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**EXPERIMENTAL ARTICLES**

# The Reduction of Cr(VI) by Bacteria of the Genus *Pseudomonas*

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**Abstract**—Non-nitrate-reducing collection bacteria from the genus *Pseudomonas* were found to be able to use hexavalent chromium as a terminal electron acceptor. The reduction of Cr(VI) was accompanied by an increase in the cell biomass. At Cr(VI) concentrations in the medium lower than 15 mg/l, the non-nitrate-reducing pseudomonads reduced Cr(VI) less efficiently than did denitrifying pseudomonads. In contrast, at Cr(VI) concentrations higher than 30 mg/l, Cr(VI) was reduced more efficiently by the non-nitrate-reducing pseudomonads than by the denitrifying pseudomonads.

*Key words*: Cr(VI) reduction, strictly aerobic and denitrifying bacteria.

In recent years, there has been increasing researcher interest in bacteria that are able to use hexavalent chromium as a terminal electron acceptor during the oxidation of various organic compounds. Cr(VI) is reduced with the formation of trivalent chromium hydroxide, which is insoluble in water.

The available biotechnologies for the purification of wastewaters from galvanizing processes employ various bacteria, either as monocultures or as associations. Such biotechnologies allow the concentration of hexavalent chromium in water to be lowered to the maximum permissible concentration in a way that is both economically and ecologically more efficient than the commonly used physicochemical methods. The sources of organic nutrition and electron donors used in these biotechnologies may be different and include agricultural wastes and sewage from the soap and other industries [1, 2].

The bioreactor system based on *Enterobacter cloacae* was shown to reduce hexavalent chromium at a rate of 10–15 mg/(l h) [3]. Chirwa and Wang [4] described a packed-bed bioreactor with immobilized *Bacillus* sp. cells that considerably lowered the concentration of Cr(VI) in wastewater. The continuously operating immobilized coculture of *Pseudomonas putida* and *Escherichia coli* with phenol as the source of organic nutrition reduced Cr(VI) at a rate of 5–21 mg/(l h) [5]. In this coculture, phenol was transformed by *P. putida*, whereas *E. coli* reduced hexavalent chromium. Sulfate-reducing bacteria grown on ethanol can diminish the concentration of Cr(VI) in the medium by 93.4% [6].

Almost all of the aforementioned chromium-reducing bacteria are obligate or facultative anaerobes or facultative aerobes capable of anaerobic respiration. Our recent studies showed that the redox potential of a mineral solution with hexavalent chromium is about +400 mV, a value typical of aerobic processes. The culture *Pseudomonas mendocina* P-13 efficiently reduced Cr(VI) at the redox potentials from +350 to +200 mV [7]. It is known that denitrifying pseudomonads reduce nitrate to $N_2$ at the redox potentials from +150 to –100 mV [8, 9]. Consequently, Cr(VI) is reduced at higher redox potentials than the oxidized forms of nitrogen. Bearing this in mind, we assumed that non-nitrate-reducing aerobic bacteria of the genus *Pseudomonas* are able to use hexavalent chromium as a terminal electron acceptor.

The present work was undertaken to verify this assumption.

## MATERIALS AND METHODS

Non-nitrate-reducing bacteria of the genus *Pseudomonas* were obtained from the Ukrainian Collection of Microorganisms (UKM) at the Institute of Microbiology and Virology, National Academy of Sciences of Ukraine (*P. fluorescens* B-53, *P. putida* strains B-117 and B-139, *P. alcaligenes* B-146, *P. pseudoalcaligenes* B-167, *P. fragi* B-184, and *P. taetrolens* B-196) and from the Collection of Microorganisms at the Dumanskii Institute of Colloid and Water Chemistry (*P. "rathonis"* P-17, *P. putida* P-15, and *P. fluorescens* P-9). The denitrifying pseudomonads *P. aeruginosa* P-1, *P. fluorescens* var. *pseudo-iodinum* P-11, *P. mendocina* P-13, and *P. stutzeri* P-19 were obtained from the latter collection.

