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Equilibrium studies of cadmium biosorption by presumed non-viable bacterial strains isolated from polluted sites

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ABSTRACT

Presumed non-viable high resistant *Pseudomonas aeruginosa* CA207Ni, *Burkholderia cepacia* AL96Co, *Corynebacterium kutscheri* FL108Hg, and *Rhodococcus* sp AL03Ni were studied for Cd^{2+} adsorption potentials. Moderate temperature, acidic pH, and high ionic strength were required for bacterial-sorption of cadmium, attaining isothermic equilibrium within 20 min. Experimental cadmium-biosorption data fitted well into biosorption isotherms. The adsorption capacities of the bacterial cell masses spanned 0.003–0.009 l mg^{-1} (Langmuir model) and 0.43–0.68 (Freundlich model), while binding capacity ranged from 1.14 to 56.16 mg gdw^{-1}, with maximum achievable cadmium uptake of 62.07–109.37 mg gdw^{-1}. The bacteria selectively removed the metal at low concentration (100.0 mg l^{-1}) with an efficiency ranging from 50.0% to 80.0%, while approximately 80.0–92.0% removal efficiency was obtained at higher ionic concentrations (450.0 mg l^{-1}). About 92.66% of the adsorbed metal was recovered from strain CA207Ni upon desorption, and approximately 91.7% of Cd^{2+} in solution was re-adsorbed onto the biomasses. In this work, effective feasible biosorption of Cd^{2+} in simulated wastewater system at harsh physico-chemistry, using non-viable resistant bacterial strains was demonstrated. The results indicate that the bacterial strains are sustainable tools for the detoxification of cadmium ions in industrial effluents via wastewater treatment, and cadmium demobilisation in contaminated ecosystem.

1. Introduction

Cadmium (Cd) is a soft, malleable, ductile, toxic, bluish-white bivalent metal. It is chemically similar in many respects to zinc and mercury but forms more complex compounds. It has various industrial applications and thus often constitute nuisance to the environment upon release of effluents generated during such industrial processes (Bertin and Averberk, 2006). Cd, as an important toxic environmental heavy metal, was rated 7th in the priority list for hazardous substances in 2007 by Comprehensive Environmental Response Compensation and Liability Act (CERCLA). Cigarette smoke as well as food, water and air contaminations are also important sources of human intoxication with Cd. It has high mobility and toxicity with no known metabolic and physiological merit to life (Todorova et al., 2007). The only exception to this is enzyme carbonic anhydrase (found in marine diatoms) that has Cd (like zinc) as the reactive centre. Upon exposure to living systems, Cd induces apoptosis in a wide variety of cell lines and has toxic effects in several tissues (Lasfer et al., 2008). Cd induces single strand breaks in DNA, chromosomal aberrations, sister chromatid exchanges and DNA-protein binding failures in several types of mammalian cells (Bertin and Averberk, 2006). Nevertheless, Cd has been shown to induce lipid peroxidation and membrane leakiness.

Detoxification of Cd-contaminated systems is a necessity in order to provide a safe, healthy environment. Conventional methods used to remove dissolved metal ions from wastewaters include chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, and electrochemical treatment. However, these high technological processes have significant disadvantages including incomplete metal ion removal, the requirement for expensive equipment and monitoring systems, high use of reagents, and pre-treated or other waste products that require disposal (Hussein et al., 2004). The adaptation to toxic metal-rich environments is resulting in microorganisms which show

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activities for biosorption, bio-precipitation, extracellular sequestration, transport mechanisms, and/or chelation. Such resistance mechanisms are the basis for the use of microorganisms in bioremediation approaches. In this case, the process of bioremediation is based on removal of Cd ions from the contaminated environments or otherwise from the sources of environmental pollution such as industrial effluents.

Biosorption is a passive uptake process, which is fast, mostly reversible and independent of cell viability (Voleski, 2007). It is a physico-chemical rather than a biological process based on mechanisms involving a large variety of binding sites of extracellular polymeric substances and bacterial cell surfaces (Guibaud et al., 2005; Voleski, 2007). It has been shown to be effective in removing metals from the environment, even at very low concentrations (Gavrilescu, 2004). Most studies of biosorption for metal removal have involved the use of either laboratory-grown microorganism or biomass generated by industries or wastewater treatment unit (Voleski, 2007). It is well recognised that microorganisms have a high affinity for metals (Voleski, 2007) and can biosorb/precipitate both heavy and toxic metals (Hussein et al., 2004) by various mechanisms despite the fact that they constitute a minor fraction of the total solid mass of the soil (Ledin et al., 1999). Heavy metal is taken up by the microbial cells but denied entrance into the cytoplasm. This involves the use of plasmid bound trait which alters cell membrane permeability to a metal such as Cd, and thus retained the metal within cell wall (Nies, 1999). Also, some cells do uptake Cd, causing redistribution of the metal from cell membrane to cell wall as seen in *Escherichia coli*.

Among bacterial species reportedly known to be potent metal biosorbents are strains of *Bacillus* (Srinath et al., 2002), *Citrobacter* (Puranik and Paknikar, 1999), *Enterobacter* (Scott and Karanjkar, 1992), *Escherichia* (Shen and Wang, 1993), *Pseudomonas* (Hussein et al., 2004) and *Streptomyces* (Selatnia et al., 2004). Other organisms, particularly eukaryotes (Voleski et al., 2003), agricultural wastes (Vijayaraghavan et al., 2005) and activated sludge (Al-Qodah, 2006) have also been reported as good biosorbents of various metals. Properties of the bacterial cell wall constituents, such as peptidoglycan, and the role of functional groups, such as carboxyl, amine and phosphonate are the basis of biosorption in bacteria (Nies, 1999; Vijayaraghavan and Yun, 2008; Pagnanelli et al., 2009).

