



Optimization of culture parameters for tannery effluent bioremediation by *Bacillus galactosidilyticus* APBS5-3

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Abstract

Wastewaters produced by tanneries contain high concentration of hexavalent chromium [Cr(VI)] and organic pollutants. In the present work, tannery effluent remediation ability of a chromium resistant *Bacillus galactosidilyticus* strain APBS5-3, isolated from tannery effluent enriched soil, was evaluated. This strain was found to be resistant up to 800 ppm Cr(VI). Optimization of pH, carbon source, nitrogen source, inoculum concentration, agitation rate and incubation temperature was performed for efficient bioremediation of tannery effluent. Highest reduction of COD and Cr(VI) was found to be 63.1 and 41.8%, respectively and final O.D. (at 600 nm) and pH were found to be 1.222 and 8.54, respectively after 72 hr under optimized conditions.

Key words

Bacteria, Bioremediation, Chromium, Tannery, Wastewater

Introduction

Wastewater from tanneries contains high amount of total solids, chlorides, sulphates, organics and chromium (Malaviya and Singh, 2011). About 80% of the tanneries in India are engaged in chrome tanning processes and use nearly 40,000 tons of basic chromium sulphate every year (Shukla *et al.*, 2007). Chromium is a highly toxic element, regulated with respect to its oxidation state between Cr(III) and Cr(VI). Out of these, Cr(VI) is the most toxic form of chromium (Chhikara *et al.*, 2010). Owing to high toxicity of Cr(VI), this metal constitutes a serious health risk and may cause mutagenesis and carcinogenesis (WHO, 1990).

For treatment of tannery effluent, various remediation options like use of chemical coagulants, oxidation ponds, trickling filters, activated sludge process, anaerobic sludge blanket etc. are available (Kaul *et al.*, 2005). However, these traditional treatment technologies possess significant drawbacks of being environmentally disruptive, requiring input of external chemical additives, as well as generating concentrated toxic sludge (Malaviya and Singh, 2011). The presence of Cr(VI) and high salinity of tannery effluent enriched environment plays a selective pressure on microflora. The process by which microorganisms interact with toxic metals enabling their removal/and recovery are

bioaccumulation, biosorption and enzymatic reduction (Farang and Zaki, 2010; Malaviya and Singh, 2012).

Reduction of Cr(VI) represents a potentially useful detoxifying process for several bacteria (Cervantes *et al.*, 2001; Pattanapitpaisal *et al.*, 2001). Bacterial reduction of Cr(VI) can be applied to remediate contaminated tannery wastewater (Malaviya and Singh, 2014). Additionally, these resistant bacteria show active growth in tannery wastewater and reduce chemical oxygen demand through metabolism and other mechanisms. In view of the above, the present study was conducted to optimize various culture parameters of Cr(VI) resistant *Bacillus galactosidilyticus* APBS5-3 for efficient reduction in Cr(VI) and COD of tannery effluent.

Materials and Methods

Chrome liquor and tannery effluent were collected manually in clean plastic cans from a private tannery located at Leather Complex, Kapurthala, Punjab, India. For artificial soil enrichment, chrome liquor was added in soil at study site for 18 months and soil from this site was collected and brought to laboratory for isolation of chromium-resistant bacteria.

pH of tannery effluent was measured by using standardized digital pH meter (Elico, India). Reduction of Cr(VI) in

the digested sample (in triacid mixture of nitric acid, sulphuric acid, and perchloric acid) of tannery effluent was determined using diphenylcarbazide (DPC) method and the resultant red-violet to purple colour complex was measured by UV-VIS Spectrophotometer Model-1800 (Shimadzu, Japan) at OD₅₄₀ (APHA, 2012). Further, chemical oxygen demand (COD) was estimated by Open Reflux Method (APHA, 2012; Malaviya and Rathore, 2007).

Tannery effluent enriched soil was centrifuged at 900 rpm for 5 min and the supernatant was used for selective enrichment in minimal salt medium containing tannery sludge as a sole carbon source. The selective enrichment was carried out in a shaker with repeated shake flask cultures (150 rpm) at 37 °C. For isolation of chromium-resistant bacterial strain, serially diluted samples were plated on Luria-Bertani (LB) agar plate spiked with 100 ppm Cr(VI), prepared from potassium dichromate solution, and incubated at 37 °C for 48 hr. For further screening and determination of minimum inhibitory concentration (MIC) for Cr(VI), morphologically different bacterial isolates were plated onto LB agar plates amended with different concentrations of Cr(VI) and incubated for 72 hr. Bacterial strain tolerating higher concentration of Cr(VI) was selected for optimization of culture parameters for tannery effluent bioremediation.

