Errata

"Polish Journal of Environmental Studies"
Vol. 21, No. 6, 2012, 1523-1527

Isolation and Identification of *Achromobacter denitrificans* and Evaluation of its Capacity in Cadmium Removal

Hajar Abyar[1]*, Alireza Safahieh[1], Hossein Zolgharnein[1], Issac Zamani[2]

IT IS:

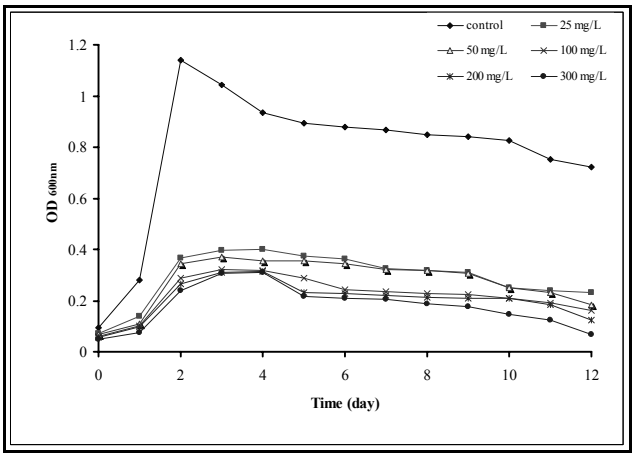

Fig. 3. Biosorption percentages of *A. denitrificans* strain PQ-1 in different concentrations of cadmium.

SHOULD BE:

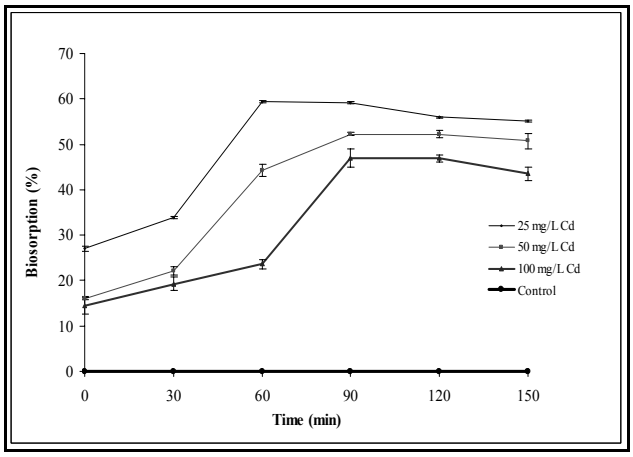

Fig. 3. Biosorption percentages of *A. denitrificans* strain PQ-1 in different concentrations of cadmium.

Pol. J. Environ. Stud. Vol. 21, No. 6 (2012), 1523-1527

Original Research

Isolation and Identification of *Achromobacter denitrificans* and Evaluation of its Capacity in Cadmium Removal

Hajar Abyar[1]*, Alireza Safahieh[1], Hossein Zolgharnein[1], Issac Zamani[2]

[1]Department of Marine Biology, Faculty of Oceanography,
Khorramshahr University of Marine Science and Technology, Iran
[2]Department of Biology, Faculty of Science, Isfahan University, Iran

Received: 14 May 2012
Accepted: 30 July 2012

Abstract

Cadmium is a persistent and toxic contaminant of aquatic ecosystems. The objectives of our present study were to isolate and identify cadmium-resistant bacteria from polluted sediment of Khor Musa (Persian Gulf) and study their capacity in cadmium biosorption. The results showed that among different isolated bacterial species, *A. denitrificans* strain PQ-1 was the most resistant bacteria against cadmium. Results also indicated that metal biosorption by *A. denitrificans* strain PQ-1 is capable of excluding 55.1%, 50.7%, and 43.5% of the cadmium from 25, 50, and 100 mg/l concentrations, respectively. It is suggested that using the biosorption ability of *A. denitrificans* strain PQ-1 could be a low-cost technique in heavy metal removal.

Keywords: *A. denitrificans* strain PQ-1, sediment, Cadmium, biosorption, Persian Gulf

Introduction

Cadmium is a persistent and toxic contaminant of the aquatic ecosystems that is non-biodegradable and causes toxicity to marine organisms as well as humans [1]. It enters the aquatic environment through municipal waste containing plastics and Ni-Cd batteries, sewage sludge, chemical fertilizers, and metal-plating industry wastes [2]. According to Egwurugwu et al. [3], the impacts of cadmium on humans include nervous system damage, destruction of liver and kidney, and metal accumulation in the food chain. The emission of this metal is increasing in the environment and it is necessary to reduce cadmium levels below the standard range [4].

