Effect of Biological Treatment on Characteristics of Soluble Organic Compounds in Hardwood KP Bleaching Effluent

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Hardwood KP bleaching effluents, before (influent) and after (effluent) biological treatment, were characterized and compared to identify the main bio-recalcitrant organic compounds in the effluent. The results indicate that biological treatment reduces COD_{cr} and BOD_5 by 67% and 88%, respectively. However, various bio-recalcitrant organic compounds were still retained in the biologically-treated effluent, which exhibits an increase in effluent color. Spectroscopic analysis suggests that colored substances are the most recalcitrant materials in the effluent. Di-n-octyl phthalate and 6,6'-di-tert-butyl-2,2'-methylenedi-p-cresol are the predominant residual materials in the biologically treated effluent. These two materials are the main recalcitrant organic compounds in the effluent and contribute to the major part of the residual COD_{cr} in the biologically treated effluent.

Keywords: Hardwood; Pulp-mill effluent; Biological treatment; Recalcitrant organic compound; Color

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INTRODUCTION

The pulp and paper industry is highly water intensive, facing increasingly more stringent effluent limitations in China. Specifically, the kraft wood pulping process generates significant amounts of highly polluted and brown colored substances which are dissolved and dispersed in process water after pulping and bleaching. The pollutants are mainly attributed to the derivatives of lignin, carbohydrates, and wood extractives. In particular, chlorinated lignin derivatives are bio-recalcitrant, toxic, and deep in color, (Saritha *et al.* 2010; Teaca and Bodirlau 2008), significantly contributing to the environment pollution when untreated or poorly treated effluents are discharged to receiving waters (Ghorbani *et al.* 2010).

Biological treatments including anaerobic and aerobic processes have been introduced in kraft pulp mills to biologically degrade organic pollutants for many years. However, lignin and its derivatives exhibit very slow degradation kinetics when using conventional biological processes because of the strong linkages within lignin molecules, especially biphenyl-type carbon to carbon linkages (Del Rio *et al.* 2002; Mohan and Karthikeyan 1997). Moreover, biological processes have little or no effect on color removal (Kreetachat *et al.* 2007; Morais *et al.* 2008). Therefore, a significant amount of color and recalcitrant substances were retained after the biological treatment (Meza *et al.* 2011), serving as the majority of the residual chemical oxygen demand (COD). Full characterization of the recalcitrant organic compounds in the biologically-treated effluents helps in designing modified technologies, *e.g.* a tertiary treatment process.

This work aims to identify the main bio-recalcitrant organic compounds in the kraft pulp effluent by using spectroscopic methods including infrared, ultraviolet, and gas chromatography–mass spectrometry. Mixed hardwoods locally grown in South China are used for pulping and generating effluents because hardwoods have become the most important feedstock for pulp and paper production in China.

EXPERIMENTAL

Effluent Sampling

Effluent samples were taken from a pulp mill located in the southern area of China. The pulp mill produces bleached chemical pulp from mixed locally grown hardwoods (60% eucalyptus, 25% chinaberry tree, and 15% *Castanopsis fissa*). Wood chips were cooked by the kraft method, followed by an oxygen delignification process. The pulp was then bleached using D_0/C-EO-P-D_1 (chlorine dioxide/chlorination, oxygen enhanced alkaline extraction, hydrogen peroxide, chlorine dioxide) sequences to 88% ISO brightness. Bleaching effluents were collected in the wastewater treatment plant where biological treatments, hydrolysis acidification combined with the activated sludge process, were performed. The hydraulic retention time of the hydrolysis acidification and the activated sludge process is 4 h and 12 h, respectively. The effluent sample before biological treatment was taken before hydrolysis acidification and the biologically treated effluent sample was taken after the secondary clarifier. Prior to use, all effluent samples were filtered with a membrane filter (pore size 0.45 μm) to remove suspended matter and particles.

