ELSEVIER

Chemical Engineering Journal 127 (2007) 59–70

Chemical Engineering Journal

www.elsevier.com/locate/cej

# Chromophores removal in pulp and paper mill effluent via hydrogenation-biological batch reactors

S.M. Ghoreishi [*], M.R. Haghighi

*Department of Chemical Engineering, Isfahan University of Technology, Isfahan 84154, Iran*

Received 18 July 2006; received in revised form 19 September 2006; accepted 23 September 2006

## Abstract

A two-phase experimental plan was designed in order to investigate a new technique to remove color from pulp mill effluents via chemical and biological reactions in series. In the first phase, the chemical characterization of chromophores using the spectra of infrared (IR), ultraviolet (UV) and nuclear magnetic resonance (NMR) analyses was carried out. The results of chemical analyses indicated that the color-causing materials are mainly unsaturated compounds, possibly conjugated double bonds on aromatic rings. Therefore, a reducing agent, sodium borohydride ($NaBH_4$) was chosen in order to change the structure of chromophores via hydrogenation reaction at ambient temperature and pressure as a means of color removal from the wastewater streams. The bench scale results of the first phase of this research demonstrated that the $NaBH_4$ reduction is a viable method for color elimination with no sludge produced. The batch kinetic study of $NaBH_4$ pretreatment indicated that 97% color decrease in 24 h followed first-order kinetics with respect to sodium borohydride consumption and the reaction rate constant was evaluated at $0.6\,h^{-1}$. Subsequently in the second phase of the experiments, the pilot plant of an innovative chemical and biological reactor system was investigated. The plant consisted of two 20-l reactors operating in the batch mode for the overall residence time of 6 days. In the first reactor, chemical hydrogenation with $NaBH_4$ was performed for 1 day and resulted in a color and COD reduction by 97% and 35%, respectively, and increased BOD up to 85%. No significant change in TSS was observed. The chemically treated effluent was then subjected to a biological oxidation reaction in the second reactor for further treatment with a residence time of 5 days. The results indicated significant decrease in BOD (99%), COD (92%), and TSS (97%). Consequently, a combined chemical and biological reaction system appears to effectively decrease the color as well as BOD, COD and TSS, in contrast to the conventional techniques such as aerated lagoon and activated sludge systems in which color reduction is not observed. The economics of the pilot scale implementation of a $NaBH_4$ color reduction treatment process for 97% color removal was estimated to be in the range of 0.001 US dollar per liter of the most highly colored wastewater sample.

*Keywords:* Chemical characterization; Chromophores; Reduction reaction; Hydrogenation-biological batch reactors; Pulp effluent; Color removal

## 1. Introduction

Wood is the major raw material in pulp industry. Lignin, which is essentially an aromatic polymer, gives the wood structural strength, and therefore it must be removed from the cellulose fibers in order to produce a high quality paper. The Kraft process is presently the dominating pulping process because it accepts without any difficulty hardwood as well as softwood of any grade and species. Kraft pulping is performed with a solution composed of sodium hydroxide and sodium sulfite, named white or cooking liquor. As much as 90% of the lignin is removed from the cellulose fibers and at this cooking stage 15% of the total effluent is produced. Lignin in wood is light yellow or cream-colored, but because of its reactivity and tendency to form chromophoric configurations, it has been pinpointed as the major cause of color in the pulp. In fact, a significant part of the pulp processes deals with the color reactions of lignin and the removal of lignin chromophores.

Lignin reactions with the chemical species of the cooking liquor has been recognized as the major source of the color in the pulp mill wastewater. In order to understand the complexity of the chromophoric structure, it is imperative to realize that lignin is an aromatic polymer, which is formed in wood by an enzyme-initiated dehydrogenative polymerization of a mixture of three different 4-hydroxyarylpropenyl alcohols (the upper portion of Fig. 1 [1]). Adler [1] has suggested a prominent structure of lignin as shown in lower portion of Fig. 1; it contains only 16 monomeric units and does not show all of the known structural

---

[*] Corresponding author. Fax: +98 311 3912677.
*E-mail address:* Ghoreshi@cc.iut.ac.ir (S.M. Ghoreishi).

1385-8947/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.cej.2006.09.022

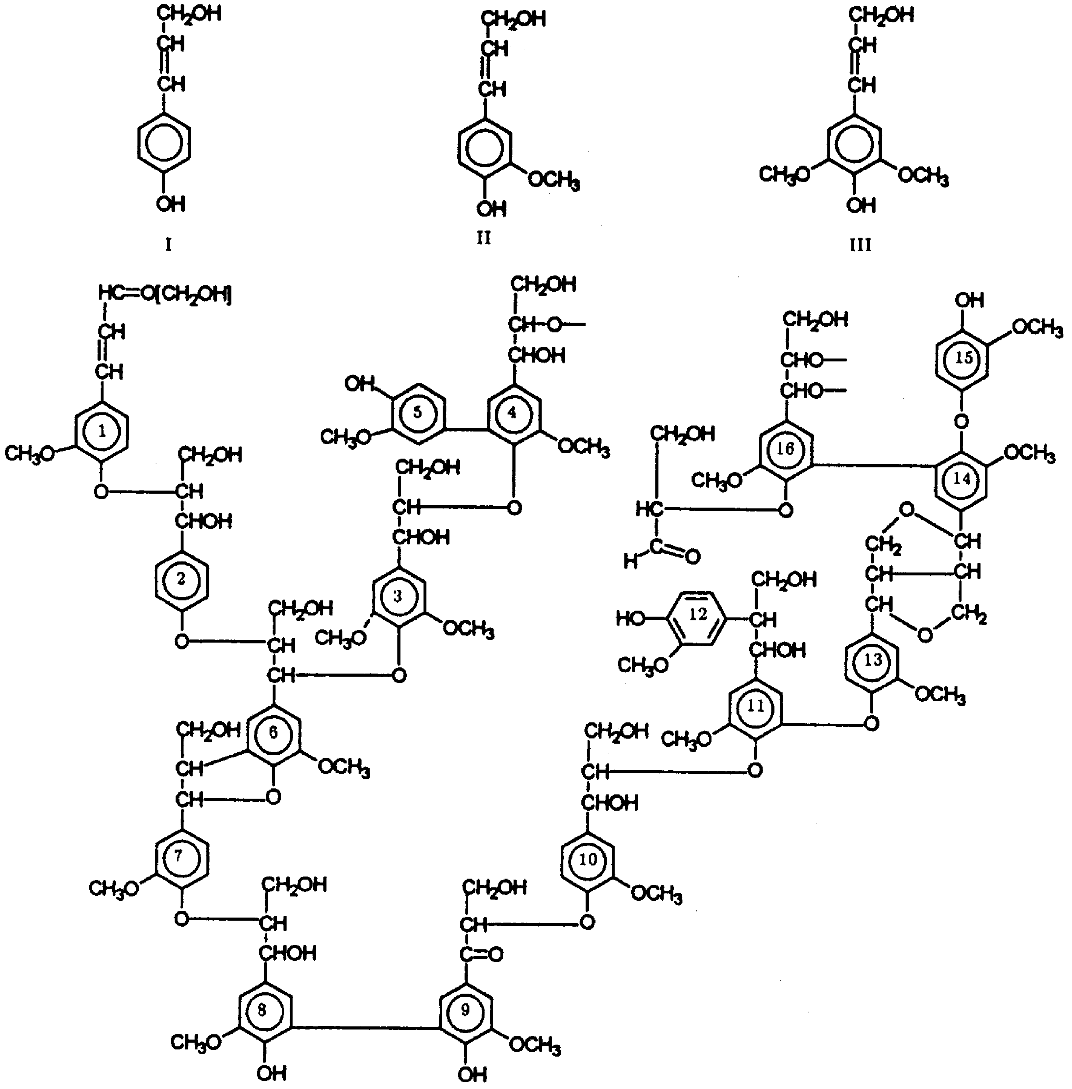


Fig. 1. Lignin precursors and prominent structures in softwood lignin.

details. It does show, however, that lignin is a branched molecule in which different types of bonds link the phenylpropane-based units. These include ether bonds of alkyl–aryl, alkyl–alkyl, and aryl–aryl configuration.

