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Immobilization of pectinase on polyethyleneimine-coated pulp fiber for treatment of whitewater from papermaking

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ABSTRACT

After being coated with polyethyleneimine (PEI), pulp fiber was used as carrier for pectinase. When PEI concentration, enzyme concentration and pH of enzyme solution were 0.01% (w/v), 0.25% (v/v) and 7, respectively, the resultant immobilized enzyme exhibited the highest enzymatic activity as about 670 $\mu g\,min^{-1}\,g^{-1}$. Higher thermo and pH stabilities than those of the free enzyme were achieved after immobilizing the enzyme on PEI-coated pulp fiber. Furthermore, the bound pectinase effectively decreased the cationic demand of whitewater in papermaking under the practical industrial conditions and still maintained high activity after operating repeatedly for 8 batches. The results presented that through a simple, facile and inexpensive method, pectinase can be effectively immobilized on pulp fiber and the immobilized pectinase showed the potential application in practical industrial treatment of whitewater in papermaking industry.

1. Introduction

It is required to increase the closure of whitewater system in papermaking industry to minimize water consumption and effluent discharge for environmental concern. However, it also leads to the accumulation of dissolved and colloidal substances (DCS) in whitewater [1]. DCS generally have anionic characters and are referred to "anionic trash" since they can form polyelectrolyte complexes with added cationic chemicals, thus interfering with the papermaking process as well as the product quality [2,3]. Usually, the level of dissolved anionic trash is identified in terms of cationic demand. Among the negatively charged troublesome substances, pectic compounds, released from the fiber structure into the effluent, are a major contributor, especially to peroxide-bleached mechanical pulp [4].

Microbial pectinase has been used as an efficient biocatalyst to reduce cationic demand in peroxide-bleached mechanical pulp since it could decompose anionic pectin or polygalacturonic acid into monomeric galacturonic acid [5–7]. In spite of its advantages over traditional physical and chemical methods including high selectivity, mild reaction conditions and environmentally-friendly process [8], enzymatic method is often disturbed by low operational stability and difficulty in recycling of the enzyme [9,10]. Therefore, enzymes are generally immobilized on support to be recycled in industrial reactors, as well as to improve its stability and activity [11].

Cellulosic material has been explored as support for enzyme by several researchers mainly due to its advantageous properties such as hydrophilicity, insolubility in water, being environment friendly and abundance in nature [12–14]. Pulp fiber, another form of cellulosic material, is readily available in papermaking and can be made into diverse shapes. Since pulp fiber is the main component in the papermaking system, the pulp fiber used as enzyme carrier for treatment of whitewater can be discharged into pulp directly after the immobilized biocatalyst was ineffective, thereby further reducing operating cost. However, little research of enzyme immobilization on pulp fiber has been reported up till now.

Polyethyleneimine (PEI), a highly branched cationic polymer, is often used in papermaking as retention and drainage aid, strength additive, system to control detrimental substances, or pitch controller [15]. Apart from that, it has been reported that PEI is effective in immobilizing enzymes [16–18]. Since PEI is a commonly used additive in pulp in papermaking, the use of PEI for pectinase immobilization will be substantially cost effective without leading to extra contamination or high equipment cost for the existing papermaking system. In the present study, PEI-coated pulp fiber was prepared and utilized, for the first time, to immobilize pectinase for diminishing cationic demand of whitewater. The influence of immobilization conditions on the enzymatic activity of

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the immobilized pectinase was investigated and the properties of the immobilized pectinase were also evaluated. Furthermore, the effectiveness of the obtained bound enzyme on lowering cationic demand of whitewater from papermaking industry was investigated to test its potential practical applicability.

2. Experimental

2.1. Materials

Bleached kraft softwood pulp board from a Chinese mill was used. The pulp board was dispersed thoroughly in a disintegrator, screened by Dynamic Drainage Jar to remove fines passing 200 mesh, and then washed three times with distilled water. The prepared fibers with a moisture content of around 70% and α-cellulose content of 88.2% were stored at 4–5 °C. Commercially available pectinase Novozym 863 was supplied by Novozymes (Denmark). Pectin from citrus peel, polyethyleneimine (PEI, MW 750 kDa) as 50% (w/v) and glutaraldehyde (GA) as 25% (w/v) aqueous solutions were purchased from Sigma–Aldrich (USA). All other commercially available chemicals of analytical grade were used without further purification. Whitewater samples were provided by a Chinese Paper Mill producing alkaline peroxide-bleached mechanical pulp. The whitewater was sampled after bleaching tower and stored at 4 °C. The solution pH was adjusted, when necessary, using HCl or NaOH solution.

2.2. Preparation of PEI-coated pulp fiber

1 g (oven dry) pulp fibers were immersed in 30 mL 0.01% (w/v) PEI aqueous solution (pH 7.0). The adsorption was carried out in a shaker for 30 min at 20 °C. After adsorption, the pulp fiber was filtrated and extensively washed with deionized water to remove excess PEI except stated otherwise. This PEI-coated sample was used for immobilization of pectinase without drying. All tests were performed in duplicate.

