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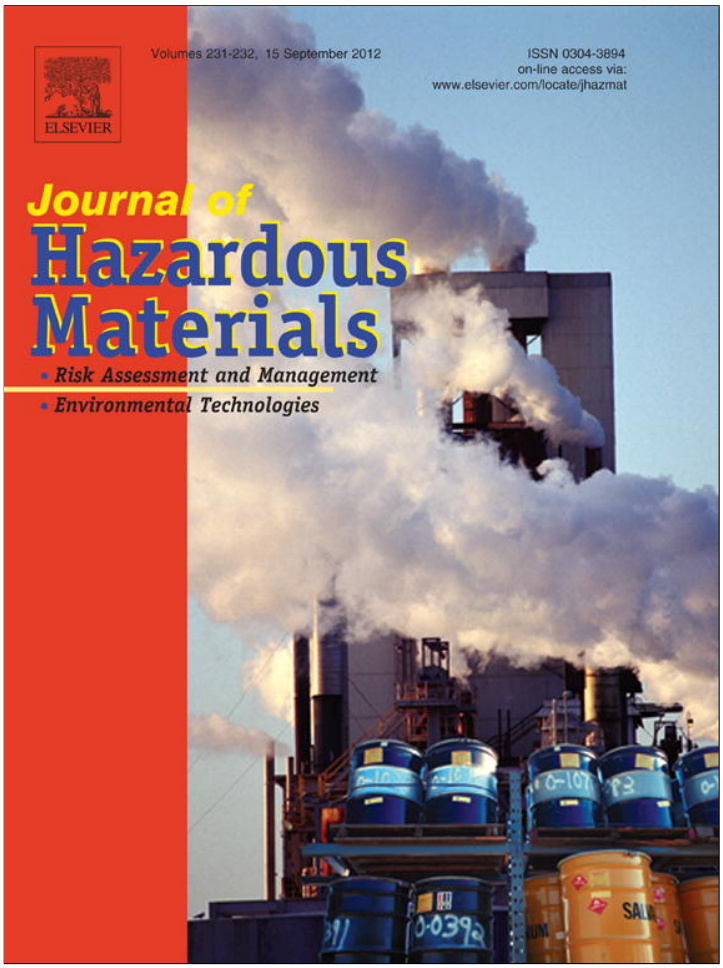

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Removal and reduction of chromium by *Pseudomonas* spp. and their correlation to rhamnolipid production

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HIGHLIGHTS

- Cr(VI) reduction ability of the bacteria was not related to chromium removal.
- There is positive correlation between chromium removal and rhamnolipid production.
- There is a negative correlation between chromium toxicity and chromium removal.
- Exposure to chromium increases the yields of rhamnolipid produced by two Pseudomonas isolates.

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ABSTRACT

Chromium removal and its association with rhamnolipid production in *Pseudomonas* spp. were investigated. Three *Pseudomonas* spp. isolates (*P. aeruginosa* 78, *P. aeruginosa* 99, and *P. stutzeri* T3) were investigated with regard to their exposure to 10 mg/L for chromium removal. *P. aeruginosa* 99 removed 16% and 20% more chromium than *P. stutzeri* T3 and *P. aeruginosa* 78 respectively. The reduction of Cr(VI) to Cr(III) by all the three isolates is more or less similar. *P. aeruginosa* 99, which removed higher chromium, also produced higher rhamnolipid (165 ± 5 mg/mL). *P. aeruginosa* 78, which removed lower chromium, also produced lower rhamnolipid (126 ± 3 mg/mL). Rhamnolipid production by *P. aeruginosa* 78 and *P. aeruginosa* 99 was increased in its exposure to 10 mg/L chromium. In the present study, results showed that rhamnolipid might play a role in chromium removal by three *Pseudomonas* spp. isolates.

1. Introduction

The pollution caused by heavy metals in wastewater has always been a very serious problem, because these elements are not biodegradable and can accumulate in living tissues [1]. Chromium is widely used in various important industrial applications including steel production, electro-plating, leather tanning, nuclear power production, textile industries, wood preservation, anodizing of aluminum, water cooling, and chromate preparation [2]. The hexavalent form of chromium, usually present in the form of chromate (CrO_4^{-2}) and dichromate ($Cr_2O_7^{-2}$), possesses significantly higher levels of toxicity than other valence states [3]. Though chromium exists in nine valence states ranging from −2 to +6, Cr(III) and Cr(VI) are of major environmental significance. In fact, Cr(VI) is more mobile and toxic than Cr(III). Hence, Cr(VI) is more important than Cr(III) in water pollution control. According to the World Health Organization (WHO) drinking water guidelines, the maximum allowable limits for hexavalent chromium and total chromium (including Cr(III), Cr(VI), and other forms) are 0.05 and 2 mg/L, respectively [4].

The conventional methods used for heavy metal removal from industrial effluents are precipitation, coagulation, ion exchange, cementation, electro-dialysis, electro-winning, electro-coagulation, and reverse osmosis [5]. These technologies are often inefficient and/or expensive, mainly when applied to dilute solutions, usually generating huge volumes of sludge containing high levels of heavy metals that have to be disposed. Due to these limitations, new technologies are necessary [6]. Biosorption of heavy metals by microbial cells has been recognized as a potential alternative to the traditional treatment technologies for waste streams and natural waters [7].