Various methods such as reverse osmosis, ion-exchange, chemical precipitation, and phytoremediation have been offered for heavy metals removal from contaminant effluents or wastes. These techniques, however, have some disadvantages like production of toxic sludge and partial metal removal [5]. In recent years, the use of micro-organisms potential in the removal of heavy metals from polluted wastewater has attracted attention [6-8]. This method is considered as a low-cost, eco-friendly, and effective technique [9]. Many studies have demonstrated that bacteria [10], fungi [11], and algae [12] have the ability to remove heavy metals from contaminated water or waste streams. It is mainly because of the presence of special chemical compounds such as carboxyl, sulfydryl, carbonyl, and hydroxyl in the cell wall of the micro-organisms biomass that are capable of binding metal ions [13, 14].

Urban and industrial developments together with oil sector activities (such as oil exploration, exploitation, and export) are responsible for heavy metal concentration of coastal waters of the north Persian Gulf in Iran. While this area is diverse in terms of aquatic life, less is known about native bacterial species, specially those that are resistant to heavy metals and capable of entrapping metal ions by their cell walls.

*e-mail: Hajar.abyar@yahoo.com

The objectives of the present study were to isolate and identify cadmium resistant bacteria from polluted sediment of Khor Musa (Persian Gulf) and study their capacity in cadmium biosorption from surrounding media.

Material and Methods

Sampling and Isolation of Cadmium-Resistant Bacteria

Sediment samples were collected from Khor Musa located in the northern Persian Gulf. One gram of sediment was added to 10 ml sterile sodium chloride solution 0.85% (w/v). The aliquots of sediments (10^{-1}, 10^{-2}, and 10^{-3}) were spread onto nutrient agar plates containing 10, 50, and 100 mg/l cadmium. Plates were incubated at 30ºC for 96 h. Among bacterial colonies formed on the cultures, the biggest colony was picked and purified [15].

DNA Extraction

DNA extraction was performed using the FTA CARD system. A clone of bacteria was suspended in 100 ml distilled water. Five milliliters of the prepared suspension were added to FTA filters and the filters were dried for 3 h. The filters were added to the Eppendorf tube containing 500 µl sterile water for seconds. Then they were put in another Eppendorf tube again and incubated at 95ºC for 15 min. The Eppendorf tube was centrifuged at 5,000 rpm for 5 min. The upper phase was extracted and frozen at -20ºC [16, 17].

PCR Amplification

PCR analysis consisted of a primary denaturation step at 95ºC for 6 min. The analysis followed by 30 cycles of 45 min at 94ºC, one min at 56ºC, 1.5 min at 72ºC, and one cycle of 7 min at 72ºC. The samples were experimented on gels containing TBE buffer (Tris 89 mM, Boric acid 89 mM, EDTA 2 mM, and pH=7) and the purification of PCR products was done by Silica gel kit (Fermentas Co) [17]. For 16S rRNA analysis, 25 µl of the PCR products had been extracted from the gel sent to Kosar Research Center, Iran.

Growth Measurements

The colony of bacteria was cultured in LB broth medium without cadmium at 30ºC with 160 rpm for 24 h. 10 ml of the medium was centrifuged and bacterial suspension was prepared [18]. In order to measure the growth of bacteria, 3 ml of the bacterial suspension was transferred into 20 ml of LB broth contaminated by 25, 50, 100, 200, and 300 mg/l concentrations of cadmium. An extra sample without the addition of cadmium was prepared as control. Samples were incubated at 30ºC for 12 days. The optical density (OD) of suspensions was monitored during 24-hour intervals. The measurement of optical density was achieved using a Spectrophotometer and 600 nm wave length [19, 20].

Biosorption Experiments

In order to evaluate the capacity of cadmium biosorption by the isolated bacteria, 1 ml of cell suspension (0.03 g of cell dry weight) was added to 100 ml cadmium solutions of 25, 50, and 100 mg/l in 250 ml flasks. The flasks were put on a rotating shaker at 30ºC with 160 rpm. Immediately after bacterial inoculation and then at 30 min intervals up to 150 min, 5 ml of metal solutions were taken and centrifuged at 4,000 rpm for 10 minutes. The supernatants were analyzed to determine cadmium concentration using an atomic absorption spectrophotometer (SavantAAS model).

