Bioresource Technology 127 (2013) 49–58

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Synergism of *Pseudomonas aeruginosa* and Fe^0 for treatment of heavy metal contaminated effluents using small scale laboratory reactor

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HIGHLIGHTS

- ▶ It shows the feasibility of developing microbial bioremediation technology.
- ▶ Present work help in the identification of the opportunity of the effluent treatment in the industrial complexes.
- ▶ Scale up demonstration of small scale laboratory reactor to industrial scale reactor for industrial effluent treatment.
- ▶ This study leads towards the development microbial technology for precocious metals recovery from the waste stream.

ARTICLE INFO

Article history:
Received 4 July 2012
Received in revised form 8 September 2012
Accepted 15 September 2012
Available online 25 September 2012

Keywords:
Common Effluent Treatment Plant
Bioreactor
Effluent treatment
Pseudomonas aeruginosa
Wastewater

ABSTRACT

In this study *Pseudomonas aeruginosa* a metal tolerant strain was not only applied for heavy metal removal but also to the solublization performance of the precipitated metal ions during effluent treatment. The synergistic effect of the isolate and Fe^0 enhanced the metal removal potential to 72.97% and 87.63% for Cr(VI) and cadmium, respectively. The decrease in cadmium ion removal to 43.65% (aeration + stirring reactors), 21.33% (aerated reactors), and 18.95% (without aerated + without stirring) with an increase in incubation period not only indicate the presence of soluble less toxic complexes, but also help in exploration of the balancing potential for valuable metal recovery. A relatively best fit and significant values of the correlation coefficient 0.912, 0.959, and 0.9314 for mixed effluent (Paint Industry effluent + CETP Wazirpur, effluent), CETP, Wazirpur, and control effluents, respectively, indicating first-order formulation and provide a reasonable description of COD kinetic data.

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1. Introduction

The contamination of the environment with hazardous and toxic compounds such as heavy metal is one of the major problems which the industrialized nations are facing these days (Masoudzadeh et al., 2011). There are several industrial processes which can generate wastes containing heavy metal ions, the most common examples are the industries with electronic components, electroplating, metal mechanic processing, and mining (Mockaitis et al., 2012). Heavy metal, such as chromium (Cr) and cadmium (Cd) are released into the environment with industrial and domestic wastewater discharge. Due to high mobility and toxicity of hexavalent chromium as compared to trivalent chromium has gained more importance (Singh et al., 2011). The presence of cadmium in water and soil, even at very low concentrations is a serious environmental problem and may cause severe health problems like decalcification, arterial-hypertension, and anemia (Shiwen et al., 1990). Treatment of wastewater containing heavy metal ions is one of the growing requirements in environmental cleaning (Atar et al., 2012).

A wide variety of living and nonliving biological materials are capable of removing toxic and precious metals from the waste streams and offer an economical and effective alternative for sorption technologies (Vijayaraghavan and Yun, 2008). Biosorption by passive binding to microorganisms (bacteria, fungi, and algae) has more potential for treating industrial effluents due to environmental friendly, less costly, elevated metal binding capacity, less sludge generation, and high removal efficiency from diluted effluents (Marcano et al., 2009; Volesky and Mayphillips, 1995). The studies performed with chromium (VI) bioaccumulating microorganisms (Donmez and Koc-Berber, 2005; Dursun et al., 2003) have

Abbreviations: CETP, Common Effluent Treatment Plant; ORP, oxidation reduction potential; COD, chemical oxygen demand; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; Cr(VI), hexavalent chromium; Cr_{add}, Cr added; Cr_{rem}, Cr removal; sCOD, soluble chemical oxygen demand; lpm, liter per minute; SVI, sludge volume index.

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http://dx.doi.org/10.1016/j.biortech.2012.09.056

shown that microbial Cr (VI) removal from solutions typically includes the following stages: (1) the binding of chromium to the cell surface, (2) translocation of chromium into the cell, and (3) reduction of chromium (VI) to chromium (III) two reaction steps have been suggested to involve in the reduction reactions (Suzuki et al., 1992). First Cr(VI) accepts one molecule of NADH and generates Cr(V) as an intermediate (Eq. (1)), and then Cr (V) accept two molecules of electron to form Cr (III) (Eq. (2)).

$$Cr^{+6} + e^{-} \rightarrow Cr^{+5} \quad (1)$$

$$Cr^{+5} + 2e^{-} \rightarrow Cr^{+3} \quad (2)$$

NADH, NADPH, and electron from the endogenous reserve are implicated as electron donors in the Cr (VI) reduction process (Appenroth et al., 2000). The recent studies suggested that Cr (VI) reduction by some bacterial strains (e.g., *Pseudomonas* sp. G1DM21 and *Pseudomonas putida*) leads to the production of soluble Cr (III) end products and not Cr $(OH)_3$ precipitates (Compos et al., 1995; Desai et al., 2008; Puzon et al., 2005). The solubility of Cr (III) species can be significantly enhanced by strong interaction of Cr (III) with microbial exudates (e.g., exopolymeric substances (EPS), citrate, alginate) in the natural system (Cetin et al., 2009; Priester et al., 2006; Puzon et al., 2005). The development of new and innovative bioremediation technologies is, therefore, crucial to achieve cleanup goals at contaminated sites and ensure an abundant source of safe water for future generations (O'Carroll et al., 2012).