Dark color in discharged pulp mill effluents is regarded as a water pollution problem. Especially in cases where the receiving stream has a low or varying flow, discharges of highly colored wastewater can diminish the quality of the receiving stream. The yellowish-brown color of Kraft pulp has been the subject of some early investigations. In Ref. [2], Falkenhag et al. evaluated potential chromophoric systems in Kraft lignin. They suggested that the exact nature of the color-causing structures of Kraft lignin is uncertain, but the following chromophores may contribute to the color: (1) CH=CH and carbonyl groups conjugated with the aromatic rings, (2) quinone structures, (3) free radicals and (4) metal/organic complexes. They have also pointed out that the two latter structures are likely to contribute to the color of Kraft lignin only to a minor extent. In Ref. [3], Sjostrom reported some chromophoric structures that confirmed Falkenhag's proposed chromophores to some extent. He confirmed that the color of unbleached pulp is caused by certain unsaturated structures such as a CH=CH and carbonyl groups conjugated with aromatic rings. A literature review indicates that aerated lagoon is still commonly used to treat the pulp mill effluents. Even though a considerable reduction in BOD, COD, and TSS occurs, no effect on color is observed because color-causing materials are not biodegradable [4–10]. Therefore, it is necessary to find an additional treatment technique to solve the color problem.

Pulp and paper wastewater treatment systems have been the subject of many current research projects [11–35] in which different techniques including physical, chemical and biological methods have been applied with variety of shotcomings. Sierra-Alvarez and Lettinga [36] studied the methanogenic toxicity effects of lignin and lignin-related compounds in which low-molecular-weight lignin derivatives in pulp industry wastewater were identified as potential inhibitors of anaerobic treatment. Bryant and Sierka [37] investigated the use of an anaerobic/aerobic biotreatment sequence that resulted in a slight reduction of color. Springer and Hand [38] considered the use of photochemical and electrochemical precipitation techniques to decolor bleached Kraft mill effluent. Their results showed that the electrochemical technique is more promising than the photocatalytic approach. Traver [39] studied the removal of color from Kraft mill wastewater using an advanced hydrogen peroxide based technology, which did not cause a significant color reduction. Ferrey and Albouy [40] used free and immobilized lignin peroxidases and horseradish peroxidase to remove color from Kraft effluent. Lignin peroxidases were shown to have considerable potential for treating Kraft effluent. A screening study of lignin-degrading fungi for the removal of color from Kraft mill effluent was performed by Esposito and Dahlin [41]. They found the lentinus edodes strain is able to remove 73% of the color in 5 days without additional carbon sources. Osterberg and Wood [42] studied oxidative treatment of bleached Kraft mill effluent, which approximately reduces the color by 50%. The COD elimination by conventional biological treatment is limited even when the process is optimized [43]. Upgrading the biological treatment through multistep processes [44], using suspended or fixed biofilm growth surfaces [45] or by addition of powdered activated carbon [46] or lignite coke [47] often fails to meet the official requirements. Several researchers [48,49] have investigated the thermo-alkaline treatment method. This method has the advantage of being extremely simple, but the disadvantage is that no COD is removed. A bleachery effluent from pulp process was treated with oxygen and hydrogen peroxide and then with fungi, *T. veriscolor*, which did not cause any degradation of effluent lignin but increased the amount of chromophores [50]. A two-stage process that combined anaerobic treatment with chemical coagulation, in contrast to anaerobic treatment alone, produced a colorless and odorless effluent [51]. But the disposal problem remained as the major disadvantage of this technique. A two-stage sequential biological treatment, viz., the fungal process and a submerged downflow anaerobic reactor, was examined [52]. The treatment resulted in a 65% color reduction in 2 days. Effluent from a Kraft pulp mill process was studied by Mohammed and Smith [53] in a batch reactor using ozone doses. It was concluded that ozone is most effective for the removal of color (62%), however, increased BOD.

Boardman et al. [54] studied color reduction of Kraft pulp and paper wastewater by means of foam separation utilizing a cationic surfactant. The results indicated that as much as 90% color could be removed. Amero et al. [55] conducted a pilot trial that evaluated the performance of a new ozone oxidation process for the destruction of color in pulp and paper mill effluents. Ozone concentration of the feed was identified as a key variable affecting ozone decolorization efficiency. Color reductions of 79% and 71% were achieved in softwood and hardwood effluents. Springer et al. [56] used a bench-scale electrochemical cell in a study that investigated the technical feasibility of electrochemical treatment as a method of removing color from Kraft effluent. Electrochemical treatment reduced effluent color by up to 90%. Haberl et al. [57] examined precipitation/flocculation, ozone oxidation and high-energy radiation as pretreatment processes for Kraft wastewater. The performance of biological treatment subsequent to these processes was documented. A combination of lime precipitation and biological treatment produced 70–85% removal of color. Boyden et al. [58] investigated the treatment of bleachery effluents from Kraft mills in which the inability to reduce color significantly via biological oxidation was concluded.

In general, several methods have been attempted for the removal of color from the pulp and paper mill effluents. These can be classified into physical, chemical and biological methods. Physical and chemical processes are quite expensive and remove high molecular weight chlorinated lignins, color, toxicants, suspended solids and chemical oxygen demand. But BOD and low molecular weight compound are not removed efficiently [59]. The advanced biological color removal process is particularly attractive since in addition to color and COD it also reduces BOD and low molecular weight chlorolignins [60,61]. Singh and Thakur [62] studied the sequential anaerobic and aerobic treatment in two steps bioreactor for removal of color in the pulp and paper mill effluent. Seventy percent color reduction was reported using anaerobic treatment in 15 days and subsequently the anaerobically treated effluent was separately applied in bioreactor in presence of fungal strain, *Paecilomyces* sp., and bacterial strain, *Microbrevis luteum*. Further color reduction resulted but change in pH of the effluent, increase in biomass of microorganisms, and long residence time in the two steps bioreactor were the shortcomings of this technique.

The main goal of this research was to find a viable treatment method to remove the color of Kraft wastewater streams. Thus a two-stage experiments were planned. In the first stage, characterization of the chemical structure of the chromophores was carried out. Using the information obtained from the chemical characterization analysis, a feasible advanced treatment method to reduce the color in situ without any disposal problem as well as BOD, COD and TSS was designed in the second stage. The innovative treatment is composed of reduction reaction in conjunction with biological oxidation.

## 2. Materials and methods

The wastewater used in this study was collected from the Chuka pulp and paper Kraft mill plant in northern Iran, which discharges its total effluent into the Caspian Sea. Two different kind of wastewaters, namely, untreated and biologically treated (aerated lagoon) samples were obtained from the Chuka plant for this study. The characterization task was carried out by IR, NMR and UV spectrometers.

For IR analysis, chromophoric materials were separated from wastewater through complete water evaporation. The potassium bromide (KBr, Fisher Scientific IR grade) was oven-dried to complete dryness and was then stored in a desiccator until use. The chromophore-KBR mixture was prepared by accurately weighing the KBr and chromophore. Dry KBr powder (380 mg) and 20 mg chromophore were weighed to obtain a total mixture of 400 mg. All amounts were adjusted to within 0.2 mg of the desired weight and weighed to a precision of 0.1 mg. The mixture was quantitatively transferred to a mortar and ground with a pestle for 20–30 min to obtain a homogenous mixture. The amount of chromophore-KBr mixture needed for one pellet (400 mg) was placed between two highly polished, stainless steel dies inside a pellet press, where it was compressed at 25,000 psi for 1 min to make a small pellet approximately 0.5 in. in diameter. Prepared pellets were stored in a desiccator to prevent the KBr from absorbing moisture. A pellet of the same mass using pure KBr was similarly prepared for obtaining background comparisons. Analyses were made for both the blank and chromophore-KBr pellets using a Shimadzu-435 model spectrometer. Prior to analysis, the instrument sample compartment was purged with nitrogen for at least 20 min.

For NMR analysis, first chromophoric materials were oven-dried to complete dryness at 100 °C and then dissolved in $D_2O$-DMSOD$_6$ (duterium oxide-dimethylsulfoxide) solvent. The NMR spectra were obtained using $H^1$ NMR Varian-EN390 (90 MHz). In order to obtain UV spectra, first color-causing organic compounds were dissolved in water and then the prepared solution was analysed by an UV-Shimadzu-240 model analyser.

The biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total suspended solids (TSS) were determined according to the American Public Health Association Standard Methods for Water and Wastewater Examination [63] methods no. 5210-B, 5220-D, and 2540-D, respectively. Glass fiber filter paper (Whatman 934-AH) was used for TSS measurement. US-EPA approved method no. 110.1 (colorimetric), which is the only applicable technique for the highly colored industrial wastewater, was used to measure the color. To obtain data reproducibility and accuracy, all experiments were triplicated with 95% precision.

The bench scale treatment system of color reduction was carried out in a 1-l continuous stirred batch reactor (CSBR). The operating conditions of CSBR were at ambient temperature (20–25 °C) and pressure (1 atm). Sodium borohydride ($NaBH_4$, Aldrich catalog no. 19807-2) was used as the reducing agent. For the color reduction study, the homogenous raw wastewater was transferred into five 1-l CSBR and, subsequently, different amounts of accurately weighed $NaBH_4$ (0.1, 0.2, 0.3, 0.4, 0.5 g) were added into the five reactors. One of the CSBR's was used for a kinetics study of chromophore reduction. The 10 ml treated samples were collected periodically during 24 h following the start of the reduction reaction. The true color of collected samples was measured after pH adjustment to 7.8 using sodium hydroxide (NaOH, Aldrich catalog no.31951-1) and hydrochloric acid (HCl, Aldrich catalog no. 33925-3).