Gas Chromatography–Mass Spectrometry (GC-MS)

An acidified (pH 2.0) and a neutralized (pH 7.0) effluent sample (100 mL) were respectively extracted with dichloromethane (30 mL) combined with diethyl ether (30 mL) in triplicate. The combined extracts were dried under vacuum conditions. The dried solid was re-dissolved in 1 mL ethyl acetate and subjected to GC-MS. The GC-MS analysis was performed using an Agilent 6890 gas chromatograph interfacing with an Agilent 5973 mass spectrograph. The analytical column connected to the system was a capillary column (DB5-30 m × 0.25 mm). Helium gas was used as carrier with a flow rate of 1 mL/min. The oven temperature was held at 60 ^{o}C for 4 min and programmed to 280 ^{o}C at a rate of 5 ^{o}C /min. The final temperature was held for another 5 min, and the inlet temperature was 250 ^{o}C. Injections were done with the split ratio of 1:1, and 1 μL of the sample was injected using a 7683B sample injector (Agilent Technologies, Santa Clara, CA, USA). The mass spectrometer was operated in the electron impact ionisation mode with an ionising energy of 70 eV. Scan data were obtained by scanning from 33 to 500 m/z.

Other Analytical Methods

The determination of the COD and the color at 465 nm was carried out on a Hach spectrophotometer (DR2800, Hach, Loveland, CO, USA) according to the standard methods (APHA 1992). The biological oxygen demand (BOD_5) was measured using samples incubated for 5 days at 20 °C on a Hach BOD detector (BOD Trak Ⅱ) following standard methods (APHA 1992). The determination of Ca^{2+}, Mg^{2+}, and Fe^{2+}/Fe^{3+} was performed on an ion chromatograph (WFX-IC, China). The pH was measured with a Sartorious PB-10 pH meter. AOX was measured using an AOX analyzer (ECS1200, Netherlands) according to the ASTM standard method. UV spectra were obtained on a Scinco S-3100 UV spectrophotometer.

For infrared (IR) spectra analysis, a 400 mL filtered sample was concentrated to 10 mL in a rotary evaporator at 60 °C and then dried in a vacuum drying oven. Five milligrams of oven-dried solid was mixed with 95 mg dry KBr, and the mixture was compressed to make pellets. FT-IR spectra in the region 4000-370 cm^{-1} (resolution 0.3 cm^{-1}) were recorded on a Bruker Vector 33 spectrometer (Germany) at a scan rate of 0.2 $cm \cdot s^{-1}$.

RESULTS AND DISCUSSION

Conventional Analysis

The characteristics of the effluent sample before biological treatment (influent) and the biologically treated effluent sample (effluent) are presented in Table 1. The influent contains a high concentration of soluble organic compounds in terms of COD_{cr} (0.99 kg/m^3) and BOD_5 (0.39 kg/m^3). The BOD_5/COD_{cr} ratio (0.39) is below the level of good biodegradability value (> 0.5) (Yaber *et al.* 1999).

Table 1. Characteristics of the Influent and the Biologically Treated Effluent

Parameter	Influent	Effluent	Discharge limit [c]
pH	7.2	8	6~9
COD_{cr} [a]/mg/L	990	325	100
BOD_5/mg/L	390	46	20
BOD_5/COD_{cr}	0.39	0.14	-
Color/C.U.	370	470	50
AOX/mg/L	2.53	0.34	12
Cl^-/mg/L	1050	800	-
$SO4^{2-}$/mg/L	220	136	-
Ca^{2+}/mg/L	47.6	41.7	-
Mg^{2+}/mg/L	35.2	28.7	-
Fe^{2+}/Fe^{3+}/mg/L	0.48	0.62	-
UV_{280} [b]	0.30	0.23	-

[a] COD value determined in accordance with dichromate method; [b] UV absorbance diluted 10 times; [c] Discharge standard of water pollutants for pulp and paper industry (GB3544-2008)

The soluble COD_{cr} and BOD_5 in bleaching effluents are efficiently removed after biological treatment by hydrolysis acidification combined with the activated sludge process (Table 1). The BOD_5/COD_{cr} ratio obtained from the biologically treated effluent decreased to 0.14, indicating an extraordinarily poor biodegradability of the residue effluent. In another words, biologically treated effluent still contains various organic compounds in terms of residual COD_{cr} of 325 mg/L and fails to satisfy the effluent standard.

Bleaching effluent contains considerable amounts of wood-derived pollutants that are released and dissolved from the reaction of raw materials with bleaching chemicals. These released materials include carbohydrates, low molar-mass organic acids, pectic acids, lignans, lipophilic extractives, and lignin-related substances (Thornton 1993). UV absorbance at 280 nm (UV_{280}) could be used to determine the content of lignin or lignin-derived compounds in the effluent (Rintala and Lepisto 1992). The UV data in Table 1 shows that only a small part of the lignin-derived material (23%, measured with the reduction of absorbance at 280 nm) was removed after biological treatment, much lower than the removal efficiency of COD_{cr} and BOD_5.

