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Biobleaching of wheat straw-rich soda pulp with alkalophilic laccase from γ-proteobacterium JB: Optimization of process parameters using response surface methodology

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Abstract

An alkalophilic laccase from γ-proteobacterium JB was applied to wheat straw-rich soda pulp to check its bleaching potential by using response surface methodology based on central composite design. The design was employed by selecting laccase units, ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) concentration and pH as model factors. The results of second order factorial design experiments showed that all three independent variables had significant effect on brightness and kappa number of laccase-treated pulp. Optimum conditions for biobleaching of pulp with laccase preparation (specific activity, 65 nkat mg^{-1} protein) were 20 nkat g^{-1} of pulp, 2 mM ABTS and pH 8.0 which enhanced brightness by 5.89% and reduced kappa number by 21.1% within 4 h of incubation at 55 °C, without further alkaline extraction of pulp. Tear index (8%) and burst index (18%) also improved for laccase-treated pulp as compared to control raw pulp. Treatment of chemically (CEH_1H_2) bleached pulp with laccase showed significant effect on release of chromophores, hydrophobic and reducing compounds. Laccase-prebleaching of raw pulp reduced the use of hypochlorite by 10% to achieve brightness of resultant hand sheets similar to the fully chemically bleached pulp.

Keywords: Biobleaching; Pulp; Alkalophilic laccase; ABTS; Response surface methodology

1. Introduction

Cooking and subsequent alkaline extraction are the two main operational processes involved in the production of wheat straw-rich soda pulp from straw and other plant materials. During alkaline extraction, most of the lignin bound to cellulose fibers gets removed due to its high solubility in hot alkaline solution. Bleaching of pulp is necessary for the whitening of the paper and is based on the removal of residual lignin from the cellulose fibers but should have no adverse effect on cellulose fiber quality (Unal and Kolankaya, 2001). However, use of chlorine-based bleaching process tends to generate toxic and highly persistent chlorinated organic by-products, which eventually pollute the water bodies. Also, the use of such chemically-treated pulp/paper in manufacturing of direct-body-contact consumables like baby diapers and food packaging, is of major concern as former is associated with chlorinated compounds including animal carcinogen, dioxin (Shoham et al., 1992). Due to increasing health awareness and growing public sensitivity towards the negative environmental impact of chlorinated pulp, there is an ever growing demand for use of chlorine-free paper products all over the world. In this regard, the world is focusing research on the development of newer health- and environment-friendly technologies. Among these, biobleaching with enzymes has shown immense potential

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in minimizing use of bleaching chemicals containing chlorine. Use of oxidative enzymes such as laccases in pulp bleaching is still on laboratory or pilot scale (Call and Mücke, 1997).

Laccases belong to the multicopper oxidases (1.10.3.2) family, which can reduce elemental oxygen to water in a four-electron step and simultaneously perform a one-electron oxidation of many aromatic substrates (Messerschmid and Huber, 1990). Laccases are widely distributed enzymes in nature. The majority of laccases characterized so far have been derived from fungi especially from white-rot basidiomycetes that are efficient lignin degraders (Wood, 1980). In contrast, little is known about bacterial laccases, although recent rapid progress in the whole genome sequence analysis suggests that laccases are wide spread in bacteria (Sharma et al., 2007). Due to its high redox potential, laccase alone can effectively oxidize phenolic-lignin structure (Bajpai, 2004). The addition of mediators (small chemical compounds) extends the substrate range of enzyme to non-phenolic lignin structures as well. It is assumed that mediator is needed because laccase molecule can not enter the secondary cell wall due to its large structure and thus cannot oxidize lignin directly (Kandioller and Christov, 2001).

Response surface methodology (RSM) is a widely practiced approach for the production and optimization of various industrially important microbiological, biochemical and biotechnological products such as chemicals and enzymes (Chang et al., 2002; Beg et al., 2003). Based on the principal of design of experiments (DoE), the methodology encompasses the use of various types of experimental designs, generation of polynomial equations and mapping of the response over the experimental domain to determine the optimum product (Box and Draper, 1987; Singh et al., 2006). The technique requires minimum experimentation and time thus proving to be far more effective than the conventional methods of developing such products.

