International Biodeterioration & Biodegradation 91 (2014) 37–44

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

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

Ganiyu Oladunjoye Oyetibo [a,*], Matthew Olusoji Ilori [a], Oluwafemi Sunday Obayori [b], Olukayode Oladipo Amund [a]

[a] Department of Microbiology, University of Lagos, Akoka, Yaba, Lagos, Nigeria
[b] Department of Microbiology, Lagos State University, Ojo, Lagos, Nigeria

ARTICLE INFO

Article history:
Received 4 June 2012
Received in revised form
18 February 2014
Accepted 3 March 2014
Available online 3 April 2014

Keywords:
Biosorption
Industrial wastewater
Heavy metal
Cadmium
Resistant bacteria

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

* Corresponding author. Tel.: +234 803 353 8860.
E-mail addresses: ganeeoyetibo@yahoo.com, goyetibo@unilag.edu.ng (G. O. Oyetibo).

http://dx.doi.org/10.1016/j.ibiod.2014.03.004
0964-8305/

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