Results

During the isolation process, the best developing colony was adherent, short rod, small in size, yellow in color, and Gram negative. The 16S rRNA gene sequence of the bacteria was amplified by PCR. Fig. 1 shows the PCR product. The comparison of 16S rRNA sequence with known bacteria sequences based on the BLAST search software determined the strain of studied species as *Achromobacter denitrificans* strain PQ-1.

The growth curve of *A. denitrificans* strain PQ-1 in LB broth is given in Fig. 2. The growth rate of *A. denitrificans* strain PQ-1 was sharply decreased in the presence of cadmium in the cultures. However, the growth rates in media enriched with different concentrations of cadmium were more or less the same. The maximum OD in cultures containing 25, 50, 100, 200, and 300 m/l cadmium were 0.4, 0.37, 0.32, 0.31, and 0.3, respectively. A lag phase was observed in all cadmium-containing cultures at first 24 h. The initial lag phase was followed by a log phase reaching to its maximum level on the 3rd day. Thereafter, a stationary phase appeared between 3rd and 4th days. The bacterial growth in LB broth media not supplemented with cadmium was remarkable and showed a maximum OD of 1.14 after 48 h.

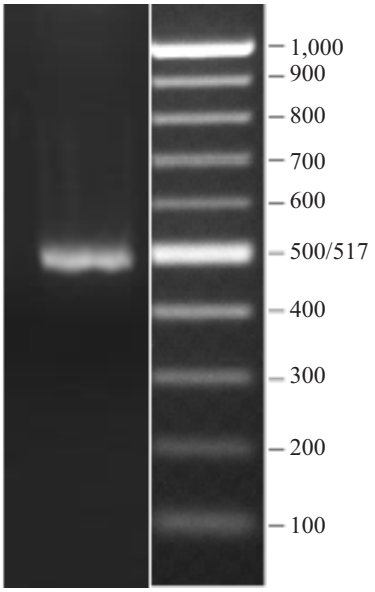

Fig. 1. PCR amplification product of *A. denitrificans* strain PQ-1.

The biosorption of cadmium by *A. denitrificans* strain PQ-1 is illustrated in Fig. 3. The total bacterial biosorption in 25 mg/l cadmium solution was 27% immediately after inoculation. The cadmium sorption in the mentioned concentration reached its maximum percentages (59.3%) after 60 min. The maximum biosorption in other solutions with higher amounts of cadmium was prolonged and appeared after 90 min of incubation. Finally, the percentages of sorbed metal in solutions containing 25, 50, and 100 m/l of cadmium were 55.1, 50.7, and 43.5%, respectively.

Cadmium removal by the bacteria in various cadmium concentrations is presented in Fig. 4. According to the results, the amount of cadmium removed by *A. denitrificans* strain PQ-1 varied between 5.1-16.5 m/l immediately after inoculation. Cadmium removal by the bacteria entered concentrations of 50 and 100 mg/l started at the beginning and continued until 90 min. The removal time for bacteria in concentrations of 25 mg/l (60 min) was shorter than what was observed for 50 and 100 mg/l. After the mentioned removal times, cadmium concentrations did not changed significantly in all solutions for 120 min. Although in 100 mg/l solution the concentration of cadmium started to increase again after this time. At the end of the experiment the final removal of cadmium by *A. denitrificans* strain PQ-1 was 13.8, 25.7, and 43.5 m/l in 25, 50, and 100 mg/l solutions, respectively.

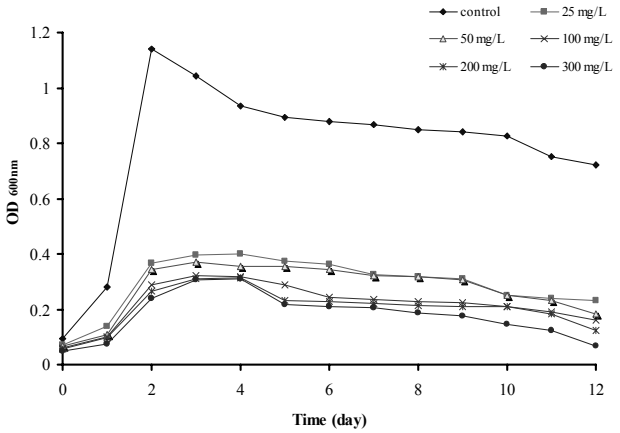

Fig. 2. Growth curve of *A. denitrificans* strain PQ-1 in different concentrations of cadmium.

