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Research article

Immobilization of chitinase on charcoal improves ability of enzyme to hydrolyze chitin

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ABSTRACT

The chitinase is an enzyme has a good ability to hydrolyze chitin in nature. Thus this enzyme play an important role in clean the environments and in industries. The activity of the free chitinase to catalyze the chitin was evaluated in previous study. The activity of immobilized chitinase on a charcoal was not studied previously. In current study, the activity immobilized chitinase to hydrolyze chitin was studies. Purified chitinase was immobilized on charcoal then the activity of immobilized was checked and compared with activity of free chitin. The results showed that the activity of immobilized chitinase was significantly higher than the activity of free chitinase. The activity of immobilized chitinase was checked at different physical conditions like pH and temperature. Maximum enzyme activity to hydrolyze chitin was observed at pH 8 and temperature 40 ^{O}C. The present study showed that the activity of chitinase increased when the enzyme was immobilized on charcoal.

Keywords: Charcoal, Chitin, chitinase, Immobilization.

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INTRODUCTION

Chitin, a homopolymer of β (1,4)-linked N-acetyl-glucosamine, is one of the most abundant, easily obtained, and renewable natural biopolymers, second only to cellulose [Jeuniaux]. Chitin is considered the second most plentiful organic resource on the earth next to cellulose and is present in marine invertebrates, insects, fungi, and yeasts. Chitin and its derivatives have high economic value owing to their versatile biological activities and agrochemical applications [2]. It is now well known that the activities of those enzymes can be, in general, stabilized under a wide range of environmental conditions such as the pH and temperature although the actual activities of enzymes are usually reduced after having been immobilized on particles [3,4]. Chen and Chang (1994) purified chitinase from *Serratia marcescens* and immobilized by covalent binding to a polymer

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(hydroxypropyl methylcellulose acetate succinate. Their results showed that the capacity of immobilized enzyme to hydrolyze soluble chitin was higher than free enzyme and the activity of enzyme to soluble chitin was higher than insoluble chitin [5]. Other researchers immobilized lipase and chitinase on superparamagnetic particles, which are subjected to a rotational magnetic field, and measure the activities of the enzymes. It was found that the activities of lipase and chitinase increase in the rotational magnetic field compared to those in the absence of a magnetic field and reach maximum at certain frequencies [6]. The chitinase was purified from Bacillus subtilis and characterized in our laboratory [7].The characterization of mobilized enzyme was evaluated in previous study [7]. In present study, we try to improve the ability of chitinase to hydrolyze the chitin by immobilizing the enzyme on charcoal.

MATERIALS AND METHODS

Enzyme extraction and purification

The standard method of Ghafil (2013) was followed to extract and purify chitinase from B. subtilis [7].

Immobilization of enzyme on charcoal

1 ml of soluble enzyme that prepared previously was mixed with 1 g of clean dried and sterilized by autoclave. The mixture was incubated at 37 ^{o}C for overnight. After that the mixture was washed three times with sterile phosphate buffer saline. The affectivity of immobilization was checked according to activity of immobilized enzyme as comparing with free enzyme.

Enzyme activity

Chitinase activity was determined colorimetrically by detecting the amount of GlcNAc released from a colloidal chitin substrate. The reaction mixture consisted of 0.3ml of crude enzyme and 0.2ml of colloidal chitin. The reaction was performed at 37 °C for 1 h. The mixture was boiled for 10 min, chilled and centrifuged to remove insoluble chitin. The resulting adduct of reducing sugars was measured by DNA method. GlcNac was used as the standard. One unit of Chitinase activity is defined as the amount of enzyme that released 1 µmol of GlcNAc from colloidal chitin per minute [8].

Effect of temperature on the enzyme activity

The reaction mixture consisted of 1 g of charcoal that was bound with enzyme (chitinase). The reaction was incubated at different temperature (20, 30, 40, 50 ^{o}C) for 30 min. The mixture was filtered with Millipore filter (0.2 µ) and the supernatant was boiled for 10 min, chilled and centrifuged to remove insoluble chitin. GlcNac was used as the standard. One unit of Chitinase activity is defined as the amount of enzyme that released 1 µmol of GlcNAc from colloidal chitin per minute.

Effect of pH on the enzyme activity

Previous study of the effect of temperature on the activity of enzyme was followed but in this section the temperature was fixed and incubated in pHs (5,6,7,8,9).

Statistical analysis

All values were calculated as means ± sd. Student t-test was followed to camper between to different groups. Origin version 8.0 software was used to calculate all values and make graphs. A value of $p < 0.05$ was considered statistically significant.

RESULTS

In present study, the chitinase was incubated with chitin substrate in to status, free and immobilized form. The results of enzyme activity showed that the activity of enzyme was increased significantly in case of immobilized enzyme as compared with free enzyme (**Fig. 1**). The current study proved that the immobilizing of enzyme increase significantly the enzyme activity.

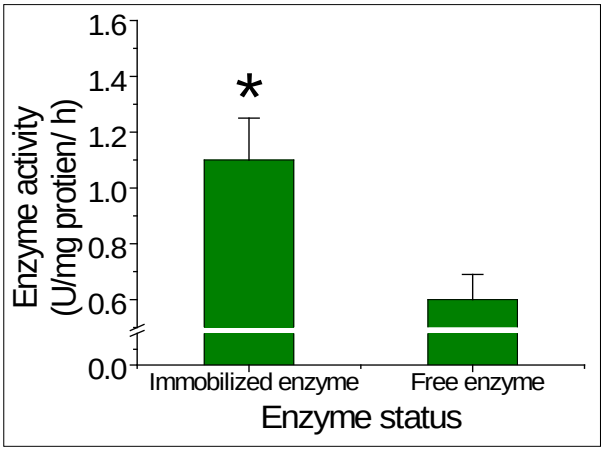

Fig. 1 The specific activity of immobilized chitinase on charcoal and free chitinase. Asterisk indicates the significant different of enzyme activity of immobilized enzyme from activity of free chitinase.

