# PAPER MILL EFFLUENT: DECOLORISATION AND DETOXIFICATION STUDIES USING CHEMICAL AND MICROBIAL METHODS

**SYNOPSIS**

**S***ubmitted in fulfillment of the requirements for the Degree of*

## DOCTOR OF PHILOSOPHY

By

N. KUMARA SWAMY

Department of Biotechnology

JAYPEE INSTITUTE OF INFORMATION TECHNOLOGY

A-10, SECTOR-62, NOIDA, INDIA

30th April 2012

## SYNOPSIS

# PAPER MILL EFFLUENT: DECOLORISATION AND DETOXIFICATION STUDIES USING CHEMICAL AND MICROBIAL METHODS

The research work carried out in the present study is structured into six chapters in thesis.
CHAPTER 1: INTRODUCTION is devoted to introduction and identification of the research problem. The chapter also defines the major objectives for the present work.

CHAPTER 2: REVIEW OF LITERATURE covers existing literature on the identified problem and explains the rationale behind the selection of current problem.

CHAPTER 3: MATERIALS AND METHODS describes the materials used and the underlying methodology adopted to achieve identified objectives in the study.

CHAPTER 4: RESULTS presents the results obtained and inferences drawn from the study.

CHAPTER 5: DISCUSSIONS discusses the results obtained, offers explanations to the observed trends and apparent contradictions. It compares results and discusses their practical implications.

CHAPTER 6: SUMMARY AND CONCLUSONS briefly summarizes the work carried out and results obtained. It also presents the conclusions drawn from the study.

These chapters are followed by REFERENCES, AUTHOR'S PUBLICATIONS and SYNOPSIS. A brief description of the work reported in the six chapters of thesis is presented below.

## 1. Introduction

The pulp and paper industry uses large quantity of freshwater and lignocellulosic materials in the process of production of paper and it generates large quantity of effluent. The generated effluent is characterized by dark color, foul odour, high organic content and extreme quantities of chemical oxygen demand (COD), biochemical oxygen demand (BOD) and pH [1]. The dark color in paper mill effluent is caused by the organic ligands such as wood extractives, resins, synthetic dyes, tannins, lignin and its degradation products [2, 3]. The dark color in untreated effluent is a major environmental concern as its discharge to water bodies inhibits the photosynthetic activity of aquatic biota by reducing sunlight, besides exhibiting the toxic effects on biota [2, 4, 5]. The chlorinated phenols generated during pulp bleaching stages of paper production are a class of harmful pollutants found in paper mill effluents. These phenols contribute considerably towards the toxicity of effluent that severely affects the fish community [3, 5, 6, 7]. The harmful environmental effects of effluents and the stricter environmental norms compel the mills to reduce color, toxic phenols and other pollutants to safe disposal levels prior to effluent discharge.

The present state of art technology adopted by paper mills to treat their effluents involves three major stages namely primary, secondary and tertiary treatment. Primary treatment methods currently used by paper mills such as sedimentation, dissolved air floatation and filtration remove only suspended solids [1]. The presently available secondary treatments such as aerated lagoons, activated sludge systems, anaerobic treatments, etc target only COD, BOD, AOX (adsorbable organo halogens) and other specific compounds such as chlorinated phenols, catechols, guiacols, etc [1, 8]. However, the success of these technologies is limited by nutrient requirement, bulking and growth of filamentous microorganisms. Therefore, tertiary treatments are often used as final polishing techniques and these technologies target the individual pollutants to meet the discharge standards [1].

Several physicochemical and electrolytic tertiary treatment methods such as rapid sand filtration, membrane processes, electro-coagulation, ozonation, chemical precipitation and adsorption have been developed to target color or phenols and the drawbacks of these methods are reported in literature [2, 9-18]. Several microbial methods employing fungi and some bacteria have also been developed by researchers to remove color and/or phenols from paper industry effluents [7, 19, 20]. However, the use of microbial methods are practically limited as many of the compounds of concern in effluents resist biological degradation and exert significant toxicity towards the mixed microbial communities within the biological treatment system [1, 10].

