Bacterial degradation of persistent organic pollutants from tannery wastewater after secondary treatment process

SUMMARY OF
Thesis

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Summary

Leather industries (LIs) are the key contributors in the economy of many developing countries because they significantly earn foreign exchange through leather exports and create employment opportunities for economically weaker sections. However, unfortunately, they are facing serious challenges from the public and governments due to the associated environmental pollution and toxicity. There is a public outcry against the industry due to the discharge of a highly polluted and potentially toxic wastewater having alkaline pH, dark brown colour, unpleasant odour, high biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), phosphate, nitrate, sulphate, phenol, chromium and a variety of persistent organic pollutants (POPs) which are used during leather processing in LIs to produce commercial leather or leather product. Various environment protection agencies and pollution control authorities have prioritized several chemicals as highly toxic and hazardous in nature and restricted their use in leather processing, however; many of these recalcitrant chemicals are used and discharged in wastewater. The highly toxic POPs applied during leather processing are not fully uptaken by the hide/skins and hence, are discharged in the TWW. Moreover, these POPs also do not degrade much during the secondary treatment process at common effluent treatment plants (CETPs) of LIs and goes into the environment where causes serious soil and water pollution along with severe toxic effects in living beings. Therefore, the biodegradation and biodetoxification of POPs in real TWW is required to protect the environment and public human.

Microbes are considered as the eco-friendly tools for the degradation and detoxification of organic and inorganic pollutants in industrial wastewaters. Therefore, in the present study, a total of ten (10) bacterial strains (GS1-GS10) were

isolated from TWW and sludge samples collected after secondary treatment process carried out at common effluent treatment plant (CETP), Unnao, Kanpur (UP), India. In addition, the purified bacterial strains (GS-GS10) were primarily screened for the ability to tolerate high salt (NaCl) concentration. Results revealed that among all the isolated bacterial strains (GS1-GS10), only seven bacterial strains i.e. GS1, GS2, GS3, GS4, GS5, GS6, and GS10 were adapted to tolerate up to 6, 4, 4, 3, 4, 3 and 8% salt (NaCl) concentration over a wide range of salinity (1-10%). Thus, it was confirmed that the isolated bacterial strains are halotolerant in nature and suitable for the treatment and detoxification of real TWW as it contains a high amount of salts. Moreover, these selected bacterial strains were further screened on the basis of pollutants (COD) removal efficiency from real TWW for the degradation and detoxification of persistent organic pollutants and to achieve better effluent treatability. Results revealed that among seven bacterial strains, only three bacteria strains i.e. GS1, GS3, and GS10 were able to remove COD up to 61.12, 54.28, and 66.32% from real TWW during its bioremediation.

The isolated bacterial strains were further characterized to confirm their identity on the basis of various morphological and biochemical tests. Results of the morphological tests revealed that the bacterial strain GS1 appeared as milky white colonies on MSM-agar plates and was gram-negative, motile, and rod-shaped. Bacterial strain GS3 appeared as white colonies on MSM agar plates and was gram-positive, non-motile, and round-shaped whereas bacterial strain GS10 appeared as greenish colonies on MSM agar plates and was also gram-negative, motile, and rod-shaped. Results of the biochemical tests revealed that the bacterial strain GS1 showed positive reactions for citrate utilization, lysine utilization, ornithine utilization, urease, catalase, nitrate reduction, glucose, adonitol, lactose whereas negative reactions for phenylalanine deamination, H_2S production, arabinose, and sorbitol. Bacterial strain GS3 showed positive reactions for malonate, citrate utilization, catalase, glucose whereas negative reactions for Voges Proskauer's, ONPG, nitrate reduction, arginine, sucrose, mannitol, arabinose, and trehalose. Bacterial strain GS10 showed positive reactions for ornithine utilization, urease, catalase, nitrate reduction, glucose, adonitol, lactose, arabinose whereas negative reactions for citrate utilization, lysine utilization, phenylalanine deamination, H_2S production, and sorbitol. On the basis of different morphological and biochemical tests, the isolated bacterial strain GS1, GS3, and GS10 were belonged to the *Ochrobactrum, Micrococcus* and *Stenotrophomonas* genera, respectively. In addition to this, the isolated bacterial strains GS1, GS3, and GS10 were identified and confirmed as *Ochrobactrum intermedium* (MK344317), *Micrococcus lylae* (MK344318), and *Stenotrophomonas acidaminiphila* (MK344319) on the basis of 16S rRNA gene sequence analysis.

Further, a new bacterial consortium, GS-TE1310 was developed using these three potential pollutants degrading bacterial strains i.e. *Ochrobactrum intermedium* GS1, *Micrococcus lylae* GS3, and *Stenotrophomonas acidaminiphila* GS10 on the basis of performance of the mono-cultures in the bioremediation of TWW. For the development of bacterial consortium, the selected bacterial strains (*Ochrobactrum intermedium* GS1, *Micrococcus lylae* GS3, and *Stenotrophomonas acidaminiphila* GS10) were checked for the compatibility test/ bio-interaction study. Results revealed that these bacterial strains (GS1, GS3 & GS10) were not inhibited the growth of each other as no zone of inhibition was formed on the MSM-agar plate and thus, the selected bacterial strains were compatible to each other and able to work together during bioremediation study. Afterward, the performance of the developed bacterial consortium GS-TE1310 was evaluated in the bioremediation of TWW on the basis of

COD removal efficiency. Results revealed that the newly developed bacterial consortium GS-TE1310 was able to remove maximum COD up 74.15% within 120 h as compared to individual bacterial strains, *Ochrobactrum intermedium* GS1 (61.12%), *Micrococcus lylae* GS3 (54.28%), and *Stenotrophomonas acidaminiphila* GS10 (66.32%).

