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Adsorption isotherms, kinetics and thermodynamic studies towards understanding the interaction between a microbe immobilized polysaccharide matrix and lead

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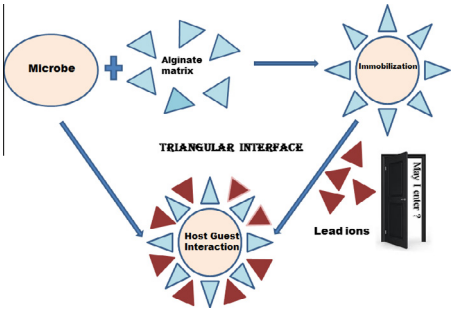
HIGHLIGHTS

- Novel *Halomonas BVR 1* strain was immobilized in sodium alginate.
- Characterization of the strain was studied in detail.
- The immobilized bacteria has an adsorption capacity of 9.68 mg g^{-1} for lead.
- Over expression of proteins under metal stress condition in bacteria.

GRAPHICAL ABSTRACT



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ABSTRACT

This work demonstrates the efficacy of a bacterium *Halomonas BVR 1* strain (isolated from an electronic industry effluent) immobilized in sodium alginate primary host matrix for enhanced adsorption of lead. The immobilized microbe-polysaccharide combination leads to an increase in the number of metal binding groups and act as a secondary host, thereby aiding in an overall improvement of metal adsorption. The characterization of the immobilized adsorbent was done through FT-IR and SEM–EDAX techniques. The obtained results suggest a physicochemical interaction between the lead ion and the microbe immobilized sodium alginate beads. The effect of various analytical parameters on the adsorption of lead was studied in detail. Lead was quantitatively adsorbed in the pH range 8–10 in accordance with pseudo second order kinetics and Langmuir isotherm model. Thermodynamic parameters were calculated and the adsorption process was found to be spontaneous and exothermic. The over expression of proteins in the bacteria under metal stressed condition has also been depicted in this work.

1. Introduction

There is a steep increase in the electronic goods production in order to meet the growing global demands [1,2]. Electronic industry is one among the major contributors of heavy metal pollution to the environment [3]. The release of untreated effluents to the environment is a matter of concern in view of the metal bioaccumulation tendency in the food chains [4]. Lead is one such hazardous heavy metal emanating from the electronic industry that affects the environment [5]. Hence, removal of these heavy metals from the effluents is of utmost importance. However, effective treatment options are still limited. Therefore, viable greener remediation techniques involving the use of microbial biomass for the removal of toxic metals assume considerable importance.

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The survival of microbes in a polluted environment depends on their structural and physiological properties, biochemical characteristics and genetic adaptations [6]. Microbes develop resistance to the habitat by several mechanisms such as bioaccumulation, biotransformation, adsorption etc., and these processes could be exploited for heavy metal removal [7]. The complex structure of the bacterial cell wall consisting of metal binding groups such as carboxylates, amines, sulfhydryl and phosphates also aids in the interaction of metal ions [8]. Intracellular accumulation, adsorption or complex formation on the cell surface could also be involved in metal binding [9].

Some of the existing methods for heavy metal removal have certain drawbacks such as secondary pollution, high sludge generation and capital investment [10]. Hence, biosorption processes are more commonly used because of their added advantages in terms of cost, less sludge production and facile regeneration of the biomass [11]. Several bacterial, fungal and algal biomasses [12–16] have been used as cation collectors for the adsorption processes. Biosorption of lead (II) from aqueous solutions by non-living algal biomasses *Oedogonium* sp. and *Nostoc* sp. has been reported [17]. Prasad et al. [18] have shown the involvement of intracellular seed proteins towards cadmium binding.

Microbial biomass cannot be used independently for a continuous scale up operation [19] and hence suitable immobilization techniques ought to be developed. The entrapment or immobilization techniques involves the confinement of microbial cells in a three dimensional mode in the lattice. The immobilization of microbe on a suitable host matrix simplifies the treatment of liquid waste and fosters the treatment of effluents to larger volumes by column studies [20,21]. Immobilization thus allows free diffusion of the metal ions in and out of the lattice and ensures effective removal of lead from the aqueous solutions [22].

