Bacterial Degradation of Residual Organic Pollutants of Pulp Paper mill Effluent by Biostimulation and Bioaugumentation Process

SUMMARY OF THE THESIS

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SUMMARY

A recent study revealed that complex organic pollutants are retained as residual organic pollutants in discharged pulp and paper mill wastewater, even after secondary treatment. Pulp and paper mill wastewater is a threat to environment for its safe disposal due to presence of residual organic pollutants, cellulose, hemicellulose, lignin, pectin, carbohydrate, extractives resins, fatty acids, terpene alcohols, phenolic compounds. The most critical reaction in the bleaching stage is chlorine oxidation, and chlorinated organic compounds or adsorbable organic halides are the main Problems with the wastewater materials. The discharged pulp paper sludge showed the presence of various unknown complex organo-metallic pollutants after secondary treatment along with androgenic compounds. Therefore it is globally reported as health hazardous for the aquatic and terrestrial ecosystem. The toxic effects of these by-products on the environment were analyzed in the wastewater. Various studies have documented the toxic/lethal effects on daphnia, shrimp, and planktons in the water bodies receiving wastewaters of the pulp and paper industry. Lignin is a major colourant of pulp and paper mill wastewater. It has also been reported that lignin has net negative charges; therefore, various heavy metals strongly binds with lignin to form large organo- metallic complex molecules. Among the different physicochemical and biological processes available for the treatment of pulp and paper mill wastewater. The wastewater after secondary treatment becomes dark brown colour due to complexation of lignin along with organic and inorganic residu rial ecosystem which ultimately affects aquatic flora and fauna. Therefore, adequate treatment al organic pollutants present in pulp paper mill wastewater. The discharged wastewater after secondary treatment causes environmental problems in the aquatic and terrest of the pulp and paper mill wastewater is warranted prior to its safe discharge into environment. Hence, the detailed chemical properties of various pollutants present in pulp paper mill wastewater before and after secondary treatment have not yet to be elucidated in detail.

The above information has been systematically elaborated in the first chapter of the thesis as introduction. Subsequently, the second chapter has mentioned objectives

of the thesis. Further, chapter three is the review of literature on the topic that has been elaborated pulp and paper mill wastewater treatment through the ligninolytic enzyme such as laccase. Ligninolytic enzymes play a key role in the degradation and detoxification of lignocellulosic waste in environment. The major ligninolytic enzymes are laccase, lignin peroxidase, manganese peroxidase, and versatile peroxidase. The structurally laccase is isoenzymes with monomeric or dimeric and glycosylation levels (10–45 %). This contains four copper ions of three different types. The enzyme catalyzes the overall reaction: 4 benzenediol + O_2 to 4 benzosemiquinone + $2H_2O$. While, lignin peroxidase is a glycoprotein molecular mass of 38–46 kDa containing one mole of iron protoporphyrin IX per one mole of protein, catalyzes the H_2O_2 dependent oxidative depolymerization of lignin. The manganese peroxidase is a glycosylated heme protein with molecular mass of 40–50kDa. It depolymerizes the lignin molecule in the presence of manganese ions. The versatile peroxidase has broad range substrate sharing typical features of the manganese and lignin peroxidase families. Although laccase enzyme has broad range of industrial applications especially the degradation and detoxification of lignocellulosic waste discharged from various industrial activities, its large scale application is still limited due to lack of limited production. Further, the extremophilic properties of laccase enzyme indicated their broad prospects in varied environmental conditions.

Chapter four has mentioned Characterization and assessment of isolated potential aerobic bacterial strains capable for ligninolytic enzyme production at different substrate and environmental conditions the result has revealed to optimize the production of Ligninolytic enzyme for degradation of complex pollutants present in pulp paper industrial effluent (PPIE). Two ligninolytic enzyme-producing bacterial strains were isolated from PPIE and identified as *Bacillus paramycoides* strain BL2 (MZ676667) and *Micrococcus luteus* strains BL3 (MZ676668). The identified bacterial strain *Bacillus paramycoides* strain BL2 showed optimum production of LiP (4.30 U/ml), MnP (3.38 U/ml) at 72 hrs of incubation, while laccase (4.43 U/ml) at 96 hrs of incubation. While, *Micrococcus luteus* strains BL3 produced maximum LiP (3.98) and MnP (3.85 U/ml) at 96 hrs of incubation and maximum laccase (3.85 U/ml) at 72 hrs of incubation, pH 7-8, and temperatures of 30-35°C. Furthermore, in

the presence of glucose (1.0%) and peptone (0.5%) as nutrient sources, the enzyme activity of consortium leads reduction of lignin (70%), colour (63%) along with COD (71%) and BOD (58%). The toxic compounds of pulp paper mill wastewater (PPMW) discharged are a major source of environmental toxicity which is not yet known. The analysis of waste showed the pollutants detected in control i.e. 3.6-Dioxa-2,7-disilaoctane , 2-Heptnoic acid ,trimethylsilyl ester, 7-Methyldinaphtho [2,1-b,1‘,2‘-d] silole , Hexadeconoic acid, trimethylysilyl ester, Methyl1(Z)-3,3-dipheny.1-4-hexenoale,2,6,10,14,18,22-Tetracosahexane,2,2-dimethylpropyl(2Z,6E)-10,11epoxy5,6 Dihyrostigmasterol, acetate were completely diminished. The toxicity of PPIE was reduced up to 75%. Hence, knowledge of this study will be very useful for industrial sector for treatment of complex wastewater.

