



# International Journal of Applied Sciences and Biotechnology

A Rapid Publishing Journal

**ISSN 2091-2609**

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**CODEN (Chemical Abstract Services, USA): IJASKD**

**Vol-3(1) March, 2015**

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Impact factor\*: **1.422**  
Scientific Journal Impact factor#: **3.419**  
Index Copernicus Value: **6.02**

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Research Article

## SIMULTANEOUS BIOREMEDIATION OF PHENOL AND Cr (VI) FROM TANNERY WASTEWATER USING BACTERIAL CONSORTIUM

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### Abstract

In the present study a consortium of four naturally isolated bacterial strains was evaluated as remediation tool for simultaneous removal of phenol and Cr (VI) from tannery effluent. Application of bacterial consortia to effluent (pH 4.6) resulted in 100 and 78% removal of initial 47 mg L<sup>-1</sup> phenol and 16 mg L<sup>-1</sup> Cr (VI), respectively at 96 h of treatment. The consortium was also active in removal of contaminants with lower removal rate in presence of extraneous higher concentrations of both phenol and Cr (VI). Treatment in static mode also resulted in removal of pollutants, however with increase in agitation speed simultaneous reduction of contaminants becomes faster. Overall it can be inferred from the study that the above formulated bacterial consortium could effectively be used for treatment of phenol and Cr (VI) laden tannery and other industrial effluents.

**Keywords:** Tannery effluent; Bioremediation; Phenol; Cr (VI), Consortium

### Introduction

Tannery is one of the oldest industries which deal with processing of animal skin and hide for manufacture of leather. Leather manufacturing involves huge quantity of toxic and hazardous chemicals that are used in different steps of processing (Chandra *et al.*, 2011; Bhattacharya *et al.*, 2014). The effluents from these industries thus contain lots of these chemical contaminants. Among different contaminants, phenol and Cr (VI) are noteworthy. In typical tannery effluents, phenolic compounds and Cr concentrations range from 0.4 to 100 and 3 to 350 mg L<sup>-1</sup>, respectively (Midha and Dey, 2008).

Both phenol and Cr (VI) are known to be highly toxic and carcinogenic pollutants. Their ease of solubility with water, high mobility, and recalcitrant nature further worsens their polluting ability. Owing to their hazardous properties, both phenol and Cr (VI) are listed as priority pollutants by the US Environmental Protection Agency (EPA) (Ontañon *et al.*, 2014). This necessitates treatment of tannery effluent for removal of these contaminants before discharging it into open environment.

Conventional physico-chemical treatment methods are generally used by tanneries for removal of phenolics and chromium. However, biological mode of treatment is preferred over physico-chemical methods due to sustainable nature of the former. Biological treatment usually involves use of microorganisms or combination of other biological

agents for removal of contaminants. There are reports of simultaneous removal of phenol and Cr (VI) using different microorganisms in synthetic or simulated system (Chirwa and Wang, 2000; Wang and Nkhalambayausi-Chirwa, 2001; Liu *et al.*, 2008; Song *et al.*, 2009; Chakraborty *et al.*, 2013; Gunasundari and Muthukumar, 2013). For instance, Gunasundari & Muthukumar (2013) reported *Stenotrophomonas* sp. isolated from tannery effluent contaminated soil for simultaneous bioremediation of phenol and Cr (VI). Similarly, Liu *et al.* (2008) have used consortia of microbes containing *Bacillus* sp. and *Pseudomonas putida* Migula for bioremediation of phenol and Cr (VI) from co-contaminated system. But very limited studies exist in which potential microorganisms were actually tested for removal of these contaminants from real industrial effluents. Industrial effluents are complex in nature as they are known to contain various organic, inorganic, and biological entities. As a result of this, very few microorganisms having multiple tolerances ability are able to survive and perform efficiently in complex effluent system. Consortium culture having number of efficient microbial strains are generally used for treatment of industrial effluents as these strains work synergistically in complex effluent.

