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KINETICS STUDY AND EQUILIBRIUM ISOTHERM MODELING DEPENDENT EVALUATION OF CADMIUM BIOSORPTION EFFICACY OF LYOPHILIZED BIOMASSES OF *LEUCOBCTER* SP. KUCD3 AND *RALSTONIA MANNITOLILYTICA* KUCD7

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ABSTRACT

A basic investigation was conducted in batch conditions to remove a hazardous heavy metal, cadmium from aqueous solutions utilizing lyophilized cell mass of two cadmium resistant bacteria, *Leucobcter* sp. KUCd3 and *Ralstonia mannitolilytica* KUCd7 separately, as biosorbents. Irrespective of initial Cd concentration of solution, both the biosorption reactions took 80 min to attain equilibrium at neutral pH and 28°C following pseudo-second order kinetics which indicated their chemisorption nature. Though the specific metal biosorption enhanced with increasing initial Cd concentration of the solution, Cd removal efficiency reduced. Maximum Cd removal efficiency of the biomasses of KUCd3 (91.8%) and KUCd7 (95.8%) was observed at 10mg/L of Cd concentration. While Cd adsorption isotherm of KUCd3 biomass followed 'Langmuir model'of monolayer sorption on a homogeneous surface, adorption by KUCd7 biomass could be described better through 'Freundlich model' of heterogeneous absorption principle. The values of separation factor between 0 to 1 and high surface coverage revealed that these reactions were favourable with considerable amount of Cd sorption sites on both these biosorbents. Therefore, KUCd3 showed maximum cadmium sorption capacity of 108.7mg/g with the Cd binding affinity of 0.083L/mg and KUCd7 having Cd binding affinity of 0.054L/mg exhibited maximum cadmium sorption capacity of 84.75mg/g as biosorbents. SEM-EDAX analysis documented cell surface morphology of KUCd3 and KUCd7 and presence of bound Cd on it. FTIR analysis revealed the involvement of different anionic functional groups presenton bacterial cell envelopin Cd^{2+} entrapment. From the *in-vitro* analysis it can be concluded that the individual call biomasses of *Ralstonia mannitolilytica* KUCd7 and *Leucobcter* sp. KUCd3 are the potent biosorbent those can be explored for cadmium removal from contaminated water body.

INTRODUCTION

Rapid urbanization and industrialization induces severe ecological crises by favouring pollution through heavy metal contamination.Cadmium (Cd) is one of the non-essential heavy metals and highly toxic pollutant at even relatively low concentrations. Anthropogenic activities such mining, disposal of sludge and effluents of metallurgic industries, incineration of plastics and batteries, application of phosphate and nitrogenous fertilizers increased its availability in the biosphere. In human, acute cadmium ingestion can cause gastrointestinal tract erosion, pulmonary, hepatic or renal injury and can eveninduce carcinogenesis depending on the route of poisoning (Paul and Datta, 2016). Cd toxicity caused serious health hazards such as itai-itai disease in human due to consumption of Cd contaminated rice (Aoshima, 2016). Its high solubility in water increases its bioavailability (Pinto *et al.*, 2004). Because of cadmium's exceptionally long half-life, provisional tolerable weekly intake (PTMI) was established as 25 µg/kg of body weight by JECFA (2011). According to WHO (2011), contamination of drinking-water may occur as a result of the presence of cadmium as an impurity in the zinc of galvanized pipes or cadmium-containing solders in fittings, water heaters, water coolers and taps. Levels of cadmium could be higher in areas supplied with soft water of low pH, as this would tend to be more corrosive in plumbing systems containing. A number of conventional physico-chemical methodologies such as ion exchange, reverse osmosis, electrochemical treatment or solvent extraction applied for cadmium removal have several disadvantages including economic and environmental aspects or ever inadequate at low permissible limit of the metal (Chakravarty and Banerjee, 2012). Generation of huge amount of metal-bearing sludge causes difficulties in disposal. The

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advent of bioremediation technology for heavy metal removal has provided an alternative to conventional methods of pollution control as it becomes more economical, eco-friendly and highly efficient process especially at low metal concentrations. Among the various bioremediation strategies of heavy metals biosorption process relies on the surface ligand dependent metal binding and removal by dead or inactivated cell biomasss. Metabolically inactive cells have precedence over living cells because it is not only a metabolism independent passive quicker method with easer metal desorption process but also independent of nutrient supplementation, growth monitoring and maintenance of culture incontamination free optimized conditions (Rezaee *et al.*, 2006; Das *et al.*, 2008; Plaza *et al.*, 2011). Due to the presence of a variety of chemical moieties and functional groups on the cell wall, which can act as metal binding sites, bacterial biomass has received considerable attention in recent days as efficient biosorbent of metals. As the biosorption capacities depend on the cell wall structure, different parameters such as affinity of surface ligands to specific metal ions, the maximum sorption capacity, as well as the rate of the metal sorption on the surface of the biosorbents are the major criteria for comparing and choosing the best type of biosorbents for specific purposes. These parameters can be evaluate by means of equilibrium isotherms and kinetic studies of biosorption process. However, other factors including type of the metal ion, contact time, microenvironment such as pH of the solution, ion strength can influence the viability of biosorption process (Joo *et al.*, 2010).

Cd tolerant, Gram positive bacteria *Leucobcter* sp. KUCd3 and Gram negative *Ralstonia mannitolilytica* KUCd7, isolated from industrial waste contaminated soil of Kalyani, Nadia, West Bengal, India, showed Cd removal efficiency from culture medium, sewage water and rhizosphere during their metabolically active growing condition through intercellular accumulation (Paul and Datta, 2016; 2017). In this study, Cd removal efficiency through passive biosorption by metabolically inactivated lyophilized cell biomasses of KUCd3 and KUCd7 was evaluated. Kinetics study and equilibrium isotherm modelling was performed along with analysis of surface morphology and characterization of Cd^{2+} binding ligands for proper evaluation of these bacterial cell biomasses as Cd biosorbents.

MATERIAL AND METHODS

Preparation of biosorbent

Cell biomasses of *Leucobcter* sp. KUCd3 and *Ralstonia mannitolilytica* KUCd7 was generated by growing them separately in 500 mL of Glucose minimal (GM) and Nutrient broth (NB) media, respectively, up to late log phase. After harvesting the cell biomasses, cell pellets was washed twice in sterile distilled water to remove residue of the culture media. As metal biosorption was a metabolism independent process, bacterial cell pellets was converted into metabolically inactive cell biomasses by means of lyophilization at -40°C and used as biosorbents.

Batch biosorption experiment

Biosorption reaction highly depends on time of contacts between sorbent and sorbet. To study the effect of contact-time on biosorption 0.1 g of lyophilized cell biomass of *Leucobcter* sp. KUCd3 and *R. mannitolilytica* KUCd7 was added in the individual set of three conical flasks of 250 mL, containing 100 mL Cd solution of three different concentrations (20, 50 and 100 mg/L). The initial pH of the solutions was adjusted to pH 7 and placed on a shaker (160 rpm) incubator at 28°C. Sampling was done at a regular time interval up to 120 min during incubation. The time after which no further increase in Cd biosorption was observed, was considered as required time for reaching that biosorption reaction to its equilibrium. Control sets, containing solutions of equal volume and Cd concentrations to their corresponding sets, was also prepared without treating them with cell biomasses. After harvesting the cells by centrifugation, cell free supernatants was digested with concentrated nitric acid (69%, Merck) at 80°C and Cd concentrations was determined using atomic absorption spectrophotometer (AAS) (Spectra AA-240, Agilent) with an air-acetylene flame and a digital readout system at a wave length of 228.8 nm as per recommendation. Calibration of the instrument, with the Cd detection limit of 0.01 mg/L, was done using standard solution prepared from 10 g/L stock solution of cadmium chloride monohydrate (98%, Merck). The amount of Cd^{2+} adsorbed per unit mass of biosorbent (q) and metal removal efficiency (%) was determined according to the following equations:

$$q= \{(C_i-C_f)/m\}v \quad \ldots\ldots\ldots\ldots (1)$$

$$\text{Cd removal efficiency (\%)}= \{(C_i-C_f)/C_i\}100 \quad \ldots\ldots\ldots (2)$$

Where, C_i and C_f = initial and final concentrations of the metal in solution (mg/L); m = dry weight of biosorbent (g); v = solution volume (L).

Biosorption kinetics study

There are several kinetic models available to understand the behaviour of biosorbent and also to examine the rate of controlling mechanism of adsorption process. In order to understand Cd biosorption by the inactive bacterial cell mass two commonly used kinetic models, pseudo-first order (Lagergren, 1898) and pseudo-second order (Ho and McKay, 1999) was investigated. The isolates was evaluated separately using the models at three different Cd concentrations mentioned above. The experimental data was fitted by linear form of the models.