Moreover, the newly developed bacterial consortium GS-TE1310 was also optimized at various environmental and nutritional parameters as well as inoculum concentration and agitation rate to further enhance the pollutants (COD) removal efficiency. Results revealed that the newly developed bacterial consortium GS-TE1310 was able to remove maximum COD up to 76.08% from real TWW at the optimized conditions within 120 h. The optimum pH, temperature, inoculum concentration, and agitation rate were found to be 7, 35 °C, 20 ml and 120 rpm, respectively and the best carbon and nitrogen source was found to be glucose and ammonium chloride, respectively, among the various carbon and nitrogen sources used for the bioremediation of real TWW.

Thereafter, the newly developed bacterial consortium GS-TE1310 was used for the degradation and detoxification of persistent organic pollutants (POPs) present in the TWW. Results revealed that an effective degradation of real TWW was attained by a newly developed bacterial consortium GS-TE1310 within 120 h (at 7 pH, 120 rpm, and 35°C) with 76.12, 85.32, 71.89, 48.59, 78.81, 69.53, 71.22, and 88.70% reduction in pollution parameters such as COD (1428 ± 5.56 mgL^{-1}), BOD (436 ± 4.58 mgL^{-1}), TDS (4064 ± 3.46 mgL^{-1}), phosphate (118.66 ± 5.03 mgL^{-1}), sulphate (6.75 ± 0.27 mgL^{-1}), nitrate (14.05 ± 0.16 mgL^{-1}), Cr (6.88 ± 0.02 mgL^{-1}), and phenol (8.68 ± 0.04 mgL^{-1}), respectively. In addition, HP-LC, FT-IR, and GC-MS techniques were used to assess the biodegradation as well as to characterize the persistent organic pollutants (POPs) and their metabolites produced during the bioremediation of TWW. Results revealed that most of the persistent organic pollutants detected (POPs) in the untreated TWW were completely mineralized/degraded into new metabolic products in the treated TWW by the newly developed bacterial consortium GS-TE1310 at the optimized conditions (7 pH, 35°C temperature, 0.5% glucose and ammonium chloride (w/v), 120 rpm (agitation rate), and 20 ml inoculum volume)). Thus, the application of this newly developed bacterial consortium GS-TE1310 instead of individual bacterial strains was highly effective in the degradation and detoxification of real TWW.

Moreover, the catabolic enzyme responsible for the degradation of persistent organic pollutants during bacterial treatment of TWW was characterized to confirm the enzymatic degradation process. Results revealed that the selected bacterial strains, *Ochrobactrum intermedium* GS1, *Micrococcus lylae* GS3, & *Stenotrophomonas acidaminiphila* GS10 exhibited catechol 1,2- dioxygenase enzyme as confirmed by the quantitative analysis. Further, the SDS-PAGE analysis also showed the molecular weight of protein around ~32 kDa in the crude extract of all the selected bacterial strains. Overall, these selected bacterial strains were able to degrade the persistent organic pollutants present in TWW and thus, the developed consortium could be used for the bioremediation of TWW.

Further, the prime objective of bioremediation is to lessen the toxicity of industrial effluents and hence, the phytotoxicity of TWW before and after treatment with the newly developed bacterial consortium, GS-TE1310 was assessed to evaluate the environmental safety. In the present study, untreated TWW was proved to be highly toxic in nature as it showed inhibitory effect on seed germination and seedling growth parameters of *Phaseolus aureus* L, which were significantly improved when seeds were irrigated with TWW treated with the newly developed bacterial consortium GS-TE1310. In addition, the phytotoxicity of TWW after treatment with the newly developed bacterial consortium GS-TE1310 was reduced significantly allowing the 70% germination of seeds as compared to seeds irrigated with untreated TWW (100%, v/v). Thus, the bacterially treated TWW could be used as a liquid fertilizer for the irrigation of agricultural crops.

Overall, the present study concludes that the newly developed bacterial consortium GS-TE1310 comprising *Ochrobactrum intermedium* GS1, *Micrococcus lylae* GS3, and *Stenotrophomonas acidaminiphila* GS10, was more effective in the treatment/detoxification of persistent organic pollutants present in the TWW to safeguard the environment and public health. This study was perhaps the first attempt on the development of a new bacterial consortium with identified potential bacterial strains and its application in the bioremediation and toxicity reduction in the TWW. This study would be useful to develop a bacteria-based bioremediation process for WWTPs treating TWW for environmental protection and to promote the sustainable development of our society with less environmental impacts.