In the present study consortia of four different bacterial strains were evaluated for simultaneous remediation of phenol and Cr (VI) from real tanning effluent. The effects of higher dosages of contaminants and agitation speeds

were also studied in order to check the efficiency of tested consortium for further industrial applications.

## Material and Methods

### Material

The media components were purchased from Hi Media Laboratories (Mumbai, India). Phenol and potassium dichromate ( $K_2Cr_2O_7$ ) which was used as source of Cr (VI) were procured from SISCO research laboratories (Mumbai, India). All other chemicals used were of analytical grade.

### Microorganism

Bacterial consortia containing three Gram -ve strains *Acinetobacter* sp. B9 (MTCC 10506), isolate B1 and C2 and one Gram + ve strain *Arthrobacter* sp. B2 (MTCC 10511) was used.

### Sample collection

The effluent was collected from local industry at Kanpur, Uttar Pradesh, India. Since the original effluent was found to contain low concentration of phenol ( $6.60 \pm 0.565$  mg L<sup>-1</sup>) and Cr (VI) ( $0.401 \pm 0.037$  mg L<sup>-1</sup>), it was amended with extraneous phenol and Cr (VI) solutions to make final 47 mg L<sup>-1</sup> phenol and 16 mg L<sup>-1</sup> Cr (VI).

### Preparation of mother culture

Loopful each of B1, B2, B9, and C2 strains were inoculated to 25 mL of sterile nutrient broth (NB) taken in 100-mL Erlenmeyer flask and incubated overnight at 30°C and 200 rpm. Entire 25 mL of mother culture comprising mixed culture was pellet down using centrifugation ( $2376 \times g$ ) for 10 min at 4°C. Pellet was first washed with saline solution and finally suspended in 2.0 mL of saline using vortexing. The resultant cell suspension (2.0 mL) was used as inoculum for the treatment of wastewater.

### Simultaneous removal of phenol and Cr (VI) from wastewater

Mother culture prepared as described above was inoculated to 50 mL of tannery wastewater sample taken in 250-mL Erlenmeyer flask, followed by incubation at 30°C and 200 rpm for 96 h. During treatment, aliquots of wastewater sample were withdrawn periodically (at an interval of 24 h) for estimation of residual phenol and Cr (VI). A control setup without any extraneous addition of any microbe was run in parallel to test sample to monitor the role of abiotic factors and native microbes on reduction of phenol and Cr (VI).

### Effect of higher Cr (VI) concentrations on simultaneous phenol and Cr (VI) removal

Substantial concurrent removal of initial 47 mg L<sup>-1</sup> phenol and 16 mg L<sup>-1</sup> Cr (VI) from tannery wastewater using tested microbial cultures encouraged us to test the potential of mixed culture for simultaneous removal of these toxicants in presence of higher concentration of Cr (VI) and phenol. To examine this, wastewater sample containing initial 47 mg L<sup>-1</sup> phenol were first amended with extraneous Cr (VI)

to make higher Cr (VI) concentrations of 25 and 32 mg L<sup>-1</sup>. This was followed by inoculation of wastewater samples with mixed culture inoculums as described before. The treatment was carried out at 30°C and 200 rpm for 96 h. Aliquots of wastewater samples were withdrawn at 24 h interval for estimation of residual Cr (VI) and phenol concentrations. A control setup without exogenous addition of any microorganism was treated similarly like test sample to monitor the role of abiotic factors and native microbes on reduction of phenol and Cr (VI).

### Effect of higher phenol concentration on simultaneous phenol and Cr (VI) removal

Wastewater sample containing initial 16 mg L<sup>-1</sup> Cr (VI) was amended with extraneous phenol to make final phenol concentration to 94 mg L<sup>-1</sup>. Wastewater sample was then inoculated with mixed culture consortium followed by incubation at 30°C and 200 rpm for 96 h. Aliquots of wastewater sample were withdrawn at an interval of 24 h for estimation of residual Cr (VI) and phenol concentrations.

