake properties. The maximum Cd^{2+} uptake capacity for immobilized live cells was about 2-fold higher than for free live cells. The Cd^{2+} uptake capacity was predominantly ion-exchange mechanism of metals adsorption.

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