CHAPTER 6

CONCLUSION

Based on the present observations of physico-chemical parameters it can be concluded that the M/s Anand paper mill (APM), effluent nature is highly toxic and require proper treatment methods before release into surroundings. Alternatively in spite of having adequate treatment system, the century pulp and paper mill (CPPM) effluent also found to be contains toxic compound mainly chlorophenols which are major concern and require an effective biological treatment method. These industries discharged their effluent without proper treatment. By this, it can be toxic to all trophic level in the aquatic ecosystem and aquatic flora; funna may be affected both in water column and sediment. Thus the effluents of Century pulp and paper mill (CPPM) and Anand paper mill (APM) paper mill effluent must be treated before disposal to protect our aquatic environment.

In this thesis we assessed, physico-chemical analysis of both paper mill Century pulp and paper mill (CPPM) and Anand paper mill (APM) showed that the effluent from Anand paper mill (APM) were dark brown-colored. Whereas in spite of using adequate treatment system, Century pulp and paper mill (CPPM) effluent was also characterized by high level of pollution parameters biological oxygen demand, chemical oxygen demand and color (BOD, COD and color) and significant concentration of various organo-chlorine In this study we were isolate some bacteria through enrichment culture technique and identified as *Paenibacillus* sp., *Aeurinibacillus aneurinilyticus*, and *Bacillus* sp. respectively.
Further, decolorization of pulp effluent was optimized by this bacterial consortium using response surface methodology. According to design, the Predicted results were close resembled to the expected experimental values under optimum conditions (Carbon source 2%, agitation speed 200 round per minute rpm and pH 8.0), which indicated good quality evaluation of experimental data. The GC-MS of analysis after bacterial treatment compared to control indicated that the consortium utilized the constituents of pulp paper effluent rather than biotransformation. Finally the toxicity and genotoxicity evaluation tests with different test models suggested that industrial effluent was detoxified by the help of bacterial treatments.

Finally, three bacterial strains PPS-S6, S6 and S8 have been found to grow in lignin amended mineral salt medium and decolourised/degraded to lignin after six days of incubation. Alternatively in spite of these bacterial strains could not bring significant growth and colour reduction when MSM was supplemented only with glucose or peptone respectively. Among of three bacterial strains PPS S6 (Paenibacillus sp.) and PPS S7 (Aneurinibacillus aneurinilyticus) are new bacterial species and no reports are available about these bacterial strains for lignin degradation. Therefore the strain PPS S8 (Bacillus sp.) has been shown to highest degradation and decolourisation of lignin. Alternatively in spite of mixed culture of these bacterial strains was found most efficient towards lignin degradation as compared to single strains. Furthermore lignin degradation by these bacterial strains at alkaline pH has great significance because the pulp and paper mill effluents pH ranged from 7.5 to 8.5. Thus these bacterial strains could be considered for the decolourisation and treatment of pulp and paper mill effluents.
SUMMARY

Continuous discharge of the organic pollutants (OPs) from pulp paper mill into the environment has increasing the levels of these pollutants in water system. In this thesis we assessed, physico-chemical analysis of both paper mill CPPM and APM showed that the effluent from Aanand paper mill (APM) were dark brown-colored. Whereas in spite of using adequate treatment system, century pulp and paper mill (CPPM) effluent was also characterized by high level of pollution parameters (BOD, COD and color) and significant concentration of various organo-chlorine (PCP, trichlorophenol, dichlorophenol etc. demonstrating ineffectiveness of existing biological treatment system. Moreover, this study tended to appraise the treatment of industrial effluent by bacterial system.

The whole work of thesis was carried out keeping the view of application of isolated strain in management of effluent discharge from pulp-paper mill. The first objective included the comprehensive analyses of physico-chemical parameters of effluents collected from two category of paper mill based on using different raw materials and treatments processes.

M/s Century Pulp-Paper industry used chlorine compounds for bleaching of pulp resulting formation of chlorinated phenols, which discharged in to Gola River. M/s Anand Paper Mill (APM), small scale mill used less bleachable raw materials, such as baggase, wastepaper rags and agriculture residues and produce brown and packaging grade paper.

