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Optimization of biobleaching of paper pulp in an expanded bed bioreactor with immobilized alkali stable xylanase by using response surface methodology

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Abstract

Purified alkali stable xylanase from *Aspergillus fischeri* was immobilized on polystyrene beads using diazotization method. An expanded bed bioreactor was developed with these immobilized beads to biobleach the paper pulp in continuous mode. Response surface methodology was applied to optimize the biobleaching conditions. Temperature (°C), flow rate of pulp (ml/min) and concentration of the pulp (%) were selected as variables in this study. Optimal conditions for biobleaching process were reaction temperature 60 °C, flow rate of 2 ml/min and 5% (w/v) of pulp. The kappa number reduced from 66 in the unbleached pulp to 20 (reduction of 87%). This system proves to be a better option for the conventional chlorine based pulp bleaching.

Keywords: *Aspergillus fischeri*; Biobleaching; Expanded bioreactor; Immobilization; Xylanase

1. Introduction

Xylan is the major hemicellulosic polysaccharide and it comprises up to 20–35% dry weight of wood and agricultural wastes. It is the major structural component of plant cell wall and the most renewable hemicellulose composed of α-1,4-linked β-D-xylopyranosyl residues. Among the annual plants, hardwoods and softwoods contain 20–25% and 7–12% xylan, respectively (Whistler and Richards, 1970). Xylanases, are pertoire of hydrolytic enzymes, facilitate the complete hydrolysis of xylan. In recent years, they have received a great deal of attention due to their potential application in food, feed, pulp and paper industries (Wong et al., 1988). Tolerant to high pH and temperature are credentials for xylanases to be effectively employed in pulp pretreatment, which improves the efficiency of conventional chemical bleaching and pollution control (Buchert et al., 1994). The environmental and legislative pressures have forced the pulp and paper industry to modify its pulping, bleaching and effluent treatment technologies to reduce the environmental impact of mill effluents (Bajpai et al., 1994). Biobleaching and bioprocessing of pulps using xylanases (Garg et al., 1998) is one of the most suitable biological applications to be used in the pulp and paper industry. Xylanases are being used, primarily, for the removal of the lignin–carbohydrate complex (LCC) that is generated in the kraft process and acts as physical barriers to the entry of bleaching chemicals (Yamasaki et al., 1981; Paice et al., 1992). Other significant benefits of this enzyme include higher brightness ceilings, a reduc-

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tion in the amounts of bleaching chemicals needed to achieve high brightness and reduced amounts of organo chlorine compounds in the bleach plant effluents (Ragauskas et al., 1994). A prerequisite in the pulp and paper industry, is the use of cellulase free xylanases that ensure minimal damage to the pulp fibres and also generate rayon grade or superior quality dissolving pulps (Jurasek and Paice, 1986). The use of abundantly available and cost-effective agricultural residues to achieve higher xylanase yields and simple, rapid purification procedures provide suitable methods to reduce the manufacturing cost of bio bleached paper, thus facilitating the adaptation of this environmentally friendly technology in the industry. The response surface method (RSM) is a statistical and mathematical method that involves main and interaction effects to account for curvature, to improve optimal process settings, and to troubleshoot process problems and weak points (Montgomery, 1996). It has been successfully utilized to optimize compositions of microbiological media, conditions of enzyme hydrolysis, and parameters for food preservation and fermentation processes (Senthilkumar et al., 2005; Lee and Chen, 1995). Previous studies have used conventional methods (such as one factor at one time) to evaluate enzymatic biobleaching of the pulp. These methods, however, require a large number of experiments to describe the effect of individual factors and are time consuming. Besides, no established statistical method has been introduced to distinguish the interaction effects from the main effects. Furthermore, up to now, there has been no reported study on the use of RSM to reduce xylan and chlorine consumption in the bleaching process. Thus, the aims of this study were to optimize the biobleaching conditions with immobilized xylanase in an expanded bed bioreactor through the response surface approach. This information will provide a better understanding of the interactions involved in biobleaching process at the industrial scale.

2. Methods

2.1. Microorganism and xylanase production

Aspergillus fischeri Fxn1 (Raj and Chandra, 1995) was propagated on potato dextrose agar medium (PDA) at 30 °C and maintained at 4 °C. The enzyme was produced in submerged fermentation using xylan as substrate at 30 °C.

2.2. Purification of alkaline stable xylanases

The two xylanases, Xyl-A and Xyl-B, were purified to homogeneity by a combination of ammonium sulphate precipitation, sephadex A-50 ion exchange chromatography, sephadex G-50 gel filtration chromatography and electro elution as described earlier (Raj and Chandra, 1996; Senthilkumar et al., 2004).

2.3. Xylanase assay

The xylanase activity was determined according to Bailey et al. (1992) using birchwood xylan (Sigma). A 50 μl of suitably diluted culture supernatant was added to 950 μl of xylan in 50 mM sodium phosphate buffer (pH 6) and incubated at 50 °C. After 10 min, the reaction was stopped by adding 1 ml 3,5-dinitrosalicylic acid reagent and the amount of reducing sugars released in the reaction was estimated by measuring absorbance at 540 nm (Miller, 1959). One unit of xylanase was defined as the amount of enzyme that is required to release 1 μmol of xylose per min under the assay conditions.

2.4. Modification of polystyrene beads

Polystyrene beads (3.5 g) were suspended in 20 ml of glacial acetic acid and stirred for 20 min at 60 °C. Twenty millilitre of 65% (v/v) nitric acid and 23 ml of 70% (v/v) sulphuric acid was prepared in an ice bath and mixed with the polystyrene beads and incubated at stirring condition. After 5 h the beads were washed with ice-cold water followed by 0.1 M NaOH and distilled water. The nitrated polystyrene denoted $PSNO_2$, was suspended in 15 ml of glacial acetic acid and mixed with 50 ml of 6 M HCl and 20 g of $SnCl_2{\cdot}2H_2O$. The hydrogenation reaction mixture was stirred for 60 h and the product was washed with 0.1 M NaOH and distilled water (Wu et al., 1998).

2.5. Immobilization of enzyme

The modified polystyrene beads were covalently coupled with xylanase by diazotization method. PS-NH_2 (3.5 g) was diazotized with 20 ml of 2 M HCl and 0.414 g of $NaNO_2$ in an ice bath for 20 min. The diazonium support obtained was immediately washed with ice-cold water and phosphate buffer, mixed with 10 ml (100,000 U) of xylanase solution. After incubation for 12 h the immobilized enzyme was collected by filtration and washed extensively with phosphate buffer (Wu et al., 1998).

2.6. Expanded bed bioreactor

The continuous biobleaching of paper pulp was carried out in an expanded bed bioreactor. The reactor body consists of a jacketed cylinder (50 cm height and 2.5 cm internal diameter). The working volume of the bioreactor was 250 ml and the bed volume was maintained at 125 ml with 86,000 U of xylanase immobilized in 3.5 g of PS-NH_2 (1.75 g of Xyl-A and Xyl-B immobilized PS-NH_2 beads). The temperature in bioreactor was controlled by continuous passing of water from temperature controlled water bath. The pulp was fed into the bioreactor using peristalistic pumps of various flow rates maintaining the expansion of the bed 165–175 ml.