The literature survey indicates that while there are treatment methods that remove either color and/or phenols from effluents, there is dearth of treatment processes that effectively remove both color and phenols from the effluent [2, 6, 9, 12-15, 19]. Therefore, some researchers found it inevitable to integrate two or more single treatment processes to explore the unique strengths of each process involved. Some integrated treatment methods involving two or more processes in sequence or in combinations have been developed and reported [11, 21]. These integrated treatments have shown higher pollutant removal efficiencies than their respective individual treatment processes. However, none of the existing integrated treatment methods were concerned with the complete removal of both color and phenols from paper mill effluents. Therefore, more studies related with to development of integrated treatment methods involving a combination of two or more treatment processes are needed to remove both color and phenols effectively from the paper mill effluents.

In the present study, an integrated two-step sequential treatment approach combining chemical precipitation process with microbial treatment process was proposed and investigated for removing both coloring matter and toxic phenols from effluent. For chemical precipitation, the metal salts of aluminum ($AlCl_3$), Iron ($FeCl_3$), copper ($CuSO_4$) and barium ($BaCl_2$) were employed. For microbial treatment, the bacteria *Pseudomonas putida* (MTCC 1194), known for its ability to degrade phenols in aqueous solutions [8, 22] was used. Further, the impact of sequentially treated effluent on germination of pea and bengal gram seeds was also studied to understand the toxicity level and assess the utility value of treated effluent in agricultural sector.

The major objectives identified for the present study were;

- To study physicochemical characteristics of the effluent and assess its pollution load
- To investigate the color and phenols removal capabilities of one-step chemical precipitation and microbial treatments processes
- To investigate the efficiency of integrated two-step sequential treatment to remove color and phenolic toxicity from effluent

- To assess the toxicity level of sequentially treated effluents through seed germination tests using the pea and bengal gram seeds and assess the utility value of treated effluent in agriculture sector

## 2. Materials and Methods

### 2.1 Chemicals

The precipitating agents ($AlCl_3$, $FeCl_3$, $CuSO_4$ and $BaCl_2$) and the other chemicals used in the study were of analytical grade and were purchased from various furnishers.

### 2.2. Effluent samples

The effluent samples were collected at different time periods over a period of one week from Madhya Bharat Paper Mill Ltd, Champa, Chattisgarh, India, pooled and stored in refrigerator at $4^oC$ until their usage.

### 2.3. Organism

The *Pseudomonas putida* (MTCC 1194), a bacterial strain capable of phenol utilization, used in investigations was purchased from MTCC, Institute of Microbial Technology, Chadigarh, India.

### 2.4. Culture media

A modified form of mineral salt culture media (MSM) was used in microbial batch studies. The mineral salt media was prepared by dissolving $KHPO_4$ (6.8 $gL^{-1}$) and $NaHPO_4$ (7.8 $gL^{-1}$) in the raw paper mill effluent/metal salt treated effluent. A trace metal salt solution of 1.0 $gL^{-1}$ was also added to MSM media and pH was adjusted to 7.0 using 2M NaOH and $H_3PO_4$.

### 2.5. Analytical methods

The physicochemical analysis of effluent was performed using the standard methods of APHA (American Public Health Association) [23].

### 2.6. Chemical precipitation experiments

For chemical precipitation experiments, 100 mL of raw paper mill effluent was taken in separate 300 mL glass stoppered bottles and the metal salt (dose 0-5.0 $gL^{-1}$) was added to it. The contents in the bottle were thoroughly mixed and the pH was adjusted to the required value. The bottles were kept in unshaken conditions for 24 hours to facilitate the formation and sedimentation of precipitates. The pollutants were analyzed after 24 hours drawing aliquots of effluent from the bottles. For the investigation of effect of pH and dose on color and phenols removal, a set of bottles at varying pH conditions or salt doses were respectively used.

### 2.7. Microbial treatment experiments

For the microbial batch experiments, 100 mL of the sterilized modified minimal salt media was taken in 250 mL Erlenmeyer flask. The media contents were first amended with desired amounts of sterilized solutions of glucose and ammonium sulfate, and later the bacterial biomass was inoculated. The culture media was incubated in an orbital shaker maintained under desired conditions. During incubation, the culture sample was removed under aseptic conditions and analyzed using standard methods. For identifying optimum conditions, the experiments were performed by varying the conditions such as pH (4.0-10.0), concentration of glucose (0-1.0g$L^{-1}$), concentration of ammonium sulfate (0-1.0g$L^{-1}$), temperature (22-34$^{o}$C), agitation rate (60-300 rpm) and inoculum concentration (0-6.0 g$L^{-1}$) in batch experiments.