During the process of pulp bleaching, lignocellulose reacts with bleaching chemicals, resulting in the formation of chlorinated organic compounds that further contribute to effluent color (Bajpai *et al.* 1999). Because of the reactivity of lignin to oxidants, the degraded chromophorous lignin released and dispersed in effluent can be a major contributor to the color. Although the removal efficiency of COD_{cr} and BOD_5 is considerably high (67% and 88%, respectively), the removal of lignin-derived substances is relatively poor (23%), suggesting that the color of the biologically treated effluent is mainly attributed to the residual lignin-derived substances.

Color values of the effluent samples show that biological treatment increases the color by 27% (from 370 C.U. to 470 C.U.). An increase in color after biological treatment was also observed by Morais *et al.* (2008) and Leiviska *et al.* (2008). Milestone *et al.* (2007) suggests that anaerobic bacteria may cause the color increase in biological treatment systems. These microorganisms use high molecular weight (MW) material from bleaching effluents as an electron acceptor for growth and in turn lead to nonreversible internal changes of the material, such as intramolecular polymerization or the formation of chromophoric functional groups. Recent studies indicate that lignin obtained from biologically treated effluent exhibited a higher average molar mass than that from the untreated effluent (Andersson *et al.* 2008). Previous studies provided experimental evidence of degradation of a low molar mass fraction as well as chemical condensation into larger molecules during biological treatment of effluent (Ganczarczyk and Obiaga 1973). Studies also indicate that high MW aromatic components, such as lignin and tannins, are simply not biodegradable in aerobic environments (Diez *et al.* 2002; Rintala and Lepisto 1992).

Infrared Spectroscopy

Infrared (IR) spectra of the influent and biologically treated effluent samples are shown in Fig. 1. Possible assignments for the infrared absorption bands of the two samples are listed in Table 2. IR spectra show that the main peaks correspond to different functional groups, such as phenols, alcohols, organic acids, ketones, acid anhydrides, ethers, alkenyls, and meta disubstituted aromatics (Table 2). These groups mainly originated from degradation products of lignin, carbohydrates, and wood extractives created during the pulping process.

Comparing the IR absorption bands of the influent sample with the biologically treated effluent sample, it can be found that absorption bands around 1164 cm^{-1} and 754 cm^{-1} are eliminated and the absorption band around 1323 cm^{-1} is weakened after biological treatment. The results demonstrate the removal or structural change of the organic pollutants after the biological treatment process. The absorption band around 3350 to 3550 cm^{-1} (O-H stretching vibration) is intensified and the absorption band around 952 cm^{-1} (O-H bending vibration) is formed after biological treatment, which suggests the formation of hydroxyl groups during the biological treatment process. The absorption bands around 1623 cm^{-1} and 1790 cm^{-1} are also intensified after biological treatment, indicating the formation of carbonyl groups after the biological treatment process. On the other hand, the absorption bands around 3350 to 3550 cm^{-1}, 1623 cm^{-1}, and 952 cm^{-1} in the spectrum of the biologically treated effluent are very intensive, indicating the abundant presence of hydroxyl groups and carbonyl groups in the effluent. It has been reported that carbonyl groups on aromatic rings are the most important chromophoric groups in lignin molecules and hydroxyl groups are the major auxochromous groups (Falkenhag 1965). It can be assumed that the color increase after biological treatment is attributed to the enhancement of these two kinds of functional groups.

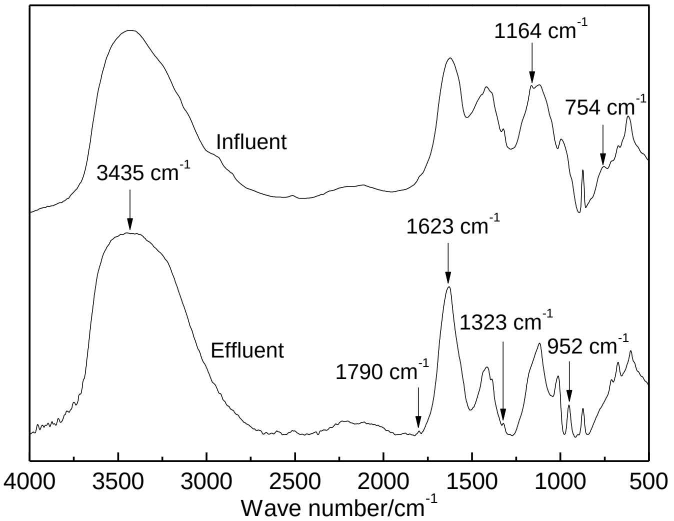
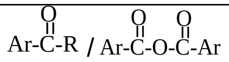