The physico-chemical analysis of both paper mill century pulp and paper mill (CPPM) and An and paper mill (APM) showed that the effluent from APM were dark brown-colored having average color unit of 18534 PtCo and lignin (413 ppm) with alkaline pH, 8.5 as well as high biochemical oxygen demand (BOD) (6033 ppm), carbon oxygen demand (COD) (15766 ppm), total dissolved solid (TDS) (1274 ppm), sulphate (405.4 ppm), phosphate (500
ppm), nitrogen (152.9 ppm), sodium (28.9 ppm), and potassium (26.5 ppm). Whereas in spite of using adequate treatment system, Century pulp and paper mill (CPPM) effluent was also characterized by high average color units (1869 Pt-Co), lignin (149.8ppm), chemical oxygen demand (COD) (1057 ppm), biochemical oxygen demand (BOD) (351 ppm), total solid (T.S) (1423 ppm) and significant concentration of chlorophenols indicating ineffectiveness of existing biological treatment system.

The second objective of this thesis was screening of potential bacterial strains for the lignin degradation and the biochemical characterization. In this study we were isolate 8 bacteria through enrichment culture technique. Three strains PPS-S6, S7 and S8 were screened on the basis of their potential for reduction of various pollution parameters and identified as *Paenibacillus* sp., *Aneurinibacillus aneurinilyticus*, and *Bacillus* sp. respectively.

The biodegradation of lignin was carried at flask level by pure and mixed bacterial culture for six days. During the course of study, bacterial growth and decrease in lignin and color were monitored spectrophotometrically. In totality mixed culture of these isolated strains is more effective than single culture.

Further the efficacies of these cultures were evaluated for the optimization of important factors which are influences the decolorization of pulp-paper mill effluents. This work tended to evaluate the statistical optimization of effluent by bacterial consortium using CCD. The degree of significance of the proposed design was evaluate using $p$-value, $F$-value, $R^2$ and adjusted $R^2$. The develop regression model explains absolutely. This fact is visualizing in graphical representation of actual verses predicted values. The observed % decolorization varied between 70-88% and the model prediction match these observational results satisfactorily. The model adequacy was further checked using ANOVA. The model $F$-values is 10.43 ($p$-value: 0.0094) in case of % decolorization imply that the model is significant.
The GC-MS of untreated and bacterial treated sample showed that a considerable utilization of pollutants by developed bacterial consortium and it has well-built for use pulp-paper ingredients as nitrogen, carbon and energy. Verities of organic pollutant were identified in untreated control sample as low-molecular-weight lignin. Therefore, this combination of bacterial consortium possibly will be used for the management of industrial effluent.

In conclusion, the isolated bacterial strains are capable for the decolorization and degradation of lignin in MSM containing simpler form of nitrogen and carbon sources at slightly alkaline pH at 30°C and agitation speed 120 rpm. However, the highest drop in color (65%) and lignin content (37%) was recorded by *Bacillus* sp. (PPS-S8) indicating ligninolytic bacterium.

On the other hand, investigations of the ligninolytic machinery in *Bacillus* sp are lacking. A complete characterization for enzyme associated with lignin degradation is now necessary in order to establish their definitive role in the breakdown of the lignin.

The Mixed culture comprises these three strains was found more efficient over single strain for lignin degradation. On the other hand *Bacillus* sp and mixed culture have been found potential for the degradation of lignin present in effluent released by pulp and paper mill even in presence of others pollutants. Besides, significant reduction in pollution parameters such as color, Total solid (TS), Biochemical oxygen demand (BOD), Chemical oxygen demand (COD) of industrial effluent by *Bacillus* sp. and mixed culture treatments reinforces the applicability of these bacterial strains for the decolorization and detoxification of the wastewater released by paper industry. The toxicity and genotoxicity evaluation tests with different test models revealed that the effluent released by pulp and paper industry was toxic which rendered non-toxic due to the bacterial treatments.