Effect of temperature on the enzyme activity

In present study, immobilized chitinase activity was checked at different incubation temperature. The present study showed that the activity of

immobilized enzyme increased dramatically with the time up to highest activity at incubation temperature 40 ^{o}C. However after this temperature the enzyme activity was decreased sharply (**Fig. 2**).

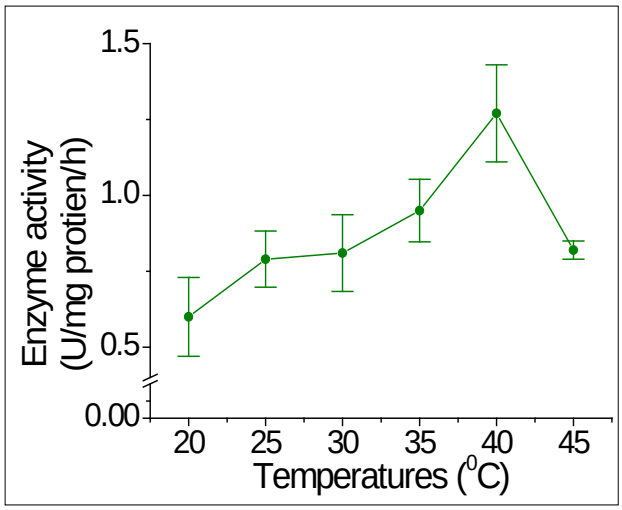

Fig. 2 Specific activity of immobilized chitinase at different incubation temperature. The maximum enzyme activity was found at temperature 40 ^{o}C.

Effect of pH on the enzyme activity

Fig. 3 shows the activity of immobilized chitinase at different pH values. The activity of enzyme increased when the level of pH increased. The maximum enzyme activity was observed at pH 8. The activity of immobilized chitinase decreased post this pH value. The present study demonstrated that the alkaline medium is the best medium for the work of immobilized chitinase.

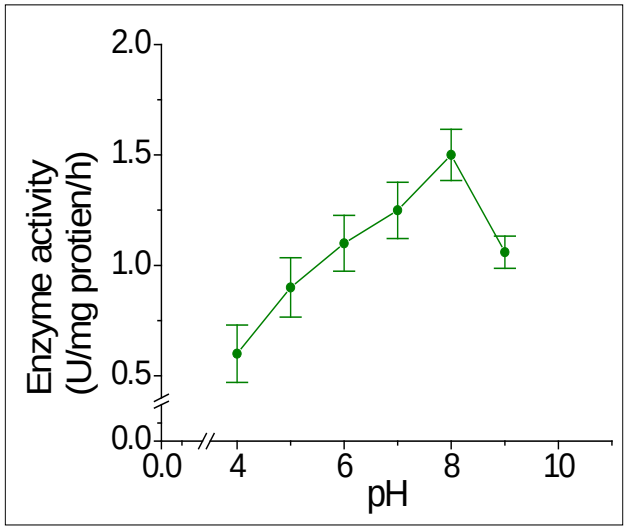

Fig. 3 Specific activity of immobilized chitinase at different incubation pH. The maximum enzyme activity was found at pH 8.

Discussion

Immobilization of enzyme is one of common way that used to improve the enzyme activity. This technology widely used in industry especially in food industries [9]. As this technology makes the enzyme work in high efficiency and specificity, moreover in this technique the controlling on the enzyme working is easily than free. The superiority of the immobilized enzyme is its easy separation with the reaction system, and can be recycled many times [10]. Several study focused on the immobilization of enzyme onto different innate surfaces [6,10]. Mizuki et al., (2013) immobilized chitinase and lipase onto superparamagnetic particles in a rotational magnetic field. They found that the enzyme activity increased decrease when the enzyme immobilized onto superparamagnetic particles but the activity of enzyme increased significantly post putting the particles in a rotational magnetic field [6]. In diagnostic field the enzyme immobilized on antibody for Appling in ELISA technique to determined levels of cytokines in body fluids such as blood [11].

In present study, the chitinase was immobilized onto charcoal particles. The enzyme activity of immobilized enzyme was compared with free enzyme. The results showed that the activity of immobilized chitinase was significantly higher than the activity of free chitinase. Further more the optimum enzyme activity of immobilized chitinase was at pH 8 and 40 ^{o}C.

As a result of enzyme immobilization, some properties of the enzyme molecule, such as its catalytic activity or thermal stability, become altered with respect to those of its soluble counterpart [12]. This modification of the properties may be caused either by changes in the intrinsic activity of the immobilized enzyme or by the fact that the interaction between the immobilized enzyme and the substrate takes place in a microenvironment that is different from the bulk solution. The observed changes in the catalytic properties upon immobilization may also result from changes in the three-dimensional conformation of the protein provoked by the binding of the enzyme to the matrix. The immobilization of enzyme results increase in the stability of enzyme and long life as well as increase in the specificity of enzyme that may explain the increase in the activity of immobilized enzyme [13]. The present study concluded that the immobilization of chitinase increased the specific activity of enzyme chitinase.

Conflict of interest

The author declares that he has no conflict of interests.

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