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Immobilization of pectin depolymerising polygalacturonase using different polymers

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ABSTRACT

Polygalacturonase catalyses the hydrolysis of pectin substances and widely has been used in food and textile industries. In current study, different polymers such as calcium alginate beads, polyacrylamide gel and agar-agar matrix were screened for the immobilization of polygalacturonase through entrapment technique. Polyacrylamide gel was found to be most promising one and gave maximum (89%) immobilization yield as compared to agar-agar (80%) and calcium alginate beads (46%). The polymers increased the reaction time of polygalacturonase and polymers entrapped polygalacturonases showed maximum pectinolytic activity after 10 min of reaction as compared to free polygalacturonase which performed maximum activity after 5.0 min of reaction time. The temperature of polygalacturonase for maximum enzymatic activity was increased from 45 °C to 50 °C and 55 °C when it was immobilized within agar-agar and calcium alginate beads, respectively. The optimum pH (pH 10) of polygalacturonase was remained same when it was immobilized within polyacrylamide gel and calcium alginate beads, but changed from pH 10 to pH 9.0 after entrapment within agar-agar. Thermal stability of polygalacturonase was improved after immobilization and immobilized polygalacturonases showed higher tolerance against different temperatures as compared to free enzyme. Polymers entrapped polygalacturonases showed good reusability and retained more than 80% of their initial activity during 2nd cycles.

1. Introduction

Advancement of biotechnology over last three decades, enzyme have drawn considerable interest of many biotechnologist because of having their excellent characteristics (high activity, selectivity and specificity under mild reaction conditions). Enzymes have been used in different industries including food, textiles, detergents and pharmaceutical industry. Enzymes are environmentally and economically more attractive as compared to organic catalyst because utilization of enzymes avoids the need of functional groups protections/activation, and provide a shorter synthetic routs for formation fine chemicals. Pectinase is a generic term used for a group of enzymes which catalyze the degradation of pectin substances by hydrolysis, trans-elimination and de-esterification reactions [1]. Among them, polygalacturonase is the most promising that completely hydrolysed pectin polymers into galacturonic acids monomers. Polygalacturonase has significant role in various industries with wide range of applications in different industrial processes such as fruit juices extraction, textile processing, paper making, pectin containing waste water treatment, degumming of plants bast fibers, wine clarification, oil extraction, coffee and tea fermentation [2–4]. But, the industrial applications of enzymes are often hampered due to low operational stability in harsh industrial conditions [5]. So there are many techniques such as protein engineering, chemical modification, adding additives and immobilization have utilized to engineer the enzymes from their native form to designed industrial bioreactor [6–9]. Immobilization not only increases the operational stability of enzymes but also makes them reusable for continuous industrial process. Immobilization is a process to confine or localize the enzyme within/onto the carrier and retained its activity for continuous uses. Different methods have been used for the immobilization of enzyme which can be categorized into three types such as binding of enzyme to a carrier, enzymes crosslinking and

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entrapment or encapsulation of enzymes within polymers. Entrapment is a simple and easy method for the immobilization of enzymes which induces no conformational modification on to the biological activities of enzymes. Up to date various supports have been used for the immobilization of polygalacturonase by different methods [10–15]. In this study, calcium alginate beads, polyacrylamide gel and agar-agar matrix were screened for the immobilization of polygalacturonase through entrapment technique. The kinetic properties of polymers entrapped polygalacturonases were evaluated with the comparison of free enzyme. The thermal and operational stabilities of entrapped polygalacturonases were investigated to evaluate the industrial feasibilities of polygalacturonase.

2. Materials and methods

2.1. Production of polygalacturonase

The production of polygalacturonase was carried out from *Bacillus licheniformis* KIBGE-IB21 in pectin containing production medium using submerged fermentation technology at 37 °C for 48 h [16]. After 48 h of fermentation crude polygalacturonase was separated from bacterial cell by centrifugation (10,000 rpm for 15 min) and precipitated through 50% ammonium sulphate saturation. The obtained precipitates were then dissolved in minimum amount of glycine–NaOH buffer (50 mM and pH 10) and dialyzed against the same buffer to remove the salts. The dialyzed enzyme solutions were used for the immobilization within different polymers.