The new combined chemical—biological treatment system in pilot scale was carried out in two 20-l continuous stirred batch reactor (CSBR) at ambient temperature and pressure. In order to carry out the chemical reaction in the first reactor, 20-l of homogeneous raw wastewater was transferred into the first CSBR and then 4.0 g of $NaBH_4$ were added and the reaction was completed after 24 h. Subsequently, 20-l of chemically treated wastewater were subjected to biological treatment for a period of 5 days in the second CSBR. Also to evaluate the effectiveness of the chemical–biological technique, the two conventional treatment systems of aerated lagoon and activated sludge were simulated in two different CSBR for the duration of 15 and 5 days, respectively. All operating conditions for each of the reactors, where applicable, such as pH, mixing, food/microorganisms, amount of reducing agent, solid retention time, hydraulic retention time, were monitored throughout the experiments.

Moreover, in order to analyse the efficiency of color removal technique utilizing sodium borohydride and its comparison with hydrogenation reaction using pure hydrogen, a stainless steel reactor at elevated temperature (25–275 °C) and pressure (200–1400 psig) was used to change the structure of color causing compounds.

## 3. Results and discussion

The results obtained in this research are classified into two main sections: (A) chemical characterization of lignin chromophores and batch kinetics study of color reduction and (B) a new combined chemical–biological treatment system for pulp mill wastewater at pilot plant scale. Based on the information derived from the experimental results of section (A), a bench scale color reduction technique and subsequently a new treatment method for pulp effluent at pilot scale was achieved.

### *3.1. Chemical characterization of lignin chromophores*

The analyses to determine the chemical structures of the chromophores at best, or the chromophoric substitutions and functional groups at least, included a combination of several instrumental methods along with reduction reaction using sodium borohydride as the reducing agent. The instruments utilized included the IR, UV and NMR. The identification of the color-causing material in the effluents initially involved three possibilities: (1) color caused by CH=CH and other groups conjugated with aromatic rings, (2) color resulting from the presence of free radicals and (3) color-causing metal organic complexes. The eventual treatment to remove the chromophores depends on which possibility, or combination thereof, is chiefly responsible for causing color.

The possibility most easily confirmed or denied was no. 1. If the color was due to unsaturation in the organic molecules, a vigorous hydrogenation should completely eliminate the color. When a sample of the colored stream was subjected to a reduction reaction using $NaBH_4$ at an ambient temperature and pressure, the resulting reaction mixture retained no apparent residual color. This result was anticipated and entirely consistent with much of the published research. The remainder of this part of the project concerned itself with identifying the chemical functional groups of the chromophores. This process involved the utilization of three types of spectra analyses.

#### *3.1.1. Infrared spectroscopy*

Infrared spectroscopy analysis of the original and hydrogenated wastewater samples are shown in Figs. 2 and 3. In order to find out if biological oxidation has any significant effect on color, two wastewater samples, before and after aerated lagoons, were obtained and the infrared absorption bands of the two samples and their intensities were compared in Table 1. Possible assignments for the absorption bands are also listed in Table 1. Comparison of IR absorption bands of wastewater

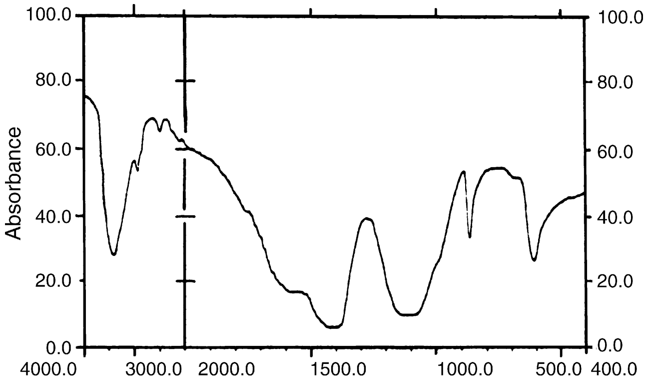


Fig. 2. IR spectrum of the aerated lagoon effluent.

chromophores, before and after biological treatment, shows that aeration is not effective in reducing color. IR absorption possible assignments indicate that the chemical functional groups of chromophoric configurations are unsaturated compounds such as phenolic, aromatic, carboxylic acid, acid anhydride, ketone, alkenyl and meta disubstituted aromatic. Figs. 2 and 3 show the results of infrared spectroscopic analysis of aerated lagoon effluent, before and after reduction reaction. The results indicate that only one absorption band (2900–3600 $\mathrm{cm^{-1}}$) is still present in the hydrogenated sample and the rest of the functional groups are eliminated. This broad peak is possibly due to presence of hydroxyl functional groups such as alcohols. Considering the fact that the original wastewater sample has 1500 color units (CU) and subsequent hydrogenation reaction using $NaBH_4$ as the reducing agent deceased the color to 50 CU, it can be concluded that the chromophores are due to unsaturated compounds possibly conjugated double bonds and carbonyl groups on aromatic rings. Furthermore, the comparison of TSS of aerated lagoon effluent (167 mg/l) and hydrogenated effluent (159 mg/l) indicates that $NaBH_4$ reduction treatment technique does not generate any excess TSS.

### 3.1.2. Nuclear magnetic resonance (NMR)

This section includes structural information, which was obtained about the make up of the chromophores as a result of the NMR application. Since this technique is sensitive to the presence of water or more specifically the hydrogen atoms in water, the dried colored material was redissovled in deuterium oxide-dimethylsulfoxide. Reduction reaction using $NaBH_4$ was also carried out in this medium for the NMR analysis. The resulting spectra can be seen in Figs. 4 and 5. The possible assignments of NMR signals of wastewater chromophores before and after biological oxidation and hydrogenated samples are presented in Table 2. Comparison of NMR chemical shifts of wastewa-

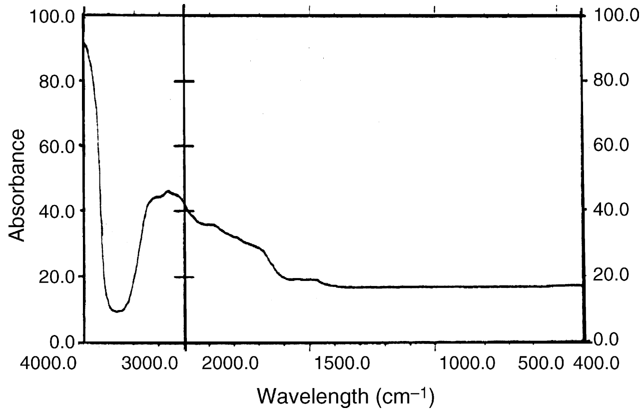
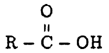
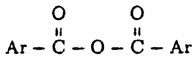
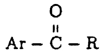


Fig. 3. IR spectrum of the aerated lagoon effluent after hydrogenation.

Table 1

IR absorptions assignments of wastewater chromophores before and after biological treatment

| Band number | $S_1$ absorption ($\mathrm{cm^{-1}}$) | Intensity | $S_2$ absorption ($\mathrm{cm^{-1}}$) | Intensity |
|---|---|---|---|---|
| 1 | 3300–3600 | S | 3200–3700 | S |
| 2 | 2000–3100 | W | 2900–3100 | W |
| 3 | 2400–2600 | M | 2400–2500 | W |
| 4 | 1780–1820 | M | 1750–1800 | M |
| 5 | 1660–1740 | M | 1650–1730 | M |
| 6 | 1300–1500 | W | 1350–1500 | S |
| 7 | 900–1050 | M | 1000–1200 | S |
| 8 | 840–890 | H | 850–880 | M |
| 9 | 620–700 | W | 650–700 | W |
| 10 | None | – | 580–520 | M |

| Absorption band number | Possible assignments | Functional group |
|---|---|---|
| 1, 6, 7 | Phenol | Ar–OH |
| 2 | Aromatic | Ar–H |
| 3, 5 | Carboxylic acid |  $\mathrm{R-\overset{\overset{O}{\|}}{C}-OH}$ |
| 4 | Acid anhydride |  $\mathrm{Ar-\overset{\overset{O}{\|}}{C}-O-\overset{\overset{O}{\|}}{C}-Ar}$ |
| 5 | Ketone |  $\mathrm{Ar-\overset{\overset{O}{\|}}{C}-R}$ |
| 8 | Alkenyl | $R_2C{=}CH_2$ |
| 9, 10 | Meta disubstituted aromatic | R–Ar–R |

Abbreviations—$S_1$: aerated lagoon influent, $S_2$: aerated lagoon effluent, S: strong, M: medium, W: weak.

