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COLOR AND PHENOLS REMOVAL FROM PAPER MILL EFFLUENT BY SEQUENTIAL TREATMENT USING FERRIC CHLORIDE AND *PSEUDOMONAS PUTIDA*

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ABSTRACT

A two-step sequential treatment method to remove color and phenols from paper mill effluent was investigated in the present study. First step in the treatment involved chemical precipitation of effluent using ferric chloride as a precipitating agent. The chemical precipitation using 2.0 gL^{-1} of ferric chloride at pH 4.0 removed 99% of color, 42% of phenols, 97% of total suspended solids (TSS) and 53% of chemical oxygen demand (COD) from the effluent. However, the treatment imparted a residual concentration of 30 mgL^{-1} of Fe(III) ions in treated water. In second step, the effluent treated with ferric chloride was subjected to further treatment using the bacteria *Pseudomonas putida*. *P. putida* adsorbed the residual Fe(III) ions to a below dischargeable level of 1.0 mgL^{-1} in two days of incubation. At the end of two-step sequential treatment 99.8% of color, 98.8% of phenols, 98% of TSS and 94% of COD were reduced from the effluent. The two-step sequential treatment effectively reduced the pollutants from paper mill effluent to the disposable levels. Further, the impact of sequentially treated effluents on germination of bengal gram seeds was also studied to understand the utility value of the treated effluents in agriculture. The bengal gram seeds grown on sequentially treated effluent showed better growth with high seed vigor index and low percentage of inhibition indicating the complete removal of toxicity from effluent during the treatment. The study concludes that the proposed sequential method may be effectively used as an alternative tertiary treatment method for remediation of paper mill effluent.

KEYWORDS

Paper mill effluent, Chemical precipitation, Sequential treatment, Bioaccumulation

INTRODUCTION

The paper mill effluent is characterized by dark color, foul odour, high organic content and extreme quantities of COD, BOD and pH [23]. The color in paper mill effluent is caused by the organic ligands such as wood extractives, resins, synthetic dyes, tannins, lignin and its degradation products [10, 17]. The dark color in effluent 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 [10, 19, 28]. The phenols generated during pulp bleaching stages of paper production are harmful pollutants that contribute significantly towards the toxicity of paper mill effluent [4, 17, 20]. The reduction of dark color and toxic phenols from effluent to levels below the discharge limits is important prior to disposal of effluent onto land or water bodies. The design of effective treatment methods to reduce the dark color and toxic phenols from effluent has been the subject of research for the past few decades.

Several physicochemical and electrolytic methods such as rapid sand filtration, membrane processes, electro-coagulation, ozonation, chemical precipitation and adsorption have also been attempted and their drawbacks have been reported in literature [5, 8, 10, 12, 13, 14, 15, 16, 30, 31, 32]. Among these methods, chemical precipitation is a technique that best removes the compounds which are polar, high in molecular weight and hydrophobic in nature. The chemical precipitation of coloring matter and its subsequent removal from paper mill effluents using lime and salts of iron and aluminum is already reported in literature [12, 30, 31]. However, the practical application of this technique is limited by the partial removal of COD and introduction of a high residual concentration of heavy metal ions in treated water leading to toxicity in water [31].

Several biological methods involving a variety of microorganisms such as white rot fungi, brown rot fungi, soft rot fungi and some bacteria have been employed by researchers to remove color and/or phenols from paper industry effluent [3, 6, 20, 21, 24, 26, 27]. The use of biological 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 [23, 31]. The inability of biological methods to treat highly concentrated and toxic effluents makes it inevitable to look beyond biological methods. Recently, the treatments combining two or more techniques have been increasingly explored to overcome the limitations of one technique by the strength of other.