Microbial heavy metal accumulation often comprises two phases [8]: (1) an initial rapid phase involving physical adsorption or ion exchange at the cell surface and (2) a subsequent slower phase involving active metabolism-dependent transport of metal into bacterial cells. During the bioaccumulation, many features of a

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living cell such as intracellular sequestration followed by localization within specific organelles, metallothionein binding, particulate metal accumulation, extracellular precipitation, and complex formation can occur [9].

Biosurfactants are biological compounds that are produced by microorganisms (bacteria or yeast), plants, animals, and even humans. These can be divided into (1) low-molecular-weight molecules that efficiently lower surface and interfacial tensions and (2) high-molecular-weight polymers. The low-molecular-weight biosurfactants are glycolipids or lipopeptides. The best-known glycolipid bioemulsifiers, rhamnolipids, trehalolipids, and sophorolipids are disaccharides that are acylated with long-chain fatty acids or hydroxy fatty acids [10,11]. Rhamnolipids are produced by *Pseudomonas aeruginosa*, *Pseudomonas* sp., and *Serratia rubidea*. Due to the anionic nature of rhamnolipids and their complexation ability, rhamnolipids can remove heavy metal ions from the soil [12]. Rhamnolipids can also be effective in the simultaneous removal of mixed hydrocarbons and heavy metals [13].

Bioremediation of soluble hexavalent chromium can be obtained by utilizing microbes in wastewater. Several bacterial strains (*Pseudomonas ambigua*, *Desulfovibrio vulgaris*, *Enterobacter cloacae* HO-1, *Alcaligenes eutrophus*, and *Dinococcus radiodurans* R1) have been described for their ability to reduce hexavalent chromium into insoluble low-valence form Cr(III) both aerobically and anerobically [14]. Hence, the chromate-reducing bacteria are most crucial for the immobility of soluble hexavalent chromium in the environment.

The main objectives of this study were (i) to investigate the chromium removal of *P. aeruginosa* 78, *P. aeruginosa* 99, and *Pseudomonas stutzeri* T3; (ii) to determine the rhamnolipid production by these three *Pseudomonas* isolates; and (iii) to investigate the reduction of Cr(VI) to Cr(III). It is also aimed to determine the effect of chromium on the rhamnolipid production of the isolates. Besides, this study was also designed to evaluate the toxicity of chromium to *Pseudomonas* isolates by biomass concentration and protein analysis. The usefulness of the findings of the present study in examining the correlation between rhamnolipid production and chromium removal was also discussed.

2. Experimental

2.1. Bacterial strains and culture growth conditions

Bacterial strains *P. aeruginosa* 78 and 99 were isolated from textile industrial wastewater (Tekirdag, Turkey), and *P. stutzeri* T3 was isolated from petroleum-contaminated soil samples. Strains were obtained from Biotechnology Laboratory Culture Collection, Gazi University. These bacterial isolates were sub-cultured on nutrient agar (Oxoid). Cultures were routinely shaken (18 h and 37 °C); and for biosorption tests, they were inoculated to acetate minimal medium (AMM) described by Badar et al. [15]. Acetate minimal medium (AMM) contained (g/L): NH_4Cl 1.0; $MgSO_4{\cdot}7H_2O$, 0.2; $FeSO_4{\cdot}7H_2O$, 0.001; $CaCl_2{\cdot}2H_2O$ 0.001; sodium acetate, 5; yeast extract, 0.5; and K_2HPO_4, 0.5 (to pH 7.0 with NaOH). The phosphate source was separately autoclaved in 10 mL of distilled water and added to the bulk medium when it was at room temperature.

2.2. Chromium removal

The removal of chromium by *P. aeruginosa* 78, 99 and *P. stutzeri* T3 isolates was evaluated using a modified method described by Matsunaga et al. [16]. A stock solution of 1000 mg/L of Cr was prepared by dissolving $K_2Cr_2O_7$ in distilled water. Then, strains were inoculated in media with and without Cr. Glassware used for experimental purposes was washed with 60% nitric acid and, subsequently, rinsed with deionized water to remove any possible interference by other metals. Isolates were exposed to 10 mg/L Cr for 48 h in acetate minimal medium at 37 °C using an incubator shaker. 1 mL samples that had been exposed to Cr were removed at 8 h intervals up to 48 h and assayed for Cr in the medium (residual), Cr adsorbed on the surfaces of the cells (surface bound), and Cr accumulated in the cells (intracellular). The concentration of Cr was measured by an atomic absorption spectrophotometer (AA-6600, Shimadzu). The percentage of chromium removal (%) was calculated as follows: (amount of removed Cr)/(amount of initial Cr) × 100.

Samples were centrifuged (10,000 rpm), and the residual Cr in the medium was determined. The pellet was further washed with 1 mL of 10 mM EDTA solution for desorption of Cr from the cell surfaces and centrifuged (10,000 rpm) once again. The Cr adsorbed onto the cell surfaces was determined from this supernatant. The amount of intracellular accumulation of Cr was determined by measuring the Cr content in the pellet, resuspended, and sonicated (Vibra Cell) at 50 MHz on ice in 1 mL of 1 N HNO_3 using an atomic absorption spectrophotometer. Hexavalent chromium was determined by measuring the absorbance at 540 nm of the purple complex of Cr(VI) with 1,5-diphenylcarbazide, in acidic solution [17]. For total Cr determination, the Cr(III) was first oxidized to Cr(VI) at approximately 100 °C, by the addition of an excess of potassium permanganate before the reaction with 1,5-diphenylcarbazide. The Cr(III) concentration was calculated by the difference between total Cr and Cr(VI) concentration.

