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Full Length Article

Effect of Chromium Forms on the Biodegradation of Reactive Black-5 Azo Dye by *Psychrobacter* and *Klebsiella* species

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Abstract

Azo dyes and chromium (Cr) are released in the wastewater of leather processing industry during tanning process. Several species of bacteria have shown ability to degrade azo dyes individually; however, presence of Cr ions in industrial effluents may affect the bacterial efficiency of removing azo dyes from waste water. In this study, biodegradation of reactive black-5 dye was studied in a mineral salts medium (MSM) liquid culture in the presence of Cr ions. Two bacterial strains *Psychrobacter alimentarius* KS23 and *Klebsiella oxytoca* N7 capable of degrading azo dyes were used individually as well as in a consortium for the degradation of azo dyes. Cr was applied in the form of $K_2Cr_2O_7$, $CrCl_3$, $Cr_2(SO_4)_3$ at different concentrations (ranging from 0-250 mg L^{-1}) of Cr. The results showed that presence of Cr in mineral salt medium significantly inhibited the azo dye decolorization. In the absence of Cr salts, complete decolorization of 100 mg dye $L^{-1}$was achieved by the bacterial strains in 8-12 h while up to 83% decolorization occurred in 72 h at 100 mg L^{-1} Cr. The rate of decolorization considerably decreased with increasing concentration of azo dye in the presence of 50 mg Cr L^{-1} medium. These findings imply that the existence of Cr in wastewater can significantly affect the bacterial efficiency in wastewater treatment systems to degrade azo dyes. © 2015 Friends Science Publishers

Keywords: Azo dye; Chromium; Textile; Tannery; Decolorization; Reactive black-5

Introduction

The use of azo dyes in modern era represents a serious pollution problem worldwide. These are extensively used in leather, textiles, cosmetics, food, pharmaceutical and paper industries (Chang *et al.,* 2004; Telke *et al.,* 2008; Erkurt, 2010). Removal of dyes and their intermediates from effluents is a serious environmental issue as they are xenobiotic in nature and found to be recalcitrant to biodegradation (Garg *et al.,* 2004; Imran et al., 2014). Problem becomes more complex with the presence of other contaminants like chromium (Cr) compounds in the wastewater along with azo dyes (Bishnoi *et al*., 2004; Mahmood *et al*., 2013).

Due to improper treatment of waste water presence of dyes and metals cause severe damage to the aquatic life. Hexavalent Cr and azo dyes exhibits same properties such as water soluble, persistence, carcinogenic and mutagenic (Moosvi *et al.,* 2007; Desai *et al.,* 2008). Hence their combine existence in tannery and textile effluents would have synergistic effect in poisoning the environment. Presence of azo dyes in wastewater is esthetically unacceptable and also affects living fauna and flora of the water bodies by affecting photosynthetic activity of plants (Bayramoglu *et al.,* 2006). Moreover, toxic effects of azo dyes results in formation of tumor, cancer and skin allergies in human; they also inhibit growth of bacteria, protozoans, algae, plants and different animals (Sponza, 2002).

Bioremediation offers an environmental friendly and cost effective substitute for azo dye treatment as they have the ability to completely mineralize organic pollutants in a natural way (Olukanni *et al.,* 2006). Many microorganisms has already been reported with developed enzymatic system for individual or simultaneous degradation of azo dyes and Cr system (Pandey *et al.,* 2007; Desai *et al*., 2008; Khalid *et al.,* 2012; Mahmood *et al*., 2013).Therefore, these bacterial strains can be helpful under such conditions to decolorize azo dyes. Hence, the present study was designed by using our two previously reported bacterial species *Psychrobacter* and *Klebsiella* to determine the decolorization behavior of these species in the presence of different forms of Cr.

