Development of Sustainable Strategies for Leather Waste Management through Bacterial Remediation

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Abstract

A new *Bacillus* species has been isolated and used for treating chrome leather wastes. The activity of *Bacillus* species is evaluated for the degradability of the Chrome Leather Waste (CLW). An initial CLW substrate concentration at 0.5 and 1%, along with the bacterial strain is studied against the control sample without bacterial strain. The higher proteolytic enzyme production and hydroxyproline release in the CLW containing medium confirms the degradation process, whereas it is significantly less in control samples. The degradation profile of CLW shows higher in 1% CLW as compared to 0.5% CLW. In 1% CLW, the protease activity of the isolated strain has been increased from 1.615 to 5.625 U/mL. In addition to protease activity, the isolated strain also expressed chromate reductase activity. Furthermore, FTIR, TGA, and SEM studies confirm the degradation of leather wastes.

Introduction

In the leather manufacture, tanning plays a major role in converting hides or skins into leather, which makes the leather more resistant to microbial attack and increases its thermal stability properties.[1-2] Among the different methods of tanning, chrome tanning is commonly used in leather manufacture as it produces leathers with high temperature tolerance and excellent washable capability.[3-7] Chrome tanning employs the trivalent heavy metal salt chromium, which generates wastes as chrome shaving, splitting, and trimming waste resulting in heavy metal pollution in the environment. The traditional way of Chrome Leather Waste (CLW) disposal is landfilling.

Biodegradation of chrome tanned leathers is of much interest to the leather fraternity. Biological degradation involves two aspects, one is remediation of chromium, and another is nutrient source utilization for bacterial growth, which offers a sustainable approach for biodegradation of CLW. Microbial management of industrial wastes for the removal of hazardous compounds can be achieved through bio-leaching, bio-mineralization, bio-accumulation, enzyme-catalyzed redox reactions, biosorption, or bioconversion to less toxic forms.[8-9] Microbial degradation is, therefore, the best alternative for processing waste materials containing heavy metal, which facilitates the recycling of CLW in an eco-benign manner. A general mechanism of chromium absorption internally occurs through the membrane sulfate transport channel present in the cells, and it spontaneously reacts with intracellular reductants to generate unstable intermediate products.[10-11] This mode of action of the bioremediation process occurs in chrome tolerant bacterial species.[12-13] Among different class of organisms bacteria is found to have a high degrading ability.[14]

In the present study, a new chromium tolerant bacterium is isolated from the tannery effluent which is used for the degradation of CLW.

Experimental

Characterization of bacterial species

Effluent from pilot tannery was used for the bacterial isolation and screened using chrome tolerance assay, most responsive strain was chosen and identified by rDNA sequencing. The resulted sequence was deposited to GenBank, NCBI and obtained the accession number, MG995009.

Evaluation of bacterial strain on chrome leather wastes

Chrome leather waste obtained from the tannery was used as a substrate for the biodegradation study. The microbial degradation of CLW was studied with the isolated bacterial culture (10^7 CFU/mL) incubated in the liquid medium for 15 days at 37°C. Samples collected every 5 days were subjected to protease assay and hydroxyproline assay.[15-16] The efficiency of Cr (VI) conversion was assessed based on the colorimetric assay, diphenyl carbazide method.[17]

ATR-FTIR of the chrome shavings

Vibrational spectroscopic study was carried out for the control and experimental CLW samples using ATR-FTIR spectrophotometer (JASCO 4200).