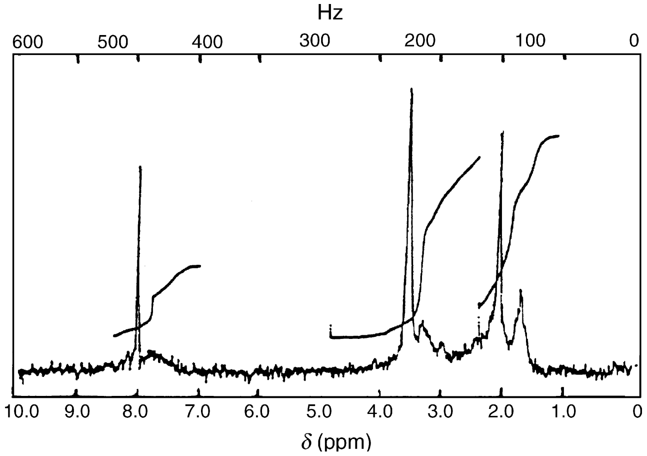


Fig. 4. NMR spectrum of the aerated lagoon effluent.

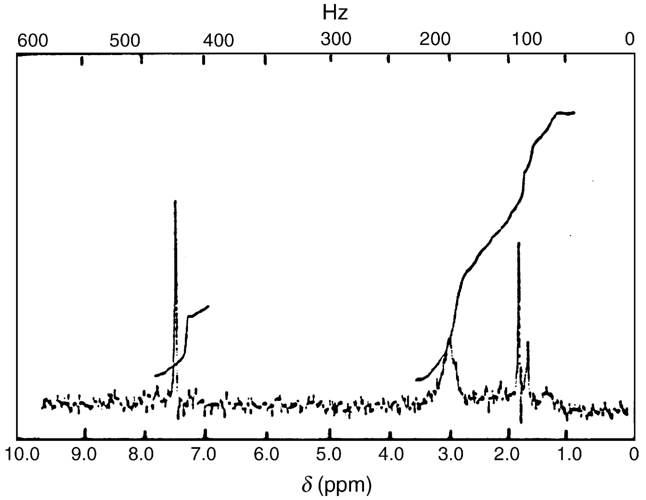
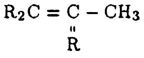
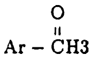


Fig. 5. NMR spectrum of the aerated lagoon effluent after hydrogenation.

ter chromophores, before and after biological oxidation, shows that aerobic treatment has not caused any significant changes in the functional groups in the two samples. This also confirms the results obtained by IR method. Moreover in Table 2, the chemical shifts of hydrogenated and unhydrogenated aerobic treatment effluent are compared and the results show that benzylic and phenolic functional groups are removed from the hydrogenated sample. It is important to realize that the NMR technique, similar to infrared analysis, indicates that the chromophoric materials are unsaturated compounds such as ketone, benzylic, phenolic and aromatics.

Table 2
The possible assignments of NMR signals of wastewaterchromophores before and after biological treatment and hydrogenated samples

| Signal number | $S_1$ signal, δ (ppm) chemical shifts | $S_2$ signal, δ (ppm) chemical shifts | $S_3$ signal, δ (ppm) chemical shifts |
|---|---|---|---|
| 1 | 1.6–2.0 | 1.7–2.0 | 1.7–2.0 |
| 2 | 2.0–2.4 | 1.9–2.2 | 2.0–2.4 |
| 3 | 2.3–2.7 | 2.3–2.6 | None |
| 4 | 3.6–3.9 | 2.7–3.1 | 3.0–3.7 |
| 5 | 4.0–4.7 | 3.2–4.5 | None |
| 6 | 8.6–9.0 | 8.0–8.5 | 8.1–8.4 |

| NMR signal number | Possible assignments | Functional group |
|---|---|---|
| 1 | Allylic |  $R_2C = \underset{R}{\overset{}{C}} - CH_3$ |
| 2 | Ketone | $Ar - C - R$ |
| 3 | Benzylic |  $Ar - \overset{O}{\overset{\Vert}{C}}H3$ |
| 4 | Alcohol | OH–$CH_2$ R |
| 5 | Phenolic | Ar–OH |
| 6 | Aromatic | Ar–H |

Abbreviations—$S_1$: aerated lagoon influent, $S_2$: aerated lagoon effluent, S3: hydrogenated aerated lagoon effluent.

Fig. 6. UV spectrum of the aerated lagoon effluent.

### 3.1.3. Ultraviolet spectroscopy

Lignin-derived chromophores not only absorb electromagnetic radiation in the infrared regions (wavelengths of 0.8–16 μm) but also in the ultraviolet (200–350 nm) and visible (350–800 nm) areas of the spectrum. The use of ultraviolet and visible absorption has been employed extensively in the study of lignin and lignin-related chemistry. Most success in terms of actually identifying organic structures has been the result of using model compounds and comparing fingerprints for similarity. A comparison was made in this study also using spectra available from the literature [2,64]. The UV spectra of the chromophoric material in the wastewater after aerobic treatment, and the hydrogenated samples are shown in Figs. 6 and 7. The results indicate a maximum absorbance in 259–263 nm, which falls within the aromatic band. Several multi-substituted aromatic structures can be concluded at the measured frequency such as *para* CHOARCl, ArCl and *ortho*, *meta* CHOArOH, CHOArOMe for the unhydrogenated sample. These substituent groups include the methoxy, hydroxy, chloro and the C=C groups. Regardless of whether there is mono-, di-, or tri-substitution, the hydroxy group has to be included because of observed great aqueous solubility

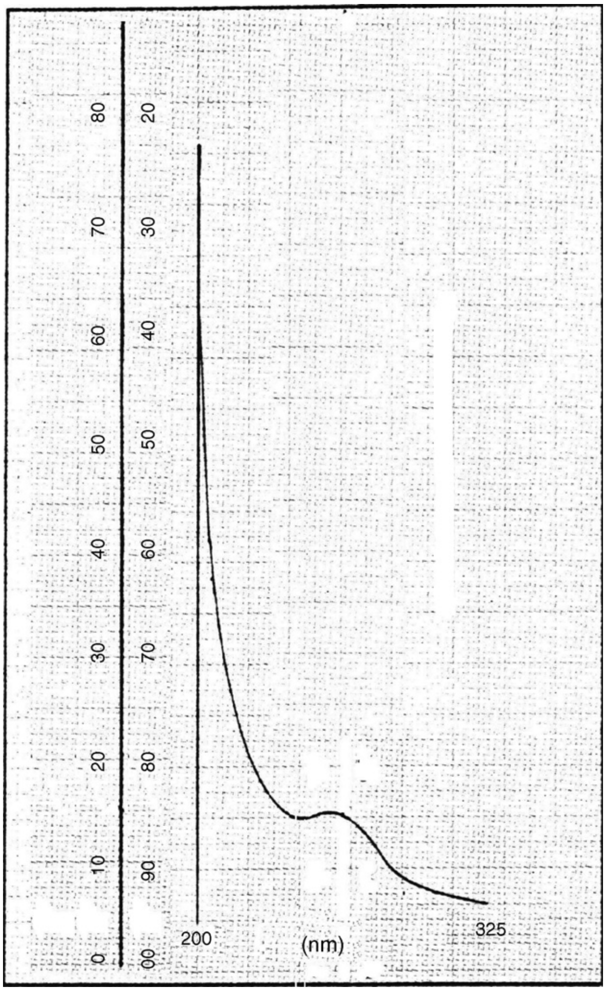


Fig. 7. UV spectrum of the aerated lagoon effluent after hydrogenation.

of the colored material. A chemical configuration, which utilizes many of the named substituent groups, which absorbs at the same UV frequency, is the stilbene structure (*cis* or *trans*-$C_6H_5CH{=}CHC_6H_5$). The stilbenes have already been shown to contribute to the chromophoric systems of Kraft lignin [3]. Comparison of Figs. 6 and 7 shows the significant absorbance reduction in the hydrogenated sample. This result may be contributed to the elimination of unsaturated functional groups due to the $NaBH_4$ reduction reaction.

### 3.2. Kinetics study of chromophore reduction

The inability to selectively reduce the carbonyl function of unsaturated compounds such as ketones, aldehydes, and acids in the presence of carbon–carbon double bonds, required the use of certain complex metal hydrides. The common commercially available salts include lithium aluminum hydride and sodium borohydride. Lithium aluminum hydride reacts violently with water and, therefore, reductions with this agent must be carried out in anhydrous ether. Sodium borohydride reductions, in contrast, can be carried out in water. Since the chromophores of Kraft pulp mill effluents are dissolved in water, $NaBH_4$ seems to be the proper reducing agent for this study. Moreover, the Sodium borohydride reaction can be conducted at an ambient temperature and pressure in contrast to hydrogenation reaction with pure hydrogen. Therefore, the metal hydride reduction using $NaBH_4$ is a feasible technique for color removal.