Materials and Methods

Reactive Black-5 (RB-5) was used as azo dye and for chromium (Cr) three different sources like $K_2Cr_2O_7$, $CrCl_3$, $Cr_2(SO_4)_3$ were used. Mineral salt medium (Khalid *et al.,* 2008) was used for conducting experiments. The study was conducted by using previously isolated bacterial

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strains *Klebsiella oxytoca* N7 (Bibi *et al*., 2012) and *Psychrobacter alimentarius* KS23 (Khalid *et al*., 2012) from textile effluents of Faisalabad and Arabian Sea sludge from Karachi, Pakistan respectively. Glassware and needed apparatus were washed and autoclaved at 121°C.

Effect of Different Cr Concentration on Reactive Black-5 Decolorization

Concentration dependent decolorization of the dye reactive black 5 was studied in the tightly sealed glass vials. Five different concentrations of the Cr (50 to 250 mg L^{-1}) with three different chromium (Cr) sources ($K_2Cr_2O_7$, $CrCl_3$, $Cr_2(SO_4)_3$). Ten mL of the mineral salt medium (MSM) spiked with 100 mg L^{-1} of reactive black-5 and respective concentration of the Cr was taken in each glass vials. Then these vials were separately inoculated with 50 µL of the inoculum of both strains with uniform optical density (0.8 ± 0.023). Glass vials were tightly sealed to provide partial anaerobic conditions and incubated at temperature of 35°C for72 h, under static conditions. In another experiment effectiveness of the individual and combination of the strains was also studied. In consortium 25 µL of each strain was used to make up the 50 µL volume of the inoculum.

Effect of Different Reactive Black-5 Concentration on Bacterial Dye Decolorizing Efficiency in the Presence of Cr

In present experiment reactive black-5 different concentrations were studied against the constant concentration 50 mg L^{-1} of the Cr. Concentrations of the reactive black-5 were 100, 200, 300, 400 and 500 mg L^{-1}. Same experimental procedure was used as described in previous experiment. All the experiments were performed in triplicate and uninoculated control was kept for comparison.

Decolorization Measurement and Calculations

Samples were periodically withdrawn after 24 h of incubation. Aliquot were collected uniformly from each test tube in to plastic vials (size 1.5 mL) and centrifuged at 10,000 x g for 10 min to spin down the suspended cell. Decolorization was measured at 597 nm by using MA 02052-USA visible spectrophotometer. Absorbance were converted to concentration unite in mg L^{-1} by drawing standard curve. Percentage decolorization was calculated by the following formula:

$$\text{Decolorization (\%)} = \frac{(C_i - C_f)}{C_i} \times 100$$

Whereas

C_i = Initial concentration; C_f = Final concentration.

Statistical Analysis

Data were presented as average of the three replicates. The mean values and standard errors were calculated by using Microsoft Excel 2010.

Results

The results showed that both strains N 7 and KS 23 were found to be capable of Reactive black-5 (100 mg L^{-1}) decolorization up to Cr concentration of 250mg L^{-1}. However reduction rate substantially decreased in the presence of Cr.

In present study, dye degradation by bacterial strain N 7 and KS13 was investigated at different Cr concentrations. The strain N 7 showed almost 100 % dye decolorization in first 12 h of incubation in the absence of Cr, but the addition of different Cr concentrations, the decolorization rate of reactive black-5 was decreased (Fig. 1a).In the presence of Cr maximum decolorization rate (91%) was observed at 100 mg L^{-1} of Cr, when $CrCl_3$ used as a Cr source after 72 h of incubation. Further increase in Cr concentration had negative impact on decolorization process and approximately 85 % decolorization was observed at higher concentrations of150 to 250 mg L^{-1} after 72 h. However variation in decolorization rate in initial 24 to 48 h of incubation was significant. About 76% of the dye was decolorized in first 24 h of incubation at 50 mg L^{-1} Cr, whereas in the same time period at higher concentrations (150–250 mg L^{-1}) the decolorization was in the range of 31 to 49% only. Similarly strain KS23 ability to degrade reactive black-5 was also observed at different concentrations of Cr (Fig. 1b). This strain exhibited maximum decolorization (88%) of reactive black-5 at 50 mg $L^{-1}$of Cr concentration after 72 h of incubation. Similarly this strain had better ability to perform at higher Cr concentrations (150-200 mg L^{-1}). At 100 and 150 mg L^{-1} of Cr ($CrCl_3$ as Cr source) concentrations about 72 and 65% reactive black-5 decolorization was observed just after 24 h of incubation. However after 72 h trend was almost similar with strain N7.

