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Biological removal of chloro-organic compounds from bagasse soda pulp bleaching effluent by *Coriolus versicolor*

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ABSTRACT

The potential of rainbow fungus (*Coriolus versicolor)* for bioremediation of bagasse soda pulp bleaching effluent containing chlorinated organic compounds was investigated without co-substrate addition. Initially, the effect of each of the operating variable including time, temperature, pH, and biomass on color reduction was investigated. The highest color reduction (percentage of the original value) was observed applying a treatment time of 6 days (58%), a pH of 3 (48%), a biomass dose of 9 g L^{-1} (59%), and a temperature of 35°C (48%). Then, the combined effect of parameters was studied to reach the best combinations of parameters leading to the highest color reduction. The highest performance of rainbow fungus on color reduction (66%) was observed when applying the combination of variables including temperature, treatment time, biomass dose, and pH combination of 35°C, 6 days, 9 g L^{-1}, and 3, respectively. At this condition, COD and BOD of effluent were decreased by 45% and 53%, respectively. It was also found that *Coriolus versicolor* can efficiently reduced various toxic compounds; chlorophenols, chloroguaiacols, and chlorocatechols present in the effluent to the levels less than their lethal concentration ($^{96}LC_{50}$) even without addition of co-substrate. In general, rainbow fungus can efficiently modify the undesirable physico-chemical characteristics of bagasse soda pulp bleaching effluent.

KEYWORDS: Rainbow Fungus, Bleach Plant Effluent, Color Reduction, Treatment Conditions, Chlorocatechols, Lethal concentration ($^{96}LC_{50}$).

1. INTRODUCTION

Application of elementary chlorine and hypochlorite to bleach non-wood pulps is probably the main factor responsible for weak environmental performance of non-woods pulping (Rolf *et al.*, 2009). Chlorinated organic compounds generated during pulp bleaching processes, especially in chlorine and hypochlorite stages, not only generate color, BOD, and COD in bleaching effluent but also initiate chronic and acute toxicity, responsible for the mutagenicity and carcinogencity of the effluent (Ali and Sreekrishnan, 2001). Therefore, for safe disposal of such bleaching effluents, in addition to modifications of pulping and bleaching processes (Rolf *et al.*, 2009), several treatment methods including aerobic, anaerobic and abiotic processes are utilized to remove color and as well as to degrade the chlorinated organic compounds (Ali and Sreekrishnan, 2001). Since the existing conventional effluent treatment methods are not sufficient and effective to remove chlorinated organic compounds, biological removal of chlorinated organic compounds originating from pulp bleaching plant has received considerable attention and interest (Taghipour and Evans, 1995; Driessel and Christov, 2001; Taseli and Gokcay, 2005). The impacts of various microorganisms on degradation of chlorinated organic substances from pulp bleaching effluents have been investigated (Eriksson, 1990; Ali and Sreekrishnan, 2001; Shintani *et al.*, 2002, Tripathi *et al.*, 2007). Among them, white rot fungi have demonstrated the capacity to completely degrade the chlorolignins (Bajpai *et al.*, 1993; Nagarathnamma and Bajpai, 1999; Driessel and Christov, 2001; Widsten and Kandelbauer, 2008). The enzyme system of white rot fungi contains a group of nonspecific extracellular enzymes which catalyzes the oxidation and degradation of aromatic and halogenated compounds (Eriksson, 1990; Fackler *et al.*, 2006).

The serious drawback associated with the fungal treatment of bleach plant effluents is the need for easily metabolisable co-substrate such as glucose needed for the growth and development of ligninolytic activity (Yin *et al.*, 1989). The application of co-substrate results in higher cost of treatment and even increase in COD and BOD of the effluents (Ali and Sreekrishnan, 2001; Bergbauer *et al.*, 1991). It needs to be mentioned that fungal treatment of pulp bleaching effluent will not usually proceed to complete removal of pollution load, and an additional treatment is required (Martin and Manzanares, 1994; Srinivasan and Murthy, 1999).

