Bacterial Degradation and Detoxification of Recalcitrant Colouring Pollutants from Textile Wastewater for Environmental Safety

SUMMARY OF THESIS

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Summary

Textile industries (TIs) play a major role by contributing to the global economy in many developing countries. TIs contribute textile trade, world export, market value and employment for urban and rural peoples. It also shares a total 13 % of exports and 5th largest source of foreign currency. But unfortunately, these are also the major sources of environmental pollution because these discharge a large volume of highly toxic wastewater having dark color, high pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), sulphate, nitrate, phosphate and phenols. Textile wastewater (TWW) also contains a variety of recalcitrant coloring pollutants (RCPs/dyes), organic compounds and heavy metals, which are used in TIs during textile production processes. Various environmental protection agencies and pollution control authorities have prioritized several chemicals as highly toxic and hazardous to nature and restricted their use in TIs. These chemicals are not fully attached to fibers and get discharged into water bodies along with the wastewater. These persist in environment for long time and cause serious threats to the water and soil ecosystem along with severe toxic effects in animal and human. However, the adequate degradation and detoxification of RCPs in real TWW is required to protect the environment and public health.

Biological treatment is a sustainable, cost-effective and eco-friendly method to effectively degrade and detoxify industrial wastewater pollutants. Therefore, in present study, a total of eight (08) morphologically distinct bacterial colonies (RKS1-RKS8) were isolated from TWW and sludge samples collected from a Handloom Bhandar, Unnao, (UP), India. Further, all the isolated purified bacterial strains (RKS1-RKS8) were screened based on the lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase enzyme activity and congo red (CR), methylene blue (MB) and methyl orange (MO) dye

decolorization efficiency. Out of eight (08) bacterial strains, only five bacterial strains i.e. RKS2, RKS4, RKS6, RKS7 and RKS8 were capable to produce a clear decolorization zone around the bacterial colonies on guaiacol, methylene blue and phenol red dye amended plates indicating the laccase, LiP and MnP enzyme activity.

In addition, these bacterial strains were further screened based on the CR, MO and MB dye decolorization efficiency and only three bacterial strains (RKS2, RKS6 & RKS7) showed the maximum dye decolorization efficiency. RKS2 showed 98% decolorization of MB dye, RKS6 showed 91% decolorization of CR dye and RKS7 showed 94% decolorization of MO dye. Thus, it was confirmed that the isolated bacterial strains are highly effective in degradation and mineralization of RCPs from TWW.

The isolated bacterial strains were further characterized to confirm their identity based on the morphological, biochemical and molecular identification tests. For morphological tests, the bacterium (RKS2) appeared as a white, circular and non-translucent colony on nutrient agar (NA) plates. It was a gram-positive, rod-shaped and non-motile bacterium. A bacterium (RKS6) appeared as milky white colonies on NA plate. It was a gram-positive, rod-shaped and non-motile bacterium. A bacterium (RKS7) appeared as milky white, concave and smooth colonies on NA plates. It was a gram-positive, motile and rod-shaped bacterium. For biochemical tests, the bacterium RKS2 showed positive reaction for catalase and VP test whereas negative for starch utilization, citrate utilization, indole production, H_2S production and urease test. RKS6 showed positive test for catalase, VP, starch utilization and citrate utilization and negative reaction for urease test, indole production and H_2S production. The bacterium RKS7 showed positive tests for urease, citrate utilization, starch utilization, catalase and MR test and negative test for VP, indole production and H_2S production.

For molecular identification, according to the 16S rRNA gene sequencing analysis,

the bacterial strains RKS2, RKS6 and RKS7 showed the highest similarity with *Bacillus albus, Bacillus megaterium* and *Bacillus paramycoides,* respectively. Thus, the isolated bacterial strains RKS2, RKS6 and RKS7 were identified as *B. albus, B. megaterium* and *B. paramycoides* with GenBank accession numbers MW407057, OK001869 and OK001866, respectively.

A new bacterial consortium RKS-TEX267 was developed using these three potential pollutants degrading bacterial strains, *Bacillus albus* (RKS2), *Bacillus megaterium* (RKS6) and *Bacillus paramycoides* (RKS7) on the basis of performance of the single cultures in treatment of TWW. For development of bacterial consortium, the selected bacterial strains were confirmed for their compatibility test/ bio-interaction study. Results indicated that all the selected bacterial strains were able to grow with each other without forming any inhibition zone.