The pseudomonads were cultivated in mineral M9 medium [10] containing (g/l) $KH_2PO_4$, 3; $Na_2HPO_4$, 6; $NH_4Cl$, 1; NaCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.1; and $CaCl_2$,

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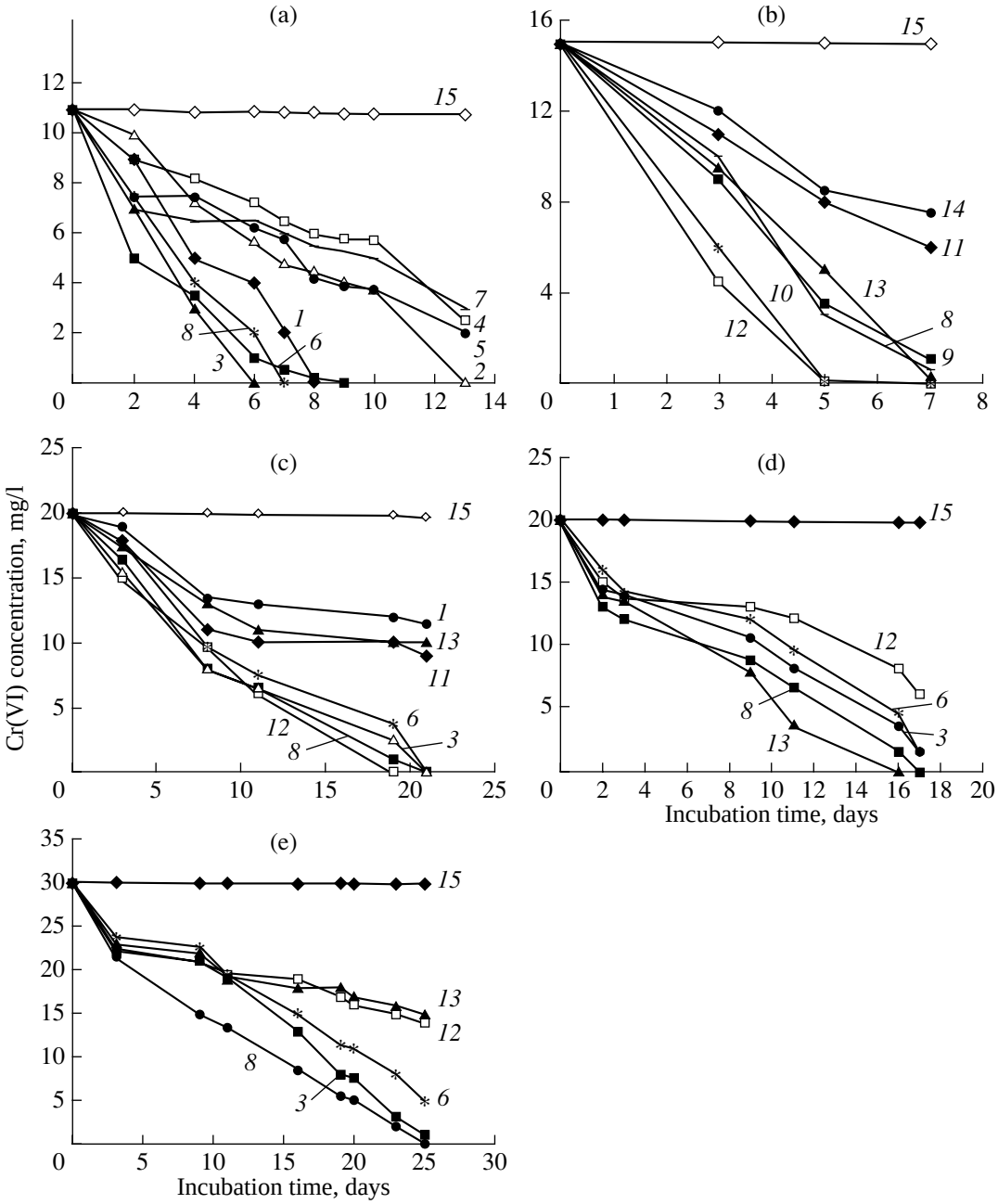