Optimization of different parameters (pH, carbon source, nitrogen source, inoculum concentration, agitation rate, and temperature) was performed by inoculating overnight grown culture of *B. galactosidilyticus* APBS5-3 to combined tannery effluent in shake flasks (150 rpm) for 72 hr. In all optimization studies, samples (collected after 72 hr) were analyzed for biomass growth, pH change, COD removal and Cr(VI) reduction. Bacterial growth was measured as optical density at 600 nm by UV-VIS Spectrophotometer Model-1800 (Shimadzu, Japan).

For optimization of pH, pH of the effluent was adjusted to 5, 7, and 9. The pH found most suitable for tannery effluent bioremediation was used for optimization of three carbon sources (0.2%) viz., glucose, sucrose and sodium acetate. Further, combination of the best pH and carbon source was used for optimization of three nitrogen sources (0.2%) viz., NH₄Cl, NH₄NO₃ and urea. Inoculum concentration of 2, 4, 6 and 8% were used for further optimization and for optimization of agitation rate, shaking was carried out at 130, 150 and 170 rpm. Finally, temperature was optimized by operating shaker at three different temperatures viz., 34, 37 and 40 °C.

Results and Discussion

Physico-chemical analysis of tannery effluent revealed basic pH 11.4 and 6.7 mg l⁻¹ Cr(VI) content. Chemical oxygen demand of the combined tannery effluent ranged from 3612.2-4078.8 mg l⁻¹, with variation in pH, carbon source and nitrogen source.

Biolog Identification System was used for identification of bacterial isolate APBS5-3 and the species showing close similarity with this strain was observed to be *Bacillus galactosidilyticus*. Minimum inhibitory concentration (MIC) for Cr(VI) was found to be 800 ppm for this bacterial strain.

During optimization of pH (5.0, 7.0, and 9.0), other process parameters were kept fixed as carbon source: 0.2% glucose; nitrogen source: 0.2% ammonium chloride; inoculum concentration: 2%; agitation rate: 150 rpm and incubation temperature: 37 °C and pH 7.0 was found to be optimum for *Bacillus galactosidilyticus* APBS5-3 (Table 1). Likewise, optimization of carbon source (0.2%) viz., glucose, sucrose and sodium acetate for single culture tannery effluent bioremediation showed glucose to be the best carbon source in terms of COD removal and Cr(VI) reduction and among different nitrogen sources (0.2%) viz. ammonium chloride, ammonium nitrate and urea, ammonium chloride was found to be the most potential nitrogen source (Table 1).

In accordance with the present findings, pH 7.0 was reported to be optimum by Humphries and Macaskie (2002); Slobodkina *et al.* (2007); Srivastava and Thakur (2007). Furthermore, Rehman *et al.* (2008), Tripathi and Garg (2010), Panda and Sarkar (2012), Rehman *et al.* (2013) and Das *et al.* (2014) also reported pH 7 to be optimum during bioremediation by different bacterial strains. pH of the medium strongly affects enzymatic processes involved in bacterial metabolism. Similar to the present results, Mishra *et al.* (2012) reported effectiveness of monosaccharides like glucose in Cr(VI) reduction and attributed it to the chemical structure of glucose as an electron donor, while disaccharides (sucrose) and sodium acetate exhibited low values of Cr(VI) reduction. Similarly, Tripathi and Garg (2010) also found glucose to be a better carbon source than sucrose.

For *Bacillus galactosidilyticus* APBS5-3, higher inoculum concentrations (4, 6 and 8%) showed slightly better performance in comparison to 2% inoculum concentration in terms of COD removal and Cr(VI) reduction. However, keeping in mind the economic aspects of the remediation system, 2% inoculum concentration was considered to be optimum due to minor difference in COD removal and Cr(VI) reduction at different inoculum concentrations (Table 2). Optimization of agitation rate (120, 150 and 180 rpm) showed best performance of *Bacillus* sp. at 150 rpm and rise or decrease in agitation rate was found to decrease bioremediation efficiency (Table 2). Further, 37 °C was found to be optimum for *B. galactosidilyticus* APBS5-3 among different incubation temperatures (34, 37, and 40 °C), both in terms of COD removal and Cr(VI) reduction (Table 2).