Different concentrations of Cr were also studied as $Cr_2(SO_4)_3$ salt to check impact of sulphate ion along with Cr. In this case with strain N7 approximately 78 and 70% of the reactive black-5 was decolorized at 50 and 100 mg L^{-1} of Cr in initial 24 h (Fig. 2a). Whereas at 250 mg L^{-1} of Cr only 8% decolorization was observed after 24 h, but after 48 and 72 h the decolorization process enhanced and 84% decolorization was achieved after 72 h at the same concentration. Maximum decolorization (85%) was noted at 50 mg L^{-1} of Cr with strain N7. Almost similar trend was observed with strain KS23 and maximum decolorization observed was 86% at 50 mg $L^{-1}$of Cr (Fig. 2b). Minimum decolorization of the reactive black with this Cr source was 77% at 250 mg L^{-1} Cr concentration. After 72 h there was non-significant difference was observed from Cr concentrations of 50 to 200 mg L^{-1}.The data for $K_2Cr_2O_7$ was not presented as there was only negligible decolorization with this salt as Cr source.

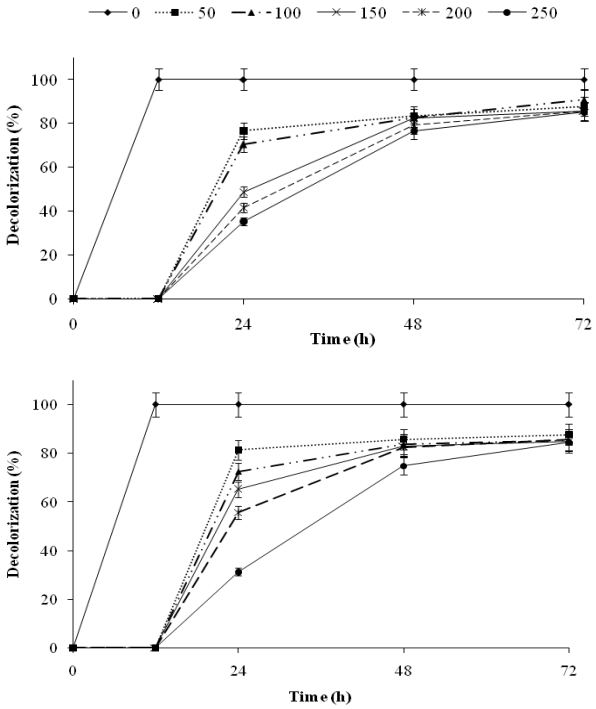

Fig. 1: Percent decolorization of reactive black-5 azo dye in liquid medium at different concentrations of chromium when $CrCl_3$ salt used as Cr source by selected bacterial N7 (above) and KS23 (below)

