Study on the bacterial degradation and detoxification of distillery wastewater pollutants for environmental safety

SUMMARY OF

Thesis

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Summary

Distillery industry is among of major sources of environmental pollution like water and soil pollution. In India, the number of distilleries has gone up to 319 with annual production of 3.25×10^9 L of alcohol and 40.4×10^{10} L of wastewater. Mainly four steps are involved in alcohol production such as feed preparation, fermentation, distillation and packaging. The DWW has dark brown colored due to the presence of melanoidins and some other coloring compounds. Molasses based distillery generates an average 15 L of spent wash in 1 alcohol production. In DWW, melanoidin is one of the major pollutants causing serious environmental and health problem. Melanoidins are dark brown to the black colored natural condensation product of sugar and amino acids, recalcitrant compounds. DWW contains a complex mixture of organic and inorganic pollutants and acts as a major source of environmental pollution. DWW causes coloration of water resources, reduces photosynthetic activities, and dissolved oxygen content, whereas, in the soil, it reduces soil fertility and seed germination. The organic and inorganic pollutants such as melanoidins and endocrine disrupting compounds (phthalates) present in DWW are well reported to have cytotoxic, genotoxic, carcinogenic and mutagenic effects on human and animal health. The physical and chemical methods suggested for treatment of wastewater are not much effective for decolorization. Biological methods like anaerobic digestion treatment reduce BOD load of the spent wash, but the substantial amount of organic components and dark brown color left behind requires secondary treatment.

Treatment through biological way is an incredible alternate for DWW pollutants due to their low cost, environmental friendly and publicly acceptable treatment. There are various biological processes such as bioadsorption and biodegradation have been reported having prospective application in color removal from spentwash by fungi such as *Coriolus, Aspergillus, Phanerochaete* and certain bacterial sp. as *Bacillus, Alcaligenes* and

Lactobacillus for the bioremediation of spent wash. Microbially treated wastewater may be less toxic and safe device for effluent management.

However, the biological treatment of DWW containing melanoidin largely depends on pH, temperature, concentration of nutrients, oxygen and inoculums size as well as there are some enzymatic system responsible for the degradation of melanoidin consists mainly sugar oxidases and peroxidases as sarbose oxidase, glucose oxidase, etc. and also ligninolytic enzyme (Laccase, MnP and LiP). Since ligninolytic enzyme (MnP and MIP) showed melanoidin decolorizing activity in the presence of H_2O_2 and the decolorizing activity of both sugar oxidases and peroxidases were found optimum at a particular pH, temperature and substrate specific. However, fate and extent of toxicity of anaerobically treated DWW remains unknown in the environment.

Thus, it requires adequate treatment before its final discharge into the environment. Physico-chemical methods available are capable of both color and organic load reduction, but these methods are highly costly and generate a large amount of sludge as secondary pollutants. Hence, biological methods are gaining its momentum in the arena of wastewater treatment methods due to cost effective and eco-friendly nature, but these methods are time-consuming. Therefore, there is an urgent need to address the limitations in existing treatment methods and to develop the integrated treatment processes that can provide a solution to DIs for the management and treatment of generated wastewater.

The first objectives of this study, which detailed in chapter three (03) isolation, screening and characterization of ligninolytic enzyme producing bacterial strains capable for the degradation of DWW pollutants. In this study initially nine (09) bacterial strains (DS, DS1-DS8) were isolated by nutrient enrichment technique, among of these four bacterial strains DS1, DS3, DS4, and DS5 screened on the basis of their growth on different concentration of DWW and manganese peroxidase activity shown on phenol red

containing GPYM agar plates amended with DWW. Further, the isolated bacterial strains were identified on the basis of morphologically/microscopically. The bacterial strains DS3 and DS4 were identified as gram positive and cell was coccus and rod shape, respectively whereas DS5 were identified as gram negative and rod shape.

Chapter four (04) of this study, result concluded that the selected bacteria strains on the basis of melanoidins resistance test and MnP enzyme production activity on plate was compatible with each other, because observed no inhibition zone found around the bacterial colony. Further, all these potential bacterial strains was selected for the consortium development. In addition, decolorization assay in axenic conditions was not much more effective for distillery wastewater decolorization or degradation. Because in axenic culture condition selected bacterial strains i.e. DS3, DS4, and DS5 showed decolorization of distillery wastewater was 52.31, 63.26, and 49.69%, respectively. Finally, it was observed that physico-chemical parameters was also not reduced significantly by the axenic bacterial treatment. Therefore, for the effective decolorization of distillery wastewater through mixed culture consortia was developed and perform decolorization experiment in laboratory.

Chapter five (05) of this study comprised that the potential bacterial strains, which were used for consortia development i.e. DS3, DS4, and DS5 were identified as *Staphylococcus saprophyticus*, *Bacillus megaterium sp.* and *Alcaligenaceae* sp. with accession number MF182113, MF967441 and MF182114, respectively. However, these bacterial strains have ability to produce ligninolytic enzyme, which may be responsible for the distillery wastewater pollutants decolorization/degradation. Subsequently, the ligninolytic enzyme laccase and MnP were identified by SDS-PAGE electrophoresis of partially purified enzyme has yield band of laccase and MnP with the molecular weight ~65 and 43 kDa, respectively. Hence, these enzyme may be involved in the pollutants

degradation such as melanoidins and other phenolic and coloring compounds present in distillery wastewater.