The release of industrial wastewater at extreme physico-chemical conditions impart negatively on the viability of potential autochthonous biotechnological tools that would have decommissioned the toxicants. Thus, the receiving ecosystem remains unabated of its contaminants. Presumed non-viable microbial tools in the polluted system that could scavenge cadmium among the components of the wastewater would be a better approach to demobilise the pollutant. Moreover, the dead microbes would equally be applicable to the treatment of industrial wastewater at high ionic strength, and low pH. With reference to the literature available, there have been several studies on biosorption of cadmium in the past 20 years globally; few of such reports were on the potentials of dead, Cd-resistant bacterial strains. So far, there is no work in Sub-Sahara Africa on bioremediation of cadmium using resistant bacteria despite the high environmental contamination. Therefore, we investigated Cd biosorption potentials of highly resistant bacterial strains previously isolated from sites contaminated with industrial effluents. The optimum physico-chemistry for Cd adsorption, adsorption capacities, removal efficiencies and affinity between Cd and the bacteria were determined. Studies of Cd biosorption equilibriums by the bacterial strains are baseline knowledge required for the selection of bacterial tools needed for biodetoxification of systems polluted with cadmium.

2. Materials and methods

2.1. Chemicals

Cadmium salt ($CdCl_2$) crystals were purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA). Stock solutions (100 g l^{-1}) of the metal was prepared and sterilized with 0.22 μm Millipore filter (Nucleopore Corp., Pleasanton, CA, USA). All other chemicals were of analytical reagent grade.

2.2. Microorganisms and culture conditions

The isolation of the bacterial strains used in this study has been reported elsewhere (Oyetibo et al., 2010). They were isolated from the water and sediments of sewerage from allied-chemical industries that have been operating for over three decades. The bacteria include *Pseudomonas aeruginosa* CA207Ni, *Burkholderia cepacia* AL96Co, *Corynebacterium kutscheri* FL108Hg, and *Rhodococcus* sp AL03Ni. The bacterial high-tolerance to Cd^{2+} has been previously reported (Oyetibo et al., 2010). The organisms were stored at −20 °C in glycerol: Luria Bertani (LB) broth (1:1). The bacteria were resuscitated by harvesting colonies on LB agar with sterile inoculating loop, pooled and transferred to screw-capped bottles containing 5 ml of physiological saline (0.9% NaCl). It was pre-cultured in Erlenmeyer flask using LB broth for 24 h at 30 °C and 175× *g*, and the biomass was harvested upon centrifugation at 7000× *g* for 10 min, washed thrice with phosphate buffer (50 mmol l^{-1} KH_2PO_4, pH 7.2) and suspended in the same buffer to approximately 10^6 cfu ml^{-1}.

2.3. Preparation of bacteria for cadmium biosorption studies

The bacterial strains were prepared for Cd biosorption as previously reported (Sprocati et al., 2006). All the bacterial strains were inoculated individually into 100 ml tryptone water in 500 ml conical flasks and incubated on a shaker at 150× *g* for 24 h at 30 °C. The cells were grown to late exponential phase, and harvested by centrifugation at 10,000× *g* for 30 min at 4 °C. Biomass concentrations in cell suspensions were determined according to Puranik and Paknikar (1999), by drying the aliquot in a pre-weighed aluminium foil container to constant weight at 85 °C. To assay the potential of dead cells to biosorb metals, the harvested cells were conditioned to pH 2.5 by repeated washing with acidified deionised water (H_2SO_4) (85 °C for 24 h) to obtain presumed non-viable (PNV) biomass, and the efficacy of this treatment was checked by plating the PNV cells on LB agar plates. The PNV cells obtained were suspended in deionised water supplemented with $CdCl_2$ (5.0 mg l^{-1}, final concentration). This pre-treatment prevents changes in the solution pH after biomass addition.

2.4. Cadmium biosorption, desorption, and resorption studies with the non-viable bacterial isolates

The ability of individual bacterial strain to remove Cd ions from simulated industrial process wastewater (SIPW) supplemented with $CdCl_2$ was studied. The SIPW contained (litre^{-1}): glucose, 10 g; NH_4Cl, 2.67 g; Na_2HPO_4, 5.35 g; 6 ml mineral salts solution ($CaCl_2 \cdot H_2O$, 0.1 g; $MgSO_4 \cdot 7H_2O$, 10 g; $MnSO_4 \cdot 7H_2O$, 0.07 g; $FeSO_4 \cdot 7H_2O$, 0.4 g; distilled water, 1000 ml). Biosorption of Cd at various ionic concentrations, pH, and temperatures were determined according to the batch equilibrium method of Miretzky et al. (2006). The PNV biomass (around 50 mg dry weight) was suspended in SIPW supplemented with various concentrations of $CdCl_2$ (0, 50, 100, 150, 200, 250, 300, 350, 400, and 450 mg l^{-1}) at constant pH (2.0) and temperature (30 °C). The setup was incubated in a rotary shaker