2.2. Immobilization of polygalacturonase

The polygalacturonase was immobilized through entrapment technique using different natural and synthetic polymers. The immobilization yield was calculated through following equation;

$$\text{Immobilization yield}(\%) = \frac{\text{Activity of Immobilized Enzyme}}{\text{Activity of Free Enyme}} \times 100$$

2.2.1. Entrapment within calcium alginate beads

The immobilization of polygalacturonase was carried out by mixing sodium alginate solution (3.0%) with partially purified enzyme solution in 1:1 ratio [17]. The calcium alginate beads with 2.0 mm diameter of size were formed by adding mixture of sodium alginate and enzyme solution in 0.2 M $CaCl_2$ solution drop-wise at 4.0 °C through needle. Substitution of calcium with the sodium due to having strong affinity to alginate leads to gelatinization of alginate to form calcium alginate beads. Beads were hardened by keeping in $CaCl_2$ solution for 30.0 min at 4.0 °C. The calcium alginate beads were then washed dried, weighted and stored in buffer (50 mM glycine–NaOH buffer, pH 10).

2.2.2. Entrapment within agar-agar matrix

Agar-agar solution (3.0%) was prepared in glycine–NaOH buffer (50 mM and pH 10) by gently heating. Equal volume of enzyme and agar-agar solution (1:1 ratio) was mixed and immediately casted in pre-assembled glass plate. After solidification at the room temperature, the gel was cut into small pieces (2.0 mm in diameter) and washed several times before use. These pieces were stored in glycine–NaOH buffer (50 mM, pH 10.0) at 4 °C for further studies [18].

2.2.3. Entrapment within polyacrylamide gel

The entrapment of polygalacturonase within polyacrylamide gel was done by polymerization of acrylamide (9.5%) and N,N′-methylenebisacrylamide (0.5%) in the presence of 4.0 ml enzyme solution by the addition of 0.375 ml ammonium persulphate (20%) and 0.003 ml commercially prepared 14.4 M tetramethylethylenediamine (TEMED). Gel was cut into pieces of equal size and washed with glycine–NaOH buffer (50 mM, pH 10) and deionized water before use.

2.3. Enzyme assay

The enzymatic activity of free and polymers entrapped polygalacturonase were estimated through DNS method using citrus pectin as a substrate (1.0%) and galacturonic acid monohydrate as standard [19]. The enzyme assay was performed by addition of 0.05 ml of partially purified enzyme solution and 0.5 g of immobilized enzyme matrixes separately into 1.0 ml citrus pectin solution of pH 10 and incubated at 45 °C for 5 min. After 5 min, 0.5 ml reaction mixtures were withdraw from each tubes and added separately into 1.0 ml DNS reagent for the estimation of reducing sugar.

One unit of polygalacturonase is defined as "*the amount of enzyme required to release* 1 μmole *of galacturonic acid per minute under standard assay conditions*".

2.4. Scanning electron microscopy

Scanning electron microscopy was used to study the surface topologies of calcium alginate beads, agar-agar and polyacrylamide gel before and after entrapment of polygalacturonase. The dried samples were sputter-coated with gold using Auto coater (Model JFC-1500 Jeol, Japan) and micrographs were taken using scanning electron microscope (JSM 6380A Jeol, Japan).

2.5. Characterization of polymers entrapped polygalacturonases

2.5.1. Effect of reaction time on the activities of polymers immobilized polygalacturonases

The effect of reaction time on the activity of calcium alginate beads, agar-agar matrix and polyacrylamide gel entrapped polygalacturonases were separately investigated by performing the enzyme assay for different time interval (5.0–60.0 min) with the comparison of free enzyme.

2.5.2. Effect of temperature on the activities of polymers immobilized polygalacturonases

The influence of temperature on the activity of entrapped polygalacturonases were determined by measuring the enzyme reaction in different incubation temperatures ranging from 30 °C to 60 °C, while keeping the pH and reaction time constant.

2.5.3. Effect of pH on the activities of polymers immobilized polygalacturonases

Effect of pH on the activities of calcium alginate, agar-agar matrix and polyacrylamide gel entrapped polygalacturonases were determined by performing the enzyme substrate reaction in various pH levels ranging from 5.0 to 12.0 at constant conditions. Different buffers such as sodium acetate buffer (pH 5.0 and pH 6.0), potassium phosphate buffer (pH 7.0 and pH 8.0) and glycine–NaOH buffer (pH 9.0–12) were used in the experiment.

2.5.4. Kinetic parameters of polymers immobilized polygalacturonases

The kinetic parameters such as maximum reaction rate (V_{max}) and Michaelis–Menten constant values (K_m) of entrapped polygalacturonases were determined by measuring the rate of reaction in different substrate concentration ranging from 1.0 to 20 mg ml^{-1} in glycine–NaOH buffer (pH 10).

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