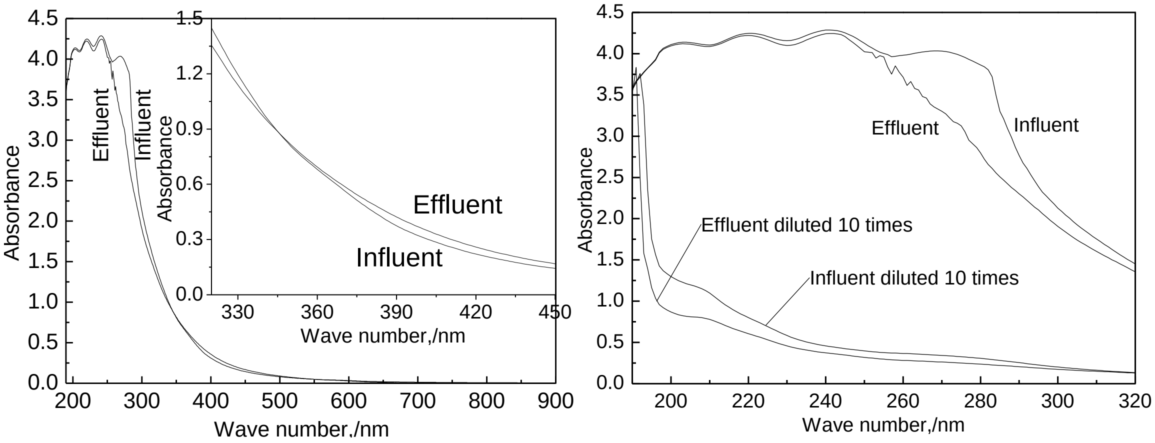
Fig. 1. FT-IR spectra of the influent and the biologically treated effluent

Table 2. Assignments of the IR Absorption Bands of the Influent and the Biologically Treated Effluent

Absorptions (cm^{-1})	Functional Groups	Possible Assignments
3350-3550; 1300-1500; 900-1180	Ar-OH/R-OH	Phenol/Alcohol
2460-2600; 1600-1700; 1300-1500	 $R\text{-}\overset{\overset{O}{\|}}{C}\text{-}OH$ / $Ar\text{-}\overset{\overset{O}{\|}}{C}\text{-}OH$	Carboxylic acid/Aromatic acid
1600-1820	 $Ar\text{-}\overset{\overset{O}{\|}}{C}\text{-}R$ / $Ar\text{-}\overset{\overset{O}{\|}}{C}\text{-}O\text{-}\overset{\overset{O}{\|}}{C}\text{-}Ar$	Ketone/Acid anhydride (Ghoreishi and Haghighi 2007)
900-1180	R-O-R	Ether
840-890	$R_2C=CH_2$	Alkenyl
610-760	R-Ar-R	Meta disubstituted aromatics (Ghoreishi and Haghighi 2007)

UV-VIS Spectroscopy

UV-VIS spectra of the influent and the biologically treated effluent samples are shown in Fig. 2. Both samples absorb electromagnetic radiation in not only the ultraviolet but also the visible areas of the spectra. Both spectra show a stronger absorbance in the region of 200 to 300 nm, while the absorbance in the region of 200 to 300 nm in the spectrum of the biologically treated effluent is weaker as compared to that of the influent. Ghoreishi and Haghighi (2007) suggest that absorbance around 259 to 263 nm in the UV spectrum of pulp-mill effluent can be attributed to several multi-substituted aromatic structures such as para CHOArCl, ArCl and ortho, meta CHOArOH, CHOArOMe, and the substituent groups include methoxy, hydroxy, chloro, and C=C groups. Previous studies indicate that absorbance in the UV region of 250 to 300 nm is attributed to π-π^* electron transitions in phenolic arenes, aniline derivatives, polyenes, and polycyclic aromatic hydrocarbons with two or more rings (Chin *et al.* 1994; Traina *et al.* 1990). The strong absorbance in the UV region of 200 to 300 nm falls within the aromatic band, which suggests the possible presence of various aromatic substances in the effluent.



