

Factors Affecting Detoxification of Hexavalent Chromium into Trivalent in Industrial Effluents by Indigenous Bacteria

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Abstract

Potassium chromate (K_2CrO_4) is a natural source of Cr(VI) and its reduction by *Bacillus pumilus*-CrK08, *Exigubacterium* sp.-CrKS1 and *Cellulosimicrobium cellulans*-CrK16 bacterial strains (reportedly chromium resistant) using varied potassium chromate concentrations (500 and 1000 $\mu g\ ml^{-1}$) at different temperature (28°C, 37°C and 45°C) and pH (5, 7 and 9) in the presence of antibiotics (ampicilline and chloramphenicol), heavy metals (Mn, Cu, Zn, As and Ni) and industrial effluents for different time intervals (24, 48 and 72 hours). Crude extract of CrK08 and CrK16 strains were also evaluated to determine the effects of K_2CrO_4 , temperature, pH and industrial effluent. Highest reduction potential was observed at 500 $\mu g\ ml^{-1}$ of K_2CrO_4 whereas ideal pH and temperature were 37°C and pH7, respectively. Increased reduction was observed in the presence of heavy metals especially $CuSO_4$. However, ampicilline suppressed reduction rate by all the strains. All three strains showed better reduction potential in the presence of wastewater after 24 hours of incubation. In addition, CrK16 extract showed comparatively better potential under these parameters.

Keywords: chromium, bioremediation, pollution, bacteria, heavy metals

1. Introduction

Industrial revolution has brought about dramatic environmental changes posing serious threats to human health and environment itself. Industrial and agricultural serpent growth changes the normal flow of substances and introducing hazardous toxins into the environment. Industries like leather tanning, electroplating, alloy, steel, wood, paints industries (Dhal et al. 2013) release their effluents rich in toxic heavy metals like lead, zinc, chromium etc. Excessive accumulation of such metals in soil and water bodies exceeds the normal levels. Exposure or ingestion of the toxic metals in food or water leads to metal intoxication in various forms of life including humans (Tamas et al. 2005). In addition, excessive metal concentrations caused decreased soil fertility and crop yield (Tripathy et al. 2014).

Chromium is a toxic heavy metal mainly found in the industrial effluent (Ozdemir et al. 2005). About 170,000 tones of chromate waste are discharged annually by these industries (Tamara and McInerney, 2001; Faisal and Hasnain, 2005). Chromium generally present in various oxidation states mainly from -2 to +6 in which two forms hexavalent (Cr-VI) and trivalent (Cr-III) are abundantly found. Oxidation states including +2, +4 and +5 are unstable and readily converted to +3, which in turn, is oxidized to +6 (Sawyer et al., 1994). Hexavalent chromium is much toxic, teratogenic than chromium three (Singh, 1998). In addition, Cr (VI) has high rate of adsorption through intestinal tract (Cheung, 2006). Whereas, Cr-III is less soluble less toxic and less bioavailable in normal conditions (Richard, 1991) and, many times less mutagenic than Cr-VI (Lofroth, 1978; Czako-Vér, 1999).

Strategies have been devised to convert hexavalent chromium Cr-VI to trivalent chromium Cr-III by means of certain chemicals which prove expensive for large-scale application with undesirable effects (Srinath et al. 2002; Tsuruta et al. 2004). Hexavalent chromium is also reduced to trivalent chromium Cr-III by various bacteria, a process called bioremediation (Ackerley et al., 2004; Park et al. 2002). Several bacterial genera like *Pseudomonas* (McLean and Beveridge, 2001; Park et al. 2000), *Bacillus* (Campos et al. 1995) and *Alcaligenes* (Peitzsch et al. 1998) have capability to adapt and colonize metal rich industrial effluents. The present study is designed to reduce or detoxify Cr-VI into less carcinogenic Cr-III and introduce efficient bacterial strains for the treatment of wastewater released by tanneries.

2. Material and Methods

2.1 Source of microorganisms

Previously isolated and identified *Bacillus pumilus*-CrK08 GQ503326, *Exigubacterium* sp.-CrKS1 GQ503330 and *Cellulosimicrobium cellulans*-CrK16 GQ503328 strains by Rizvi and Faisal (2009) from different tanneries of Kasur, were used. These strains were sustained on Luria agar with 1000 $\mu g\ ml^{-1}$ of K_2CrO_4 .