The Characterization of thermotolorant autochthonous bacterial community in biostimulation process for degradation of pulp paper mill effluent. The pulp and paper mill effluent is reported to discharge out various recalcitrant and androgenic compounds even after secondary treatment, but detail knowledge is not available yet regarding the properties of organic pollutants and their bioremediation process described in chapter five. Therefore, the study has been focused to detect the residual organic pollutants of pulp and paper mill wastewater after secondary treatment and their degradability in the biostimulation process The physico-chemical characteristics of the wastewater sample collected from the discharged site of paper mill discharged wastewater showed high pollution parameters along with various heavy metals. Due to various pollutants in wastewater, it contributed high BOD and COD which were beyond the permissible limit (CPCB, 2015). The high values of COD (untreated 20559 ± 207.5 and treated 4335 ± 12.94) and BOD (untreated 26946±120) and treated

(6108±541) and TDS (untreated 559 ± 11.10 and 120 ± 1.44 treated) and TSS (untreated 58± 2.10 and 19.22 ± 2.06 treated).Moreover, high content of soluble lignin (untreated 45568±13.20 and treated 1528±73) and its derivatives pH (untreated 8.7 ± 0.12 and treated 6.7 ± 0.18), The higher sulfate present in untreated paper mill wastewater 1654±11.70 and treated 1211±45). The total phenol (untreated 404±11.24 and (treated 246.33±28.72) and its derivatives induce toxicity as a carcinogen, immunotoxic, genotoxic, and a physiological effect on fish has been reported by Michael et al., (2010). The other pollution parameters like total solids, dissolved

solids, suspended solids, fixed solids, volatile solids, chloride, phosphate, nitrate, and various heavy metals were also detected above the permissible limits. The major identified compounds were The major peaks at RT 8.09, 14.54, 20.91, 21.54, 26.51,
32.36 and 37.31 were characterized as Octadecanoic acid, 8-AZABICYCLO[5,1,0] OCATANE , Decanal, 4-(3-methylstyrl)naptho[2,1-b] thiophene, 1,2-Benzene dicarboxylic acid , dibutyl ester (CAS) , 1,2-Benzene dicarboxylic acid , dibutyl ester (CAS) and 4,5,6,7-tetrakis (P-chlorophenoxyl) 1-2-diiminoiso indoline. While the majority of these compounds are known as environmental toxicants as endocrine-disrupting chemicals (EDCs). Some of these compounds were lignin monomer which revealed the necessity of treatment for the detoxification of discharged effluent. The supplementation of carbon (glucose 1.0%) and nitrogen (peptone 0.5%) stimulated the degradation process. Therefore, degraded samples after the biostimulation process showed either disappearance or generation of metabolic products at optimized conditions i.e. rpm (150), temp (37±1ºC) after 3 and 6 days of bacterial incubation. Isolated potential autochthonous bacteria were identified as indentified thermotolorant autochthonous bacterial community *Acinetobacter Colocae PC7* (OQ672613), *Bacillus sp.strain* PCB (OK614116) and *Geo bacillus stearothermophillus strain PC8* (OQ788357) In addition, the study also revealed that there was a generation of some value-added products during the detoxification of effluent in the biostimulation process from residual chloro-lignin compounds. This also supported the commercial importance of this process.

Chapter six has described this research has sought to examine the ability of a built bacterial consortium to evaluate the pulp and paper industrial wastewater detoxification process by bioaugmentation method. This study examines how native bacteria, combined with two specific aerobic strains (*Acinetobacter baumannii* PC4 and *Bacillus cereus* PC10), contribute to the detoxification of pulp and paper industrial wastewater (PPIW) through biostimulation and bioaugmentation processes. The growth of two native bacteria were stimulated in pulp paper industry wastewater by the addition of carbon (1% glucose) and nitrogen (0.5% peptone w/v). After the growth of these native bacteria in biostimulation process showed significant reduction in various physico-chemical parameters i.e. lignin (53%), color (46%), COD (76%),

and BOD (86%). Similarly, reductions in various heavy metals were also observed, such as Fe (73%), Zn (36%), Cr (41%), Cu (22%), and Ni (65%). The growing native bacterial co-culture were identified as *Enterobacter cloacae* strain PC7 (OQ672613) and *Bacillus* sp. strain PCB (OK614116). However, the addition of *Acinetobacter baumannii* (OK582199) PC4 and *Bacillus cereus* (OK582201) PC10 enhanced the reduction of various physico-chemical parameters in range of 10 - 28%. Consequently, the UV-Vis absorption peak showed reduction due to the decolorization of wastewater and degradation of various pollutants. Moreover, GC-MS analysis indicated that most of the compounds detected in the control were diminished after bacterial treatment. However, compounds such as Pentadecane, 2-Methyl, Silane (dodecylosy) trimethyl, Octadecanoic acid, trimethylsilyl ester, and Docasane were noted as recalcitrant. Additionally, new metabolic products such as 1-ethyl-5,6-dimethoxy-2,3-dihydroindole, Trimethylsily ether of glycerol, and 1-(Trimethylsilylmethyl1) dimethylsilyloxy tetradecane were detected. Due to degradation of various pollutants toxicity reductions were found up to 76% during a comparative evaluation between control and bacterial treated wastewater using the *Phaseolus mungo* L. seed germination test. Hence, it was concluded that the biostimulation and bioaugmentation process would be an effective technique for degrading residual organic pollutants from pulp and paper industrial wastewater (PPIW) for safe disposal.