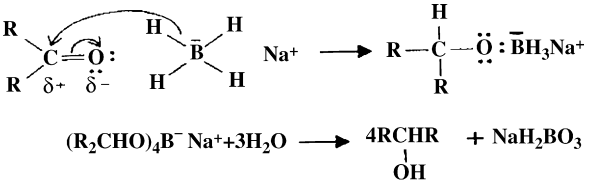


Fig. 8. The reduction mechanism of unsaturated compound by sodium borohydride.

The reduction mechanism of the chromophores by sodium borohydride is shown in Fig. 8. The key step in the mechanism for the reduction of unsaturated compounds present in the Kraft effluent, such as ketone, by $NaBH_4$ is the transfer of a hydride ion from the metal to the carbonyl carbon. In this transfer the hydride ion acts as a nucleophile. Since compounds of trivalent-boron are Lewis acids, they react as electrophiles at the carbonyl oxygen and facilitate the hydride transfer. A series of hydrogen ion transfer to the unsaturated carbonyl carbon results in the formation of a boron complex which, subsequently decomposes to generate some low-molecular weight biodegradable products such as the secondary alcohols and inorganic salts.

To determine the reaction rate of $NaBH_4$ reduction, 0.2 g of sodium borohydride was added to the 1-l wastewater CSBR. Periodically, a 10 ml sample was collected and after pH adjustment to 7.8, the true color was measured. The color reduction versus time data was obtained and different reaction orders were investigated. The test for the first-order reaction in terms of $NaBH_4$ consumption is shown in Fig. 9. Since the experimental data fall on a reasonably straight line with $R^2$ value of 0.98 for the least squares, it can be concluded that the rate of color removal follows the first-order with respect to sodium borohy-

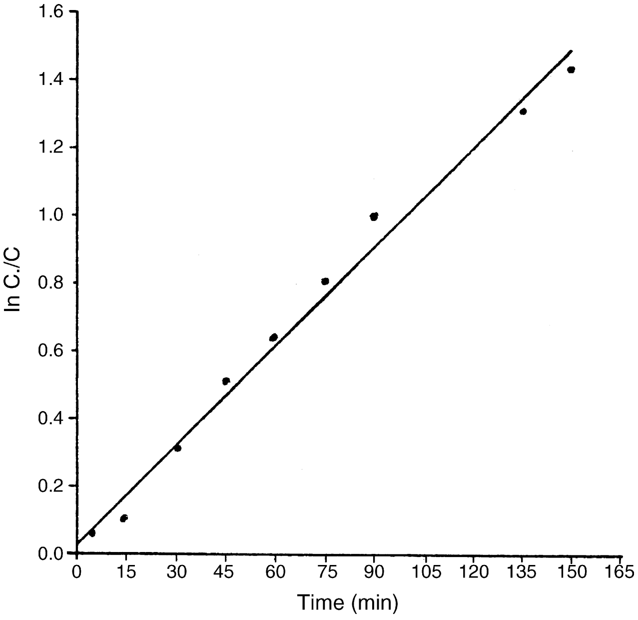


Fig. 9. Kinetics study of color reduction for first-order reaction.

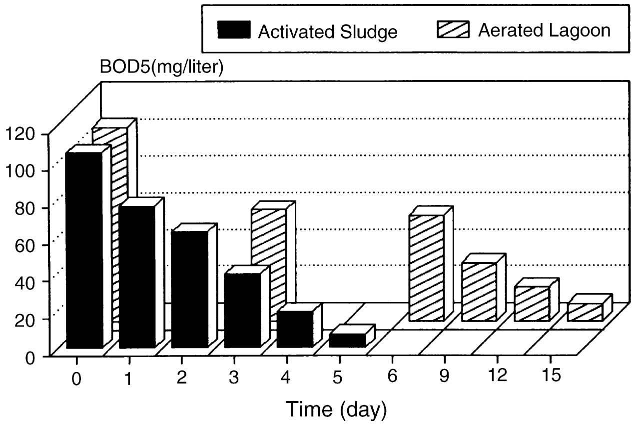


Fig. 10. Effect of aerated lagoon and activated sludge on BOD of Kraft mill effluent.

dride concentration. Applying the linear least square technique, the reaction rate constant (*k*) was evaluated as the slope of the obtained straight line to be 0.6 $h^{-1}$.

### 3.3. Conventional and proposed new treatment system for pulp mill wastewater

In this section the results obtained from the experimental simulation of two conventional and a new treatment system for pulp wastewater is presented.

#### 3.3.1. Aerated lagoon and activated sludge

The two widely used treatments in pulp industry were carried out in two different reactors for the purpose of evaluating their color removal effectiveness. The obtained results on BOD, COD, and color are shown in Figs. 10–12. Both methods reduced the BOD substantially and a moderate decrease in COD was observed; however, there was no change in color. This shows that the color-causing materials are mostly non-biodegradable. This shortcoming of the two conventional treatment techniques is mainly due to the complex nature of the chromophoric molecular structure, which makes the biodegradation ineffective for color removal in contrast to the BOD reduction.

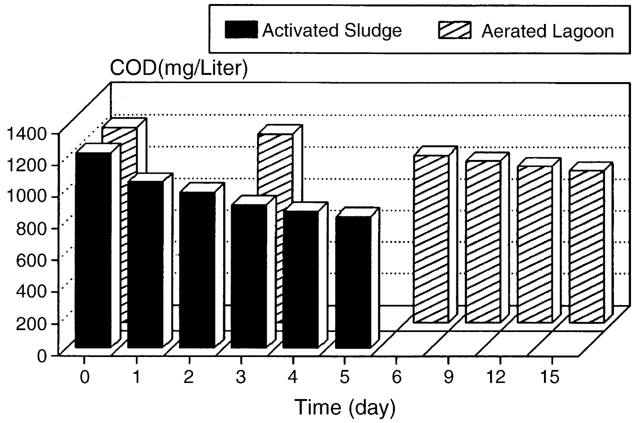


Fig. 11. Effect of aerated lagoon and activated sludge on COD of Kraft mill effluent.

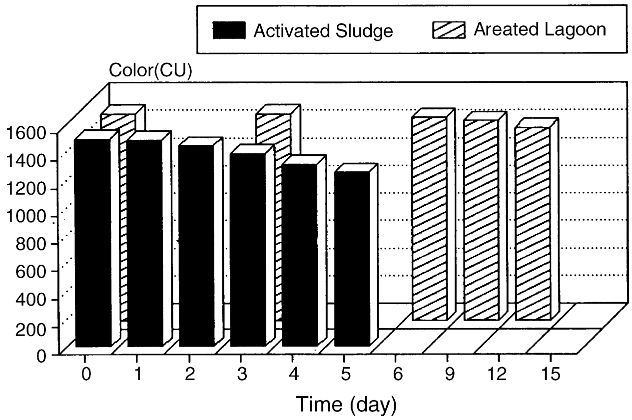


Fig. 12. Effect of aerated lagoon and activated sludge on color of Kraft mill effluent.

All operating conditions for both treatment systems, such as pH, temperature, mixing, and food/microorganism, were maintained constant, except the solid residence time (SRT) and aeration technique. The mechanical surface aeration and 15 days SRT were used for lagoon in contrast to the subsurface diffusion aeration and a SRT of 5 days in the activated sludge system. A more efficient aeration method for activated sludge process obviously increased the rate of oxidation reaction and subsequently decreased the required SRT. Also the original total suspended solids (TSS) of 167 mg/l was reduced by aerated lagoon and activated sludge systems up to 46% and 82%, respectively. In order to provide an optimum operating condition for living bacteria, the pH was adjusted to about 7.0.

#### 3.3.2. New combined chemical-activated sludge treatment system

The results obtained from the chemical analyses of Kraft pulp mill wastewater indicated that the color-causing materials are mainly some unsaturated organic compounds. Since the results of the aerated lagoon and the activated sludge experimental study indicated that these materials are naturally non-biodegradable, therefore changes in the chemical structure of chromophores via $NaBH_4$ reduction and hydrogenation reaction with pure hydrogen were investigated as an alternative technique to remove color without producing any sludge.

The chemical reduction reaction with $NaBH_4$ and hydrogenation reaction using pure hydrogen were carried out in two different reactors. The results of color elimination by both methods are shown in Table 3. The operating conditions of hydrogenation with pure hydrogen at pressure of 200 psig and room temperature reduced the color by 58%. To obtain a higher color removal, the selection of 500 psig and 136 °C resulted in 69% color disappearance. Finally, it was found that for almost complete color elimination (99%) a pressure of 1400 psig and temperature of 275 °C was required. The same level of color reduction (97%) was also obtained using $NaBH_4$ at ambient temperature and pressure. A comparison of the required operating conditions for the two chemical treatment techniques indicated that $NaBH_4$ reduction method is economically and technically more feasible and cost effective.

