Bull Environ Contam Toxicol (2009) 82:597–600
DOI 10.1007/s00128-009-9662-3

Evaluation of Consortia of Microorganisms for Efficient Removal of Hexavalent Chromium from Industrial Wastewater

Bushra Muneer · Abdul Rehman · Farah R. Shakoori · Abdul R. Shakoori

Received: 24 October 2008 / Accepted: 16 January 2009 / Published online: 30 January 2009

Abstract The Chromium (Cr) uptake ability of Cr-resistant bacterium *Bacillus thuringiensis*, yeast *Candida etschellsii*, and a protozoan *Stylonychia mytilus,* isolated from industrial waste water, was evaluated individually and in different combinations. It was found that the three types of microorganisms grown together in a culture medium could collectively uptake 90% of Cr^{6+} from the culture medium as against 82% by bacterium + protozoan or yeast + protozoan combined culture, each. Consortium of bacterium, yeast and ciliates therefore could make much more efficient inoculum for remediation of Cr-contaminated industrial waste water.

Keywords Metal uptake · Bioremediation · Hexavalent chromium · Industrial waste water

Chromium (Cr) occurs naturally at trace levels in most soils and water, but disposal of industrial waste and sewage sludge containing Cr compounds has created a number of contaminated sites, which could pose a major environmental threat. Cr primarily exists as a soluble, highly toxic Cr^{6+} anion as against its reduced form Cr^{3+}, which is less soluble and less toxic. Reduction/oxidation reactions between the two states are thermodynamically possible under physiological conditions (Arias and Tebo 2003) and hence reduction of Cr^{6+} to Cr^{3+} is potentially useful for remediation of Cr^{6+}-affected environments (Michel et al. 2001).

Conventional methods for removing toxic Cr include chemical reduction followed by precipitation, ion exchange, and adsorption on activated coal, alum, kaolinite, and ash. However, most of these methods require high energy or large quantities of chemical reagents (Komori et al. 1990). Several bacteria possess the ability to convert chromate to Cr^{3+} (Camargo et al. 2003; Francis et al. 2000). Microbial reduction of toxic Cr^{6+} has practical importance, because biological strategies provide green technology that is cost-effective (Ganguli and Tripathi 2002). Cr resistance and bioaccumulation has been studied in bacteria (Shakoori et al. 1999, 2000), algae (Rehman and Shakoori 2001, 2003) and protozoan (Haq et al. 2000; Shakoori et al. 2004). Twenty Cr-resistant yeast strains isolated from industrial effluents have been shown to tolerate high concentrations of Cr^{6+}, and the strain CMBLY3 and CMBLY4 have been shown to reduce 97% of Cr^{6+} from the ambient medium after 96 h of incubation (Dar and Shakoori 1999). A ciliate, *Vorticella microstoma*, has been reported to tolerate Cr^{6+} at a concentration of 260 μg/mL, and has the ability to reduce 48% of Cr^{6+} after 192 h in a culture medium containing 100 μg/mL of Cr^{6+}. Frequent occurrence of ciliates in wastewater or industrial effluents indicates that they are able to withstand the heavy metal contaminated environment. This property makes protozoa excellent candidate for exploitation in metal detoxification and bioremediation (Haq et al. 2000; Shakoori et al. 2004).

In an ecosystem of industrial wastewater, a variety of microorganisms including bacteria, yeast, algae,

B. Muneer · A. R. Shakoori (✉)
School of Biological Sciences, University of the Punjab, New Campus, Lahore 54590, Pakistan
e-mail: arshak@brain.net.pk; arshakoori@sbs.pu.edu.pk; arshaksbs@yahoo.com

A. Rehman
Department of Microbiology and Molecular Genetics, University of the Punjab, New Campus, Lahore 54590, Pakistan

F. R. Shakoori
Department of Zoology, GC University, Lahore, Pakistan

protozoans etc. thrive in the contaminated water, as they have developed strategies to resist, tolerate, metabolize and detoxify these substances (Shi et al. 2002). Different mechanisms involved in processing heavy metals can be exploited to decontaminate the waste water bodies.

This paper deals with evaluation of Cr-resistant bacteria, yeast and protozoa alone and in combination for decontamination of wastewater of Cr^{6+}, a strategy which could be adopted for remediation of industrial waste water.