2.3. Isolation and quantification of rhamnolipids

The strains producing biosurfactants (rhamnolipids) were grown in nutrient media with and without chromium (48 h, 37 °C, and 120 rpm). Samples were collected every 8 h beginning from initial incubation (0 h) up to 48 h and rhamnolipids were analyzed in the culture medium. The pH of the samples was adjusted to 8.0 (using 10 M NaOH), and biomass was removed by centrifugation for 20 min at 10,000 × *g*. The obtained supernatant was treated by acidification to pH 2 using a 6 M HCl solution, and the acidified supernatant was left overnight at 4 °C for the complete precipitation of the biosurfactant [18]. After centrifugation, the precipitate was dissolved in a 0.1 M $NaHCO_3$ solution, followed by the biosurfactant extraction step with a solvent having a 2:1 CH_3Cl–C_2H_5OH ratio at room temperature (25–27 °C) [19]. The mixture was shaken for 10 min. Centrifugation was performed for 10 min at 10,000 × *g*, and the organic phase was removed. The organic phase was transferred to a round-bottom flask connected to a rotary evaporator (Heidolph, Laborota 4000) to remove the solvent at 40 °C, thereby yielding a viscous, honey-colored biosurfactant product. The product was dissolved in methanol, filtered (Sterivex-GV 0.22 mm, Millipore, Bedford, MA, USA), and concentrated again using the rotary evaporator [20]. Rhamnolipid concentration was determined according to Dubois et al. [21] by the colorimetric phenolsulphuric acid method at 480 nm by the spectrophotometer Hitachi UV-VIS.

2.4. Viability by biomass concentration and protein analysis

Optical density was quantified by using a spectrophotometer at 600 nm [22]. Determination of the protein content is based on the Bradford method [23] by using the Coomassie Protein Assay Reagent (Pierce, Rockford). Bovine serum albumin (BSA) was used as a standard. The extent of tolerance was compared, and the "normalized" biomass was calculated, that is, biomass at chromium concentration per biomass using a control.

2.5. Experimental design and statistical analysis

All experiments were done in triplicate, and mean values are presented. Statistical analysis was performed on the data using SPSS 13.0 Bivariate Correlation Analysis. The Pearson rank-order coefficient was determined for the comparison of Cr tolerance between rhamnolipid production and Cr removal by *P. aeruginosa* 78, 99 and *P. stutzeri* T3 isolates. One-way ANOVA was used for the detection effect of Cr on rhamnolipid production. Individual differences were detected by Dunnett and Tukey grouping tests. A value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Chromium removal and reduction

P. aeruginosa 78, 99 and *P. stutzeri* T3 isolates were exposed to 10 mg/L chromium for 48 h in AMM medium; and they also showed different abilities for Cr removal. One part of the total chromium removed by the cells was intracellularly accumulated, and the other part was adsorbed onto the cell surfaces for both isolates. *P. aeruginosa* 99 removed more Cr than *P. aeruginosa* 78 and *P. stutzeri* T3. At the end of the 48th hour, *P. aeruginosa* 99 adsorbed 22% of 10 mg/L Cr onto the cell surfaces and intracellularly accumulated 23% of 10 mg/L Cr. *P. stutzeri* T3 adsorbed 14% of 10 mg/L Cr onto the cell surfaces and intracellularly accumulated 19% of 10 mg/L Cr (Fig. 1). On the other hand, *P. aeruginosa* 78 adsorbed 12% of 10 mg/L Cr onto the cell surfaces and intracellularly accumulated 13% of 10 mg/L Cr. The proportions of the remaining total chromium in the medium were determined as 76% for *P. aeruginosa* 78, 55% for *P. aeruginosa* 99, and 67% for *P. stutzeri* T3 (Fig. 1).

Studies developed by Park et al. [24] suggested that Cr(VI) can be reduced to Cr(III) by the biomass through two different mechanisms: in the first mechanism, Cr(VI) is directly reduced to Cr(III) in the aqueous phase by contact with the electron-donor groups of the biomass, and the second mechanism consists of three steps:

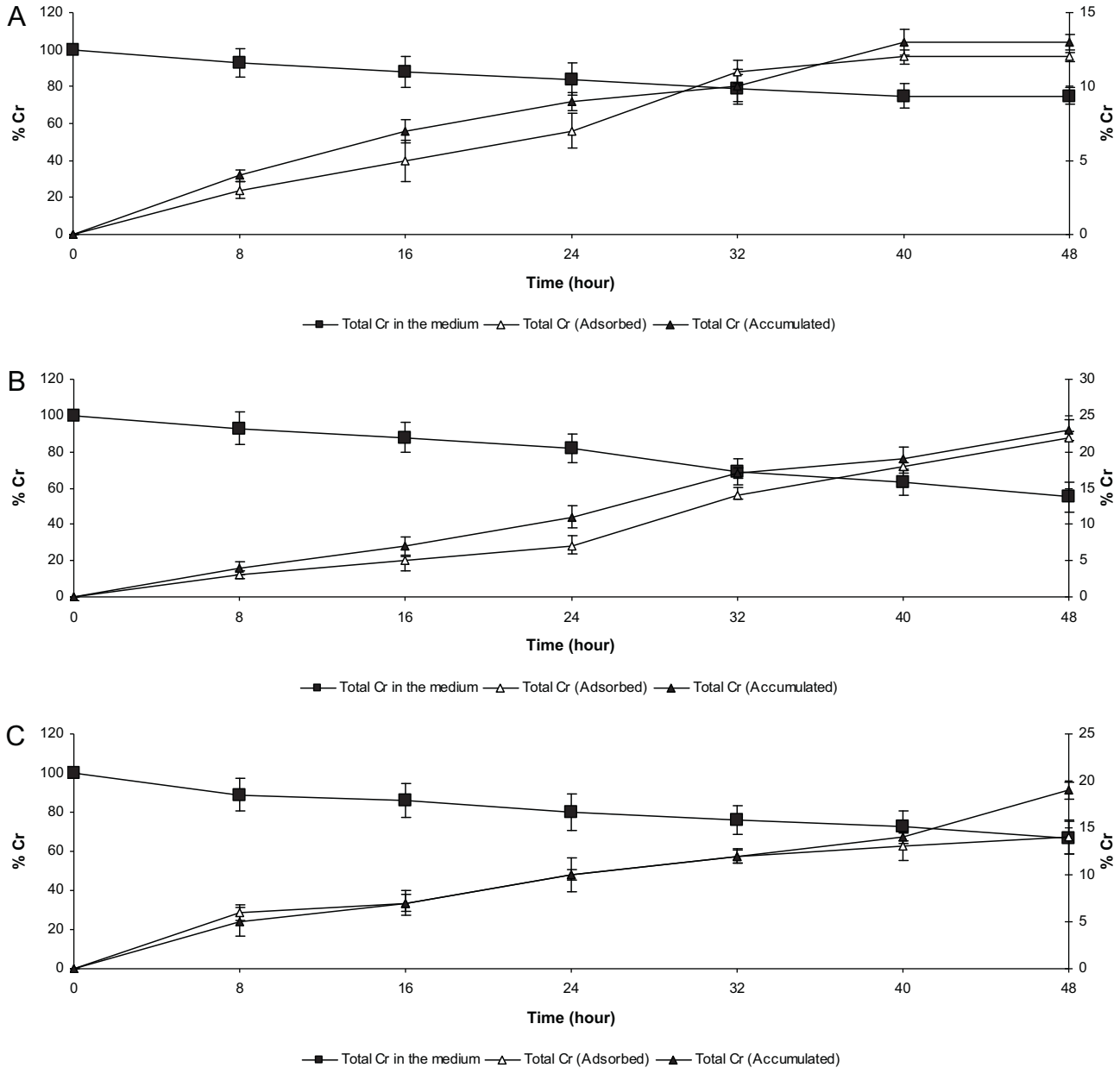

Fig. 1. Cr removal of *P. aeruginosa* 78 (A), *P. aeruginosa* 99 (B), and *P. stutzeri* T3 (C). *Y*-axis (left): Cr in the medium; *Y*-axis (right): adsorbed and accumulated Cr.

(1) binding of anionic Cr(VI) ion species to the positively charged groups present on the biomass surface; (2) reduction of Cr(VI) to Cr(III) by adjacent electron-donor groups; and (3) release of the Cr(III) ions into the aqueous phase due to electronic repulsion between the positively charged groups on the cell's surface and the Cr(III) ions, or the complexation of the Cr(III) with adjacent groups capable of Cr-binding.

The ability to reduce Cr(VI) and to resist high Cr(VI) concentrations were found to be independent properties of bacteria especially *Pseudomonas* sp. [25]. Using classical biochemical techniques, a novel soluble enzyme (ChrR) with chromate reductase activity was previously purified to homogeneity from *Pseudomonas putida* [26]. Additionally, efficient and rapid reduction of Cr(VI) to Cr(III) has been measured for a soluble flavoprotein (ChrR) purified and kinetically characterized from *P. putida* MK1 [27] and for soluble crude fractions prepared from *P. putida* PRS2000 [28], suggesting that *P. putida*, if present in the indigenous microbial community, is likely an important contributor to the bioreduction of chromate in polluted soil environments.

The Cr(VI) reduction of the isolates was expressed as Cr(VI) and Cr(III) concentrations in the medium. *P. aeruginosa* 78, 99 and *P. stutzeri* T3 isolates were exposed to 10 mg/L chromium for 48 h in AMM medium. In the present study, each of the isolates converted Cr(VI) to Cr(III) as shown in Fig. 2. These results indicate that Cr(VI) reduction ability of the bacteria was not related to chromium removal.

