Development of a bacterial consortium for lignin degradation from pulp and paper mill effluent

SUMMARY OF

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Summary

Pulp and paper mills are one of the main water and energy intensive industries as it is sixth largest water polluting sector in the world. In India about 75% of total fresh water supplied to pulp and paper industries and emerges as waste water. Fresh water requirement in pulp and paper industry is quiet high (150-250 m^3 per ton of product) in comparison to other industries. The problems associated with pulp and paper mill effluents are pH, colour, high levels of biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS), adsorbable organic halides (AOX) etc. Pulp and paper manufacturing process release lignin and chlorinated organic compounds which are the major contaminants formed in the pulp and paper mill effluent. Pollutants released from pulp and paper mills poses harmful effects on aquatic as well as terrestrial environment. Furthermore some pollutants of pulp and paper mill effluent are resistant to biodegradation and can bioaccumulate in the aquatic food chain which indirectly affects human beings. Due to the high chemical diversity of the pollutants of pulp and paper mill effluent, a wide variety of toxic effects on recipient water bodies have been observed. Pulp and Paper mill wastewater treatment is of immense concern for the environment due to this various conventional treatment processes such as adsorption, membrane filtration, ion exchange, chemical precipitation etc have been evaluated. However, these methods are highly cost-intensive and generate enormous amount of toxic sludge and secondary pollutants. Thus, the study of lignin degrading microorganisms for the degradation and detoxification of pulp and paper mill effluent has recently gained importance and offers a safe and eco-friendly option to achieve bioremediation of effluent contaminated environments. So, the aim of present investigation was to isolate and characterize lignin degrading bacterial strains from pulp and paper mill effluent and

develop a potent bacterial consortium that was capable to reduce lignin and colour to explore the reduction potentiality of bacterial consortium. Thus, the findings of the thesis present are summarized below:

1. The pulp and paper mill effluent and sludge sample was collected from Century pulp and paper mill, Lalkuan, Uttarakhand, India. From the collected pulp and paper mill effluent and sludge samples a total of twenty one bacterial isolates were isolated and screened on the basis of their tolerance limit with increased concentration of lignin on L-MSM agar plates. Out of twenty one, six bacterial isolates were selected for ligninolytic enzyme production by using dye decolorization plate assay method, and out of six, three bacterial strains (PLP 6, PNP 13 and PNP 19) were found to decolorize the dyes as PLP 6 decolorize azure B while PNP 13 and PNP 19 changed the colour of phenol red dye. These three bacterial strains were well thought-out for the characterization, based on the Gram's reaction, cell morphology, colony morphology, growth pattern and biochemical tests Further, these bacterial strains i.e. PLP 6, PNP 13 and PNP 19 were characterized and identified on the basis of cellular morphology, biochemical and 16S rDNA gene sequencing analysis as *Bacillus aryabhattai*, *Bacillus megaterium* and *Pseudomona* sp. with their accession no. MG966493, MG966669 and MH045500, respectively. The phylogenetic relationship between the identified isolated bacteria and other intimately related bacteria found in the GenBank database was performed.
2. After screening these three bacterial strains were checked for their compatibility to each other for the development of bacterial consortium. All the three isolated strains were compatible to each other and hence, selected for

development of bacterial consortium and used throughout the study for the effective degradation and detoxification of pulp and paper mill effluent.

3. The reduction ability of bacterial consortium was found better than the individual bacterial strains. The treated pulp and paper mill effluent clearly confirms that, the developed bacterial consortium is capable to remove pollutant parameters such as colour, lignin, BOD and COD. Thus, the use of bacterial consortium instead of single strain is highly effective and active approach for the remediation of pulp paper effluent without any extra additional requirement. The effect of various environmental (pH and temperature) and nutritional (carbon and nitrogen sources) parameters were also studied to achieve much maximum reduction in pollutant parameters by the developed bacterial consortium. The optimization studies revealed that, pH 7.0 and 35 ºC temperature was the most optimum environmental conditions for reduction experiments whereas, glucose (1.0%) and peptone (0.25%) were found optimum carbon and nitrogen sources supplement to broth medium for the effective degradation of pulp paper effluent by consortium culture. In addition to that, the effect of resting and shaking condition was also studied. The results from such studies revealed that, maximum reduction was achieved by bacterial consortium culture under shaking condition. Earlier studies showed that the optimization of environmental (i.e. pH and temperature) and nutritional parameter (carbon and nitrogen sources) is a pragmatic approach to enhance the reduction capability of consortium cultures. But, when all the optimized conditions or parameter were applied at one time on the same medium of bacterial consortium it increases the rate of the degradation process. Thus, degradation and detoxification of pulp paper effluent at all

optimized conditions at the same time by developed bacterial consortium could be a more rapid and useful way to remediate effluent contaminated environments.

4. During the treatment of pulp and paper mill effluent, an increase in the production of ligninolytic enzymes was found indicating its involvement in the degradation process of pulp paper effluent. Thus, the isolated lignin degrading bacterial strains were also studied for ligninolytic enzyme activity. Results obtained from the present investigations, showed that all three bacterial strains *Bacillus aryabhattai* showed lignin peroxidase activity while, *Bacillus megaterium* and *Pseudomona* sp. exhibits manganese peroxidase activity.
5. The results of physico-chemical analysis of pulp and paper mill effluent revealed that it was alkaline in nature, deficient in dissolved oxygen, having high BOD, COD, suspended and dissolved solids values. The tannery wastewater also contained various heavy metals like Cr, Cd, Pb and Mn. Most of the pollutants present in the effluent were more than its recommended permissible limit. But after the treatment by a developed consortium, pollutants of pulp and paper effluent reduced upto a significant level and came under the standard limit set by the central pollution control board.
6. The metabolites produced during the treatment of pulp and paper effluent by the developed bacterial consortium was determined by GC-MS analysis. This study concluded that GC-MS analysis confirmed the presence of various organic compounds in pulp and paper mill effluent, in which some compounds were reported as hazardous for aquatic as well as terrestrial environments. But, after bacterial treatment toxicity of pulp paper effluent reduces. The results showed that the number of peaks reduced or disappeared in bacterial degraded

sample as compared to untreated samples. In treated samples the high molecular weight compounds metabolized into low molecular weight compounds. Some compounds such as furan and its derivatives were not degraded by bacterial consortium as detected in both untreated and treated effluent samples which shows its recalcitrant nature.

7. The toxicity assessment of pulp and paper mill effluent before and after bacterial treatment was also investigated by phytotoxicity on the seeds of two important agricultural pulses viz. *Vigna radiata* and *Cicer arietinum* to show toxic effects of untreated pulp and paper mill effluent. The results from phytotoxicity investigation showed that the germination was significantly reduced in untreated effluent. The seeds that were grown in untreated effluent had shown no or very little shoot and root growth compared to those seeds that grown in control and bacterially treated pulp paper effluent. This indicates that untreated pulp paper effluent had inhibitory effect on seed germination. But, the seeds exposed to bacterial treated pulp paper effluent showed better development of both root and shoot. Thus, treatment of pulp and paper mill effluent with bacterial consortium is an effective approach to reduce the pollutants present in the effluent as well as phytotoxicity.

Thus, the whole work concludes that the developed bacterial consortium from three bacterial strains; *Bacillus aryabhattai*, *Bacillus megaterium* and *Pseudomona* sp. was more effective and promising approach to reduce the toxic pollutants of pulp and paper effluent. Hence, it can be used as a potential agent for the effective bioremediation of pulp and paper mill effluent and also their contaminated environment.