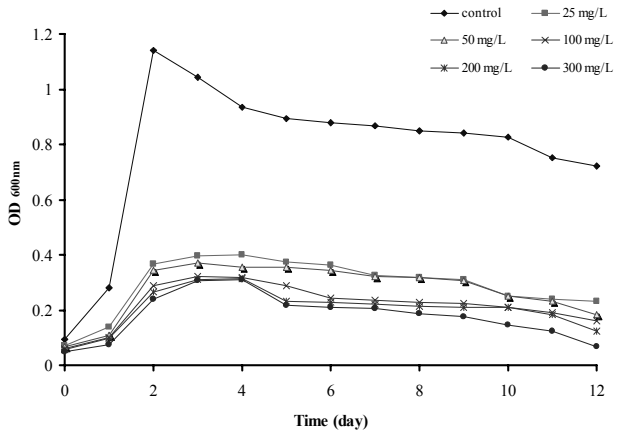

Fig. 3. Biosorption percentages of *A. denitrificans* strain PQ-1 in different concentrations of cadmium.

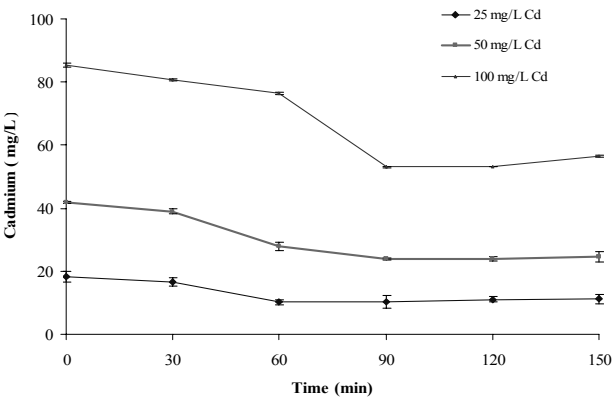

Fig. 4. Cadmium biosorption by *A. denitrificans* strain PQ-1 in different concentrations.

Discussion

Many bacterial species such as *Pseudomonas*, *Bacillus*, *Alcaligenes*, *Geobacillus*, and *Desulfococcus* have been identified as resistant to cadmium [19, 21-23]. However this ability was not previously documented for *A. denitrificans* strain PQ-1. The present study shows that *A. denitrificans* strain PQ-1 is a cadmium-resistant species that could grow in cadmium concentrations up to 300 mg/l. However, it has been reported that three marine bacteria *Pseudomonas putida* PTCC 1664, *Bacillus cereus* PTTC 1665, and *Pseudomonas pseudoalkaligenes* PTCC 1666 could grow in the presence of 80-100 mg/l cadmium [19], and *Alcaligenes eutrophus CH34* is tolerant to 200 mg/l concentration of cadmium [21]. This study showed that *A. denitrificans* strain PQ had better tolerance compared to other studied species.

After 48 hours of incubation the growth rate of *A. denitrificans* strain PQ-1 in control showed OD value of 1.14. This value was markedly higher than OD values found for cadmium exposed bacteria (0.3 to 0.4). This finding means that all cadmium concentrations examined in the present work have been toxic to *A. denitrificans* strain PQ-1, which adversely affected bacterial growth. Similarly, Andreoni et al. [24] showed *Pseudomonas putida* B14 exposed to cadmium concentration of 97 mg/l grow in the lower rate than non-exposed bacteria.

The lag phase observed during the initial 24 h is another sign of cadmium toxicity to *A. denitrificans* strain PQ-1. In fact when a microorganism is inoculated into a new culture containing toxic substances, the cells will damage due to pollutant toxicity. In this condition the microorganism expands energy to repair cell damage and adapt its enzymatic pathway to a new condition [25]. The lag phases at the beginning step of growth could be due to the mentioned adaptation.