Equation of the pseudo-first order model is generally expressed as:

$$dq_t/dt=k_1(q_e- q_t) \quad \ldots\ldots (3)$$

Where q_t = Cd adsorbed at any time t and q_e=Cd adsorbed per gram of cell biomass at equilibrium (mg/g) also designated as specific Cd biosorption (Oh *et al.*, 2009) respectively; k_1 = the equilibrium rate constant of pseudo-first order (1/min).

The linearized form of pseudo-first order equation is:

$$\log(q_e-q_t)=\log q_e-(k_1/2.303)t \quad \ldots\ldots (4)$$

The plots of log (q_e-q_t) versus t for different Cd concentrations give straight lines. From the plot, k_1 and q_e was determined from the slope and the intercept, respectively.

Equation of the pseudo-second order model is generally expressed as:

$$dq_t/dt= k_2(q_e - q_t)^2 \quad \text{..…… (5)}$$

Where, k_2 = the equilibrium rate constant of pseudo-second order (g/mg.min).

By integrating this equation between the limits t = 0, q_e =0 and t =t, q_e = q_e, the following linearized form is obtained.

$$t/q_t= 1/k_2q_e^2 + 1/q_et \quad \text{……. (6)}$$

By plotting t/q_tversus t, the values of q_e and k_2 can be obtained from the slope and the intercept, respectively. $k_2q_e^2$or h = the initial adsorption rate according to pseudo-second order reaction;.

Study on the effect of pH on Cd biosorption

100 mL of Cd solution of two different concentration i.e. 20 mg/L and 100 mg/L was prepared in 250 mL conical flasks. For each concentration the pH of the solutions was adjusted 4, 5, 6 and 7 in separate flasks.0.1 g of lyophilized cell biomasses of KUCd3 and KUCd7 was separately inoculated in each set of solutions. These experiments was conducted in a shaker (160 rpm) incubator at 28°C and sampling was done after 120 min of incubation. Control sets was prepared maintaining equal volume, concentration and pH of Cd solutions with their corresponding sets untreated with cell biomass. Sampling and estimation of Cd solution in cell free supernatant using AAS was done as mentioned previously. The amount of Cd^{2+} adsorbed (q) per unit mass of biosorbent was calculated according to the formula (1).

Study on Cd biosorption equilibrium isotherm

The equilibrium isotherm study is of particular importance because it represents the interactions between the biosorbents and adsorbed metal ions and also is very useful to compare the efficiency and sorption capacity of several biosorbents. These equilibrium experiments was conducted by exposing 0.1 g of lyophilized cell biomass of two selected isolates individually in 100 mL of Cd solution under a wide range of Cd concentrations (10, 20, 50, 100, 200 and 300 mg/L) at pH 7 for biosorption in a shaker (160 rpm) incubator up to the specific time needed to reach the biosorption reaction to its equilibrium. Langmuir isotherm and Freundlich isotherm are two widely used models and have been used to interpret the equilibrium sorption data in this study (Langmuir, 1918 and Freundlich, 1906).

Langmuir model is based on the assumption that a maximum adsorption corresponds to a saturated monolayer of the solute molecules on the adsorbent surface, that the energy of adsorption is constant and there is no transmigration of adsorbate in the plane of the surface. General equation of Langmure model is as follows:

$$q_e = q_{max}bC_e /1+bC_e \quad \text{…….(7)}$$

Where, C_e = metal concentration of solution at equilibrium (mg/L), q_e = specific Cd biosorptionor amount of Cd uptake per gram of cell biomass at equilibrium (mg/g), q_{max} = the maximum metal uptake (mg/g) to form a complete monolayer on the surface, and b = the Langmuir equilibrium constant (L/mg) that represents the affinity between the sorbent and sorbet. The linear form of the equation of Langmuir's isotherm model isas follows:

$$C_e/q_e= 1/q_{max}b + C_e/q_{max} \quad \text{…… (8)}$$

The q_{max} and b was determined by plotting C_e/q_e versus C_e. An efficient biosorbent was judged according to the magnitudeof the values of q_{max} and b. High b values are usually reflected in the steep initial slope of a sorption isotherm.

The main characteristics of Langmuir isotherm is expressed by separation factor, R_L, as was given in equation-

$$R_L = 1/(1+bC_i) \quad \text{…….. (9)}$$

Where, b = the affinity between sorbent and sorbate; and C_i = the initial Cd concentration.

The value of R_L>1 shows an unfavourable sorption, R_L=1 a linear sorption, 0<R_L<1 a favourable sorption and R_L =0 indicates an irreversible sorption (Jafari and Cheraghi, 2014).

Surface coverage (Ø) is number of adsorption sites occupied divided by number of adsorption sites available. The adsorption behaviour (bC_i) of the metal ions on the biomass was determined by the formula:

$$bC_i = Ø/(1-Ø) \quad \text{……. (10)}$$

From above equation, surface coverage (Ø) was calculated as:

$$Ø =bC_i/(1+ bC_i) \quad \text{…….. (11)}$$

Freundlich model has been proven well-fitted for metal adsorption by sorbent which has heterogeneous distribution of metal binding active sites on it. General form of Freundlich model is:

$$q_e= K_FC_e^{1/n} \quad \text{…….. (12)}$$

Where, k_F (mg/g) and n = the Freundlich constant which is the indicator of the biosorption capacity and Freundlich exponent indicates sorptive intensity, respectively. 1/n represents the surface heterogeneity. The linear form of equation for the Freundlich isotherm model is:

$$\log q_e = \log K_F + (1/n) \log C_e \quad \text{…….. (13)}$$

k_F and n was calculated from the intercept and slope of a plot of $\log q_e$ versus log C_e, respectively(Freundlich, 1906).

Study on the effect of initial Cd concentrations on Cd biosorption

Two sets of 100 mL of Cd solution was treated individually with 0.1 g of lyophilized cell masses of KUCd3 and KUCd7 under a wide range of Cd concentrations (10, 20, 50, 100, 200 and 300 mg/L) at 28°C and pH 7 for biosorption in a shaker (160 rpm) incubator up to the specific time needed to reach the biosorption reaction to its equilibrium. Thereafter, percentage of Cd biosorption was estimated according to the formula (3) mentioned previously.

Scanning electron microscopy (SEM) with energy dispersive X-ray analysis (EDAX)

A scanning electron microscope equipped with an energy dispersive X-ray spectrometer (SEM-EDX) was used to

observe the bacterial cell surface characteristics after Cd treatment and to detect the accumulation of Cd on cell surface, respectively. Lyophilized cell biomasses of KUCd3 and KUCd7 incubated in presence or absence of Cd(100 mg/L) was harvested through centrifugation at 5000 rpm for 10 min at 4°C. The samples for SEM was prepared following standard techniques (Chakravarty and Banerjee, 2008). Bacterial cell biomasses was washed several times with Na-phosphate buffer (0.1 M, pH 7.2) followed by fixation with 2.5% glutaraldehyde in the same buffer for 3 h at 4°C. The cells was then washed with 0.1 M phosphate buffer thrice and post-fixed with 1 ml 1% (w/v) osmium tetroxide for 8 h at room temperature. The sample was then washed twice with buffer and Cell biomasses was dehydrated through a gradient of ethanol concentration of 20-100% (v/v). 3µL of fixed cells was dried on glass pieces of 1cm^2, coated with silver and studied under scanning electron microscope coupled with EDAX system (FEI Quanta 200F with Oxford-EDS system IE 250 X Max 80).

Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR was used to identify the main chemical nature of the functional groups of these biosorbents involved in adsorption of Cd on the cell biomass. For this purpose, lyophilized cell biomasses of KUCd3 and KUCd7 was incubated in presence or absence of Cd (100 mg/L), harvested through centrifugation and 2.5 mg of each dried bacterial biomass was mixed and ground with 75 mg of KBr in an agate mortar separately. The translucent discs was prepared by pressing the ground material with the aid of 8 tonnes of pressure bench press. The tablet was immediately analysed with a Fourier transform infrared spectrophotometer (FTIR) (Perkin Ekmer, Sperrum One: L120-000A) in the range of 1000-4000 cm^{-1} with a resolution of 5 cm^{-1}. The influences of atmospheric water and CO_2 was always subtracted.

Statistical Analysis

Data was the mean of three replications and standard error (±SE) was calculated using standard formula and 'Microsoft Office Excel 2013' software.