### Effect of different agitation speeds on simultaneous phenol and Cr (VI) removal

To observe the effect of agitation speed on phenol and Cr (VI) removal efficiency of mixed culture, wastewater samples containing initial 47 mg L<sup>-1</sup> phenol and 16 mg L<sup>-1</sup> Cr (VI) was inoculated with mixed culture. This was followed by incubation of inoculated samples at 30°C under different agitation speeds (0, 100, and 200 rpm). During incubation, samples were taken at every 24 h interval and analyzed for phenol and Cr (VI) removal. A control setup mentioned as above was run in this case also.

### Determination of phenol and Cr (VI) concentration

Phenol and Cr (VI) concentrations in the samples were determined colorimetrically at 500 and 540 nm, respectively using UV-Vis spectrophotometer (APHA, 1998). Estimation of total chromate concentration in the sample was done using atomic absorption spectrophotometer (AAS) after acid digestion of the sample (Bhattacharya and Gupta, 2013).

Each experiment was done at least two times and mean values are reported. Standard deviations are shown as error bars in the figures.

## Results and Discussion

Tanning industries are known as one of the most polluting industries causing severe environmental pollution through its effluent discharge (Chandra *et al.*, 2011; Verma and Singh, 2012). The tannery effluent especially originating from tanning processes contains a huge quantity of pollutants including organic matter, phenolics, tannins, and chromium and possesses serious health and environmental threats (Chandra *et al.*, 2011; Bhattacharya *et al.*, 2014). Thus, treatment of tannery wastewater is a prerequisite before discharging it into the environment. The present

study deals with simultaneous remediation of phenol and Cr (VI) from tannery effluent by using consortium of four bacterial isolates. Inoculated consortium culture along with the indigenous microbes of the wastewater resulted in a significant removal of phenol and Cr (VI) compared to the control, i.e., in the absence of any exogenous microorganisms or consortia. The removal of contaminants from undiluted wastewater and in the absence of any external nutrient source shows the capability of culture for tannery effluent treatment.

**Simultaneous remediation of phenol and Cr (VI) from industrial wastewater**

Fig. 1 shows the simultaneous removal of phenol and Cr (VI) using consortium culture. After 96 h of treatment with consortium, overall 100% removal of phenol and 78% reduction of Cr (VI) could be achieved. Removal of phenol was slow in the first 24 h while at 48 and 72 h, 37 and 85% reduction of phenol were detected followed by complete reduction at 96 h. The removal of Cr (VI) on the other hand was observed to be faster in the first 48 h; thereafter, the reduction became slower with very small differences at further time periods of 72 and 96 h. The control set showed 13 and 60% reduction of phenol and Cr (VI) at 96 h, respectively. The reduction in case of control might be due to activity of indigenous microorganisms of the wastewater. Zahoor and Rehman (2009) have also observed about 52-62% reduction of Cr (VI) in control set up (effluent without

any extraneous addition of microorganism) due to native microflora of wastewater.

There was also overall 81% reduction of initial total Cr content ( $1525 \text{ mg L}^{-1}$ ) after 96 h treatment of wastewater with mixed culture. As shown in Fig. 2 industrial wastewater was also found to be decolorized after treatment. Similar microbial treatment of tannery wastewater for the simultaneous removal of chromate and phenolics has been reported by Srivastava *et al.* (2007) using consortia of microbes, whereas individual removal of either phenol or Cr (VI) from tannery wastewater has been demonstrated by Paisio *et al.* (2012) using *Rhodococcus* strain and Panda and Sarkar (2012) using *Enterobacter aerogenes* T2, respectively.