In contrast to conventional wood chemical pulping, bagasse soda pulping generates different effluent streams with unique characteristics. Bagasse preparation stage generates effluent loaded with carbohydrates and some amounts of lignin, and although the pollution load is high in bagasse preparation stage effluent, it can be easily treated applying conventional treatment processes (Chinnaraj and Rao, 2006; Bocchini *et al.*, 2005). However, the effluent stream originating from bleaching plant contains chlorinated organic compounds which are hard to treat. Since it is not possible to mix bleaching plant effluent with bagasse preparation stage effluent, this portion of the effluent need to be pretreated to reach an acceptable level of pollutants and then mixed with other streams to be treated under conventional processes.

This paper investigates the biological pretreatment of bagasse soda pulp bleach plant effluent applying different operating conditions in terms of decolorisation, BOD and COD reduction and dechlorination utilizing *Coriolus versicolor* without co-substrate addition. This procedure will offer an alternative to the conventional and capital intensive treatment of bagasse chemical pulping effluent.

2. MATERIALS AND METHODS

2.1. Material

The bleaching effluent used in this study was collected from Pars Paper Mill bleach plant (Iran). The effluent was stored at 4°C and then filtered through a 0.5 mm sieve to remove large suspended particles. The characteristics of effluent as collected from bagasse soda pulp bleaching effluent using hypochlorite bleaching sequence are presented in Table 1.

Table 1. Initial characteristics of soda bagasse pulp bleaching effluent

Parameter	Unit	Value
pH	-	8
Temperature	ºC	30-35
Color	PCU	470
COD	mg L^{-1}	2300
BOD_5	mg L^{-1}	350
TDS	mg L^{-1}	1200
TSS	mg L^{-1}	1800

2.2. Fungal Strains and Growth

Coriolus versicolor, a lignin degrading fungus known as rainbow fungus, was provided by Alborz Research Center, Institute of Forest and Rangelands, Karaj, Iran. The preparation of culture media, sterilization, and growth of *Coriolus versicolor* were performed according to Karimi *et al.*, (2007).

Treatment of effluent with a known amount of the sterilized strains of *Coriolus versicolor* was performed according to Bergbauer *et al.* (1991). Several pre-treatment trials were carried out to determine the optimum growth condition of *Coriolus versicolor* in bagasse soda pulp bleach plant effluent without co-substrate addition. The parameters in this stage were; time, 7 periods (1 -7 days), temperature, 10 levels (20 to 65°C), pH, 9 levels (2 to 10), and biomass dose, 5 levels (3 to 15 g L^{-1}). To determine the effect of each parameter on the color reduction, one parameter was varied and the other parameters were kept constant in the best condition (biomass dose 5 g L^{-1}; pH 4; 4 days; 30°C) according to Bergbauer *et al.* (1991). Then, to study the combined effect of the four parameters on the color reduction and finding the optimum growth condition of *Coriolus versicolor* leading to the highest color reduction, two levels of each parameter showing better performances in the pre-treatment stage were selected for further experiments.

2.3. Analytical Methods

The effluent color was measured spectrophotometerially using a Hach spectrophotometer, and expressed as Platinum Cobalt Units (PCU). To derive the calibration curve, 1g of $CoCl_2.6H_2O$ (equivalent to 250 mg Co), 1.246 g of K_2PtCl_6 (equivalent to 500 mg Pt) and 10 ml concentrated HCl were added to distilled water, and the total volume was made up to 1000 ml with distilled water to yield a standard solution of 500 Pt.Co color units (PCU). Then, solutions of different Pt.Co units were prepared by dilution. The pH of different standard solutions was adjusted to 7.6, and their absorbance was determined at 465 nm, and calibration curve was then plotted. To determine the effluent color before and after treatment, the pH of the effluent was adjusted to 7.6, and then the effluent was centrifuged for 5 min at 1500 rpm to remove the suspended particles. The absorbance of this solution was determined at 465 nm, and the color was computed from the calibration curve (Clescerl *et al.*, 1991; Sharma *et al.*, 1997).

The effect of fungus growth on COD, BOD_5, TSS, and TDS of the effluent (at the optimum combination of variables) was also studied. BOD_5 and COD (open reflux method), TSS and TDS of effluent before and after treatment were determined using corresponding standard methods (Clescerl *et al.*, 1991).