(a) (b)

Fig. 2. UV-VIS spectra of the influent and the biologically treated effluent for: (a) 190 to 900 nm, (b) 190 to 320 nm

Biological treatment causes an obvious decrease in UV absorbance (190 to 300 nm) of effluent (Fig. 2). There is a 20.8% reduction in UV_{254} and 23.0% reduction in UV_{280} absorbance after biological treatment (Table 3). The UV absorbance at 254 nm and 280 nm is mainly due to the soluble aromatic derivatives and lignin-derived compounds (Ko *et al.* 2009) that are commonly present in pulp-mill effluent. These reductions indicate that removal or structural change of aromatic compounds and lignin-derived substances occurred during the biological treatment. Our results agree with previous reports (Zimmermann 1990; Pandila 1973; Lewis *et al.* 2011) in which up to 13% to 35% of lignin-related compounds were removed by microbiological treatment.

Table 3. Percentage Changes in UV_{254}, UV_{280}, and Color after Biological Treatment

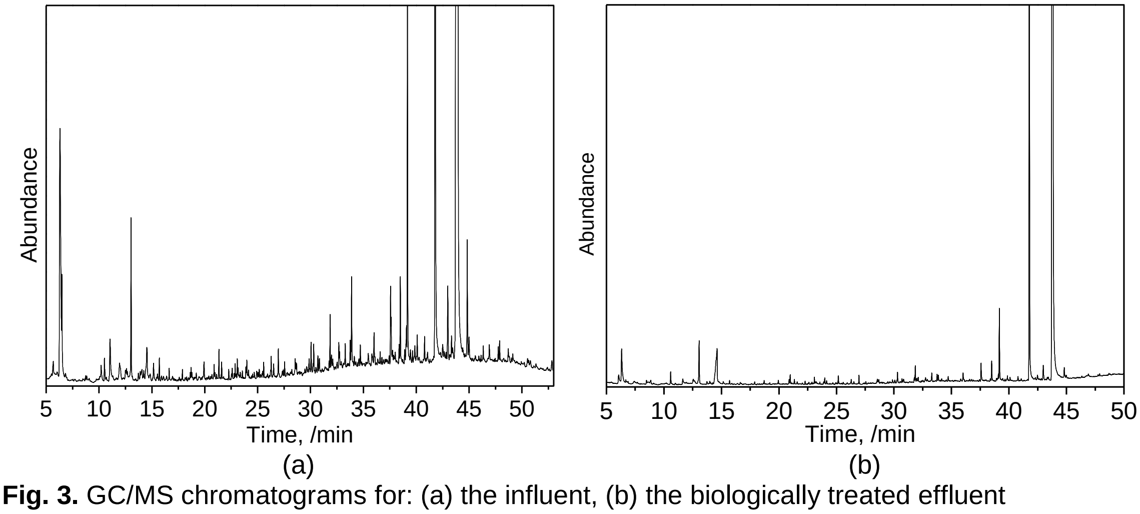
Parameter	Influent	Effluent	Change (%)
COD_{cr} /mg/L	990	325	−67 [b]
UV_{254} [a]	0.37994	0.30081	−20.8
UV_{280} [a]	0.30687	0.23624	−23.0
$Color_{456}$	0.13264	0.15536	+17.1 [c]
Color $_{Pt\text{-}Co}$ /C. U.	370	470	+27
Specific UV_{254} (UV_{254}/COD_{cr})	3.84×10^{-4}	9.26×10^{-4}	+141
Specific UV_{280} (UV_{280}/COD_{cr})	3.10×10^{-4}	7.27×10^{-4}	+134
Specific $Color_{456}$ ($Color_{456}/COD_{cr}$)	1.34×10^{-4}	4.78×10^{-4}	+257
[a] UV absorbance diluted 10 times; [b] (−)decrease; [c] (+)increase			

When compared with the removal efficiency of COD_{cr} and BOD_5, the reductions of aromatic compounds are relatively lower after biological treatment. Moreover, when expressed as specific absorbance, the specific UV_{254} (UV_{254}/COD_{cr}) and UV_{280} (UV_{280}/COD_{cr}) increased by 141% and 134%, respectively, after biological treatment. Thus, it can be concluded that aromatic compounds and lignin-related substances in the bleaching effluent are mineralized to a lesser extent during the biological treatment process than other compounds such as cellulose and hemicellulose-degraded products and contribute to the major part of residual COD_{cr} of the biologically treated effluent. Considering the fact that the biodegradability (BOD/COD ratio) of the bleaching effluent decreases from 0.39 to 0.14 after biological treatment, it is reasonable to conclude that aromatic compounds and lignin-related substances are the main bio-recalcitrant substances in the pulp-mill effluent.