Chapter six (06) summarized that the distillery wastewater was deep brown in color carrying very high values of BOD, COD total solids, phosphate, phenolics, and sulfate. In addition DWW also contains heavy metals such as Fe, Zn, Cd, Pb, and Ni etc. Further, by the treatment of bacterial consortium (DS3+DS4+DS5) was found effective to decolorize DWW upto 76.12% in comparison to axenic culture conditions with considerable reduction in BOD, COD values, total solids, sulfates, phosphates and phenolic metal content. Further, the decolorization of distillery wastewater pollutants was also studied by the various environmental factor such as pH and temperature and several carbon source (glucose, fructose, sucrose, galactose, maltose) and nitrogen source (yeast extract, peptone, urea, ammonium sulphate, and sodium nitrate) for the optimum decolorization of distillery wastewater. Hence, it was observed that potential bacterial consortium showed the maximum decolorization was 76.12% in presence of glucose (0.5%) and peptone (0.1%) at pH 7.0 and temperature 35 °C. This study proves that in distillery wastewater decolorization environmental factor and various nutritional sources are play their role effectively. Further, it was also observed that the coloring compounds such as melanoidins etc. present in distillery wastewater may increases the cell size of identified bacterial strains *Staphylococcus saprophyticus*, *Bacillus megaterium sp.* and *Alcaligenaceae* sp. under morphological observation with SEM analysis.

Chapter seven (07) of this study, concluded that FT-IR and LC-MS/MS analysis confirmed the presence of various organic compounds in DWW, in which some compounds were reported as hazardous for living organisms. Bacterial treatment reduces the toxicity of DWW. FT-IR and LC-MS/MS shown the presence of various organic compounds with molecular weight such as 270, 182, 165, 154, 150, 135, 126, 98, 86 that

were identified as Diethyl-3,4-ethylenedioxypyrrole-2,5-dicarboxylate, Trans-2-Tridecenal, Dihydroxyconiferyl alcohol ($C_{10}H_{14}O_3$), 2-nitroacetophenone ($C_8H_7NO_3$), Anhydrohexose from $[M-H]^-$, 2,6-dimethoxyphenol ($C_8H_{10}O_3$), 4-vinyl-2- methoxyphenol ($C_9H_{10}O_2$), p-chloroanisol (C_7H_7ClO), 4-methyl guaicol, N-methyl indane, 5-(hydroxymethyl)-2-furfural ($C_6H_6O_3$), Furfuryl alcohol ($C_5H_5O_2$) (98), and Butenoic acid ($C_4H_6O_2$), respectively and some other compounds before and after bacterial treatment. Hence, it was clearly observed that after secondary treatment process several toxic chemicals remains in distillery wastewater. Thus, there is an urgent need to address the limitations in the existing treatment methods and to develop the integrated treatment processes that provide a complete solution to the treatment of distillery wastewater.

Finally chapter eight (08) comprised a reports that examined a comparative study at two contaminated sites i.e. distillery and tannery wastewater/sludge contains high concentration of undesirable PTEs (Co, Cr, Ni, Mn, Zn, Fe, Pb etc.) and physico-chemical parameters (pH, EC, organic matter, moisture, and chloride) beyond the standard/permissible limit. It can be also concluded that wheat and mustard plants growing at distillery and tannery wastewater contaminated site have high metals accumulation potential in different parts i.e. root, shoot, and leaves. This high metal accumulation in plants may directly or indirectly hamper various metabolic activities as well as oxidative damage by altering the structure of enzymes, transporters or regulatory enzymes owing to their strong affinity as ligends to sulfhydryl and carboxylic groups. The biochemical analysis of the selected plant i.e., wheat and mustard revealed increased lipid peroxidation, non-enzymatic and enzymatic antioxidant activities such as MDA, H_2O_2, ASC, SOD, APX, CAT and GPX. Thus, it can be concluded that continuous monitoring is too needed near distillery and tannery area of water and soil. However, ecotoxicological analysis/ observation need to be more attention prior its application in crop fields.

In addition, the main objective of this study to check that bacterial treatment reduces the toxicity of DWW or not. This was confirmed by the animal test model (*C. elegans*) with untreated and bacterial treated distillery wastewater. This test organism are well reported for environmental toxicity, which clearly showed the toxicity of DWW pollutants in terms of alteration in mRNA expression of genes related to Ach transmission and lipid content. The overall study concluded that inadequate disposal of untreated DWW can cause the transfer of toxic substances into the environment, disturbing the biological ecosystem near the industry as well as receiving aquatic resources.

Thus, an urgent need to address the limitations in existing treatment methods and to develop the integrated treatment processes that could be provide a solution to DIs for the management and treatment of generated wastewater. In this connection, this study helps to know the nature and characteristics of the recalcitrant organic pollutants in distillery wastewater that remained even after the secondary treatment process. Further, the potential bacterial strains could be useful for the new knowledge generation and technology development for the effective treatment and complete removal of organic pollutants also to know the toxicological effects of such types of wastewaters in soil as well as in animal, which is a serious threat to environment. That's why this study could be useful for the eco-friendly and cost effective treatment of distillery wastewater with sustainable and safe treatment.