The culture media without bacterial inoculums were used as controls in all the experiments.

### 2.8. Sequential treatment experiments

In first step of sequential treatment, the chemical precipitation experiments were performed using the optimum dose and pH conditions that produced highest color reduction in effluent. The precipitate produced was removed by centrifugation and the 100 mL supernatant was used to prepare the minimal salt medium required for second step of treatment.

For second step, 100 mL of the above minimal salt media was taken in 250 mL Erlenmeyer flask and the contents were amended with desired amounts of glucose and ammonium sulfate. The media was later inoculated with bacterial biomass (0-6.0 g$L^{-1}$) and incubated on orbital shaker at agitation rate of 180 rpm at 30$^{o}$C. During incubation, the culture sample was removed under aseptic conditions and analyzed for pollutants by standard analytical methods.

The culture media without bacterial inoculums were used as controls in these experiments.

### 2.9. Toxicity evaluation by seed germination tests

The toxicity evaluation of treated effluent samples was conducted in as recommended by Environmental Protection Agency (EPA), US, using the seed germination bioassay tests [24-27]. For seed germination experiments, dry bengal gram seeds were used. Seeds were first soaked overnight and placed on the petri plate containing moist cotton. They were later maintained in an incubator at 28$^{o}$C by supplying appropriate quantity of test samples (treated and untreated effluent) at regular intervals. After 48 hours, the germination percentage was calculated by counting the number of germinated seeds (emergence of root and shoot). On the sixth day, the shoot length (S) and root length (R) were measured. The seed germination inhibition percent (SGI) and seed vigor index (SVI) were computed using relationships given by Rao *et al*. [24] and Abdul-Baki and Anderson [25]. For these experiments, drinking water (Bisleri water) was used as control.

## 3. Results and Discussions

### 3.1. Physicochemical analysis

The paper mill effluent used in study had a dark brown color and a turbid colloidal appearance with a large amount of solid particulate matter present in it. The analysis of effluent showed high presence of color (6285 CU) and lignin (4188 $mgL^{-1}$) in effluent. The effluent hosted high phenol content (79.5 $mgL^{-1}$) and its measured pH was 7.18. The effluent was also highly deficient in dissolved oxygen (0.2 $mgL^{-1}$) with extreme quantities of COD (4306 $mgL^{-1}$) and BOD (980 $mgL^{-1}$) associated with it. The effluent was rich in organic solids with a high total suspended solids (TSS) level of 3263 $mgL^{-1}$. The effluent had total suspended solids (TDS) level of 1046 $mgL^{-1}$ which is within permitted range for disposal. The maximum permitted safe disposal levels for pH, color, phenols, COD, BOD, TSS and TDS are 6.5-8.5, 100 CU, 1-5 $mgL^{-1}$, 250 $mgL^{-1}$, 100 $mgL^{-1}$, 100 $mgL^{-1}$ and 3500 $mgL^{-1}$ respectively as per Indian standards.

The analysis of pollution load in treated effluent samples was mainly restricted to five parameters namely color, phenols content, residual metal ions, COD and TSS. The COD was measured to account for both the biologically oxidizable (BOD component) and non-oxidizable organic components in effluent. The TDS was within the permitted disposal levels and so it was not monitored.

## 3.2. Chemical precipitation

Four chemical precipitation treatments using metal salts namely aluminum chloride, ferric chloride, copper sulfate and barium chloride were attempted in the present study. The brief summary of results and the observations made during each of these treatments is given below.

### *3.2.1. Aluminum chloride*

The precipitation of color and phenols by aluminum chloride showed high sensitivity towards pH and metal salt dose. The color removal was effective in acidic conditions (pH 4.0-7.0) and the pH conditions close to 5.0 were found to be highly suitable for color removal. The highest color removal of 99.8% was achieved using aluminum chloride at pH 5.0 for the dose of 4.0 $gL^{-1}$. Under the same conditions, about 39% of phenols, 96% of TSS and 59% of COD were removed from the effluent and 31.5 $mgL^{-1}$ of residual Al(III) ions were introduced to effluent.

The aluminum chloride was found to be effective in the removal of phenols under highly alkaline conditions in the pH range of 9.0-12.0. The highest phenols removal of 99.0% was achieved at pH 12.0 for the dose rate of 4.5 $gL^{-1}$of aluminum chloride but the observed color, TSS and COD removals under these conditions were 83%, 84% and 48.5% respectively. The treatment also left 43.6 $mgL^{-1}$ residual ions in treated effluent. These results clearly suggest that the one-step treatment using aluminum chloride alone is not effective enough for the treatment of effluent as it remove either color or phenols but not both under any given treatment condition.

