

Novel application of fungal *Phanerochaete* sp. and xylanase for reduction in pollution load of paper mill effluent

Author Details

Ravi Dutt Yadav (Corresponding author)	Research and Development Division, Ballarpur Industries Limited (BILT), Yamuna Nagar - 135 001, India e-mail: rdy01@yahoo.com
Smita Chaudhry	Institute of Environmental Studies, Kurukshetra University, Kurukshetra - 136 119, India
Sanjeev Gupta	Environment Group, Thapar Centre for Industrial Research and Development, Yamuna Nagar - 135 001, India

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Abstract

Four different strategies of pulping and bleaching were carried out to develop alternative mechanistic ecoenvironmental friendly approaches and generated effluent was characterised. Strategy-I included *Phanerochaete* sp. fungal pretreatment followed by conventional bleaching, whereas in strategy-II, fungal pretreatment was followed by enzyme xylanase aided bleaching. Strategy-III also included xylanase supplement but without prior fungal pretreatment. Chemically driven pulping and bleaching was the IV strategy. Conventional C_DE_{OP}D₁D₂ sequence of bleaching was used for strategy-I and IV whereas XC_DE_{OP}D₁D₂ sequence was applied to strategy-II and III. Strategy-II was responsible for 27.5% reduction in Kappa no. whereas the maximum (27.5%) reduction in refining energy was observed with strategy-II. Biobleaching strategies- II and III were helpful in saving 37.3 and 20.3% of elemental chlorine (Cl₂) and 30.8 and 23.1% of chlorine dioxide (ClO₂), respectively. In comparison to control (strategy-IV), strategy II resulted in maximum pollution load reduction of chemical oxygen demand (COD), biological oxygen demand (BOD), color and adsorbable organic halides (AOX) upto 57, 60, 30 and 43.6%, respectively.

Key words

Adsorbable organic halides, Biobleaching, Biopulping, White rot fungus, Xylanase

Introduction

Pulp and paper industry is one of the major polluting industries in the developed and developing countries (Battan *et al.*, 2007). In recent past, environment protection groups have taken strict actions to restrict the release of toxic components to water bodies. Therefore, in order to cope-up with the increasing pressure to protect the environment and to develop the cleaner technologies, various trials have been carried out by employing numerous enzymes (Dhiman *et al.*, 2008), mediators (Suurnakki *et al.*, 2010) and microorganisms (Yadav *et al.*, 2010) for pulp and paper industry. Physio-chemical treatments have also been applied to remove the contaminants from effluent (Chakrabarti *et al.*, 2006), but it results in generation of extra chemical sludge.

Use of fungal inoculum, prior to pulping, offers an attractive opportunity for conventional pulping mechanism. This augmentation could save energy at refining stage of mechanical process (Shukla

et al., 2004). Likewise, incorporation of biological method in case of chemical pulping facilitates the removal of lignin by modifying its structure for easier extraction in subsequent bleaching processes. Use of suitable lignin-degrading fungi can lead to energy efficient pulping procedure and strength properties of paper can also be improved (Kondo *et al.*, 1994).

Most frequently used pulping procedure around the world is 'chemical pulping', also known as the sulphate (Kraft) process (Damiano *et al.*, 2003). After the first report by Viikari *et al.* (2001), use of enzymes in paper industry has been revolutionized. A number of reports have been published on the application of hydrolyzing enzyme for biobleaching (Ahlawat *et al.*, 2007; Dhiman *et al.*, 2009). In present investigation, schematic study was done under four different strategies. Strategy-I included *Phanerochaete* sp. fungal pretreatment followed by conventional bleaching, whereas in strategy-II, fungal pretreatment was followed by enzyme xylanase

aided bleaching. Strategy-III also included xylanase supplement but without prior fungal pretreatment. Chemically driven pulping and bleaching was the IV strategy. The pulp sample at different stages were analysed for various physical and optical properties. Effluent from these strategies was also characterized to investigate their remedial efficiency in term of pollution load.

