

Study on the bacterial population and residual organic pollutants present in tannery wastewater after secondary treatment process and its toxicity assessment

SUMMARY OF Thesis

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Summary

The leather industry is an important economic sector in many developing countries including India. However, it is also a major source of environmental pollution due to the discharge of huge volume of potentially toxic and hazardous wastewater into the receiving water body, which negatively affect societies. The wastewater discharged from leather industries are characterized by high pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solids (TDS), chromium, sulfate, phosphate, chloride and highly toxic organic pollutants that makes the wastewater unfit for irrigation and poses serious damage to plants and human being.

The majority of small-scale tanneries cannot afford their own effluent treatment plant, instead they depends on a central facility, the Common Effluent Treatment Plant (CETP), to manage their wastewater. In the CETP, combined effluent from nearby tanneries is brought to a central place for the treatment. More than 150 have been set-up so far under the Indian government scheme for the treatment of industrial wastewater. The continuous discharge of the tannery wastewater into the environment is of serious eco-toxicological concerns. The chemicals used in tannery for the tanning process include synthetic organic pollutants like tannins, phthalates, phenolic compounds, azo dyes, surface active compounds, pesticides, sulphonated oils and grease that are not completely degraded through secondary CETP and are released untreated.

Continuous releases of residual organic pollutants in tannery wastewater into the Ganga River through the drains have been a growing environmental concern and require urgent attention for the protection of environment and human health. The nature and characteristics of the residual organic pollutants in tannery wastewater, which are not significantly, degrade during the secondary treatment process at CETP, have yet to fully investigated.

The tannery wastewater samples were collected from the Common Effluent Treatment Plant (CETP) located at Unnao, Uttar Pradesh, India. The wastewater samples viz. raw effluent (a-before treatment), after secondary treatment were collected for physicochemical analysis and pollutant detection as well as bacterial population analysis. This is an activated sludge process (ASP) based CETP, treating ~1.9 MLD wastewater received from a cluster of ~ 30 tanneries located in nearby areas against a design flow of ~2.15 MLD Unnao is one of the major industrial towns adjacent to Kanpur having most of the leather, slaughterhouse, textile, steel and other industries.. Unnao industrial area is situated near Kanpur in northern side of River Ganga having more than 40 industrial units mainly tannery units and the wastewater discharged by the tannery industries after treatment at CETP is finally discharged into the River Ganga. The quality of the treated wastewaters often fails to conform to the prescribed limits recommended by the pollution control bodies of India (CPCB, 2013). Therefore, we have chosen this site for the study.

Over the years, many new chemicals has been introduced in tanning processes. Hence, detail analysis of wastewater generated from tanneries are essential for better understanding of the toxicity and chemical nature of the effluents. The discharge of partially treated wastewater from CETP, affects the flora and fauna of the aquatic ecosystem by blocking the sunlight penetration in receiving water bodies and photosynthetic activity, thus, negatively affects the aquatic life .

The wastewater was found to have high concentration of BOD (680.00 ± 20 mg/L), COD (1300.00 ± 45 mg/L), EC (4.40 ± 0.2 Ms/cm), TDS (3850.00 ± 10.0 mg/L), (566.00 ± 12.5 mg/L), sulfate (8.64 ± 0.42 mg/L), phosphate (26 ± 2 mg/L), nitrate (12.3 ± 0.3 mg/L), chloride (1434 ± 12 mg/L) and phenolic (10.5 ± 0.5 mg/L). The nature of wastewater was alkaline of pH (8.45 ± 0.18). Besides, high

concentration of total chromium (7.39 ± 0.06 mg/L) was also observed. The values of the various physico-chemical parameters were higher than the permissible limits for effluent discharge as suggested by CPCB and USEPA. High Cr level causes toxic, genotoxic, mutagenic, and carcinogenic effects on humans, animals, plants, and microbes.

The wastewater released from tannery industries may contain a variety of organic pollutants and millions of pathogenic and non-pathogenic bacteria per milliliter including coliform, fecal coliform, anaerobic spore forming bacilli, and many other types of health hazards organisms. The presence of microbial pathogens in treated wastewater as well as in polluted aquatic resources poses a considerable health risk to the general public health. Despite large advances in water and wastewater treatment processes, waterborne diseases still pose a major world-wide threat to public health.