3.2. Rhamnolipid production

Pseudomonas species is well known for its capability to produce rhamnolipid biosurfactants with potential surface-active properties when grown on different carbon substrates. Rhamnolipid biosurfactants produced by *P. aeruginosa*, in particular, offer special advantages because of their potent emulsifying activity and low,

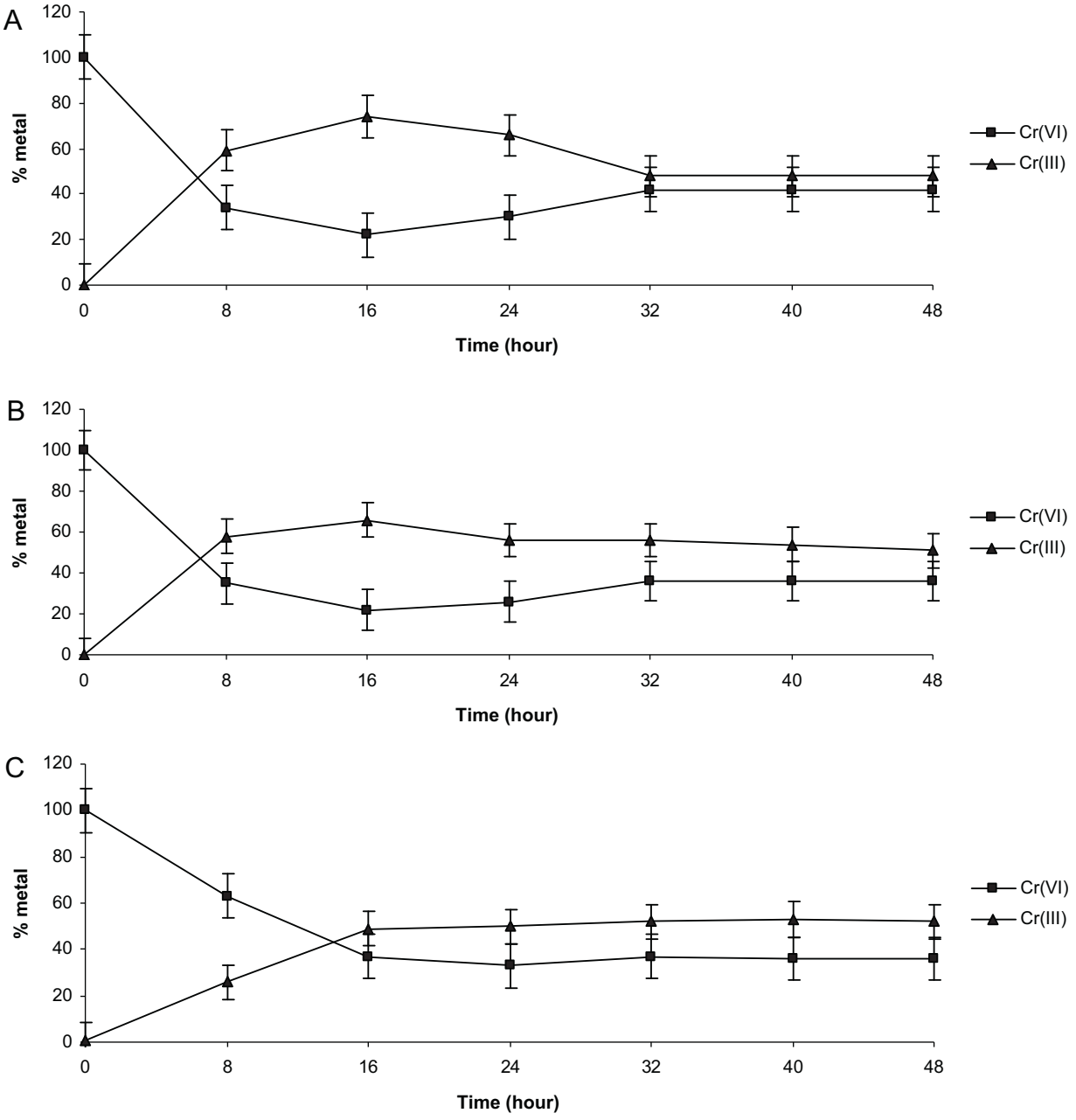

Fig. 2. Chromium reduction of *P. aeruginosa* 78 (A), *P. aeruginosa* 99 (B), and *P. stutzeri* T3 (C).

critical micelle concentration [29]. Biosurfactants have potential due to their low toxicity and biodegradability [30]. The biosurfactant that was used in this study, a rhamnolipid, is a glycolipid that was produced by two selected isolates of *Pseudomonas* sp. according to their chromium removal ability. *P. aeruginosa* 78 (lower Cr removal) and *P. aeruginosa* 99 (higher Cr removal) were exposed to 10 mg/L Cr. The rhamnolipid production of each isolate was determined in the presence of Cr and compared with controls. Maximal rhamnolipid concentration was reached at the 48th hour. At the end of the 48th hour, a significant and regular increase was observed in the rhamnolipid production by both *P. aeruginosa* 78 (ANOVA; $F_{6,14} = 5.048$; $p = 0.0001$) and *P. aeruginosa* 99 (ANOVA; $F_{6,14} = 13.381$; $p = 0.0001$) exposed to 10 mg/L Cr. As shown in Table 1, *P. aeruginosa* 99 not only removed more chromium but also produced high amounts of rhamnolipid; whereas *P. aeruginosa* 78 not only removed less chromium but also produced low amounts of rhamnolipid. In this study, a positive correlation was significantly determined between chromium removal and rhamnolipid production ($p < 0.05$). In the present study results showed that rhamnolipid might play a role in chromium removal.