Materials and Methods

Fungus culture and pulp: Pure culture of white rot fungus *Phanerochaete* sp. was provided by Biotechnology Department, Kurukshetra University, Kurukshetra, India. Culture was maintained on potato dextrose agar (PDA) slant at $30 \pm 1^\circ\text{C}$ and as glycerated frozen stock at -20°C . Mixture of bamboo (*Bambusa ventricosa*) and eucalyptus (*Eucalyptus globulus*) chips for pretreatment and pulp were obtained from Ballapur Industries Limited (BILT), Yamuna Nagar, Haryana, India.

Enzyme assay: Enzyme activity was determined through modified Bailey's method (1992) by measuring release of reducing sugars during enzyme substrate reaction according to Miller's method (1959). One unit of enzyme activity was defined as amount of enzyme that catalyzes the release of $1\mu\text{mol}$ of reducing sugars from respective substrates in 1 min under standard assay conditions.

Analytical studies: The different properties of pulp and paper like Kappa number, viscosity, holocellulose content, brightness and strength properties were determined according to TAPPI test methods (1996). Effects of different strategies on the properties of effluent were also investigated. Chemical oxygen demand (COD) was determined by open reflux method as per APHA (2005). Biochemical oxygen demand (BOD) was determined as per IS:3025, 1993 (part 44). Color was determined by spectrophotometric technique as per APHA (2005). Adsorbable organic halides (AOX) content of effluent was estimated as per ISO 9562, (1989) using Euroglas make AOX analyzer (Model ECS, 2000).

Pretreatment and bleaching of wood chips: Twelve day old, thick fungal mat, grown on liquid media (2.4% of potato dextrose broth, 0.75% of yeast extract; $30 \pm 2^\circ\text{C}$) was recovered by washing with sterilized saline solution using Buchner funnel. Homogenized fungal mycelium was diluted and used as inoculum for sterilized mixture of hardwood and bamboo chips (eucalyptus 80% and bamboo 20%) having 12% moisture. The chips were screened through 12×12 mm followed by 4×4 mm screens for the study.

Aerated bed bioreactor (25 l) was used for 1000 g oven dried mixed chips. Mixed chips were sterilized in an autoclave at temperature of 121°C and 15 psi pressure for 30 min. After this, the chips were kept for cooling and it was inoculated in a laminar air flow chamber by using stainless steel ladder, containing comprehensively mixed corn steep liquor (CSL, 0.6% on d.wt. basis) and fungal suspension (7.0 mg kg^{-1} ; d.wt. basis). Bioreactor was aerated with humidified air (30 l hr^{-1}) and maintained at $30 \pm 2^\circ\text{C}$ for 3 week. To avoid any contamination, compressed air was passed through a sterilized air filter. Before pulping, pretreated wood chips

were again characterized to determine their cellulosic, hemicellulosic, holocellulosic and lignin contents according to the standard methods (Parsons, 1963; Updegraff, 1969; Deschatelets and Yu, 1986). Kraft pulping of untreated and pretreated wood chips was carried out with 15% active alkali as Na_2O at 21.8% sulphidity and wood-to-liquor ratio of 1:3 at 160°C for 120 min with the H-factor of 870.

The biobleaching experiments were carried out in polythene bags and optimized for different parameters like: Dose of enzyme xylanase (450 to 550 g t^{-1}), pH (5.5 to 10.5), retention time (60 to 105 min), and temperature (45 to 70°C). The maximum reduction of Kappa no. was found with enzyme dose of 500 g t^{-1} , pH 9.0, retention time 90 min at 55°C . After extensive washing of pulp with distilled water, unbleached pulps from different strategy were refined to 31-32°SR (degrees of Schopper Riegler) for strength properties determination.