In the proposed two-step sequential treatment, the effluent was first subjected to chemical precipitation using ferric chloride followed by the microbial treatment with *Pseudomonas putida*. For chemical precipitation, ferric chloride was selected as precipitating agent, as it is commonly favored precipitating agent and cheaper in terms of cost. *P. putida* was used in microbial treatment as it is known to degrade phenols and some of its strains are also known for uptake of heavy metals like Pb, Cu, Zn, etc [3, 11, 22]. The expected role of *P. putida* in sequential treatment involve the removal of residual Fe(III) ions as well as the degradation of residual phenols from effluent. Further, the impact of sequentially treated effluents on germination of seeds was examined to understand the utility value of the treated effluents for agriculture purpose.

The major objectives identified for this study were: a) To assess the effectiveness of two-step sequential treatment with ferric chloride and *P. putida* to remove color, phenols and the generated Fe(III) ions from the effluent, and b) To examine the utility value of the treated effluents for agriculture purposes by assessing the impact of sequentially treated effluent on the germination and growth of bengal gram seeds.

MATERIALS AND METHODS

***(i) Chemicals and instruments*:**

The analytical grade chemicals obtained from various furnishers were used in the experiments. The UV-Visible Spectrometer (Spectronic 20, Model: 336001, Spectronic Instruments) and Inductively Coupled Plasma (Model: JY2000, Horiba Jobin Yvon, France) were used for optical density and heavy metal concentration (Iron) measurements respectively.

The statistical analysis of the experimental data was performed using GraphPad InStat (Version 3.05) statistical software.

(ii) Effluent samples:

The combined effluent from bleaching and pulping stages was collected from local paper mill and it was stored in refrigerator at $4^{o}C$ until further investigation was carried out.

(iii) Organism:

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

(*iv) Culture media*:

A modified form of minimal salt media (MSM) was used in batch studies.

For the microbial treatment of raw effluent, the media was prepared by dissolving $KHPO_4$ (6.8 gL^{-1}) and $NaHPO_4$ (7.8 gL^{-1}) in the paper mill effluent. A trace salt stock solution of 1.0 mLL^{-1} was added to the above solution and final pH of media was adjusted to 7.0 using NaOH (2M) and phosphate buffer. The prepared media was autoclaved, cooled to room temperature and used in batch experiments. The composition of metal salts in 100 mL of trace salt stock solution was $CaCl_2.2H_2O$ (4.7g), $FeSO_4.7H_2O$ (0.37g), $CoCl_2.6H_2O$ (0.37g), $MnCl_2.4H_2O$ (0.1g) and $Na_2MoO_4.2H_2O$ (0.02g).

For the sequential treatment of chemically precipitated effluent, minimal salt media (MSM) was prepared using paper mill effluent pre-treated with 2.0 gL^{-1} of ferric chloride in place of raw effluent.

***(v) Physicochemical characterization*:**

The characteristics of water samples such as pH, COD, TSS, total dissolved solids (TDS) and lignin were determined using standard methods of APHA (American Public Health Association)[1].

(vi) Determination of color:

The color was determined according to CPPA standard method [20]. Before measurement, the pH of sample was adjusted to 7.6 by the addition of 2N NaOH and then it was centrifuged to remove suspended solids. The supernatant was used for the measurement of absorbance at 465 nm against distilled water. The platinum-color units (CU or Pt-CU) was calculated according to the equation

$$CU = 500 \times \left(\frac{A_2}{A_1} \right)$$

Where A_1 is absorbance of 500 CU platinum-cobalt standard solution (A_{465}=0.132) and A_2 is the absorbance of effluent sample.

(vii) Total phenol content:

The total phenol content was determined by antipyrene method [1]. For the measurement, 100 mL water sample was mixed with 2.5 mL of NH_4OH (0.5N) solution and its pH was adjusted to 7.9±0.1 using phosphate buffer. Subsequently, 1.0 mL of 4-aminoantipyrene and 1.0 mL of potassium ferric cyanide solutions were added to the sample mixture and left for 15 minutes. The absorbance of sample mixture was then measured at 500 nm and phenol content was determined using standard calibration curves.