### *3.2.2. Ferric chloride*

Ferric chloride, like aluminum chloride, was more effective in removing color under acidic conditions in the pH range of 3.0-6.0. The conditions close to pH 4.0 were found to be highly effective in removing color from effluent. The highest color removal of 99.0% was observed at pH 4.0 for the dose of 2.0 $gL^{-1}$of ferric chloride and the observed phenols, TSS and COD removals in these conditions were about 42%, 97% and 53% respectively. The treatment also added 30.0 $mgL^{-1}$ of residual Fe(III) ions to treated effluent.

The ferric chloride showed effective phenols removal under highly alkaline pH conditions in the pH range of 9.0-12.0 with a maximum removal of 98.0% at pH 12 for the dose of 2.5 $gL^{-1}$. But, the observed color, TSS and COD removals were 76%, 85.5% and 43% respectively under these conditions. The residual Fe(III) ions produced under these conditions were 18.3 $mgL^{-1}$. These results suggest that under no conditions treatment by ferric chloride alone is effective enough to remove both color and phenols completely from the effluent.

***3.2.3. Copper sulfate***

The copper sulfate was found to be effective in removing color from effluent in the pH range of 6.0-12.0. The pH conditions close to 10.0 were found to be highly effective in removing color from the effluent. The highest color removal of 96% was achieved at pH 10.0 for the dose of 2.5 $gL^{-1}$of copper sulfate and under the same conditions 48% phenols, 95% TSS and 62% COD removals were observed. This treatment left about 65 $mgL^{-1}$ residual Cu(II) ions in effluent.

The copper sulfate was effective in phenols removal under highly alkaline pH conditions (pH 9.0-12.0) with the highest removal of 94% recorded in pH 12.0 for the dose rate of 2.5 $gL^{-1}$of copper sulfate. The same treatment removed about 70% of color, 81.7% of TSS, 52% of COD from effluent and induced 18.3 $mgL^{-1}$ of residual Cu(II) ions into effluent.

***3.2.4. Barium chloride***

The one step precipitation using barium chloride showed highest removal of both color and phenols under highly alkaline conditions in the pH range of 9.0-12.0. The highest removal of 96% color, 90% phenols, 94% TSS and 52% COD was observed at pH 12.0 for the barium chloride dose of 3.0 $gL^{-1}$. However, the treatment using barium chloride failed to remove phenols to below disposal levels thus suggesting the need for further treatment.

The mechanism of removal of coloring matter and phenols is well documented in literature [9, 10, 28]. The color in paper mill effluent is caused by the presence of organic ligands such as lignins, synthetic dyes, tannins and their degradation products. These organic compounds have a local negative charge due to which they show characteristics of strong ligands. The addition of metal salts to effluents hydrolyses the metal ions which result in the formation of monomeric and polymeric metal hydroxide species. These species have large surface area and net positive charge. The positively charged metal hydroxide products adsorb the color causing organic ligands from effluent which eventually leads to removal of coloring matter through precipitation.

The effect of pH on precipitation can be explained as a balance of two competitive forces; (i) the competitive force between $H^+$ and metal hydrolysis products for organic ligands and (ii) the competitive force between hydroxide ions and organic ligands for metal hydrolysis products. In case of aluminum and iron salts, the high charge on their metal ions help to overshadow the competition from $H^+$ ions and push the effective pH range into acidic regime. However, this effect was weak in case of divalent metal salts of copper and barium due to the lower charge; hence these salts show higher precipitation rates in alkaline pH range.

The mechanism of removal of phenolic compounds from effluent is also established. The polar phenolic compounds under alkaline conditions tend to ionize into negatively charged phenolate ions. The negative phenolate ions combine with metal ions to form metal phenolates when metal salts are added to effluent [28]. The removal of phenols as metal phenolates is also pH and metal salt dose sensitive. The phenols removal is highest in all cases of metal ions at pH 12 due to high rate of formation of phenolate ions under these conditions.

It was also observed that the chemical precipitation treatments partially remove COD and induce residual metal ions into treated waters there by making the treatment incomplete requiring further treatment. Therefore, it was concluded that one-step chemical precipitation using metal salts was alone not efficient enough to effectively treat the effluent to disposal levels.