It is important to note that all experiments were performed without co-substrate addition to the effluent, each was repeated three times, and the results are reported as average value.

The quantity of the toxic chlorinated compounds present in the effluent was determined prior to and after fungal treatment. Chlorinated organic compounds from the effluent (prior to and after fungal treatment) were extracted using a modified method of the procedure suggested by Lindstrom and Nordin (1976). The pH of 1000 ml of effluent was adjusted to 2.5 and extracted twice with 500 ml of diethyl ether and then 500 ml of ethyl acetate applying intermittent shaking.

Gas chromatography and mass detection were used to determine the type and quantity of chlorinated organic compounds after extract derivatization with BSTFA according to Hong *et al.*, (2003). GC data were collected on an Agilent 6890 plus GC under the set-up presented in Table 2.

To estimate the effect of fungal treatment on the effluent toxicity, the concentrations of detected toxic compounds were compared with their reported ${}^{96}LC_{50}$ values in the literature. ${}^{96}LC_{50}$ is an indication of the toxicity of a particular compound and it is the lethal concentration at which 50% of the test organisms will be killed when the test organisms are exposed to the toxicant for a period of 96 h under standard conditions. The ${}^{96}LC_{50}$ value of the toxic compounds found in pulp bleaching plant effluents such as chlorinated derivatives of phenols, catechols, guaiacols, and syringaldehydes have been estimated as 0.5-0.6 mg L^{-1} (Sharma *et al.*, 1996; Sharma and Kumar, 1999).

Table 2. Set-up for detection of chlorinated compounds in effluent prior to and after biotreatment

Parameter	**Column (HR -1)**
Detector	FID
Detector range	5º
Chart speed (cm min^{-1})	5
Sample size (μL)	1
Column dimensions	30 mm×25 mm×0.25 mm
Film thickness	0.25 μm
Injection and detector temp (ºC)	250
Column temperature (ºC)	50 for 5 min, 50-280 ºC at 5 ºC min^{-1}
Total Run Time (min^{-1})	56

3. RESULTS AND DISCUSSION

3.1. Effect of Parameters

The color of bleaching plant effluent using chlorine compound mainly originates from high molecular weight chloro-organic compounds which are hard to remove or degrade (Driessel and Christov, 2001). Therefore, in this study we initially examined the effect of individual operating parameters on the color reduction to establish the optimum operating conditions to treat bagasse soda pulp bleaching effluent applying *Coriolus versicolor.*

The results revealed that as the temperature of effluent increased to above 35°C, the color reduction significantly decreased, and the lowest reduction value (18%) was observed at 65°C. The effect of operating temperature on color reduction of effluent was pronounced at the temperature range between 20°C to 35°C (48% reduction was reached at 35°C treatment temperature). According to the previous studies (Bergbauer *et al.*, 1991; Martin and Manzanares, 1994; Srinivasan and Murthy, 1999), at the temperatures higher and lower than 20°C to 35°C range, the growth of this fungus reduced decreasing the color reduction efficiency.

The other important factor affecting on color reduction is the pH of the effluent. The results revealed that the maximum color removal was observed applying pH of 3, leading to the highest color reduction (48%). The color removal ability of this fungus was gradually reduced as the pH values increased from 3 to 10. As reported in literature, the optimum pH for *Coriolus versicolor* growth and activity in effluent *treatment* is the acidic pH range between 3 and 4 (Srinivasan and Murthy, 1999; Sevlam *et al.*, 2002; Driessel and Christov, 2001).

The independent effect of the biomass dosage on color reduction indicated that the maximum color reduction (59%) was reached at biomass dose of 9 g L^{-1}. The effect of treatment time indicated that the initial color of the effluent (470 PCU) gradually reduced as incubation time increased. The highest color reduction (58%) was observed on the 6^{th} day, and there was no statistically significant difference between the color reduction after 6^{th} and 7^{th} days of incubation. These results are in agreement with the reported results in which the best decolorisation of bleach plant effluent employing *Coriolus versicolor* was achieved at the biomass dose and treatment time ranges of 5 to 10 g L^{-1} and 4 to 7 days respectively (Bergbauer *et al.*, 1991; Martin and Manzanares, 1994 and Srinivasan and Murthy, 1999).