(viii) Estimation of Fe (III):

The Fe(III) concentration in water samples was analyzed using the inductively coupled plasma (ICP). For calibration, a series of the standard solutions of Fe(III) ions in the range of 0.1-100 mgL^{-1} were used.

***(ix) Chemical precipitation with ferric chloride*:**

The preliminary chemical precipitation studies for color removal were performed by mixing 1.0 gL^{-1}, 2.0 gL^{-1} and 3.0 gL^{-1} of ferric chloride respectively into three separate glass stoppered bottles containing 100 mL raw effluent. The contents were mixed immediately by repeated inversion to ensure proper dissolution of ferric chloride and the bottles were left unshaken for 24 hours at room temperature to facilitate the formation of precipitate and its sedimentation. The ferric chloride dose of 2.0 gL^{-1} was selected for investigating the effect of pH as it produced the highest decolorization (50%) amongst the three doses.

For studying the effect of pH, 2.0 gL^{-1} of ferric chloride was thoroughly mixed into 100 mL of effluent in a set of glass stoppered bottles and their pH was adjusted to pH ranging from 2.0 to 12.0. The bottles were left unshaken for 24 hours to facilitate the completion of reaction and the samples were analyzed for color, total phenols and residual Fe(III) ions.

(x) Microbial treatment with P. putida:

The modified minimal salt media required for microbial treatment was prepared by dissolving media components in raw effluent. For the 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 sterilized solutions of glucose (0.05%) and ammonium sulfate (0.05%), and later inoculated with 5.0 gL^{-1} bacterial biomass. The culture media was later incubated at 30°C in an orbital shaker at 180 rpm up to 3 days. During incubation, the culture sample was removed under aseptic conditions and analyzed. The growth of the culture was determined by measuring optical density at 620 nm at suitable intervals during incubation. The color, total phenols, TSS, COD and Fe(III) were analyzed using standard analytical methods.

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

(xi) Sequential treatment:

In first step of sequential treatment, the raw effluent was treated with 2.0 gL^{-1} dose of ferric chloride at pH 4.0. In second step, the effluent treated with ferric chloride was subjected to microbial treatment with *P. putida*. The minimal salt media was prepared using paper mill effluent treated with 2.0 gL^{-1} ferric chloride in place of distilled water.

For second step, 100 mL of the above minimal salt media was taken in 250 mL Erlenmeyer flask and the contents were amended with sterilized solutions of glucose (0.05%) and ammonium sulfate (0.05%). The media was later inoculated with 5.0 gL^{-1} bacterial biomass. The culture media was later incubated at 30°C in an orbital shaker at 180 rpm up to 3 days. During incubation, the culture sample was removed under aseptic conditions and analyzed for color, total phenols, TSS, COD and Fe(III) content using standard analytical methods.

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

(xii) Seed germination test:

For seed germination experiments, bengal gram dry seeds were used. They were soaked overnight and placed on petri plate containing moist cotton. They were kept in an incubator at

28°C and appropriate quantity of test samples (treated and untreated effluent) were added separately 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 below [2, 25].

$$\text{Germination (\%)} = G = 100\left(\frac{N_g}{N}\right)$$

$$\text{Seed Germination Inhibition (\%)} = SGI = 100\left(\frac{G_c - G_t}{G_c}\right)$$

$$\text{Seed Vigor Index} = SVI = 100\left(\frac{N_g S}{N}\right)$$

Where N is the total number of seeds used (Average of three replicates), N_g is the total number of seeds germinated in 2 days (Average of three replicates), G_t is germination percent in test sample, G_c is germination percent in control and S is seedling length in mm.

The seed germination results obtained for sequentially treated effluent samples were compared with those of raw effluent sample and the control. The results from experiments conducted using drinking water (Bisaleri Water) were used as control.

RESULTS AND DISCUSSION

1. Physicochemical characterization of effluent:

The paper mill effluent characteristics were analyzed and the results of analysis are shown in Table-1.

Table-1
Physicochemical characteristics of paper mill wastewater.