RESULTS

Batch biosorption experiments

The effect of time on biosorption process was determined by incubating 0.1 g of lyophilized cell masses of KUCd3 and KUCd7 individually up to 120 mininthree batches of Cd solution (20mg/L, 50mg/L and 100mg/L) at pH 7 and in 28°C on shaker at 160 rpm. A sharp increase of Cd biosorption was observed in both the biosorbents up to 10 min from the initiation of biosorption reaction and then the reaction became slower gradually over time. Maximum Cd biosorption was observed at 80 min after which no significant increase in Cd biosorption was observed for both the bacterial biomass (Fig1). Therefore, 80 min was considered as time required reaching Cd biosorption at its equilibrium. It was also found that initial rate of cadmium adsorption (h)and experimentally derived values of specific Cd biosorption($q_{e,exp.}$) given in table 1, both increased with the increase of initial Cd concentration of the solution.

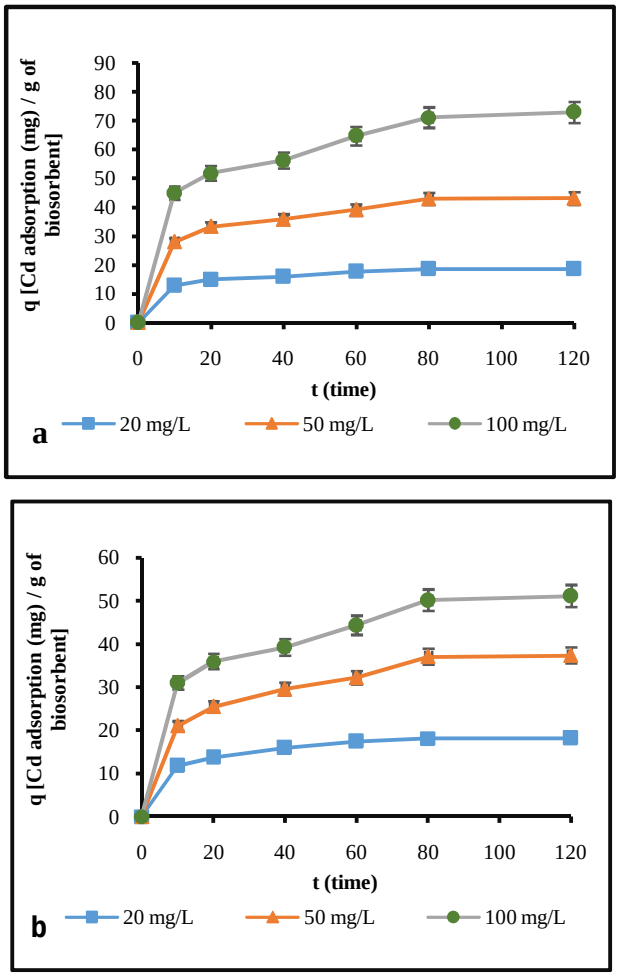

Figure 1 Kinetics of Cd biosorption from the solution with initial Cd concentration 20 mg/L, 50 mg/L and100 mg/L by the bacterial biosorbents prepared from (a) KUCd3 and (b) KUCd7. Bars represent Mean ±SE

Cd biosorption kinetics study

To evaluate whether Cd biosorptions by the baterial biosorbents KUCd3 and KUCd7 from solutions of three different Cd concentrations were following pseudo-first order kinetics model (Lagergren, 1898), experimental values of $\log(q_t\text{-}q_e)$ was calculated and plotted against time (Fig 2) to derive linear equations. Those equations was then compared with standard linearized form of equation of pseudo-first order kinetics [equation (4)] to deduce the separate values of k_1, the equilibrium rate constant and $q_{e,\ model}$, the specific Cd biosorption value according to pseudo-first order kinetics (Table1). Similarly, for evaluating these Cd biosorption reaction according to pseudo-second order kinetics model (Ho and McKay, 1999), experimental values of t/q_t was calculated and plotted against time of reactions (Fig3) to derive another set of linear equations. The linear equations for KUCd3 and KUCd7 mediated Cd biosorption was compared with standard linearized form of equation of pseudo-second order kinetics [equation (6)] to deduce the separate values of k_2, the equilibrium rate constant and $q_{e,model}$, specific Cd biosorption according to pseudo-second order kinetics for both the biosorbents (Table 1).

Then the values of $q_{e,model}$ of both types of kinetics models at different Cd concentrations was compared with their corresponding experimentally achieved $q_{e,exp}$. values, it was found that irrespective of treatment regime of Cd concentration, $q_{e,model}$ values of pseudo-second order kinetics models were very much closer to $q_{e,exp.}$ than $q_{e,model}$ values of pseudo-first order kinetics (Table 1). Again, it is clear from the Table1 that R^2, the correlation coefficient values calculated from linear equation of pseudo-second order kinetics models were much closer to 1 than derived from linear equation of pseudo-first order kinetics. Considering all these analysis it could be assumed that all the Cd biosorption reactions carried out by KUCd3 and KUCd7 at different Cd concentrations following the reaction kinetics of pseudo-second order reaction more effectively than pseudo-first order reaction. So, according to pseudo-second order reaction it can be assumed that Cd absorption by both of the biosorbents were chemisorption in nature. A gradual increase of the initial adsorption rate (h or $k_2q_e^2$) and decrease of the rate constants of pseudo-second order model (k_2) was observed with increase of

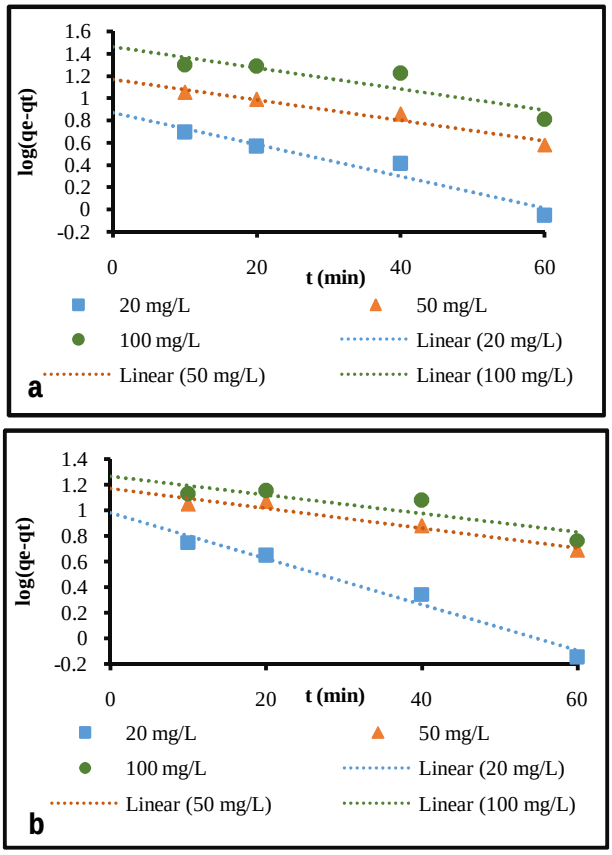

Figure 2 Kinetics of Cd biosorption by the bacterial biosorbents prepared from (a) KUCd3 and (b) KUCd7 for comparing with pseudo-first order model

initial Cd concentrations (Table 1). It suggested that the increase in initial Cd concentration led to increase the initial driving force where more Cd^{2+} took rush on the surface of the biosorbents at the same time period. But the possibility of collision of Cd^{2+} and repulsive force acted between these on the surface of the biosorbent was more at higher Cd concentrations which might reduce the rate of Cd^{2+} diffusion toward the surface of the biosorbent (Kumar and Kirthika, 2009).