Materials and Methods

Water samples of the industrial effluents from ponds getting wastes of tanneries in Kasur (Pakistan) were collected in sterilized screw capped glass bottles. Physical parameters of wastewater viz., pH and temperature were also recoded. A large number of bacteria, yeast and protozoa were present in the wastewater.

Luria Bertani agar medium (1% NaCl, 1% tryptone, 0.5% yeast extract and 1.5% agar) was used for the growth of bacteria. YEPD medium (1% yeast extract, 0.5% peptone, 0.2% glucose and 1.5% agar) was used for the growth of yeast. Bold basal medium [$NaNO_3$ (0.250 g/L), $CaCl_2 \cdot 2H_2O$ (0.0250 g/L), $MgSO_4 \cdot 7H_2O$ (0.0750 g/L), K_2HPO_4 (0.075 g/L), KH_2PO_4 (0.175 g/L), NaCl (0.025 g/L), EDTA (0.050 g/L), KOH (0.031 g/L), $FeSO_4 \cdot 7H_2O$ (0.0498 g/L), H_2SO_4 (0.001 ml/L), H_3PO_3 (0.01142 g/L), $ZnSO_4 \cdot 7H_2O$ (0.00881 g/L), $MnCl_2 \cdot 4H_2O$ (0.00144 g/L), MoO_3 (0.00071 g/L) $CuSO_4.5H_2O$ (0.00157 g/L) and $Co(NO_3)_2 \cdot 6H_2O$ (0.00049 g/L)], diluted 1:1,000 with distilled water was used for culturing protozoa (Shakoori et al. 2004).

Several biochemical tests, besides Gram's staining such as catalase, Voges Proskauer, citrate utilization, nitrate reduction, tyrosine decomposition, casein and starch hydrolyse, growth on media containing 7% NaCl, Sabouraud Dextrose agar and 0.001% lysozyme, and acid production from glucose were performed for identification of bacteria. Sporulation, formation of mycelium, carbon assimilation, acid production from different sugars, growth on 5% glucose and 10% NaCl containing medium, starch hydrolysis and ester production, Diazonium blue B and urease tests were used for identification of yeast (Cheesbrough, 1993; Collee et al. 1989). Ciliates were identified on the basis of their shape and size (Edmondson 1966; Curds et al. 1983).

The effect of Cr on the growth of bacteria and yeast was determined by counting the number of cells in the medium. Bacterial, yeast and protozoan cells were grown in their respective media with 100 μg/mL of Cr^{6+} at 37°C for 48 h. Aliquots (2 mL) of incubated culture were taken out every hour for 48 h and the extent of growth was determined by O.D. The growth was compared with that of control culture, which contained no added Cr ions.

Metal processing ability of microorganisms was checked in single and in different combinations, such as bacteria + yeast, yeast + protozoa, bacteria + protozoa, and bacteria + yeast + protozoa. For determination of metal processing ability the calculated amount of bacteria and yeast cells (bacteria 10×10^7 cells/mL and yeast 10×10^5 cells/mL), yeast and protozoan (10×10^5 cells/mL of yeast and 10×10^3 cells/mL of protozoa), bacteria and protozoan (10×10^9 cells/mL of bacteria and 10×10^3 cells/mL of protozoa), bacteria, yeast and protozoa (bacteria 10×10^9 cells/mL, yeast 10×10^5 cells/mL and protozoa 10×10^3 cells/mL) were added in water having glucose as a carbon source containing 100 μg/mL of Cr^{6+} and grown at optimum pH and temperature in culture flasks. A control was also run having 100 μg/mL of Cr^{6+} but without microorganisms. The samples to be used for estimation of Cr^{6+} were taken out after 0, 12, 24 and 48 h, centrifuged at 3,000 rpm for 15 min to spin down the cells, and the supernatant was used to estimate Cr with the help of AA1275 atomic absorption spectrophotometer at wave length 357.9 nm. A graph was plotted between the time interval and the concentration of Cr^{6+}.

All observations were made and estimations done in triplicate. At least three flasks were maintained, each for control and metal treatment. The average of control and experimental groups were compared and significant differences evaluated by using Student's *"t"* test of significance.