**Fig. 1.** Declines in the concentration of hexavalent chromium in the culture liquids of *Pseudomonas* bacteria at the initial Cr(VI) concentrations of (a) 11 mg/l (the initial culture turbidity, ICT, is 0.03), (b) 15 mg/l (ICT = 0.03), (c) 20 mg/l (ICT = 0.03), (d) 20 mg/l (ICT = 0.06), and (e) 30 mg/l (ICT = 0.03). Curves: (*1*) *P. fluorescens* B-53, (*2*) *P. putida* B-117, (*3*) *P. putida* B-139, (*4*) *P. alcaligenes* B-146, (*5*) *P. pseudoalcaligenes* B-167, (*6*) *P. fragi* B-184, (*7*) *P. taetrolens* B-196, (*8*) *P. "rathonis"* P-17, (*9*) *P. putida* P-15, (*10*) *P. fluorescens* P-9, (*11*) *P. aeruginosa* P-1, (*12*) *P. fluorescens* var. *pseudo-iodinum* P-11, (*13*) *P. mendocina* P-13, and (*14*) *P. stutzeri* P-19; (*15*) control without microorganisms.

0.1. The medium was supplemented with a trace element solution (1 ml per l medium) containing (mg/l) $FeSO_4 \cdot 7H_2O$, 25; $MnSO_4 \cdot 2H_2O$, 5; $CoSO_4$, 1; $ZnSO_4$, 1; $CuSO_4 \cdot 6H_2O$, 0.1; $H_3BO_3$, 0.1; $Na_2MoO_4$, 25; and $NiCl_2 \cdot 6H_2O$, 0.1. The carbon source was glucose at a concentration of 5 g/l.

The pseudomonads were cultivated in 100-ml flat-bottom flasks. Dissolved oxygen was not removed from the cultivation medium; however, to prevent the diffusion of additional oxygen from the air, the medium was overlaid with a 2-cm layer of sterile mineral oil. The medium was inoculated with cells grown on nutrient agar. The initial culture turbidity was 0.03–0.06. The initial concentration of Cr(VI) in the medium was 11, 15, 20, and 30 mg/l. In the course of cultivation, the cultures were sampled using a sterile syringe. The samples

were centrifuged at 8000 *g* for 30 min, and the chromium concentration in the supernatant was determined photometrically at 450 nm by reaction with a mixture of diphenylcarbazide and sulfuric acid (1 : 1) [11]. When the concentration of chromium in the medium was close to zero, samples were not centrifuged before the chromium assay. Control 1 was sterile M9 medium with Cr(VI). Control 2 was the pseudomonad-inoculated M9 medium supplemented with the trace elements and glucose but not with Cr(VI) and overlaid with mineral oil. The microbial biomass in control 2 increased due to glucose metabolism at the expense of the dissolved oxygen initially present in the medium.

## RESULTS AND DISCUSSION

Figure 1a shows a decline in the concentration of hexavalent chromium in the cultivation medium in which the initial concentration of Cr(VI) was 11 mg/l and the initial culture turbidity was 0.03. It can be seen that the most active strains *P. putida* B-139, *P. fragi* B-184, *P. fluorescens* B-53, and *P. "rathonis"* P-17 completely reduced hexavalent chromium within 6, 7, 8, and 9 days of incubation, respectively. *P. putida* B-117 completely reduced this amount of Cr(VI) within 13 days of incubation, after which the residual concentrations of Cr(VI) in the culture liquids of *P. pseudoalcaligenes* B-167, *P. alcaligenes* B-146, and *P. taetrolens* B-196 were 2, 2.5, and 3 mg/l, respectively.

The ability of denitrifying pseudomonads to use hexavalent chromium as a terminal electron acceptor is widely recognized [12, 13]. For this reason, it was of interest to compare the chromium-reducing capabilities of the collection strains of denitrifying bacteria and non-nitrate-reducing bacteria, which are believed to be obligate aerobes.