**3.3. Microbial treatment**

The experimental results showed that the removal of pollutants was influenced by all culture conditions. It was found that the maximum removal of pollutants occurred under the conditions of pH 7.0, glucose concentration of 0.5%(w/v), ammonium sulfate concentration of 0.5%(w/v), agitation rate of 180 rpm, temperature of 30$^{o}$C, biomass concentration of 5.0 $gL^{-1}$ and contact time of 48 hours. The microbial treatment performed under these optimized conditions showed the removal of 3% of color, 75% of phenols, 95% of TSS and 72% of COD from the raw effluent within 48 hours of incubation. It was observed that the treatment removed the color, phenols and

COD from the effluent only to a partial or incomplete level. This showed that bacterial treatment alone was incapable of removing the pollutants to levels as permitted by environmental agencies. Therefore, one-step bacterial treatment alone can not be recommended for treatment of the effluent.

It is interesting to note that some of the pollutants that were unremoved in chemical precipitation were removed effectively in the microbial treatment. This clearly indicates the possibility of effective removal of pollutants from effluent when the chemical precipitation is combined with microbial treatment. Keeping this in mind, a two-step sequential treatment approach by combining the chemical precipitation with microbial treatment was proposed and investigated.

### 3.4. Chemical-microbial sequence treatments

Four two-step sequential treatments namely $AlCl_3$+*P.putida*, $FeCl_3$+*P.putida*, $CuSO_4$+*P.putida* and $BaCl_2$+*P.putida* were attempted and investigated in the study.

#### *3.4.1. $AlCl_3$+P.putida*

In $AlCl_3$+*P.putida* sequential treatment, the effluent was first treated using 4.0 $gL^{-1}$dose of aluminum chloride at pH 5.0. The effluent obtained after treatment with aluminum chloride was subject to further treatment with bacteria *P. putida*. This two step treatment as a whole reduced the color, phenols, COD and TSS to 100%, 40%, 60% and 97% of their initial values in the effluent respectively. Though the color, TSS and Al(III) were reduced to below the safe disposal limits, the two-step treatment a whole was a failure as it failed to remove toxic phenols and COD to below dischargeable levels. The failure in treatment was due to inability of bacteria to survive the culture conditions.

#### *3.4.2. $FeCl_3$+P. putida*

In the sequential treatment $FeCl_3$**+ *P*.** *putida*, the effluent was first treated using 2.0 $gL^{-1}$ dose of ferric chloride at pH 4.0. This treatment was followed by microbial treatment with bacteria *P. putida*. The treatment with 2.0 $gL^{-1}$ ferric chloride at pH 4.0 has removed 99% of color, 42% of phenols, 53% of COD and 97% of TSS from the effluent and it imparted 30.0 $mgL^{-1}$ of residual Fe(III) ion concentration to treated water. The treatment using *P. putida* further reduced the pollutants to still lower concentrations. In the treatment with *P. putida*, Fe(III) ions concentration was reduced from 30.0 $mgL^{-1}$ to 1.0 $mgL^{-1}$.

The two-step sequential treatment as a whole reduced the color, phenols, COD and TSS to 99.2%, 99%, 94% and 98% of their initial values in effluent. These results showed that the pollutants including Fe(III)ion concentrations are reduced to below standard disposal levels after two-step treatment. The treatment as a whole was successful in removing pollutants from effluent.

### *3.4.3. $CuSO_4$+ P. putida*

In the two-step sequential treatment $CuSO_4$+ *P. putida*, the effluent was first treated using 2.5 $gL^{-1}$dose of copper sulfate at pH 10.0. This treatment was followed by microbial treatment with bacteria *P. putida*. The treatment using copper sulfate has removed 96% of color, 48% of phenols, 62% of COD and 95% of TSS from the effluent and it also imparted a residual Cu(II) ion concentration of 65 $mgL^{-1}$ to treated water. During treatment using *P. putida*, the organism survived the culture conditions and it further reduced the pollutants from effluent. In microbial treatment, Cu(II) ions concentration were reduced from 65 $mgL^{-1}$ to 2.3 $mgL^{-1}$.