Results and Discussion

Chromium resistant bacterium, yeast and ciliate were isolated from the wastewater samples. The temperature of the wastewater harboring the microorganisms was 30°C and pH was 8.6. On the basis of physical and biochemical characterization bacterium was identified as *B. thuringiensis,* yeast as *Candida etchellsii* and the ciliate as *S. mytilus.*

Figure 1 shows the effect of Cr^{6+} on the growth of bacteria and yeast. It shows the characteristic phases during the growth of culture. It is clearly indicated that microorganisms without metal treatment (control) showed lag phase of 1 h, and accelerated growth of 12–21 h during the log phase. The microorganisms with Cr^{6+} stress (treated), however, showed lag phase of 4–6 h and log phase of 16–18 h.

Figure 2a shows the ability of Cr^{6+} resistant *B. thuringiensis* and *C. etchellsii* present together in the medium to decrease 78% of Cr^{6+} from the medium after 48 h of incubation, whereas *C. etchellsii* and *S. mytilus* used

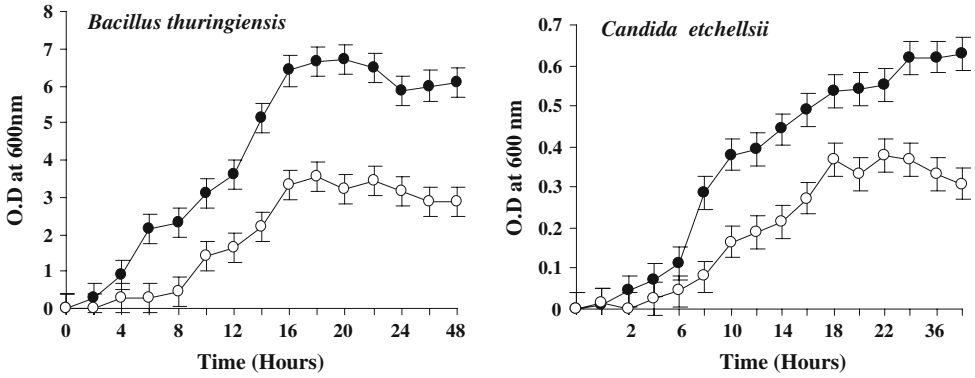

Fig. 1 Growth curves of *B. thuringiensis* and *C. etchellsii* in Cr^{6+} containing medium (*solid circles*). Control cultures (*open circles*) did not contain any metal ions