Gnanamani et al. [31] studied the bioremediation of chromium(VI) by biosurfactant-producing, marine isolate *Bacillus* sp. MTCC 5514. The remediation carried out by this strain proceeded via two processes: reduction of Cr(VI) to Cr(III) by extracellular chromium reductase and entrapment of Cr(III) by the biosurfactants. The first process transforms the toxic state of chromium into a less-toxic state, and the second process prevents the bacterial cells from the exposure of chromium(III). Both reactions keep bacterial cells active all the time and provide tolerance and resistance toward high hexavalent and trivalent chromium concentrations [32]. Many authors reported that highest rates of Cr(VI) reduction by *Pseudomonas* sp. occurred at aerobic conditions and not related to Cr(VI) resistance or removal [33,34]. In

Table 1
Rhamnolipid production by *P. aeruginosa* 78 and *P. aeruginosa* 99 grown in the presence of 10 mg/L chromium.

Time (h)	Rhamnolipid production (mg/mL)			
	P. aeruginosa 78		*P. aeruginosa* 99	
	Control	With Cr	Control	With Cr
0	50 ± 2	51 ± 3	49 ± 4	49 ± 4
8	75 ± 3	68 ± 3	97 ± 2	78 ± 5
16	84 ± 3	69 ± 2	97 ± 2	99 ± 3
24	87 ± 2	88 ± 4	104 ± 4	104 ± 2
32	102 ± 2	99 ± 3	125 ± 3	153 ± 2
40	122 ± 4	171 ± 3	160 ± 4	193 ± 3
48	126 ± 3	172 ± 4	165 ± 5	194 ± 4

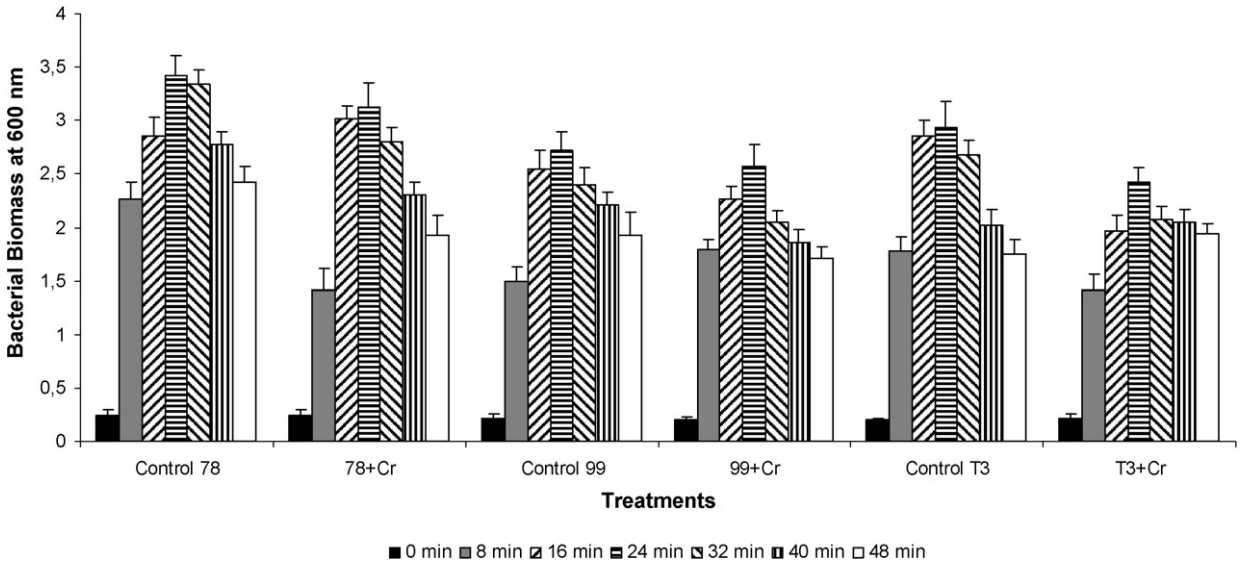

Fig. 3. Effect of 10 ppm Cr on the biomass of *Pseudomonas* isolates.

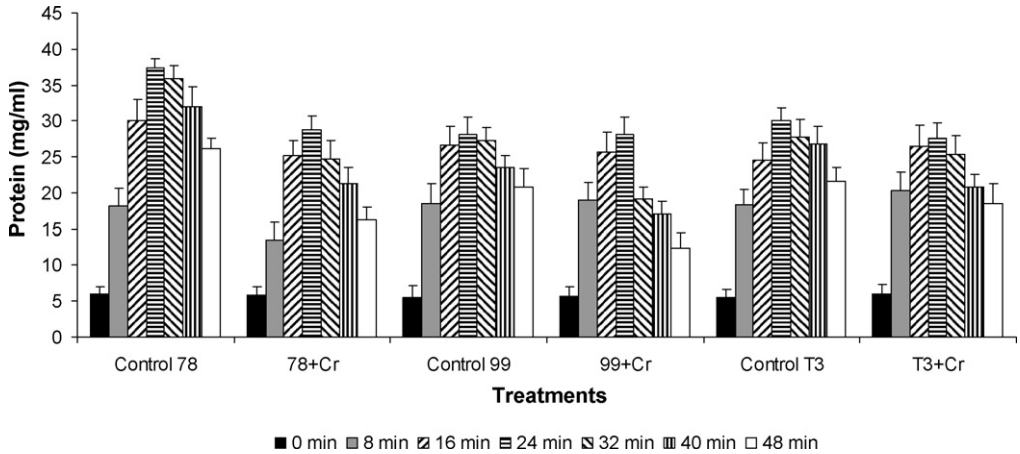

Fig. 4. Effect of 10 ppm Cr on the total protein of *Pseudomonas* isolates.

the present study, Cr(VI) reduction by each of the isolates was determined. Results of the present study confirmed the results of Gnanamani et al. [31].