Afterward, the newly developed bacterial consortium RKS-TEX267 was optimized at various environmental and nutritional parameters as well as inoculum concentration and agitation rate for the effective degradation of RCPs from TWW. Results revealed that the newly developed bacterial consortium RKS-TEX267 showed 99.28% decolorization of RCPs at optimized conditions within 24h. The optimized conditions were found to be pH 7, temperature 30±2 °C, inoculum size 10%, salt concentration 1%, static condition and best carbon and nitrogen sources were found glucose and yeast extract for the effective degradation RCPs in real TWW.

The untreated real TWW showed dark color (ADMI 1354) with high values of pH (9.56), temperature (39 ºC), EC (6.36 us/m), COD (1746 mg/L), BOD (699 mg/L), TOC (3801 mg/L), TDS (7203 mg/L), TSS (501 mg/L), TS (7101 mg/L), phenol (2.27 mg/L), nitrogen (11.13 mg/L), surfactant (9.80 mg/L), chloride (1731 mg/L), sulphate (1605 mg/L) and phosphate (9.33 mg/L). It also showed high values of Cr (1.70 mg/L), Ni (4.23

mg/L), Cd (1.10 mg/L), As (2.55 mg/L), Fe (3.15 mg/L) and Pb (0.31 mg/L). All the values of various physicochemical parameters are found to be higher than permissible limit for the Central Pollution Control Board (CPCB 2013) and the National Environment Quality Standard.

After bacterial treatment, the quality of TWW improved significantly with 99%, 83.80%, 88.77%, 93.99%, 72.53%, 71.85%, 76.44%, 71.89% 85.90%, 65.58%, 34.83%, 41.05% and 46.83% reduction in color (ADMI), EC, COD, BOD, TOC, TDS, TSS, TS, phenol, nitrogen, chloride, sulphate and phosphate, respectively. The developed bacterial consortium RKS-TEX267 also removed Cr (72.35%), Cd (79.09%), Ni (83.21%), As (65.88%), Fe (32.69%) and Pb (83.87%) from real TWW.

Fourier Transform Infrared (FT-IR) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis were used to identify the RCPs and their metabolites produced during the treatment process. FT-IR results showed various functional groups present in RCPs and after treatment, these functional groups convert/transform into new metabolites. Further, GC-MS results revealed that most of the RCPs present in real untreated TWW were degraded and transformed into metabolites by the newly developed bacterial consortium RKS-TEX267 at optimized conditions. Thus, results indicated that the developed bacterial consortium RKS-TEX267 can be used effectively in treatment of industrial wastewater pollutants.

Further, the degradation of RCPs are only possible due to the ligninolytic enzymes present in microorganisms. Laccase, LiP and MnP enzyme was detected during the decolorization of RCPs. Results revealed that the selected bacterial strains, *Bacillus albus* (RKS2) showed LiP, *Bacillus megaterium* (RKS6) showed MnP and *Bacillus paramycoides* (RKS7) showed laccase enzyme as confirmed by the quantitative analysis. Further, MnP, LiP and laccase enzymes were characterized by SDS-PAGE analysis and

found to have molecular weight of around 126 kDa, 58 kDa and 97 kDa, respectively in the crude enzymes extract. Overall, the synergistic role of LiP, MnP and laccase enzymes may be key potential for the effective degradation of RCPs from real TWW.

In addition, the toxicity test of TWW before and after bacterial treatment was performed by using *Vigna radiata* and *Vigna mungo* as a terrestrial model. Results revealed that the untreated TWW was highly toxic in nature as it showed inhibitory effects on seed germination, root length, shoot length and biomass production of *Vgna radiata* and *Vigna mungo*. The treated TWW showed significant improvement in seed germination (100-90%), root length, shoot length and biomass production as compared to untreated TWW. Results indicated that TWW may be degraded into less/non-toxic metabolites by developed bacterial consortium RKS-TEX267. Thus, the bacteria 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 RKS-TEX267 comprising *Bacillus albus* (RKS2), *Bacillus megaterium* (RKS6), *Bacillus paramycoides* (RKS7) were more effective in degradation and detoxification of RCPs from real TWW. This study was perhaps the first attempt on the development of a new bacterial consortium with identified potential bacterial strains and its application in degradation and detoxification of RCPs from real undiluted TWW. Therefore, this study can be useful to develop a bacteria-based treatment process for the degradation of industrial wastewater pollutants for environmental safety and to promote the sustainable development of our society with less environmental impacts.