2.2 Chromate reduction medium

Hexavalent chromium Cr(VI) reduction was followed using DeLeo and Ehrlich (1994) medium (10gm tryptone, 5.0 gm yeast extract, 5 gm NaCl, 1 gm citric acid and 6.9 gm Na_2HPO_4 , each per liter of distilled water) at 37°C, shaking at 150 rpm for 24 hours. Residual contents of hexavalent and trivalent chromium were measured

calorimetrically by plotting a standard curve.

2.3 Chromate reduction

Chromium reduction potential of the individual bacteria was evaluated in chromium reduction broth having $500 \mu\text{g ml}^{-1}$ K_2CrO_4 . A loop full of bacterial culture was suspended in distilled water in a microfuge tube. This bacterial suspension was inoculated in 5ml of chromium reduction broth incubated for 48 hours at 37°C . One milliliter of the cultured broth was put in a microfuge tube and then centrifuged (4000 rpm for 5 minutes). Hexavalent chromium reduction was detected at a spectrophotometer at 540 nm wavelength (Clesceri *et al.* 1998).

2.4 Determination of initial concentration, temperature and pH effect

Two initial chromate concentration (500 and $1000 \mu\text{g ml}^{-1}$ of K_2CrO_4) in DeLeo and Ehrlich medium were used in shaking incubator at 150 rpm at 28°C , 37°C and 45°C and, 5.0, 7.0 and pH9 separately for 24-48 hours to check chromate reduction.

2.5 Determination of heavy metals effects

A total of $50 \mu\text{g ml}^{-1}$ each of MnSO_4 , Na_2HAsO_4 , NiCl_2 , CuSO_4 and ZnSO_4 were added in culture medium separately, amended with $500 \mu\text{g ml}^{-1}$ of K_2CrO_4 . Cultures were incubated at 37°C and pH7 for 48 hours and, processed for measuring reduction by spectrophotometric method at 540nm.

2.6 Determination of effects of inhibitors

Certain antibiotics inhibit the growth of these bacteria and reducing the reduction potential. Ampicilline ($10 \mu\text{g ml}^{-1}$) and chloramphenicol ($30 \mu\text{g ml}^{-1}$) with potassium chromate ($500 \mu\text{g ml}^{-1}$) were added in the culture medium. Cultures were incubated at 37°C with pH 7.0 for 48 hours and processed for measuring Cr-VI reduction.

2.7 Determination of effect of time duration

Chromium reduction potential was also monitored at various time durations of 24, 48 and 72 hours. For this purpose, reduction medium was amended with potassium chromate at a concentration of $500 \mu\text{g ml}^{-1}$ and incubated at 37°C . Harvest was taken after regular intervals.

2.8 Determination of effect of industrial effluent

K_2CrO_4 at a quantity of $200 \mu\text{g ml}^{-1}$ was taken in one liter wastewater (autoclaved). This wastewater was then mixed with DeLeo and Ehrlich medium in 1:1 ratio. The cultures were incubated at 150 rpm with 37°C for 24 hours. The reduction of chromate was measured by spectrophotometric method.

2.9 Cell-free assay

Over-night grown bacterial culture in 200ml of LB-broth was taken with centrifugation at 6000 rpm at 4°C for 20min. Pellet was twice washed with Tric HCl. Bacterial cells were disrupted by sonication (Sonics VC 500 USA) for 5 min under cold (4°C) conditions. Supernatant was taken after 8000rpm shaking. Whereas, the extract heated at 100°C for 30min was used as negative control. Cr(VI) reduction was evaluated using crude extract through four parameters including potassium chromate concentration, pH, temperature and industrial effluent.