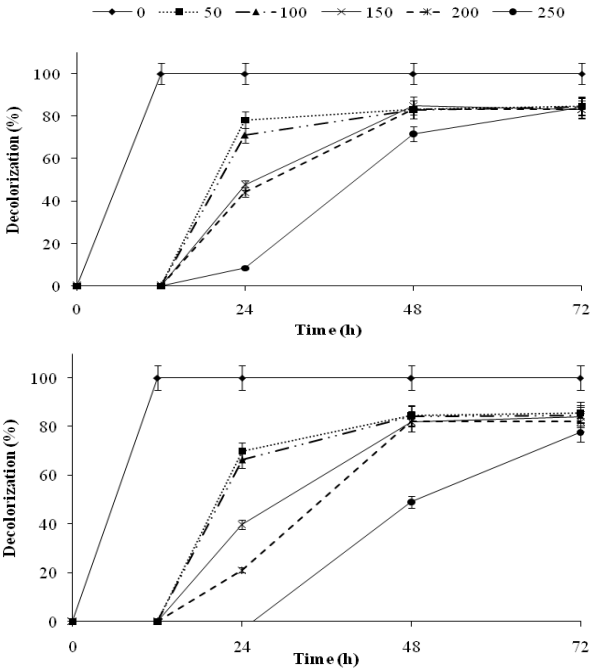

Fig. 2: Percent decolorization of reactive black-5 azo dye in liquid medium at different concentrations of chromium when $Cr_2(SO_4)_3$ salt used as chromium source by selected bacterial N7 (above) and KS23 (below)

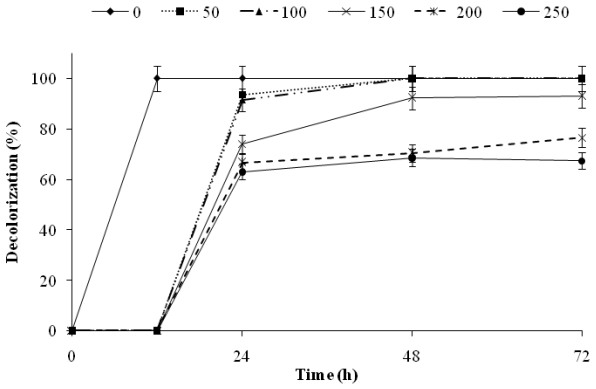

Fig. 3: Percent decolorization of reactive black-5 azo dye in liquid medium at different concentrations of chromium when $CrCl_3$ salt used as Cr source by consortium

In another experiment consortium of the both strains was developed to see the effectiveness of these strains in combination (Fig. 3). Consortium of these strains showed enhanced decolorization potential and even 93 and 92% of the dye was decolorized in first 24 h of incubation at 50 and 100 mg L^{-1} of Cr ($CrCl_3$ as Cr source). Whereas 100 % decolorization was achieved at these concentration in 48 h. Also in case 150 mg L^{-1} of Cr 100% decolorization was occurred after 72 h.

Effect of different concentration of Cr was also studied at constant 50 mg L^{-1} of Cr concentration with both salt $CrCl_3$ and $Cr_2(SO_4)_3$. In the case of $CrCl_3$ as a Cr salt the strain N7 had approximately 88% of dye decolorization at concentration of 100 mg L^{-1} dye (Fig 4a). Further increase in reactive black had negative impact on decolorization process and at highest concentration of reactive black-5 i.e., 500 mg L^{-1} only 57% reactive black-5 decolorization was observed. Whereas other concentrations of the dye from 200 to 400 mg L^{-1} were decolorized in the range of 62 to 71% only after 72 h of growth period. In the same way other strain KS23 showed 91% reactive black-5 degradation at 100 mg L^{-1} of the dye with constant 50 mg L^{-1} of Cr with same salt as previous discussed in this paragraph (Fig 4b). Further increase in dye concentration had negative correlation with the reactive black-5 degradation. At 200 mg L^{-1} dye about 20% reduction in decolorization was observed as compared to its lower concentration of 100 mg L^{-1}. This gap further widened with more increase in concentration and only 58% decolorization was occurred at 500 mg L^{-1} of dye concentration.