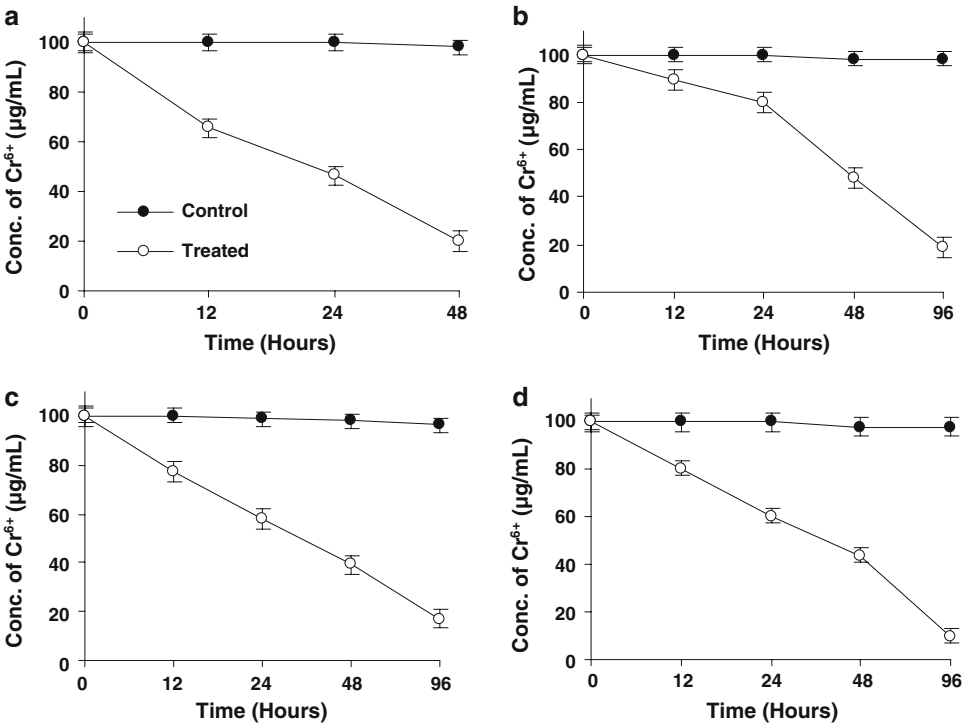

Fig. 2 The heavy metal processing ability of microorganisms used in combination; **a** bacteria (*B. thuringiensis*) and yeast (*C. etchellsii*), **b** yeast (*C. etchellsii*) and protozoa (*S. mytilus*), **c** bacteria (*B. thuringiensis*) and protozoa (*S. mytilus*), **d** bacteria (*B. thuringiensis*), yeast (*C. etchellsii*) and protozoa (*S. mytilus*) isolates from industrial wastewater. The isolates were grown in medium containing 100 µg/mL of Cr^{6+}. The control culture medium contained heavy metal, but no organism

simultaneously removed 82% Cr^{6+} from the medium after 96 h of incubation (Fig. 2b). Figure 2c shows the ability of Cr^{6+} resistant *B. thuringiensis* and *S. mytilus* to reduce Cr^{6+} from the medium after 96 h of incubation. The three categories of microorganisms when used simultaneously removed 90% of Cr^{6+} after 96 h of incubation (Fig. 2d).

Individual microorganisms were also used to determine their efficiency to remove Cr^{6+} from the medium. Bacteria and yeast removed 82 and 80% of Cr, respectively, after 72 h of incubation. The protozoan alone removed 60% of Cr after 96 h of incubation. In the case of combination of organisms, bacteria and yeast removed 78% of metal after 48 h, yeast and protozoa 82%, bacteria and protozoa 82%, and bacteria, yeast and protozoa 90% of after 96 h.

Several studies have reported improvements in metal removal by immobilization of protozoa, yeast or bacterial cells (Zeroual et al. 2001). Bacteria and yeast communities are central to the functioning of terrestrial ecosystem and consist of a large number of different bacterial and yeast type (O-Muter et al. 2002; Boenigk and Arndt 2000). Remediation of sites contaminated with heavy metals is a complex problem (Sandrin et al. 2000). Bioremediation can be effective where environmental conditions permit microbial growth and activity (Vidali 2001). Microorganisms have important role in biogeochemical cycling of toxic metals (Lloyd and Lovley 2001).

It was observed that protozoa may not be important in large scale processing of wastes containing heavy metals, but they share the capability of resisting this toxic metal ion with other microorganisms like bacteria and yeast. Mixed culture is considered to be important in an ecosystem due to cooperative actions. It would not be advisable to

use a pure culture of a microorganism due to disturbances in population structures in an ecosystem.

References

Arias YM, Tebo BM (2003) Chromium reduction by sulfidogenic and non sulfidogenic microbial consortia. Appl Environ Microbiol 69:1847–1853. doi:10.1128/AEM.69.3.1847-1853.2003

Boenigk J, Arndt H (2000) Comparative studies of the feeding behavior of two heterotrophic nanoflagellates: the filter-feeding choanoflagellate *Monosiga ovata* and the raptorial-feeding kinetoplastid *Rhyncomonas nasuta*. Aquat Microb Ecol 22:243–249. doi:10.3354/ame022243

Camargo FAO, Bento FM, Okeke BC, Frankenberger WT (2003) Chromate reduction by chromium-resistant bacteria isolated from soils contaminated with dichromate. J Environ Qual 32:1228–1233

Cheesbrough M (1993) Medical laboratory manual for tropical countries, vol II: microbiology. University Press, Cambridge

Collee JG, Fraser AG, Marmion BP, Simmons A (eds) (1989) Mackie and McCartney practical medical microbiology, 14th edn. Churchill Livingstone, Singapore

Curds CR, Gates MA, Roberts D-MCL (1983) British and other freshwater ciliated protozoa, Part II. Cambridge University Press, London