At the end of two-step sequential treatment $CuSO_4$+*P. putida*, 98% of color, 97% of phenols, 95% of COD and 95% of TSS were removed from the effluent. These results showed that the pollutants including Cu(II)ion concentrations are reduced to below standard disposal levels after two-step treatment. Therefore, the treatment was a successful in reducing pollutants desired levels.

### *3.4.4. $BaCl_2$+ P. putida*

In the two-step sequential treatment $BaCl_2$**+ *P.*** *putida*, the effluent was first treated using 3.0 $gL^{-1}$dose of barium chloride at pH 12.0. The resulting effluent was further subject to microbial treatment with *P. putida*. In this treatment, the bacteria did not survive the treatment conditions resulting in incomplete removal of pollutants from effluent. The overall reduction of 97% of color, 91% of phenols, 53% of COD and 94% of TSS was observed during this treatment. The treatment failed to remove phenols, COD and Ba(II) ions to below disposal levels.

Interestingly, in all the four treatments, the bacterium was effective in removing residual metal ions from effluent by biosorption. The experimental data for biosorption of Al(III), Fe(III), Cu(II) and Ba(II) ions on bacterial cell surface was modeled and analyzed using Langmuir and Freundlich adsorption models. In all the cases, the correlation coefficients ($R^2$) for experimental data clearly obeyed Langmuir adsorption model ($R^2$=0.9958).

### 3.5. Toxicity evaluation by seed germination tests

The seed germination tests were conducted to assess the toxicity levels and the suitability of treated effluent for safe disposal and for agricultural utilization. The assessment of toxicity and suitability of treated effluent from all the four sequential treatments was made using pea and bengal gram seeds. The seeds grown on drinking water were used as control. The growth characteristics, germination inhibition percent and seed vigor index of pea and bengal gram seeds were studied. It was observed that both pea and bengal gram seeds when grown on drinking water (control) showed a high percentage of germination, low percentage of seed germination inhibition and a high seed vigor index. The shoot and root growth were also high in control samples. On the other hand, the pea and bengal gram seeds grown on raw effluent showed low germination percentage, high growth inhibition and low seed vigor index than the seeds grown on control. The poor germination and growth characteristics of seeds on raw effluent were due to high toxicity and pollution load associated with the effluent.

The growth of seeds on sequentially treated effluents showed varying results. It was observed that the seeds grown on effluent samples from treatments $FeCl_3$+*P.putida* and $CuSO_4$+*P.putida* showed a better germination and growth characteristics with a high germination, low seed germination inhibition and a high seed vigor index comparable with those of control. The root and shoot growth was also comparable to those of control samples in both type of seeds. These results indicate that the sequential treatments $FeCl_3$+*P.putida* and $CuSO_4$+*P.putida* have reduced pollution load in effluent to low levels that are not toxic to plants. On the other hand, the seeds grown on effluent samples from treatments $AlCl_3$+*P.putida* and $BaCl_2$+*P.putida* showed poor germination and growth characteristics with a low germination percent, high seed germination inhibition and low seed vigor index than control. The poor germination and growth characteristics may be due to incomplete removal of toxic pollutants. The seed germination tests clearly endorse the results of sequential treatments. Therefore, it may be claimed that the sequential treatments $FeCl_3$+*P.putida* and $CuSO_4$+*P.putida* successfully remove the color and toxicity (phenolic) from the paper mill effluent and may be recommended for pilot scale operations to confirm its efficiency to treat effluents at large scale.

## 4. Conclusions and directions for future work

The results of investigations on various one-step precipitation treatments and also microbial treatment conclude that one-step treatments alone are incapable of removing pollutants to below disposal levels and therefore multi-step sequential treatments are inevitable for better polishing of effluent. On the basis of overall pollutant reductions achieved and also the results of seed germination tests, it may be concluded that the two-step sequential treatments $FeCl_3$+*P.putida* and $CuSO_4$+*P.putida* are more efficient and also cost effective than the two-step sequential treatments $AlCl_3$+*P.putida* and $BaCl_2$+*P.putida*. Therefore, the sequential treatments $FeCl_3$+*P.putida* and $CuSO_4$+*P.putida* may be adopted for tertiary treatment of paper mill effluent to achieve targeted removal of color and toxic phenols.

From the results of the study, it may be concluded that the present study is a right step in the direction of addressing the problem of color and toxic phenols from paper mill effluent and also the results of study adds to the knowledge of tertiary treatment techniques. The present study also calls for more sequential treatment studies employing various other combinations apart from chemical precipitation and microbial treatments in near future to enrich the technologies of tertiary treatment.