3.3. Viability by biomass concentration and protein analysis

The time-course data on cellular growth and total protein were observed for each isolate under its optimal pH and temperature conditions. To investigate the heavy metal resistance of the isolates, each isolate was grown in medium containing 10 mg/L Cr. Growth and protein release was determined at 8, 16, 24, 32, 40, and 48 h. The results showed a maximal release of protein in the exponential phase (24 h) and a significant decrease during the stationary phase. Growth of *Pseudomonas* isolates in medium with or without chromium is presented in Fig. 3. In medium without Cr (control), each isolate produced more biomass than in medium with Cr. Among the tested isolates (chromium treated), highest growth and protein release was determined in *P. aeruginosa* 78 exposed to 10 mg/L Cr (Figs. 3 and 4). In the present study, a negative correlation was significantly determined between chromium removal and viability of each of the isolates ($p < 0.05$). Chromium removal of the most resistant strain *P. aeruginosa* 78 was the lowest among the others. As a result, chromium removal is not related to chromium toxicity. Also, previous studies confirmed the results of the present study [35,36].

4. Conclusions

Chromium removal of *Pseudomonas* spp. in terms of Cr(VI) reduction and rhamnolipid production was demonstrated, for the first time.

Results of the present study indicate that (1) Cr(VI) reduction ability of the bacteria was not related to chromium removal; (2) there is a positive correlation between chromium removal and rhamnolipid production and a negative correlation between chromium toxicity and chromium removal; (3) rhamnolipid production of *P. aeruginosa* 78 and *P. aeruginosa* 99 isolates was significantly affected by 10 mg/L of chromium; and (4) exposure to 10 mg/L of chromium increases the yields of rhamnolipid produced by two *Pseudomonas* isolates.

References

[1] L. Deng, Y. Su, H. Su, X. Wang, X. Zhu, Biosorption of copper (II) and lead (II) from aqueous solutions by nonliving green algae *Cladophora fasciculares*: equilibrium, kinetics and environmental effects, Adsorption 12 (2006) 267–277.

[2] U.K. Garg, M.P. Kaur, V.K. Garg, D. Sud, Removal of hexavalent chromium from aqueous solution by agricultural waste biomass, J. Hazard. Mater. 140 (2007) 60–68.

[3] C. Quintelas, B. Fonseca, B. Silva, H. Figueiredo, T. Tavares, Treatment of chromium(VI) solutions in a pilot-scale bioreactor through a biofilm of *Arthrobacter viscosus* supported on GAC, Bioresour. Technol. 100 (2009) 220–226.

[4] V.K. Gupta, A. Rastogi, Biosorption of hexavalent chromium by raw and acid-treated green alga *Oedogonium hatei* from aqueous solutions, J. Hazard. Mater. 163 (2009) 396–402.

[5] S.S. Ahluwalia, D. Goyal, Microbial and plant derived biomass for removal of heavy metals from wastewater, Bioresour. Technol. 98 (2007) 2243–2257.

[6] E.S. Cossich, E.D. da Silva, C.R.G. Tavares, L.C. Filho, T.M.K. Ravagnani, Biosorption of chromium (III) by biomass of seaweed *Sargassum* sp. in a fixedbed column, Adsorption 10 (2004) 129–138.

[7] A. Ozer, D. Ozer, H.I. Ekiz, The equilibrium and kinetic modelling of the biosorption of copper (II) ions on *Cladophora crispate*, Adsorption 10 (2004) 317–326.

[8] T. Srinath, T. Verma, P.W. Ramteke, S.K. Garg, Chromium(VI) biosorption and bioaccumulation by chromate resistant bacteria, Chemosphere 48 (2002) 427–435.

[9] M. Enshaei, A. Khanafari, A. Akhavan Sepahey, Metallothionein induction in two species of *Pseudomonas* exposed to cadmium and copper contamination, Iran. J. Environ. Health Sci. Eng. 7 (2010) 287–298.

[10] N. Christofi, I.B. Ivshina, A review: microbial surfactants and their use in field studies of soil remediation, J. Appl. Microbiol. 93 (2002) 915–929.

[11] S. Wang, C.N. Mulligan, Rhamnolipid foam enhanced remediation of cadmium and nickel contaminated soil, Water Air Soil Pollut. 157 (2004) 315–330.

[12] Y. Asci, M. Nurbas, Y. Sag Acikel, Investigation of sorption/desorption equilibria of heavy metal ions on/from quartz using rhamnolipid biosurfactant, J. Environ. Manage. 91 (2010) 724–731.

[13] K. Urum, T. Pekdemir, Evaluation of biosurfactants for crude oil contaminated soil washing, Chemosphere 57 (2004) 1139–1150.

[14] M. Faisal, S. Hasnain, Microbial conversion of Cr(VI) in to Cr(III) in industrial effluent, Afr. J. Biotechnol. 3 (2004) 610–617.