In these experiments, the concentration of hexavalent chromium in the medium was raised to 15 mg/l at the same initial culture turbidity (0.03). As can be seen from Fig. 1b, *P. fluorescens* var. *pseudo-iodinum* P-11, *P. mendocina* P-13, *P. fluorescens* P-9, and *P. putida* P-15 reduced this amount of hexavalent chromium within 5–7 days, whereas *P. stutzeri* P-19 and *P. aeruginosa* P-1 reduced about half the quantity of Cr(VI) initially present in the medium over the same incubation period.

When the initial concentration of hexavalent chromium was elevated to 20 mg/l, it was completely reduced by the denitrifying bacterium *P. fluorescens* var. *pseudo-iodinum* P-11 in 19 days, while in 21 days by the non-nitrate-reducing bacteria *P. "rathonis"* P-17, *P. putida* B-139, and *P. fragi* B-184 (Fig. 1c). In this case, the culture turbidities increased to 0.09–0.11. The other strains under study reduced Cr(VI) considerably more slowly. In control medium 2, the culture turbidity increased from 0.03 to 0.043–0.063 at the expense of the dissolved oxygen initially present in the medium. Consequently, the increase in the biomass of the strains in the presence of both terminal electron acceptors, dissolved oxygen and Cr(VI), was about twofold higher than in the presence of dissolved oxygen alone.

The rise in the initial culture turbidity from 0.03 to 0.06 led to faster hexavalent chromium reduction (Fig. 1d). In this case, the denitrifying bacterium *P. mendocina* P-13 and the non-nitrate-reducing bacteria *P. "rathonis"* P-17, *P. putida* B-139, and *P. fragi* B-184 completely reduced the hexavalent chromium in the medium within 16–18 days of incubation, their culture turbidities increasing to 0.095 (*P. mendocina* P-13) and 0.143 (*P. "rathonis"* P-17). After 17 days of incubation in the presence of 20 mg/l Cr(VI), the culture turbidity of *P. fluorescens* var. *pseudo-iodinum* P-11 rose from 0.06 to 0.1.

At the initial concentration of hexavalent chromium equal to 30 mg/l (Fig. 1e), the non-nitrate-reducing bacteria *P. "rathonis"* P-17, *P. putida* B-139, and *P. fragi* B-184 completely or nearly completely reduced the hexavalent chromium in the medium within 25 days, whereas the denitrifying bacteria *P. mendocina* P-13 and *P. fluorescens* var. *pseudo-iodinum* P-11 reduced about half the amount of hexavalent chromium initially present in the medium over the same cultivation period. The greatest rise in the culture turbidity (from 0.03 to 0.16) was observed for *P. "rathonis"* P-17. In control medium 2, the culture turbidity of this strain increased to 0.094.

Thus, our experiments showed that the collection strains of non-nitrate-reducing pseudomonads, which are believed to be obligate aerobes, exhibit anaerobic respiration and are able to use hexavalent chromium as a terminal electron acceptor. At low initial concentrations of Cr(VI), the non-nitrate-reducing pseudomonads reduce hexavalent chromium with the same efficiency as the denitrifying pseudomonads under study, but with greater efficiency when the initial concentration of hexavalent chromium in the medium is high. The better growth of pseudomonads in the media containing both dissolved oxygen and hexavalent chromium as compared with their growth in control medium 2 without Cr(VI) confirms the supposition that these pseudomonads are capable of anaerobic respiration with Cr(VI) as a terminal electron acceptor. The ability of the pseudomonads under study, which are believed to be obligate aerobes, to reduce hexavalent chromium can be explained by the fact that the standard potential of the reaction of hexavalent chromium reduction to trivalent chromium ($E_0$ = 1333 mV) is considerably higher than that of the reduction of the oxidized forms of nitrogen and is almost the same as that of the reduction of oxygen ($E_0$ = 1228 mV) [14]. The view that the pseudomonads studied in this work are obligate aerobes seems to be mistaken.

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