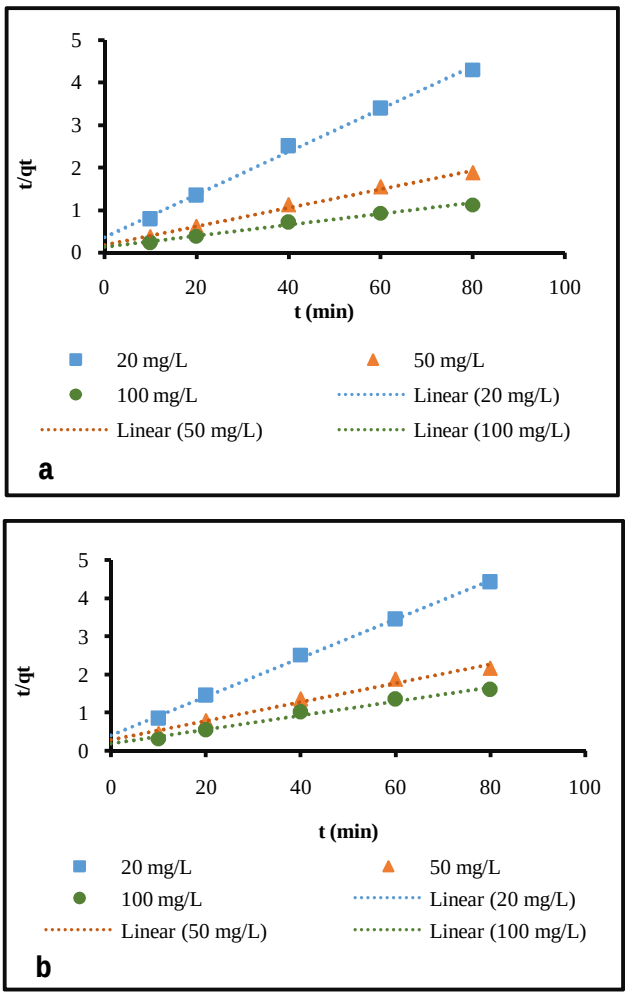

Figure 3 Kinetics of Cd biosorption by the bacterial biosorbents prepared from (a) KUCd3 and (b) KUCd7 for comparing with pseudo-second order model

$q_{e,exp}$. and $q_{e,\ model}$ = Experimentally derived value of Cd biosorption per gram of biosorbent obtained at equilibrium according to the kinetics models; k_1 and k_2 = the equilibrium rate constants of the two kinetic reaction models; $k_2q_e^2$ **or**h = the initial adsorption rate according to pseudo-second order reaction; R^2= the correlation coefficient values calculated from linear equations of the respective kinetics model.

Effect of pH on Cd biosorption reaction

When lyophilized cell masses of KUCd3 and KUCd7 was separately incubated in two batches of Cd solution (20mg/L and 100mg/L) at varying pH (pH 4 to pH 7) for 120 min, it showed that with increase of the initial pH of the solution, the Cd biosorption capacity of both the isolates increased significantly irrespective of the initial Cd concentration of the solution, although maximum Cd biosorption by these two biosorbents was observed within the range of pH 6 -7 (Fig4).

Equilibrium isotherm modelling of Cd biosorption

When 0.1 g of lyophilized cell masses of KUCd3 and KUCd7 was treated individually in Cd solution under a wide range of Cd concentrations (10, 20, 50, 100, 200 and 300 mg/L) with pH 7, Cd biosorptions in each cases reached their equilibrium after 80 min.

Table 1 Comparison of Cd biosorption values at equilibrium of two kinetics models with experimental data at three Cd concentrations by *Leucobacter* sp. KUCd3 and *Ralstonia mannitolilytica*. KUCd7

Biosorbents	Initial Cd concentration $[C_i]$ (mg/L)	$q_{e,exp.}$ (mg/g)	Pseudo-first order kinetics			Pseudo-second order kinetics			
			k_1 (1/min)	$q_{e,model}$ (mg/g)	R^2	k_2 (g/mg.min)	$q_{e,model}$ (mg/g)	$k_2q_e^2$ or h (mg/g.min)	R^2
Leucobacter sp. KUCd3	20	18.58	0.033	7.36	0.94	0.007	19.88	2.87	0.996
	50	42.9	0.021	14.68	0.964	0.002	45.87	5.59	0.992
	100	71.1	0.021	28.78	0.802	0.001	77.52	7.58	0.987
Ralstonia mannitolilytica KUCd7	20	18.11	0.041	9.41	0.974	0.006	19.80	2.40	0.998
	50	37	0.018	14.78	0.946	0.002	40.82	3.34	0.985
	100	50.1	0.017	18.42	0.796	0.002	54.35	5.08	0.983

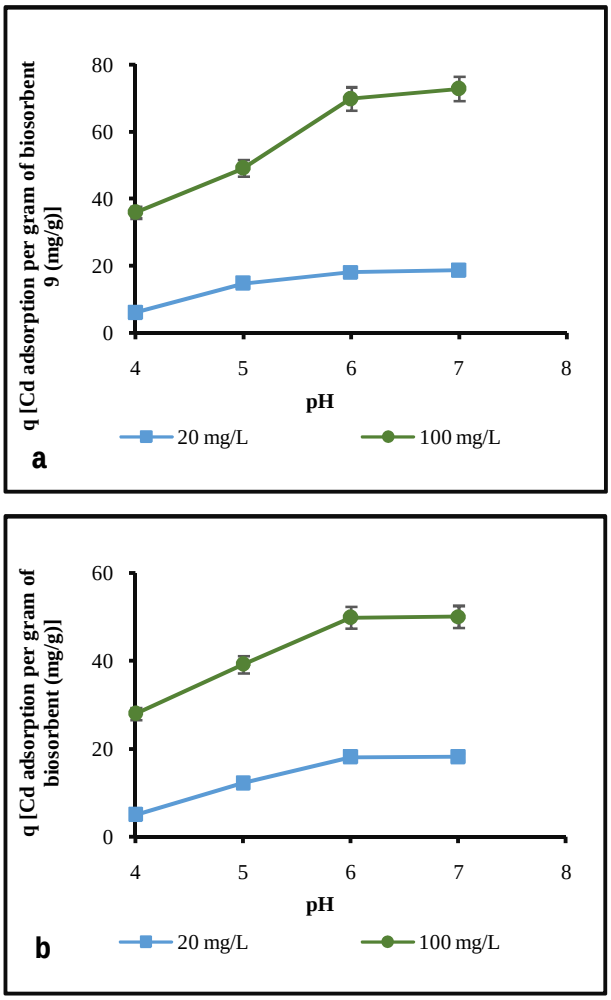

Figure 4 Effects of pH on Cd biosorption by the bacterial biosorbents prepared from (a) KUCd3 and (b) KUCd7 after 120 min. Bars represent Mean ±SE

To interpret the biosorption reaction following Langmuir model (Langmuir, 1918), final Cd concentration of the solutions at equilibrium (C_e) and Cd biosorption at equilibrium (q_e) by lyophilized cell masses was estimated. Then, experimental values of C_e/q_e was calculated and plotted against C_e(Fig 5)to derive linear equations from the plots. After comparing these linear equations for the biosorbents KUCd3 and KUCd7, individually with the standard equation of Langmuir model [equation (7)] q_{max}, the maximum Cd biosorption capacity on the surface and b**,** the Langmuir equilibrium constant representing the affinity between the sorbent and sorbet was deduced.

It was observed that both these values for KUCd3 biosorbent (q_{max} = 108.7 mg/g; b = 0.083 L/mg) were greater than those of KUCd7 biosorbent (q_{max} = 84.75 mg/gand b = 0.054 L/mg) (Table 2).

Again, to interpret these following Freundlich model (Freundlich, 1906), experimentally obtained values of $\log q_e$ was plotted against $\log C_e$ (Fig 6) and linear equations wasderived from the plots.

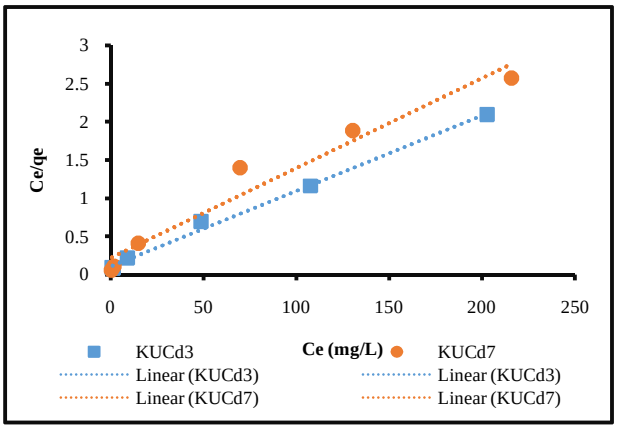

Figure 5 Cd biosorption at equilibrium by the bacterial biosorbents for comparing with Langmuir isotherm model

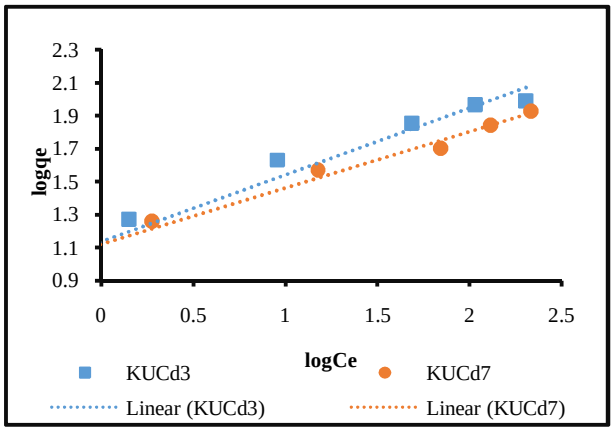

Figure 6 Cd biosorption at equilibrium by the bacterial biosorbents for comparing with Freundlich isotherm model

After comparing these equations with the standard equation of Freundlich model [equation (13)] K_F, the indicator of the biosorption capacity, and n, the Freundlich exponent was deduced. The value of K_F related to biosorption capacity according to Freundlich model was greater in case of Cd biosorption by KUCd3 [K_F = 13.61 (mg/g)(L/mg)$^{1/n}$] than that of KUCd7 [K_F = 13.17 (mg/g)(L/mg)$^{1/n}$] (Table 2). The value of

Freundlich exponent, n should be within 1 and 10. The value of n was greater in case of KUCd7 (n = 2.92) than that of KUCd3 (n = 2.46). A greater value exhibited a stronger interaction between metal ions and its ligands on the surface of the biosorbents (Li *et al.*, 2010).