In another study conducted by Ge *et al.* (2013) higher inoculation volume failed to elicit further increase in Cr(VI) reduction, suggesting that competition for limited nutritional resources restricts the bacterial metabolic activity. An optimum

Table 1 : Optimization of pH, carbon source and nitrogen source for bioremediation of tannery effluent by *Bacillus galactosidilyticus* APBS5-3

Parameters for optimization	COD (mg l ⁻¹)	Cr(VI) (mg l ⁻¹)	pH	cell growth (A ₆₀₀ nm)
pH				
5.0	2565.9 (29.7)	4.5 (32.8)	5.76	0.464
7.0	1387.0 (63.1)	3.9 (41.8)	8.54	1.222
9.0	1944.3 (49.1)	4.2 (37.3)	8.78	0.873
Carbon source				
Glucose	1387.0 (63.1)	3.9 (41.8)	8.54	1.222
Sucrose	1474.8 (62.7)	3.9 (41.8)	8.50	1.092
Sodium acetate	1561.4 (56.8)	4.1 (38.8)	8.49	0.923
Nitrogen source				
Ammonium chloride	1387.0 (63.1)	3.9 (41.8)	8.54	1.222
Ammonium nitrate	1424.6 (62.6)	4.0 (40.3)	8.50	1.283
Urea	1488.9 (60.2)	4.0 (40.3)	8.49	1.147

Values in parenthesis are percentage reduction of COD and Cr(VI) as compared to their initial concentration in tannery effluent; Incubation period: 72 hr; inoculum concentration: 2%; temp.: 37°C; rate of agitation: 150 rpm; initial COD: 3763.2 mg l⁻¹ at 7 pH with glucose as carbon source and ammonium chloride as nitrogen source; initial Cr(VI) concentration: 6.7 mg l⁻¹

Table 2 : Optimization of inoculum concentration, agitation rate and incubation temperature for bioremediation of tannery effluent by *Bacillus galactosidilyticus* APBS5-3

Parameters for optimization	COD (mg l ⁻¹)	Cr(VI) (mg l ⁻¹)	pH	cell growth (A ₆₀₀ nm)
Inoculum concentration (%)				
2	1387.0 (63.1)	3.9 (41.8)	8.54	1.222
4	1312.8 (65.1)	3.8 (43.3)	8.57	1.398
6	1296.0 (65.6)	3.8 (43.28)	8.58	1.423
8	1283.8 (65.9)	3.6 (46.3)	8.57	1.410
Agitation rate (rpm)				
130	1706.5 (54.7)	4.5 (32.8)	8.12	0.974
150	1387.0 (63.1)	3.9 (41.8)	8.54	1.222
170	1899.4 (49.5)	4.8 (28.4)	7.94	0.972
Incubation temperature (°C)				
34	1454.4 (61.4)	4.4 (34.3)	8.54	1.176
37	1387.0 (63.1)	3.9 (41.8)	8.54	1.222
40	1405.3 (62.7)	4.1 (38.8)	8.52	1.209

Values in parenthesis are percentage reduction of COD and Cr(VI) compared to their initial concentration in the tannery effluent; Incubation period: 72 hr; initial COD: 3763.2 mg l⁻¹ at 7 pH with glucose as carbon source and ammonium chloride as nitrogen source; initial Cr(VI) concentration: 6.7 mg l⁻¹

agitation of medium is essential for proper oxygen supply to bacterial cells. However, reduction in bioremediation efficiency above 150 rpm in the present study was attributed to change in cell morphology and damage to bacterial cells caused by shearing effect, which reduced metabolic activities, and hence bioremediation efficiency of bacterial cells (Garg *et al.*, 2013).

Furthermore, improved performance of bacterial strains at 37°C reported in the present study is corroborated by the findings of Humphries and Macaskie (2002), Sultan and Hasnain (2007) and Xu *et al.* (2011). Likewise, Mclean *et al.* (2000); Liu *et al.* (2006); and Mishra *et al.* (2012) reported decrease in Cr(VI)

reduction with increase in temperature (from 35°C to 45°C) and attributed this to loss of viability or metabolic activity of bacterial cells on prolonged incubation at higher temperatures. High temperature in synergism with high salt concentration has also been reported to induce cell lysis, resulting into reduced biomass growth (Camargo *et al.*, 2003).

During optimization of different parameters for bioremediation of tannery effluent by *Bacillus galactosidilyticus* APBS5-3, rise in pH of tannery effluent after 72 hr is also supported by the observations of Xu *et al.* (2005). Such increase in pH was correlated with Cr(VI) reduction.

The present study concludes that *Bacillus galactosidilyticus* APBS5-3 was capable of tolerating highly toxic tannery effluent and 63.1 and 41.8% reduction in COD and Cr(VI) after 72 hr indicated a potential application of bacterial strain APBS5-3 for bioremediation of tannery effluent.

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