Kinetics and mechanisms of mercury biosorption by an exopolysaccharide producing marine isolate *Bacillus licheniformis*

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Abstract Eight exopolysaccharide (EPS) producing metal-removing marine bacteria were screened for mercury (Hg) sorption. *Bacillus licheniformis* with the highest MIC values and Hg sorption ability was selected for further study. Biosorption of Hg from aqueous solution by *Bacillus licheniformis* was studied with respect to the metal concentration, adsorbent concentration, pH, different contact times, and in the presence of other metal ions. Under optimum conditions, more than 70% mercury was removed by 25 mg dried biomass of *Bacillus licheniformis* at pH 7.0 after 1 h of contact time. Freundlich adsorption isotherm was acceptable at studied Hg concentrations as compared to Langmuir isotherm model. Pseudo-second-order kinetic model was found to be more suitable for data presentation in contrast to pseudo-first-order kinetic model. Involvement of external mass transfer was prominent as compared to intraparticle diffusion model. Desorption of Hg was more effective with acids from all the studied eluents, showing 49.36 and 33.8% eluting capacity for 0.1 N HCL and 0.1 N HNO₃, respectively. Scanning electron microscopy exhibited altered cell surface morphology of the cells under the influence of mercury. The spectral images of energy dispersive spectroscopy showed the presence of metal ions on the surface of cells.

Keywords Mercury · Biosorption · Adsorption isotherm · Kinetic models · Desorption

Introduction

Pollution of heavy metals has become a serious global problem as a result of rapid industrialization. Metallic pollutants severely affect the ecosystem and cause health hazard due to their toxicity. Amongst the myriad of environmental pollutants, cadmium, chromium (VI), lead, and mercury merit a special attention due to their potential health hazard to human as well as wild life (Rizzuti et al. 2015). Mercury is a prevalent contaminant having high toxicity and rapid accumulation. Minamata incident triggered awareness about the hazardous effect of mercury among the people. Industries manufacturing pesticides, batteries, scientific instruments, explosives, electronics, catalysis, chloroalkali paints, thermometers, dental preparations and fluorescent as well as ultraviolet lamps are facing the major problem of mercury containing waste disposal (Modi and Dave 1995; Raza et al. 2015). In aquatic world, the presence of mercury ranges between 1–3 ng/L in rivers and lakes, 0.5–3 ng/L in open Ocean, and 2–15 ng/L in coastal seawater (EI-Agroudy and Elektorowicz 1999). Green-Ruiz (2006) has reported the mercury concentration for uncontaminated coastal sediments in the range from 0.39 to 1.62 µg/g. High concentrations of mercury cause serious neurological and renal disturbances leading to the death in severe cases (Huang and Lin 2015). For all these reasons, removal of mercury needs a special attention. Several conventional methods have been developed to remove metal ions from aqueous solutions such as ion exchange, reverse osmosis, filtration, precipitation, coagulation, and biosorption (Bayramoglu and Yakup

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Arica 2009). The high-cost, operational problems, non-eco-friendly nature, and lower recovery of metal by the conventional methods are the main hurdles in their practical applications (Amoozegar et al. 2012). Biosorption is sorption-type phenomenon which occurs because of the electrostatic attraction between the metal cations and the negative charge on the surface of the microbial cell (Rossi 1990). The mechanisms by which microorganisms remove or accumulate metals is an important parameter to be understood for the development of microbial processes for the concentration, removal, and recovery of metals from aqueous solutions. Metal sequestration by different parts of the cell can occur via complexation, ion exchange, coordination, chelation of metals, adsorption, and ionic microprecipitation (Volesky 1986; Brierley et al. 1986).