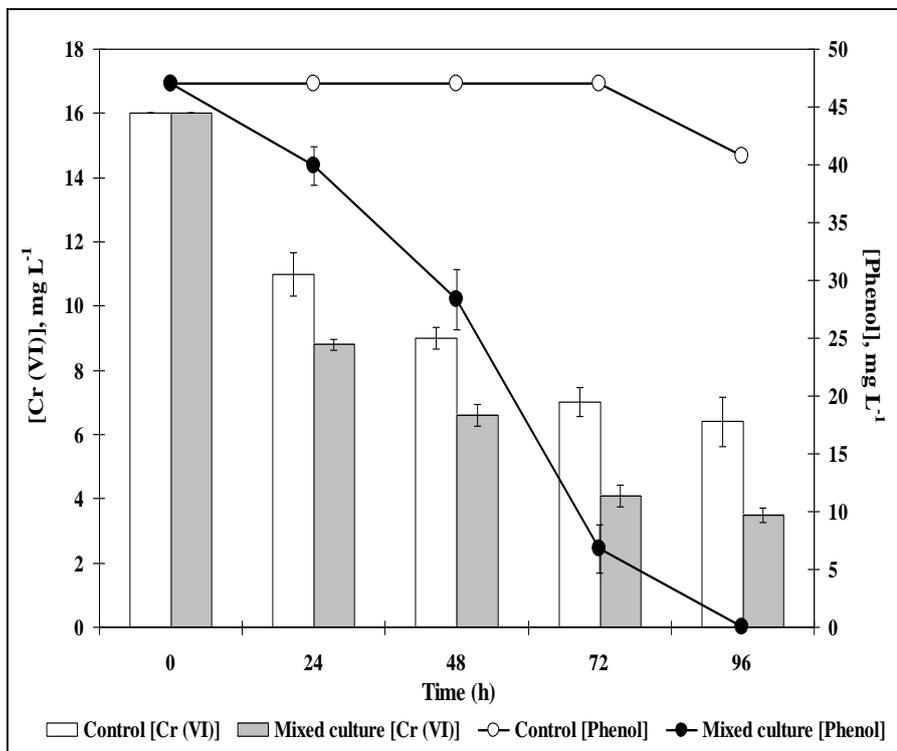


**Fig. 2:** Color of the wastewater before and after treatment

- 1- Original wastewater before treatment
- 2- After treatment with mixed culture

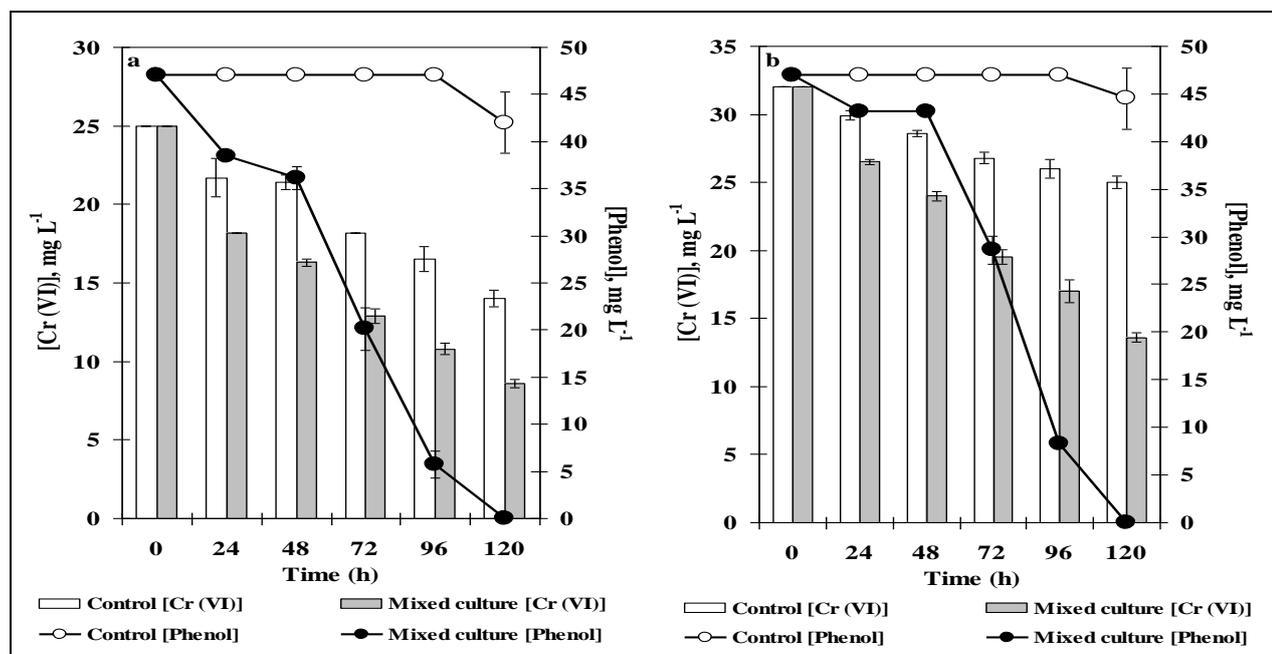
**Simultaneous removal of phenol and Cr (VI) from wastewater in presence of higher concentrations of Cr (VI)**