Even though each variable is independently effective on color removal of the effluent as well as removing other pollutants, the combined effect of the four aforementioned parameters under study on the ability of *Coriolus versicolor* to reduce effluent color can be pronounced. Therefore, in further step of our study, we examined the combined effects of the variables under study to find the best combination of operating parameters leading to the highest color reduction. Consequently, the most suitable combinations of the variables (two values for each variable with the highest performance and the total of 16 combinations) were selected for further work. These combinations of variables were applied on the effluent as the final treatment. As the results summarized in Table 3 indicate, the optimum conditions of variables indicating the highest effect on undesired characteristics of baggase soda bleach plant effluent were the same as those obtained from the individual effects indicating that there was no distinct interaction between the individual parameters affecting on color reduction.

It was demonstrated that rainbow fungus *(Coriolus versicolor)* showed the highest effect on color reduction (66%) in the biomass dose, temperature, pH and treatment time of 9 g L^{-1}, 35°C, 3 and 6 days, respectively (known as T5 in Table 3). As observed in Table 3, even in the absence of co-substrate, *Coriolus versicolor* was able to efficiently decrease bleaching effluent color, COD, and BOD by 66.4%, 45%, and 53%, respectively. Moreover, TDS and TSS of effluent were decreased by 51% and 63%, respectively (T5 in Table 3).

Martin and Manzanares (1994) studied the potential of *C. versicolor* to decolorise straw alkaline pulping liquor and reported that maximum decolorisation was obtained for effluents supplemented with 10 g L^{-1} of glucose, where about 70% of the initial color (measured as absorbance at 465 nm) was removed after 4 days. Srinivasan and Murthy (1999) showed that *C. versicolor* could best decrease effluent color (82.5%) when supplemented with glucose and ammonium chloride (15 and 0.5 g L^{-1} respectively). Ortega *et al.*, (2007) reported that the mentioned fungus could decrease COD and color of pulp and paper effluent by 47%.

Table 3. Optimum levels of biomass dose, temperature, pH, and time for biotreatment of bagasse soda pulp bleaching effluent and the results

Trial	Biomass dose ($g\ L^{-1}$)	Temp. (°C)	pH	Time (day)	Color reduction (%)	COD reduction (%)	BOD reduction (%)
T1	9	30	3	6	63.8	37.9	37.1
T2				7	63.3	24.6	22.3
T3			4	6	62.6	8.7	45.8
T4				7	62.9	12.9	46.9
T5		35	3	6	66.4	45	53.1
T6				7	64.9	33.5	41.4
T7			4	6	63	30.5	45.7
T8				7	63.6	34	32.9
T9	12	30	3	6	61.4	25.7	41.4
T10				7	63	20.4	40
T11			4	6	62.8	15.8	30.6
T12				7	59.6	12.6	37.2
T13		35	3	6	62.6	43.5	45.2
T14				7	63.4	12.7	37.2
T15			4	6	63.4	30	50
T16				7	61.5	19.9	20

At the laboratory scale experiment, *C. versicolor* exhibited the potential to decrease effluent color and COD by 63.9% and 59.3% in 4 days, respectively (Sevlam *et al.*, 2002). As was observed above, even without adding any amount of cost intensive co-substrates, the results reached in our study are comparable to, and even in some cases better than, those mentioned in the literature. It can be concluded that at the given combination of variables, *Coriolus versicolor* can efficiently and economically reduce the undesirable characteristics of bagasse soda pulp bleach plant effluent.

