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Indian
Journal of Advances in
Chemical Science

Indian Journal of Advances in Chemical Science S1 (2016) 217-220

A Two-step Reduction of Color and Phenols from Paper Industry Wastewater using Copper Sulfate and *Pseudomonas putida*

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Received 18th April 2016; Revised 2nd May 2016; Accepted 10th May 2016

ABSTRACT
In the present work, a two-step sequential method employing copper sulfate ($CuSO_4$) and Pseudomonas putida as treatment materials was investigated to remove color and phenols from paper industry wastewater. The wastewater was first subjected to precipitation treatment using $CuSO_4$, which removed 96% and 52% of initial color and phenols, respectively. However, this treatment induced 65 mgL^{-1} of residual Cu(II) concentrations in treated water. The water treated by $CuSO_4$ was later subjected to treatment with the bacteria, P. putida, which further reduced the color and phenols to below disposable levels. Interestingly, the latter treatment also reduced residual Cu(II) ions to below safe disposal level of 1.0 mg L^{-1}. The study concludes that the proposed two-step sequential treatment involving $CuSO_4$ and P. putida may be effectively used as an alternative tertiary treatment method for the remediation of color and toxic phenols from paper industry wastewater.

Key words: *Chemical precipitation, Adsorption, Sequential treatment, Paper industry wastewater.*

1. INTRODUCTION

The dark color in paper industry effluent caused by the organic ligands such as lignin, resins, synthetic dyes, and tannins is a major environmental concern as the discharge of dark colored untreated effluent to water bodies inhibits the photosynthetic activity of aquatic biota by reducing sunlight, besides exhibiting the toxic effects on biota [1,2]. The chlorinated phenols generated during pulp bleaching stages of paper production are a class of harmful pollutants found in paper industry effluents, which contribute considerably toward the toxicity of effluent [3,4]. The design of effective treatment methods to reduce the dark color and toxic phenolic compounds from effluent has been the subject of focus of researchers for the past few decades.

Many researchers have focused on the removal of color [1,4-9] while some studies have explored on the removal of phenolic compounds from effluent [3,10]. The lack of a single treatment technique that effectively removes both color and phenols from paper industry effluent makes it inevitable to look for a combination of two or more techniques. In the present study, we propose a two-step sequential treatment method employing copper sulfate ($CuSO_4$) and the bacteria. *Pseudomonas putida* to remove both color and phenols from paper industry effluent.

2. EXPERIMENTAL SECTION

2.1. Reagents and Effluent Samples

The chemicals used in the study were purchased from Sigma chemicals. The combined effluent of bleaching and pulping stages was collected from the local paper mill, and it was stored in refrigerator at 4°C until further investigation was carried out.

2.2. Organism and Culture Media

The organism *P. putida* (MTCC 1194) was purchased from IMTECH, Chandigarh, India. A modified mineral salt culture media was prepared by dissolving $KHPO_4$ (6.8 g/L) and $NaHPO_4$ (7.8 g/L) in paper mill effluent pre-treated with $CuSO_4$. A trace metal salt solution of 1.0 g/L was added to the above solution, and final pH of media was adjusted to 7.0. The media was later autoclaved, sterilized, and used for batch experiments in sequential treatment.

2.3. Physicochemical Characterization

The color and total phenol content were determined using to CPPA and 4-aminoantipyrene standard methods, respectively [11,12]. pH, chemical oxygen

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demand (COD), total suspended solids (TSS), total dissolved solids (TDS), and lignin were determined using standard APHA methods [12]. The copper (II) in water samples was analyzed using the instrument inductively coupled plasma.

2.4. Treatment with $CuSO_4$

The color removal tests were performed by adding $CuSO_4$ doses to effluent taken in glass-stoppered bottles. The contents in the bottles were mixed immediately by repeated inversion to ensure dissolution of $CuSO_4$, and the bottles were left unshaken for 24 h at room temperature to facilitate the formation and sedimentation of precipitate.