In presence of initial 25 and  $32 \text{ mg L}^{-1}$  Cr (VI), mixed or consortium culture showed complete removal (100%) of phenol at 120 h of treatment (Figure 3). Whereas, at both the Cr (VI) concentrations, overall 66 and 57% reduction of initial chromate content, respectively, at 120 h were recorded (Fig. 3). Control set up which was not exogenously supplied with consortia or any microorganism achieved 10 and 44% removal of phenol and Cr (VI), respectively in presence of initial Cr (VI) content of  $25 \text{ mg L}^{-1}$  after 120 h. At  $32 \text{ mg L}^{-1}$  Cr (VI) content, control set up overall showed 5 and 22% reduction of phenol and Cr (VI), respectively at 120 h. The lower phenol and Cr (VI) reduction is due to the toxicity of higher Cr (VI) concentrations on microbial culture (Song *et al.*, 2009; Gunasundari and Muthukumar, 2013). Lower and slower simultaneous reduction of phenol and Cr (VI) in presence of higher chromate content have also been observed by Song *et al.* (2009) using *Pseudomonas aeruginosa* CCTCC AB91095 culture.



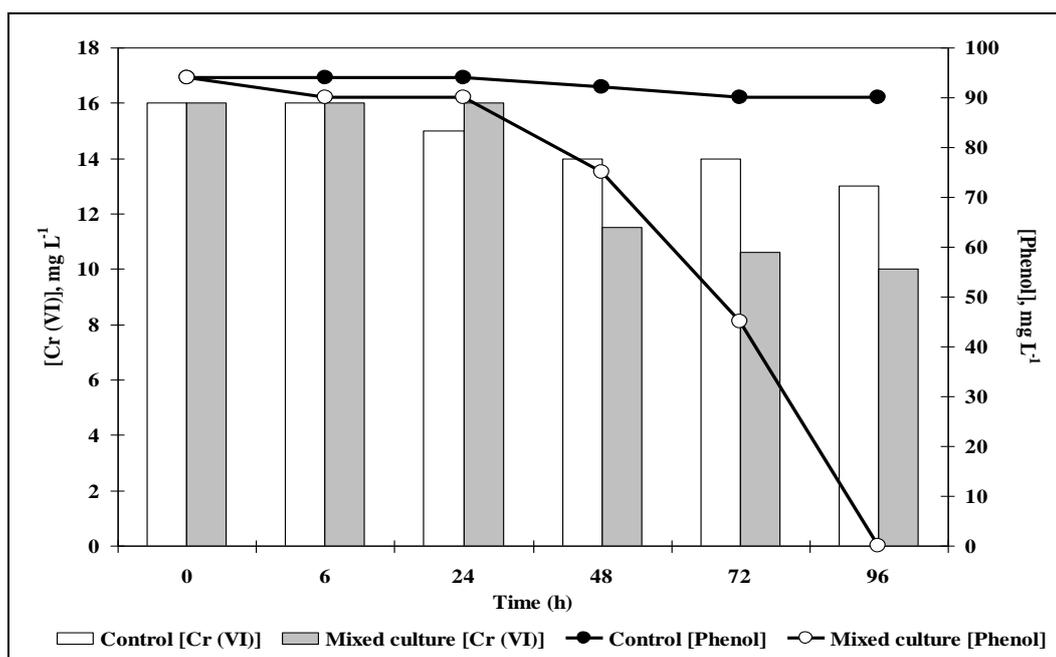
**Fig. 1:** Simultaneous remediation of phenol and Cr (VI) from tannery wastewater using mixed consortium culture.