(150× *g*) at 30 °C for 2 h with periodic sampling every 5 min to determine the adsorption equilibrium. The experiment was repeated at constant ionic concentrations (100 mg l^{-1}) and temperature (30 °C) but varied pH (2, 3, 4, and 5); and at varied temperature (25, 30, 35, 40, and 45 °C) while ionic concentration (100 mg l^{-1}) and solution pH (2) remained constant. The experimental setup was further incubated for 18 h to determine Cd removal efficiency of the biomasses. At the end of the agitation period, samples were filtered with 0.22 μm acetate cellulose membrane (Micro Separations Inc.). The initial and final concentrations of Cd in the broth were determined. Controls were blanks performed under the same conditions but without Cd, while loss of Cd due to abiotic factor was monitored in sterile Cd-amended SIPW. Cadmium recovery (desorption) from the cell surfaces upon adsorption, and reusability (resorption) potentials of the PNV biomasses to adsorb Cd were also investigated. Desorption was achieved with the slightly modified protocol of Goyal et al. (2003), where aqueous 0.1 mol l^{-1} Na_2CO_3 was used as desorption solution instead of 0.1 mol l^{-1} NaOH. The bacterial cell mass that was previously loaded with Cd upon biosorption, was re-suspended in physiological phosphate buffer and treated with desorption solution (50.0 ml) at 30 °C, with constant shaking (200× *g*) for 20 min. The supernatant was separated upon centrifugation at 5000× *g* for 10 min, and kept for Cd assay. Resorption was according to Sprocati et al. (2006). The desorbed cells obtained were washed with excess 0.1 mol l^{-1} NaOH, followed by extensive washing with saline solution, and inoculated in Cd amended SIPW (100.0 mg l^{-1} Cd) adjusted to pH 2.0 with 0.1 mol l^{-1} NaOH and 0.1 mol l^{-1} HNO_3. It was incubated by shaking (150× *g*) at 30 (±2) °C for 1 h. Aliquots of the cells were collected by centrifugation (5000× *g*, for 10 min) and Cd content in the biomass was determined (Section 2.5). All values of Cd^{2+} adsorption, desorption, or resorption were mean of triplicate experiments in mg Cd per dry weight, and the standard error of the mean as error bars.

2.5. Analyses of cadmium

A 10.0–100.0 mg of oven-dried biomass was subjected to acid digestion with 5.0 ml of 69.0% HNO_3 (Aristar BDH, England) and 2.0 ml of 30.0% H_2O_2 (SKG Pharma Ltd., Nigeria) in screw-capped tubes using a microwave oven. The program of mineralisation was performed in three steps of 10, 10 and 5 min at 100, 300, and 600 W, respectively. The solutions were completely transferred to a volumetric flask after cooling and made up to the final volume with ultra pure water. In the final solutions, Cd was measured with appropriate metal lamp by flame atomic absorption spectrophotometer (AAS) (Perkin–Elmer, Canada). The calibration curves were obtained from aqueous calibrating solutions of 1000.0 mg l^{-1} of Cd (BDH, England).

2.6. Cadmium removal efficiency

The experimental data generated from effect of metal concentration gradient on the sorption potential of the bacterial strains were used to determine the metal removal efficiency (RE) of bacteria. This was calculated, according to Zhou et al. (2007), by:

$$R = \left(\frac{C_i - C_{eq}}{C_i}\right)$$

where R is the metal RE, C_i is the initial metal ion concentration (mg l^{-1}), C_{eq} residual metal ion concentration at equilibrium.

2.7. Biosorption isotherm models

Equilibrium isotherms were measured to determine the capacity of the biosorbent for Cd ions as previously described (Vijayaraghavan and Yun, 2008). Biosorption data were fitted to Langmuir and Freundlich isotherm models. The Q_{eq} versus C_{eq} sorption isotherm relationship was mathematically expressed by linearised Langmuir ($1/Q_{eq} = 1/Q^o b \times 1/C_{eq} + 1/Q^o$) model as *Qo* and *b* were Langmuir constants. Q^o corresponds to the maximum achievable uptake by the isolates (moles of solute sorbed per unit weight of isolate to form a complete monolayer), and was used to compare the performance of the PNV isolates (biosorbents); while *b* was related to the affinity between the metal (sorbate) and the PNV isolates (sorbents), which characterised the initial slope of the isotherm. Linear Freundlich ($In\ Q_{eq} = b_F\ In\ C_{eq} + In\ K_F$) model was also used to fit the biosorption equilibrium as b_F and K_F (mg gdw^{-1}) are Freundlich constants that were determined from slope and intercept, respectively, in a straight line plot of $In\ Q_{eq}$ versus $In\ C_{eq}$. K_F corresponded to the binding capacity (maximum adsorption capacity), and b_F characterises the affinity (adsorption intensity) between the isolates (sorbent) and Cd (sorbate). Generally, C_{eq} is the Cd concentration in solution at equilibrium, and Q_{eq} is the moles of Cd sorbed per unit weight at concentration C_{eq}.

2.8. Analysis of functional groups by Fourier transforms infrared spectroscopy

The infrared absorption spectra were recorded within the range of 4000.0–400.0 cm^{-1} with a resolution of 6.0 cm^{-1} on a Fourier transform infrared spectrometer (WGH-30A, Tianjin, China) with a DTGS detector. The samples were dried for 10 h at 60 °C under reduced pressure. The product was ground in a mortar and pestle, and each powdered sample (2.0 mg) was blended with IR-grade KBr (200.0 mg) in an agate mortar. The sample-KBr mix was pressed into tablet and analysed by the Fourier transform infrared spectrometer. Over 60 scans were collected for each measurement, and the spectra were recorded.

2.9. Statistical analysis

All statistical tests were performed using the Prism 5 software program (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Effect of physico-chemical conditions on cadmium adsorption