The effect of pH on removal color and phenols was investigated by adding 2 g/L of $CuSO_4$ into a set of glass stoppered bottles and setting pH in the pH range of 2-12. The samples were analyzed for color, total phenols, and residual Cu(II) ions after 24 h. The effect of $CuSO_4$ dose was studied by adding varying doses of $CuSO_4$ in the range of 1-4 g/L into a set of bottles. The effluent samples without $CuSO_4$ addition were used as controls.

2.5. Treatment with P. putida

For the batch experiments, 100 ml of the sterilized minimal salt media was inoculated with 5% (v/v) enriched inoculums optical density (OD value 0.5 at 620 nm) and incubated at 30°C in an incubator shaker at 180 rpm up to 3 days. During incubation, the growth of the culture was measured by monitoring OD at 620 nm at regular intervals. The culture media without bacterial inoculum was used as a control in these experiments.

3. RESULTS AND DISCUSSION

3.1. Wastewater Characteristics

The raw paper mill effluent sample was analyzed for its physicochemical characteristics. Some of the analyzed effluent parameters are shown in Table 1. The raw effluent was dark brown in color, and its pH was 7.18. The analysis of effluent showed the presence of high levels of color (6285 CU), TDS (1046 mg/L), TSS (3263 mg/L), total phenols (79.5 mg/L), and COD (4306 mg/L). Cu (II) was not detected in effluent. Among the estimated pollution parameters, color, TSS, total phenols, and COD were found above the maximum permitted limit of disposal prescribed by minimal national standards.

3.2. Treatment with $CuSO_4$

The chemical precipitation of coloring matter and phenols from paper mill effluent was brought about by mixing a known quantity of $CuSO_4$ to 100 mL raw effluent at a particular pH. The addition of $CuSO_4$ initially produced fine bluish gray precipitate which eventually formed larger aggregates and settled down as sludge. The supernatant obtained upon centrifugation was clear and showed reduced color. It was observed that the addition of $CuSO_4$ dropped the pH of effluent from 7.18 to 5.2. The readjustment of pH to its initial value of 7.18 showed an improved color reduction thereby indicating role of pH on chemical precipitation.

3.2.1. Effect of pH

The effect of pH on the removal of color and phenols by $CuSO_4$ was investigated by treating the 100 mL of effluent with a 2 g/L dose of $CuSO_4$ under different pH conditions. The results showed a strong dependence of color and phenols removal on pH (Figure 1). The $CuSO_4$ was found to decolorize the effluent effectively over a wide range of alkaline pH conditions from pH 7-12. The removal of color from effluent increased with increase in pH and reached a maximum value of 76% at pH 10 for $CuSO_4$ dose of 2 g/L.

The removal of phenols by $CuSO_4$ was found to be very effective above pH 10 (Figure 1). The experiments showed increased phenol removal from 30% at pH 10

Table 1: Physicochemical characteristics of paper mill effluent. Readings are average of triplets±SEM.

Parameter measured	Unit	Parameter value+SEM
pH	-	7.18±0.3
Color	Pt-CU	6285±62.3
TDS	mg/L	1046±11.3
TSS	mg/L	3263±23.5
Lignin	mg/L	4188±70.7
COD	mg/L	4306±72.5
Cu (II)	mg/L	BD±1.5*
Total phenols	mg/L	79.5±0.7

*BD=Below detection level (<1 mg/L), TSS=Total suspended solids, TDS=Total dissolved solids, COD=Chemical oxygen demand, SEM=Standard error of the mean

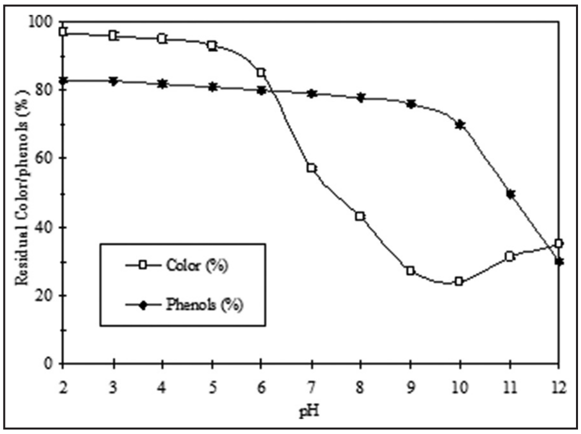

Figure 1: Effect of pH on the removal of color and phenols from paper mill effluent by copper sulfate ($CuSO_4$) (dose of $CuSO_4$ used=2 g/L).

