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Immobilization of pectinase on oxidized pulp fiber and its application in whitewater treatment

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ABSTRACT

Modified pulp fiber was originally used as a new type of carrier for pectinase immobilization. Pulp fiber was oxidized by sodium periodate to produce aldehyde groups for covalently binding with amino groups of pectinase. Results showed that the enzymatic activity of immobilized pectinase on pulp fiber reached 65 $\mu g\, g^{-1}\, min^{-1}$ when immobilization pH value, temperature and time were of 7.0, 20 °C and 15 min, respectively. The immobilized pectinase showed higher thermo stability in a wider temperature range of 40–70 °C than its free type and its optimal pH shifted from 8.0 to 8.8. Furthermore, the immobilized pectinase exhibited good operational stability. When employed in whitewater treatment of papermaking industry, it still efficiently decreased the cationic demand after operating repeatedly for six batches. The results obtained demonstrate a promising route to prepare available, cheap and biodegradable carrier for immobilizing enzymes with potential application in wastewater treatment in papermaking industry.

1. Introduction

The uses of enzymes are increasingly being exploited for industrial applications due to high selectivity, mild reaction conditions and environmentally friendly process (de Carvalho, 2011). However, the practical application of free enzymes is often hampered by low operational stability and difficulties in recycling (Idris & Bukhari, 2011; Sheldon, 2007). Immobilized enzymes provide various advantages over free enzymes including rapid termination of reactions, facile separation of the enzymes and products from reaction media, repeated or continuous usage and simplification of the design of the reactors (Mateo, Palomo, Fernandez-Lorente, Guisan, & Fernandez-Lafuente, 2007; Sheldon, 2007). Due to these properties, immobilized enzymes have been successfully applied in many areas, such as heterogeneous biocatalysis, selective adsorption, controlled release of protein drugs, analytical device and solid phase protein industries (Cao, 2005).

The properties of the carrier materials have an overwhelming influence on the performance of the immobilized enzyme system. Many attempts have been made for the development of desirable support materials to achieve high catalytic activity and enzyme loading (Huang, Yu, & Xu, 2008; Sheldon, 2007). A variety of synthetic and natural organic polymers, as well as inorganic materials, have been explored for immobilization purpose. Of particular interest is the use of cellulose and their derivatives. Cellulose is the most abundant natural, renewable and biodegradable polymer in the world. Its long chains and loose mesh structure make it accessible for macromolecular infiltration and adsorption. Apart from that, there are a great number of hydroxyl groups in cellulose which impart cellulose chemical variability with high donor reactivity. Cellulosic materials are generally hydrophilic, insoluble in water, stable to chemicals, nontoxic, inexpensive and biodegradable, which makes them useful supports for enzyme immobilization (Klemm, Heublein, Fink, & Bohn, 2005).

To broaden its application, researchers have modified cellulose in many ways. Among the chemical modification methods, periodate oxidation is highly specific by cleaving the C2—C3 bond of the anhydroglucose unit and resulting in two aldehyde groups (Kim, Kuga, Wada, Okano, & Kondo, 2000; Kristiansen, Potthast, & Christensen, 2010; Princi et al., 2004). The aldehyde groups can couple with primary amines via a facile Schiff base reaction (Kim & Kuga, 2001; Maekawa & Koshijima, 1991). Thus enzymes would be immobilized on the cellulosic carrier. By using this method, several kinds of natural cellulosic materials, including cotton yarn (Nikolic et al., 2010), bagasse (Varavinit, Chaokasem, & Shobsngob, 2001) and cotton gauze bandage (Seabra & Gil, 2007), have been modified and successfully applied for enzyme immobilization. Pulp fiber, another form of cellulosic material, is readily available in

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papermaking and can be made into diverse shapes. Since pulp fiber is the main composition in the papermaking system, the immobilized enzyme on pulp fiber can be discharged into pulp directly after its activity decreases to some ineffective extent, thereby further reducing operating cost. However, till now no efforts have been made for the application of pulp fibers in enzyme immobilization.

As one of the most important industries, pulping and papermaking industry provides paper products for our daily use (Žarković, Todorović, & Rajaković, 2011). However, it also generates large quantities of by-products. In particular as a result of alkaline treatment steps, pectic compounds are released from the fiber structure into the effluent, being a major contributor to a phenomenon known as anionic trash. Usually, the level of dissolved anionic trash is identified in terms of cationic demand. Microbial pectinase has been used as an efficient biocatalyst in papermaking to reduce cationic demand in peroxide-bleached mechanical pulp (Liu, Li, Li, He, & Zhao, 2010; Ricard, Orccotoma, Ling, & Watson, 2005). It could decompose anionic pectin or polygalacturonic acid which account for much of the cationic demand in peroxide-bleached mechanical pulp. However, major limitations of free enzyme used in papermaking industry are the expensive process and the low operational stability of the biocatalyst (Sheldon, 2007; Mateo et al., 2007).

