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Immobilization of pectinase and lipase on macroporous resin coated with chitosan for treatment of whitewater from papermaking

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HIGHLIGHTS

- The dual-enzymes (pectinase and lipase) were immobilized on the macroporous resin coated with chitosan.
- The immobilized dual-enzymes exhibited high activities and excellent operational stability.
- The immobilized dual-enzymes were effective in removing anionic residues and pitch deposits in the whitewater from papermaking.

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ABSTRACT

Anionic residues and pitch deposits in whitewater negatively impact the operation of paper-forming equipment. In order to remove these substances, a macroporous resin based on a methyl acrylate matrix was synthesized and coated with chitosan of various molecular weights through glutaraldehyde cross-linking. Pectinase from *Bacillus licheniformis* and lipase from *Thermomyces lanuginosus* were immobilized on the resin coated with chitosan by a Schiff base reaction. The highest hydrolysis activities of the immobilized enzymes were achieved by using chitosan with 10×10^5 Da MW for coating and 0.0025% glutaraldehyde for cross-linking chitosan. The cationic demand and pitch deposits in whitewater were reduced by 58% and 74%, respectively, when treating whitewater with immobilized dual-enzymes for 15 min at 55 °C and pH 7.5. This method is useful for treatment of whitewater in the papermaking industry.

1. Introduction

Pectins (commonly called "anionic trash") and lipophilic extractives (commonly called "pitch" or "resin stickies") originating from the alkaline peroxide bleaching of mechanical pulps during papermaking tend to accumulate in whitewater and reduce the effectiveness of cationic retention aids, such as cationic polyacrylamide, polyethyleneimine, used to improve the retention of fibers, fines, and fillers in paper sheets (Yu and Deng, 2004; Boegh et al., 2001). The triglycerides in the pitch deposits can degrade product quality and impair the production process when they are deposited on exposed parts of the paper machine, such as on wires or airfoils. Consequently, anionic trash and pitch deposit controls in whitewater are important. In paper mills, the traditional strategy for reducing the contents of these troublesome substances has been the use of cationic polyelectrolytes for neutralization and of talc (Ho and Mckay, 2000; Bengtsson et al., 2008). Submerged membrane reactors have been explored for the treatment of paper mill wastewater, but membrane fouling limits their application (Lin et al., 2011; Simstich et al., in press; Qu et al., 2012). Due to the effects of pollution by the pulp and paper industry, replacement of the traditional processes with "green" processes are desirable.

Paper mills have begun to use enzymes to solve many problems that arise in the paper making process. For example, free pectinase is effective in decreasing the cationic demand of whitewater while also solving the retention problems (Ricard and Reid, 2004; Reid and Ricard, 2000) and lipase is able to degrade pitch deposits and destabilize colloidal particles (Rundlof et al., 2002). However, free enzymes dissolved directly in water not only can contaminate the paper, but are also difficult to recover. Compared to free enzymes, immobilized enzymes are more rigid and resistant to environmental changes, thereby increasing their thermal operational stability and recoverability (Bayramoglu et al., 2011; Zhao et al., 2011; Gao et al., 2009; Busto et al., 2006).

Therefore, in the present study enzyme immobilization was carried out. Although chitosan beads can be used as enzyme carriers

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(Liu et al., 2010), their poor mechanical strength makes them not very useful. Thus, chitosan was attached to a macroporous resin prior to enzyme immobilization. Since whitewater treatment is ideally done both with pectinase and lipase (“dual enzymes”), these enzymes were immobilized together. The operational stability of the immobilized enzymes and their efficacy for treating whitewater were also studied.

2. Methods

2.1. Materials

Yuhuan Ocean Biochemical Co., Ltd (Zhejiang, China) provided the chitosan (MW 1×10^5, 3×10^5 and 10×10^5 Da, degree of deacetylation 95%). Pectin from citrus peel, and bovine serum albumin (BSA) used as the standard for the protein assay were purchased from Sigma–Aldrich (USA). Pectinase from *Bacillus licheniformis* and lipase from *Thermomyces lanuginosus* were purchased from Novozymes (Denmark). Diaion HP2MGL and HP20 resins were purchased from Mitsubishi Chemical Corporation (Japan). Whitewater samples were provided by a Southern China Paper Mill producing high-brightness mechanical specialty grades from peroxide-bleached softwood thermo-mechanical pulp. The whitewater was sampled at the exit of the bleaching tower. The pulp had a consistency of about 10%. The fines in whitewater samples were removed with a 400-mesh sieve. The samples were stored at 4 °C. All other chemicals were of analytical grade and used without further purification.