Halomonas BVR1 was reported for the first time by our group as a novel strain for the adsorption of cadmium [23]. The success achieved using this strain prompted us to explore its utility for immobilization in a polysaccharide matrix for the adsorption of lead. Although, several immobilization techniques are known for heavy metal adsorption, there are no literature reports till date concerning the immobilization of *Halomonas BVR1* in a polysaccharide matrix such as sodium alginate. Alginate consists of a linear chain of (1–4) linked residues comprising D -mannuronic and guluronic acids and serves as an effective host to immobilize or encapsulate different cells [24,25]. The present study deals with the comparison between the use of the *Halomonas BVR 1* strain (free cells) and its subsequent immobilization in sodium alginate to achieve an enhanced lead removal.

2. Materials and methods

2.1. Isolation of bacteria from effluent

The microorganisms were isolated from an electronic industry effluent containing heavy metals. The effluent samples were collected from the entry points of Common Effluent Treatment Plant (CETP) and Reverse Osmosis (RO) units of an electronic industry. *Halomonas BVR 1* microbe was isolated and selected based on its high level of resistance to different heavy metals. The detailed processes of isolation and their biochemical and molecular characterisations have been reported previously [23].

2.2. Minimum inhibitory concentrations (MIC) of lead

The metal tolerance was evaluated through turbidometric analysis by varying the concentrations of lead ion that prevented the growth of bacteria [26,27]. The sorption efficiency of *Halomonas BVR 1* was tested against lead and the lowest concentration of lead that prevented the bacterial growth was considered as the MIC. Varying concentrations of Pb (II) ranging from 50 mg L^{-1} to 450 mg L^{-1} prepared using lead nitrate (Merck) were added to the nutrient broth. About 1 mL of 1.7×10^5CFU/mL concentration of culture was inoculated into 50 mL of the above mentioned solutions and incubated at 37 °C for 24 h. After the incubation period, triplicate measurements of the optical density (OD) values were recorded at 600 nm to check for the bacterial growth.

2.3. Protein expression assays in heavy metal-resistant microorganisms

The proteomes from the *Halomonas BVR 1* were extracted by sonication process. The control set of samples (bacterial cells without the exposure of lead) and the treated set (bacterial cells with the exposure of lead) from mid-log phases of cellular growth (Optical Density 0.3–0.4) were taken and centrifuged separately at 7300 rpm for 10 min. Subsequently, the cells were suspended in 100 mM Tris HCl (pH 8.0) buffer for lysis. The sonication process was then carried out for 8 min at 40 s pulse with a 30 s break time and the pattern of proteomic expression was analyzed by 12% SDS–PAGE using Laemmli's method [28].

2.4. Entrapment of cell suspension in sodium alginate beads

The microbial strain isolated from the effluent was inoculated into 100 mL LB medium containing 0.5 mol L^{-1} NaCl and incubated at 37° C for 48 h till they reached the exponential phase [23]. The cell suspension was harvested by centrifuging a 100 mL culture at 7200 rpm for 10 min. The pellet was washed twice with Milli Q water and then resuspended in 5 mL of autoclaved Milli Q water. Equal volumes of this cell suspension were mixed with 0.5% (w/v) sodium alginate (Himedia-MB-114 (Molecular Biology grade) and stirred for an hour [29]. This mixture of the cell suspension and sodium alginate was added dropwise into 1% calcium chloride solution and maintained for 2 h at room temperature. The beads obtained were dried at 90 °C and 0.4 g of the immobilized bacterial strain was used for batch sorption studies.

The adsorption capacity of the free *Halomonas BVR 1* strain and microbe immobilized sodium alginate beads can be obtained using the equation

$$q_e = \frac{(C_o - C_e)V}{W} \quad (1)$$

where V is the volume of aqueous phase and W is the weight (g) of the adsorbent used in the batch adsorption study, C_o and C_e are the initial and final concentrations of lead in solution.

2.5. Surface characterisation of the adsorbent

The surface morphology of the sodium alginate beads immobilized with *Halomonas BVR 1* strain was analyzed through SEM–EDAX and FT-IR techniques.

The infrared spectra of the *Halomonas* strain immobilized in sodium alginate was obtained using a Fourier Transform IR Spectrometer (Jasco 4200) in order to investigate the functional group changes in the adsorbent before and after adsorption. The surface images of the adsorbent were taken using a Scanning Electron Microscope (SEM). The EDAX spectral analysis was used to ascertain the presence of lead after adsorption.

An Li- 127 model pH meter (Elico, India) was used for pH optimization. An HI98185 ion meter equipped with lead ion selective electrode (Hanna Instruments, USA) was used to measure the concentration of lead. The amount of lead adsorbed was also