Since the 1970s, the microorganisms are being used as a potential alternative for heavy metal removal and recovery. Marine bacteria may offer direct use of organisms in adverse conditions without any genetic manipulation (Dash et al. 2013). Marine microorganisms were found to produce nearly half of the discovered bioactive compounds including exopolysaccharides (EPS). Exopolysaccharides (EPS) are high-molecular weight polymers consisting mainly of carbohydrates. EPS occur as either capsular polysaccharides in which the polymers are covalently bound to the cell surface or slime polysaccharides that remain attached (loosely bound) to the cell surface. EPS produced by marine bacteria are supposed to play a role in the protection against extreme physico-chemical conditions of marine environment such as low or high temperature, high pressure, low nutrient concentration, high salinity, and heavy metal presence. EPS also present a wide range of industrial applications including emulsification, thickening, gel formation, anticancer treatment, and metal biosorption (Zhang et al. 2017). Heavy metal removal by EPS is a passive phenomenon in which the metal cations bind to the negative charges of acidic groups present on EPS. High salt concentrations in wastewater treatment systems have inhibitory effects on chemical treatments, while the conventional biological treatments cannot function well at such high salt concentrations. Halophilic and halotolerant microorganisms are suitable candidates for bioremediation processes, since they are able to grow on a wide range of salt concentrations (Amoozegar et al. 2012). This study emphasizes on the metal sorption behaviour of *Bacillus licheniformis* and its optimization. Characteristics of biosorbent were studied by SEM-EDS analysis. In addition, equilibrium and kinetic studies were performed.

Materials and methods

Screening of bacterial strains for metal removal

One hundred and forty one marine bacterial strains were isolated from samples collected from the coastal region of Alang, Bhavnagar, India (Upadhyay et al. 2016). Amongst these, eight EPS-producing bacterial isolates, namely, *Bacillus licheniformis* (SR5) (KP178603), *Bacillus subtilis* (SR6) (KJ830756), *Micrococcus flavus* (SR40) (KM406437), *Xanthonas* sp. (SR48) (KP178613), *Kocurea turfenensis* (SR55) (KM406442), *Cellulosimicrobium funkei* (SR57) (KM406443), *Pontibacter korlensis* (SR83) (KM406444), and *Pseudomonas aeruginosa* (E3) (KM406424) with more than 25% mercury removal ability, were screened for the copper, cadmium, lead, and mercury removal at 50 mg/L concentration in 50 mL system with 25 mg biomass.

Mercury removal in the presence of other metal ions

Experiments were conducted to remove mercury by 25 mg dried biomass of *Bacillus licheniformis* (SR5) (KP178603) from the sea-water samples (sample 2 and 3) collected from the Alang, Bhavnagar. Sample 1 was the control system containing 50 mg/L Hg in double distilled water. Sample 2 contained Fe at 6 mg/L concentration, whereas sample 3 had the presence of 18 mg/L Fe, 16 mg/L Cu, 17 mg/L Zn, and 4 mg/L Ni. Mercury (50 mg/L) was also added to samples 2 and 3 to check its removal in the presence of these cationic metal ions. Simulated waste (sample 4) was prepared in double distilled water by adding the Fe, Cu, Zn, and Ni in the concentrations similar to the present in sample 3 to mimic the sea-water system along with Hg. The residual metal ions along with residual mercury were estimated from the supernatant after 1 h of contact time.

Biosorption studies

Stock solution of 1000 mg/L Hg was prepared by dissolving analytical grade salt of HgCl_2 (HiMedia, India) in double distilled water.

Bacillus licheniformis was grown in EPS broth medium (Atlas 1993) for 120 h on environmental orbital shaker at 125 rpm and $30 \pm 2^\circ\text{C}$. The growth was harvested by centrifugation (Remi R-23, India) at $10,000 \times g$ for 20 min. The cell pellet was washed free of medium using sterile distilled water. The biomass was dried in oven at 60°C until constant dry weight was obtained.

Optimization study

To get the detailed insight of influencing factors on mercury removal, specific experiments had to be performed. As these combinations were very high in numbers, influence of selected parameters viz., reaction pH, mercury concentration, amount of biomass, additional dose of biomass, fresh and old biomass, and pretreatment to biomass was studied as outlined below.

To study these physico-chemical parameters, if otherwise mentioned, all the experiments were run in triplicates in 250 mL Erlenmeyer flask having 50 mL of system, 25 mg biomass, and 50 mg/L mercury concentration. All the flasks were kept on orbital shaker at 125 rpm at $30 \pm 2^\circ\text{C}$ for 2 h. Samples were withdrawn at the regular interval of time and analysed for Hg removal in aqueous system. For each experiment, parallel control flasks without biomass were run.