Generally, the bioremediation rate of living microorganisms strongly dependent on the population of the cells (Guo et al., 2010). In order to understand the metal binding mechanism with microorganism it is essential, to purify the isolate and the latter is applied for investigation in small scale reactors. The small scale reactors were selected so that we could control allthe influential environmental parameters in well-organized manners. The optimum conditions evaluated under flasks study were applied in small scale reactors, which can further be applied in pilot scale reactors. This reactor study not only help to explore the applications of the isolated microbes in the toxic wastewater treatment study but also aided in understanding the mechanism of the metals bioaccumulation.

2. Methods

2.1. Microbes and their sources of isolation

2.1.1. Isolation of microbes

The soil samples for the isolation of the microorganisms were collected in the sterilized zip lock polybags to prevent environmental contamination. The samples were immediately transported to the lab and maintained below 4 °C, till their utilization.

The metal resistant microbes were isolated from metals contaminated samples collected from various sites through enrichment culture techniques. For isolation of microbes, 1 g of contaminated soil sample was inoculated in a 250 ml Erlenmeyer flask containing 100 ml of broth medium amended with 100 mg/l Cr(VI) concentration. Nutrient Agar medium (g/l): Peptone 5.0, NaCl 8.0, Agar 15, Beef extract 3.0, pH 7.0, and Tris minimal medium (Michel et al., 1986) modified as (g/l): Tris base 6.0, KH_2PO_4 0.67, $(NH_4)_2SO_4$ 4.0, KCl 0.62, $MgSO_4$ 0.063, $FeSO_4$ 0.003, Glucose 6.0 were used for the isolation and further metals removal study, respectively.

During screening, growth was observed at shaking speed of 120 rpm, incubation temperature 37 °C and pH 7.0. The harvesting time for the microbes during the screening was kept 72 h. The serial liquid dilutions were used in isolation attempts using the standard spread plate method. The different dilutions ranging from $10^{-1}$to 10^{-6} and 20–30 ml sterilized medium were taken into petri plates for bacterial isolation under sterilized conditions. Other essential requirements were also sterilized in the autoclave for 15 min at 15-psi pressure. Hundred microliters of all serial dilutions were spread on solidified agar-plated medium and grown at 37 °C and purified using standard isolation techniques. The particular healthy small colony was selected and streaked starting from top of petri plate and streaked to the whole of the plate in the zig-zag manner. By repeating the process, pure culture of desired isolate was achieved and it was preserved on slants at 4 °C. These isolates were characterized and further employed for heavy-metal removal and tolerance studies.

Isolated and purified bacterial strain was identified based on biochemical and morphological characteristics from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh (India).

2.1.2. Bacterial inoculums

A loop of the isolate was inoculated into 250 ml conical flask containing 100 ml sterilized Nutrient Broth. The inoculated flasks were incubated ina BOD shaker cum incubator at 120 rpm for 48 h at 37 °C. Optical density (DO) was measured using a UV spectrophotometer at 620_{nm}. The culture grown almost fully within 48 h and 1% freshly grown culture was used as inoculum.

2.2. Chromate reductase enzyme assay

The samples withdrawn from the reactors were also used for Cr(VI) reductase enzyme assay as described by Elangovan et al. (2006). The samples for the enzyme assay were centrifuged at 7500 rpm at 4 °C for 10 min in 1.5 ml centrifuge tubes. Chromate reductase activity was determined by measuring the decrease in Cr(VI) by the s-diphenylcarbazide method in the presence of NADH as an electron donor. The assay was carried out in 1.5 ml reaction mixture containing 100 µl crude enzyme, 500 µl Cr(VI) (5–10 µg/ml), 500 µl NADH (0.1 mM), and 400 µl phosphate buffer (pH 6). The assay mixtures were incubated at 37 °C for 30 min. The enzyme blank was carried out without NADH to find out the residual hexavalent chromium in the medium. Concentrated H_2SO_4 (0.1 µl) was added to reaction mixture and was diluted to 10 ml with double distilled water. To find residual hexavalent chromium, 0.2 ml of the 1,5-diphenylcarbazide reagent (dissolved in acetone) was added to final 10 ml reaction mixture and kept for developing color before taking $OD540_{nm}$. One unit of the enzyme activity is defined as the change (µg/min) in Cr(VI) at 37 °C.

2.3. Analytical methods

Total heavy metal ions concentration was analyzed using AAS 6300. The Cr(VI) concentration was analyzed by 1,5-diphenylcarbazide methods at 540 nm with a UV/VIS spectrometer. COD was determined using the dichromate COD Titrator Model CT-15 based on the use of measurement for high-range COD (0–500 mg/l) using potassium hydrogen phthalate solutions as a standard. The COD results were then corrected by adding the oxygen corresponding to the added chromium. The masked COD due to the presence of Cr(VI) in the sample was calculated using following equation (Trunfio and Crini 2010).

$$COD = Cr(VI) * 3 * 32/52 * 4 \quad (3)$$

where Cr(VI) is the concentration, in mg/l, of hexavalent chromium previously detected in the solution, 52 is the molecular weight of chromium and 32 is the molecular weight of O_2.

pH, ORP, Cr(VI) and protein contents were measured immediately, whereas the filtrate was preserved as per standard methods

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