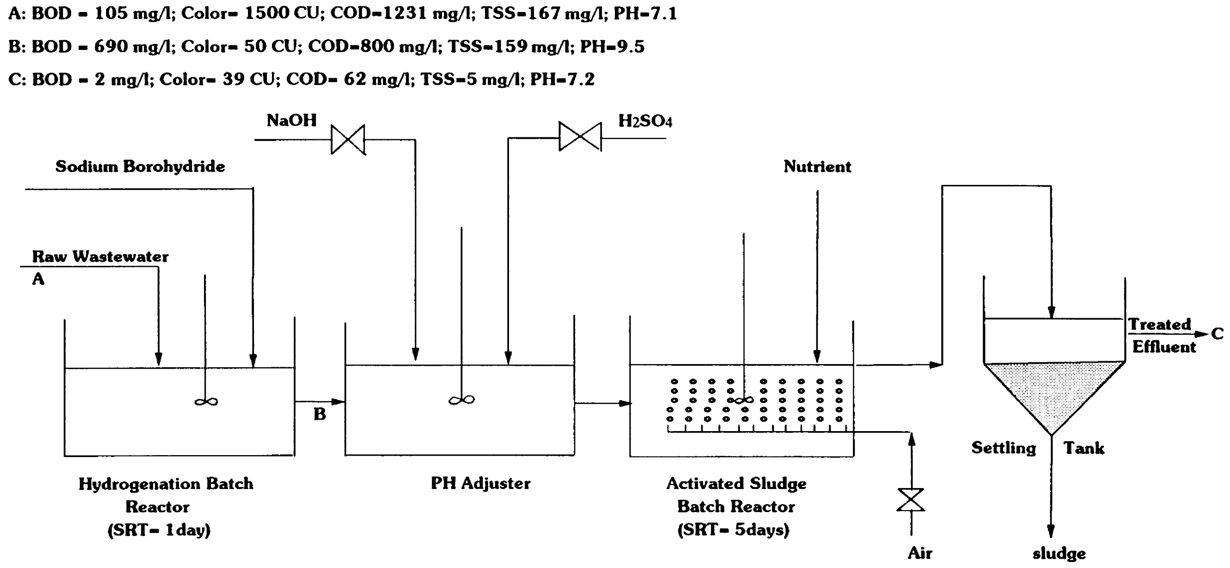


Fig. 13. Schematic diagram of new chemical-activated sludge treatment system for pulp mill wastewater.

Table 3
The results of selective hydrogenation with $NaBH_4$ and pure hydrogen

| Conditions | Color units (pH = 7.8) | Color reduction (%) |
|---|---|---|
| (1) Untreated sample | 1500 | 0 |
| (2) $NaBH_4$ reduction reaction (1 atm, 25 °C) | 45 | 97 |
| (3) Hydrogenation reaction | | |
| 200 psig, 25 °C | 630 | 58 |
| 500 psig, 136 °C | 465 | 69 |
| 1400 psig, 275 °C | 15 | 99 |

Fig. 13 shows the schematic diagram of the innovative combined chemical-activated sludge treatment process. This system is composed of two 20-l CSBR’s. The chemical reduction reaction with $NaBH_4$ was carried out in the first reactor. Subsequently after pH adjustment, the chemically treated effluent is subjected to activated sludge process in the second reactor. A major color reduction was achieved in the first reactor after 1-day retention time. Significant reductions in BOD (Fig. 14), COD (Fig. 15), and TSS were observed with a 5 days retention time in the second reactor. The effect of chemical reaction on color is shown in Fig. 16. For 1-day retention time, a 97% color reduction was observed. Another significant result of this color removal process is 85% increase in BOD in contrast to 35%

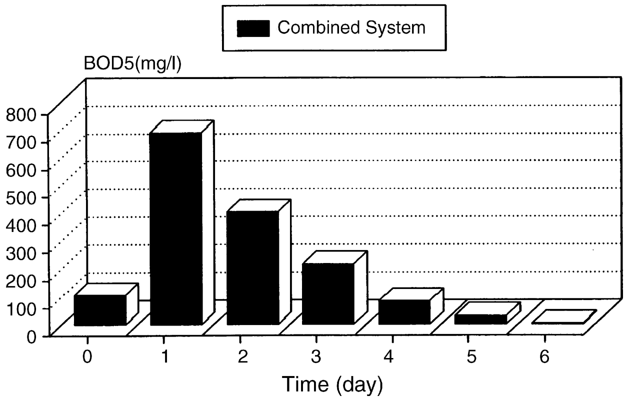


Fig. 14. Effect of combined chemical-activated sludge treatment system on BOD of pulp mill wastewater.

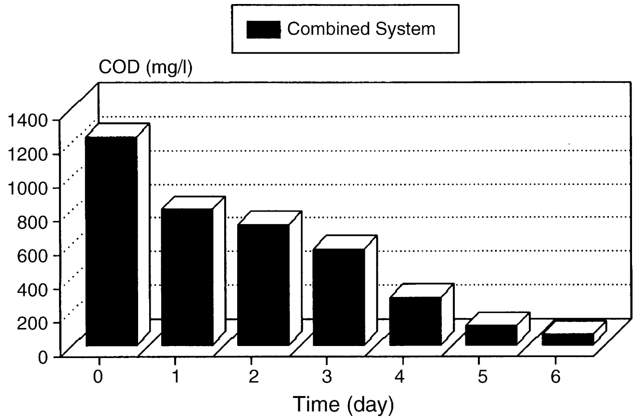


Fig. 15. Effect of combined chemical-activated sludge treatment system on COD of pulp mill wastewater.

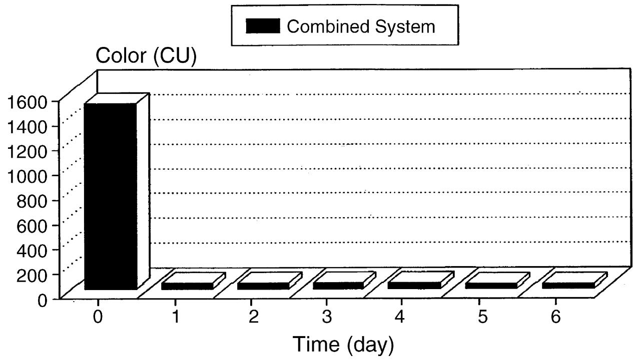


Fig. 16. Effect of combined chemical-activated sludge treatment system on color of pulp mill wastewater.

reduction of COD. This shows that some non-biodegradable chromophores are converted to biodegradable organic compounds such as secondary alcohols in the first reactor. The experimental TSS measurements in the first reactor indicate that the new proposed technique eliminates the environmental sludge disposal problem compared to the conventional methods, such as chemical precipitation and coagulation. Further treatment by the activated sludge process in the second reactor decreased BOD, COD, and TSS by 99%, 92%, and 97%, respectively. The experimental results of this study are reported within 5% error. The major difference between this newly developed treatment method with other conventional systems is the key step of converting non-biodegradable chromophores into biodegradable compounds via reduction reaction with sodium borohydride.

### 3.3.3. *Safety and environmental assessment of treated effluent via new technique*

The products of reduction reaction in the first reactor are some low-molecular weight biodegradable products such as the secondary alcohols and inorganic salts ($NaH_2BO_3$) which are biologically oxidized and decomposed in the second reactor. Thus the final discharged effluent into environment contains environmentally compatible products. But high concentration of boron can be considered as an environmental risk. Specifically in this regard, there is a concern over the amount of boron entering the ecosystem and its harmfulness to some plants. The highly colored wastewater streams comprising 15% of the total effluent flow in a Kraft pulp mill are responsible for 90% of the total effluent color, which can be treated separately with $NaBH_4$. After color reduction, dilution of the chemically treated wastewater with the remaining 85% effluent would finally result in about 3 ppm boron concentration in the discharged wastewater into the Caspian Sea. According to US-EPA irrigation standards [65], the maximum allowable boron concentration for resistive (grasses, alfalfa), semi-resistive and sensitive (citrus) plants are 10, 2.5 and 1.25 ppm, respectively. Therefore, the proposed new treatment system does not seem to create any significant water pollution problem in this regard. Another safety and environmental issue of using $NaBH_4$ is the possible air pollution. Sodium borohydride can be dangerous when it is heated to decomposition because it emits toxic fumes. But since the new treatment method is carried out at ambient temperature and pressure, therefore the proposed technique does not make any air pollution problem either. The third safety issue is that sodium borohydride is poison by ingestion. In this regard it is imperative to realize that since sodium borohydride is the limiting reactant in the chemical reaction, therefore, the conversion of the reducing agent in the reduction reaction is 100% and thus it is completely consumed and decomposed into environmental compatible products in the first batch reactor, which have no safety nor environmental risks.