Results indicate that the growth of *A. denitrificans* strain PQ-1 was reduced by the increase of cadmium concentration. In the other words, the maximum and minimum growth of cadmium-exposed bacteria were observed in 25 and 300 mg/l concentrations, respectively. Similar results were reported by Kader et al. [26], who revealed that the

growth rate of six Gram-negative and three Gram-positive bacteria are decreased as a result of increasing cadmium concentration in their culture. As the cadmium content of media increases, the amount of metal biosorption by the bacteria increased. Therefore the maximum cadmium biosorption was observed in 100 mg/l. The biosorption of metal ions by the bacterial cell wall is an equilibrium reaction whose direction depends on the concentration of free metal ions in the surrounding media and the amount of metal ions bound to the cell wall. When cadmium concentration in the media increases, the reaction shifts toward production of much bonded cadmium ions [27]. Similarly, Green-Ruize et al. [28] found that the sorption of mercury by *Bacillus* sp. in different initial concentrations increase significantly with increasing metal concentration.

The percentage of cadmium biosorption by *A. denitrificans* strain PQ-1 was increasing from the beginning until 90 min. Thereafter it remained constant finally at 150 min and a slight reduction of cadmium was observed in some cultures. Some investigators believed that when metal biosorption reaches its maximum level, growth would decrease and the number of viable cells in the culture would be reduced [29, 30]. If so, it's suggested that glycoprotein materials components in the cell wall of dead bacteria might be degraded by viable cells. This condition leads to the release of the absorbed metals into the aqueous solution.

Conclusion

Results of the present study showed that *A. denitrificans* strain PQ-1 isolated from Khor Musa is a cadmium-resistant species that could absorb a considerable amount of metal in 25 m/l concentrations. The conventional methods of heavy metals removal from the solutions are sometimes ineffective at low metal concentration (< 100 mg/l). This is suggested that *A. denitrificans* strain PQ-1 could be used to remove cadmium concentrations from metal-contaminated wastes.