## REFERENCES

[1] Pokhrel D., Viraraghavan T.," *Treatment of pulp and paper mill wastewater-a review*", Science of the Total Environment, vol.333, pp.37-58, 2004.

[2] Deilek F. B., Bese S, "*Treatment of pulping effluents using alum and clay-color removal and sludge characteristics*", Water SA, vol.27, No.3, pp.361-366, 2001.

[3] Lacorte S., Lattorre A., Barcelo D., Rigol A., Malmqvist A., Welander T., "*Organic compounds in paper mill process waters and effluents*", Trends in Analytical Chemistry, vol.22, No.10, pp.725-737, 2003.

[4] Singh A, Agrawal S. B., Rai J.P.N., Singh.P, "*Assessment of the pulp and paper mill effluent on growth, yield and nutrient quality of heat (Triticum aestivum L)*", Journal of Environmental Biology, vol.23, No.3, pp.283-288, 2002.

[5] Merilainen P, Oikari A., "*Exposure assessment of fishes to modern pulp and paper mill effluents after black liquor spil*", Environmental Monitor Assessment, vol.144, pp.419-435, 2008.

[6] Bajpai P., Bajpai P.K, "*Reduction of organochlorine compounds in bleach plant effluents*", Advances in Biochemical Engineering and Biotechnology, vol.57, pp.213-259, 1997.

[7] Nagarathnamma R., Bajpai P., Bajpai P.K., "*Studies on decolorization, degradation and detoxification of chlorinated lignin compounds in kraft bleaching effluents by Ceriporiopsis subvermispora*", Process Biochemistry, vol.34, pp.939-948. 1999.

[8] Annadurai G., Juang R., Lee D., "*Microbial degradation of phenol using mixed liquors of Pseudomonas putida and activated sludge*", Waste Management, vol.22, pp.703-710, 2002.

[9] Stephenson R.J, Duff S.J.B, "*Coagulation and precipitation of mechanical pulping effluents-I. Removal of carbon, color and turbidity*", Water Research, vol.30, No.4, pp.781-792, 1996a.

[10] Stephenson R.J, Duff S.J.B., "*Coagulation and precipitation of mechanical pulping effluents-II. Toxicity removal and metal salt recovery*", Water Research, vol.30, No.4, pp.793-798, 1996b.

[11] El-Bestawy E., El-Sokkary I., Hussein H., Abu-Keela A. F., " *Pollution control in pulp and paper industrial effluents using integrated chemical-biological sequences*" Journal of Industrial Microbiology and Biotechnology, doi: 10.1007/s10295-008-0453-3, 2008.

[12] Ugurlu M., Gurses A., Dogar C., Yalcin M.; "*The removal of lignin and phenol from paper mill effluents by electrocoagulation*", Journal of Environmental Management, vol.87, pp.420-428, 2008.

[13] Jain C. K., Kumar A., Izazy H.M, "*Color removal from paper mill effluent through adsorption technology*", Environmental Monitor Assessment, vol.149, pp.343, 2009.

[14] Hanafi F., Assobhei O., Mountadar M., "*Detoxification and discoloration of Moroccan olive mill wastewater by electrocoagulation*", Journal of Hazardous Materials., vol.174, No.1, pp.807, 2010.

[15] Chaudhari P.K., Majumdar B., Choudhary R., Yadav D.K, Chand S., "*Treatment of paper and pulp mill effluent by coagulation*", Environmental Technology, vol.31, No.4, pp.357-363, 2010.

[16] Charkraborty A., Deva D., Sharma A., Verma N., "*Adsorbents based on carbon microfibres and carbon nanofibres for the removal of phenol and lead from water*", Journal of Colloids and Interface Science, vol.359, No.1, pp.228, 2011.

[17] Ghaly M. Y., Jamil T. S., El-Seey I. E.., Souaya E. R.., Nasr R. A., "*Treatment of highly polluted paper mill wastewater by solar photocatalytic oxidation with synthesized nano* $TiO_2$," Chemical Engineering Journal, vol.168, No.1, pp.446, 2011.

[18] Kilic M., Apaydin-Varol E., Putu A. E., "*Adsorptive removal of phenol from aqueous solutions on activated carbon prepared from tobacco residues: Equilibrium, Kinetics and Thermodynamics*", Journal of Hazardous Materials, vol.189, No.1, pp.397, 2011.

