SUMMARY AND CONCLUSION

An enormous industrial growth has taken place throughout the world in the past few decades. It has become so vast that, the environment has totally changed from what it was earlier. Due to increasing human needs of natural resources, the level of pollution in environment has raised to devastating extends leading to disastrous consequences. Pollution today is found in each and every thing that we need the most viz. air, water, soil, etc. Water being one of the most important natural resources, is required in huge amount to fulfill all human needs. Apart from personal usage, the amount of water utilized by various industries is very large. Pulp and paper mills are one of the main water and energy intensive industries as it is sixth largest water polluting sector. Typically in India around 75% of total fresh water supplied to pulp and paper industries emerges as waste water. In comparison to other industries fresh water requirement in pulp and paper industry is quiet high (150-200 m$^3$) per ton of product.

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. Paper manufacturing process release chlorinated lignosulphonic acids, chlorinated resin acids and chlorinated phenols are the major contaminants formed in the effluent of pulp and paper mill. Pollutants released from pulp and paper mills into the environment pose numerous problems and physiological destruction. Furthermore some compounds in the effluents are resistant to biodegradation and can bio-accumulate in the aquatic food chain.

Due to the high chemical diversity of the organic pollutants in paper and pulp mill waste water, a wide variety of toxic effects on aquatic communities in recipient watercourses have been observed. Pulp and Paper mill wastewater treatment is of immense concern for the environment due to its after effects.

Present work involves the detailed study of the pulp and paper mill wastewater generated from various processing units. A review of different existing technologies available for the treatment of pollution exerted by such wastewater was also carried out.
Isolation of naturally occurring bacterial strains and their utilization for the treatment of wastewaters emanating from the industry was the main focus of this work. An effort to tackle different problem areas engulfing this industry in the present day scenario like color, biochemical oxygen demand, chemical oxygen demand, dissolved solids etc. in terms of meeting discharge regulations, was made through this work. Characterization of wastewater collected from different units was carried out. Bioremediation of pulp and paper wastewater and the reduction of its organic pollution index by bacterial strains isolated, purified and identified during the course of the study were the highlights of this study.

Physico-characterization of wastewater produced by different processing units of pulp and paper mill like chlorination, extraction, hypochlorite, combined effluent bleach plant, Primary Clarifier inlet, Primary Clarifier outlet and Secondary Clarifier outlet) was conducted. Different parameters like temperature, pH, colour, biochemical oxygen demand, chemical oxygen demand, solids were analysed and compared. All analysis was carried out in triplicate. The study revealed that there was immense variability in all the samples for all the parameters. This propounded the fact that no two units are similar in their wastewater composition. On the basis of results obtained, it was felt that knowledge of the pollution index of each of the unit could form a base to devise appropriate strategies for mitigating potential environmental hazards arising due to these. During the study, it was observed that pulp and paper mill selected in our study has effluent treatment facility but improvements are still needed to increase their efficiencies for treating discharge wastewater. This also indicated that there is a lot of scope in improvising the existing activated sludge system, which possibly could be done by utilizing the natural wealth of bacteria, specific for the particular pollution problem. It was concluded that the PC outlet was the best unit for treatment for further study.

Based on the biodegradability, the industrial waste water has been classified as low, moderate and highly biodegradable. This is primarily due to presence of microorganisms used for the biodegradation of different constituents present in individual industries. Major problems in pulp and paper industry wastewater are lignocelluloses and processing chemicals. Lignocellululosic compound degrading
bacteria were isolated from different sources within the industrial premises. The bacteria were isolated and screened on the basis of their degrading capability of lignocellulosic compounds. Ten bacteria were isolated from three soil samples (wood yard section, final discharge point combined effluent discharged point). The bacteria were characterized on the basis of their colony morphology.

Ten bacterial isolates were screened; out of them six were selected on their capability to degrade lingo-cellulosic compounds. Two bacterial isolates (PNP 1, PNP 3) showed the presence of lignin degrading enzymes, two isolates (PNP 5, PNP 6) were showing capability to degrade cellulose and rest of the two isolates (PNP 8, PNP 9) were selected on the basis of xylan degradation capability.