[Mixed culture was extraneously inoculated to raw wastewater containing initial  $47 \text{ mg L}^{-1}$  phenol and  $16 \text{ mg L}^{-1}$  Cr (VI) and incubated at  $30^\circ\text{C}$  and 200 rpm as described in the text. The control set was not inoculated with any extraneous microorganisms.]



**Fig. 3.** Simultaneous remediation of phenol and Cr (VI) from tannery wastewater in presence of higher Cr (VI) concentrations of a) 25 mg L<sup>-1</sup> and b) 32 mg L<sup>-1</sup>.

[Mixed culture was separately inoculated to raw wastewater containing initial 47 mg L<sup>-1</sup> phenol and higher Cr (VI) content of 25 and 32 mg L<sup>-1</sup> Cr (VI) and incubated at 30°C and 200 rpm as described in the text. The control sets were not inoculated with any microorganisms.]



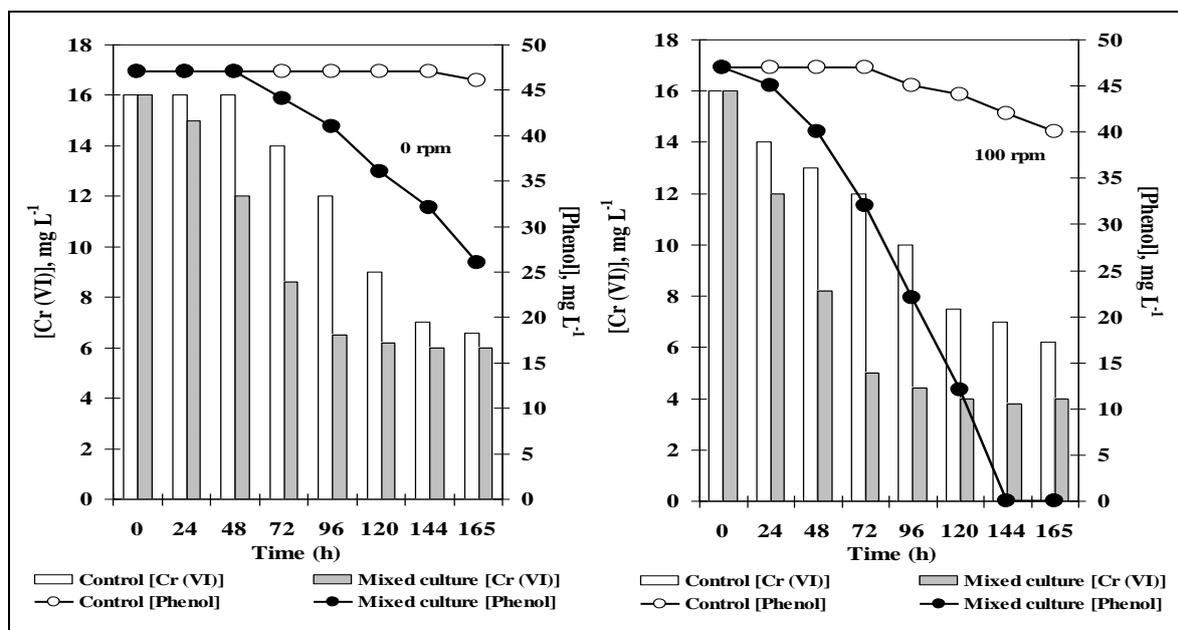
**Fig. 4.** Simultaneous remediation of phenol and Cr (VI) from tannery wastewater in presence of higher phenol concentration of 94 mg L<sup>-1</sup>.