2.2. Preparation of macroporous resin coated with chitosan and immobilization of dual-enzymes

Chitosan solution was prepared by dissolving 0.2 g of chitosan flakes in 25 ml of 5% (v/v) acetic acid. Then 2 g of macroporous resin was added and stirred for 10 h at a stirring rate of 150 rpm at 25 °C. After removing excess chitosan solution through vacuum filtration, the macroporous resin was suspended in 200 ml of 2.0 M NaOH/20% ethanol and stirred for 3 h. After separating the macroporous resin coated with chitosan and washing with deionized water, 10 ml of 0.0025% (v/v) glutaraldehyde solution was added and allowed to react for 30 min. Then 20 ml of dual-enzymes solution (containing 10 ml of 1% (v/v) pectinase and 10 ml of 0.1% (v/v) lipase) was added. After 3 h, the modified macroporous resin was washed thrice in deionized water to remove unbound enzymes.

2.3. SEM analysis and BET surface area measurement

The surface morphology of the macroporous resin with and without coated chitosan of 10×10^5 Da MW was analyzed with a scanning electron microscope (SEM, Hitachi S-3700 N). The BET surface areas of macroporous resin with and without coated chitosan were determined by N_2 adsorption using a Micrometrics model ASAP-2020 analyzer.

2.4. Pectinase activity

The activity of pectinase was assayed by measuring the amount of D-galacturonic acid liberated from citrus pectin. One gram of resin with immobilized dual-enzymes was added to the reaction mixture containing 0.5% (w/v) pectin in 5 ml of 0.05 M sodium phosphate buffer (pH 8.5). The reaction mixture was incubated for 30 min at 55 °C. The end product was analyzed using dinitrosalicylic acid (DNSA) reagent. One unit (U) of pectinase was defined as the amount of enzyme required to release 1 μg of D-galacturonic acid per min per milliliter under the assay conditions.

2.5. Lipase activity

The activity of lipase was assayed by titrating the fatty acid produced in the hydrolysis of olive oil. The substrate consisted of 4% (w/v) polyvinyl alcohol and olive oil (volume ratio 3:1). Four ml of substrate and 6.0 ml of sodium phosphate buffer (50 mM, pH 8.5) were mixed and placed at 55 °C, 0.5 g of resin with immobilized dual-enzymes was added and stirred. After reacting for 15 min, 15 ml of ethanol was added to terminate the reaction. The produced fatty acid was titrated using 0.05 M NaOH. Similarly, a blank experiment with added 0.5 g of resin without immobilized dual-enzymes was carried out. According to the amount of alkali consumed, the produced amount of fatty acid was calculated. One unit (U) of lipase was defined as the amount of enzyme required to release 1 μmol of free fatty acid per min under the assay conditions.

2.6. Protein assay

Protein content was estimated by the method of Bradford using Bio-Rad protein dye reagent concentrate. Bovine serum albumin was used as the standard (Bradford, 1976).

2.7. Operational stability of the immobilized dual-enzymes

For assessment of operational stability, the immobilized dual-enzyme system was used to hydrolyze poly-galacturonic acid or olive oil in sodium phosphate buffer (50 mM, pH 8.5). The conditions were the same as those used for the pectinase or lipase activity assay. After each cycle, the immobilized enzyme was filtered and washed with sodium phosphate buffer (pH 8.5).

2.8. Treatment of whitewater with immobilized pectinase(IP), lipase(IL) and dual-enzymes(IDE)

Five-hundred ml of whitewater was stirred for 5 min and maintained at 55 °C and pH 7.5. Then 40 g of IDE, IP, or IL was added at a stirring rate of 100 rpm and incubated for 15 min. The anionic trash content in whitewater was estimated by the cationic demand which was measured as follows. One ml of whitewater was extracted with a pipette, diluted 10-fold with distilled H_2O and titrated with 0.001 N poly diallyl dimethyl ammonium chloride (poly-DADMAC) by using a Mütek Particle Charge Detector (PCD-04) and Titrator (PCD-T). The size of the pitch deposit in whitewater before and after treatment with immobilized dual-enzymes was determined by using a Malvern-Sizer laser diffraction particle size analyzer. The turbidity of the whitewater samples was measured with a 2100AN turbidity meter (Hach). The depositable pitch contents of whitewater samples were measured according to TAPPI method RC 324. In this method, two stainless steel plates are vibrated rapidly in a sample of whitewater for 30 min at 55 °C and pH 7.5, the plates are removed and kept in the drying oven at 105 °C for 120 min. The net weight gain of the plates is an index of the amount of pitch deposit present in the whitewater.

3. Results and discussion

3.1. Screening of macroporous resin

Two types of macroporous resins (Diaion HP20 and HP2MGL resins) were investigated. The enzymes immobilized on resin HP2MGL coated with chitosan exhibited higher activities and the activities of pectinase and lipase reached 84.3 and 87.3 U/g-resin, respectively. However, the activities of pectinase and lipase immobilized on resin HP20 coated with chitosan were only 47.6 and