3. Results and discussion

3.1 Effect of initial concentration, temperature and pH effect

Although potassium chromate was reduced at both concentrations but maximum reduction was 50.48 percent by CrK16 and 48.99% by CrKS1 strains at $500 \mu\text{g ml}^{-1}$. Whereas lowest reduction obtained was 40 percent by CrK08 and 35 percent by CrKS1 strains at $1000 \mu\text{g ml}^{-1}$ (Fig. 1). Chromate reeducation rate was increases using *Bacillus* sp. and starts decreasing after certain level (Wang and Xiao, 1995). It can tolerate about 600mM chromate and reduces 0.2mM completely into less toxic chromate in 24 hours under aerobic conditions (Amoozegar *et al.* 2007). Bacterial resistance to chromium at high concentration was also reported by Shakoori *et al.* (2000). Chromium reduction is an enzyme-mediated process in bioremediation and any change in pH affects enzymatic activity (Mayo and Noike 1996). After 48 hours of incubation, maximum reduction was shown by all strains at pH7 i.e. 51, 57 and 48 percent by CrK08, CrKS1 and CrK16, respectively. pH9 was found second most favorable value for reduction by all the three strains, whereas, minimum reduction was observed at pH7 i.e. 22, 27 and 18% by CrK08, CrKS1 and CrK16, respectively (Fig. 2). CrK08, CrKS1 and CrK16 showed maximum reduction at 37°C . However, optimum temperature for reduction was found to be 28°C because it stimulated growth of each strain (Fig. 3). Most of the bacterial strains reduce chromate at 30 to 37°C and optimally at 30°C (Wang *et al.* 1989).

3.2 Effect of heavy metals effects

Majority of strains showed higher reduction rate with CuSO_4 and NiCl_2 as compared to Na_2HASO_4 and MnSO_4 . Only CrK16 showed 36, 51, 34, 32 and 13% reduction in the presence of NiCl_2 , CuSO_4 , ZnSO_4 , Na_2HASO_4 and MnSO_4 , respectively. CrK16 showed maximum reduction potential i.e. 51% in the presence of CuSO_4 . It was observed that the ability of chromium resistant bacteria decreased with addition of metals especially MnSO_4 (Fig. 4). Living organisms including humans require metal ions like Co, Cu, Mn, Zn (Nies *et al.* 1992). Excessive amounts of these metals beyond optimal level prove toxic to the organisms. Any imbalance in their quantity

results reduced growth of bacteria (Rathnayake *et al.* 2013). Small quantity of heavy metals such as arsenic ($60 \mu\text{g ml}^{-1}$) and copper ($20\text{-}40 \mu\text{g ml}^{-1}$) does not affect the reduction potential of *Pseudomonas* (McLean and Beveridge, 2001). However, addition of $200 \mu\text{g ml}^{-1}$ and $100 \mu\text{g ml}^{-1}$ of zinc has been found to decrease chromium reduction by 16 and 33 percent by *E. coli*, respectively (Shen and Wang, 1994).

3.3 Effects of inhibitors

Ampicilline was found to suppress the reduction potential of all of the three strains as compared to chloramphenicol. CrK08 and CrK16 showed comparatively better reduction potential in the presence of ampicilline i.e. 20 and 19%, respectively (Fig. 5).

3.4 Effect of time duration

Reduction potential was found directly proportional to time interval provided. CrK08, CrKS1 and CrK16 strain showed 52, 49 and 56% reduction after 24 hours of incubation, respectively. But after 72 hours strains CrK08, CrKS1 and CrK16 showed 80, 81 and 85% reduction, respectively. This increase in reduction potential is associated to gradual increase in cell mass with the passage of time. This suggests that for complete reduction of chromate by these bacterial strains, the incubation time should be more than 72 hours (Fig. 6).

3.5 Effect of industrial effluent

All of the three strains showed higher reduction potential which was 57.9, 56.3 and 52.9% by CeK08, CrKS1 and CrK16 after 24 hours of incubation, respectively (Fig. 7). Bioremediation proves to be an effective and inexpensive approach for recycling wastewater. This approach in conjunction of plants e.g. *Eichhornia crassipes* (phytoremediation) founds to be more efficient for chromate reduction (Jinxia *at al.* 2009). Introduction of various microbes offers environmental friendly conversion of chromate into insoluble Cr-III and sewage treatment.

3.6 Outcomes of cell free assay

Strains CrK08 and CrK16 bacterial strains were cultured to evaluate the effects of these parameters. CrK16 showed comparatively better growth under different parameters (Fig. 8-11).