### 3.3.4. *Economics*

The perspective of the capital and operating costs of the proposed new treatment method is a major concern for the pulp and paper industry. The total wastewater of a typical pulp mill plant is composed of streams with different flow rates and color intensities. In the pilot scale experiments of this study, the operating cost of $NaBH_4$ for reducing the color up to 97% in a 1-l of the most highly colored wastewater sample (1500 CU) was estimated to be 0.001 US dollar. To implement this technique in a large-scale environmental application with less cost, it is proposed to reduce the color of the highly colored wastewater streams (15% of the total effluent with color intensity of 1500 CU) with $NaBH_4$ up to 50%. Then subsequently dilute the chemically treated sample (color intensity of 750 CU) with the remaining 85% effluent (color intensity of 200 CU). The resulted dilution would naturally decrease the color further and then the total wastewater is subjected to biological oxidation in order to decrease the BOD and COD. This would finally result in a discharged total effluent with substantially reduced color intensity with 50% cost of the pilot scale experiment. Therefore, implementing the proposed technique at large-scale treatment plant, it is expected to have an extra operating cost of about $ 0.0005 per 1-l of total effluent for the reducing agent in order to remove the color almost completely. The other operating costs of the new technique are lower than those of conventional treatment methods. The main economic advantage of the proposed technique is to eliminate the sludge disposal problems caused by the conventional methods such as adsorption with activated carbon and chemical precipitation with different coagulants such as alum. The operating cost due to disposal problems and color removal efficiency of the developed method of this study was compared with three conventional treatment methods (biological oxidation, adsorption with activated carbon, coagulation with alum) as shown in Table 4. Not only does the new combined chemical–biological treatment system have higher color removal efficiency and capability, it also does not create any sludge disposal problems in contrast to the chemical precipitation with activated carbon and alum.

Table 4
Comparison of reduction–biological technique with other conventional treatment methods

| Treatment technique | Sludge disposal problem | Color removal |
|---|---|---|
| (1) Reduction–biological technique | Slight | High |
| (2) Biological oxidation | Medium | Low |
| (3) Coagulation with alum | Severe | Low–medium |
| (4) Adsorption with activated carbon | Severe | Medium–high |

## 4. Conclusions

The results of chemical analyses (IR, NMR, UV) indicate that the color-causing materials in the Kraft pulp mill effluents are due to unsaturated compounds, possibly conjugated double bonds and carbonyl groups on the aromatic ring. Based upon the functional group identification, obtained from the chemical analyses, an advanced treatment technique for color removal at bench-scale was developed and tested. The results show that a reduction reaction using sodium borohydride as the reducing agent is a viable method for almost complete color elimination without any potential disposal problems. The batch study of $NaBH_4$ reduction indicated that the color decrease followed first-order kinetics with respect to sodium borohydride consumption with the reaction rate constant of $0.6\,h^{-1}$. Therefore, subsequently a new method, combined chemical–biological treatment system, was investigated at pilot scale. This technique seemed to effectively decrease the color as well as BOD, COD and TSS in contrast to the conventional techniques such as aerated lagoon and activated sludge systems in which no significant color and COD reduction occurs. Another major advantage of this new method with respect to other methods, namely, adsorption with activated carbon and coagulation with alum, is to remove color without causing any serious disposal problems and consequently much less operating condition cost. The activated sludge and aerated lagoon were simulated with the residence time of 5 and 15 days, respectively, and no effect on color was observed in both treatment systems. But the new chemical–biological method in a total residence time of 6 days decreased the color, COD, BOD, and TSS up to 97.5%, 95%, 98%, 97%, respectively. The operating cost of the proposed chemical reduction reaction treatment system using $NaBH_4$ was estimated to be 0.001 US dollar per 1-l of the most highly colored wastewater for 97% color elimination.

## Acknowledgement

The financial support for this project by the Isfahan University of Technology is gratefully acknowledged.

## References

[1] E. Adler, Advances in chemistry of wood and pulping processes, Wood Sci. Technol. 11 (1977) 169–181.

[2] S.I. Falkenhag, et al., Chromophores in Kraft Lignin, in: Symposium of the 150th Meeting of ACS, Atlantic City, NJ, USA, 1965.

[3] E. Sjostrom, Wood Chemistry, Fundamentals and Applications, Academic Press, New York, USA, 1981.

[4] C.W. Bryant, A.D. Reeves, Organic Halide and Organic Carbon Distribution and Removal in a Pulp and Paper Wastewater Lagoon, J. Water Pollut. Control Fed. 59 (1987) 89–96.

[5] C.W. Bryant, G.L. Amy, Seasonal and in-mill aspects of organic halide removed by an aerated stabilization basin treating a Kraft mill wastewater, Water Sci. Technol. 21 (1988) 231–242.

[6] R. Saunamaki, Upgrading of aerated lagoon treatment plants, in: Proceedings of the TAPPI Environ. Conference, 1990, pp. 175–186.

[7] R. Sherwood, Wetland treatment, in: TAPPI Environ. Conference Proc., Richmond, Va., 1992, pp. 395–398, Book 2.

[8] J.W. Graves, J.L. Join, Effect of chlorine dioxide substitution, oxygen delignification, and biological treatment on bleach plant effluent, TAPPI J. 76 (7) (1993) 153–158.

[9] R.L. Knight, W.E.H. Blum, Design and performance of the champion pilot-constructed wetland treatment system, TAPPI J. 77 (1994) 240–251.

[10] J.A. Moore, M. Chapman, Wetland treatment of pulp mill wastewater, Water Sci. Technol. 29 (1994) 241–251.

[11] J.C.T. Dias, R.P. Rezende, C.M. Silva, V.R. Linardi, Biological treatment of Kraft pulp mill foul condensates at high temperatures using a membrane bioreactor, Process Biochem. 40 (3–4) (2005) 1125–1129.

[12] X. Fujian, H. Chen, L. Zuohu, Solid state production of lignin peroxidase (LiP) and manganese peroxidase (MnP) by phanerochaete chrysosporium using steam exploded straw as substrate, Biores. Technol. 80 (2001) 149–151.

[13] M.S.K. Hossain, M. Das, S.H. Ibrahim, Aerobic studies on pollution abatement of sulfite pulp bleaching effluent using phanerochaete chrysosporium (MTCC-787), J. Ind. Pollut. Control 17 (2) (2001) 191–200.

[14] P.R. Jayaramraja, T. Anthony, R. Rajendran, K. Rajkumar, Decolorisation of paper mill effluent by aspergillus fumigatus in bioreactor, Poll. Res. 20 (3) (2001) 309–312.

[15] D. Pokhrel, T. Viraraghavan, Treatment of pulp and paper mill wastewater—a review, Sci. Total Environ. 333 (3) (2004) 7–58.

[16] P. Singh, I.S. Thakur, Removal of colour and detoxification of pulp and paper mill effluent by microorganisms in two step bioreactor, J. Sci. Ind. Res. 63 (2004) 944–948.

[17] I.S. Thakur, Screening and identification of microbial strains for removal of colour and adsorbable organic halogens in pulp and paper mill effluent, Process Biochem. 39 (2004) 1693–1699.

[18] G. Thompson, J. Swain, M. Kay, C.F. Forster, The treatment of pulp and paper mill effluent: a review, Bioresour. Technol. 77 (2001) 275–286.

[19] M. Ali, T.R. Sreekrishnan, Aquatic toxicity from pulp and paper mill effluents: a review, Adv. Environ. Res. 5 (2001) 175–196.

[20] S. Lacorte, A. Latorre, D. Barceló, A. Rigol, A. Malmqvist, T. Welander, Organic compound in paper mill process waters and effluents, Trends Anal. Chem. 22 (2003) 725–737.

[21] S. Young, D.W. Smith, Effect of pulp mill chemicals on flocculation in river water, Water Sci. Technol. Water Supply 1 (2001) 251–258.

[22] T. Rana, S. Gupta, D. Kumar, S. Sharma, M. Rana, V.S. Rathore, B.M.J. Pereira, Toxic effects of pulp and paper mill effluents on male reproductive organs and some systemic parameters in rats, Environ. Toxicol. Pharm. 18 (2004) 1–7.

[23] J. Wu, Y.Z. Xiao, H.Q. Yu, Degradation of lignin in pulp mill wastewaters by white-rot fungi on biofilm, Bioresour. Technol. 96 (2005) 1357–1368.