In this study Four (04) bacterial strains were isolated on HiCrome Bacillus Agar (TWW-2) and Klebsiella Selective agar base selective media (TWW-3) Vibrio agar and (TWW-4) from CETP- treated tannery. The isolates bacterial strains were further characterized to confirm their identified on the basis of morphological and biochemical test. Result of the microscopic observation of the isolated pathogenic and non-pathogenic bacteria has revealed that isolates (TWW-1, TWW-2 and TWW-3) were rod shaped, gram negative, except TWW-2, which was gram positive. Further, all isolates also showed positive reaction for motility and catalase activity. The tannery wastewater act as enriched media to many microbes including pathogens. It also spread growth of bacterial species resistant to may metals and antibiotic.

Based on the results obtained in this study, it was concluded that the contamination of soil and water resources with industrial wastewaters containing toxic

metals and a variety of residual organic pollutants are the major sources of multi-drug and multi-metal resistant pathogenic microbes in environment.

Further, the PCR amplified 1500 bp long 16S rRNA gene sequences of bacterial isolates have shown the closest relatedness (99%) with newly *Bacillus tropicus* (TWW-2) and *Klebsiella pneumonia* (TWW-3) respectively. The partial 16S rRNA gene sequences of 566 bp, and 1427 bp were submitted to GenBank and an accession number MH762877 and MH559818 were assigned to TWW-2 and TWW-3 strains, respectively.

NGS high-throughput sequencing technologies have been developed to compressively identify the microbial diversity in different samples including activated sludge, soil, wastewater and food. In this study, microbial community in treated tannery wastewater from CETP was investigated by NGS. This study was conducted in order to elucidate the bacterial community structure associated with tannery wastewater treatment. Total number of amplicon sequences reads obtained from tannery wastewater was 1,113,718 reads. The operational taxonomy unit (OTU) recorded was 9,525.

Tannery wastewater has complex mixture of various chemicals pollutant, which is not possible to extract by single solvent extraction method. Therefore, combinations of different solvent system were used to extract majority of organic pollutants through GC-MS analysis. The GC-MS/MS analysis of the CETP treated tannery wastewater was also performed. In GCMS/ MS analysis of tannery wastewater sample, we have detected many peaks of harmful compounds. The result of the present studies suggests that combination of DCM + n-hexane organic solvents was able to extract maximum number of ROPs and thus, might be useful in the extraction of organic pollutants from tannery and other wastewater. Almost 16 types of residual organic pollutants were

detected by GC-MS, which indicates incomplete treatment of tannery effluent by CETP.

Based on the results, it was concluded that the collected CETP treated tannery wastewater had very high values for BOD, COD, TDS, sulfate and phenolics along with a variety of residual organic pollutants such as benzoic acid, 3-[4-(T-butyl) Phenyl] furan-2-5-dione, benzeneacetamide, resorcinol, dibutyl phthalate, benzene-1,2,4-triol, and 1-Phenyl-2-phenylthio etc. In the last few years, leather-tanning has adapted an eco-friendly, non/less toxic and biodegradable chemicals as per strict regulations to limit the pollution level. However, the quality of treated wastewater has not yet improved, which is apparent from present study.

The toxicity tests combined with physico-chemical analysis are essential in the evaluation of treated tannery using *Vigna radiata* L. and *Allium cepa* L. The *A. cepa* test, a relatively easy, rapid, sensitive and highly reproducible plant model has been strongly recommended for the toxicity/genotoxicity evaluation of environmental contaminants present in water, wastewater, sludge and soils. The phytotoxicity using seed germination test is considered as one of the simplest short-term, sensitive and cost effective method of toxicity evaluation for wastewaters.

The toxicological studies showed the phytotoxic nature of wastewater as it inhibited the seed germination in *Vigna radiata* L. and root growth of *A. cepa*. Genotoxicity was evidenced in root tip cell of *A. cepa* where chromosomal aberrations (stickiness, chromosome loss, C-mitosis, and vagrant chromosome) and nuclear abnormalities like micronucleated and binucleated cells were observed. Thus, results suggested that the discharge of partially treated wastewater into the environment is not safe for environment.

The chemically contaminated wastewater discharged into the environment causing serious soil and water pollution along with serious threat to human health in different ways. Thus, it becomes very essential to develop and optimize a biological process (bacterial process) for the adequate degradation and detoxification of toxic pollutants present in tannery wastewater for the safety of aquatic resources. Over all the study indicated that there is a need to adopt a proper treatment and bioremediation strategies to reduce the pollution load of tannery wastewater for the safety of environment.