Our previous researches showed that immobilized pectinase on cross-linked chitosan beads may significantly lower the cationic demands of polygalacturonic acid solution (Liu et al., 2010). In order to further improve the activity/stability but lower the cost of the immobilized pectinase system for practical application in waste water clean-up, a new immobilization method by using modified pulp fiber was developed in this research. Pulp fiber was, for the first time, used as carrier for enzymatic immobilization in this research. The modification process of pulp fiber via oxidization was evaluated. The immobilization procedure and the properties of immobilized pectinase on pulp fiber were also investigated for the first time.

2. Materials and methods

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 51019 was supplied by Novozymes (Denmark). Pectin from citrus peel was purchased from Sigma–Aldrich (USA). Sodium periodate, glycerol and all other chemicals were purchased from local market, of analytical grade and 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. Buffer solutions used were 0.05 mol L^{-1} phosphate ($KH_2PO_4 + Na_2HPO_4$).

2.2. Oxidation of pulp fibers with sodium periodate ($NaIO_4$)

1 g (oven dry) pulp fibers were immersed in 50 mL $NaIO_4$ aqueous solutions of various concentrations (2 g L^{-1}/8 g L^{-1}/25 g L^{-1}). The pH value was adjusted to below 4 using sulfuric acid. The mixture was then stirred in the absence of light at 45 °C. The reaction was terminated by removal of $NaIO_4$ and addition of 40 mL 0.1 mol L^{-1} glycerol. Then the oxidized fibers were washed with deionized water several times. This oxidized sample was used for immobilization of pectinase without drying. All the oxidation tests were performed in duplicate.

2.3. Effects of reaction conditions on the oxidation rate of pulp fibers

Three different $NaIO_4$ concentrations (2 g L^{-1}, 8 g L^{-1}, and 25 g L^{-1}) were utilized to evaluate the effect of $NaIO_4$ concentration on the oxidation rate of pulp fibers. Different lengths of oxidation time were employed to study the effect of oxidation time on the oxidation rate of pulp fibers.

The $NaIO_4$ consumption was determined by UV absorbance at 290 nm. The formation of soluble fragments, as a result of cellulose destruction (i.e. cellulose chain scission caused by subsequent reaction, not by oxidation itself), was determined by measuring the weight loss of oxidized fibers by applying the direct gravimetric method (Janjic et al., 2009; Koblyakov, 1989).

2.4. Determination of aldehyde group contents

The aldehyde content present in the oxidized fiber was measured according to the method described in literature (Clift & Cook, 1932; Parkinson & Wagner, 1934). Oxidized fiber (0.3000 g) was added into a conical flask of 50 mL, followed by adding 10 mL deionized water and 10 mL sodium bisulfate (4.4 g L^{-1}). After being mixed, the flask was transferred to a gas bath rotary shaker under 180 r min^{-1}. The reaction was protected from light and lasted for 1 h at 25 °C. Then, after adding 0.5 mL starch indicator into the mixture, titration was immediately carried out using standard solution of I_2/KI (0.1000 mol L^{-1}). Blank test was conducted as the same except for adding oxidized fiber. The aldehyde group content was calculated according to the following formula:

$$\text{aldehyde group content (mmol g}^{-1}) = \frac{(v_0 - v)c}{m}$$

v_0 is the consumption of I_2/KI in blank test, mL; v is the consumption of I_2/KI, mL; c is the concentration of I_2/KI solution, mol L^{-1}; and m is the mass of sample, g.

2.5. Immobilization of pectinase on oxidized fiber

The oxidized fiber was immersed in 25 mL of buffer solution (pH 7.0) in a conical flask of 100 mL, and then 25 mL enzyme solution diluted by 400 times was added while stirring. The mixture was incubated in a gas bath rotary shaker with constant shaking at 20 °C. Subsequently, the fiber was filtered to remove unbound enzyme and washed with 300 mL distilled water. The filtrate and the washings were collected for protein measurements. The resultant immobilized pectinase on fiber was stored in refrigerator at 4 °C. All immobilizing tests were performed in duplicate.

2.6. Assay of protein loading

The amount of pectinase coupled onto the oxidized fibers was estimated from the initial protein amount present in the enzyme-coupling solution subtracting the final total protein amounts present in the remaining coupling solution and the washing solution by means of a mass balance, following the UV absorption method (John, 2002). The immobilization efficiency of the protein was defined as the amount of protein per gram of the support.

2.7. Assays of catalytic activities of the free and immobilized enzymes

The activity of free and immobilized pectinase was measured by assaying the amount of reducing sugars. One unit 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 method (Miller, 1959).