Influence of biomass concentration

Influence of dried biomass concentration was studied in the range from 10 to 100 mg/50 mL system (dry weight).

Influence of initial mercury concentration

Influence of the initial mercury concentration upon its removal with dried bacterial biomass was studied within the range of 5–1000 $\mu\text{g/mL}$ in a system of 50 mL, with 25 mg biomass/system.

Influence of reaction pH

To check the influence of reaction pH upon mercury removal, a wide range of pH between 2 and 10 was selected. The pH was adjusted with HCl/NaOH.

Influence of contact time

To study the maximum mercury removal at particular time, samples were withdrawn at the regular interval of time ranging from 5 to 120 min and analysed for Hg removal in aqueous system.

Influence of pretreatment to the biomass

For pretreatment, the biomass was exposed to 1 N solution of HCl, HNO_3 , NaOH and KOH as well as to organic solvents such as ethanol, methanol, acetone, and formaldehyde for 1 h. The biomass was washed with distilled water thrice and it was collected by centrifugation at $10,000 \times g$ for 20 min. The pre-treated biomass was then used for Hg removal experiment.

Desorption study

Desorption studies were performed to elute the metal from biomass. A 25 mg of loaded biomass was suspended in 50 mL of 0.1 N HCl, 0.1 N HNO_3 , 0.1 N NaOH, and 0.1 M EDTA, respectively (Saleem and Bhatti 2011). Flasks were kept on shaker for 2 h. Eluted Hg was estimated and calculations were done to find out the efficacy of the eluent.

Analysis

Biosorption ability of the cell mass was determined by analysing the residual mercury content in the system with spectrophotometer (Systronics, India) method using Malachite Green as a complexing agent (De et al. 1983). Copper, cadmium, and lead estimations were done by double beam Atomic Absorption Spectrophotometer (AAS) (Elico SL-243, India). Significance of data was calculated by statistical analysis.

SEM–EDAX analyses were performed to examine the sorption of the metal on the biomass of the isolate and its effect on cell morphology. The untreated and treated bacterial biomass with Hg(II) as well as the desorbed biomass with 0.1 N HCl were washed with double distilled water and smeared onto glass slides and dried. The samples were subsequently mounted on aluminium stubs and sputter coated with gold using a sputter-coater to increase electron conduction and quality of the micrographs. Specimens were examined using SEM equipped with an energy dispersive spectroscopy (Suriya et al. 2013).

Adsorption isotherms

The data were modelled by Langmuir (Eq. 1) and Freundlich isotherms (Eq. 2) (Dave et al. 2009):

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

where q_e is uptake of metal per unit weight of biosorbent, $q_{\max}$ is the maximum amount of metal ion per unit weight of biomass, C_e is the final equilibrium concentration of metal remaining in the solution, and b is the Langmuir constant:

$$q_e = K_F \times C_e^{(1/n)}, \quad (2)$$

where K_F and n are Freundlich constants.

Kinetic models

To analyse the Hg sorption rate for dried biomass of *B. licheniformis*, pseudo-first-order equation (Eq. 3), pseudo-second-order equation (Eq. 4), and Elovich kinetic models (Eq. 5) were used (Dave et al. 2012):

$$dq_t/dt = k_{ad}(q_e - q_t), \quad (3)$$

where q_e is uptake of metal per unit weight of biosorbent (mg/g), q_t is the amount of metal sorbed at any time (mg/g), and k_{ad} is the rate constant (per min):

$$dq_t/dt = k_2(q_e - q_t)^2, \quad (4)$$

where k_2 is the equilibrium rate constant (mg/g/min).

Elovich equation used to describe chemisorption performance (Liu and Liu 2008):

$$q = \alpha \ln(\beta\alpha) + \alpha \ln(t), \quad (5)$$

where α is the initial adsorption rate and β is the sorption constant. A plot of q versus $\ln(t)$ gives the slope equivalent to the initial adsorption rate.