2.3. Immobilization of pectinase on PEI-coated pulp fiber

The PEI-coated pulp fiber was immersed in 50 mL of 0.25% (v/v) pectinase solution (pH 7.0) and the mixture was incubated in a shaker for 1 h at 20 °C. Then glutaraldehyde (GA) was added (or not) to a final concentration of 0.1% (w/v) and the mixture was further cultured for 10 min. Subsequently, the fiber was filtered to remove unbound enzyme and then washed with deionized water. The resultant immobilized pectinase on PEI-coated pulp fiber was stored at 4 °C between uses.

2.4. Assays of pectinase activity

The activity of free/immobilized pectinase was measured by assaying the amount of reducing sugars released using pectin as substrate. 1 mL of 1% (v/v) free pectinase or 0.6 g of the immobilized pectinase was added to 5 mL 0.5% (w/v) pectin in 0.05 mol L^{-1} citrate buffer (pH 3.5) and the total volume was made up to 10 mL by adding buffer used. The reaction was carried out at 35 °C for 30 min. One unit (1 U) of enzyme activity was expressed as the amount of enzyme required to release 1 μg of galacturonic acid per min as quantified by the dinitrosalicylic acid (DNS) method [19].

2.5. Desorption of enzyme from PEI-coated pulp fiber

The desorption was performed in batch experiment. 1 g of the PEI-coated pulp fiber carrying enzyme was immersed into 20 mL buffer (pH 4.0) and the mixture was kept at 35 °C. The enzymatic activity in the solution was measured at different times.

2.6. Elemental analysis

The contents of carbon (C), hydrogen (H), and nitrogen (N) (%) were measured using an elementar Vario EL cube equipment (Germany). The amount of PEI adsorbed on pulp fiber was calculated according to the following equation:

$$(N/C)_{PEI-KP} = (N/C)_{PEI}x + (N/C)_{KP}(1 - x)$$

where $(N/C)_{PEI-KP}$, $(N/C)_{PEI}$ and $(N/C)_{KP}$ are the mass ratio of N to C in PEI-coated fiber, PEI and pulp fiber, respectively, and x (%) is the content of PEI in PEI-coated fiber. Then the obtained data could be converted to the amount of PEI adsorbed on pulp fiber (mg g^{-1}). Similarly, the enzyme loading was determined using the equation

$$(N/C)_{IE} = (N/C)_{E}y + (N/C)_{PEI-KP}(1 - y)$$

in which $(N/C)_{IE}$ and $(N/C)_{E}$ are the mass ratio of N to C in immobilized enzyme on PEI-coated fiber and enzyme, respectively, and y (%) is the content of enzyme. And y could also be converted to the amount of enzyme on per gram dried pulp fiber (mg g^{-1}).

2.7. Scanning electron microscopy (SEM) analysis

The pulp fiber, PEI-coated pulp fiber and PEI-coated fiber with immobilized enzyme were freeze-dried, sputtered with gold and examined by SEM using Hitachi S-3700 Scanning Electron Microscope (Japan).

2.8. Stability of the enzymes

pH stability: 0.6 g of the immobilized enzyme or 1 mL of the free enzyme was added to pectin in buffer of different pH values and incubated at 35 °C for 30 min. The relative activity was expressed as the ratio of the retained activity to the maximal activity of the enzyme.

Thermal stability: 0.6 g of the immobilized enzyme or 1 mL of the free enzyme was added to pectin in buffer (pH 3.5) and incubated at various temperatures (25–65 °C) for 30 min. The relative activity was expressed as the ratio of the retained activity to the maximal activity of the enzyme.

2.9. Treatment of whitewater with pectinase

Enzyme treatments and subsequent analyses were carried out without adjusting the pH of the whitewater samples; these samples had pH values of 7.2–7.3. The pectinase dosage was around 4000 U/L whitewater. 50 mL of whitewater was stirred for 5 min and maintained at 55 °C. Then 0.3 g (oven dry) of immobilized pectinase or 2.5 mL of 0.25% (v/v) free pectinase was added at a stirring rate of 100 rpm. The cationic demand was measured at intervals.

To evaluate the operational stability of the immobilized enzyme in whitewater, the whitewater treatment tests by the enzyme were performed repeatedly (20 min per batch). Between cycles the immobilized enzyme was filtered from the whitewater mixture, washed twice with deionized water and then added to an untreated batch of whitewater solution (50 mL).

2.10. Cationic demand measurement

Cationic demand was measured using the Mütek Particle Charge Detector (PCD-04) and titrator (PCD-T). The samples were diluted with deionized water and then titrated with 0.0001 N poly diallyl dimethyl ammonium chloride (poly-DADMAC).