3.2. Toxicity

The chemical analysis of the effluent showed that three categories of chlorophenolic compounds including various chlorophenols, chlorocatechols, and chloroguaiacols are present in bagasse soda pulp bleach plant effluent from Pars Paper Mill (Iran), using hypochlorite as the bleaching agent. As seen in Table 4, the concentration of all chlorophenolic compounds decreased after fungal treatment. Various studies have shown that fungal reactivity toward chlorophenols is due to the production of lignin-degrading enzymes, particularly laccase and Mn-peroxidase that are secreted in increased quantities by the fungus during ligninolytic activity (Evans *et al.*, 1994). Archibald and Roy (1992) have reported that laccase plays the primary role in decolorization of bleaching effluent by *C. versicolor*. It has been demonstrated that *C. versicolor* laccase, in the presence of phenolic substrates, was able to generate Mn(III) chelates similar to those produced by MnP. These compounds are responsible for the oxidation and consequent detoxification of bleaching effluents (Lackner *et al.*, 1991).

The concentrations of the 12 detected chlorophenolics were reduced to the level less than $^{96}LC_{50}$ limit values, and two compounds, e.g., 2-chloroguaiacol and 4,5,6-trichloroguaiacol were 100% degraded during the fungal treatment. However, despite the degradation and reduction of three chlorophenolic compounds, e.g., 2,3,5- trichlorophenol by 49.6%, 3,6- dichlorocatechol by 4.3%, and 3,5- dichlorocatechol by 45.4%, their concentrations still remained slightly above the $^{96}LC_{50}$ limit. Considering the results, we can conclude that rainbow fungus (*Coriolus versicolor)* even without any co-substrate addition can detoxify bagasse soda pulp bleach plant effluent to a large extent. Absence of co-substrate not only reduces the overall cost of effluent treatment, but also reduces the BOD_5 of the system which in turn facilitates further treatment procedure.

Table 4. Concentration of different chlorophenolics detected in bagasse soda pulp bleaching effluent prior to and after fungal treatment with *Coriolus versicolor*

Compound	Untreated effluent ($mg\ L^{-1}$)	Treated effluent ($mg\ L^{-1}$)	Removal (%)
2,6-chlorophenols	0.13	0.06	54
2,5-chlorophenols	0.48	0.08	83.4
2,3,5-chlorophenols	1.35	0.68	49.6
2,4,6-chlorophenols	0.55	0.15	72.7
Tetra-chlorophenols	0.18	0.09	50
Penta-chlorophenols	0.8	0.42	47.5
2-chloroguaiacols	0.29	0	100
4,6-chloroguaiacols	0.1	0.05	50
3,6-chloroguaiacols	0.09	0.04	55.6
3,4,5-chloroguaiacols	1.32	0.1	92.5
3,4,6-chloroguaiacols	0.16	0.1	37.5
4,5,6-chloroguaiacols	0.03	0	100
3,6-chlorocatechols	1.61	1.54	4.3
3,5-chlorocatechols	1.5	0.82	45.4
3,4,5-chlorocatechols	0.6	0.42	3

4. CONCLUSIONS

The study of the potential of each effective variable on the ability of *Coriolus versicolor* to decolorise bagasse soda pulp bleaching effluent showed that the highest color reduction was reached when applying a treatment time of 6 days (58%), a pH of 3 (48%), a biomass dose of 9 g L^{-1} (59%), and a temperature of 35°C (48%) respectively. The combined effect of the operating parameters revealed that *Coriolus versicolor* exhibited the highest effect on color reduction (66%) applying temperature, treatment time, biomass dose, and pH combination of 35°C, 6 days, 9 g L^{-1}, and 3, respectively. It is observed that *Coriolus versicolor* was able to significantly decrease the effluent COD and BOD (in the optimum combination of operating parameters) by 45%, 53%, respectively. Moreover, it was found that even without adding co-substrate, *Coriolus versicolor* could efficiently degrade and reduce the various toxic compounds including chlorophenols, chloroguaiacols, and chlorocatechols in Pars Paper Mill bleach plant effluent (Iran), to the extent lower than their lethal concentration ($^{96}LC_{50}$) in most cases. Based on the results of this study, it can be concluded that rainbow fungus (*Coriolus versicolor)*, can efficiently reduce the undesirable physico-chemical properties of a bagasse soda pulp bleach plant effluent to the level acceptable as pretreatment for chlorinated effluent. Since bagasse soda pulping generates different streams of effluents with varying characteristics, it is advisable to apply suggested pretreatment on bleach plant effluent prior to mixing with other streams.

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