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Biosorption of cadmium using a novel bacterium isolated from an electronic industry effluent

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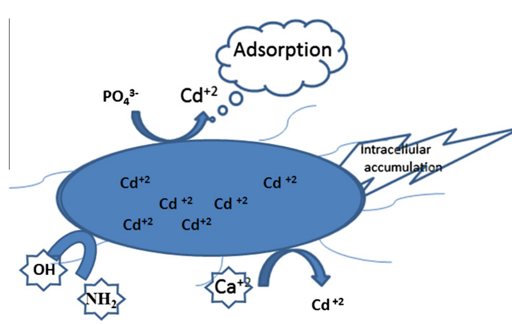
HIGHLIGHTS

- Novel *Halomonas* strain was isolated from electronic industry effluent.
- Characterization of the strain was studied in detail.
- The bacterium has an adsorption capacity of 12.023 mg g^{-1} for cadmium.
- Removal of cadmium is by surface adsorption.
- The adsorption suits Langmuir isotherm and pseudo second order models.

GRAPHICAL ABSTRACT



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ABSTRACT

Heavy metal clean-up from electronic industry effluents is an important issue to be addressed. In this work, we report for the first time the isolation of a novel bacterial strain of *Halomonas* from electronic industry effluent and its subsequent utility for the adsorption of cadmium. The microbial community was characterised using various biochemical and molecular techniques. The bacterial surface possessing hydroxyl, carboxyl and amino groups play a vital role in this host guest interaction. Three strains of *Halomonas* species were isolated from these effluents, among which *Halomonas BVR 1* was found to be an excellent host to the divalent cadmium. The influence of various analytical parameters on the adsorption of cadmium was studied in detail. The surface characterization of the adsorbent was done through FT-IR and SEM–EDAX techniques. Adsorption thermodynamics was spontaneous and exothermic. *Halomonas BVR 1* sp. was found to be highly resistant to cadmium with a minimal inhibitory concentration of 250 mg L^{-1}. Cadmium was quantitatively adsorbed above pH 8 in accordance with the pseudo second order kinetics and Langmuir isotherm model with an adsorption capacity of 12.023 mg g^{-1}. The obtained results indicated that *Halomonas BVR 1* species is very effective in adsorbing cadmium thereby opening its doors to the clean-up process of heavy metals from electronic industry effluent.

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1. Introduction

The increasing impact of information technology, leading to an increment in production of electronic goods is a rising global problem causing environmental pollution. Electronic sector is one of the prime contributors of heavy metals to the environment. Electronic manufacturing units involve the manufacture of passive components such as resistors, capacitors, semiconductor components, printed circuit boards and wiring assemblies [1,2]. Environmental issues related to this industry gain impetus since they involve hazardous wastes like heavy metals, cyanides, sulphates and phosphates [3]. Leaching of these toxic ingredients due to the indiscriminate dumping results in land and water

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pollution [4]. The presence of the heavy metals such as cadmium, chromium, lead, zinc, copper and mercury in the environment pose a major threat in view of their bioaccumulation tendency and toxicity [5]. Hence, removal of these heavy metals from the environment is of paramount importance. Cadmium is one such hazardous heavy metal which does not degrade easily [6,7], and is known to bind to the essential respiratory enzymes leading to oxidative stress and cancer [8,9]. Conventional chemical processes such as precipitation, ion exchange, chemical oxidation and reverse osmosis are known for the removal of these heavy metals. However, the overall process involves huge capital for its operation and is influenced by several criteria and therefore there is a need for an economic and safe approach towards remediation of heavy metals. In this regard, use of microorganisms for the removal of heavy metals gains significance [10–12]. Thus bioremediation techniques provide an effective and alternate strategy to accelerate the clean-up processes [13,14]. Bacteria and other microorganisms help in catalysing various chemical reactions by way of their physiological processes [15]. Microbes possess the ability to bind to metal ions present in the external environment, at their cell surface through the carboxyl, hydroxyl and amine functional groups [16]. The metal adsorption could involve a surface phenomenon through physisorption or ion adsorption on the surface of dead or live bacteria. Alternatively, microorganisms could also accumulate these metals quite slowly according to their metabolic activity [17–19]. They develop resistance mechanisms to these toxic heavy metals and hence heavy metal resistant microorganisms has paved the way to alleviate the problems of heavy metal contaminants in various effluents [20]. Studies involving the use of various fungi and yeasts for the removal of toxic metals like lead and cadmium have been reported [21]. Kirkelund et al. [22] recently reported the electrodialytic removal of cadmium from biomass combustion fly ash. Reports suggest the use of *Bacillus subtilis*, *Rhizopus arrhizus*, *Saccharomyces cerevisiae*, algae and various halophilic bacteria for the adsorption of heavy metals from industrial wastes [23]. All these studies illustrate that cadmium is known to bind to the microbial cell surface through various biosorption mechanisms [24,25]. Hence, isolation of heavy metal resistant microorganisms for environmental remediation assumes considerable importance. To the best of our literature survey, there are no reports till date on the isolation and characterization of bacterium from e-waste effluents for heavy metal removal. Herein, we report for the first time a novel heavy metal resistant bacterial strain which is capable of removing cadmium as high as 80 mg L^{-1} cadmium.