to 70% at pH 12 for $CuSO_4$ dose of 2 g/L. From these experiments, it was concluded that the pH 10 was more effective for removal of color, whereas the pH 12 was effective for the removal of phenols from paper mill effluent. The controls showed no change in the level of color and phenols for all the pH values studied which indicate that the pH change alone is not enough for the removal of color and phenols.

3.2.2. Effect of dose rate

The dependence of decolorization on the dose of $CuSO_4$ was studied in the pH range of 6-12 by varying doses of $CuSO_4$. The results obtained are shown in Figure 2. The precipitation treatment showed improved decolorization with an increase in dose rate for all pH conditions studied. The color reduction of 48 % (pH 6.0), 60 % (pH 8.0), and 70 % (pH 12) was recorded for the $CuSO_4$ dose of 3 g/L, 2.5 g/L, and 2.5 g/L, respectively. However, the most effective decolorization of 96% was observed at pH 10 for the $CuSO_4$ dose of 2.5 g/L. Under same conditions, the removal of phenols was observed to be 48%.

The dependence of phenols removal on the dose of $CuSO_4$ was studied in the pH range of 10-12 by varying dose of $CuSO_4$. The results obtained are shown in Figure 3. The phenols reduction of 69% (pH 10), 85% (pH 11), and 94% (pH 12) was observed for the $CuSO_4$ dose of 4 g/L, 3.5 g/L, and 2.5 g/L, respectively. The highest phenol removal of 94% was observed at pH 12 for the $CuSO_4$ dose of 2.5 g/L.

At pH 10, for the $CuSO_4$ dose of 2.5 g/L, the treatment removed 96% of color, 52% of phenols, 95% of TSS, and 62% of COD from the effluent. However, the chemical precipitation resulted in huge sludge formation, and there was also residual 65 mg/L of Cu(II) concentration left in the treated water. The residual Cu(II) may cause toxicity of the treated water. Hence, the removal of residual Cu(II) concentrations becomes essential. This was achieved by subjecting the effluent treated with $CuSO_4$ to further treatment with the bacteria *P. putida*.

3.3. Treatment with *P. putida*

The treatment of $CuSO_4$ treated effluent with *P. putida* showed a further reduction in color, phenols, and Cu(II) ions and improved the characteristics of effluent. The removal of color, phenols, and Cu(II) from effluent at different incubation periods is shown in Figure 4.

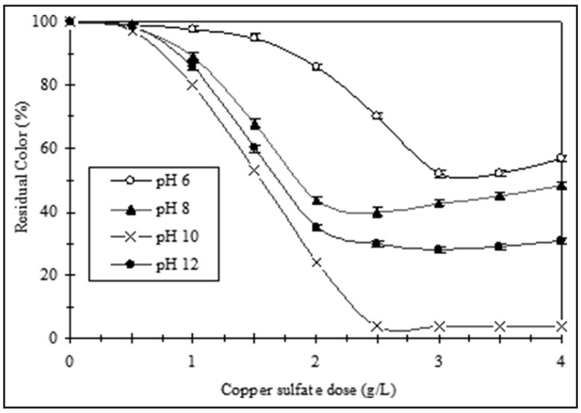

Figure 2: Effect of dose of copper sulfate on the removal of color from effluent under different pH conditions.