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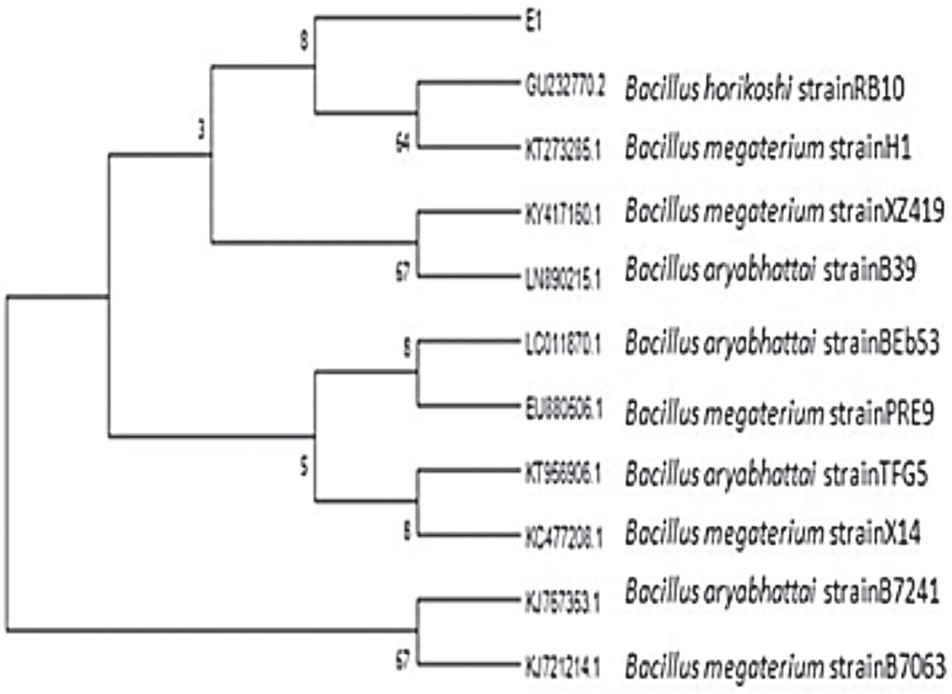

Figure 1. Phylogenetic tree of strain MG995009

TGA of the chrome shavings

TGA study was performed for control and experimental CLW using Q50 thermogravimetric analyzer in a N_2 atmosphere with a heating range from 25° to 800°C at 10°C/min.

Microscope Evaluation

Samples of 5 × 2mm sizes were cut and mounted on aluminum stubs. Then coated with gold using an Edwards E-306 sputter coater and introduced into the chamber of PHENOM ProX Scanning Electron Microscope. This was done to understand the structural morphology of the control and *Bacillus aryabhattai* (MG995009) treated CLW.

Results and Discussion

The present research unravels the biodegradation efficiency of the newly isolated bacterial species *Bacillus aryabhattai* (MG995009) on CLW which possess both hydrolytic and chrome reductase activities (Figure 1)

The newly isolated strain sequence was submitted in NCBI and obtained the accession number, MG995009. Based on the phylogenetic information, the newly isolated strain belongs to the *Bacillus* genus which is commonly present in the skins and hides.

The isolated bacterial strain was tested for its proteolytic activity to understand the biodegradation profile of CLW, and the results are given in Table I. It is observed that 0.5% of the substrate showed 3.12 U/mL, and 1% of the substrate showed 5.63 U/mL on 15th day. At higher substrate concentration, the proteolytic activity has found to be a little higher, which ascertains the effective utilization of substrate by the new bacterial isolate. From the results, it can be ascertained that 1% of CLW was chosen to understand the biodegradation in further studies.

Table I

Release of protease during the degradation process of CLW

Time intervals (Days)	Protease activity (U/mL)		
	Control	0.5%	1%
0	1.25±0.002	1.27±0.002	1.65±0.003
5	2.45±0.004	2.86±0.005	3.11±0.006
10	2.48±0.004	2.85±0.005	3.96±0.007
15	2.58±0.005	3.12±0.006	5.63±0.011

Hydroxyproline release is quantified, to understand the biodegradability of leather, and the results are given in Table II. Hydroxyproline in 1% CLW liquor increased from 18 to 84 µg/mL during the degradation period of 0 to 15 days. From the results, it can be inferred that *Bacillus sp.* utilized the CLW, which ensures chrome tolerance ability of the newly isolated species.