Parameter Measured	Unit	Parameter value ±SEM
pH	__	7.18 ± 0.1
Color	Pt-CU	6285 ± 37
TDS	mgL^{-1}	1046 ± 23
TSS	mgL^{-1}	3263 ± 13
Lignin	mgL^{-1}	4188 ± 31
COD	mgL^{-1}	4306 ± 41
Fe(III)	mgL^{-1}	*BD
Total Phenols	mgL^{-1}	79.5 ± 0.3

* BD-Below detection level (<1 mgL^{-1})

The effluent used in the study was dark brown in color and its pH was 7.18. The analysis showed the high presence of color (6285 CU), TDS (1046 mgL^{-1}), TSS (3263 mgL^{-1}), total phenols (79.5 mgL^{-1}) and COD (4306 mgL^{-1}) in the effluent. Most of the estimated parameters in effluent exceed the maximum permitted limit (MPL) set for the disposal of effluent by Minimal National Standards (MINAS, India)(Table-2).

Table-2
Comparison of pollution parameters in effluent before and after treatment with ferric chloride and Pseudomonas putida. Readings are average of triplicates ±SEM

Pollution Parameter (Unit)	Raw Effluent	Pollutant concentrations achieved on treatment with			MPL**
		Ferric chloride	P. Putida	Seq. Treatment	
pH	7.18 ± 0.1	4 ± 0.2	7.0 ± 0.2	6.7 ± 0.1	6.5-8.5
Color (CU)	6285 ± 37	63± 43 (1%)	6096 ± 38(97%)	50 ± 32(0.8%)	clear
TSS (mgL^{-1})	3263 ± 13	98± 15 (3%)	163 ± 17 (5%)	65 ± 18 (2%)	100
COD(mgL^{-1})	4306 ± 41	2011± 45(47%)	1206± 51(28%)	264± 37(6%)	250
Fe(III) (mgL^{-1})	*BD	30±1.5	BD*	1± 1.2 (3.3%)	—
Total Phenols(mgL^{-1})	79.5 ± 0..3	46± 0.7(58%)	20± 0.6(25%)	1± 0.5(1.2%)	1-5.

- **BD-Below Detection Level (< 1.0 mgL^{-1}).**
- **** MPL-Maximum Permitted Level (Indian Standards)**

2. Chemical precipitation using ferric chloride:

The precipitation of coloring matter and phenols from paper mill effluent was brought about by mixing 2.0 gL^{-1} of ferric chloride to 100 mL of raw effluent separately under a pH in the range of 2.0 to 12.0. The addition of ferric chloride initially produced dark brown flock which eventually formed larger aggregates and settled down as sludge. The supernatant obtained upon centrifugation was clear and lighter in color. It was observed that the addition of ferric chloride reduced the pH of effluent to acidic values closer to pH 3.0. However, the pH was readjusted to its initial value to facilitate the effective precipitation. The pH readjustment showed an improved color reduction thereby indicating role of pH on chemical precipitation.

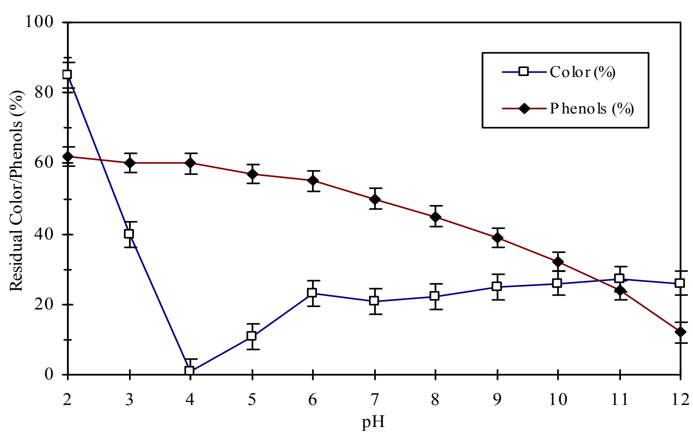

Figure-1
Effect of pH on color and phenols content of paper mill effluent during treatment with 2.0 gL^{-1} of ferric chloride.