Dar N, Shakoori AR (1999) Chromium tolerant yeast strains isolated from industrial effluents and their possible use in environmental clean-up. Bull Environ Contam Toxicol 63:744–750. doi:10.1007/s001289901042

Edmondson WT (1966) Freshwater biology. Wiley, New York

Francis CA, Obraztosovar AY, Tebo BM (2000) Dissimilatory metal reduction by the facultative anaerobe *Pantoea agglomerans* SPI. Appl Environ Microbiol 66:543–548. doi:10.1128/AEM.66.2.543-548.2000

Ganguli A, Tripathi AK (2002) Bioremediation of toxic chromium from electroplating effluent by chromate reducing *Pseudomonas aeruginosa* A2Chr in two bioreactors. Appl Microbiol Biotechnol 58:416–420. doi:10.1007/s00253-001-0871-x

Haq RU, Rehman A, Shakoori AR (2000) Effect of dichromate on population and growth of various protozoa isolated from industrial effluents. Folia Microbiol 45:275–278. doi:10.1007/BF02908959

Komori K, Rivas A, Toda K, Ohtake H (1990) A method for removal of toxic chromium using dialysis-sac cultures of a chromate-reducing strain of *Enterobacter cloacae*. Appl Microbiol Biotechnol 33:117–119. doi:10.1007/BF00170582

Lloyd JR, Lovley DR (2001) Microbial detoxification of metals and radionuclides. Curr Opin Biotechnol 12:248–253. doi:10.1016/S0958-1669(00)00207-X

Michel C, Brugma M, Aubert C, Bernadac A, Bruschi M (2001) Enzymatic reduction of chromate: comparative studies using sulfate-reducing bacteria. Appl Microbiol Biotechnol 55:95–100. doi:10.1007/s002530000467

O-Muter I, Lubinya D, Millers L, Grigorjeva E, Ventinya A (2002) Rapport, Cr(VI) sorption by intact and dehydrated *Candida utilis* cells in the presence of other metals. Process Biochem 38:23–131

Rehman A, Shakoori AR (2001) Heavy metal resistant *Chlorella* spp., isolated from tannery effluents, and their role in remediation of hexavalent chromium in industrial wastewater. Bull Environ Contam Toxicol 66:542–547. doi:10.1007/s00128-001-0041-y

Rehman A, Shakoori AR (2003) Isolation, growth, metal tolerance and metal uptake of the green alga, *Chlamydomonas* (Chlorophyta) and its role in bioremediation of heavy metals. Pak J Zool 35:337–341

Sandrin TR, Chech AM, Maier RM (2000) A rhamnolipid biosurfactant reduces cadmium toxicity during naphthalene biodegradation. Appl Environ Microbiol 66:4585–4588. doi:10.1128/AEM.66.10.4585-4588.2000

Shakoori AR, Tahseen S, Haq RU (1999) Chromium tolerant bacteria from industrial effluents and their use in detoxication of hexavalent chromium. Folia Microbiol 44:50–54. doi:10.1007/BF02816221

Shakoori AR, Makhdoom M, Haq RU (2000) Hexavalent chromium reduction by a dichromate-resistant gram-positive bacterium isolated from effluents of tanneries. Appl Microbiol Biotechnol 53:348–351. doi:10.1007/s002530050033

Shakoori AR, Rehman A, Haq RU (2004) Multiple metal resistance in the ciliate protozoan, *Vorticella microstoma*, isolated from industrial effluents and its potential in bioremediation of toxic wastes. Bull Environ Contam Toxicol 72:1046–1051. doi:10.1007/s00128-004-0349-5

Shi W, Becker J, Bischoff M, Turco RF, Konopka AE (2002) Association of microbial community composition and activity with Pb, Cr and hydrocarbon contamination. Appl Environ Microbiol 68:3859–3866. doi:10.1128/AEM.68.8.3859-3866.2002

Vidali M (2001) Bioremediation. An overview. Pure Appl Chem 73:1163–1172. doi:10.1351/pac200173071163

Zeroual Y, Moutaouakkil A, Blaghen M (2001) Volatilization of mercury by immobilized bacteria (*Klebsiella pneumoniae*) in different support by using fluidized bed bioreactor. Curr Microbiol 43:322–327. doi:10.1007/s002840010310