[Mixed culture was separately inoculated to raw wastewater containing initial 94 mg L<sup>-1</sup> phenol and Cr (VI) content of 16 mg L<sup>-1</sup> Cr (VI) and incubated at 30°C and 200 rpm as described in the text. The control set was not inoculated with any microorganisms.]

**Simultaneous removal of phenol and Cr (VI) from wastewater in presence of higher phenol concentration**

Fig. 4 represents concurrent removal of phenol and Cr (VI) by mixed culture in presence of higher phenol concentration of 94 mg L<sup>-1</sup>. Complete phenol removal was observed to take place at 96 h of treatment. However, the rate of reduction was slower compared to lower phenol concentration of 47 mg L<sup>-1</sup>. Chromate reduction also

becomes slower in presence of 94 mg L<sup>-1</sup> phenol. On the whole 34 and 37% reduction of Cr (VI) were observed at 72 and 96 h, respectively. Control setup on the other hand showed 4 and 19%, reduction of phenol and Cr (VI), respectively at 96 h of treatment. Tziotzios *et al.* (2008) too reported lower phenol and Cr (VI) removal in presence of higher phenol content.



**Fig. 5:** Simultaneous removal of phenol and Cr (VI) from wastewater using mixed consortium culture under stationary or 0 rpm and 100 rpm.

[Wastewater containing initial 47 mg L<sup>-1</sup> phenol and 16 mg L<sup>-1</sup> Cr (VI) was inoculated with mixed consortium culture as describe in the text followed by incubation at 30°C under different agitation speed. Control was not inoculated with any microorganisms.]

#### **Effect of varying agitation speed on concomitant removal of phenol and Cr (VI) from wastewater**

During the analysis of effect of agitation on removal efficiency, it was observed that with increase in agitation speed, phenol and Cr (VI) removal got enhanced and faster using mixed culture (Fig. 5). At 0 rpm, up to 47% phenol and 62% Cr (VI) reductions were observed at 165 h of treatment. Subsequent increase in agitation speed to 100 and 200 rpm enhanced the removal rate of both phenol and Cr (VI) (Fig. 5 and Fig. 1). At 100 rpm, 100 and 70% deduction of initial phenol and Cr (VI) content were observed at 120 h of treatment, respectively. Similarly at 200 rpm, complete phenol removal and up to 78% removal of Cr (VI) were detected at 96 h of treatment. Verma and Singh (2013) have observed increased reduction of pentachlorophenol and Cr (VI) with increased in agitation speed up to 120 rpm using *Brevibacterium casei*. However, the authors reported lower reduction of contaminants with further increase in agitation speed which is contrary to our findings. In the present study, though there was lower simultaneous removal of phenol and Cr (VI) under static condition, but it shows the competence of consortium in removal of both the contaminants without the help of any mechanical aeration or agitation. This property of above used bacterial consortium can help in economic remediation of phenol and Cr (VI) from the wastewater.

#### **Conclusion**

Application of bacterial consortia containing four naturally isolated bacterial strains including *Acinetobacter* sp. B9 and *Arthrobacter* sp. B2 in tannery wastewater treatment resulted in significant removal of both phenol and Cr (VI).

Thus the formulated consortia may find suitable application in contemporaneous bioremediation of phenol and Cr (VI) from tannery and other industrial wastewaters.

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