[24] H.S. Shin, Y.R. Kim, B.S. Han, I.E. Makarov, A.V. Ponomarev, A.K. Pikaev, Application of electron beam to treatment of wastewater from paper mill, Rad. Phys. Chem. 65 (2002) 539–547.

[25] C.R. Tavares, M. Vieira, J.C.C. Petrus, E.C. Bortoletto, F. Ceravollo, Ultrafiltration/complexat-ion process for metal removal from pulp and paper industry wastewater, Desalination 144 (2002) 261–265.

[26] O. Milstein, A. Haars, A. Majcherczyk, J. Trojanowski, D. Tautz, H. Zanker, A. Hutterman, Removal of chlorophenols and chlorolignins from bleaching effluents by combined chemical and biological treatment, Water Sci. Technol. 20 (1988) 161–179.

[27] R.J. Stephenson, S.J.B. Duff, Coagulation and precipitation of a mechanical pulping effluent-i. removal of carbon, colour and turbidity, Water Res. 30 (1996) 781–792.

[28] Y.D. Yan, S.M. Glover, G.J. Jameson, S. Biggs, The flocculation efficiency of polydisperse polymer flocculants, Int. J. Miner. Process. 73 (2004) 161–175.

[29] V.C. Srivastava, I.D. Mall, I.M. Mishra, Treatments of pulp and paper mill wastewaters with poly aluminium chloride and bagasse fly ash, Colloids Surf. A 260 (2005) 17–28.

[30] D. Solberg, L. Wågberg, Adsorption and flocculation behavior of cationic polyacrylamide and colloidal silica, Colloids Surf. A 219 (1) (2003) 61–172.

[31] M.S. Burgess, J.E. Curley, N. Wiseman, H. Xiao, On-line optical determination of floc size. Part I. Principles and techniques, J. Pulp Paper Sci. 28 (2002) 63–65.

[32] D. Berryman, F. Houde, C. DeBlois, M. O'Shea, Nonylphenolic compounds in drinking and surface waters downstream of treated textile and pulp and paper effluents: a survey and preliminary assessment of their potential effects on public health and aquatic life, Chemosphere 56 (2004) 247–255.

[33] K.R. Munkttrick, M.R. Servos, J.H. Carey, G.J. Van Der Kraak, Environmental impact of pulp and paper wastewater: evidence for a reduction in environmental effects of north American pulp mills since, Water Sci. Technol. 35 (1997) 329–338.

[34] J. Rintala, J.L.S. Martin, G. Lettinga, Thermophilic anaerobic treatment of sulphate rich pulp and paper integrate process water, Water Sci. Technol. 24 (1991) 149–160.

[35] E. Rodriguez, M.A. Pickard, R. Vazquez-Duhalt, Industrial dye decolorization by laccase from ligninolytic fungi, Curr. Microbiol. 38 (1999) 27–32.

[36] R. Sierra-Alvarez, G. Lettinga, The effect of aromatic structure on the inhibition of acetoclastic methanogenesis in granular sludge, Appl. Microbiol. Biotechnol. 34 (1991) 544–561.

[37] C. Bryant, R.A. Sierka, Enhancement of Kraft effluent quality by increased chlorine dioxide substitution and membrane treatment of the Kraft extraction waste stream prior to aerobic and anaerobic biotreatment, in: TAPPI Environ. Conf. Proc., Boston, Mass., USA, 1993, pp. 383–388, Book 1.

[38] A.M. Springer, V.C. Hand, Analysis of the potential of photochemical and electrochemical techniques for decolorization of bleached Kraft mill effluent, in: TAPPI Environ. Conf. Proc., USA, Richmond, Va., 1992, pp. 831–842, Book 3.

[39] M.L. Traver, The removal of color from pulp and paper- mill effluents using an advanced hydrogen-peroxide based technology, Abstr. Pap. Am. Chem. Soc. 204 (1995) 43-ENVR.

[40] L. Ferrey, Y. Albouy, Decolorization of Kraft effluent by free and immobilized lignin peroxidases and horse radish peroxidase, Biotechnol. Lett. 13 (8) (1991) 577.

[41] E. Esposito, T. Dahlin, Screening of lignin degrading fungi for removal of color from Kraft mill wastewater with no addition as extra carbon source, Biotechnol. Lett. 13 (8) (1991) 571.

[42] F. Osterberg, M. Wood, Oxidative treatment of bleached Kraft effluents, in: Can. Pulp Paper Assoc. Tech. Section Conf., vol. 159, no. 5, Vancouver, BC, 1994, pp. 345–360.

[43] C.H. Mobius, M. Cordes-Tolle, Advanced treatment paper mill wastewaters, Water Sci. Tech. 29 (1988) 273–282.

[44] R. Huster, C.H. Mobius, Performance increase of aerobic biological wastewater treatment plants, Water Sci. Tech. 22 (1990) 287–301.

[45] W. Hegemann, Neuere erkenntnisse uber das belebungsverfahren mit festen, Allgemeine Papier-Rundschau 50/51 (1987) 1476–1480.

[46] A. Gianetto, Powdered activated carbon in an activated sludge treatment plan, Water Res. 18 (1984) 133–137.

[47] P. Ehrler, R. Erken, Unterstutzung der aeroben biologischen Abwasserreinigung, Abwasser Korrespondenz 34 (2) (1987) 129–138.

[48] M. Nystrom, Reducing aox by nonbiological external treatment methods, in: TAPPI Envi. Conf. Proc, Richmond, Va, 1992, pp. 843–848.

[49] P. Yu, T. Welander, A comparison of biological and thermo-alkaline treatment for the removal of chlorinated organic compounds from Kraft plant effluent, Nord. Pulp Paper Res. J. 8 (2003) 360–369.

[50] M. Bergbauer, C. Eggert, Degradability of chlorine-free bleachery effluent lignins by 2 fungi effects on lignin subunit type and on polymer molecular weight, Can. J. Microbiol. 40 (1994) 192.

[51] W.Z. Liang, L.Y. Ye, Anaerobic treatment of bagasse pulp mill effluent, in: CTAPI Seventh International Symposium Wood Pulping Chem. Proc., 3, Beijing, China, 1993, pp. 633–636.

[52] D.Y. Prasad, T.W. Joyce, Removal of chlorinated organics from Kraft softwood bleached plant effluent by sequential biological treatment using white-rot fungus and an anaerobic reactor, in: 46th Ind. Waste Conference Proc., Purdue University, USA, 1992, pp. 299–305.

[53] A. Mohammad, D.W. Smith, Effect of ozone on Kraft process pulp mill effluent, Ozone-Sci. Eng. 14 (1992) 461–485.

[54] G.D. Boardman, et al., Treatment of Kraft pulp and paper wastewaters by means of foam separation, Chem. Eng. Commun. 114 (1992) 89–102.

[55] B. Amero, J. Hilleke, Advancement in effluent decolorization using ozone, in: TAPPI Environ. Conf. Proc., Boston, Mass., USA, 1993, pp. 597–608.

[56] A.M. Springer, et al., Electrochemical removal of color and toxicity from bleached Kraft effluents, in: TAPPI Int. Environ. Eng. Conf. Proc., 1994, p. 271, Book 1.

[57] T. Haberl, et al., Anaerobic-aerobic treatment of organic high strength industrial wastewater, Water Sci. Technol. (G.B.) 23 (1991) 1909.

[58] B.H. Boyden, et al., Treatment of bleachery effluents from Kraft mills pulping mature eucalyptus, Water Sci. Technol. 29 (1994) 274.

[59] P. Singh, A. Singh, Physiochemical characteristics of the disttillery effluent and its chemical treatment, J. Nature Sci. Technol. 3 (2) (2004) 205–208.

[60] R. Nagarathnamma, P. Bajpai, P.K. Bajpai, Studies on decolourization, degradation and detoxification of chlorinated lignin compoundsin Kraft bleaching effluents by ceriporiopsis subvermispora, Process Biochem. 34 (1999) 939–948.

[61] D.A. Barton, J.W. Lee, D.B. Buckley, S.W. Jett, Biotreatment of Kraft mill condensates for reuse, in: Proceedings of the Tappi Minimum Effluent Mills Symposium, Atlanta, GA, USA, 1996, pp. 270–288.

[62] P. Singh, I.S. Thakur, Clour removal of anaerobically treated pulp and paper mill effluent by microorganisms in two steps bioreactor, Biores. Technol. 97 (2006) 218–223.

[63] Standard Methods for the Examinations of Water and Wastewater, 17th ed., American Public Health Association and Water Environment Federation, Washington, DC, USA, 1992, pp. 213–272.

[64] D.H. Williams, I. Fleming, Spectroscopic Methods in Organic Chemistry, McGraw-Hill Book Company, UK, 1973.

[65] Environmental Protection Agency, Guidelines for Water Reuse, EPA/625/R-92/004, Washington, DC, USA, 1992.