Only a few studies have been performed to develop alternative biobleaching systems using fungal laccases from *Trametes versicolor* (Archibald et al., 1997), *Coriolus versicolor* (Balakshin et al., 2001), *Pleurotus eryngii,* (Camarero et al., 2004) and *Pycnoporus cinnabarinus* (Georis et al., 2003) at acidic pH. Only one prokaryotic laccase from *Streptomyces cyaneus* (Arias et al., 2003) has been used for bleaching of kraft pulp at laboratory level, at pH 5.0. The present study is an application of alkalophilic laccase from γ-proteobacterium JB, in chlorine-free bleaching of wheat straw-rich soda pulp without further alkaline extraction. It is an attempt to derive an eco-friendly and chemical-free process for bleaching of pulp using RSM approach for optimizing various process parameters viz. enzyme dose, mediator concentration and pH involved in the bleaching process. The RSM may prove to be more productive and beneficial than the conventional technique by virtue of investigation of the effect of all the parameters simultaneously.

2. Methods

2.1. Pulp

Raw wheat straw-rich soda pulp [kappa number (measure of lignin content) 9–10; brightness 39–40 ISO% and consistency (% age of pulp in water) 10%] used in the bleaching experiments, was obtained from Shreyan paper mill (Punjab, India). The pulp was composed of (w/w) wheat straw (*Triticum astivum*) (78.8%), sarkanda (*Saccharum spontaneum*) (10.6%) and candy (*Eragrostis* sp.) (10.6%) cooked at 165–175 °C for 30 min at a pressure of 7.0–7.5 kg m^{-3}.

2.2. Microorganism and cultural conditions

γ-Proteobacterium JB, isolated from industrial waste water and identified previously in our laboratory (Bains et al., 2003) was maintained as a suspension in 20% glycerol at −70 °C and was routinely cultured on M162 medium (g L^{-1}: $CaSO_4 \cdot 2H_2O$, 0.4; $MgCl_2 \cdot 6H_2O$, 2.0; nitrilotriacetic acid, 1.0; 0.01 M ferric citrate solution, 5 mL; micronutrient solution (g L^{-1}: H_2SO_4, 0.5 mL; $MnSO_4 \cdot H_2O$, 2.28; $ZnSO_4 \cdot 7H_2O$, 0.5; H_3BO_3, 0.5; $CuSO_4 \cdot 5H_2O$, 0.025; $Na_2MO_4 \cdot 2H_2O$, 0.025; $CoCl_2 \cdot 6H_2O$, 0.045), 10 mL; yeast extract, 2; tryptone, 2 (Degryse et al., 1978). One milliliter of inoculum (overnight culture) was used to inoculate 100 mL of M162 medium, incubated at 37 °C and 150 rpm for 24 h. Culture supernatant obtained by centrifugation at 10,000 × g, 4 °C for 10 min was used as crude extracellular preparation with enzyme activity 7.8 nkat mL^{-1} and specific activity 65 nkat mg^{-1} protein.

2.3. Enzyme assays

Bacterial laccase activity was determined using 2 mM guaiacol as substrate, at 55 °C in 0.1 M phosphate buffer (pH 6.5). The change in absorbance due to oxidation of guaiacol in the reaction mixture was monitored at 465 nm ($\varepsilon = 48{,}000\ M^{-1}\ cm^{-1}$) for 10 min of incubation. Enzyme units were expressed in nkat (nmol of substrate converted $s^{-1}\ mL^{-1}$ of enzyme) (Singh et al., 2007). Assays for lignin peroxidase and manganese peroxidase were performed as described by Tien and Kirk (1988). Xylanase and cellulase assays were performed by dinitrosalicyclic acid method (Miller, 1959).

2.4. Pulp tests

Kappa number and brightness of pulp were determined according to Tappi (Technical Association of pulp and paper industry) test methods T 236 and T 452, respectively (Anonymous, 1991). Tear index (resistance of paper fibers to tear, $Nm^3\ g^{-1}$) and burst index (pressure required to rupture a standard sample of paper divided by its basis weight, KPa $m^2\ g^{-1}$) were determined using facilities available at mill. Denatured and active laccase-treated pulp