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The experimental results for different pH conditions showed a strong dependence of color and phenols removal on pH (Fig-1). The removal of color by ferric chloride was found to be very effective under acidic conditions (pH 3.0-6.0) where as phenols removal was more effective in alkaline conditions (pH 10.0-12.0). The experiments showed an extremely significant reduction in color (p=0.0028) on treatment. The observed color reduction varied from 15% at pH 2.0 to 74% at pH 12.0 for the ferric chloride dose of 2.0 gL^{-1}. The highest color reduction of 99% occurred at pH 4.0. Under same dose conditions, the phenol removal also increased from 38% at pH 2.0 to a maximum of 88% at pH 12.0. The highest phenols removal of 88% was recorded with 2.0 $gL^{-1}$of ferric chloride at pH 12.0. In addition to removal of color and phenols, treatment at pH 4.0 also removed 97% of TSS, 53% of COD and imparted a residual Fe(III) ion concentration of 30 gL^{-1} to water. The residual Fe(III) ions may possibly cause toxicity in treated water and prevent its utilization effectively for agricultural purposes. Hence, the removal of residual Fe(III) ions and other residual pollutants was essential. This was achieved in the study by subjecting the effluent treated with ferric chloride to further sequential treatment.

In precipitation experiments, effluent samples without ferric chloride at different pH conditions were used as the control samples. The controls showed no change in the level of color or phenols for all the pH values studied indicating that the addition of ferric chloride was responsible for the removal of color and phenols from effluent.

3. *Microbial Treatment with Pseudomonas putida:*

For microbial treatment *Pseudomonas putida* was selected taking into consideration its reported ability to treat phenolic waters and uptake heavy metals [3, 11, 22].

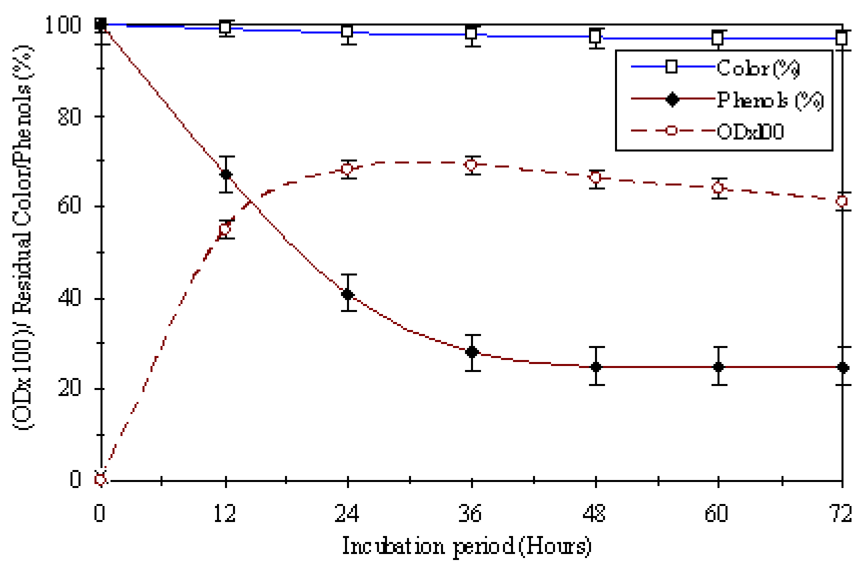

Figure-2

Plot showing the changes in optical density (OD) during growth of organism and the percentage of removal of phenols and color during treatment of paper mill effluent with bacteria P. putida.

In microbial treatment, the organism was grown on modified MSM-raw effluent media amended with 0.05% glucose and 0.05% ammonium sulfate. The organism grew in 24 hours and it significantly (p<0.05) removed phenols from the effluent. The phenols and COD respectively reduced to 75% and 72% of their initial values in 3 days of incubation. However, there was no significant reduction in color of effluent ($p>0.1$). The growth and pollutant removal by organism is shown in Fig-3. The results show that the microbial treatment of raw effluent using *P. putida* alone was incapable of removing pollutants to below dischargeable levels.