Surface coverage (Ø) value of biosorbentsKUCd3 (Ø = 0.96) and KUCd7 (Ø = 0.94) biomasses calculated using equation (10) were very high which indicates the substantial amount of surface availability on inactive biomass of both bacteria for Cd^{2+}biosorption (Table 2).

Table 2 Langmuir and Freundlich equilibrium isotherm parameters for Cd biosorption by *Leucobacter* sp. KUCd3and *Ralstonia mannitolilytica* KUCd7

Biosorbents	Langmuir model					Freundlich model		
	q_{max} (mg/g)	b (L/mg)	R^2	R_{L300}	Ø	K_F (mg/g)(L/mg)$^{1/n}$	n	R^2
Leucobacter sp. KUCd3	108.7	0.083	0.996	0.04	0.96	13.61	2.46	0.949
Ralstoniamannitolilytica KUCd7	84.75	0.054	0.958	0.06	0.94	13.17	2.92	0.987

q_{max} = the maximum metal uptake to form a complete monolayer on the surface; b = the Langmuir equilibrium constant; R^2= the correlation coefficient values calculated from linear equations of the respective isotherm models; R_{L300} = separation factor at Cd concentration 300 mg/L; Ø = surface coverage; K_F = Freundlich constant; n = Freundlich exponent.

As can be seen in table 2 both of the models described the experimental data with a reasonable precision for both the biosorbents. But correlation coefficient value, R^2 calculated through Langmuir isotherm model (R^2 = 0.994) was much closer to 1 than R^2 value calculated following Freundlich isotherm model (R^2 = 0.949) in case of KUCd3. So it can be inferred that *Leucobacter* sp. KUCd3 followed 'Langmuir isotherm' model which described the biosorption by complete monolayer formation. Reverse condition was true for Cd biosorption by KUCd7 as R^2 value calculated following Freundlich isotherm model (R^2 = 0.987) was much closer to 1 than R^2 value calculated through Langmuir isotherm model (R^2 = 0.958) which implied that biosorption of Cd by *Ralstonia mannitolilytica* KUCd7 could be described better through 'Freundlich model' of heterogeneous absorption principle.

The values of separation factor, R_L calculated using equation (8) decreased with increasing initial Cd concentration (Fig 7). The obtained values for this parameter were in the range of 0.03 to 0.55 for biosorption by KUCd3 and 0.06 to 0.65 for biosorption by KUCd7. As the values of R_L ranging between 0 and 1 indicated favourable absorption, both KUCd3 and KUCd7 mediated sorption were favourable biosorption process.

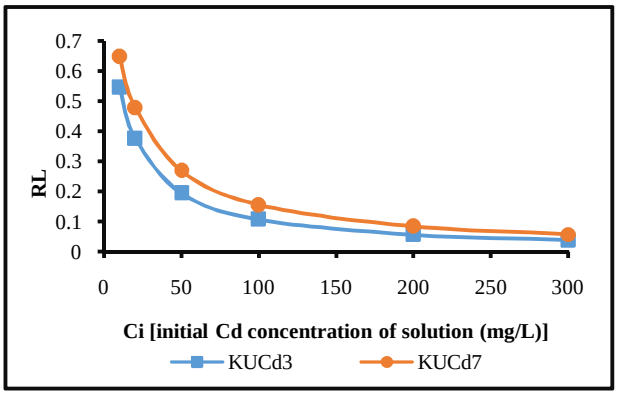

Figure 7 Separation factors (R_L) for Cd bisorption by the bacterial biosorbents with the increasing Cd concentrations

Effect of initial Cd concentrations on Cd biosorption capacity

At equilibrium, Cd removal efficiency of biomasses of KUCd3 and KUCd7 declined from 91.8% to 32.37% and from 95.8% to 28.03%, respectively, with the increase of initial Cd concentrations from 10mg/L to 300mg/L (Fig 8). This might be due to the saturation of limited biosorption sites on the biomasses. As these sites on the surface of bacterial biomasses were getting saturated with a certain amount of Cd^{2+}, further increase in the initial Cd concentrations caused increasing concentration of unabsorbed Cd^{2+} which retained in the solution. As a result, percentage of Cd removal decreased, comparatively.

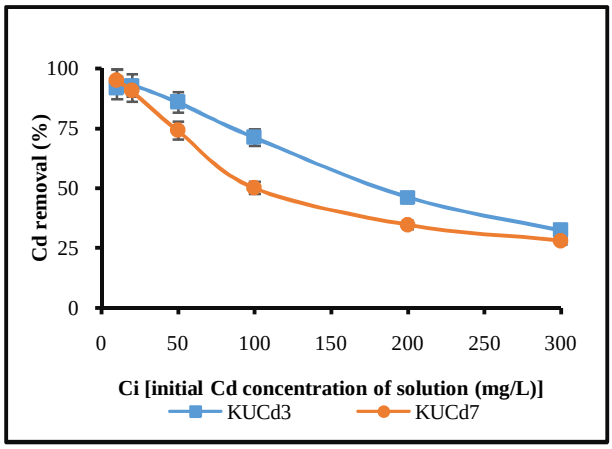

Figure 8 Effect of initial Cd concentrations on biosorption by the bacterial biosorbents. Bars represent Mean ±SE

SEM-EDAX analysis

Modification of cell surface and precipitation of Cd on biomasses was revealed in SEM- EDAX study. Rough cell surface and membrane indentations were clearly evident in the micrographs of Cd treated (100 mg/L) cell biomasses of KUCd3 (Fig. 9a) and KUCd7 (Fig. 10a). EDAX spectrum analysis in Fig. 9cand 10cexhibited distinct Cd-L peaks in the Cd treated samples that confirmed the presence of surface-bound Cd. These peaks were absent in Cd untreated control set. However, some unrelated peaks like C-K, N-K, O-K, Na-K, Mg-K, P-K, K-K Ca-K and Au-K were also found which were contributed by the cell surface components, elements presented in buffer solutions, metallic coat.

Fourier transform infrared (FTIR) spectroscopy to detect functional groups involved in Cd biosorption

FTIR spectra of Cd untreated and treated biomasses of *Leucobacter* sp. KUCd3 and *Ralstoniamannitolilytica* KUCd7, in the range of 1000-4000 cm^{-1}, was analyzed to understand the surface binding mechanism of Cd^{2+} by identifying the chemical functional groups involved inof Cd^{2+} binding on the cellsurface. A number of peaks in the spectra was found to be shifted in Cd treated cells of KUCd3 (Fig 11) and KUCd7 (Fig 12) when compared with corresponding Cd untreated cells. Probable functional groups on the cell surface of KUCd3 (Table 3) and KUCd7 (Table 4)

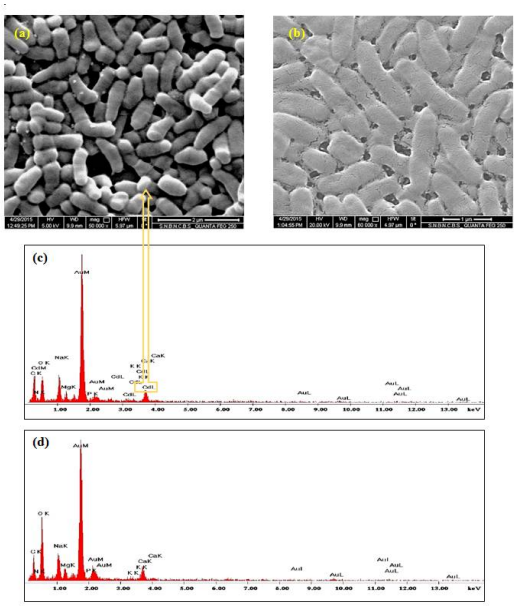

Figure 9 Scanning electron micrographs of the cell biomass of KUCd3 at (a) Cd treated (b) Cd untreated conditions. EDAX analysis revealed the presence of Cd on the surface of Cd treated cell biomasses by its characteristic Cd-Lpeak formation (c), which was absent in the EDAX spectra of Cd untreated cell biomass (d)