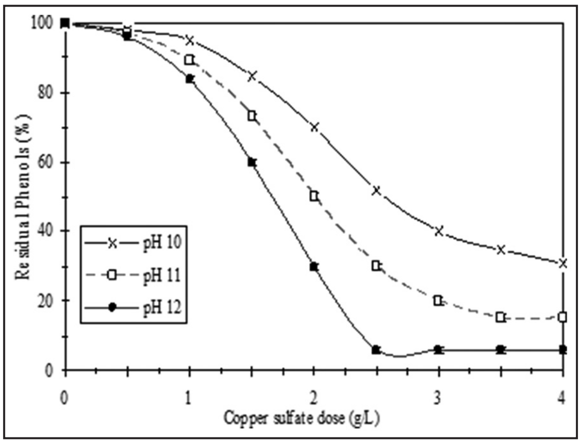

Figure 3: Effect of dose of copper sulfate on the removal of phenols from effluent at different pH conditions.

Table 2: Comparison of pollutant levels in effluent before and after sequential treatment. Readings are average of triplets±SEM.

Pollution parameter	Unit	Untreated effluent	After treatment		MPL**
			$CuSO_4$	*Pseudomonas putida*	
pH	-	7.18±0.3	10.0±0.29	6.7±0.31	6.5-8.5
Color	CU	6285±62.3	251±62.4	125±62.2	clear
TSS	mg/L	3263±23.5	163±23.6	146±23.3	100
COD	mg/L	4306±72.5	1636±72.1	215±72.4	250
Cu (II)	mg/L	BD±1.5*	65±1.6	2.3±1.3	5
Phenols	mg/L	79.5±0.7	38±0.68	2.4±1.71	1-5

*BD=Below detection level (<1 mg/L). **MPL=Maximum permitted level as per MINAS standards (India), TSS=Total suspended solids, COD=Chemical oxygen demand, MINAS=Minimal national standards, SEM=Standard error of the mean

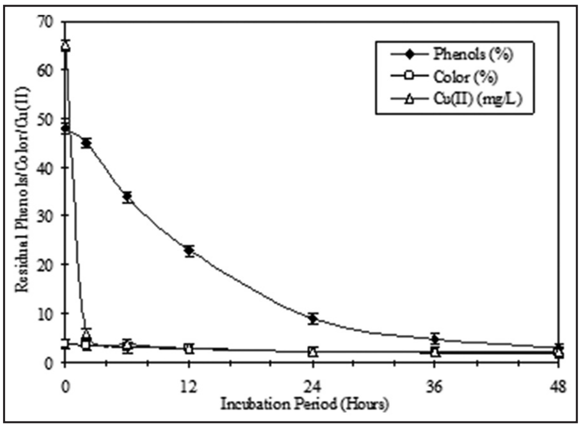

Figure 4: Removal of total phenols, color, and Cu(II) ions during treatment of copper sulfate pre-treated paper mill effluent with bacteria *Pseudomonas putida*.

The results show that the treatment with *P. putida* did not have any impact on the color of the effluent. However, phenols were reduced to a below disposable level of 2.4 mg/L (3%) in a span of 48 h of incubation. The treatment was also very effective in removing Cu(II) ions within a short period of time. The Cu(II) concentration was reduced to 2.3 mg/L (3.5%) in <2 h of incubation. The characteristics of the effluent before and after treatment with *P. putida* are shown in Table 2. The results show that the color and phenols were reduced to below disposal levels thus suggesting the effectiveness of the combined treatment with $CuSO_4$ and *P. putida*.

4. CONCLUSIONS

A study on two-step treatment using $CuSO_4$ and *P. putida* was carried out on paper mill effluent with an objective of removing color and phenols from effluent. The results of the study showed that it is possible to remove color and phenols from paper mill effluents to below discharge levels by adopting the proposed two-step sequential treatment. This sequential treatment is advantageous in the sense that it gets rid of residual metals from effluent which otherwise remain in treated water when $CuSO_4$ alone is used. The cost of copper is cheaper than that of commonly used precipitating agent aluminum chloride. The residual $CuSO_4$ after treatment may function as a good fungicide and a micronutrient for plants. Therefore, this method may be used as an effective alternative technique for the tertiary treatment of paper industry wastewater.

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***Bibliographical Sketch**

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