4. Sequential Treatment using Fe(III) salt and P.putida:

In sequential treatment, the paper mill effluent treated with 2.0 gL^{-1} of ferric chloride was used for further treatment with *P. putida*. The culture media required for sequential treatment with *P. putida* was prepared by dissolving the constituents of MSM media in paper mill effluent treated using 2.0 gL^{-1} of ferric chloride. The growth of *P. putida* and its impact on color, phenols and Fe(III) concentrations during sequential treatment are shown in Fig-3 and Fig-4.

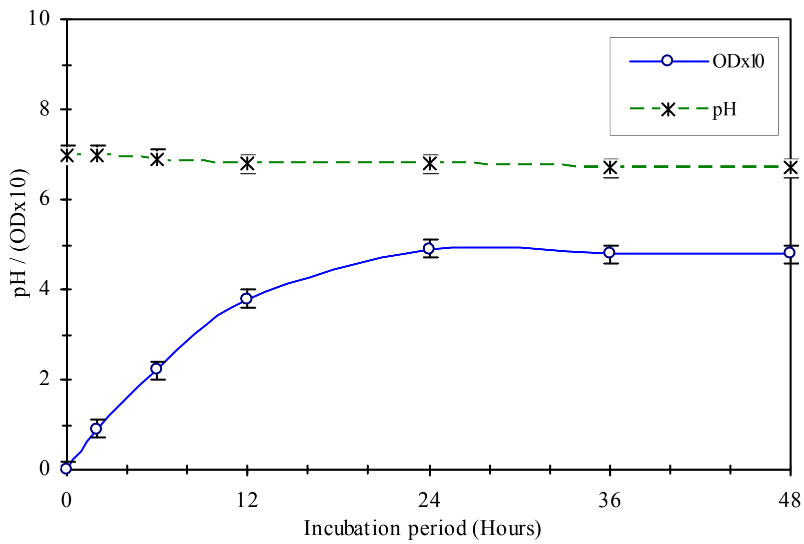

Figure-3
***Plot showing the changes in pH and optical density (OD) during growth of Pseudomonas putida on the modified MSM culture medium containing ferric chloride pretreated paper mill effluent*.**

The growth of *P. putida* reached a maximum in about 24 hours of incubation time. The maximum removal of pollutants from effluent also occurred during this period (Fig-4). The microbial treatment did not show any significant changes ($p=0.1$) in the color of effluent. However, the phenols and COD content in effluent were reduced to near disposable level of 1.0 mgL^{-1} (1.2%) and 264 mgL^{-1} (6%) within 48 hours of incubation.

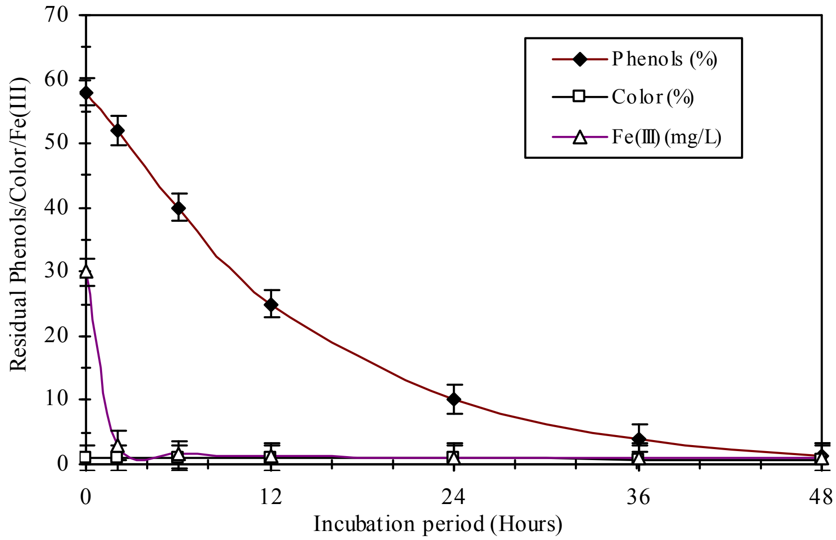

Figure-4

Removal of total phenols, color and Fe(III) ions during the sequential treatment of ferric chloride pretreated paper mill effluent with bacteria Pseudomonas putida.