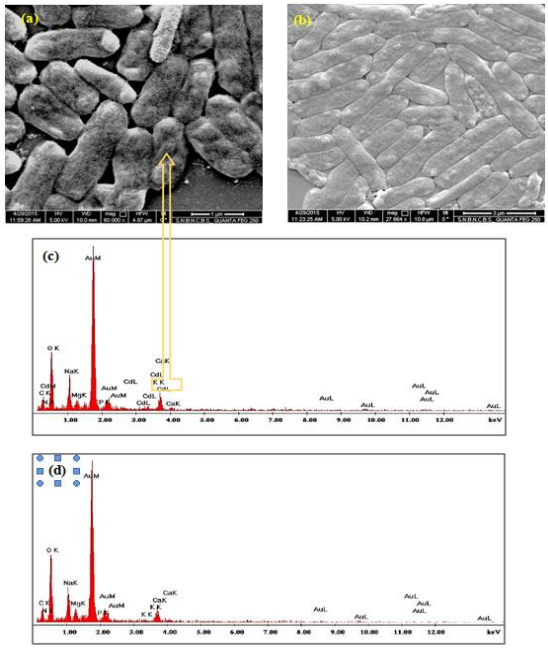

Figure 10 Scanning electron micrographs of the cell biomass of KUCd7 at (a) Cd treated (b) Cd untreated conditions. EDAX analysis revealed the presence of Cd on the surface of Cd treated cell biomass by its characteristic CdL peak formation (c), which was absent in the EDAX spectra of Cd untreated cell biomass (d)

Involved in Cd^{2+} binding was anticipated according to their corresponding peaks which was recognized as characteristic features of certain functional groups within specific spectrum ranges. FTIR analysis showed the formation of varying spectra following adsorption of the Cd^{2+} on both the bacterial biomass validated the contribution of functional groups like amine, amide (amino acids, proteins, glycoproteins etc.), hydroxyls, alkyl, carboxyl (fatty acids, lipopolysaccharides, etc.), and phosphate in Cd binding.

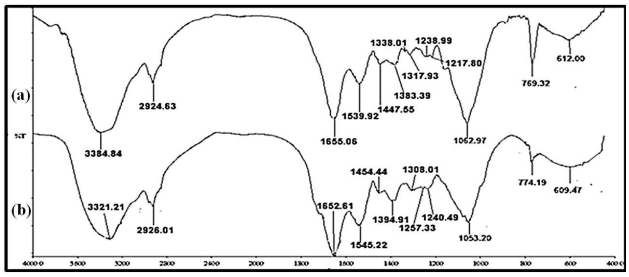

Figure 11 Spectra obtained by FTIR analysis of (a) Cd untreated and (b) Cd treated cell mass of KUCd3

Table 3 Characterization of FTIR spectra absorbance peaks of Cd untreated and treated biomass of *Leucobacter* sp. KUCd3

Frequency (cm^{-1})		Functional Groups
Cd untreated	Cd treated	
3384.84	3321.21	Bonded-OH stretching vibration, stretching vibration of-NH groups
2924.63	2926.01	Alkyl chains-CH stretching vibration
1655.06	1652.61	Amide I band, C=O stretching vibration
1539.92	1545.22	Amide II band combining both the-NH bending and the-CN stretching [-C(= O)-NH-], C= O stretching
1447.55	1454.44	$-CH_2$ scissoring or-CH_3 anti-symmetrical bending vibration
1383.39	1394.91	$-CH_2$ and $-CH_3$ deformation [(C-H) bending vibration] of alkane group, C(= O)-O− symmetric stretching vibration in carboxylate is overlapped in the wavenumbers
1317.93	1308.01	Amide III band vibration, C-N stretch of aromatic amines
1238.99	1257.33	C-O stretch of carboxylic acids, alcohol, esters (C-O-C), C-N stretch of aliphatic amines, P=O asymmetric stretching vibrations of PO^{2-} phosphodiester
1217.80	1240.49	
1062.97	1053.20	C-O stretching of alcohol (C-OH), aliphatic amine (C-N stretch), C-C stretching, P-O-C links of the organic phosphated groups and P-O vibration of (C-PO_3^{2-}) moiety
< 1000		Phosphates and sulfur groups (‘‘Fingerprint” zone)

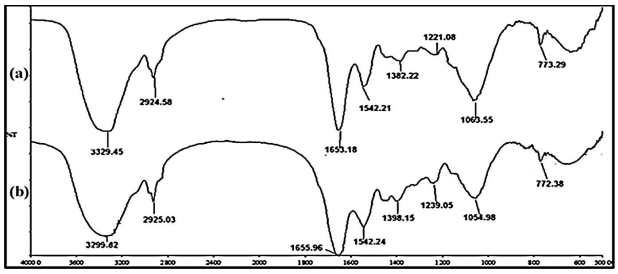

Figure 12 Spectra obtained by FTIR analysis of (a) Cd untreated and (b) Cd treated cell mass of KUCd7

Table 4 Characterization of FTIR spectra absorbance peaks of Cd untreated and treatedbiomass of *Ralstoniamannitolilytica* KUCd7

Frequency (cm^{-1})		Functional Groups
Cd untreated	Cd treated	
3329.45	3299.82	Bonded –OH stretching vibration, stretching vibrationof –NH groups
2924.58	2925.03	Alkyl chains –CH stretching vibration
1653.18	1655.96	Amide I band, C=O stretching vibration
1542.21	1542.24	Amide II band combining both the –NH bending and the –CN stretching [–C(= O)–NH–], C= O stretching
1382.22	1398.15	CH_2 and CH_3 deformation [(C-H) bending vibration] of alkane group, C(= O)–O− symmetric stretching vibration in carboxylate is overlapped in the wavenumbers
1221.08	1239.05	C-O stretch of carboxylic acids, alcohol, esters (C–O–C), C–N stretch of aliphatic amines, P=O asymmetric stretching vibrations of PO^{2-} phosphodiester
1063.55	1054.98	C-O stretching of alcohol (C-OH), aliphatic amine (C-N stretch), C-C stretching, P–O–C links of the organic phosphated groups and P–O vibration of (C–PO_3^{2-}) moiety
< 1000		Phosphates and sulfur groups (‘‘Fingerprint” zone)