Unlike the absorbance reduction in the ultraviolet region, the absorbance in the visible region (350 to 550 nm) increases after biological treatment (Fig. 2). There is a 27% increase in $Color_{pt\text{-}Co}$ and a 17.1% increase in the visible absorbance at 465 nm ($Color_{465}$) after biological treatment (Table 3). When expressed as specific absorbance at 465 nm ($Color_{465}/COD_{cr}$), there is a 257% increase after biological treatment. Based on these results, it can be deduced that colored substances are the most recalcitrant materials in the effluent samples.

GC-MS Analysis

Gas chromatograms corresponding to the organic compounds extracted from the influent and the biologically treated effluent samples are shown in Fig. 3. The compounds detected in the extracts obtained from the influent sample consist of 59 organic compounds, and up to 84.5% (relative percentage of areas, similarly hereinafter) of the detected compounds are attributed to aromatic compounds. These compounds include 11 esters (74.69%), 10 phenols (10.63%), 17 alkanes (8.53%), and 5 organic acids (2.81%), out of which 5 phthalates contribute to a 69.91% relative percentage of peak areas. Moreover, 10 organochlorides are detected in the effluent sample before biological treatment and contribute to a 5.63% relative percentage of areas.



(a) (b)

Fig. 3. GC/MS chromatograms for: (a) the influent, (b) the biologically treated effluent

Chromatogram obtained from the biologically treated effluent sample indicates that 25 compounds previously detected in the influent sample disappeared. The peak area and intensity of the retained compounds markedly decreased after biological treatment (Fig. 3b). There are 34 organic compounds detected in the extracts obtained from the biologically treated effluent sample including 9 esters (82.59%), 4 phenols (10.94%), 11 alkanes (2.76%), and 2 organic acids (1.97%). In total, up to 95.48% of the detected materials are attributed to aromatic compounds. The content of aromatic compounds that are mainly attributed to lignin-derived substances was increased by 10.98% after biological treatment. It can be concluded that lignin-derived materials are resistant to biological degradation and contribute to the residual COD in the biologically treated effluent. On the other hand, the content of the organochlorides decreases from 5.63% to 1.76% and only 3 organochlorides are detected after biological treatment, indicating effective removal of these toxic pollutants.

According to the chromatograms, the predominant residual compounds in the biologically treated effluent are di-n-octyl phthalate and 6,6'-di-tert-butyl-2,2'-methylenedi-p-cresol, which contribute to 79.73% and 10.36% relative percentage of areas, respectively. These two typical lignin-derivatives are the main recalcitrant organic compounds in the effluent and contribute to the major part of the residual COD in the biologically treated effluent. Di-n-octyl phthalate has engendered great environmental concern in recent years and has been listed as a priority environmental pollutant in both China and the USA (Wang *et al.* 1995; US EPA 1992). Since the biologically treated effluent still contains such environmentally harmful substances, the removal of these substances by using more effective methods, *e.g.* tertiary treatment, is important for clean production in hardwood kraft pulp mills.

CONCLUSIONS

1. The removal of soluble COD_{cr} and BOD_5 from pulp mill effluent is 67% and 88%, respectively, after the biological treatment. The biologically treated effluent still contains various bio-recalcitrant organic compounds, which are mainly attributed to the aromatic compounds and lignin-related substances. These aromatic compounds and lignin-related substances are mineralized to a lesser extent during the biological treatment process than other compounds such as cellulose and hemicellulose-degraded products and contribute to the major part of the residual COD_{cr} in the biologically treated effluent.
2. The aromatic compounds and lignin-related substances in the pulp-mill effluent were removed or structurally modified during the biological treatment process.
3. The biological treatment of hydrolysis acidification combined with the activated sludge process is not effective to reduce the color of the effluent and the color of the effluent was increased instead. This suggests that colored substances are the most recalcitrant materials. The color increase may be attributed to the enhancement and abundant presence of carbonyl groups and hydroxyl groups after biological treatment.
4. Di-n-octyl phthalate and 6,6'-di-tert-butyl-2,2'-methylenedi-p-cresol are the predominant residual materials in the biologically treated effluent. These two materials are the main recalcitrant organic compounds in the effluent and contribute to the major part of the residual COD in the biologically treated effluent.

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