The microbial treatment was very effective in removing residual Fe(III) ions from effluent. The concentration Fe(III) was reduced very significantly ($p<0.001$) from 30 mgL^{-1} to 1.0 mgL^{-1} (3.3%) within two hours of incubation. During microbial treatment, the change in pH was not considerable ($p>0.1$) and it slightly changed from pH 7.0 to pH 6.7.

The comparison of pollutants removed in individual treatments and sequential treatment is given in Table-2. The tabulated results indicate that the removal of color, phenols, TSS, COD and Fe(III) from effluent was only partial in individual one step treatments. Contrary to this, the color, phenols, TSS, COD and Fe(III) were reduced to below disposal levels from paper mill effluent in two-step treatment which suggests the effectiveness of two-step treatment.

5. Seed germination tests:

The seed germination tests were conducted by growing bengal gram seeds on test and control samples for 6 days. The seeds grown on different water samples showed varying growth characteristics (Table-3 and Plate-1). The bengal gram seeds showed higher root and shoot growth, seed germination percent and seed vigor index in both control and test samples. There was no significant difference in the characteristics of bengal gram seeds grown on control and sequentially treated effluent ($p<0.05$). The seed vigor index for the seeds grown on sequentially treated samples was slightly higher than that of control. On the other hand, compared to control and sequentially treated effluent, the raw effluent showed highly significant inhibition percent and low seed vigor index indicating the presence of high toxicity in raw effluent ($p<0.001$). From the results of seed germination experiments, it may be concluded that the sequential treatment reduces toxicity level in effluent to a level that favors seed germination and plant growth.

Table-3

Observed seedling length (S±SEM), root length (R±SEM), germination percent, seed germination inhibition percent and seed vigor index of pea seeds in 6 days of growth on drinking water (DW), raw effluent (WW) and effluent samples sequentially treated using ferric chloride and Pseudomonas putida (B*).

Water sample fed to Pea seeds	Seedling Length S (mm)	Root Length R (mm)	Germination Percentage (%)	Seed Germination Inhibition (%)	Seed Vigor Index
Control (DW)	68 ± 14	80 ± 16	100	0	6800
Raw effluent (WW)	40 ± 11	50 ± 14	85	15	3400
Treated with (Fe+Putida)	70 ± 17	88 ± 13	100	0	7000

Plate-1

Photograph showing the growth of bengal gram seeds on raw effluent (WW), drinking water (DW) and sequentially treated wastewaters B*-($FeCl_3$+P.Putida), after 6 days.

6. Mechanism of pollutant removal:

The possible mechanism of removal of coloring organic matter and phenols during precipitation is discussed in literature [7, 18, 30]. Accordingly, in aqueous medium, the trivalent metal cations hydrolyses to form positively charged monomeric and polymeric species that have a very large surface area and they tend to adsorb onto surface of negatively charged coloring organic matter and forms insoluble precipitates which eventually settle down as sludge [7, 31].

$$xFe^{3+} + yH_2O \longrightarrow Fe_x(OH)_y^{(3x-y)+} + yH^+$$

The removal of phenols occurs via adsorption of phenolate ions by metal ions to form metal phenolates [18]:

$$X_nC_6H_{6-n}O-H + OH^- \longrightarrow (X_nC_6H_{6-n}O)^- + H_2O$$

$$3(X_nC_6H_{6-n}O)^- + Fe^{3+} \longrightarrow (X_nC_6H_{6-n}O)_3 - Fe\downarrow$$

During chemical precipitation, the phenols removal rate increased with increase in pH. This may be attributed to increased ionization of phenols into phenolate ions under highly alkaline conditions. The higher availability of phenolate ions at higher pH conditions increases the conversion of metal ions into metal phenolates [18].