The selected isolates (PNP 1, PNP 3, PNP 5, PNP 6, PNP 8 and PNP 9) were screened for their capability to degrade the pulp and paper wastewater. Pulp and paper industrial wastewater is complex in nature. It is difficult for single microorganism to degrade the complex pulp and paper wastewater. Therefore, in the present study different permutation combinations (consortia were formulated) were made of bacterial isolates in order to treat the pulp and paper wastewater holistically. Microbial consortium was used to treat the wastewater because it has long been known that they are much more efficient at degrading complex organic wastes than single isolate. In a microbial consortium one might find any number of organisms with different metabolic capabilities. These different metabolic capabilities allow the consortium to work together in degrading a variety of complex waste streams. Current study clearly showed that, application of consortia can be used for the treatment of large pulp and paper mills effluents. Twenty consortia were formulated and screened, out of these consortium 9 was selected for further studies. The successful approach of cooperator cheater showed the synergism between the three bacteria present in the consortium 9. The said consortium was selected for the further studied in order to increase the degradation rate.

In the present study the process of bioremediation was optimized, by Taguchi approach. The Taguchi method utilizes orthogonal arrays for design of experiments to study a large number of variables with a small number of experiments. An orthogonal array significantly reduces the number of experimental configurations to be studied. In this
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study the sequential batch reactor was used for the removal of pollutants from the waste water of pulp and paper mill by using bacterial consortium. After the optimization of parameters for different levels the combination selected was F/M ratio (0.75), nutrient dosing (100:5:1), dissolved oxygen (2.0), pH (7.0) and hydraulic react time was 16 hrs.

The selected consortium was able to cater the problem of sludge bulking. It was observed from the results that SVI value is about 60 - 80 which is quite low as compared to the obtained value for the conventional process. The characteristic of pulp and paper industry effluent was varied depending upon the raw material used and the manufacturing process. The treatment was studied at different pollution load, in order to check the performance of the consortium. The bioremediation experiments were performed at four different temperatures i.e., 25°C, 30°C, 35°C and 40°C. It was observed that the consortium was acting effectively at different pollution loads at all the temperatures which are taken as an advantage of this selected consortium. So, it is possible to increase the bioremediation rate significantly using the proposed statistical technique.

One of the most fascinating aspects of the microbial world is its extraordinary diversity. Because of this biodiversity, it is desirable to classify and arrange bacteria into groups based on their mutual similarities. In science, accurate and standardized names are essential. All biologists must agree on the names of the organisms. Faced with the great number and diversity of organisms, biologists use the characteristics of different organisms to describe specific forms of life and to identify new ones. The grouping of related organisms together is the basis of classification. In our study we have identified the bacteria on the basis of 16S rDNA sequence analysis. The consortium 9 consisting of three bacteria isolates (PNP 3, PNP 6 and PNP 8) were identified by using 16S ribosomal RNA gene sequence analysis. It was found that PNP 3 was identified as Klebsiella sp., PNP 6 was Alcaligenes sp., and finally PNP 8 was identified as Cronobacter sp. The 16S rRNA gene sequence of PNP3, PNP6 and PNP8 has been submitted to GenBank under accession numbers KF531636, KF531637 and KF531638 respectively.

It is necessary to check that the bacteria of our selected consortium are surviving or not in the effluent during treatment process. Morphologically it is difficult to identify the
bacteria; therefore a molecular typing method (ERIC PCR) for identification of bacteria was used in the study. ERIC stands for Enterobacterial Repetitive Intergenic Consensus (ERIC) sequences which are 127-bp imperfect palindromes that occur in multiple copies in the genomes of enteric bacteria. ERIC PCR generate specific DNA fingerprint of bacteria. It was observed that survival of bacterial consortium in the effluent during the treatment process played an important role in the reduction of pollution. It was concluded that a synergistic consortium formulated from the three bacterial strains can lead to holistic degradation of the wastewater in pulp and paper mill and therefore prove to be very useful for bioremediation purposes.