[19] Nair I. C., Jayachandran K., Shashidhar S., " *Treatment of paper factory effluent using a phenol degrading Alcaligenes sp. under free and immobilized conditions*" Bioresource Technology, vol.98, pp.714-716, 2007.

[20] Ramchandra, Raj A., Yadav S., Patel D. K., "*Reduction of pollutants in pulp paper mill effluent treated by PCP degrading bacterial strains*", Environmental Monitor Assessment, vol.155, pp.1-11, 2009.

[21] Foelkel C., "*Advanced waste treatment*", Pulp and Paper, vol.63, No.4, pp.173–184. 1989.

[22] Deeb B.L., Altlhi A.D., "*Degradative plasmid and heavy metal resistance plasmid naturally coexist in phenol and cyanide assimilating bacteria*", American Journal of Biochemistry and Biotechnology, vol.5, No.2, pp.83-84, 2009.

[23] APHA "*Standard methods for examination of water and waste water*", $19^{th}$ Ed, American Public Health Association, Newyork, 1995.

[24] Rao J.V.S., Rama M., Rao K., Murthy S. S, "*Allopathic effect of some weed of crops on the germination and early seedling growth of bajra*", Tropical Ecology, vol.20, No.1, pp.5-8, 1979.

[25] Baki A.A., Anderson J.D., "*Vigor determination in soybean by multiple criteria*", Crop Science, vol.22, pp.630-633, 1973.

[26] Siddiqui A. H., Tabrez S., Ahmed M., "*Validation of plant based bioassays for the toxicity testing of Indian waters",* Environmental Monitoring and Assessment, vol.179, pp.241-253, 2011.

[27] Mitelut A.C., Popa E.P., *"Seed germination bioassay for toxicity evaluation of different composting biodegradable materials",* Romanian Biotechnological Letters, vol.16, No.1, pp.121-129, 2011.

[28] Lin S.H., Wang C. S.," *Treatment of high strength phenolic wastewater by a new two step method*", Journal of Hazardous Materials, B 90, pp.205-216, 2002.

## AUTHOR'S PUBLICATIONS

1] Kumara Swamy. N, Pratibha Singh, Indira P. Sarethy, *"Color and phenols removal from paper mill effluent by sequential treatment using ferric chloride and Pseudomonas putida",* International Journal of Pharma and Bioscience, vol.3, No.2, pp.380-392, 2012.

2] Kumara Swamy. N, Pratibha Singh, Indira P. Sarethy, "*Precipitation of phenols from paper industry wastewater using ferric chloride*", Rasayan Journal of Chemistry, vol.4, No.2, pp.452-456, 2011.

3] Kumara Swamy. N, Pratibha Singh, Indira P. Sarethy, "*Aerobic and anaerobic treatment of paper industry wastewater*"**,** Research in Environment and Life Sciences, vol.4, No.4, pp.141-148, 2011.

4] Kumara Swamy. N, Pratibha Singh, Indira P. Sarethy, *"Removal of phenols from paper industry wastewater using aluminum chloride",* (Manuscript communicated to Research Journal of Chemistry and Environment, 2011, Manuscript No-766).

5] Kumara Swamy. N, Pratibha Singh, "*Pulp and paper mill wastewater and Its treatment approaches*", A chapter in the book "**Recent Progress in Environmental Biology**", published by Oxford Book Co., New Delhi, Year 2011.

6] Kumara Swamy. N, Indira P. Sarethy, Pratibha Singh, **"***Aerobic and anaerobic treatment of paper industry wastewater*", Paper presented in National Conference on **Reducing Carbon Foot Prints and its Impact on Global Warming** held at Rajastan College of Engineering, Jaipur, during August 20-21$^{st}$ , 2010.

7] Kumara Swamy. N, Indira P. Sarethy, Pratibha Singh, "*Physicochemical characterization of paper mill effluent and its treatment using* $AlCl_3$ *and* $CaOCl_2$" Poster presented in National symposium on **Contemporary Environmental Problems and Biotechnological applications in their Management** held on March 7-8$^{th}$ , 2008, at School of Environmental Sciences, J.N.U, New-Delhi.

| Name and Signature of the Student | Name and Signature of Supervisor(s) |
|---|---|
| N. Kumara Swamy | Dr. Indira P. Sarethy |
| | Dr. Pratibha Singh |