Diffusion process

Intraparticle diffusion was studied using Weber Morris equation (Weber and Morris 1963; Wu et al. 2013):

$$q = Kt^{1/2}. \quad (6)$$

Intraparticle diffusion rate constant, K , was deduced from a graph of qt versus $t^{1/2}$.

The external mass transfer was calculated using the following equation:

$$\left[\frac{dC/dC_0}{dx} \right]_{t=0} = -\beta_L S. \quad (7)$$

Stoichiometry analysis

A 25 mg of Hg-loaded biomass was digested in 20 mL aqua-regia (3:2—HNO₃:HCl) at 150–180 °C temperature until dried. Further digestion was carried out in 10 mL concentrated H₂SO₄ at the same temperature for 15 min and finally diluted to 250 mL by double distilled water (Vogel 1962). The metal contents were analysed using AAS (Elico SL-243, India) by the standard procedures.

Results and discussion

Screening of bacterial isolates

On the basis of our previous work, eight EPS-producing marine bacteria were selected for the metal removal study (Upadhyay et al. 2016). The biosorption study of Cu, Cd, and Pb has been described by number of researchers, whereas less data available for Hg removal by marine bacteria which led to study the Hg removal using these marine bacteria (Parungao et al. 2007; Priyalaxmi et al. 2014; Dorian et al. 2012). Amongst these eight isolates, five isolates gave above 50% Hg removal, of which only

two were found to give Hg removal above 65%. The highest Hg removal was observed with SR 5 followed by SR 83, SR 40, SR 6, SR 48, SR 55, SR E3, and SR 57 (results not shown).

Mercury removal in multimetal system

Efficacy of biomass as sorbent for Hg was reduced considerably in the presence of other metal ions (Table 1). Under the experimental conditions, used biomass showed mercury loading capacity of 72 µg/mg biomass which decreased significantly in the presence of other metals. As the number of other metals increased the loading, capacity decreased. In the presence of only Fe (sample 2), the decrease was 12 µg/mg, but in multimetal system (samples 3 and 4), the decrease was more prominent and it was 1.5- to 2.0-fold higher. Different cationic metals compete with each other at different rates for the sorption site present on the surface of the biomass (Bohli et al. 2013; Shaheen et al. 2012). Sar et al. (1999) have investigated marginal decrease in Ni sorption and slight inhibition of Cu sorption by *Pseudomonas aeruginosa* in the presence of Co, Cr, Pb, and Zn. Say et al. (2001) have reported lower sorption for Cd, Pb, and Cu by *Phaenerochaete chrysosporium* in competitive conditions. Hence, it is always desirable to carry out an experiment in composite condition, where the effect of the presence of other metals with the candidate metals can be studied.

Influence of various parameters on Hg sorption

The results of influence of various parameters on Hg removal process are depicted in Fig. 1. Mercury removal increased as the amount of the biosorbent increased. The rise in mercury removal was rapid up to 25 mg/system biomass concentration thereafter no considerable increase in removal of Hg (Fig. 1a). The loading capacity of Hg increased as high as 200 µg of Hg/mg biomass, beyond which the removal pattern did not change and it got steady with increase in the initial Hg concentration (Fig. 1b). This is supported by the fact that the concentration of metal and availability of metal binding sites are directly proportional and limit their interaction after certain concentration of either metal or biomass (Al-Homaidan et al. 2014). The percent sorption of Hg gradually increased to a certain limit starting from 5 to 60 min. The maximum mercury removal was attained at 60 min of the contact time. It remained almost constant with further increase in contact time (Fig. 1c), which could be explained by non-availability of sorption sites on biomass after exposure of certain contact time (Huang and Lin 2015; Akpomie et al. 2015). Reaction pH is one of the most important parameter, as at lower pH, the protonation of surface reduces the sorption of metal