Further, as per the objective four of the study, Optimization of carbon and nitrogen ratio for bacterial growth and degradation residual organic pollutants on discharged pulp paper mill effluent. The physic-chemical analysis and identification of various residual organic pollutants were present in PMMW before and after secondary treatment. The result has revealed that i.e. (mg/L^{-1}) i.e. pH (8.4 ± 0.10), Total solid (814 ± 115), COD (24659 ± 112.5), BOD (5820 ± 104), Lignin(854±20.20), sulphate (1632 ± 10.98), totalphenol (536 ± 17.25) and heavy metals i.e.Fe (75.02 ± 0.06), Ni (5.10 ± 0.75) and Zn(12.08 ± 0.12). However, a sharp reduction pH (6.5± 0.10), Total solid (102 ± 1.24), COD (3025 ± 14.94), BOD (2032 ± 13.05),Lignin (231±1.57), s u l phate (1202 ± 3.24), total phenol (217 ±16.45) and heavy metals i.e. Fe (15.01 ± 0.65), Ni (0.97 ± 0.55) and Zn (3.7 ± 0.67) was noted. The main focus of this work is to study the

potential producing bacterium Bacillus cereus strain PP2 (OK582201) for biodegradation and toxicity reduction from pulp paper mill wastewater. The *cereus strain* PP2 (OK582201) exhibited up to
78.67 % of decolorization and degradation capability for hazardous residual organic pollutants at different nutritional (glucose: 1.0 %, peptone: 0.5 %) and environmental conditions (pH: 7.0, temperature: 37^0C, agitation: 180 rpm, incubation period: 120 h). The absorption peak of the UV-Vis spectral scan identified the decolorization and degradation pattern for pollutants present in wastewater during treatment. The reduction of toxicity was measured up to 70 % for treated wastewater with *Phaseolus mungo* L seeds. From these findings, it is concluded that the isolated bacterium may be used in the biodegradation process for the further detoxification and degradation of discharged pulp paper mill wastewater for environmental safety.

The eighth chapter has focused on Development and optimization of tertiary stage detoxification technique of pulp paper mill effluent. Residual organic pollutants are responsible for an array of health hazards and environmental abuses. The remediation of such compounds is remaining as an area of interest for scientist community. The present study aimed for isolation and screening of lignin degrading bacterial strains optimized at various environmental and nutritional requirement to assess their potential for lignin degradability. The result have revealed that isolated strain C1 and C2 bacterial consortia showed degradation of lignin 86% and 82.1%, respectively at temperature 37 °C and pH 9 in presence of glucose(1%) and peptone(0.5%) within 15 days of incubated at 140 rpm in temperature controlled shaker. The variation of temperature and pH from optimized condition decrease the performance of isolated strains. The metabolite characterization through GCMS showed strain C1 9,12-octadecadienoic acid, Oxido-4,17-cholestadian-3a,16a-diol, Heptadecane,2,6,10,15-tetramethyl, Heptacosane, 1,2-benzenedicarboxylic acid and Octadecane 3-ethyl-5-(2-ethylbutyl) as major product while C2 showed their major product as Disiloxane,hexamethyl-(CAS), Benzene -1,2,4-triol(trimethylsilyl) ester, Octahydroquinoline-6-carboxylic acid(phenylethyl amide) and 4-methyl1,4-hydroxy-6-(2,5,6,6′-tetramethylcyclohex-1-en-1-yl)hex-2-ynyl acetate after 15 days degradation. Many compounds detected in control were completely removed. The isolated strains were identified as PC4 *Acinetobacter baumannii*, PC10 *Bacillus*

cereus, PCB *Bacillus sp.* based their 16s-rRNA sequencing. The gene bank's accession numbers for each strain's nucleotide sequences are OK582199 and OK582201 and OK614116. These potential strains may be useful for detoxification of lignin containing pollution in soil and water ecosystem.

The chapter nine has summarized the whole thesis work and findings with research output in a systematic manner. The chapter ten has compiled the all cited references of thesis which are relevant to the topic and they are cited in each chapter. Each chapter has cited the recent references based on methodology and results. The available references are cited on synthesis of lignin, physico-chemical properties of distillery wastewater, impact of pulp and paper wastewater at polluted sites. The complete thesis has 200 cited references.

The last chapter has listed all the scientific output based on the thesis work. There is two original research paper published in peer reviewed journal of international repute with impact factor