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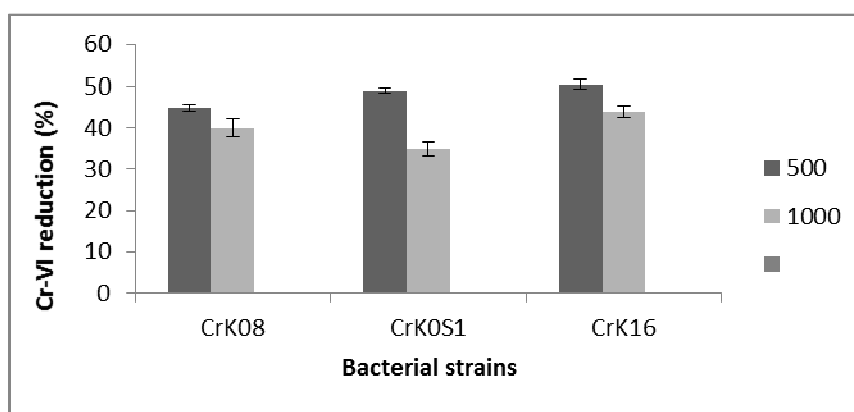


Figure-1: Effect of initial concentration (500 µg ml⁻¹ and 1000 µg ml⁻¹) of Cr-VI (K₂CrO₄) on reduction potential of bacterial strains CrK08, CrK0S1 and CrK16.

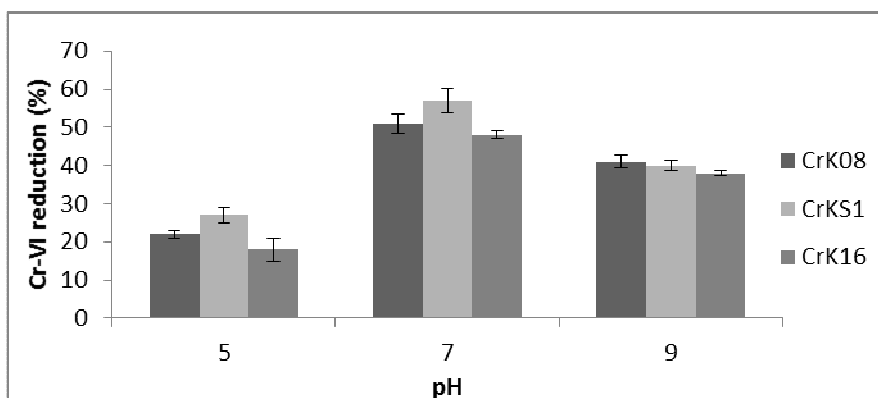


Figure-2: Effect of pH (5, 7 and 9) on Cr-VI (K₂CrO₄) reduction potential of bacterial strains CrK08, CrK0S1 and CrK16.

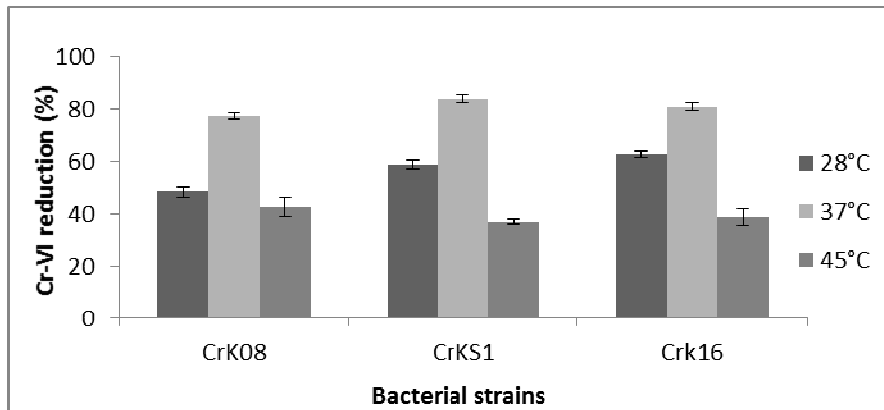


Figure-3: Effect of temperature (28°C, 37°C and 45°C) on Cr-VI (K_2CrO_4) reduction potential of bacterial strains CrK08, CrK0S1 and CrK16.