The correlation between the $Cr_2(SO_4)_3$ salt as a Cr source and different concentrations of reactive black was also studied. This salt had more negative impact as compared to the $CrCl_3$ salt as a Cr source. The maximum decolorization observed in this experiment was 81% with strain N7, about 7% less as compared to $CrCl_3$ as a Cr source (Fig 5a). Similarly at highest concentrations of the reactive black-5 the condition become worse and only 18

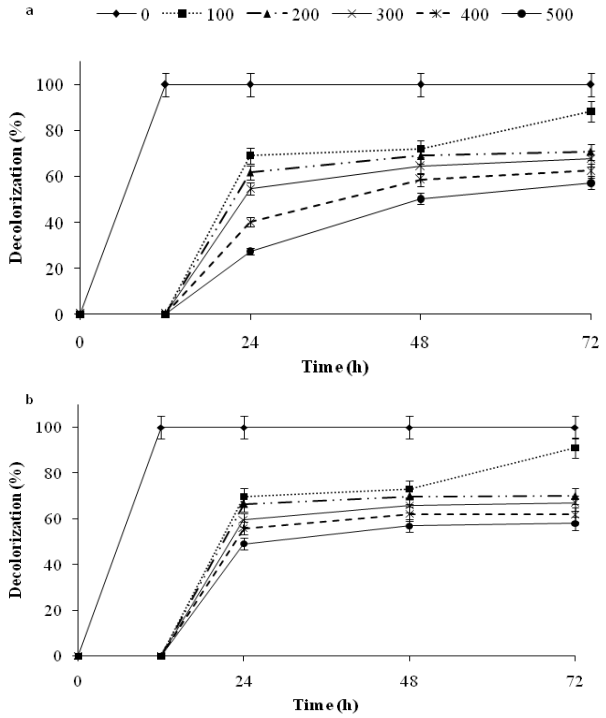

Fig. 4: Percent decolorization of reactive black-5 azo dye in liquid medium at different concentrations of reactive black-5 when $CrCl_3$ salt used as chromium source by selected bacterial N7 (a) and KS23 (b)

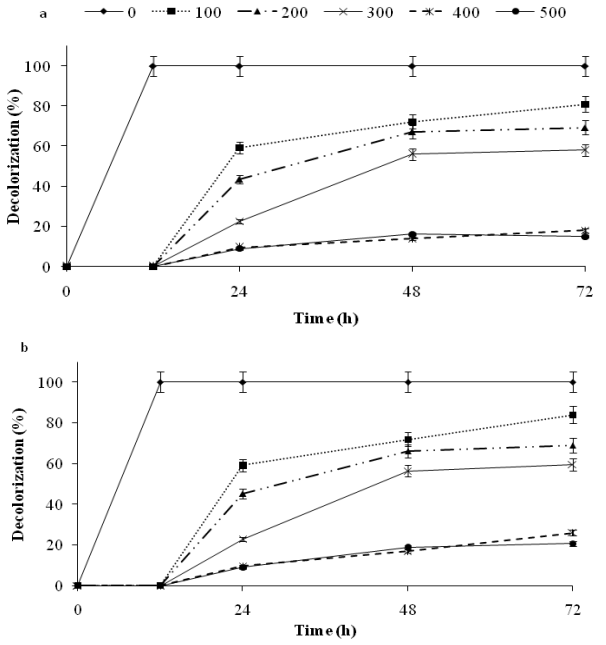

Fig. 5: Percent decolorization of reactive black-5 azo dye in liquid medium at different concentrations of reactive black-5 when $Cr_2(SO_4)_3$ salts used as chromium source by selected bacterial N7 (a) and KS23 (b)

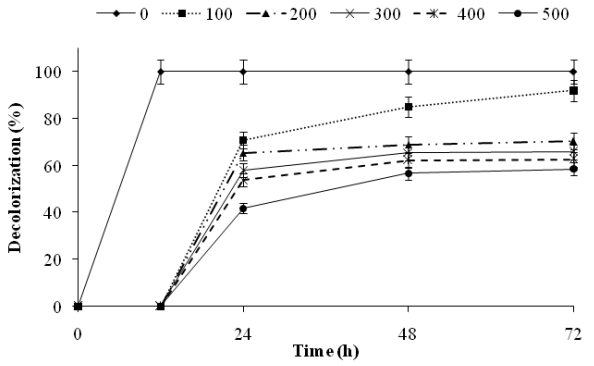

Fig. 6: Percent decolorization of reactive black-5 azo dye in liquid medium at different concentrations of reactive black-5 when $CrCl_3$ salt used as Cr source by consortium

and 15% of dye removal was observed at 400 and 500 mg L^{-1} dye, respectively. The strain KS23 performance was better as compared to N7 and approximately 84% reactive black-5 decolorization was observed at lowest concentration of reactive black-5 (Fig 5b). Likewise at higher concentration 400 and 500 mg L^{-1}decolorization of dye was 26 and 21% respectively.