[15] U. Badar, A. Ahmed, A.J. Beswick, P. Pattanapipitpaisal, L.E. Macaskie, Reduction of chromate by microorganisms isolated from metal contaminated sites of Karachi, Pakistan, Biotechnol. Lett. 22 (2000) 829–836.

[16] T. Matsunaga, H. Takeyama, T. Nakao, A. Yamazawa, Screening of marine microalgae for bioremediation of cadmium-polluted seawater, J. Biotechnol. 70 (1999) 33–38.

[17] D. Eaton, L.S. Clesceri, A.E. Greenberg, Standard Methods for the Examination of Water and Wastewater, American Public Health Association (APHA), Washington, 1995.

[18] M.M. Yakimov, H.L. Fredrickson, K.N. Timmis, Effect of heterogeneity of hydrophobic moieties on surface activity of lichenysin A, a lipopeptide biosurfactant from *Bacillus licheniformis* BAS50, Biotechnol. Appl. Biochem. 23 (1996) 13–18.

[19] Y. Zhang, R.M. Miller, Enhancement of octadecane dispersion and biodegradation by a *Pseudomonas* rhamnolipid surfactant (biosurfactant), Appl. Environ. Microb. 58 (1992) 3276–3282.

[20] S. Pansiripat, O. Pornsunthorntawee, R. Rujiravanita, B. Kitiyanana, P. Somboonthanatea, S. Chavadej, Biosurfactant production by *Pseudomonas aeruginosa* SP4 using sequencing batch reactors: effect of oil-to-glucose ratio, Biochem. Eng. J. 49 (2010) 185–191.

[21] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric methods for determination of sugars and related substances, Anal. Chem. 28 (1956) 350–356.

[22] S. Congeevaram, S. Dhanarani, J. Park, M. Dexilin, K. Thamaraiselvi, Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates, J. Hazard. Mater. 146 (2007) 270–277.

[23] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding, Anal. Biochem. 72 (1976) 248–254.

[24] D. Park, Y-S. Yun, J.M. Park, Studies on hexavalent chromium biosorption by chemically-treated biomass of *Ecklonia* sp., Chemosphere 60 (2005) 1356–1364.

[25] M. Christl, E. Imseng, J. Tatti, C. Frommer, L. Viti, R. Giovannetti, Kretzschmar, Aerobic reduction of chromium(VI) by *Pseudomonas corrugata* 28: influence of metabolism and fate of reduced chromium, Geomicrobiol. J. 29 (2012) 173–185.

[26] C.H. Park, M. Keyhan, B. Wielinga, S. Fendorf, A. Matin, Purification to homogeneity and characterization of a novel *Pseudomonas putida* chromate reductase, Appl. Environ. Microbiol. 66 (2000) 1788–1795.

[27] D.F. Ackerley, C.F. Gonzalez, C.H. Park, R. Blake, M. Keyhan, A. Matin, Chromate-reducing properties of soluble flavoproteins from *Pseudomonas putida* and *Escherichia coli*, Appl. Environ. Microbiol. 70 (2004) 873–882.

[28] Y. Ishibashi, C. Cervantes, S. Silver, Chromium reduction in *Pseudomonas putida*, Appl. Environ. Microbiol. 56 (1990) 2268–2270.

[29] H. Rashedi, M. Mazaheri Assadi, E. Jamshidi, B. Bonakdarpour, Optimization of the production of biosurfactant by *Psuedomonas aeruginosa* HR isolated from an Iranian southern oil well, Iran. J. Chem. Chem. Eng. 25 (2006) 25–30.

[30] C.N. Mulligan, S. Wang, Remediation of a heavy metal-contaminated soil by a rhamnolipid foam, Eng. Geol. 85 (2006) 75–81.

[31] A. Gnanamani, V. Kavitha, N. Radhakrishnan, G.S. Rajakumar, G. Sekaran, A.B. Mandal, Microbial products (biosurfactant and extracellular chromate reductase) of marine microorganism are the potential agents reduce the oxidative stress induced by toxic heavy metals, Colloids Surf. B 79 (2010) 334–339.

[32] M. Pacwa-Płociniczak, G.A. Płaza, Z. Piotrowska-Seget, S.S. Cameotra, Environmental applications of biosurfactants: recent advances, Int. J. Mol. Sci. 12 (2011) 633–654.

[33] J.M. Chen, O.J. Hao, Microbial chromium(VI) reduction, Crit. Rev. Environ. Sci. Technol. 28 (1998) 219–251.

[34] E. Parameswari, A. Lakshmanan, T. Thilagavathi, Chromate resistance and reduction by bacterial isolates, Aust. J. Basic Appl. Sci. 3 (2009) 1363–1368.

[35] S. Ozturk, B. Aslim, Z. Suludere, Evaluation of chromium(VI) removal behaviour by two isolates of *Synechocystis* sp. in terms of exopolysaccharide (EPS) production and monomer composition, Bioresour. Technol. 100 (2009) 5588–5593.

[36] S. Ozturk, B. Aslim, Z. Suludere, Cadmium(II) sequestration characteristics by two isolates of *Synechocystis* sp. in terms of exopolysaccharide (EPS) production and monomer composition, Bioresour. Technol. 101 (2010) 9742–9748.