2. Materials and methods

2.1. Collection, characterisation and isolation of microbes from effluents

The effluent samples were collected from various discharge areas involving the access sites at the Reverse Osmosis (RO) unit and the Common Effluent Treatment (CET) points in the electronic industry located near Hyderabad, India. In the collected effluent cadmium is present in the range of 1–10 mg L^{-1}. The collected sample was stored at 4 °C before analysis. Analytical grade chemicals were used in the preparation of the required medium for the growth of microorganisms. The reagents were procured from Himedia laboratories, Sigma Aldrich and New England Biolabs. Bacterial strains were isolated from the effluents using Luria bertani medium (LB medium). LB broth was prepared by using Caesin enzymic hydrolysate (10 g L^{-1}), Yeast extract (5 g L^{-1}) and Sodium chloride (5 g L^{-1}) and 1.5 g agar for 100 mL medium. To isolate strains, standard pour plate technique was performed for which the serially diluted effluents were plated onto a LB agar plate. The inoculated plates were incubated at 37 °C for 48 h. After the incubation period distinct colonies were isolated from each plate and subjected to the morphological, biochemical and molecular characterisation.

2.2. Identification of bacteria

Out of the twelve colonies obtained on the plate, three isolates were selected based on their unique yellow cream spherical morphology and labelled as *BVR 1*, *BVR 2*, and *BVR 3*. These strains were analysed by morphological (Grams reaction, motility, and presence of endospores) and biochemical characterizations involving catalase test, carbohydrate fermentation, indole tests, methyl red/voges proskauer, citrate utilization and starch hydrolysis tests in accordance to Bergey's Manual of Determinative Bacteriology [26–28].

2.3. Minimum inhibitory concentrations (MIC) of heavy metals and antibiotic resistance of the isolates

The MIC of the cadmium metal ion was determined by turbidometric analysis [29,30]. The lowest concentration that prevented the bacterial growth was considered as the MIC. Stock solutions of the metal salt ($CdCl_2$) were added to the nutrient broth in various concentrations ranging from 50 mg L^{-1} to 250 mg L^{-1}. Approximately 1.5×10^5 CFU/mL cells were inoculated into each of the tubes. After incubation at 37 °C for 24 h the optical density was measured at 600 nm to measure the bacterial growth. The susceptibility of microorganisms to antimicrobial agents was carried out using the disc agar-diffusion method [31]. Resistance to Penicillin (10 units/disc) Ampicillin (10 mcg/disc), Chloramphenicol (30 mcg/disc), Tetracyclin (30 mcg/disc) and Streptomycin (10 mcg/disc) was determined on LB agar plates using antibiotic sensitivity test. Zone of inhibition was noted after 48 h of incubation. This zone of inhibition was compared with the standard interpretative chart of antibiotic susceptibility given by Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). These experiments were performed in triplicates. Among the three strains, *BVR 1* strain was found to be most resistant to cadmium, hence it was chosen for the present study and further confirmed by molecular characterisation.

2.4. Molecular characterisation

2.4.1. Isolation of genomic DNA

Genomic DNA was isolated by the standard DNA extraction procedure [32]. Isolated microorganisms were grown in 5 ml LB medium at 37 °C for overnight by constant agitation. The culture was spun down and DNA pellet was lysed using GET buffer (Glucose-20%, Tris-50 mM and EDTA-50 mM). This was followed by lysozyme treatment at 37 °C for 30–40 min. Subsequently extraction was carried out using 10% SDS at 37 °C for 1–2 h. Extraction, by adding equal volumes of PCI (Phenol:Chloroform:Isoamylalcohol) was done and the lysate was then subjected to ethanol treatment and the precipitate was dissolved in 30 μL of TE (Tris–EDTA buffer). Isolated Genomic DNA was visualized using 1% agarose gel electrophoresis.

2.4.2. PCR amplification of 16S rDNA

Amplification of 16S rDNA were carried out using universal primers (27 F 5′-AGAGTTTGATCMTGGCTCG-3′ and 1492R 5′-GGTTACCTTGTTACGACTT-3′) by using genomic DNA as a template [33,34]. 100 ng/μL of the extracted DNA was used as a

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