Refrences

1. MALIK A. Metal bioremediation through growing cells. Environ. Int. **30**, 261, **2004**.
2. JARUP L. Hazards of heavy metal contamination. Brit. Med. Bull. **68**, 167, **2003**.
3. EGWURUGWU J.N., UFEARO C.S., ABANOBI O.C., NWOKOCHA C.R., DURUIBE J.O., ADELEYE G.S., EBUNLOMO A.O., ODETOLA A.O., ONWUFUJI O. Effects of ginger (*Zingiber officinale*) on cadmium toxicity. Afr. J. Biotechnol. **6**, 2078, **2007**.
4. ÖZTÜRK M., ÖZÖZEN G., MINARECI O., MINARECI E. Determination of heavy metals in fish, water and sediments of avsar dam lake in turkey. Iran. J. Environ. Health Sci. Engineer. **6**, 73, **2009**.
5. AZZA A.A., WESAM A.H., HEDAYAT M.S., GHADA A.A.F. Biosorption of some heavy metal ions using bacterial species isolated from agriculture waste water drains in Egypt. J. Appl. Sci. Research. **5**, (4), 372, **2009**.
6. YAHAYA Y.A. Biosorption of selected heavy metals by free and immobilized *Pycnoporus sanguineus*: batch and column studies. Malaysia University, pp. 1-47, **2008**.
7. TARANGINI K. Biosorption of heavy metals using individual and mixed cultures of *Pseudomonas aeruginosa* and *Bacillus subtilis*. Depart. chem. Engineer, pp. 2-70, **2009**.
8. ZOLGHARNEIN H., KARAMI K., MAZAHERI ASSADI M., DADOLAHI SOHRAB A. Molecular characterization and phylogenetic analyses of heavy metal removal bacteria from the Persian Gulf. Biotechnol. **9**, (1), 1, **2010**.
9. LEUNG W.C., WONG M-F., CHUA H., LO W., YU P.H.F., LEUNG C.K. Removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal wastewater. Water Sci. Technol. **14**, 233, **2000**.
10. WEON B., CINDY H., WU J.K., ASHOK M., Wilfred C. Enhanced Hg biosorption by bacterial cells with surface-displayed MerR. Appl. Environ. Microb. **69**, 3176, **2003**.
11. RUCHI G., SAXENA RK., RANI G. Fermentation waste of *Aspergillus terreus*: A promising copper bio-indicator. Process Biochem. **39**, 1231, **2003**.
12. PARK D., YEOUNG-SANG Y., JONG MP. Studies on hexavalent chromium biosorption by chemically-treated biomass of *Ecklonia* sp. Chemosphere. **60**, 1356, **2005**.
13. AHALYA N., RAMACHANDRA T.V., KANAMADI R.D. Biosorption of heavy metals. J. Chem. Envir. **7**, (4), 71, **2003**.
14. ALLURI H.K., RONDA S.R., SETTALLURI V.S., BONDILI J.S., SURYANARAYANA V., VENKATESHWAR P. Biosorption: An eco-friendly alternative for heavy metal removal. Afr. J. Biotechnol. **6**, (25), 2924, **2007**.
15. DZAIRI F.Z., ZEROUAL Y., MOUTAOUAKKIL A., TAOUFIK J., TALBI M., LOUTFI M., LEE K., BLAGHEN M. Bacterial volatilization of mercury by immobilized bacteria in fixed and fluidized bed bioreactors. Ann. Microbiol. **5**, (4), 353, **2004**.
16. WALLACE D.F., DICKINSON D.J. 16S rRNA analysis of the bacteria associated with biocide degradation. The Int. Research Group on Wood Preserv, pp. 1-12, **2004**.
17. PEREZ-LUZ S., ADELA YANEZ M., CATALAN V. Identification of waterborne bacteria by the analysis of 16S-23S rRNA intergenic spacer region. J. Appl. Microbiol. **97**, 191, **2004**.
18. KIM S.U., CHEONG Y.H., SEO D.C., HUR J.S., HEO J.S., CHO J.S. Characterisation of heavy metal tolerance and biosorption capacity of bacterium strain CPB4 (Bacillus spp.). Water Sci. Technol. **55**, 105, **2007**.
19. SHIRDAM R., KHANAFARI A., TABATABAEE A. Cadmium, nickel and vanadium accumulation by three strains of marine bacteria. Iran. J. Biotechnol. **4**, 180, **2006**.
20. MALATOVA K. Isolation and characterization of hydrocarbon degrading bacteria from environmental habitats in western New York State. Instit. Technol. Rochester, pp. 1-93, **2005**.
21. MAHVI A.H., DIELS L. Biological removal of cadmium by *Alcaligenes eutrophus* CH34. Int. J. Environ. Sci. Technol. **1**, (3), 199, **2004**.
22. NAZ N., HILARY K.Y., AHMED N., GEOFFERY M.G. Cadmium Accumulation and DNA Homology with Metal Resistance Genes in Sulfate-Reducing Bacteria. Appl. Environ. Microbiol. **71**, (8), 4610, **2005**.
23. HUSSEIN H., MOAWAD H., FARAG S. Isolation and characterization of pseudomonas resistant to heavy metals contaminants. Arab. J. Biotechnol. **7**, (1), 13, **2003**.

24. ANDREONI V., COLOMBO M., COLOMBO A., VECCHIO A., FINOLI C. Cadmium and zinc removal by growing cells of *pseudomonas putida* strain B14 isolated from a metal-impacted soil. Ann. Microbiol. **5**, (3), 135, **2007**.
25. GIKAS P., SENGOR S.S., GINN T., MOBERLY J., PEYTON B. The effects of heavy metals and temperature on microbial growth and lag. Global NEST J. **11**, 325, **2009**.
26. KADER J., SANNASI P., OTHMAN O., ISMAIL B.S., SALMIJAH S. Removal of Cr (VI) from aqueous solutions by growing and non-growing populations of environmental bacterial consortia. Global J. Environ. Research. **1**, 12, **2007**.
27. KING P., RAKESH N., BEENALAHARI S., KUMAR Y.P., PRASAD V.S.R.K. Removal of lead from aqueous solution using *Syzygium cumini* L. equilibrium and kinetic studies. Environ. Pollut. Contr. Engineer. 8, 340, **2006**.
28. GREEN-RUIZ C. Mercury (II) removal from aqueous solutions by nonviable *Bacillus* sp. from a tropical estuary. Bioresource Technol. **97**, 1907, **2006**.
29. SINHA S., MUKHERJEE S.K. *Pseudomonas aeruginosa* KUCD1, a possible candidate for cadmium bioremediation. Braz. J. Microbiol. **40**, 655, **2009**.
30. KHANAFARI A., ESHGHDOOST S., MASHINCHIAN A. Removal of lead and chromium from aqueous solution by *Bacillus Circulans* biofilm. Iran. J. Health Sci. Eng. **5**, (3), 195, **2008**.