Changes in incubation temperature, pH, and metal concentration influenced, very sharply, the biosorption rates of Cd^{2+} by the PNV bacterial strains. The sorption equilibrium for Cd^{2+} were at 30 °C for CA207Ni, and AL96Co with an uptake equilibrium at 92.0 (±2.5) and 56.0 (±2.3) mg gdw^{-1}, respectively. Unlike strain FL108Hg that reached sorption equilibrium of 63.0 (±2.9) mg gdw^{-1} at 35 °C, that of AL03Ni was 50.0 mg gdw^{-1} at 40 °C (see Fig. 1, panel a). Acidic pH 2.0 facilitated the highest Cd^{2+} sorption equilibrium for all the isolates as depicted in Fig. 1, panel b. It was revealed that the bacterial strains CA207Ni, AL96Co, FL108Hg, and AL03Ni had Cd^{2+} sorption capacity of 80.0 mg gdw^{-1}, 37.0 (±2.9) mg gdw^{-1}, 70.0 mg gdw^{-1}, and 55.0 mg gdw^{-1} respectively at pH 2.0. Biosorption of Cd^{2+} were at 300.0 mg l^{-1} by AL96Co (40.0 mg gdw^{-1}), and AL03Ni (50.0 ± 0.6 mg gdw^{-1}), while CA207Ni (94.0 ± 1.2 mg gdw^{-1}), and FL108Hg (61.0 ± 1.2 mg gdw^{-1}) were at 400.0 mg l^{-1} and 450.0 mg l^{-1} respectively (Fig. 1, panel c). Maximal Cd loading capacity was achieved within 20 min of contact between metal and the bacterial strains.

3.2. Metal removal efficiency, desorption and resorption

Fig. 2 shows the suitability of the bacterial strains for treatment of dilute metal solutions at low and high concentrations. At low

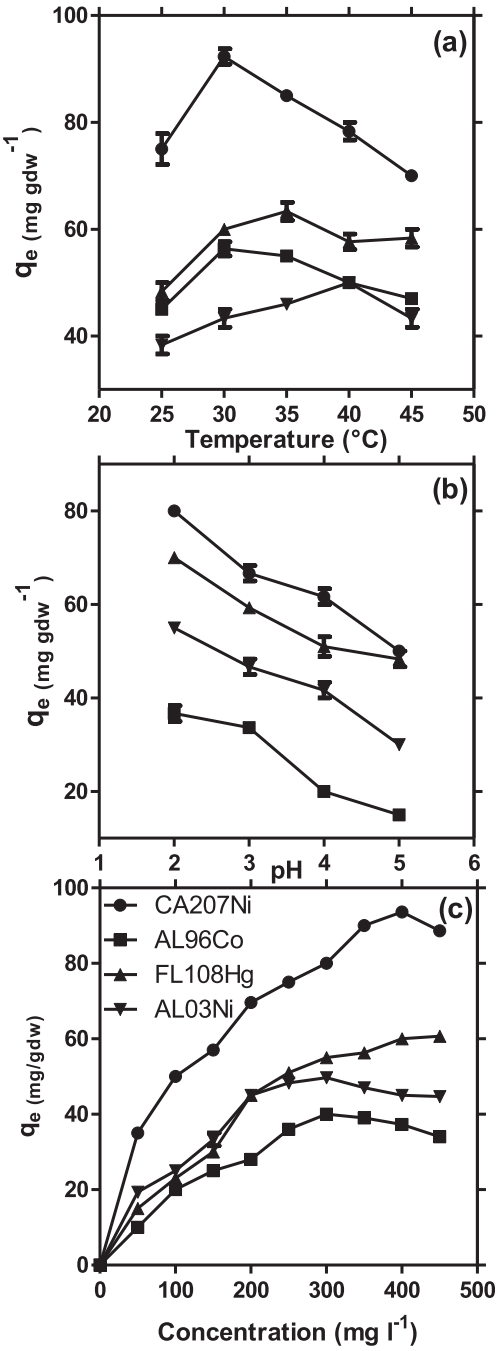

Fig. 1. Effect of physico-chemical parameters on the adsorption of Cd^{2+} ion by *Pseudomonas aeruginosa* CA207Ni, *Burkholderia cepacia* AL96Co, *Corynebacterium kutscheri* FL108Hg, and *Rhodococcus* sp. AL03Ni. Temperature (panel a) (pH 2; Cd^{2+}, 100 mg l^{-1}), pH (panel b) (temperature 30 °C; Cd^{2+}, 100 mg l^{-1}) and ionic concentration (panel c) (pH 2; temperature 30 °C), a cell mass of 0.75 g l^{-1} and agitation at 150× *g* were used. Error bars are calculated based on triplicate runs at 95% confidence intervals.

metal concentrations, CA207Ni had Cd^{2+} removal efficiency of 50.0 ± 1.2%, while 77.0 ± 1.5% was observed with FL108Hg. Bacterial strain AL96Co was more effective in removing Cd^{2+} (80.0%), whereas, 75.0% of Cd^{2+} was efficiently removed from the solution at low metal concentration. Metal removal was more efficient at higher Cd concentration than at lower concentration. Cd removal efficiency was the least with CA207Ni (80.0 ± 0.3%), but the highest with AL96Co (92.0 ± 0.2%) at high ionic concentration. Other strains including FL108Hg and AL03Ni effectively removed

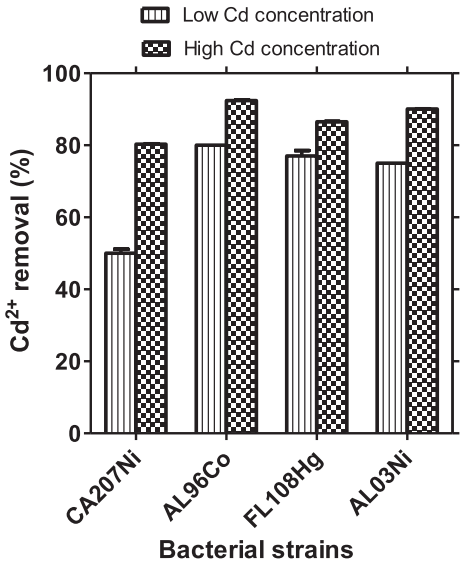

Fig. 2. Removal efficiencies of Cd by bacterial strains, at low and high metal concentrations (temperature, 30 °C; pH 2; cell mass, 0.75 g l^{-1}; agitation, 200× *g*). Low Cd concentration was 100 mg l^{-1}, while high concentration was 450 mg l^{-1}. Errors of the mean ($n = 3$) are calculated based on triplicate analyses at 95% confidence intervals.