Most extensively used bleaching sequence, $\text{C}_0\text{E}_{\text{op}}\text{D}_1\text{D}_2$ (C_0 -chlorine and chlorine dioxide; E_{op} -extraction with oxygen and hydrogen peroxide; D_1 and D_2 -chlorine dioxide) was used for strategy-I and IV. Experiments were designed with different dosages of Cl_2 at the C_0 stage by applying a Kappa factor of 0.25 for calculating Cl_2 dose. The E_{op} stage was carried out in stainless steel vessel at $75 \pm 1^\circ\text{C}$ after adding the required caustic (25 kgt^{-1}) and hydrogen peroxide (H_2O_2 ; 5 kg t^{-1}). Pressure of oxygen was maintained at 5.0 and 2.5 kg cm^{-2} for initial 30 and final 90 min, respectively. The D_1 and D_2 bleaching stage treatments were carried out by using chlorine dioxide (ClO_2). Final pH in the reaction vessel was maintained by adding dilute H_2SO_4 before the addition of ClO_2 .

All experiments were carried out independently in triplicates and results presented are the mean of three. The \pm value represent the standard deviation (S.D).

Results and Discussion

Pretreatment of wood chips: There was 1.8% increase in holocellulose content and 3.9% reduction in lignin content after fungal pretreatment in comparison to control. It was due to the fact that lignin oxidizing enzymes secreted by *Phanerochaete* sp. deteriorated the lignin structure. Oxidation of lignin due to fungal pretreatment leads to its easier extraction which was reflected by increase in NaOH solubility of lignin by 13.8% in comparison to control. A similar observation has also been reported by Myers et al., (1998); Ferraz et al., (2000); Van Beek et al., (2007). Pretreated kraft pulp was subjected to C_0 , E_{op} , D_1 , D_2 bleaching steps. Optimum Cl_2 dose was calculated by multiplying Kappa number with 0.25. There was less consumption in Cl_2 (9.4%) and ClO_2 (15.4%) to obtain the same brightness as compared to control.

Process development for biobleaching ($\text{XC}_0\text{E}_{\text{op}}\text{D}_1\text{D}_2$): The enzymatic pretreatment resulted in better optical and strength properties of pulp. The brightness improved by 12.0% and there was significant improvement in viscosity (4.2%), reflecting better strength properties of the pulp.

Table - 1: Pollution load of effluent with different Strategy*

Parameters	Strategy -I	Strategy -II	Strategy -III	Strategy -IV
COD (mg l ⁻¹)	650	385	600	895
BOD (mg l ⁻¹)	250	130	215	325
Colour (PE-Co Unit)	410	385	400	550
AOX (mg l ⁻¹)	49.0	31.0	43.0	55.0

*Mixed effluent of all the stages (C_D, E_{OP}, D₁, D₂) in the ratio – 3.25:1:1:1.

Chlorination step along with extraction stages was generally viewed as continuation of delignification process and further lignin removal was more selective in chlorination and extraction stages than the pulping. Pulping to a bleachable grade removed over 90-95% of the original lignin and some portion of the cellulose and hemicelluloses also got dissolved (Hong *et al.*, 1989), hence 4-5% lignin still constituted the dry weight of unbleached pulp (Battan *et al.*, 2007). After xylanase pretreatment (X-stage), pretreated kraft pulp was subjected to C_DE_{OP}D₁D₂ bleaching steps. Optimum Cl₂ dose was calculated by multiplying Kappa number with 0.25 for strategy-III, IV and for strategy-II, it was 0.21.

With an optimized enzyme dose (500 kg t⁻¹), there was reduction in Cl₂ and ClO₂ in strategy-II and III. There was 37.4 and 20.3% less consumption of Cl₂ and 30.8 and 23.1% less consumption of ClO₂ to obtain the same brightness in strategy-II and III, respectively as compared to control strategy -IV, (Fig. 1). Numerous reports were available on the reduction in Cl₂ consumption by using enzymes (Beg *et al.*, 2000).