Table II

Release of hydroxyproline during the degradation process of CLW

	Hydroxyproline release (µg/mL)
Days	**1%**
0	18±0.036
5	33±0.040
10	40±0.080
15	84±0.126

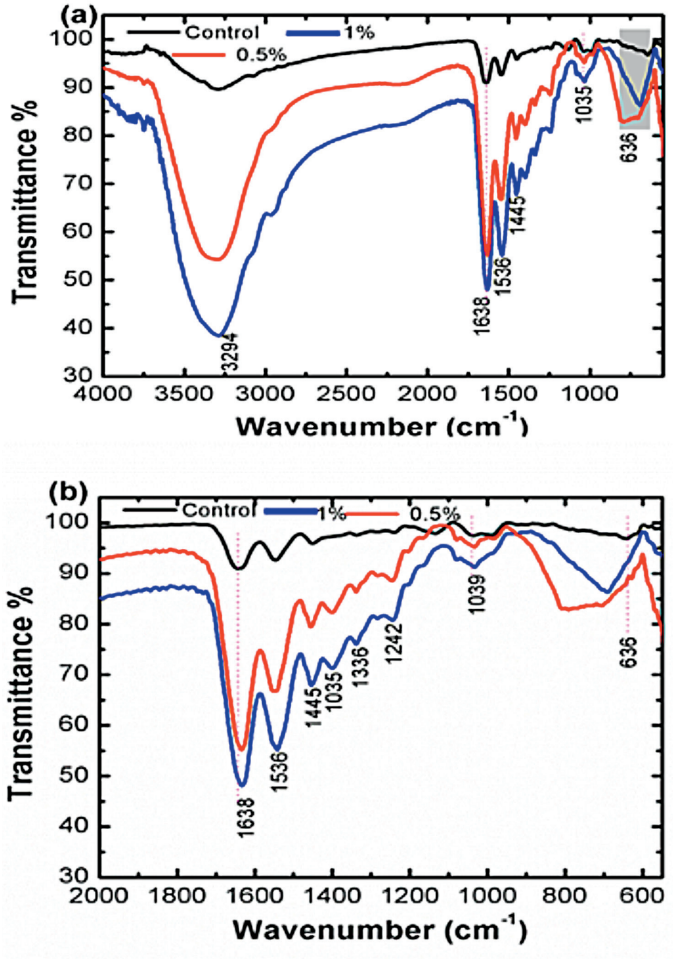

Figure 2. **(a)** FT-IR spectra for treated and untreated samples, **(b)** Magnified image of changes in wave numbers of treated and untreated samples

Chrome tolerant bacterial strain *Bacillus aryabhattai* isolated in this study have been evaluated for its chromate reductase activity, which would help in reducing toxic chrome species.[18] Isolated bacterial strain reduced the 100 µM Cr (VI) to 64.88 µM Cr (VI) within 20 min at the volume of 250 µg of the crude enzyme. However, at a lower concentration of 50 µg crude enzyme, the reduction is significantly less.

Vibrational spectrums of the control and experimental samples are represented in Figure 2. From the spectrum, it can be observed that the signatory peaks of amide I, II, and III are prominent in control, and the amide peaks are less intense in experimental samples (0.5 and 1.0%), which is due to the biodegradation process. These peaks represent the protein material, which was reduced in wave numbers after degradation due to the blue shift. In 0.5 and 1% CLW samples, peaks exerted at 1445, 1035, and 1331 cm^{-1} with reduced intensities and these peaks have corresponded to methylene (CH_2), ester, and OH groups.

Thermal analysis of control and experimental CLW showed distinct mass losses have been inferred from the thermograms as seen in Figure 3.

As seen in Figure 3, the mass loss percentage of the experimental samples 0.5 and 1% CLW are found to be 76.96 and 89.61%, respectively, whereas control shows about 68.83%.[19] The reduction in thermal stability and increase in mass loss indirectly indicate the degradation of CLW samples.