DISCUSSION

Bacterial cell surface adsorption is considered to be one of the basic mechanisms for the interaction with Cd^{2+}. The Cd^{2+} bioadsorption capacities mainly depend on morphology and chemical composition of the bacterial cell wall. The process can be achieved by extracellular deposition mediated by functional groups (i.e.,-OH,-NH, and-PO_4^{3-}) present in the cell wall components peptidoglycan, glycoprotein, teichoic acid, and teichuronic acid; the last two compounds are characteristic of Gram-positive bacteria (Hoyle and Beveridge, 1983). The complex forms of Cd^{2+} with surface ligands are generally not readily transported into the cell because of their structure and complexity. In this study, the Cd biosorption capacity of two biosorbents prepared from lyophilized biomassesof Gram positive bacteria, *Leucobcter* sp. KUCd3 and Gram negative bacteria, *Ralstonia mannitolilytica* KUCd7was evaluated. Different parameters such as tendency toward the metal ions, the maximum sorption capacity, as well as the rate of the metal sorption on the surface of the biosorbents are the major criteria for comparing and choosing the best type of biosorbents for the purpose. Equilibrium isotherms and kinetic studies are commonly used for the calculation of these parameters. However, many factors including type of the metal ion, cell wall components of microorganisms, pH of the solution, ion strength, contact time, and the metal ion concentrations can influence efficiency and quality of the process (Joo *et al.*, 2010). A series of batch experiments for biosorption of Cd by the two biosorbents individually at three differentCd concentrations (20mg/L, 50mg/L and 100 mg/L) showed that about 70% of initial metal was rapidly adsorbed within the first 10 min of contact followed by slow gradual increase of biosorption to reach the equilibrium after about 80 min of contact and no Cd removal occurred after this time (Fig 1). Initial short time period of sorption process was important for a high rate of metal sorption (Oves *et al.*, 2013). Similar results had also been determined for biosorption of Ni and Pb (Gabr *et al.*, 2008). Availability of maximum vacant metal binding sites in the biosorbent might be the major driving force for rapid biosorption of the metal at the initial 10 min of contact followed by slower sorption rate whichwas attributed to the depletion of vacant Cd^{2+} binding sites, increase of the repulsive forces between Cd^{2+} on the surface of the biosorbent and slow interior penetration (Ertugay and Bayhan, 2010; Akar *et al.*, 2012).With the increase of initial metal concentration from 20 to 100 mg/L, as the initial rate of biosorption increased, the specific Cd biosorption value also increased. But the equilibrium time remained unaffected. While *Bacillus* sp. was reported to take120 min to reach equilibrium state during bisorption of Cd, Cr, Mn and Pb from aqueous solutions (García *et al.*, 2016), lyophilized biomass of *Pseudomonas stutzeri* took only 30 min to reach the biosorption of Pb, Cd and Cu to the equilibrium (Oh *et al.*, 2009). Thought rate of Cd biosorption increased, increase of initial Cd concentration of the solution from 10 to 300 mg/L decreased Cd removal percentage from 91.8% to 32.37% and 94.8% to 28.03% for KUCd3 and KUCd7 biosorbents, respectively (Fig 8). At lower concentrations, maximumCd^{2+} present in the solution could interact with free Cd binding sites on the biosorbent and thus the biosorption percentage was higher. But with the increase of initial Cd^{2+} concentration those binding sites became more and more saturated with Cd^{2+}.At high Cd^{2+} concentration, lower percentage of Cd removal was observed might be attributed to a lack of sufficient free sites for Cd^{2+}biosorption.Similar kinds of results have been reported alsoby other researchers (Oves *et al.*, 2013; Huang *et al.*, 2013; Oh *et al.*, 2009). Biosorption kinetics can provide valuable information on the reaction pathways and the mechanism of the biosorption process (Aryal and Liakopoulou-Kyriakides, 2015). Several kinetic models have been applied to correlate the kinetics data of different metal ions in various bacterial cells. Comparison of correlation coefficients values (R^2) calculated from linear equations of the Cd biosorption kinetic data of KUCd3 and KUCd7 under three different Cd concentrations revealed that the pseudo-second order model was best fitted to describe the experimental biosorption data than the pseudo-first order model for both the biosorbents (Table 1) which also led to the assumption that the chemisorptions (e.g. ion-exchange or complex formation) type of reactions might be therate-controlling stepin these biosorption process (Jafari and Cheraghi, 2014). Similar kinds of Cd biosorption experimental data following pseudo-second-order kinetic model have been reported for *Pseudomonas aeruginosa* PU21 (Chang *et al.*, 1997), *Streptomyces rimosus* (Selatnia *et al.*, 2004), *Pantoea* sp. (Ozdemir *et al.*, 2004), *Pseudomonas veronii* 2E (Vullo *et al.*, 2008), *Rhodobacter sphaeroides* (Bai *et al.*, 2008), *Geobacillus thermoleovorans* sub.sp. *stromboliensis*, *Geobacillus toebii* sub. sp. *decanicus* (Ozdemir *et al.*, 2009), *Escherichia coli* HD701 (Morsy, 2011), *Brevundimonas* sp. ZF12 (Masoudzadeh *et al.*, 2011), *Acidiphilium symbioticum* H8 (Chakravarty and Banerjee, 2012), *Bacillus cereus* RC-1 (Huang *et al.*, 2013), *Corynebacterium glutamicum* (Mao *et al.*, 2013). However, in some instances such as metal biosorption by *Pseudomonas stutzeri* (Hassan *et al.*, 2009) pseudo-first-order equation also used to explain experimental kinetic data and assumes that metal ions bind only to one active site of bacterial biomass surface.

The pH of the solution highly influences metal biosorption since it determines surface charge of the adsorbent, solubility of the metal ions and degree of ionization and chemical speciation of adsorbate (García *et al.*, 2016). At low pH values, negatively charged metal binding ligands on the biosorbent surface are blocked or closely associated with H^+ or H_3O^+ ions present in the medium and restricted the approach of metal cations competing for the same binding sites as a result of the repulsive force (Oh *et al.*, 2009). As the pH increased, more ligands such as carboxyl, phosphate, imidazole and amino groups would be exposed and carried negative charges with a subsequent attraction of positively charge Cd^{2+} and biosorption onto the cell surface. Increase in solution pH permits a significant increase in the sorption ofCd^{2+}. Further increase in pH did not favour greater sorption due to low solubility of Cdat alkaline pH. Moreover, beyond pH 7.0, Cd exists as $[Cd(OH)_3]$- or $[Cd(OH)_4]^{-2}$ form (Chakravarty and Banerjee, 2012) which causes repulsion with the negatively charged binding sites of the adsorbent. In this study, Cd biosorptions increased with the gradual increase of pH from 4 to 6 and maximized within the pH range pH 6 -7, irrespective of the initial Cd concentration of the medium (20mg/L and 100 mg/L) (Fig 4). Similar findings were also observed in *P. aeruginosa* PU21 (Chang *et al.*, 1997) and *P. pudita* (Pardo *et al.*, 2003) where maximum Cd biosorption occurred at pH 6 and in *Bacillus cereus* M116 pH 6.5 favoured maximum biosorption (Ganguly *et al.*, 2011). Biosorption isotherm is the relationship betweenquantities of metal ions per unit of bacterial biomassand the concentration of these metal ions in thesolution (Wang and Chen, 2009). The isotherm parameters give informationabout pattern of distribution of metal binding sites, their affinity towards metals which determines the capacity ofmetal biosorption process.

Among several isotherm models, Langmuir and Freundlichmodels have been widelyused for heavy metal sorption with bacteria, sincethey are simple and explain the experimental equilibriumdata efficiently.

The theoretical basis of the Langmuir model relies that there are a finite number of binding sites on the adsorbent surface with the same affinity for adsorption of a single molecular layer and there is no interaction between adsorbed molecules, whereas Freundlich model assumes that the adsorption energy of a metal binding to a site on an adsorbent depends on whether the adjacent sites are already occupied or not. Comparison of correlation coefficients value (R^2) derived from Langmuir and Freundlich models for KUCd3 and KUCd7revealed that Cd biosorption by KUCd3 biomass was better described by the Langmuir model implying a monolayer sorption on a homogeneous surface, whereas the Cd biosorption by KUCd7 biomass could be demonstrated better through Freundlich model representingheterogeneous biosorption process (Table 2). Bacterial biosorbents followed Langmuir isotherm model during biosorption of Cd include *Pseudomonas aeruginosa* PU21 (Chang *et al.*, 1997), *Ochrobacterium anthrobi* (Ozdemir *et al.*, 2003), *Aeromonas caviae* (Loukidou *et al.*, 2004), *Streptomyces rimosus* (Selatnia *et al.*, 2004), *Pantoea* sp. (Ozdemir *et al.*, 2004), *Paenibacillus jamilae* (Perez *et al.*, 2008), *Pseudomonas veronii* 2E (Vullo *et al.*, 2008), *Escherichia coli* HD701 (Morsy, 2011), *Brevundimonas* sp. ZF12 (Masoudzadeh *et al.*, 2011), *Bacillus cereus* RC-1 (Huang *et al.*, 2013), *Corynebacterium glutamicum* (Mao *et al.*, 2013), *Anoxybacillus amylolyticus*, *Geobacillus thermantarcticus*(Ozdemir *et al.*, 2013). On the other hand, bacterial biosorbentssuch as *Staphylococcus xylosus* (Ziagova *et al.*, 2007), *Bacillus* sp., *Actinomyces* sp., *Streptomyces* sp. (Karakagh *et al.*, 2012) and *Pseudomonas aeruginosa* PAO1

Table 5 The comparison of Langmuir and Freundlich models' parameters for Cd biosorption by several bacterial biosorbents

Biosorbents	Langmuir parameters		Freundlich parameters		References
	q_{max} (mg/g)	b (L/mg)	K_F (mg/g) $(L/mg)^{1/n}$	n	
Leucobacter sp. KUCd3	108.7	0.083	13.61	2.46	This study
Ralstonia mannitolilytica KUCd7	84.75	0.054	13.17	2.92	This study
Pseudomonas aeruginosa CA207Ni	109.37	0.009	65.16	0.45	
Burkholderia cepacia AL96Co	71.74	0.003	1.52	0.59	Oyetibo *et al.* (2014)
Corynebacterium kutscheri FL108Hg	103.55	0.003	1.14	0.68	
Rhodococcus sp. AL03Ni	62.07	0.009	21.09	0.43	
Anoxybacillus amylolyticus	18.72	0.29	5.97	4.09	Ozdemir *et al.*(2013)
Geobacillus thermantarcticus	33.78	0.3	7.91	3.00	
Pseudomonas aeruginosa PAO1	158.25	0.027	14.60	2.20	Konig-Péter *et al.* (2013)
Pseudomonas sp. LKS06	27.69	0.1711	11.04	5.61	Huang and Liu (2013)
Bacillus cereus RC-1	31.95	0.2825	6.7771	2.1291	Huang *et al.* (2013)
Bacillus thuringiensis OSM29	59.17	0.031	2.576	1.36	Oves *et al.* (2013)
Pseudomonas stutzeri	47.86	0.055	9.29	3.84	Oh *et al*. (2009)
Geobacillus thermoleovorans sub.sp. *stromboliensis*	38.8	0.180	7.3	2.7	Ozdemir *et al.* (2009)
Geobacillus toebii sub.sp. *decanicus*	29.2	0.2	6.7	3.2	
Staphylococcus xylosus	250	8	7.00	0.547	Ziagova *et al.* (2007)
Enterobacter sp. J1	46.2	0.004	0.883	1.68	Lu *et al.* (2006)
Pantoea sp.	58.1	0.057	10.34	0.34	Ozdemir *et al*. (2004)

q_{max}= maximum Cd biosorption capacities; b = the Langmuir equilibrium constant;
K_F = Freundlich constant; n = Freundlich exponent

