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Optimizing bioscouring condition of cotton knitted fabrics with an alkaline pectinase from *Bacillus subtilis* WSHB04-02 by using response surface methodology

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Abstract

This present study was undertaken to find optimum conditions of pH, temperature, incubation time and enzyme concentration for bioscouring of cotton knitted fabrics with an alkaline pectinase isolated from *Bacillus subtilis* WSHB04-02 by use of response surface methodology (RSM). A central composite design was used as an experimental design for the analysis of the allocation of treatment combination. A second-order polynomial regression model was fitted and was found adequate with a determination coefficient R^2 of 0.9844 ($P < 0.001$). The effect of pH was the most significant factor influencing cotton bioscouring and no significant interactions between different factors were found. Estimated optimum parameters were as follows: pH 9.1, temperature 57 °C, incubation time 1.25 h and pectinase concentration 1.0 g/l. Under these conditions, a desired pectin removal percentage companied with adequate wettability was reached. In addition, a boiling water pretreatment of 30 min before enzymatic scouring was found to be useful for subsequent pectinase degradation due to the improvement of the accessibility of pectinase to the pectins in cotton fibers.

Keywords: Optimization; Bioscouring; Cotton; Pectinase; Response surface methodology

1. Introduction

Cotton contains some non-cellulosic impurities such as pectins, waxes, proteins, ashes and others which mostly locating on the outer surface of the fibers [1]. The content and distribution of these impurities, especially waxes and pectins, are responsible for hydrophobic property of cotton fibers. The removal of the impurities present in raw cotton to obtain good absorbency or wettability is necessary for subsequent dyeing and finishing processes. This treatment is referred to as scouring processing. Conventional scouring is carried out at higher temperatures with caustic soda solutions, which has accompanying disadvantages such as high energy consumption and large amounts of strongly basic wastewater pollution and treatment problems. Many textile chemists have investigated the feasibility of replacing conventional alkaline scouring with environmentally friendly enzymatic scouring (i.e. bioscouring) during the last few years. Although a few enzymes such as pectinases [2–10], cellulases [2,3,9], proteases [9,11], cutinases [12], xylanases [3,13] and lipases [3,14] were screened and evaluated for their applicability for bioscouring, pectinases have been proven to be the most effective for cotton enzymatic scouring in all kinds of enzymes. The mechanism of pectinase scouring is believed to be the removal of pectic substances which adhere to cellulose and hydrophobic waxy materials as cements [3]. Pectic substances, commonly known as pectins, are basically complex mixtures of polysaccharides whose major components consist of α-D-galacturonic acid units linked by α-1,4-glycosidic bonds [15]. The degradation and removal of pectins will make cotton fibers achieve higher hydrophilic properties without fiber deterioration.

Degradation of pectins is catalyzed by a number of enzymes, which differ in their specificities, reaction mechanism, and action pattern [10,16]. Pectin lyase (EC 4.2.2.10) and pectate

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lyase (EC 4.2.2.2) can catalyze the cleavages of α-1,4-glycosidic linkage in pectin and pectic acid, respectively. Polygalacturonase (EC 3.2.1.15) hydrolyzes randomly α-1,4-glycosidic linkage in pectic acid. All the enzymes described are characterized by a random (endodepolymerase) type of action on the polymeric substrate. In addition, the methyl ester bonds in pectin are hydrolyzed by a pectinesterase (EC 3.1.11.1). So far different types of pectinases including acidic pectinases (hydrolases) [2,3,10] and alkaline pectinases (lyases) [4–9] have been applied in cotton bioscouring and positive results are obtained. Nowadays the most commonly used commercial pectinase is Bioprep 3000L from Novozymes, an alkaline pectinase with a standard activity of at least 3000 APSU (Alkaline Pectinase Standard Unit). However, the industrial scale application in China was greatly restricted because of its high cost and inadaptability for the cotton fabrics with inferior quality. Recently, we have successfully isolated and optimized the production of an alkaline pectinase by *Bacillus subtilis* WSHB04-02, which has better degradation ability of pectins in cotton under moderate temperature and buffered, mildly alkaline conditions and shows great application potential in bioscouring of cotton fabrics.