Table 1 Mercury removal in multimetal system

Metals (mg/L)	Loading capacity (μg metal/mg biomass)			
	Samples			
	1	2	3	4
Cu	–	–	31.52	44.04
Ni	–	–	1.04	2.34
Zn	–	–	21.2	12.78
Fe	–	4.4	27.4	11.56
Hg	72	60	48	54

ions. Increase in the pH removes protonation of the surface and allows it to attain negative charge, which increases the affinity of metal ion to get adsorbed on the surface of biomass (Huang and Lin 2015; Akpomie et al. 2015). *B. licheniformis* showed the highest Hg removal at pH 7. Fall in metal sorption was observed on either side of pH 7.0 (Fig. 1d), which could be due to lack of interaction between groups present on biomass and metal (Pabst et al. 2010). All the pretreatments to biomass proved detrimental and they lowered down the sorption capacity as compared to untreated biomass. The biomass showed decline in Hg removal from 13.29 to 38.29% when treated with physical and chemical agents. Amongst the selected treatments, the alkaline treatment given to biomass using NaOH showed 69% Hg removal, which was 3.09% less as compared to untreated biomass. Therefore, in all sets of experiment, the untreated biomass was used. The observed decrease in mercury removal after organic solvent treatment may be due to the complexity of reactions with a wide variety of reactive groups present on cell wall (Fig. 1e). There are many reports on enhancement of metal sorption with alkaline treatments and decline in metal sorption with acid treatments (Sar et al. 1999; Javaid et al. 2011; Alluri et al. 2007; Yan and Viraraghavan 2000; Puranik and Paknikar 1999). The reported negative influence of alkaline treatment in this study might be the result of solubilization and subsequent loss of biomass components having mercury binding sites. Kapoor and Viraraghavan (1998) have reported 45% decline in adsorption of Ni(II) ions due to treatment with NaOH. They stated that deproteination reduced metal retention and reported the unacceptable loss of biomass due to caustic treatments. Therefore, treatment with alkali required strict control so as to prevent loss of biomass.

Desorption study

Desorption study also revealed the adverse effect of acid on sorption of Hg as acids help in desorption not in sorption (Roy et al. 1993; Saeed et al. 2005). As it can be seen from

the Fig. 1f, a maximum of 49.36 and 33.8% Hg eluted out with 0.1 N HCl and 0.1 N HNO₃ when Hg-loaded biomass was subjected to desorption. The desorption was observed to be less with alkaline solutions as compared to the acidic solutions, which supports the previous data of decreased sorption at acidic pH and pretreatment of biomass with HCl and HNO₃ (Sar et al. 1999). Thus, it is clear that the acidic pH and acids interfere with the metal sorption, but helps in desorption.

Adsorption isotherms

As observed, from isotherm studies, the maximum sorption capacity (q_{max}) predicted by Langmuir isotherm data was 74.2 mg/g. K_f obtained from Freundlich model was 1.4 mg/g indicated the better adsorptive capacity of metal from the solution (Table 2).

The values of $n > 1$, suggested the favourability of Hg(II) sorption by *Bacillus licheniformis* dried biomass. The observation of R^2 values suggested the suitability of Freundlich isotherm model as compared to Langmuir isotherm model. The Langmuir model represents monolayer sorption, whereas Freundlich isotherm deals with heterogeneous surface sorption (Dave et al. 2012; Langmuir 1916; Freundlich 1906). These results represent the multilayer coverage of biosorbent molecules at studied Hg concentration (Fig. 2a, b).

Kinetic models

Kinetic model parameters (Table 2) were evaluated by calculating the rate constant and linearity as indicated by correlation coefficient (R^2). As observed from Fig. 3a–c, Hg(II) sorption by *B. licheniformis* showed greater linearity when modelled to pseudo-second-order kinetic model than the pseudo-first-order kinetic and Elovich kinetic models. The correlation coefficient values obtained from the pseudo-first-order model and Elovich kinetic model were lower as compared to that of pseudo-second-order kinetic model.

Fig. 1 Influence of various parameters on Hg sorption: **a** biosorbent concentration. **b** Sorbate concentration. **c** Contact time. **d** pH. **e** Pretreatment. **f** Eluent treatment. *Significant, ***most significant, ns = non-significant. D.W. = distilled water

Diffusion process

The external mass transfer and intraparticle diffusion models (Weber–Moris plot) were studied to investigate the diffusion mechanism of Hg(II) biosorption. It was observed that the plots were neither linear nor passed through the

origin (Fig. 4a, b). Thus, it can be inferred that intraparticle diffusion and external mass transfer were not the sole rate-limiting factors for biosorbent under study. Ho and McKay (2003) and Das et al. (2013) have shown that if the intraparticle diffusion is the sole rate-limiting step, then it is essential for q_t versus $t^{1/2}$ plots to pass through the origin.