Again the consortium of the strain N7 and CKB3 was also studied with varying concentrations dye and 50 mg L^{-1} of Cr in the form of $CrCL_3$. It showed enhanced decolorization as compared to individual strains and maximum of 94% removal of reactive black-5 was found at concentration of 100 mg L^{-1}(Fig 6). However at higher concentrations the results were compatible with individual strains. Overall it was observed that increased concentrations of the both Cr and reactive black-5 had severed negative impact on decolorization process. Similarly comparison of the different Cr salts indicated that the $K_2Cr_2O_7$ had most negative impact (data not presented) as almost no decolorization was observed with this salt. However in the presence of salts $CrCl_3$ and $Cr_2(SO_4)_3$ decolorization of the reactive black-5 can occur.

Discussion

This study exhibited that the increasing concentrations of the Cr had profound negative impact on bacterial decolorization ability of the azo dye reactive black-5. Previously it was also reported that the Cr can be a potential problematic pollutant commonly coupled with azo dyes in waste water released from dying industries (Yusuff and Sonibare, 2004). Few species were reported to have the ability to tolerate the very high levels of Cr (Kathiravan *et al.*, 2010; Dey and Paul, 2012; Christl *et al.*, 2012). However elevated levels of Cr negatively influence their survival and growth (Oleszkiewicz and Sharma, 1990). Therefore based on this data we designed a study to see the impact of Cr on azo dye degradation ability of the

previously reported strains having the efficient dye degradation process.

In present study, it was observed that Cr had crucial effect on decolorizing efficiency of bacteria relatively to its normal degrading ability. Similarly sources of Cr also very important as in our study the maximum degradation of the reactive black was observed with $CrCl_3$ followed by $Cr_2(SO_4)_3$ as a Cr source. Both the strains were not able to decolorize dye even at very low concentration (30 mg L^{-1}) of Cr as $K_2Cr_2O_7$.Hexavalent Cr is highly toxic even at very low concentration (Mahmood *et al*., 2013), so decolorization was not observed when $K_2Cr_2O_7$was used as Cr source. It was well reported that in case of $K_2Cr_2O_7$ almost all the Cr were in hexavalent form and other sources like $Cr_2(SO_4)_3$ and $CrCl_3$ also contains minor quantities of the hexavalent Cr that use of $K_2Cr_2O_7$ during dyeing process may produce thriving condition for bacterial growth in MSM. Hence provides attention to research more with $K_2Cr_2O_7$.

Similarly ability of the consortium of these strains to decolorize the reactive black was also studied and it was observed that consortium had little bit enhanced degradation ability compared to the individual strains. This trend may be due to the synergistic effect of the strains in consortium form (Khehra *et al.,* 2005; Saratale *et al.,* 2009).Similarly another important finding of our study is that at higher concentrations of both Cr and azo dye reactive black-5, there was significant reduction in decolorization process occurred. The inhibiting effect on the decreasing the performance of bacteria might be due to the reason of toxic effect of these contaminants at increased concentration for bacterial cells (Dhal *et al*., 2010; Dey and Paul, 2012). Another important factor for this reduction can be the due to the production of morphological changes and oxidative stress on bacterial cell (Desai *et al.,* 2008).

Conclusion

The strains *Psychrobacter alimentarius* KS23 and *Klebsiella oxytoca* N7 were capable of degrading azo dyes individually as well as in consortium under higher Cr and dye condition with varying potential.

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