87.0 ± 0.1% and 90.0 ± 0.07% of Cd^{2+} from highly concentrated solution. The regeneration of Cd^{2+} from the PNV cell mass laden with $CdCl_2$ upon treatment with sodium carbonate is shown in Fig. 3. Of the adsorbed Cd, 87.0 ± 0.3, 37.0 ± 0.4, 56.0 ± 0.6, and 46.0 ± 0.2% was recovered from cell masses of CA207Ni, AL96Co, FL108Hg, and AL03Ni, respectively. Resorption of the metal was at the best with CA207Ni (80.0 ± 1.3%), while AL96Co had the least potential of re-usage for Cd sorption with 32.0 ± 0.8% efficiency. Other strains, FL108Hg and AL03Ni, had 49.0 ± 0.8% and 38.0 ± 1.5% competence, respectively, when desorbed cell mass were treated with $NaOH–HNO_3$ and introduced to $CdCl_2$ amended SIPW.

3.3. Biosorption isotherms

Fittings of the overall experimental data of Cd^{2+} biosorption into the Langmuir and Freundlich sorption isotherms were summarized in Table 1. From the calculated maximum metal uptake (*Qo*) in

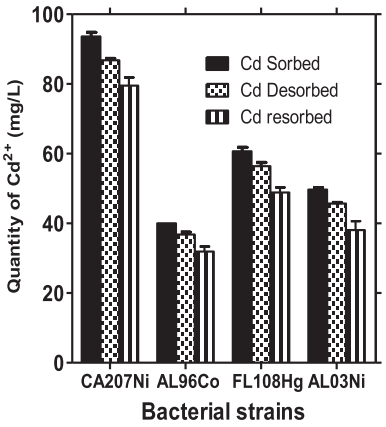

Fig. 3. Desorption of Cd^{2+} from metal-laden bacterial isolates, and resorption of Cd^{2+} with desorbed bacterial isolates. Desorption medium consisted of 0.1 mol l^{-1} sodium carbonate at 30 °C, shaken at 200× *g* for 20 min while desorbed cells were treated with 0.1 mol l^{-1} NaOH before resorption (see Section 2.4 for details). Errors are calculated based on triplicate analyses at 95% confidence intervals.

Table 1
Fitness of experimental cadmium adsorption data into biosorption isotherms.

Bacterial strains	Langmuir isotherm				Freundlich isotherm			
	Q^o (mg gdw^{-1})	b (l mg^{-1})	r^2	P	K_F (mg gdw^{-1})	b_F	r^2	P
CA207Ni	109.37	0.009	0.9784	<0.0001	65.16	0.45	0.9844	<0.0001
AL96Co	71.74	0.003	0.9775	<0.0001	1.52	0.59	0.8942	0.0001
FL108Hg	103.55	0.003	0.9790	<0.0001	1.14	0.68	0.9696	<0.0001
AL03Ni	62.07	0.009	0.9355	<0.0001	21.09	0.43	0.8576	0.0003

Note: Q^o = maximum achievable metal uptake by bacterial isolates; b = affinity constant between metals and the isolates; K_F = binding capacity (adsorption intensity) between the bacterial isolates and the metals; b_F = affinity (adsorption capacity) constant between the bacterial isolates and the metals.
All the experimental values did fit into model since correlation coefficients (r^2) were more than 0.5.

Langmuir isotherm, the sequence of achievable Cd uptake among the bacteria was in the following order: CA207Ni (109.37 mg gdw^{-1}) > FL108Hg (103.55 mg gdw^{-1}) > AL96Co (71.74 mg gdw^{-1}) > AL03Ni (62.07 mg gdw^{-1}). The affinity between Cd^{2+} and the isolates as depicted by Langmuir affinity constant b revealed that AL96Co and FL108Hg demonstrated equal capacity (0.003 l mg^{-1}), and were better attracted to Cd than CA207Ni and AL03Ni. It should be noted that CA207Ni and AL03Ni established equal affinity to Cd (0.009 l mg^{-1}) according to the Langmuir isotherms. The evaluated adsorption intensity (K_F) as predicted by Freundlich isotherm revealed that strain CA207Ni had higher binding capacity to Cd^{2+} (65.16 mg gdw^{-1}) than other isolates, while FL108Hg had the least (1.14 mg gdw^{-1}). Affinities (b_F) for Cd^{2+} by the bacterial cells was in the sequence FL108Hg (0.68) > AL96Co (0.59) > CA207Ni (0.45) > AL03Ni (0.43). This correlates with prediction of Langmuir isotherm stated earlier. It was observed that all correlation coefficients (r^2) in Langmuir isotherm were >0.9000 unlike in Freundlich isotherm where r^2 was <0.9000 for AL96Co and AL03Ni. It confirms that the Langmuir isotherm performed better than Freundlich isotherm because of their higher r^2. On this basis, Langmuir isotherm model with r^2 of 0.9790 indicated that FL108Hg performed better than other bacteria studied in the sorption of Cd^{2+} (Q^o = 103.55 mg gdw^{-1}; b = 0.003).

3.4. FTIR analysis

The metal binding functional groups in bacterial cell wall as determined by infrared absorbance spectra (IAS) with the peak stretching in infrared (IR) absorbance implies that corresponding molecular groups are functional. It is apparent that the bacteria differed in functional molecular groups used for Cd^{2+} adsorption. Changes in some important IR peaks and corresponding molecular groups are summarised in Table 2.