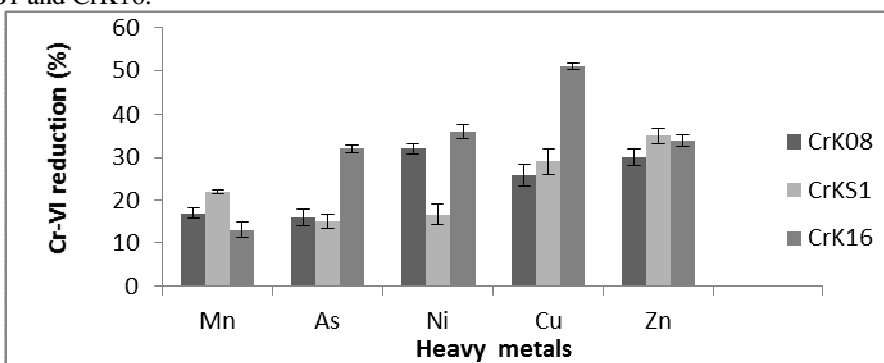


Figure-4: Effect of heavy metals (Mn, As, Cu and Zn) on Cr-VI (K_2CrO_4) reduction potential of bacterial strains CrK08, CrK0S1 and CrK16.

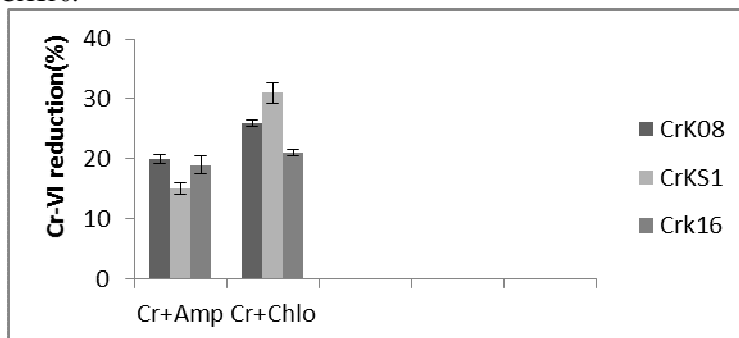


Figure-5: Effect of antibiotics (Ampicilline and chloramphenicol) on Cr-VI (K_2CrO_4) reduction potential of bacterial strains CrK08, CrK0S1 and CrK16.

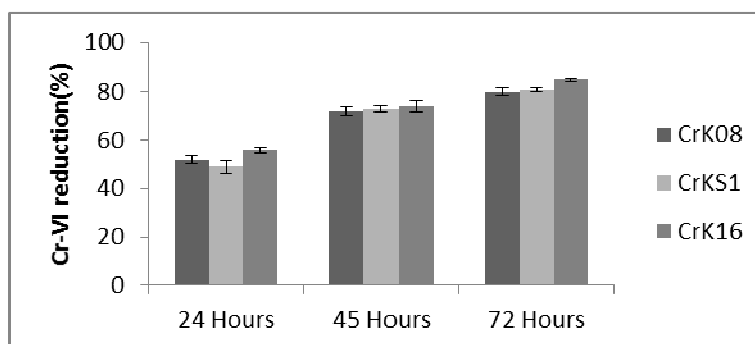


Figure-6: Effect of time intervals (24, 45 and 72 hours) on Cr-VI (K_2CrO_4) reduction potential of bacterial strains CrK08, CrK0S1 and CrK16.

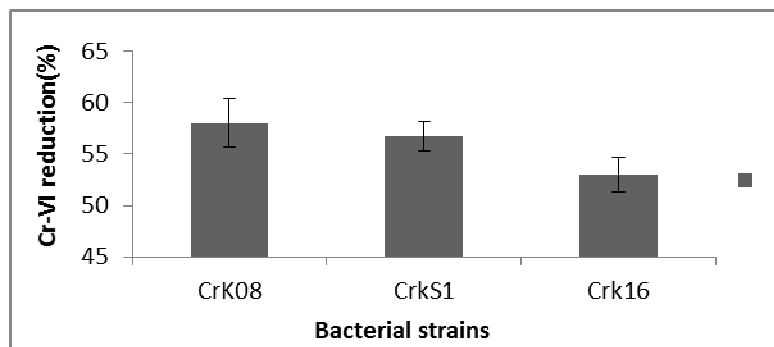


Figure-7: Effect of industrial effluent (wastewater) on Cr-VI (K_2CrO_4) reduction potential of bacterial strains CrK08, CrK0S1 and CrK16.