The conventional method used for optimization is the "change-one-factor-at-a-time" method in which a single factor or one independent variable is varied while fixing all others at a specific level. This is not a precise method and may lead to unreliable results and less accurate conclusions because of the existence of factor interactions [17]. Response surface methodology (RSM), which includes factorial designs and regression analysis, can better deal with multifactor experiments and assess the individual effect and interactions of different processing parameters. The predicted optimal value can be found from the estimated surface if the surface is shaped like a simple hill or a valley. If the estimated surface is more complicated, or if the predicted optimum is far from the region of experimentation, then the shape of the surface can be analyzed to indicate the directions in which new experiments should be performed [18]. Additionally, RSM decreases the number of experimental trials needed to evaluate multiple parameters and their interactions. It is less laborious and time-consuming than other approaches.

In recent years, although RSM has been successfully applied in many aspects of biotechnology [17,19,20], no reports focused on optimization of processing parameters of cotton bioscouring. This present investigation has been carried out with an aim to reveal the interactions of different processing parameters and optimize them for cotton knitted fabrics with an alkaline pectinase with lyase activity isolated from *B. subtilis* WSHB04-02 by use of RSM.

2. Materials and methods

2.1. Microorganism and enzyme production

B. subtilis WSHB04-02, isolated by our laboratory was used in the present study as source of pectinase.

The seed culture contained, per liter, 40 g infusion of soybean cake meal, 0.2 mol phosphate (KH_2PO_4–$K_2HPO_4 \cdot 3H_2O$ buffer, pH 7.0), 5 g glucose, 5 g yeast extract, 1 g $MgSO_4 \cdot 7H_2O$, 2.5 g $(NH_4)_2SO_4$, and 3 g corn steep liquor. The fermentation culture contained, per liter, 40 g infusion of soybean cake meal, 0.2 mol phosphate (KH_2PO_4–$K_2HPO_4 \cdot 3H_2O$ buffer, pH 7.0) and 8 g $CaCO_3$.

The inoculum was prepared in a 250 ml Erlenmeyer flask containing 25 ml of medium, while fermentation was done in 500 ml Erlenmeyer flasks containing 50 ml of medium. Cultures were started by the addition of slant contents and were incubated at 37 °C on a rotatory shaker maintained at 200 rpm for 14 h (inoculation) and 25 h (fermentation).

After 25 h of culture, when the pectinase activity was maximal, the contents of the flask were gauze-filtered to remove the mycelium. Ammonium sulphate was added to the supernatant to 60% saturation with mild agitation to remove miscellaneous proteins. The extract was then centrifuged at 10 000 × *g* for 10 min. The supernatant was removed and the precipitate was re-suspended in 20 mM phosphate buffer, pH 7.0 and dialyzed against the same buffer for 24 h. An ion exchange treatment with cellulose ion exchange chromatograph was then performed followed by further purification with Sephadex G-75 gel filtration chromatography and ultrafiltration. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) of this pectinase was carried out by using a 12.0% gel. The gel containing standard proteins with a molecular weight ranging from 14.4 to 97.4 kDa was stained with Coomassie brilliant blue R-250 (CBB) solution. SDS–PAGE showed only one band, corresponding to a molecular weight of about 43 kDa. The enzyme was stored in a sterile container at 4 °C until further use.

2.2. Assay for pectinase

The activity of pectinase using pectin as substrate were determined as previously described by Bruhlmann [21] with slight modification. One unit of enzyme activity was defined as the amount of the pectinase which formed 1 μmol/ml of unsaturated product from the pectins on cotton fibers per minute under the standard assay conditions. The activity of pectinase used in this work was 50 U/ml according to above assay.

2.3. Scouring of cotton knitted fabrics

Cotton knitted fabrics (14.5 tex, 150 g/m^2, supplied by Wuxi Knitting Factory, China) of 10 g were subjected to a boiling water pretreatment prior to the enzymatic scouring. The bioscouring was carried out in an enzyme bath with 0.05 mol/l glycine buffer, and a liquor ratio of 20:1, under designed experimental conditions. All experiments involved a WHYF-2F thermostatic bath (Shanghai Yuejin Co. Ltd., China) with a moderate stirring speed (90 rpm) in the presence of 0.1% non-ionic surfactant (TX-10) as a wetting agent. The samples were then washed in boiling water for 2 min to deactivate pectinase followed by washing with distilled water for several times. The treated cotton knits were air dried in the end. All the scouring experiments were duplicated.