The following can be deduced in an attempt to compare between the IR spectra of the bacteria before and after Cd^{2+} loading:

a. The stretching of the bands assigned transition metal carbonyls indicate the interaction of Cd^{2+} with C═O groups on the cell wall.
b. Cd^{2+} adsorption leads to disappearance of some molecular groups in the bacteria. For example, −OH at 3570.32 cm^{-1}, dimeric OH at 3514.33 cm^{-1}, heterocycline amine at 3485.59 cm^{-1}, and −C−H from tertiary amine disappeared after Cd^{2+} adsorption onto strain CA207Ni. However, there were appearances of some functional groups on the cell masses after Cd^{2+} loading as found with amide at 1658.91 cm^{-1} on strain CA207Ni surface.
c. Shifting and broadening of bands (for example, −OH from phenol shifted from 3570.32 cm^{-1} before Cd^{2+} adsorption to 1365.99 cm^{-1} after Cd^{2+} adsorption; and −N-H from secondary amine at 1558.99 cm^{-1} before Cd^{2+} loading got broadened to 1577.63–1558.16 cm^{-1} after Cd^{2+} loading on CA207Ni) were due to the loading effect of the metal.
d. Cd^{2+} interaction with sulphate groups was attributable to stretching of the bands that appeared at 1113.15 cm^{-1}, 1101.91 cm^{-1}, and 1114.51 cm^{-1} on the surfaces of CA207Ni, FL108Hg, and AL03Ni, respectively.

4. Discussion

Industrial processes can generate wastewaters that are laden with heavy metals, which can subsequently poison microbes in the receiving ecosystem. Moreover, the processes contributing to industrial wastewater generation are diverse. The physico-chemical composition of industrial wastewater affects the rate and extent of toxic metals leaching into ecosystems receiving these wastewaters. Biosorption is the most suitable and reliable approach for the removal of toxic metals from wastewaters, and ecosystems that receive such industrial effluents. In essence, when harsh wastewater inactivates indigenous microbiota upon discharge, the PNV biomasses in the affected environment would mop up the toxic metals from the ecosystem. Adsorption of Cd onto the PNV cell masses of bacterial strains, CA207Ni, AL96Co, FL108Hg, and AL03Ni, were observed to increase with incubation temperature, and had optimal Cd^{2+} adsorption at 30–40 °C (Fig. 1, panel a). The increase in metal uptake at increased temperature is attributable to increased surface activity and kinetic energy of the metal (Vijayaraghavan and Yun, 2007), which resulted into either higher affinity of bacterial surface sites for metal or an increase in binding sites on the relevant cell mass. It has been shown that at higher temperature (25–45 °C), the energy of the system facilitates metal attachment on the surface. However, there is a decrease in metal sorption at extreme temperatures (>45 °C) due to distortion of some sites on the cell surface available for metal biosorption (Goyal et al., 2003; Zhou et al., 2007). Moreover, an increase in temperature beyond the optimal range has been found to reduce the biosorption capacity of biomass in some exothermic adsorption processes (Zhou et al., 2007.).

Maximum sorption potential of the isolates was obtained at pH 2.0. This is not surprising as acidic pH has been reported as critical for metal biosorption among microorganisms (Goyal et al., 2003; Atifet et al., 2004; Tsui et al., 2006; Zhou et al., 2007). It is evident from Fig. 1 (panel b) that the adsorption of Cd^{2+} increased with decreasing pH of the medium. This is related to the mechanism of metal adsorption on the surfaces of microorganisms and reflects the nature of the pH-dependent physico-chemical interaction of both the ions in solution and the nature of cell adsorption sites. For most metal ions, maximum biosorption were observed at weak acidic pH due to involvement of carboxyl group and other acidic functional groups (Vijayaraghavan and Yun, 2008). It follows from the theory of acid-base equilibria that, in the pH range 2.0–4.0, the binding of heavy metal cations is determined primarily by the state of dissociation of the weak acidic groups. Sensitivity of

Table 2
Stretching changes of IR absorbance peaks of important molecular groups of the bacterial cells before and after Cd^{2+} adsorption.

Bacterial strains	Before adsorption		After adsorption	
	IR peak (cm^{-1})	Assigned molecular group	Stretching or status of IR peak (cm^{-1})	Assigned molecular group
CA207Ni	3570.32	–OH from phenol	Disappeared	
	3514.33	Dimeric OH	Disappeared	
	3485.59	Heterocycline amine	Disappeared	
	2032.36–1828.48	Transition metal carbonyls	2033.53–1860.46	
	1803.56–1778.91	Aryl carbonate	1804.38–1780.44	
	1742.91	Ester	1742.43	
	No peak		1658.91	Amide
	1558.99	–N–H from secondary amine	1577.63–1558.16	
	No peak		1365.99	–OH from phenol
	1197.22–1155.6	–C–H from tertiary amine	Disappeared	
	No peak		1165.81–1148.56	–C–H from secondary amine
	1117.46	Sulphate ion	1113.15	
	1083.61–1056.88	–C–H from primary amine	1089.12–1048.7	
AL96Co	2036.59–1883.62	Transition metal carbonyl	2032.25–1827.67	
	1803.66	Aryl carbonate	1809.85	
	1745.08	Ester	1743.9	
	1678.00	Amide	Disappeared	
	1293.65–1211.85	–C–OH from polysaccharide	Disappeared	
	1154.22–1138.55	–C–N from secondary amine	1164.29	
FL108Hg	3120.67–3067.8	–N–H from NH_3	3124.1	
	1881.58–1859.11	Transition metal carbonyls	1958.31–1839.5	
	1782.37	Aryl carbonate	1804.08–1781.26	
	1678.18	Amide	1675.7	
	1642.82	–N–H from primary amine	Disappeared	
	1343.59	–OH from polysaccharide	Disappeared	
	1194.77	–C–N from amine	Disappeared	
	1091.92	Sulphate ion	1101.91	
	1066.18–1020.01	Phosphate ion	Disappeared	
	No peak		1063.1–1045.51	–C–N from primary amine
	878.99	Carbonate ion	Disappeared	
AL03Ni	3040.84	Ammonium ion	3038.92	
	2032.21–1830.17	Transition metal carbonyl	1959.1–1858.7	
	1804.44–1779.65	Aryl carbonate	1803.68–1779.79	
	No peak		1677.25–1656.78	Amide
	1655.69	–N–H from secondary amine	Disappeared	
	1425.8	Carbonate ion	1420.23	
	1365.37	–OH from phenol	Disappeared	
	1197.8	–C–N from tertiary amine	1195.9	
	No peak		1162.06–1134.97	–C–N from secondary amine
	1100.5	–C=O from secondary alcohol	Disappeared	
	1080.28	Sulphate ion	1114.51	
	876.332	Carbonate ion	877.563	