Improvement in various properties of pulp: Significant improvement in various pulp properties was observed with fungal pretreatment and biobleaching, compared to control. An increase of 9.3, 21.3, and 14.7% was observed in Tear index with strategy-I, II and III, respectively. On the other hand, reduction of 20.0, 27.5 and 22.5% in beating time (refining energy) was observed with strategy-I, II and III, respectively. The reduction in refining energy revealed the requirement of less energy for pulp to paper processing. Beating and refining were mechanical processes that enhance fibrillation and inter-fiber bonding. Properly applied, microbial enzymes enhanced the fiber strength, reduced beating time, and increased inter-fiber bonding through fibrillation.

Characterization of effluent: Efficiency of fungal pretreatment and potentials of biobleaching had been already reported (Baraznenok *et al.*, 1999; Maheshwari and Chandra, 2000; Damiano *et al.*, 2003). Most of these investigations were restricted to the study on improvement in physical properties of pulp. However, in present investigation, effluent of different bleaching strategies were also characterized to study the after effects of fungal pretreatment, enzyme aided bleaching and coupling of both on concentration of various toxic organic components (Table 1).

Effluent color, indicator of lignin content in effluent, was reduced maximum upto 30%. Through strategy-II. The lignin

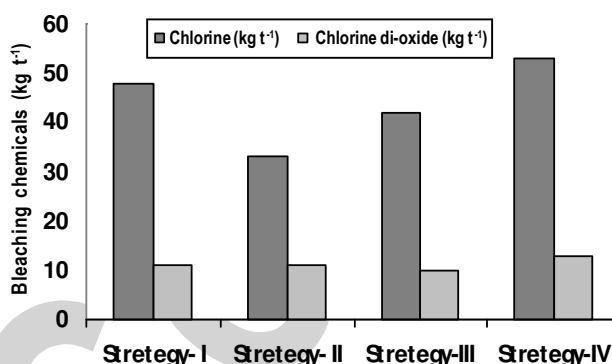


Fig. 1: Consumption of bleaching chemicals in different strategies

oxidizing enzymes produced by white rot fungus degraded lignin structure and its residual concentration was extracted through enzyme aided bleaching. Reduction in effluent colour was also reported by (Gutierrez *et al.*, 2000) using different white rot fungi.

As strategies-I, II and III were supplemented with biological methods, therefore less concentration of oxidizing chemicals were consumed and toxic compounds were released in low concentration during the treatments. Low values of COD in effluent clearly indicated the remedial efficiency of these strategies compared to control. Reduction of 27.3, 57.0 and 33.0% in COD was observed through strategy I, II and III, respectively. Like COD values, results were further supported by BOD values, where lowest (23.0%) and highest (60.0%) reduction was observed through strategy-I and II, respectively. The low generation of COD and BOD would result in reduction in power requirement for aeration during biological oxidation. Similar types of results were also reported by other authors where significant reduction in COD and BOD was observed (Wang *et al.*, 1994; Choi *et al.*, 2004).

Due to abridged utilization of chlorinated material, subsequent generation of halogenated compounds got reduced. It was supported by AOX (indicator of presence of halogenated compounds in effluent) values for bleaching strategies coupled with biological methods. Enzyme aided bleaching sequences followed in strategy-II and III were responsible for 43.6 and 21.8% reduction in the AOX, respectively. However 65% reduction in AOX contents was reported by Barroca *et al.*, (2001) using different chemical bleaching sequences. Similar result was also reported by Kengo *et al.*

al., (2006) where extended ClO₂ treatment at higher temperature was responsible to reduce the AOX content.

Comparative analysis of different bleaching sequences showed significant reduction in release of toxic organic compounds. Fungal pretreatment was successfully coupled with enzyme aided bleaching to achieve the highest removal of oxidizing chemicals. In comparison to control (strategy-IV), strategy-II resulted in reduction of COD, BOD, color and AOX upto 57, 60, 30 and 43.6% respectively. This novel approach can be used as bleach boosting agent and can be potentially used to develop the green technology for pulp and paper industry.

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