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The majority of phenols found in effluent are chlorinated phenolic derivatives. The degradation and removal of these chlorinated derivatives by bacteria occurs by several mechanisms and distinct pathways that are dependent on degree of chlorination in phenols.

In case of lower chlorinated phenolic derivatives (less than 2 chlorines), the bacterial mono-oxygenases initially attack phenols and produce chlorocatechols as intermediates which subsequently undergo ring cleavage and dechlorination[29]. On the other hand, the polychlorinated phenols (2-5 chlorines) are first converted to chlorohydroquinones as initial intermediates and subsequent reactions remove chlorines from ring prior to cleavage of rings[29].

The removal of Fe(III) from effluent occurs either via biosorption or bioaccumulation in living bacterial cells. In biosorption, the bacterial cell wall is the first component that comes in contact with the metal ions. The metal ions then interact with the chemical components at the cell wall surface. The anionic functional groups such as carboxyl, hydroxyl, phosphates, amines located on peptidoglycan phospholipids, teichoic acids, teichuronic acids and lipopolysaccharides of cell wall surface attracts positively charged metal cations and bind to them to eventually get transferred on to cell wall surface. In bioaccumulation, the binding process is same as in biosorption. But, the bound metal cations subsequently get transported across the cell wall and cell membrane [9].

CONCLUSION

In the present study, the removal of color and phenols from paper mill effluent by chemical precipitation and microbial methods and their sequential combination was investigated.

In chemical precipitation method, the treatment of paper mill effluent was performed using ferric chloride as a precipitating agent. The chemical precipitation when performed alone, using 2.0 gL^{-1} of ferric chloride at pH 4, removed 99% of color, 42% of phenols content, 97% of total suspended solids (TSS) and 53% of chemical oxygen demand (COD) from effluent. The sludge formation, incomplete removal of phenols and COD from effluent, and induction of residual concentration of 30 gL^{-1} of Fe(III) ions to treated water were the major disadvantages of chemical precipitation by ferric chloride.

In microbial method, the treatment of paper mill effluent was performed using the bacteria *Pseudomonas putida*. Microbial treatment with *P. putida*, reduced 75% of phenols and 72% of COD from the effluent in 3 days of incubation. However, the treatment was ineffective in removing color from the effluent.

In two-step sequential method, the treatment of paper mill effluent was performed by combining chemical precipitation and microbial methods in a sequence. The paper mill effluent was first treated with 2.0 $gL^{-1}$of ferric chloride followed by a treatment with *P.putida*. The sequential treatment reduced 99.8% of color, 98.8% of phenols and 94% of COD were reduced from the effluent. Interestingly, the residual Fe(III) ions introduced to water during chemical precipitation were also removed from effluent possibly through sorption onto bacterial cell surface. The two-step sequential treatment, in comparison to individual one step treatments, was more efficient in reducing the pollutants from paper mill effluent and it reduced them to the below disposable levels set by minimal national standards (MINAS, India).

Further, the impact of sequentially treated effluents on germination of seeds was also studied to understand the utility value of treated effluents for agriculture purpose. The bengal gram seeds grown on sequentially treated effluent showed better growth with high seed vigor index and low percentage of inhibition than those grown on raw effluent. The seeds grown on sequentially treated water samples showed similar characteristics as control samples thereby indicating complete absence of toxicity in treated effluents. The seed germination experiments confirms that the proposed two-step sequential treatment reduces the toxicity level in effluent to a level that favors seed germination and plant growth, and the treated effluent may find utility value in agriculture. The study concludes that the two-step sequential treatment is simple, cost effective and may be used as an alternative method in treatment of paper mill effluent.

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