(Konig-Péter *et al.*, 2013) followed Freundlich isotherm model during biosorption of Cd. Both Langmuir and Freundlich models were suitable for biosorption of Cd in *Enterobacter* sp. J1 (Lu *et al.*, 2006), *Bacillus thuringiensis* OSM29 (Oves *et al.*, 2013), and *Pseudomonas* sp. LKS06 (Huang and Liu, 2013). Values of separation factor laid between 0 and 1 for both the biosorbents indicated sorption processes were favourable (Fig 7). A list of several bacterial biosorbents studied previously for Cd biosorption from solutions, is presented in Table 5 in order to compare their equilibrium isotherm parameters. According to the Langmuir model, KUCd3 and KUCd7 belong in fourthand sixth positions among the biosorbentsin the table respectively in terms of maximum Cd biosorption ability (q_{max}). Comparison of the data in the table also suggests that the value of Langmuir constant, b which indicates the affinity of Cd toward biosorbent is in moderate range for KUCd3 and KUCd7. The Freundlich exponent, n should give the value between 1 and 10. A bigger value shows a stronger interaction between metal ions and ligands on the surface of the biosorbents (Li *et al.*, 2010). KUCd3 and KUCd7 showed considerable strength in the interaction with the metal during biosorption. Based on the Freundlich model, biosorption capacity, K_F, KUCd3 and KUCd7 was given the fourth and fifth places, respectively among the other biosorbents in the table. So, the lyophilized biomass of KUC3 and KUCd7 can be considered as potent biosorbents for Cd removal from aqueous solutions due to their high efficiency especially at low Cd concentrations.

The first mechanism of heavy metal removal by bacteria is the binding of cationic heavy metals on cell surface (Clark *et al.*, 1987). This bounded metal retain for long time on cell surface due to the structural complexity. SEM-EDAX analysis was essential for further validation of cell surface interaction with Cd as it provided excellent photographic evidence of cell surface modification along with electron dense dark patches which might be the effect of extracellular adsorption, such as surface complexation which could change the surface architecture and increased cell surface roughness. Presence of Cd detected through EDAX analysis, indicated strong cell surface binding which, if adhered loosely, would have washed out during the washing treatment with buffers during sample preparation. These results were in good agreement with Chakravarty and Banerjee (2008) and Huang *et al.* (2014) as they also found similar kind of results.

Microbial metal binding process involves complex chemical, physical, biological interactions, and the presence of a multitude of functional groups on the cell wall facilitates this kind of interaction. Reaction between a cationic metal ion and an anionic moiety of the surface polymer plays important role in metal binding process (McLean *et al.*, 1996). In Gram positive bacterial cell wall, carboxylate groups of peptidoglycan and phosphate and carboxylate residues of teichoic acids and teichuronic acids were major binding sites of the metals (Beveridge and Murray, 1980). In Gramnegative bacteria such as *E. coli*, phosphate residues in the lipo-polysaccharidesalongwith thinner peptidoglycan layer was primarily responsible for metal binding character (Hoyle and Beveridge, 1984). So, carboxyls and phosphoryls are the most effective metal binding ligands. Further, phosphate-containing moieties can act as precipitation nucleation sites that facilitate continuous formation of metal crystal aggregates and thus increase metal loading capacity (Sannasi *et al.*, 2009). Acetyl groups have also been shown to influence the metal-binding specificity of extracellular polysaccharides (Corzo *et al.*, 1994). The functional groups responsible for the Cd biosorption process can be identified through FTIR spectra analysis in the range of 400-4000 cm^{-1}. The region 3700-3300 cm^{-1} is characteristic for O-H and N-H stretching vibrations (Guo and Zhang, 2004), the region from 3000 to 2700 cm^{-1} is dominated by the C-H stretching vibrations of CH_3, CH_2, CH and CHO functional groups (Stuart, 1997; Dumas and Miller, 2003), the region between 1800 and 1500 cm^{-1} shows characteristic bands for proteins, wherein 1700 to 1600 cm^{-1} is specific for amide-I bands (Dumas and Miller, 2003),which is mainly due to C = O stretching vibrations of peptide bond (Backmann *et al.*, 1996). On the other hand the region from 1600 to 1500 cm^{-1} is specific for amide-II bands, which is due to N-H bending vibrations (Fischer *et al.*, 2006). The bands in the 1500-1200 cm^{-1} region arise mainly from the C-H bending vibrations of CH_3, CH_2 and CH functional groups (Wolkers *et al.*, 2004; Yee *et al.*, 2004). Information on phosphodiester functional groups can be obtained in the region between 1250 and 1200 cm^{-1} which corresponds to -P=O asymmetric stretching frequencies (Dumas and Miller, 2003; Yee *et al.*, 2004). The region from 1200 to 900 cm^{-1} is mainly dominated by a sequence of bands due to C-O, C-C, C-O-C and C-O-P stretching vibrations of polysaccharides (Wolkers *et al.*, 2004). Comparative study between the FTIR spectrum data of Cd treated biomass of KUCd3 and KUCd7 to their corresponding untreated (control) cells revealed the shifting of the major characteristic peaks belonging to the amine ($R\text{-}NH_2$) and amide [-C(=O)-NH-] groups which found in amino acids, proteins, glycoproteins; carboxylic acid (C-O), ester (C-O-C), alkyl ($-CH_2$ and $-CH_3$), hydroxyl (OH) groups present in fatty acids, lipopolysaccharides and phosphate-containing metabolites, probably the sugar phosphate esters from the cell wall (Tables 3 and 4). The transmittance of the peaks in the Cd loaded biomass was substantially lower than those in the untreated sample of the bacterial biomass. This indicates that bond stretching occurs to a lesser degree due to the presence of Cd, and following peak transmittance was reduced. Therefore it can be concluded that varying spectra of above mentioned functional groups following adsorption of Cd by the biomasses of KUCd3 and KUCd7 validated their contribution in biosorption. These results are in good agreement with those obtained by other authors (Nivens *et al.*, 1993; Jiang *et al.*, 2004; Parikh and Chorover, 2005; D'Souza, 2008; Ueshima *et al.*, 2008; Sannasi *et al.*, 2009; Oh *et al.*, 2009; Oves *et al.*, 2013; Suriya *et al.*, 2013) who also considered that these functional groups were responsible for biosorption of Cd. The results of FTIR analysis indicating chemical modification on bacterial cell surface due to Cd treatment can be related to the SEM mediated observation of cell surface modification.In support of FTIR data, Chakravarty and Banerjee (2012) further established the interaction mechanism of those anionic functional groups on the biomass of *Acidiphilium symbioticum* H8with Cd^{2+} bychemical group modification study.

CONCLUSION

From the experimental observation demonstrated above it was evident thatkinetics of biosorption study showed lyophilized

cell biomass of KUCd3 and KUCd7 followed pseudo-second order reaction kinetics, which implies absorption in both the cases are chemisorption in nature. These biosorption reactions are time dependent process which reached to their equilibrium after 80 min of incubation. Increasing initial Cd concentration highly favoured initial Cd biosorption process and Cd accumulation by unit cell biomasses. Cd removal efficiency of these biosorbent was very high at low Cd concentration. Neutral pH favoured the highest biosoption of Cd by these two biosorbants.While *Leucobacter* sp. KUCd3 followed 'Langmuir isotherm' model which described the biosorption by complete monolayer formation, biosorption of Cd by *Ralstonia mannitolilytica* KUCd7 could be described better through 'Freundlich model' of heterogeneous absorption principle. To support the occurrence of Cd biosorption reaction, SEM-EDX analysis provided strong micrographic evidence of cell surface binding of Cd. Formation of varying spectra in FTIR analysis validated the contribution of anionicfunctional groups of cell envelop macromolecules in complex formation and binding with Cd^{2+}.The results demonstrate that lyophilized cells of *R. mannitolilytica* KUCd7 and *Leucobacter* sp. KUCd3 could be used as promising biosorbents for the removal of Cd from aqueous environment.

Acknowledgments

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