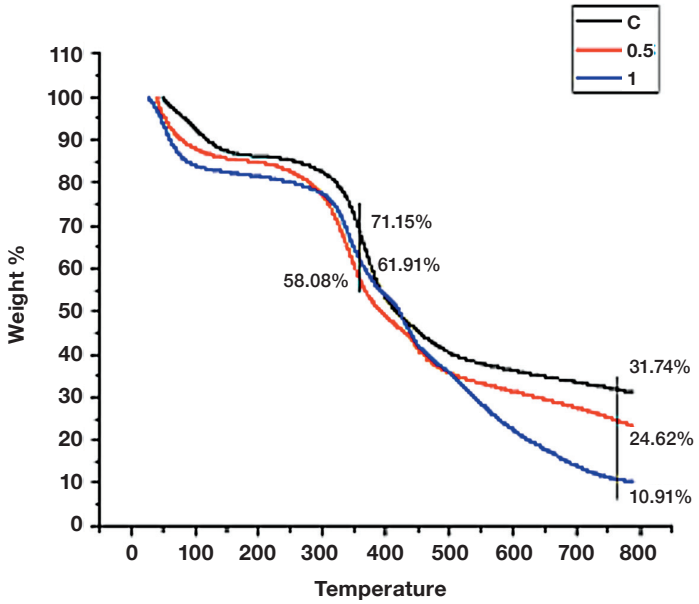

Figure 3. Thermogravimetric Analysis of treated and untreated CLW

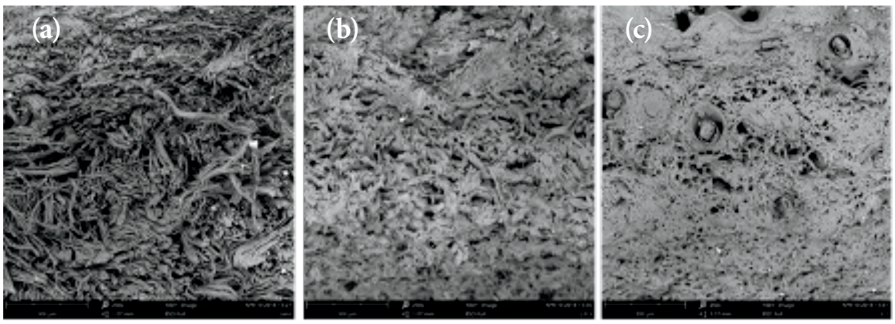

Figure 4. SEM images of treated and untreated CLW of **(a)** Control **(b)** 0.5% **(c)** 1%

The morphological study helps in the visual understanding of the biodegradation process. As seen in Figure 4a, the control CLW has defined and ordered fibrous structure.

Moreover, the compactness of fibers is well maintained in the control samples, which confirm that the substrate has not undergone degradation. From Figure 4b, the effect of bacterial strains on the CLW (0.5%) has been well pronounced, and it can be observed from the swollen and coalesced fibrous network. Moreover, fiber compactness and orientation are changed owing to the effect due bacterial enzyme. Whereas, as seen from Figure 4c in 1% CLW sample, the degradation is faster with the broken fragments and formation of grooves. The definite fiber orientation and compactness are disappeared in 1% CLW completely as compared to 0.5% CLW.

Conclusion

Biodegradation of tanned leather is of a great need to achieve eco-sustainability. In the current research, an attempt has been successfully addressed in identifying new bacterial *Bacillus* sp., which possesses dual characteristics viz., degradation of chrome leathers, and the ability to reduce the chromium. From the collagen content measurement, it is observed that the hydroxyproline released in the 1% CLW has gradually increased towards 15th day. Furthermore, thermal, vibrational and morphological studies confirm the degradation of CLW by *Bacillus aryabhattai*. The study holistically addresses the major challenge towards the disposal of CLW.

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Authors Contribution

All the authors have shared their knowledge and contributed to frame the manuscript. AS and CK performed the experiments, GCJ contributed towards framing the manuscript and structured the methodology of the work, AY contributed towards the analytical assay and SVK framed the conceptual idea and structured the final version of the manuscript.

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