microorganisms has been linked to isoelectric properties of metals (Goyal et al., 2003), where the non-viable cells will have net negative charge at pH values above the isoelectric point and thereby develop ionic bond to net positive charges of the metals. Consequently, Cd^{2+} being more reactive than H_3O^+, will displace hydrogen from the carboxyl group to form oxides with the COO^- in a strong ionic bond.

Maximum metal sorption by the bacterial cells was observed at concentrations ranging from 300.0 mg l^{-1} to 450.0 mg l^{-1}. The equilibrium biosorption of cadmium ions in all the organisms increased with increasing ionic strengths. This could be ascribed to the competition between ions, changes in the metal activity, or in the properties of the electrical double layer. When cell mass surface and heavy metal in aqueous solution are in contact, they are bound to be surrounded by an electrical double layer owing to electrostatic interaction. Goyal et al. (2003) reported decrease in biosorption of metals with increasing ionic strength when brown seaweed was used as biosorbent. The observed enhancement of cadmium sorption in this study could be due to the increase in electrostatic interactions, in relation to covalent interactions, involving sites of progressively lower affinity for metal ions in corroboration with previous report (Puranik and Paknikar, 1999). At low cadmium concentrations, AL96Co was more effective in removing Cd^{2+} (80%), but more removal efficiency was observed at higher ionic concentration where as much as 92.44% (±0.1) efficiency was observed. Previously, metal removal efficiencies of 82.5% at pH 2.5 have been reported for *Bacillus licheniformis* (Zhou et al., 2007). It is important to note that the removal efficiency is independent of the biosorbent mass; rather, it is inversely proportional to initial metal concentrations. This was also affirmed by Tsui et al. (2006). Therefore, a good biosorbent is expected to have high metal removal efficiency.

Metal desorption is required when metal recovery is targeted or when the cost of the biosorbent limits the process economy. Approximately 92.66% desorption of Cd from CA207Ni cell mass is noteworthy. This was close to the order of 100.0% desorption efficiency reported previously (Munoz et al., 2006). Desorption is possible since sodium carbonate normally attracts the positive charged metals, thus making the cells to carry net positive charge that equally repel the protons. Bacterial biomasses pose problems during desorption due to their microscopic structure. They tend to be affected by the presence of both strong acidic and alkaline conditions which are often used during desorption processes (Vijayaraghavan and Yun, 2008). Desorption processes have been reported successful in a number of biomasses (Puranik and Paknikar, 1999; Beolchini et al., 2003; Goyal et al., 2003).

Resorption of Cd ions were achieved back into *P. aeruginosa* CA207Ni (91.66%) when desorbed cell mass were treated with NaOH–HNO_3 and introduced to Cd-amended solution. This is better than values previously reported (Puranik and Paknikar, 1999; Beolchini et al., 2003; Goyal et al., 2003).

Biosorption mechanisms are based on the use of dead biomass to passively uptake toxicants. It is due to a number of metabolism-independent processes that essentially take place in the cell wall, where the mechanisms responsible for pollutant uptake differ according to the biomass type (Vijayaraghavan and Yun, 2008). It was observed in this study that Cd-adsorption intensity was best with CA207Ni. The extent of Cd^{2+} biosorption depends on the bacterial genus, due to variations in the cellular constituents. Furthermore, the role of environmental factors in influencing adaptive features in autochthonous microorganisms via genomic modification cannot be overemphasized. Genetic modification of bacteria in extreme environments, such as heavy metal polluted systems, often manifest as cell types with higher intrinsic capability and specificity, as well as greater resistance to ambient conditions. It is worthwhile that the hyper-tolerance of the bacterial strains understudied has been reported (Oyetibo et al., 2010). Moreover, the viable cells of the strains investigated have been reportedly displayed intrinsic capability and specificity for degradation of hydrocarbons associated with industrial wastewater in medium enriched with toxic metals (Oyetibo et al., 2013). The excellent performance of the PNV bacterial strains to adsorb cadmium, in comparison with data available in literature, must be due to the adaptive traits like metal binding sites enshrined on the bacteria from the environment. The binding sites of bacterial cell mass were chemically modified to enhance the biosorption capacities by multiple folds since the number of binding sites available is directly proportional to the density of the functional groups in the cell wall and relatively corresponds to their sorption capacities. Contrary to conditioning of cell mass by repeated washing with acidified deionised water at 85 °C for 24 h used in this study, Jeon and Holl (2003) used chloroacetate to introduce carboxyl in the place of hydroxyl groups, and then treated with enediamine followed by carbodiimide to form aminated biomass.