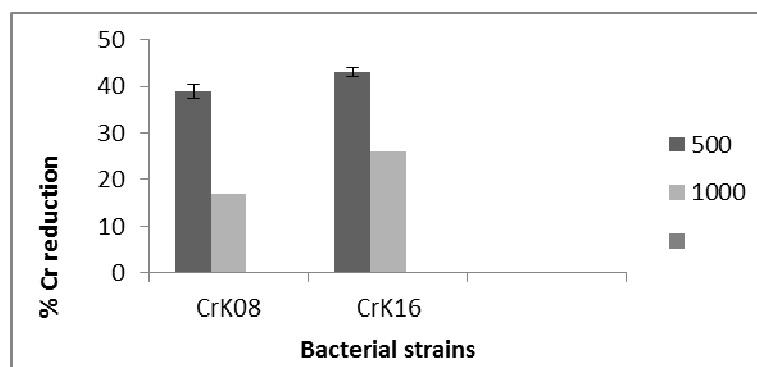


Figure-8: Effect of initial concentration ($500\ \mu g\ ml^{-1}$ and $1000\ \mu g\ ml^{-1}$) of Cr-VI (K_2CrO_4) using bacterial crude extract on reduction potential of bacterial strains CrK08 and CrK16.

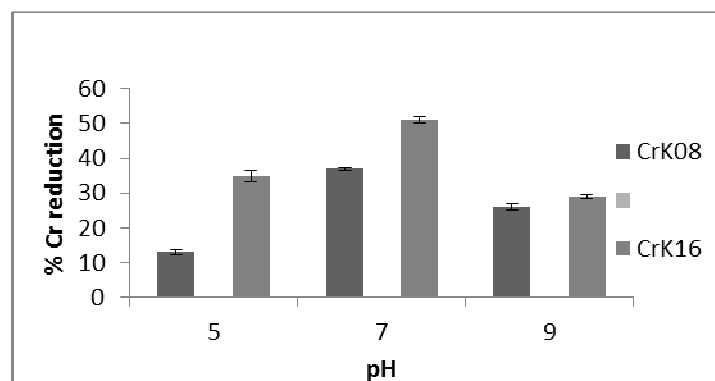


Figure-9: Effect of pH (5, 7 and 9) on Cr-VI (K_2CrO_4) using bacterial crude extract on reduction potential of bacterial strains CrK08 and CrK16.

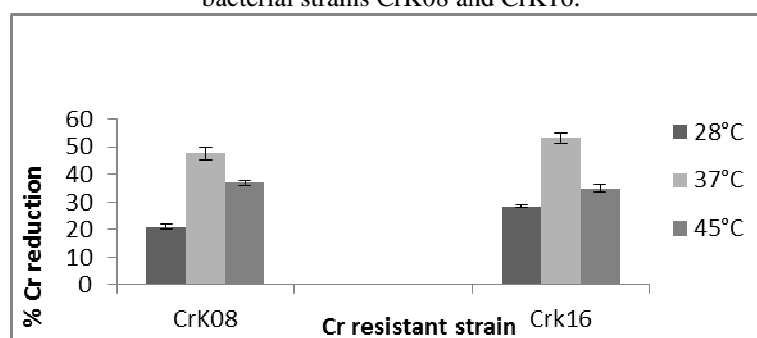


Figure-10: Effect of temperature ($28^\circ C$, $37^\circ C$ and $45^\circ C$) on Cr-VI (K_2CrO_4) using bacterial crude extract on reduction potential of bacterial strains CrK08 and CrK16.

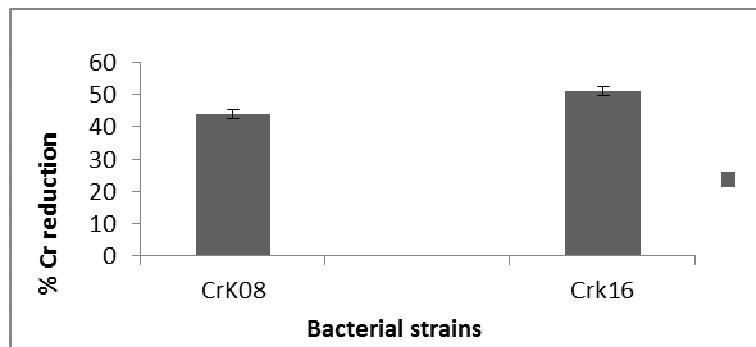


Figure-11: Effect of industrial effluent (wastewater) on Cr-VI (K_2CrO_4) using bacterial crude extract on reduction potential of bacterial strains CrK08 and CrK16.

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