Table 2 Rate constants of isotherm, kinetic models, external mass transfer, and intraparticle diffusion for Hg removal by SR 5

Metal concentration (mg/L)		Isotherms				
		Langmuir		Freundlich		
		<i>B</i>	<i>R</i> ²	<i>n</i>	<i>K_f</i>	<i>R</i> ²
5–50		11.79	0.748	2.5	1.4	0.923
<i>C_o</i> (mg/L)		Kinetic models				
		First-order kinetics		Second-order kinetics		Elovich kinetic model
		<i>k</i> ₁ (mg/g/min)	<i>R</i> ²	<i>k</i> ₂ (mg/g/min)	<i>R</i> ²	<i>α</i> <i>β</i> <i>R</i> ²
5		1.3	0.936	1	0.988	2.36 0.1 0.764
10		1.41	0.986	1.2	0.989	0.46 4.16 0.652
25		1.29	0.982	2.3	0.991	0.12 0.65 0.511
50		1.42	0.891	2.4	0.996	0.1 3.44 0.683
Metal concentration (mg/L)		Diffusion process				
		External mass transfer		Intraparticle diffusion		
		<i>β_LS</i>	<i>R</i> ²	<i>K</i>	<i>R</i> ²	
5		11.8	0.936	6.25	0.811	
10		14.9	0.986	3.13	0.587	
25		14.4	0.987	0.79	0.652	
50		12.1	0.891	0.3	0.648	

Fig. 2 Application of adsorption isotherms to experimental data: **a** Langmuir isotherm. **b** Freundlich isotherm

According to Rao et al. (2010), if the plot is nonlinear or linear, but does not pass through origin, then the process is controlled by film diffusion or chemisorptions.

SEM analysis

The SEM and EDS spectral images of metal loaded biomass showed the altered cell morphology after biosorption as compared to the unloaded biomass (Fig. 5). Swelling and deformation on the surface are also reported upon adsorption of metal (Ghoneim et al. 2014). The EDS analysis showed the peaks of metal ions as an evidence of

binding of metal ions on the biomass. Metal ions peaks were found to be removed due to desorption.

Stoichiometry analysis

Acid digestion experiment suggested the stoichiometry of metal adsorbed and left unadsorbed. In this study, 2500 µg Hg was incorporated in the system. Total 2125 µg Hg was estimated from the control flask (without biosorbent). Residual Hg was 900 µg, while Hg from acid digested biosorbent was found to be 1075 µg resulting in total gap of 150 µg (7.05%). The observed anomaly in the metal concentration may be due to no detection of all salts of Hg

Fig. 3 Kinetic models: **a** pseudo-first-order equation and **b** pseudo-second-order equation. **c** Elovich model

Fig. 4 **a** Intraparticle diffusion plot. **b** External mass transfer

or may be due to volatility of Hg in its oxidized state during the digestion process.

Conclusion

Amongst the studied EPS-producing marine isolates, *Bacillus licheniformis* found to be the best organism for Hg removal. It indicates the effective, eco-friendly, and

efficient sorption of Hg(II). The studied biosorbent has maximum loading capacity as high as 200 $\mu\text{g}/\text{mg}$ of biomass under the experimental setup. Biosorbent:bi sorbate ratio of 1:10 was found to be optimum for sorption. Sorption process fits Freundlich isotherm model and follows pseudo-second-order rate kinetics. Desorption of Hg from the loaded biomass was about 88%. The obtained results suggest that the *B. licheniformis* could be used for remediation of Hg from contaminated water.

Fig. 5 SEM–EDS profile of biomass: **a** Untreated biomass, **a'** EDAX of untreated biomass. **b** Hg-treated biomass, **b'** EDAX of Hg-treated biomass. **c** Desorbed biomass, **c'** EDAX of desorbed biomass

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Compliance with ethical standards

Conflict of interest We do not have any conflict of interest for the research carried out and data presented in this paper.

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