Anionic functional groups present in the peptidoglycan, teichoic acids and teichuronic acids of Gram-positive bacteria, and the peptidoglycan, phospholipids, and lipopolysaccharides of Gram-negative bacteria are the components reportedly responsible for the anionic character and metal-binding capability of the cell wall. Since the mode of solute uptake by dead/inactive cells is extracellular, the chemical functional groups present on the bacterial cell wall, including carboxyl, phosphate, amine and hydroxyl groups play a vital role in binding of metal cations (Vijayaraghavan and Yun, 2008). The IAS analysis of the bacterial strains in this study showed that the presence of several of these functional groups (amine, carboxyl, sulphate, hydroxyl and phosphate groups) are likely responsible for the binding of Cd^{2+} onto the surface of the bacterial strains. These molecular groups have been implicated in metal adsorption onto the surfaces of biosorbents in previous reports (Wang et al., 2010; Xu et al., 2011). The functional groups must have been negatively charged in the media and may therefore interact with the positively charged Cd^{2+} electrostatically. Carboxylic and amino groups were reported to be the main active sites responsible for the binding properties of biomass (Pagnanelli et al., 2009). Amine groups do not only chelate cations, but also adsorb anionic metal species via electrostatic interaction or hydrogen bonding in processes of the metal sorption as earlier reported by Vijayaraghavan and Yun (2007). Amine groups were observed in species of *Corynebacterium* to protonate at pH 3.0 and attract negatively charged chromate ions via electrostatic interaction (Kang et al., 2007; Vijayaraghavan and Yun, 2007). Enhanced Cd uptake by *P. aeruginosa* CA207Ni (93.67 ± 1.2), better than other bacteria studied, is not unconnected with the presence of amine groups in their cell wall in agreement with previous report (Vijayaraghavan and Yun, 2008).

In this study, the adsorption equilibrium occurs within 20 min at the end of rapid physical adsorption. The rapid transfer of the metal from solution to the surfaces of the bacteria explains the homogeneity of the cell mass studied. Previously, adsorption equilibrium time of 30–40 min has been reported (Goyal et al., 2003). A homogeneous cell mass would have similar functional groups available for metal sequestration. On the contrary, a heterogeneous biosorbent, such as biosolids that reportedly exhibited slower reaction rates (Norton et al., 2004), have a host of different functional groups present due to the different components in the microorganism cell wall. These different groups providing adsorption sites may have differing rates of metal adsorption onto sites, resulting in different rates of metal uptake by the biosorbent and a longer time than the PNV bacterial cell masses to reach equilibrium.

The equilibrium can be represented by Langmuir and Freundlich adsorption isotherm equations. Langmuir isotherm fitting of the experimental adsorption data of Cd^{2+} by FL108Hg is based on the assumptions that respective metal ions are chemically adsorbed at a fixed number of well-defined sites. Each site could only hold one ion as all sites are energetically equivalent, and there is no interaction between the ions. The experimental fittings into Langmuir isotherm compares well with the reports of Hussein et al. (2004), and Zhou et al. (2007). The linearized Langmuir isotherm allows the calculation of adsorption capacities, which indicated that FL108Hg was better than other isolates with the potentials to uptake 103.55 mg gdw^{-1} Cd at high affinity (0.003 l mg^{-1}) with the metal. Freundlich isotherm fittings in adsorption of Cd^{2+} by CA207Ni assume there are infinite supplies of unreacted sites on the heterogeneous surfaces of the bacteria involved. The adsorption capacities (b_F) (0.43–0.68) and adsorption intensity (K_F) (1.14–65.16 mg gdw^{-1}) compared well with previous reports of 0.01–447.92 l g^{-1} for macrophytes sorption of Cd (Miretzky et al., 2006). The biosorption isotherm parameters indicate that favourable adsorption exists between the cell masses and the metal. For example, the Freundlich constant, b_F, of the experimental data further expressed that sorption of the metal on the cell masses was not just by physical bonding but involvement of different phenomena including ion exchange, complexation and chelation. On the basis of high maximum metal uptake values, high values of adsorption intensity between the bacteria and corresponding low values of affinity constants between the metals and the bacteria obtained in this study, the bacterial strains are excellent biosorbents suitable for the treatment of cadmium-laden industrial effluent streams. Cd biosorption properties of the bacterial strains studied are of higher order of magnitude than what has been reported for using other bacteria to the best of our knowledge. It is noteworthy that lesser cadmium uptake capacities were reported for strains of *Bacillus circulans* (Yilmaz and Ensari, 2005), *Enterobacter* sp J1 (Lu et al., 2006), *P. aeruginosa* PU21 (Chang et al., 1997), *Pseudomonas putida* (Pardo et al., 2003), *Pseudomonas stutzeri* (Hassan et al., 2009), *Streptococcus pimprima* (Puranik et al., 1995), *Ochrobactrum anthropi* (Ozdemir et al., 2003), and *Ochrobactrum cytisi* Azn6.2 (Rodriguez-Llorente et al., 2010). Interestingly, Ziagova et al. (2007) and Loukidou et al. (2004) reported seemingly higher biosorption potentials for the bacterial biosorbent they worked on, but ironically they applied higher biosorbent load of 1.0 g l^{-1} while just 0.1 g l^{-1} was applied in this study.

5. Conclusion

Presumed non-viable cells of highly resistant bacterial strains isolated from environments polluted with industrial wastewater in Nigeria demonstrated knack to adsorb cadmium more efficiently

than most biosorbents previously reported. Ambient temperature, acidic pH, and high ionic strength were effective for the dead/inactive biomass of the organisms to passively uptake cadmium ions from the solution. Moreover, Langmuir and Freundlich isotherms established better Cd^{2+} affinities and capacities in the bacterial strains than almost all other biosorbents that have been reported. The efficient Cd^{2+} adsorption in the organisms were characterized with the repertoire of functional molecular groups on their surfaces. The cells were reusable during cadmium detoxification, having up to 86.0% desorption and 79.0% resorption potentials. Thus, the presumed non-viable bacterial strains showed a great potential for decommissioning systems laden with toxic cadmium at extreme physico-chemistry.

Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.ibiod.2014.03.004.

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